Universidad
de Jaén

Tesis Doctoral

Disrupción de los mutualismos planta-polinizador de *Ziziphus lotus* (L) Lam por pérdida de hábitat y degradación del paisaje:

Consecuencias para el flujo génico y la conservación de sus poblaciones en el sureste semiárido de España

Ana González Robles

Directores

Dr. Pedro J. Rey Zamora
Dr. Antonio J. Manzaneda Ávila

Jaén, 2019

UNIVERSIDAD DE JAÉN
Facultad de Ciencias Experimentales
Departamento de Biología Animal, Vegetal y Ecología

Disrupción de los mutualismos planta-polinizador de *Ziziphus lotus* (L) Lam por pérdida de hábitat y degradación del paisaje:

Consecuencias para el flujo génico y la conservación de sus poblaciones en el sureste semiárido de España

---

TESIS DOCTORAL

Ana González Robles

Jaén, 2019

Programa Oficial de Doctorado en Ciencia y Tecnología de la Tierra y del Medio Ambiente

UNIVERSIDAD DE JAÉN

Disrupción de los mutualismos planta-polinizador de *Ziziphus lotus* (L) Lam por pérdida de hábitat y degradación del paisaje:

Consecuencias para el flujo génico y la conservación de sus poblaciones en el sureste semiárido de España

Memoria presentada por la Licenciada en Biología Ana González Robles
para optar al título de Doctor por la Universidad de Jaén,
con la Mención de Doctorado Internacional.

Ana González Robles

Esta tesis ha sido realizada bajo la dirección de:

Dr. Pedro J. Rey Zamora   Dr. Antonio J. Manzaneda Ávila

Grupo de Investigación "*Ecología, Evolución y Conservación de la Vegetación Mediterránea*"

UNIVERSIDAD DE JAÉN

Dr. Pedro J. Rey Zamora, Catedrático de Ecología, y Dr. Antonio J. Manzaneda Ávila, Profesor Contratado Doctor, del Departamento de Biología Animal, Vegetal y Ecología de la Universidad de Jaén

CERTIFICAN:

Que el trabajo recogido en la presente memoria, titulada "*Disrupción de los mutualismos planta-polinizador de Ziziphus lotus (L) Lam por pérdida de hábitat y degradación del paisaje: Consecuencias para el flujo génico y la conservación de sus poblaciones en el sureste semiárido de España"* presentada por Ana González Robles, ha sido realizado bajo nuestra dirección y autorizamos su presentación y defensa para optar al grado de Doctor por la Universidad de Jaén, con la Mención de Doctorado Internacional.

Jaén, Abril de 2019

Fdo. Dr. Pedro José Rey Zamora   Fdo. Dr. Antonio J. Manzaneda Ávila

Este trabajo de tesis ha podido realizarse gracias a la Universidad de Jaén, por conceder a la autora una de sus *Ayudas para la Formación de Personal Investigador* del Plan Propio de Apoyo a la Investigación, Desarrollo Tecnológico e Innovación (Acción 16, convocatoria 2013); a la Junta de Andalucía, por la financiación del Proyecto de Excelencia RNM-766 (*Disrupción de los mutualismos de polinización y dispersión de semillas por fragmentación de hábitat: consecuencias para la conservación de poblaciones vegetales y hábitats en el sureste semiárido)*; y a los Fondos Europeos de Desarrollo Regional (FEDER) de la Unión Europea.

A José Luís, Ana Belén,

Feli y Mey,

mis cuatro pilares

Los cortijos comienzan a esparcirse.
A las huertas embarradas suceden los alijares
y las ramblas arenosas y desérticas.
La vegetación se reduce a su expresión más mínima (…)
A la derecha, la llanura se extiende hasta los médanos del golfo,
difuminada por la calina.
Los atajos rastrean el pedregal y se pierde entre
las zarzas y matorrales, chamuscados y espinosos.
Las nubes coronan las sierras del Cabo de Gata.
En el horizonte, el mar es sólo una franja de plomo derretido (…)
El camión atraviesa un arroyo de piedras, subimos la cuesta
y arriba, el paisaje es casi lunar. Alberos, páramos y canchales se suceden
hasta perderse de vista casi en el horizonte (…)
"Parece África, ¿verdad?" – dice leyéndome el pensamiento.

JUAN GOYTISOLO

Campos de Níjar (1960)

ÍNDICE

AGRADECIMIENTOS

Parecía mentira, pero como siempre he dicho, “esto tiene que terminar en algún momento, ¿no?”. Y finalmente, ese momento ha llegado. Ha necesitado 5 años de campo, laboratorio y ordenador pero… ¡¡aquí está!!

En fin, todos los que me conocéis sabéis que hablar, lo que se dice hablar…hablo un rato. Y que siempre que puedo intento idear algo que permita desconectar un poco de este trajín de vida que es el doctorado. Porque trabajar hay que trabajar, y mucho! Pero también hay que desconectar, para poder volver con las pilas cargardas los lunes. Así que bueno… 5 años, dan para hablar mucho y con mucha gente, y por supuesto para cargar la mochila de experiencias con todas ellas. Este trabajo no hubiera podido realizarse sin esa gente…sin todos vosotros. Por ello, anticipo que el apartado de agradecimientos PROMETE no ser corto, ya sabeís…si no, no serían MIS agradecimientos.

En primer lugar, tengo que agradecer a mis directores, ***Pedro J. Rey*** y ***Antonio J. Manzaneda***, la oportunidad de poder realizar este trabajo de tesis. Gracias por todo el esfuerzo que habéis empleado en mí. Por la paciencia. Por darme la oportunidad de aprender de vosotros y de mí misma, de superarme y crecer, no solo como científica si no también como persona. Siempre os estaré agradecida. Especialmente a ***Pedro***, por todas esas horas sentados delante del ordenador; por las correcciones siempre tan rápidas; por la comprensión (si bueno….como todo jefe también ha habido broncas, pero…siempre con cariño, que lo sé yo); por acompañarme en mis inicios a las tierras almerienses, donde el horizonte se pierde en un mar de plásticos y donde unos descampados y unas ramblas no son un lugar muy propio para que una “chiquita”(como dicen por Jaén) vaya sola; por las risas en los muestreos y por mostrarme esa otra versión de “jefe” que solo se ve fuera del despacho (y es que el jefe gana mucho en los muestreos de campo ☺). ***Antonio***, gracias por esos “que pasada, tíoo!!!” (jajaja) que tanto te caracterizan y por ese ambiente distendido que siempre creas, para un pre-doctoral es muy motivador ver que su coodirector se apasiona por su trabajo y por los resultados; gracias por enseñarme siempre con interés.

A ***Paco Valera*** (o mi “jefe infiltrado”, como yo le digo a veces) y, como no podía ser de otra forma, al queridísimo ***Perruni*** (“ for its assistance and advice!” (jajaja), Leo siempre tiene un hueco para mí, ya sabes que es mi debilidad). Por los ratos buenos en el campo, por tu inmensa ayuda y disponibilidad siempre que lo he necesitado, por acogerme cuando he estado sola por esas tierras, aconsejarme cuando he estado algo más perdida, y por supuesto por las innumerables risas que nos das siempre. Tengo tantas anécdotas contigo que me es difícil escoger alguna. Sin duda, una de las grandes oportunidades que me ha dado esta tesis es conocerte y poder trabajar contigo.

A ***Jesús***, "¡Yisuuuss, que lo he acabado!". Parecía que no iban a acabar nunca las 12 horas a 36-40°C en el fresquito almeriense, ¿eh? Ni que nuestros *Ziziphus* nos matasen a arañazos con sus caricias de amor cuando les cogíamos hojas. O como olvidar, ese "*Eau de zorro*" en los sobres, que luego invadia todo el Suzuki. Las hemos pasado "peluas" *Yisus*... tantas horas y tantos días juntos… pero que buenas conversaciones, risas y cantos hemos echado. Creo que tenemos el record de eficacia en multitarea la verdad (pero guárdame el secreto). Gracias por todo compi. Por ayudarme a entender (eres una gran cabeza pensante), por el apoyo y los ánimos, por todo el asesoramiento y la ayuda.

A ***Jorge***, mi Makoki preferido y uno de mis "niños" Life. Nos conocimos cuando yo llevaba menos de un año de doctorado, y aunque inicialmente entraste como estudiante de fin de grado, me venías a echar una mano siempre que podías con mis *Ziziphus*, a deshoras e incluso los domingos. Llenaste el campo y el laboratorio de buenos momentos. Los muestreos no hubieran sido lo mismo sin esa cabeza de oveja que tenías, sin tus pantalones pitillo o tus zapatos de *skaters* (si, ya sé que eso duró poco, y ya tienes botas…pero en su momento era tu sello de identidad ☺), tus clases de conducir, tu música en el suzuki, tus locuras y tu risa melón!!

A super-***Tere***, mi chica del GIS, sinceramente por todo. Por tu trabajo, tu compañía, tus ideas para quedar con el grupo, tu risa, tu humor. Por tu Pablo Alborán y Carlos Herrera sonando en el laboratorio (jajaja). Por esos días en los que todos llegábamos a la UJA esperando la hora del café porque Tere traía bizcocho o tarta (a esas reuniones no faltaba nadie eh! ☺). Siempre has sabido como quitarle preocupación a las cosas, dar buenos consejos y poner una gran sonrisa al día. Has sido mucho más que una amiga.

A ***Julio M. Alcántara***, junto con Pedro es la primera persona del grupo a la que conocí, en esa "entrevista" que ambos me hicieron para saber si era apta o no para el contrato. Por todas esas veces en las que he llamado a tu puerta diciendo "Julio, te puedo preguntar una cosilla..."; la respuesta siempre ha sido positiva aunque estuvieras liado. Muchas gracias por tu tiempo y por ese buen rollo que siempre transmites.

A ***Rafita***, nuestro querido Dr. House (by Paqueras), por el latazo que das en el laboratorio! (jajajaja) La verdad que has sido un puntazo desde el primer momento. Creo que el humor-onubense ha compajinado bien con el humor-granaino. Por tus frases con doble sentido, tus piques (a ver quien da más, pero siempre de “buenro” eh”), tu “oye me puedes coger esto” o “oye morena, échate un centímetro para allá que aquí no entro (cuando tienes ocupada toda la encimera)” o “oye ¿pero donde vais…me dejáis solo aquí?” Nunca olvidaré nuestra sesión de “los grandes existos de ayer y de hoy” en el laboratorio (junto con Paqueras). Gracias por esa forma de ser que tienes, personaje. Por cierto, como nos conocemos “Rafa por dios subete el pantalón” ☺ .

A ***Paco Camacho***, nuestro Paqueras/Pacorro (by Dr. House), por dejarte conocer, majo! y por todos esos momentos con los #EcólogosTrendingTopic! Esas PCRs nunca hubieran salido sin ti. Gracias por todo la ayuda en el laboratorio y por cuidar a los peque-*Ziziphu*s como si fueran tus hijos en mi ausencia portuguesa. Sin ti, esa parte no hubiera podido hacerse.

Al resto de ***Ujeño***s, o #EcólogosTrendingTopic, porque sois geniales e indiscutiblemente he tenido una suerte infinita de coincidir con todos durante estos años. Entre ellos, especialmente, a ***Lucía***, mi morena y mi gran compañera de sala, quien iba a decir que un primer día en Jaén lleno de cucarachas nos depararía esta amistad eh! (jajajaja). Sabes que has sido uno de mis mayores apoyos. Por tu positivismo, por la estadística y las charlas científicas, nuestras tardes de gimnasio, y nuestras ganas de volver locos al resto de #Ecólogos organizando salidas ujeñas. A ***Luisa***, mi Wendy, mi otra gran compañera, pero en este caso de laboratorio y de grupo. Por esas idas y venidas al jardín, a las cámaras, por tu musicote en el laboratorio, por las risas y por los momentos más agrios que ambas nos tomábamos con humor juntas, por ponerle siempre buena cara a todo. A ***Gäel***, nuestro gran cocinillas, por tus buenos ánimos, por el squash, las clases de Master Chef, por el buen ambiente que creas… ah y por esa feria! Sin ti nuestra sala estaría incompleta. A ***Dayana***, mi argentina indispensable, por tus ideas y tus ánimos, y por siempre estar ahí cuando lo he necesitado mi yo-yo, sabes que para mí eres familia. A ***Carlos***, el Charly del grupo, por preocuparse de si seguía viva durante este último año o me había subido ya por las paredes. Gracias por tus ánimos y charlas! A ***Manu*** por las buenas conversaciones. A ***Perea*** y ***Victor***, por los momentos distendidos y sacar siempre hueco para tomar algo.

José Luis, nuestro “Horse” y otro de mis “Life”, por todo el tercer año de tesis, por sacarme y desconectarme de este trajín mental, por incluirme como una más de los #Arvenses, por siempre acordarte de mí ☺. A ***Gemma***, por arrastrame al gimnasio a desconectar, por enseñarme a respirar más y preoparme menos, y junto con ***Miguel*** (El Negro) por quitarle peso al doctorado y darme muy buenos ratos sevillanos.

A la ***Dra. Cristina García*** y mi "jefa" portuguesa durante la estancia en el CIBIO (Vairão, Porto), por su indiscutible implicación en este trabajo de tesis. Por todo el tiempo que me has dedicado (durante y después de la estancia), por enseñarme y darme la oportunidad de aprender contigo, por todo Vila do Conde y por tu apoyo; eres única Cris! Y por supuesto, a ***Carol***, mi brasileña, eso último también va por ti. Sin el apoyo, la ayuda y los momentos con ambas la estancia hubiera sido muy distita. ☺.

A ***Lore*** (que incluso se animó a venir de muestreos *Ziziphus*) y ***Marta***, mis amigas desde el colegio, y con las que sería imposible haber realizado todo esto, porque ambas son mi familia. Por todo los momentos, y porque estos cinco años, como todos los anteriores, hubieran sido imposibles sin vuestro apoyo, aguante y cariño. A ***Sabe***, por seguir ahí "después", junto con esos Skype que tanto necesitaba, por interesarte por mi trabajo, por los momentos veraniegos y por tener siempre palabras de ánimo. A ***Lore*** (***la Gemela***), porque sin tú apoyo y tus charlas esto tampoco hubiera sido posible, gracias por sacarme siempre una sonrisa morena. A ***Javi***, el canario, porque aunque estés lejos siempre estás para mí. A ***Cherter***, por acogerme en Barcelona como si nos conociéramos de toda la vida, por tus dobles sentidos, tus whatsapp siempre a tiempo y por supuesto…por las viñetas de dinosaurios y curiosidades de animales que siempre sacan una gran sonrisa! A mi ***Jose Luís Postigo***, mi Capi del máster, sin ti no hubiera podido conseguir este contrato pre-doctoral, así que gracias por pensar en mí y por la oportunidad que me diste. A ***Rosita***, mi alumna de primero y ayudante de laboratorio cuando las clases se lo permitían, por enseñarme a ser profe, y por todo el esfuerzo que le pusiste a tu trabajo.

A ***José Luis*** y ***Ana Belén***, mis padres, no solo por sus consejos, su compañía y ayuda en los muestreos y durante todo el transcurso de esta tesis (os habéis pinchado mucho), si no por ser mi mayor ejemplo científico y personal. Gracias por los abrazos. A ***Feli***, mi abuela, porque igual que hace suyas todas mis caídas, también lo son todos mis logros, así que esto es tuyo. Siempre estás conmigo Buelus. A mi ***Mey***, mi hermana, que nunca deja que me caiga. Por decirme que siempre puedo y por darle el sentido correcto a la cosas :3

A ***Paco*** y ***Carmen***, mis ***tíos***, y por supuesto a ***Ñorton***, por todos esos sábados en Pinos cuando necesitaba aire; a mi ***prima Carmen***, por nuestros viajes gallegos, por aceptar mi limitada disponibilidad de tiempo y por quitarle importancia a los problemas; a ***mis primos Paco*** y ***Manuel***, por todas las horas de campo que habéis pasado conmigo para que no fuera sola por las dunas y plásticos, que han sido muchas (pero que muchas, muchas!) sin vosotros el primer año de muestreos de campo hubiera sido imposible.

A ***Charly*** y ***Lili***, mis tíos argentinos, por las charlas y consejos durantes los meses que pasasteis con nosotros. A la ***Dra. Concha Morales***, por descubrirme el amor por la botánica y el trabajo de campo, al igual que el ***Dr. Julio Peñas*** y ***Dr. Juan Lorite***, todos del departamento de Botánica de la Universidad de Granada, porque en su día me dieron la oportunidad de aprender y sin ellos no hubiera llegado aquí. A la ***familia del Hotel Los Arcos de El Alquián***, por hacerme sentir siempre como en casa aunque estuviera lejos de ella durante muchos días.

A ***Rubén***, mi último chico Life, y sin duda el mayor descubrimiento de esta tesis. Moreno, esto sin duda es tuyo, y todo lo que pueda decir sobre ti se queda corto. Por tener siempre las palabras indicadas y ser mis hombros, por enseñarme a ver la ciencia desde tus ojos y querer verla conmigo, por cruzarte en mi camino y molestarte en conocerme. Por quererme como soy.

A vosotros, y a todos aquellos que me habéis acompañado y aguantado durante estos cinco largos (y cortos) años, GRACIAS. Porque, como digo, esto no es solo mío, es de todos.

RESUMEN

La pérdida y degradación de hábitats constituye una de las mayores amenazas de los ecosistemas naturales, siendo la intensificación antrópica de los usos del suelo su principal responsable. Numerosas investigaciones han constatado cómo estas pérdidas de calidad y superficie de los hábitats naturales están estrechamente ligadas a disrupciones en las interacciones mutualistas planta-animal. Así procesos ecológicos indispensables para la regeneración y viabilidad de los ecosistemas, tales como la dispersión de polen y semillas, están experimentando graves limitaciones, con las subsecuentes consecuencias que eso tiene para la viabilidad de las poblaciones de especies vegetales. Cuanto menor es el tamaño de las poblaciones y mayor su aislamiento, más acentuadas son las consecuencias de estos cambios, donde aumenta también la susceptibilidad que éstas presentan a la pérdida de diversidad genética, cuellos de botella y deriva genética. Todo ello, pone en riesgo la persistencia de las poblaciones naturales, que ante cambios muy intensos y/o continuados puede llevarlas incluso a la extinción.

La estrecha relación existente entre los ecosistemas mediterráneos y el hombre desde tiempos prehistóricos ha hecho que los efectos que la pérdida y degradación de hábitats tienen sobre estos ecosistemas y sus especies sean el foco de numerosas investigaciones. Sin embargo, a pesar de ser una de las regiones más afectadas por la intensificación en los usos del territorio (principalmente con fines agrícolas y urbanísticos), pocos estudios de este tipo han sido llevados a cabo en sudeste semiárido de la Península Ibérica. El uso desmedido y mal gestionado de este territorio ha llevado a convertirla en la mayor superficie agrícola de invernaderos del mundo. Dentro del escenario de cambio global en el que nos encontramos, y siendo una de las prácticas agrícolas que más está en expansión por todo el globo, resulta indispensable entender sus efectos sobre el funcionamiento y viabilidad de los ecosistemas naturales adyacentes.

Esta tesis doctoral se centra en las poblaciones de *Ziziphus lotus* del sudeste semiárido peninsular (provincias de Almería y Murcia). Esta especie es considerada la especie clave de un hábitat prioritario para su conservación según la *Directiva Hábitats* de la Unión Europea, *Hábitat 5220*- Matorrales arborescentes con Ziziphus lotus.* Al menos desde la década de los 50 del pasado siglo, este hábitat ha experimentado elevadas tasas de pérdida y seria degradación y el proceso no ha parado a pesar de su catalogación. El objetivo general de este trabajo es evaluar en qué medida esta pérdida de calidad de hábitats, a escala local y de

paisaje, está influyendo sobre el ensamblaje de polinizadores silvestres, diversidad genética y flujo génico de las poblaciones de *Z. lotus* del sudeste de la Península Ibérica. Para abordar este trabajo, en el ***Capítulo 1*** se analizan los efectos que la degradación de hábitats y alteración del paisaje tienen sobre el ensamble de visitantes florales de *Z. lotus*, evaluando también el impacto que el uso agrícola (principalmente en invernaderos) de abejas manejadas tiene sobre el ensamblaje de visitantes florales silvestres. Todos estos aspectos se abordan desde una perspectiva taxonómica y funcional de los distintos ensamblajes. Nuestros resultados muestran que el sistema de polinización de *Z. lotus* es muy generalista y que la abundancias de abejas melíferas y sus visitas florales se asocian negativamente con la abundancia y visitas de insectos silvestres en las poblaciones de *Z. lotus*. La densidad de flores y las distancias entre poblaciones cercanas (escala local de degradación) muestran también efectos sobre la abundancia y visitas florales de visitantes silvestres, mientras la diversidad de usos del suelo (escala de alteración de paisaje) es el factor principalmente implicado en las diferencias de riqueza de visitantes encontradas entre poblaciones de *Z. lotus*. Los insectos silvestres no pertenecientes al grupo de las abejas resultan ser el grupo más sensible a todos estos efectos. En cualquier caso el impacto sobre los ensamblajes de polinizadores es más manifiesto a nivel taxonómico que a nivel de grupo funcional, lo que sugiere que el elevado generalismo del ensamblaje de polinizadores y el alto número de especies en los grupos funcionales pueda estar actuando como seguro para el cumplimiento de la función de polinización.

El ***Capítulo 2*** recoge los resultados del aislamiento y caracterización de 14 marcadores moleculares del tipo microsatélite (*SSRs*) específicos para *Z. lotus*, necesarios para llevar a cabo la evaluación de la diversidad y estructura genética de las poblaciones ibéricas de *Z. lotus,* la cual se realiza en el ***Capítulo 3***. Al mismo tiempo, este tercer capítulo analiza si la variabilidad genética de las poblaciones de *Z. lotus* es debida a aislamiento por distancia geográfica o consecuencia de la degradación, a escala de hábitat y paisaje, de las áreas naturales de *Z. lotus*. La región más alterada muestra los valores más bajos de diversidad genética. Según nuestros resultados, a pesar de que el 87.3% de la variación genética de las poblaciones de *Z. lotus* es detectada dentro de población, tres grupos genéticos definen la estructura genética de las poblaciones del sudeste peninsular: Oeste, Centro y Este. El aislamiento por distancia parece ser el principal factor de explicación de las diferencias genéticas poblaciones, manteniéndose una clara estructuración geográfica de la variación genética. Sin embargo la cobertura de hábitats naturales (variable a escala de paisaje) muestra también correlación con estas diferencias genéticas. La combinación de ambos resultados sugiere que, si bien las

características de historia de vida de la especie y sus mecanismos de dispersión de polen y semillas mantienen en gran medida la estructura y diversidad genética histórica de la especie, existe ya una señal de efectos genéticos adversos de la intensa degradación y pérdida de hábitat, que puede agravarse en el futuro.

Finalmente, en el ***Capítulo 4*** se analizan, para tres poblaciones de *Z. lotus*, representativas de diferentes niveles de alteración local del paisaje, las variaciones en los patrones de apareamiento y flujo génico dentro y entre las poblaciones, asociadas a la variación en características fenotípicas y ecológicas de sus individuos, presuntamente inducidas por la distinta degradación antrópica de sus poblaciones. A pesar de la gran alteración antrópica de su área de distribución en la Península Ibérica, las poblaciones de *Z. lotus* muestran una gran estabilidad en los patrones de cruzamiento, siendo la altura de los individuos y la densidad de flores, junto con la tasas de visitas florales por insectos, los principales factores determinantes de estos patrones. La distancia de dispersión de polen muestra sus valores mayores en aquellas poblaciones más grandes y continuas, pero sorprendentemente la mayor tasa de migración es mostrada en la población más alterada por agricultura intensiva bajo invernadero.

Considerados conjuntamente, nuestros resultados concluyen que: i) el uso de abejas melíferas con fines agrícolas, junto con la degradación de hábitats a escala local y de paisaje, suponen una amenaza para las comunidades de insectos silvestres que visitan y polinizan las poblaciones de *Z. lotus*, constatándose que un diverso ensamblaje de visitantes florales asegura una mayor estabilidad funcional del sistema; ii) existe un importante movimiento de polen a larga distancia, que promueve el flujo génico, el cual se ve favorecido por la estructura abierta de las poblaciones de *Z. lotus* y la gran movilidad y diversidad de su ensamblaje de polinizadores; iii) a pesar de que las características de vida larga y rebrote de nuestra especie de estudio y su sistema de polinización parecen estar retrasando la erosión en la diversidad genética de las poblaciones de *Z. lotus*, la intensa pérdida y degradación de su hábitat natural empieza ya a mostrar consecuencias genéticas en ellas, consecuencias que es esperable que se agraven en el futuro por el uso desmedido y la degradación continuada de su hábitat.

Asegurar la conectividad entre las áreas naturales y parches remanentes supone una de las principales medidas de gestión encaminadas a la mejora de la calidad de hábitats dentro de paisajes profundamente alterados por el ser humano debido al uso intensivo del terreno. Esperamos que este trabajo sirva para entender los efectos negativos que las prácticas agrícolas intensivas tienen sobre las poblaciones de plantas naturales, a la vez que para cubrir el hueco de conocimiento existente sobre este tema en los hábitats áridos/semiáridos.

SUMMARY

Habitat loss and landscape degradation, mainly by land-use intensification, are currently one of the major threats of natural ecosystems. There are growing evidences that the loss of natural habitat quality is closely linked to disruptions of plant-animal interactions. In this way, essential ecological processes for the plant regeneration and viability of ecosystems, as pollen and seed dispersal, are suffering serious limitations with negative consequences for plant population viability. Smaller and more isolated populations are also more sensitive to suffer genetic diversity loss and other genetic problems associated with bottlenecks and genetic drift. All these factors jeopardize the persistence of natural populations, leading even toward population extinction if these threats continue in long term.

Since prehistoric times a close relation between Mediterranean ecosystems and humans exists. Many studies have approached the effects of habitat loss and landscape degradation on Mediterranean ecosystems and their species. However, despite being one of the most affected regions by land-use intensification (mainly for agricultural and urban expansion), few works have been carried out in the semiarid southeast of Iberian Peninsula. Poor management of this territory has led it to keep the largest greenhouse area of the World. Being the greenhouse agriculture one the most expanding practices around the World, it is essential to understand its effects on the functioning and viability of surrounding natural ecosystems.

This Ph. D. dissertation is focused in *Ziziphus lotus* populations from the semiarid southeast of Iberian Peninsula (Almería and Murcia provinces). *Z. lotus* is a keystone species of a European priority habitat according to *Habitat Directive*, *Habitat 5220**- *Arborescent scrubs with Ziziphus lotus*. Since 1950s, this habitat has suffered a severe degradation rate, which has continued in spite of its rating as priority habitat for conservation. The main aim of this research is assessing how the loss of habitat quality, at local and landscape scales, is affecting wild pollinator assemblages, genetic diversity and gene flow of *Z. lotus* populations in the semiarid southeast of Iberian Peninsula. In this way, ***Chapter 1*** analyzes the effects of habitat degradation and landscape disturbance on *Z. lotus* floral visitor assemblage and assesses the impact of the abundance and visitation rates of managed honey bees on its wild floral visitors. Both issues are examined from a taxonomic and functional perspective. Our results show that *Z. lotus* has a very generalist pollination system and that the abundance of honey bees and their floral visits are negatively associated with the abundance and floral visits of wild insects.

Flower density and distance between nearby populations (associated to local-scale degradation) also show effects on the abundance and floral visits of wild visitors, while wild visitor richness differences among populations of are mainly explained by land-use diversity (alteration at landscape scale). Non-bee taxa are the insect groups most sensitive to land-use change. To a large extent, functional pollinator assemblages exhibit greater stability to the loss of habitat-quality than taxonomic assemblages. This suggests that the generalist pollinator assemblage, with high species number, could be acting as insurance for the pollination function.

Chapter 2 shows the results of the isolation procedure and characterization of 14 specific microsatellites markers (SSRs) for *Z. lotus*, which were necessary to carry out genetic diversity and structure analyses of Iberian *Z. lotus* populations that are subsequently presented in ***Chapter 3***. This third chapter also assesses if the genetic variability of the *Z. lotus* populations is due to geographic isolation or consequence of *Z. lotus* habitat degradation (at local and landscape scale). The most altered region shows the lowest values of genetic diversity. Three main clusters define the genetic structure of *Z. lotus* populations in our study area: West, Core and East region. Isolation by distance seems to be the main factor explaining population genetic differences, provoking a clear geographic genetic structure of the species. However, natural habitat cover (an indicator of the degradation at landscape level) is also correlated with these genetic differences. These results suggest that long life of this species and its pollen and seed dispersal systems have preserved high genetic diversity within the *Z. lotus* population. However, the severe habitat degradation is already showing negative genetic effects on its populations, which could be exacerbated in the future.

Finally, ***Chapter 4*** analyzes, in three *Z. lotus* populations representative of different levels of alteration at landscape level, the variations in mating pattern and gene flow within and among populations, and its relationships with variations in phenotypic and environmental features. Despite the huge alteration degree of its Iberian distribution area, *Z. lotus* populations show a great stability of the mating pattern, being individual height, flower density and floral visitation rates by insects the main determinants of these patterns. Larger and more continuous population display higher pollen dispersal distance, but surprisingly the highest values of migration rates are exhibited in the most altered populations by greenhouse agriculture.

Our results conclude that: i) the use of managed honey bees for agricultural purpose, together with the habitat degradation (at local and landscape scales) are threats for the wild insect assemblages that visit and pollinate to *Z. lotus*; it seems that a diverse taxonomic assemblage assures a great functional stability of the pollination function of this species; ii) an important long-distance pollen movement exits in the studied populations that promotes the gene flow and which is favored by the open spatial structure of the *Z. lotus* populations and the great mobility and diversity of its insect pollinator assemblages; iii) although some life-history traits of the species, as its long-life span, sprouting ability and pollination system, are delaying the erosion of the genetic diversity of *Z. lotus* in the region, signals of the genetic impact of the intense habitat loss and degradation on its populations are already detectable, which are expected to aggravate in the future.

Ensuring the connectivity among natural areas and habitat remnants is one of the main management measures to improve natural habitat quality within highly-altered landscapes. We hope this work will help to anticipate the negative effects that intensive agricultural practices and land conversion have on native plant populations, as well as to fill the gap of knowledge that exist in this subject in arid/semiarid ecosystems.

INTRODUCCIÓN GENERAL

Los cambios acelerados en los usos del suelo, debido principalmente a prácticas agrícolas intensivas o urbanísticas, están produciendo severas modificaciones de los ecosistemas terrestres a distintas escalas (Foley et al. 2005; ver **Fig. 1**). El juego combinado de la pérdida y fragmentación de hábitat representan uno de los principales promotores del Cambio Global y la crisis actual de biodiversidad (Sala et al. 2000). Hábitat naturales extensos son divididos en fracciones más pequeñas. De este modo, paisajes continuos son transformados en un conjunto de teselas de menor tamaño (parches naturales remanentes) inmersas en una matriz antrópica (Fahrig 2003, concepto fragmentación de hábitat). Sin embargo, estos cambios estructurales suelen ir acompañados de cambios composicionales, donde además de la subdivisión del hábitat natural en un mayor número de parches, ocurre una reducción en su área de ocupación (pérdida de hábitat) y consecuentemente un aumento de la distancia entre parches (Jackson y Fahrig 2016, ver **Fig. 2**).

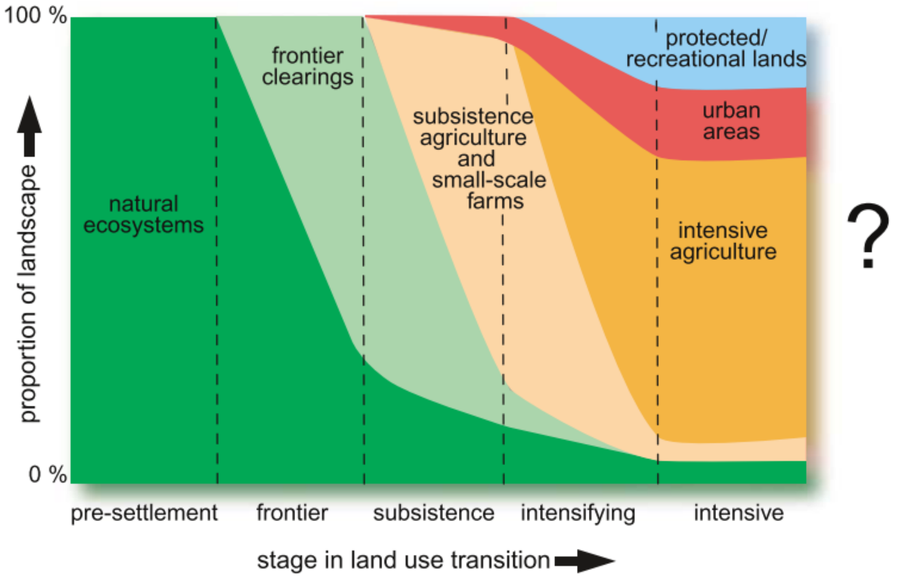


Fig. 1: Transición en los usos del suelo que una región puede experimentar a lo largo del tiempo, desde un paisaje 100% natural (izquierda) hasta un sistema donde las prácticas antrópicas intensivas dominan el paisaje (derecha). El tiempo de permanencia en una etapa y la velocidad de cambio dependen del área geográfica de estudio. Algunas regiones permanecerán más tiempo en estados intermedios, mientras que otras pueden experimentar más rápidamente la transición a etapas intensivas de los usos del suelo. [Extraído de Foley et al. 2005]

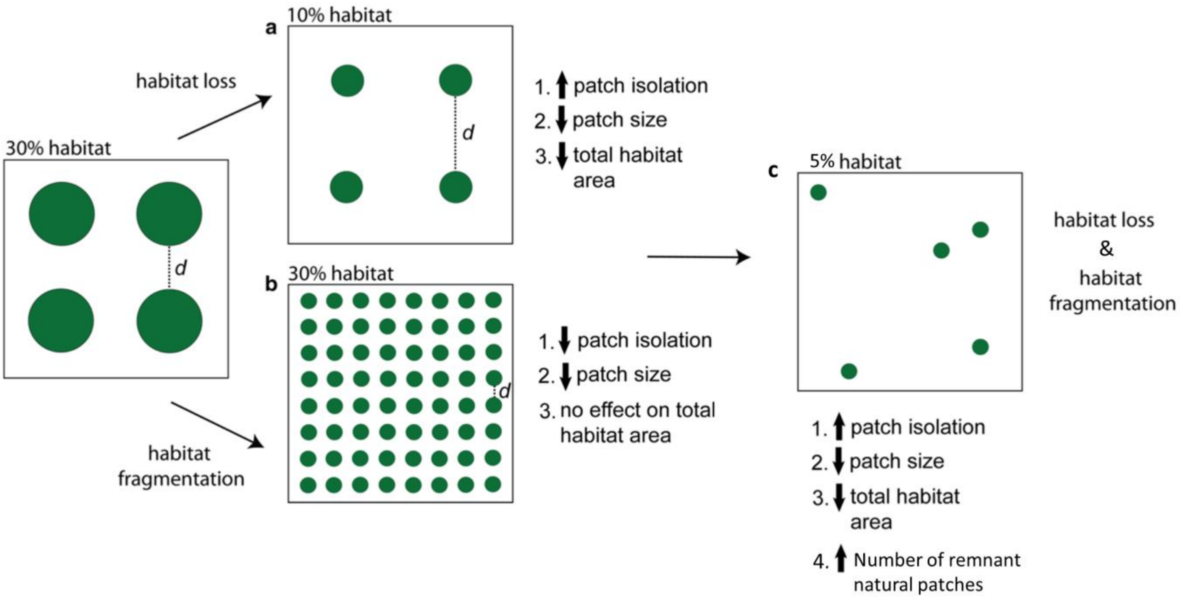


Fig. 2: Efectos de la pérdida (cambios composicionales) y fragmentación (cambios de configuración) de hábitat en ecosistemas naturales (verde = vegetación natural, blanco = matriz sin vegetación): (**a)** la pérdida de hábitat aumenta la distancia (*d*) entre parches remanentes, disminuyendo el tamaño medio de los parches y con ello la cantidad de hábitat disponible para una especie; (**b**) la fragmentación no conlleva una disminución en la cantidad de hábitat disponible, si no en el tamaño de los parches remanentes, pudiendo disminuir la distancia entre ellos; (**c**) cuando pérdida y fragmentación de hábitat ocurren al mismo tiempo la cantidad de hábitat natural disminuye y se produce un aumento del número de parches remanentes, los cuales se definen por un menor tamaño y mayor aislamiento entre ellos (caso típico de un sistema afectado por la intensificación agrícola y urbana). [Modificado de Jackson y Fahrig 2016]

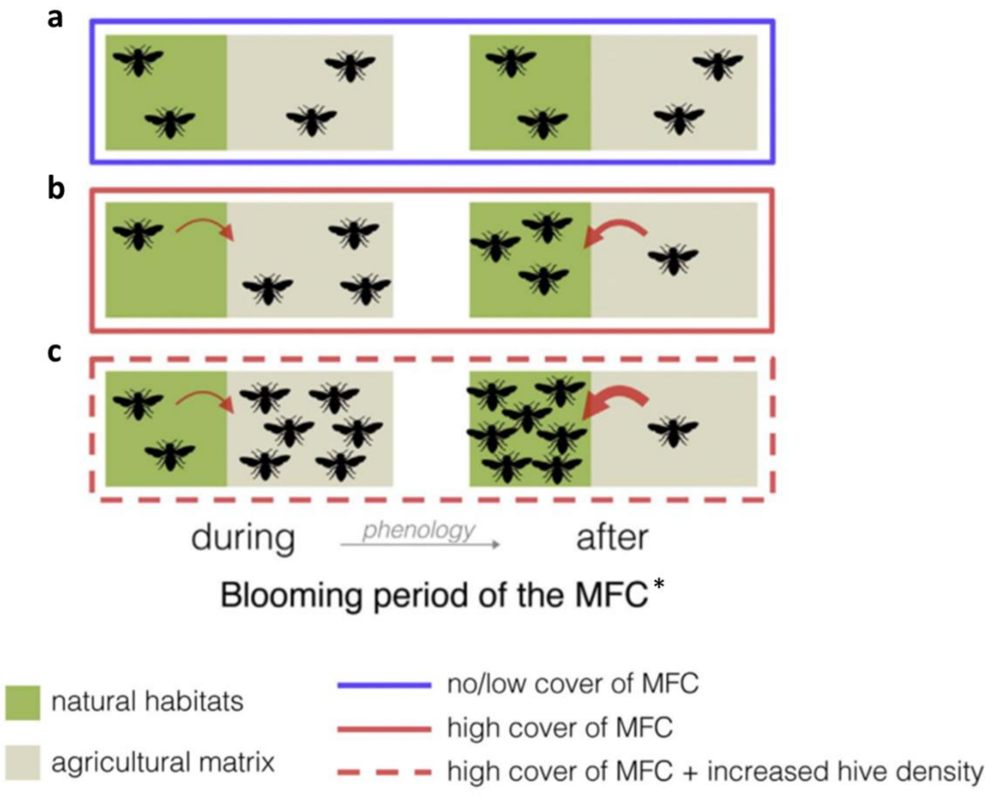
De este modo, la intensificación en el uso del territorio da como resultado un paisaje antrópico en el cual el aislamiento entre parches remanentes de hábitats naturales, con frecuencia de reducido tamaño, es cada vez mayor, lo cual a largo plazo acarrea graves consecuencias para las comunidades naturales que subsisten en ellos. La mayoría de las especies animales, cuando son incapaces de hacer frente a las nuevas situaciones, recurren al desplazamiento o cambio de hábitat (desde zonas más aisladas y degradadas a zonas naturales mejor conservadas), de forma que en qué medida se verán afectas es función de su capacidad de desplazamiento (Wereszczuk et al. 2017). Sin embargo, debido a su limitado movimiento, esto es algo a lo que las especies de plantas no pueden recurrir, haciéndolas uno de los grupos más sensibles a los cambios severos de usos del suelo (Young et al. 1996). Alteraciones en importantes procesos ecológicos para la demografía, dinámica y flujo génico de las poblaciones vegetales, como son la dispersión de polen y semillas, han sido detectados en distintas especies de plantas dentro de ambientes degradados como consecuencia de disrupciones en las interacciones planta-animal (Benton et al. 2003, Jordano 2010, Pott et al. 2010). Poblaciones más pequeñas y aisladas ven así dificultado su flujo génico con otras zonas, lo que las hace más propensas a sufrir problemas de erosión genética y, a largo plazo, más susceptibles a diferenciación por deriva genética o incluso a la extinción (Frankham 2003, Lowe et al. 2005, Browne y Karubian 2018).

Efectos de la pérdida de hábitat, degradación del paisaje e intensificación agrícola sobre comunidades vegetales

Visitantes florales y polinización

Las comunidades de insectos definen unas de las interacciones ecológicas más importantes de los sistemas naturales y agrosistemas, son las llamadas relaciones mutualistas planta-polinizador. Debido a su incapacidad de movimiento, las plantas emplean recompensas florales, como el néctar o el polen para atraer a insectos visitantes hasta ellas (Kearns et al. 1998). Estos insectos a cambio de alimento se encargaran de transportar los granos de polen de unos individuos a otros, posibilitando así la polinización a media y larga distancia (Klein et al. 2007). En general, los insectos son considerados uno de los grupos animales más eficaces en la polinización de especies vegetales, por lo que su presencia en los sistemas naturales resulta indispensable. Sin embargo, las continuas modificaciones y cambios en los usos del suelo, especialmente hacia sistemas altamente intensificados, están produciendo graves alteraciones en sus niveles poblacionales (Winfree et al. 2011; Kenedy et al. 2013).

En los últimos años, numerosas investigaciones han puesto de manifiesto cómo la degradación y pérdida de hábitat debida a prácticas intensivas son responsables de disminuciones de diversidad y riqueza de especies en la comunidad de polinizadores (Aizen et al. 2009, González-Varo et al. 2013, Garibaldi et al. 2013), siendo reconocidos como uno de los principales responsables de la actual crisis de polinizadores. En principio, parece clara la idea de que la expansión urbana y el uso de productos agrícolas tienen efectos adversos sobre las comunidades de insectos silvestres. Aunque se ha visto que determinados grupos de insectos son capaces de sobrevivir en hábitat antrópicos, la mayoría de ellos siguen teniendo una mayor predilección por los hábitat naturales/semi-naturales (Collado et al. 2019). Por un lado, el aumento de la superficie urbanizada supone una disminución de áreas naturales, y por tanto de los recursos disponibles para estas especies silvestres. Por otro lado, el uso agrícola de pesticidas y productos químicos de amplio espectro produce una disminución no solo de los insectos plaga sino también de toda la entomofauna asociada a esos sistemas, afectando así a los insectos de hábitats colindantes menos alterados (Potts et al. 2010, Gill et al. 2016). Dada la escasez de polinizadores, y con la finalidad de aumentar el rendimiento de los cultivos, el uso de colmenas comerciales, principalmente de *Apis mellifera* (abeja doméstica de la miel), se ha convertido en una práctica agrícola ampliamente extendida (González-Varo y Vilà 2017). Sin embargo, hoy en día, no está claro el efecto que el uso no regulado de estas prácticas puede tener sobre las comunidades de insectos silvestres, existiendo una gran controversia en el mundo científico sobre ello. *A. mellifera* se caracteriza por tener una alta tasa de visitas florales, siendo especialmente atraída por aquel recurso más dominante en el medio. Distintos autores han constatado escapes de abejas manejadas desde los cultivos, una vez ha terminado su periodo de floración, hacia hábitat naturales contiguos (fenómeno denominado "spillover" Holzschuh et al. 2016, González-Varo y Vilà 2017, Magrach et al. 2017; ver **Fig. 3**). Para los polinizadores silvestres estos escapes suponen una competencia importante por recursos alimenticios (Torné-Noguera et al. 2016), lo que pone en peligro no solo la diversidad taxonómica de estos sistemas, sino también su funcionalidad. La pérdida de taxones claves o minoritarios lleva consecuentemente a la pérdida de funciones desempeñadas por ellos (diversidad funcional), que de no poder ser remplazadas por otros taxones, debilitan y ponen en peligro la estabilidad de estos ecosistemas (Loreau et al. 2003). Donde la pérdida y degradación de hábitat naturales se combine con el manejo de abejas para la polinización de cultivos intensivos, los ensamblajes de polinizadores naturales, y con ello la polinización de especies vegetales en los remanentes de hábitat natural, podrían verse seriamente afectados por efectos aditivos o sinérgicos de ambos.



* MFC = Mass flowering crop

Fig. 3: Patrones espacio-temporales esperados en la densidad de abejas melíferas en cultivos y hábitats naturales colindantes durante y después del período de floración de los cultivos: (**a**) muestra un escenario en el cual, al haber una baja cobertura de cultivos, los niveles de abejas melíferas en ambos períodos y hábitats se mantienen constantes; (**b**) muestra un escenario con una alta cobertura de cultivos, donde se produce un aumento de la densidad de abejas melíferas en hábitat naturales después del período de floración de los cultivos; (**c**) muestra un escenario en el cual además de una alta cobertura de cultivos hay una alta densidad de colmenas comerciales, produciéndose una sobrecarga del número de abejas melíferas en hábitat naturales tras el período de floración de los cultivos. La dirección de las flechas alude al sentido de los movimientos de las abejas de un hábitat a otro, mientras que su grosor alude a la magnitud de esos movimientos [Extraído de González-Varo y Vilà 2017]

Estructura y diversidad genética

La reducción de áreas naturales conlleva una disminución en el número de individuos que conforman sus poblaciones de plantas (Jackson y Fahrig 2016), por lo que una de las principales preocupaciones de la ecología y biología de la conservación son las implicaciones que la degradación y pérdida de hábitat tienen sobre el componente genético de las poblaciones vegetales (Templeton et al. 1990, Wang y Bradburd 2014). Sin embargo, la eliminación de individuos por pérdida de hábitat no es selectiva. Esto ocasiona cambios en la diversidad genética de los nuevos parches, generalmente hacia valores menores (pérdida alélica), produciendo un aumento de la homocigosis (Young et al. 1996, Frankham 2003, Lowe et al. 2005, Browne y Karubian 2018). Determinados alelos raros o deletéreos que antes se encontraban en bajo número en poblaciones naturales extensas, pueden llegar a fijarse en las nuevas poblaciones más reducidas a través de procesos de cuello de botella y deriva genética, con el consecuente detrimento del potencial adaptativo que eso supone para sus individuos (Templeton et al. 1990, Sork et al. 2002, Aguilar et al. 2008). Al mismo tiempo, hábitats extensos al quedar subdivididos en parches más aislados y de menor tamaño, verán dificultado los flujos de polen entre sus individuos (Ellstrand 2014). Cuando las matrices antrópicas en las que se ven inmersos estos parches remanentes de hábitats son muy extensas y poco permeables para esos organismos, estos movimientos pueden verse totalmente limitados. Cuanto mayor es la distancia entre parches remanentes de hábitats, menos probable es que se produzcan intercambios de polen entre ellos (Sork y Smouse 2006, Hahn 2018), quedando los más pequeños y aislados más expuestos a sufrir procesos de endogamia y deriva genética (Lander et al. 2010, Aparicio et al. 2012). A largo plazo si se supera el riesgo de extinción, esto desemboca en divergencia genética entre parches, lo que da como resultado un nuevo paisaje genético, definido por la estructura genética de estas nuevas poblaciones.

Sin embargo, la magnitud de los efectos que la degradación y pérdida de hábitat tienen sobre las poblaciones vegetales parece depender de numerosos factores, tales como el tipo y tiempo de actuación de la perturbación o la estructura de la matriz donde quedan embebidos los parches remantes de hábitat natural, junto con particularidades de cada especie (compatibilidad polínica, modo de dispersión de polen y semillas, forma de vida, longevidad, entre otras) (Hamrick y Godt 1996, Young et al. 1996, Lowe et al. 2005, Aguilar et al. 2008). Por ejemplo, tras la pérdida de hábitat, especies anuales suelen mostrar efectos más rápidos sobre la variabilidad genética de sus poblaciones que especies de vida larga, ya que, debido a sus rápidos ciclos de vida, cualquier cambio genético que ocurra como consecuencia de una alteración severa del medio es más probable que quede fijado en la siguiente generación (Hamrick et al. 1992, Vranckx et al. 2011). En especies de vida larga, los cambios de diversidad genética o diferenciación poblacional suelen necesitar mucho más tiempo y más generaciones (puesto que en ese caso las generaciones no son discretas, no se sustituyen unas a otras completamente). Sin embargo cuando los dispersores de semillas/polen a larga distancia han sido eliminados, los efectos sobre la estructura genética de las poblaciones y su diferenciación suelen ocurrir antes de lo esperado (Wang et al. 2009, Browne et al. 2015). Por tanto, a la hora de diseñar medidas de gestión y conservación efectivas en sistemas altamente modificados por el hombre resulta indispensable conocer cómo estos cambios de hábitat están afectando a las características genéticas de las poblaciones vegetales y a los mecanismos que erosionan o impulsan su diversidad genética. De lo contrario, y tal como ocurre en muchos planes de restauración y conservación de especies amenazadas, los esfuerzos pueden resultar insuficientes o incluso ineficientes (Frankham 2003).

Dispersión de polen y patrones de cruzamiento

En general, los patrones de cruzamiento en las poblaciones de una especie vegetal vienen determinados por características propias de ella, tales como la compatibilidad polínica o el sistema de cruzamiento (zoofilia, anemofilia o hidrofilia). Sin embargo, factores ecológicos, ambientales y particulares de cada individuo interactúan entre sí e intervienen también en la definición de estos patrones. La distancia entre individuos/poblaciones cercanas, el tamaño y la densidad de flores de cada individuo o la fertilidad de machos y hembras son algunos de esos otros elementos claves que pueden condicionar que un individuo de una especie en una población determinada llegue a formar semillas con una mayor o menor probabilidad (Barret y Harder 2017, **Fig. 4**).

Como ya hemos comentado anteriormente, la degradación y pérdida de hábitat tienen efectos importantes sobre muchos de estos factores, por lo que también producen cambios en los patrones de cruzamiento de poblaciones vegetales (Young et al. 1996, Leimu et al. 2006, Breed et al. 2012). En general, y el grado de aislamiento condicionan los cruzamientos entre individuos dentro y entre poblaciones de plantas, y por tanto su flujo génico (Lowe et al. 2005). La reducción del área de ocupación de hábitats naturales y el aumento de las distancia entre parches remanentes de esos hábitats, hace que los movimientos de polen, especialmente a larga distancia, se vean dificultados por tener desplazarse dentro de una matriz antrópica (Breed et al. 2012). Distintos estudios han reportado problemas en los patrones de cruzamiento y dispersión de polen en distintas poblaciones de plantas de ambientes antrópicamente alterados (Young et al. 1996, Browne and Karubian 2018, Chybicki y Oleksa 2018, Honnay y Jacquemyn 2017), como bajas tasas de migración de polen o incrementos en las tasas de autopolinización. Una disminución en las tasas de migración se traduce en nubes de polen con menor diversidad polínica, que junto con la mayor formación de semillas por procesos de autofecundación o entre individuos más estrechamente emparentados va erosionando genéticamente las poblaciones y afectando su viabilidad (Breed et al. 2012, 2015).

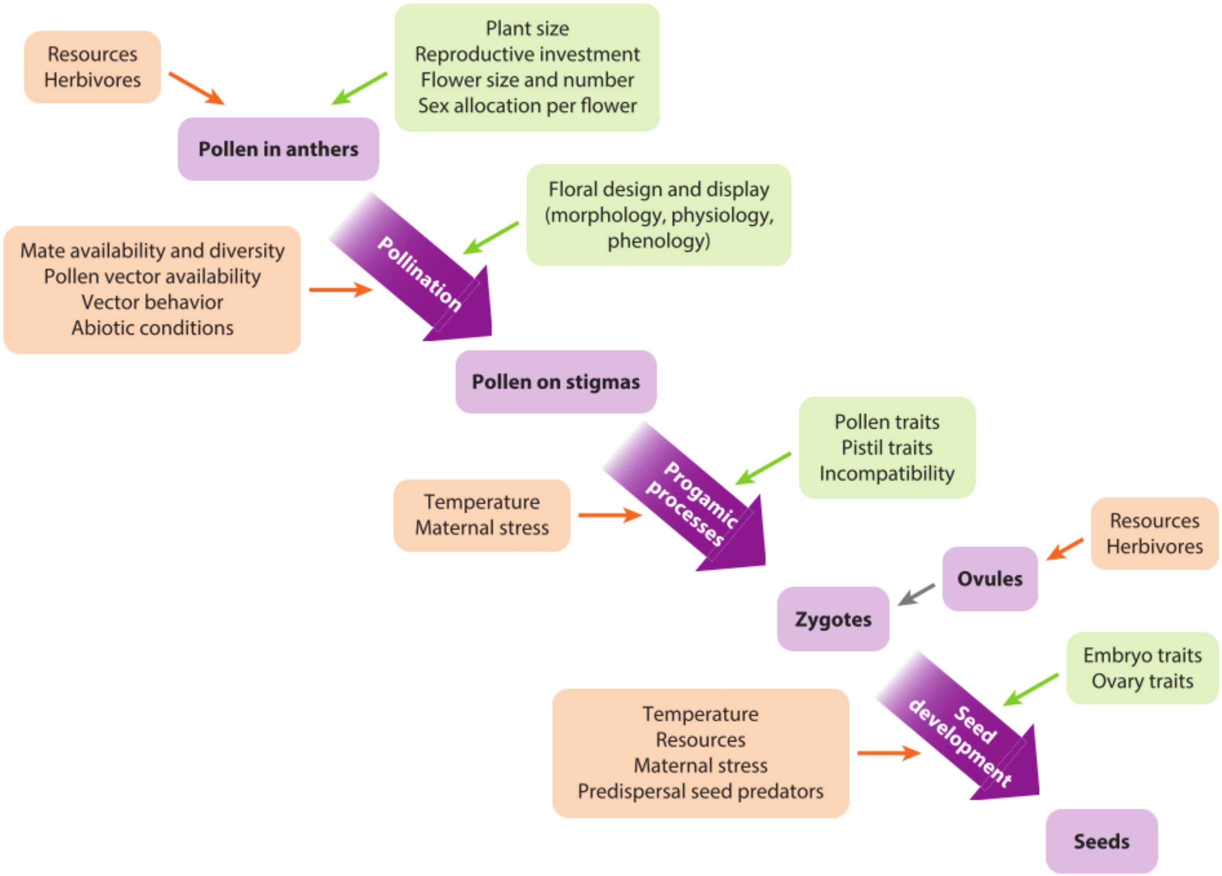


Fig. 4: Principales factores ecológicos y ambientales (naranja) y propios de cada individuo (verde) que condicionan los procesos de cruzamiento en especies vegetales (morado). [Extraído de Barret y Harder 2017]

De esta forma, entender qué factores gobiernan los patrones de cruzamiento de las especies vegetales, junto con los efectos que los cambios en los usos del suelo tienen sobre ellos, es otro de los objetivos que la genética del paisaje y la conservación de especies vegetales tienen hoy en día.

ESPECIE Y ÁREA DE ESTUDIO

La estrecha relación existente entre los ecosistemas mediterráneos y el hombre desde tiempos prehistóricos permite entender gran parte de su actual estructura y funcionamiento. En sus paisajes y hábitats quedan patentes las profundas modificaciones que estos territorios han experimentado con el paso del tiempo, desde prácticas tradicionales hacia otras más intensivas (Valladares 2014). Sin embargo, en la última mitad del siglo XX, la internacionalización de los mercados y el crecimiento del turismo junto con las favorables condiciones climáticas de estas áreas han ocasionado un deterioro acelerado de estos ecosistemas, especialmente en las zonas costeras. Concretamente, el sudeste de la Península Ibérica ha sido una de las zonas más afectadas por la intensificación en los usos del suelo. Desde finales de la década de los 50, el deterioro y degradación de sus hábitat naturales ha ido en aumento, dejando paso a un paisaje profundamente marcado por prácticas agrícolas intensivas (principalmente bajo invernadero) y zonas urbanizadas (Mota et al. 1996, Mendoza-Fernández et al. 2015; ver **Fig. 5**). Actualmente, en la provincia de Almería existen aproximadamente 31 614 ha de cultivos bajo plástico, de las cuales sólo las 21 545 ha del Campo de Dalías (poniente almeriense) definen la estructura humana más grande capaz de ser observada desde el espacio (Consejería de Agricultura, Pesca y Desarrollo de la Junta de Andalucía 2018).

El sudeste semiárido peninsular define un área de gran interés florístico y biogeográfico, ya que alberga hábitats relictos para Europa que datan de la época del Mesiniense (hace más de 5 millones de años) cuando las floras del norte de África y sur de Europa estaban conectadas (ver **Fig. 6**) (Blondel et al. 2010). Estas particularidades hacen de las zonas semiáridas de la Península importantes centros de diversificación de especies, caracterizados por una gran cantidad de endemismos (Blanca y Valle 1986). Las especies vegetales que conforman sus ecosistemas se encuentran altamente especializas, siendo capaces de soportar condiciones climáticas hostiles (principalmente alta insolación y escasez de precipitaciones) y donde destacan principalmente la presencia de matorrales y arbustos arborescentes (Morales 1993). La singularidad de estos hábitats ha hecho que muchos de ellos sean considerados hábitats de interés para su conservación por la Unión Europea (*Directiva Habitat*) (Anon 1992), algunos incluso con carácter prioritario, debido al estado de gran degradación en el que se encuentran. Entre estos últimos destaca el caso del *Habitat 5220*: matorrales arborescentes con Ziziphus lotus* (Tirado 2009), el cual es objeto de estudio de este trabajo de tesis (**Imagen 1**).

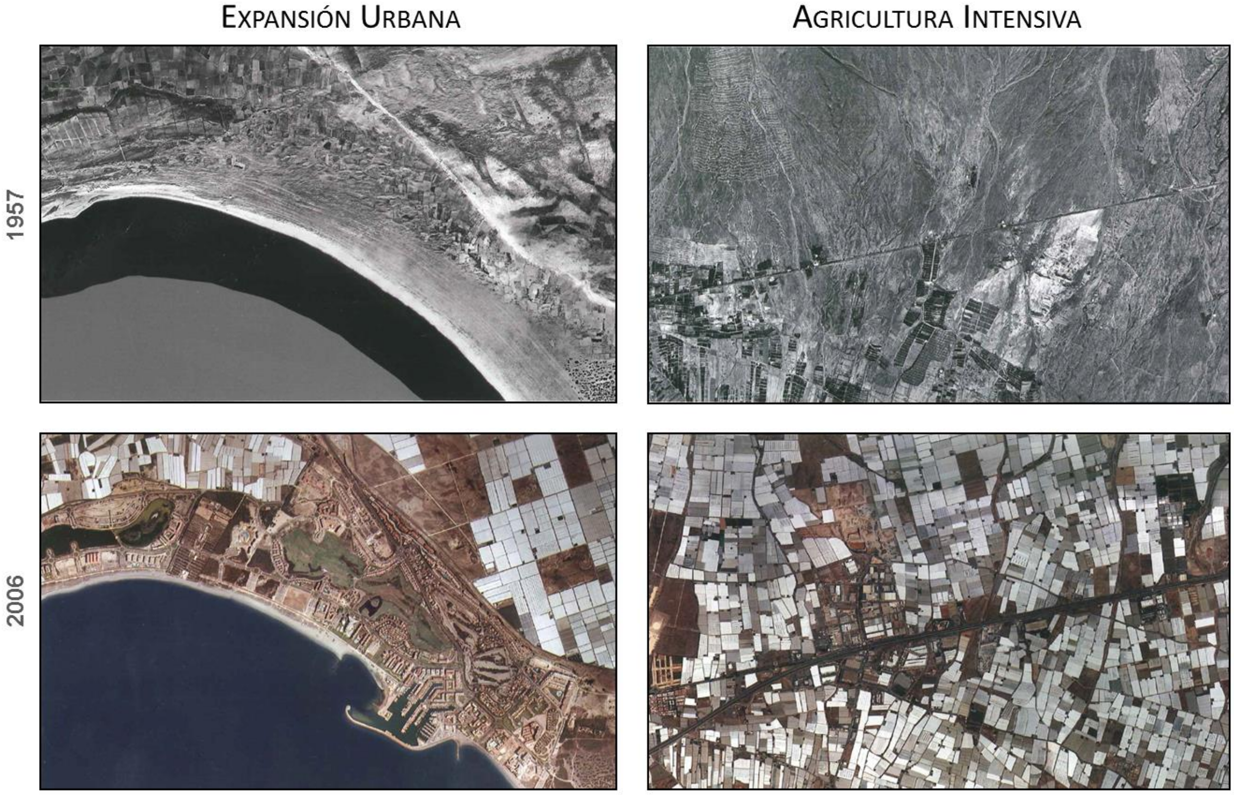


Fig. 5: Fotografías áreas de 1957-2006 de la zona de poniente almeriense (concretamente de El Ejido-Almerimar) que ejemplifican las grandes y rápidas modificaciones que ha sufrido el sudeste semiárido de la Península Ibérica (principalmente por expansión urbana [izquierda] e intensificación agrícola [derecha]), al mismo tiempo que la disminución y deterioro de sus áreas naturales. [Fotografías extraídas del libro "*50 años de transformaciones territoriales en Almería: Aniversario del "Vuelo Americano" (1957-2006)*", Instituto de Estudios Almeriense 2008]

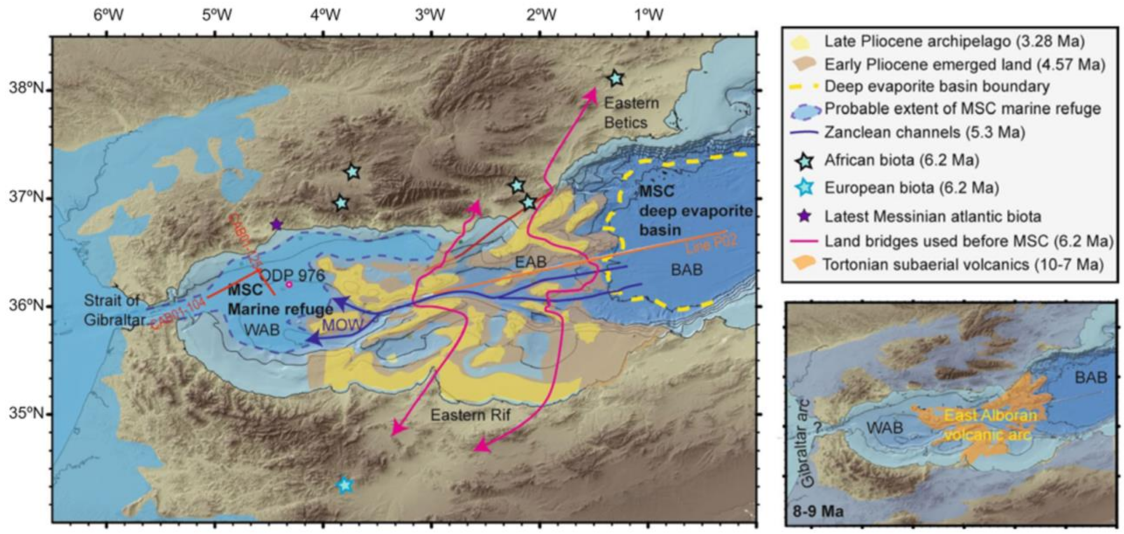


Fig. 6: Mapa del Mediterráneo Occidental durante la crisis de salinidad del Mesiniense (MSC), donde se muestran características de antes y después de los eventos de desecación. Pueden verse los contactos entre el norte de África y sur de la Península Ibérica por la existencia de distintos puentes continentales. El contacto más al este representa la unión entre la zona del Cabo de Gata (Almería) y Marruecos-Argelia que explicaría por qué actualmente existen elementos florísticos compartidos entre ambos territorios. El mapa inferior muestra una reconstrucción del archipiélago volcánico este del Mar de Alborán hace 8-9 ma. *Acrónimos*: BAB = Balearic-Algelian Basin; EAB = East Alborán basin; WAB =West Alborán Basin. [Extraído de Booth-Rea et al. 2018]

Z. lotus es un arbusto arborescente de gran tamaño (hasta 30 m de diámetro y 3 m de alto; Rey et al. 2018) con distribución circumediterránea, principalmente en el Norte de África, pero también es encontrado en el Sahara y en la Península Arábica. En Europa, aunque su presencia es escasa, encuentra su máxima ocupación en el sudeste de la Península Ibérica (más del 90% de su área europea; provincias de Almería y Murcia, mayoritariamente), pudiendo encontrarlo también en Chipre y Sicilia con pocas poblaciones severamente reducidas (European Environmental Agency https://eunis.eea.europa.eu/habitats/10099). En España esta especie ocupa principalmente zonas costeras dunares y ramblas (Morales 1993), donde gracias a su especial sistema radicular (raíces pivotantes principales con numerosas raíces secundarias más superficiales) capta agua de niveles freáticos. Las raíces secundarias hidratan las capas superficiales del suelo, lo que permite que otras especies vegetales que no pueden captar agua de zonas profundas crezcan en su vecindario (tales como *Lycium intricatum*, *Launaea arborescens*, *Thymalaea hirsuta*, *Thymus hyemalis*, *Salsola oppositifolia*, *S. vermiculata*, *S. gesnistoides*, *Artemisia barrelieri*, *Whitania frutescens*, *Androcymbium europaeum*, *Asparagus albus*, *A. horridus*, *Teucrium sp*., entre otras) (Tirado 2009). Esta característica, junto con el cobijo y alimento que otorga a gran parte de la fauna de estos hábitats, es lo que hace que sea considerada la especie clave de estos ecosistemas. A pesar de su importancia, la severa degradación de los hábitats del sudeste semiárido ha arrastrado a las poblaciones de *Z. lotus* a una situación dramática (Mota et al. 1996), pudiendo encontrar poblaciones con tamaños muy variados, de 10 a cientos de individuos (generalmente menores a 100), dependiendo del grado de alteración de cada zona (Rey et al. 2018). La gran capacidad de rebrote de sus individuos es una de las principales características que ha evitado la desaparición de la especie en estos ambientes tan degradados.

Imagen 1: Matorrales arborescentes con *Ziziphus lotus* (*Habitat 5220**) bien conservados donde puede observarse las característica estructura espacial dispersa que toman los individuos de *Z. lotus* dentro de las poblaciones (El Toyo, Almería). [Fotografía de Ana González Robles]

Este trabajo centra sus objetivos en poblaciones Ibéricas de *Z. lotus* con distinto grado de alteración y distribuidas a lo largo de las provincias de Almería y Murcia, donde la especie encuentra su máximo rango de distribución en Europa (**Fig. 7**). Dentro del área de estudio la degradación y pérdida de hábitat de las poblaciones de *Z. lotus* puede ser entendida a distintas escalas: local (dentro de los remanentes de hábitat natural/poblaciones); de paisaje (define una escala mayor de alteración, en términos de usos del territorio en el entorno de los remanentes de hábitat natural); y regional (definida por contexto geográfico de alteración del paisaje y degradación de los remanentes a lo largo del rango de distribución este hábitat en la Península ibérica; ver **Fig. 7**). La degradación a escala local contempla cambios en de estructura y tamaño de los parches remanentes de hábitat. Principalmente es debida al abandono de usos agrícolas tradicionales, pero también a la utilización de estas zonas como vertederos ilegales y, en menor medida, al sobrepastoreo. La alteración a escala de paisaje es principalmente consecuencia de la intensificación agrícola y expansión urbanística en toda la región, que ha ocasionado una reducción de la cobertura de hábitats naturales/semi-naturales y una importante transformación en los usos del suelo.

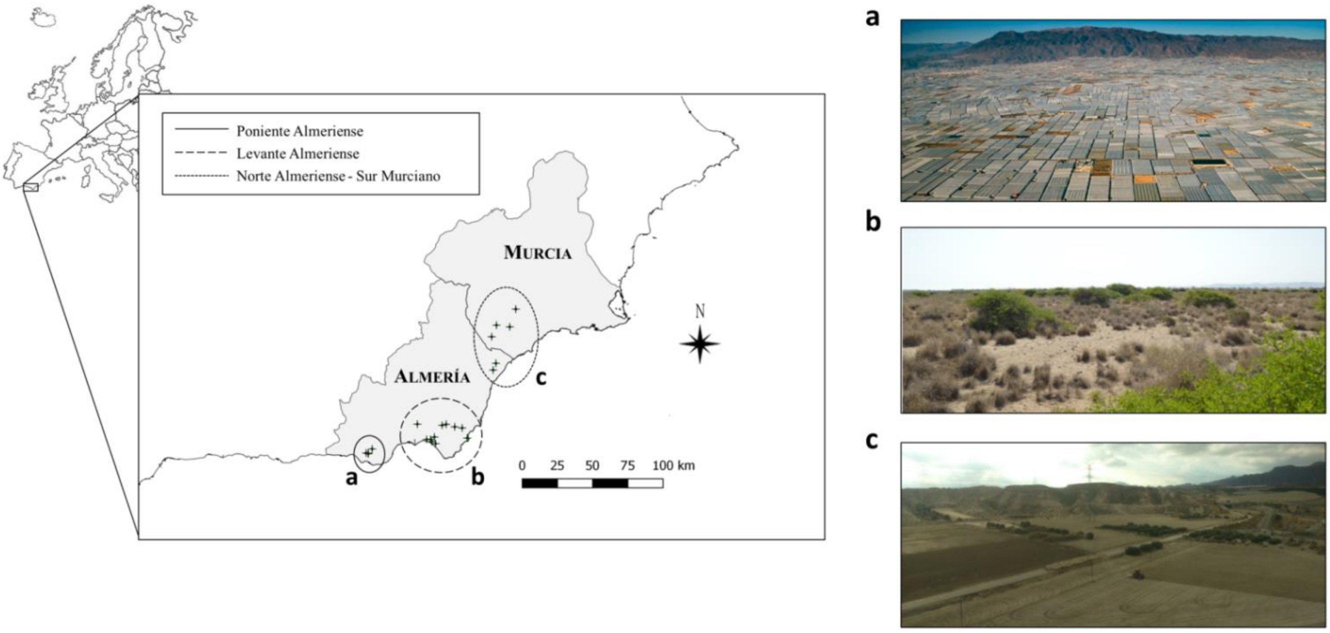


Fig. 7: Área de muestreo en el sudeste semiárido de la Península Ibérica (provincias de Almería y Murcia), donde además se muestra la distribución de las 21 poblaciones de *Z. lotus* estudiadas en este trabajo de tesis (*cruces*). Este área puede quedar subdividida en tres zonas geográficas (escala regional): (**a**) el poniente almeriense, donde la mayor degradación del territorio es debida a la agricultura intensiva bajo plástico (fotografía aérea de la zona del Campo de Dalías); (**b**) el levante almeriense, donde la mayor parte del territorio se encuentra protegido dentro del Parque Natural Cabo de Gata-Níjar, siendo la zona donde se encuentras las poblaciones de *Z. lotus* mejor conservadas (fotografía de la población de El Toyo); (**c**) el norte de la provincia de Almería y sur de Murcia, donde predomina el uso del terreno para cultivos intensivos de regadío. Aquí las poblaciones de *Z. lotus* han quedado relegadas a los bordes y límites de cultivos, y están formadas por muy pocos individuos, siendo la distancia entre poblaciones/individuos dispersos generalmente muy elevada (fotografía del paisaje que rodea la población de Cuevas de Almanzora). [Fotografías de (**a**) GRUPOMSC.COM; (**b** y **c**) Ana González Robles]

ANTECEDENTES

Los efectos que la pérdida y degradación de hábitats tienen sobre los ecosistemas mediterráneos y sus especies ha sido el foco de numerosas investigaciones (Luiselli et al. 1997, Santos et al. 2002, González-Varo et al. 2009, Rey y Alcántara 2014, Valladares et al. 2014, Nora et al. 2015, Vulliamy et al. 2016). Sin embargo, a pesar de ser una de las zonas mediterráneas más degradas por los cambios acelerados en los usos del suelo, pocos estudios de este tipo han sido llevados a cabo en los ecosistemas semiáridos del sudeste de la Península Ibérica (no obstante ver Martínez-López et al. 2017). Mota et al. (1996) advertían a finales del siglo pasado sobre las graves consecuencias que la intensificación en los usos del territorio estaba ya teniendo sobre la biodiversidad de los hábitats del sudeste peninsular. La agricultura bajo invernaderos es actualmente una de las prácticas agrícolas que más está en expansión por todo el mundo, por lo que entender de qué manera estas prácticas intensivas afectan al funcionamiento y viabilidad de los ecosistemas naturales adyacentes es indispensable dentro del escenario de cambio global en el que nos encontramos.

Al ser uno de los hábitats más singulares de esta área, los matorrales arborescentes con *Z. lotus* han sido estudiados desde distintos puntos de vista por diversos autores. Tirado y Pugnaire (2003, 2005) fueron los primeros que estudiaron la estructura espacial de estas comunidades y en qué medida los individuos de *Z. lotus* establecían procesos de facilitación con otras especies del mismo hábitat. Tirado (2009) sentó las bases sobre la ecología y el estado de conservación de estos hábitats de interés prioritario. Guirado (2013) y Guirado et al. (2018) constataron el carácter freatófito de *Z. lotus*, mostrando cómo su presencia se encuentran estrechamente ligadas a la disponibilidad de aguas subterráneas y a su salinidad, por lo que clasificaron a sus comunidades como ecosistemas dependientes de aguas subterráneas. De esta forma, los individuos de *Z. lotus* parecen exhibir una mayor agregación en las zonas de fallas y una mayor separación en zonas donde la competitividad por el agua es mayor (como por ejemplo, los lugares cercanos a la costa). Sin embargo, no es hasta Cancio et al. (2016, 2017) y Rey et al. (2017) cuándo se describe la fuerte disrupción planta-agentes dispersantes de semillas que existe en las poblaciones Ibéricas de *Z. lotus*, a consecuencia de la fuerte degradación a escala de hábitat y de paisaje que sufren estos ecosistemas. En las zonas más alteradas y aisladas, las poblaciones de *Z. lotus* muestran una gran limitación en cuanto a los procesos dispersivos de semillas a larga distancia, a consecuencia de una disminución del consumo de semillas por mamíferos carnívoros de mediano tamaño (principalmente por zorro, *Vulpes vulpes,* el cual parece ejercer también un cierto efecto regulador sobre la densidad de conejos del medio – principales depulpadores de semillas de *Z. lotus* y que interfieren en su dispersión). Estos autores enfatizan la necesidad de mejorar la conectividad entre poblaciones/parches remanentes, al mismo tiempo que su área de ocupación, con la intención de restaurar los servicios dispersivos que actualmente se encuentran erosionados. Al mismo tiempo, Rey et al. (2018) revelaron los grandes problemas de regeneración y reclutamiento de juveniles de *Z. lotus* que existen en estos ecosistemas, a consecuencia de estas disrupciones en las interacciones planta-animal. Estas alteraciones mantienen en un estado casi estático a las poblaciones de *Z. lotus*, las cuales aparentemente perduran gracias a la capacidad de rebrote y gran longevidad característica de sus individuos.

Todas estas modificaciones ecológicas corroboran el fuerte efecto que la pérdida y degradación del paisaje tienen sobre estos ecosistemas singulares, y concretamente sobre las poblaciones de *Z. lotus* del sudeste peninsular. Sin embargo, cómo la intensificación en los usos del suelo puede estar afectando a (1) las interacciones mutualistas planta-insecto y por tanto a la comunidad de polinizadores y su servicio de polinización; (2) la diversidad y estructura genética; (3) y los procesos de cruzamiento y flujo génico de las poblaciones de *Z. lotus*, es aún desconocido. Entender la situación actual de las poblaciones de *Z. lotus* en estos aspectos, resulta esencial no solo para el diseño de medidas de gestión y conservación adecuadas a nuestro hábitat de estudio que aseguren su continuidad, sino también para anticipar las consecuencias que la expansión de la intensificación agrícola bajo invernadero, en conjunto con la expansión urbanista, pueden tener sobre los sistemas naturales y/o semi-naturales sobre los que se asientan.

Individuos de *Z. lotus* en límites de cultivos, Campohermoso – Almería *[Fotografía Ana González Robles]*

HIPÓTESIS Y OBJETIVOS

A pesar de que los matorrales arborescentes con *Z. lotus* se encuentran dentro de los hábitats de interés prioritario para su conservación, el conocimiento existente de los mismos es muy inferior al que se tiene de otros hábitats de interés comunitario (Tirado 2009). Estas particularidades, junto a la acusada situación de regresión en la cual se encuentran las poblaciones de *Ziziphus* a causa de la pérdida y alteración de sus hábitats, explicarían el interés y la necesidad del estudio que aquí planteamos.

Así, el **objetivo general** que este trabajo de tesis se plantea es evaluar en qué medida la drástica y rápida degradación de los matorrales arborescentes con *Z. lotus*, a escala local y de paisaje, está influyendo sobre el ensamblaje de polinizadores silvestres, diversidad genética y flujo génico de las poblaciones de *Z. lotus* del sudeste semiárido peninsular.

La **hipótesis de partida** es que la intensa pérdida y degradación del hábitat ocurrida a lo largo de los últimos 60 años, y que ha generado una reducción de efectivos poblacionales de la especie de estudio, ha provocado además una reducción de la diversidad y abundancia del ensamblaje de polinizadores, limitando sus movimientos y produciendo una reducción del flujo génico entre poblaciones/parches remanentes. Esta pérdida de conectividad sería especialmente grave en aquellas áreas más alteradas (poniente almeriense). A su vez, el aislamiento y pérdida de hábitats habría acabado reflejándose a nivel genético, erosionando su diversidad genética principalmente en las zonas más alteradas e incrementando la diferenciación genética entre poblaciones.

Por el contrario, la **hipótesis alternativa** es que la diversidad y/o la abundancia de polinizadores visitantes de *Z. lotus* estaría siendo positivamente afectada (o no afectada) debido a la mayor disponibilidad y diversidad de nichos que supondría la diversificación de usos del territorio y/o el manejo de abejas en la agricultura intensiva. El flujo génico histórico y procesos poblacionales pretéritos habrían conformado la diversidad y estructura genética detectada actualmente en las poblaciones de *Z. lotus.* Así, la longevidad de la especie habría amortiguado los potenciales mecanismos de erosión genética dentro de las poblaciones, manteniendo elevada la diversidad genética en estas a pesar de la degradación a nivel local y de paisaje.

Los **objetivos específicos** que se pretenden abordar para examinar el objetivo general de la investigación son:

- Analizar los efectos de la degradación de hábitat y alteración del paisaje sobre el ensamblaje (taxonómico y funcional) de visitantes florales de *Z. lotus*, evaluando además el impacto que el uso agrícola de abejas manejadas tiene sobre el ensamblaje de visitantes florales silvestres – ***Capítulo 1***.

- Evaluar en qué medida la diversidad y estructura genética de las poblaciones ibéricas de *Z. lotus* está siendo afectada por la alteración y pérdida, a escala local, de paisaje y regional, de sus áreas naturales – ***Capítulo 2 y 3***.

- Analizar las posibles variaciones en los patrones de apareamiento y flujo génico dentro y entre las poblaciones de *Z. lotus*, como consecuencia de la variación en características individuales y ecológicas inducidas por la intensa degradación antrópica del área de estudio – ***Capítulo 4***.

REFERENCIAS

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches. *Mol Ecol*, 17, 5177–5188.

Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2009) How much does agriculture depend on pollinators? Lessons from long- term trends in crop production. *Ann Bot*, 103, 1579–1588.

Anon (1992) Directive 92/43 of the council of the European community on the conservation of habitats and wild fauna and flora. European Community, Brussels.

Aparicio A, Hampe A, Fernández-Carrillo L, Albaladejo RG (2012) Fragmentation and comparative genetic structure of four mediterranean woody species: Complex interactions between life history traits and the landscape context. *Divers Distrib*, 18, 226–235.

Barrett SCH, Harder LD (2017) The Ecology of Mating and Its Evolutionary Consequences in Seed Plants. *Annu Rev Ecol Evol Syst*, 48, 135–157.

Blanca G, Valle F (1986) Las plantas endémicas de Andalucía Oriental. *Monografías de Flora y Vegetación Bética*, 1, 1-53.

Blondel J, Aronson J, Bodiou JY, Boeuf G (2010) *The Mediterranean Region: Biological Diversity in Space and Time* (2nd edition). Oxford, New York, 384 pp.

Booth-Rea G, Ranero CR, Grevemeyer I (2018) The Alboran volcanic-arc modulated the Messinian faunal exchange and salinity crisis. *Sci Rep*, 8, 1–14.
doi: https://doi.org/10.1038/s41598-018-31307-7

Benton TG, Vickery JA, Wilson JD (2003) Farmland biodiversity: Is habitat heterogeneity the key? *Trends Ecol Evol*, 18, 182–188.

Breed MF, Marklund MHK, Ottewell KM, Gardner MG, Harris JBC, Lowe AJ (2012) Pollen diversity matters: revealing the neglected effect of pollen diversity on fitness in fragmented landscapes. *Mol Ecol*, 21, 5955–5968.

Breed MF, Ottewell KM, Gardner MG, Marklund MHK, Dormontt EE, Lowe AJ (2015) Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. Heredity, 115, 108–114.

Browne L, Karubian J (2018) Habitat loss and fragmentation reduce effective gene flow by disrupting seed dispersal in a neotropical palm. *Mol Ecol*, 27, 3055–3069.

Browne L, Ottewell K, Karubian J (2015) Short-term genetic consequences of habitat loss and fragmentation for the neotropical palm *Oenocarpus bataua*. *Heredity*, 115, 389–395.

Cancio I, González-Robles A, Bastida JM, Isla J, Manzaneda AJ, Salidos T, Rey PJ (2017) Landscape degradation affects red fox (Vulpes vulpes) diet and its ecosystem services in the threatened Ziziphus lotus scrubland habitats of semiarid Spain. *J Arid Environ*, 145, 24–34.

Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Salido T, Rey PJ (2016) Habitat loss exacerbates regional extinction risk of the keystone semiarid shrub *Ziziphus lotus* through collapsing the seed dispersal service by foxes (*Vulpes vulpes*). *Biodivers Conserv*, 25, 693–709.

Chybicki IJ, Oleksa A (2018) Seed and pollen gene dispersal in *Taxus baccata*, a dioecious conifer in the face of strong population fragmentation. *Ann Bot*, 122, 409–421.

Collado MÁ, Sol D, Bartomeus I (2019) Bees use anthropogenic habitats despite strong natural habitat preferences. *Divers Distrib*, 1–12.

Consejería de Agricultura, Pesca y Desarrollo (2018) Cartografía de invernaderos en Almería, Granada y Málaga. Junta de Andalucía, Sevilla, España, 25 pp.

Ellstrand NC (2014) Is gene flow the most important evolutionary force in plants? *Am J Bot*, 101, 737–753.

Fahrig L (2003) Effects of Habitat Fragmentation on Biodiversity. *Annu Rev Ecol Evol Syst*, 34, 487–515.

Frankham R (2003) Genetics and conservation biology. *C R Biol*, 326, 22–29.

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, et al. (2005) Global consequences of land use. *Sci*, 309, 570–574.

Garibaldi LA, Steffan-Dewenter I, Winfree R, et al. (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Sci*, 339, 1608–1611.

Gill RJ, Baldock KCR, Brown MJF, Cresswell JE, Dicks LV, et al. (2016) Protecting an Ecosystem Service: Approaches to Understanding and Mitigating Threats to Wild Insect Pollinators. *Adv Ecol Res*, 54, 135–206.

González-Varo JP, Arroyo J, Aparicio A (2009) Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (*Myrtus communis*). *Biol Conserv*, 142, 1058–1065.

González-Varo JP, Biesmeijer JC, Bommarco R, et al. (2013) Combined effects of global change pressures on animal-mediated pollination. *Trends Ecol Evol*, 28, 524–530.

González-Varo JP, Vilà M (2017) Spillover of managed honeybees from mass-flowering crops into natural habitats. *Biol Conserv*, 212, 376–382.

Guirado E (2013) Factores que afectan a la distribución espacial de vegetación freatófita (*Ziziphus lotus*) en el acuífero costero de Torre García (sureste de España). *Trabajo fin de máster*, Universidad de Almería, 60 pp.

Guirado E, Alcaraz-Segura D, Rigol-Sánchez JP, Gisbert J, Martínez-Moreno FJ, Galindo-Zaldívar J, González-Castillo L, Cabello J (2018) Remote-sensing-derived fractures and shrub patterns to identify groundwater dependence. *Ecohydrology*, 11, e1933.
doi: https://doi.org/10.1002/eco.1933

Hahn MW (2018) *Molecular Population Genetics*. Sinauer Associates, New York

Hamrick JL, Godt MJW (1996) Effects of Life History Traits on Genetic Diversity in Plant Species. *Philos Trans R Soc B Biol Sci,* 351, 1291–1298.

Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New For*, 6, 95–124.

Holzschuh A, Dainese M, González-Varo JP, Mudri-Stojnic S, Riedinger V, et al. (2016) Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecol Lett*, 19, 1228–1236.

Honnay O, Jacquemyn H (2007) Susceptibility of Common and Rare Plant Species to the Genetic Consequences of Habitat Fragmentation. *Conserv Biol*, 21, 823–831.

Instituto de Estudios Almeriense (ed.) (2008) “*50 años de transformaciones territoriales en Almería: Aniversario del << Vuelo americano >> (1957-2006)*”. Artes Gráficas, Almería, España, 83 pp.

Jackson ND, Fahrig L (2016) Habitat amount, not habitat configuration, best predicts population genetic structure in fragmented landscapes. *Landsc Ecol*, 31, 951–968.

Jordano P (2010) Pollen, seeds and genes: The movement ecology of plants. *Heredity*, 105, 329–330.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. *Annu Rev Ecol Syst*, 29, 83–112.

Kennedy CM, Lonsdorf E, Neel MC, Williams NM, Ricketts TH, Winfree R, Bommarco R, Brittan C, Burley AL, Cariveau D, et al. (2013) A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters*, 16(5), 584–599.

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B Biol Sci*, 274, 303–313.

Lander TA, Boshier DH, Harris SA (2010) Fragmented but not isolated : Contribution of single trees , small patches and long-distance pollen flow to genetic connectivity for *Gomortega keule*, an endangered Chilean tree. *Biol Conserv*, 143, 2583–2590.

Leimu R, Mutikainen P, Koricheva J, Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? *J Ecol*, 94, 942–952.

Loreau M, Mouquet N, Gonzales A (2003). Biodiversity as spatial insurance in heterogeneous landscapes. *Proc Natl Acad Sci USA*, 100, 12765–12770.

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005) Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity*, 95, 255–273.

Luiselli L, Capizzi D (1997) Influences of area, isolation and habitat features on distribution of snakes in Mediterranean fragmented woodlands. *Biodiversity & Conservation*, 6, 1339–1351.

Magrach A, González-Varo JP, Boiffier M, Vilà M, Bartomeus I (2017) Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. *Nat Ecol Evol*, 1, 1299–1307.

Martínez-López V, de la Rúa P, Zapata VM, Robledano F (2017) Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest. *Urban Ecosyst*, 20, 1161–1168.

Mendoza-Fernández AJ, Martínez-Hernández F, Pérez-García FJ, et al. (2015). Extreme habitat loss in a Mediterranean habitat: *Maytenus senegalensis* subsp. *europaea*. *Plant Biosyst,* 149, 503–511.

Morales-Torres C (1993) Flora amenazada y de interés de la provincia de Almería. En: *Regeneración de la cubierta vegetal: actas de la V Aula de Ecología. Las ramblas mediterráneas: actas de la VI Aula de Ecología*, 69-76. Instituto de Estudios Almerienses, Almería, España

Mota JF, Peñas J, Castro H, Cabello J, Guirado J (1996) Agricultural development versus biodiversity conservation: the Mediterranean semiarid vegetation in El Ejido (Almería, southeastern Spain). *Biodivers Conserv*, 5, 1597–1617.

Nora S, Albaladejo RG, Aparicio A (2015) Genetic variation and structure in the Mediterranean shrubs Myrtus communis and Pistacia lentiscus in different landscape contexts. *Plant Biol*, 17, 311–319.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, et al. (2010) Global pollinator declines: Trends, impacts and drivers. *Trends Ecol Evol*, 25, 345–353.

Rey PJ, Alcantara JM (2014) Effects of habitat alteration on the effectiveness of plant-avian seed dispersal mutualisms: Consequences for plant regeneration*. Perspectives in Plant Ecology, Evolution and Systematics*, 16, 21–31.

Rey PJ, Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Valera F, Salido T, Alcántara JM (2017) Local-scale and landscape disturbances impact through distinct pathways on the regional variation in seed dispersal by mammals in threatened semiarid habitats. *Perspect Plant Ecol Evol Syst*, 28, 10–18.

Rey PJ, Cancio I, Manzaneda AJ, González-Robles A, Valera F, Salido T, Alcántara JM (2018) Regeneration of a keystone semiarid shrub over its range in Spain: habitat degradation overrides the positive effects of plant – animal mutualisms. *Plant Biol*, 20, 1083-1092.

Sala OE, Chapin III FS, Armesto JJ, Berlow E, Bloomfield J, et al. (2000) Global Biodiversity Scenarios for the Year 2100. *Sci*, 287, 1770–1774.

Santos T, Telleria JL, Carbonell R (2002) Bird conservation in fragmented Mediterranean forests of Spain: effects of geographical location, habitat and landscape degradation. *Biological Conservation*, 105, 113–125.

Sork VL, Davis FW, Smouse PE, Apsit VJ, Dyer RJ, Fernandez JF, Kuhn B (2002) Pollen movement in declining populations of California Valley oak, *Quercus lobata*: where have all the fathers gone? *Molecular Ecology*, 11, 1657–1668.

Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landsc Ecol*, 21, 821–836.

Templeton AR, Shaw K, Routman E, Davis S (1990) The genetic consequences of habitat fragmentation. *Ann Missouri Bot Gard*, 77, 13–27.

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus* (*). En: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, 68 p.

Tirado R, Pugnaire FI (2003) Shrub spatial aggregation and consequences for reproductive success. *Oecologia*, 136, 296–301.

Tirado R, Pugnaire FI (2005) Community structure and positive interactions in constraining environments. *Oikos*, 111, 437–444.

Torné-Noguera A, Rodrigo A, Osorio S, Bosch J (2016) Collateral effects of beekeeping: Impacts on pollen-nectar resources and wild bee communities. *Basic Appl Ecol*, 17, 199–209.

Valladares F, Benavides R, Rabasa SG, Díaz M, Pausas JG, Paula S, Simonson WD (2014). Global change and Mediterranean forests: current impacts and potential responses. En: DA Coomes, DFRP Burslem, WD Simonson, *Forests and global change*, pp 47–75.

Vranckx G, Jacquemyn H, Muys B, Honnay O (2011) Meta-Analysis of Susceptibility of Woody Plants to Loss of Genetic Diversity through Habitat Fragmentation. *Conserv Biol,* 26, 228–237.

Vulliamy B, Potts SG, Willmer PG (2016) The Effects of Cattle Grazing on Plant-Pollinator Communities in a Fragmented Mediterranean Landscape. *Oikos*, 114, 529–543.

Wang IJ, Bradburd GS (2014) Isolation by environment. *Mol Ecol*, 23, 5649–5662.

Wang R, Ai B, Gao BQ, Yu S, Li YY, Chen XY (2009) Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var. *pumila* (Moraceae). *Popul Ecol*, 51, 307–315.

Wereszczuk A, Leblois R, Zalewski A (2017) Genetic diversity and structure related to expansion history and habitat isolation: Stone marten populating rural-urban habitats. *BMC Ecol*, 17, 46. doi: https://doi.org/10.1186/s12898-017-0156-6

Winfree R, Bartomeus I, Cariveau DP (2011) Native Pollinators in Anthropogenic Habitats. Annu Rev Ecol Evol Syst, 42, 1–22.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol Evol*, 11, 413–41.

CAPÍTULO 1

MANAGED HONEY BEES AND HABITAT AND LANDSCAPE DEGRADATION IMPACT ON THE WILD INSECT VISITOR ASSEMBLAGES AND POLLINATION SERVICES IN IBERIAN THREATENED SEMIARID SCRUBLANDS

Ana González-Robles[1], Teresa Salido[1], Antonio J. Manzaneda[1], Francisco Valera[2] and Pedro J. Rey[1]

[1]Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, E-23071, Jaén, Spain.

[2]Estación Experimental de Zonas Áridas, EEZA-CSIC, E-04120, Almería, Spain.

***under review, Biodiver Conserv* (2019)**

ABSTRACT

The great expansion of intensive agriculture has caused severe landscape alterations but also an increase in managed honey bee (*Apis mellifera)* as the main pollinator of crops worldwide. However, few studies have considered together landscape degradation and managed bee effects on wild insect visitors of native plant species. Here, we analyze how habitat and landscape degradation and managed honey bee introduction for agricultural purposes affect wild insect assemblages visiting *Ziziphus lotus* flowers, a keystone scrub of threatened semiarid habitats from southeastern Spain. We describe its floral insect visitor assemblages and their potential pollination service (the number of visited flowers) in 21 populations distributed across an ample landscape degradation gradient in its whole Iberian range, and relate them with population, habitat remnant and landscape features. Differences in wild visitor abundance and their floral visits were negatively associated with differences in honey bee abundance and its floral visits. Population differences in wild visitor abundance and floral visits were also explained by population characteristics (flower density and distance between nearby populations), while variables linked to landscape alteration (land-use diversity) and local degradation (distance between nearby patches) affect more to visitor species richness. Honey bee abundance and floral visits were not associated either with population or with landscape disturbance features. Wild visitor sensitivity to habitat and landscape disturbance and to honey bee abundance was higher for taxonomic than for functional composition and richness. This study highlights the wild pollinator fragility and the necessity to establish adequate regulation and management measures of invasive agricultural practices.

INTRODUCTION

Pollinator communities play a fundamental role in the functioning of ecosystems, being responsible for pollinator service of numerous plant species (Rathcke and Jules 1993, Gill et al. 2016). Pollinator services are provided by many different animal groups (insects, birds, bats or lizards) (Ratto et al. 2018), yet insect species are usually considered the most relevant pollinators at higher latitudes (Klein et al. 2007). In fact, wild insect pollination is considered one of the most vulnerable ecosystem services (Garibaldi et al. 2013). In recent years, researchers are drawing attention to the loss of insect pollinators as a part of the biodiversity crisis, studying how different plant-insect interactions and native insect communities are being affected by the disturbance of natural habitats (Aizen and Feinsinger 2003, Klein et al. 2007, Steffan-Dewenter and Westphal 2008, Gonzalez-Varo et al. 2009, Tylianakis 2013, Martins et al. 2015, Traveset et al. 2017). Intensive agriculture, land-use changes and landscape alteration are associated with population declines of insect pollinators (Aizen et al. 2009, González-Varo et al. 2013, Garibaldi et al. 2013). Bees, wasps, ants, flies, beetles, butterflies (among others) are different wild pollinator groups, and the effects of human disturbances on them depend on several factors (Stavert et al. 2018), as the type of natural habitat surrounding anthropogenic habitats and agricultural management.

Honey bee management (mainly *Apis mellifera*) has become a key practice of intensive farming to achieve massive pollination and hence the highest crop yield (Rucker et al. 2012, Breeze et al. 2014, Garibaldi et al. 2013, Magrach et al. 2017). This is the main reason why its stock has been increasing in the last decades (Aizen and Harder 2009). As a reference, the global stock of managed honey bee hives has increased ~45% since 1960s, raising until ~120%, ~250% and ~360% in countries like China, Spain or Argentina (respectively) (Aizen and Harder 2009, González-Varo and Vilà 2017). In 2016, ca. 16 million hives (600 000 beekeepers) were recorded only in Europe (Rossi 2017). Several studies have addressed the effect of intensive agriculture and landscape alteration on honey bees (González-Varo et al. 2013), associating pesticides and other chemicals with the great losses of beekeeping sector and the pollinator crisis in the last decades (Potts et al. 2010). Nonetheless, in the last years, different researchers (Holzschuh et al. 2016, González-Varo and Vilà 2017, Magrach et al. 2017, Geldmann and González-Varo 2018) warned about the risk that the unregulated use of managed honey bees poses on wild pollinators, mainly due to honey bee spillovers into nearby natural habitats. González-Varo and Vilà (2017) highlighted the negative impact that these spillovers can have into natural ecosystems, provoking variation in wild bee and other taxa communities. The massive introduction of non-native insects in natural habitats can even cause serious damage to the functional diversity of wild pollinators (Morales et al. 2017) and thus affect ecosystem stability (Tscharntke et al. 2005). This issue has provoked a huge controversy (Geldmann and González-Varo 2018, González-Varo and Geldmann 2018, Kleijn et al. 2018, Saunders et al. 2018). Some researchers suggest that the honey bee increase in natural ecosystems suppose a great competition for wild insect pollinators (Magrach et al. 2017), in terms of food resources, making honey bees partly responsible of wild pollinator decline. Others authors defend that these negative effects have not been demonstrated, and that honey bee conservation is beneficial for wild insects too (Breeze et al. 2014). These authors argue that the negative effects of landscape disturbance are more important than the effects of managed honey bees on wild insect pollinators. Thus, contrasting evidence underlines the importance of carrying out more studies about how natural habitat alteration affects, taxonomically and functionally, wild insect pollinator communities (Steffan-Dewenter and Westphal 2008, González-Varo et al. 2013, Holzschuh et al. 2016), to distinguish between effects from habitat structural changes (large or local scale) and those caused by managed honey bee spillovers into natural ecosystems (Geldmann and González-Varo 2018). However, few studies have evaluated these spillover effects on wild pollinators (Geldmann and González-Varo 2018), and its differential effect on taxonomical and functional composition of wild floral visitors. The goal of this study is to shed light on these issues.

Here, we analyze the variation induced by several drivers of global change on the insect visitor assemblage of an arborescent scrub confined in Europe to the semiarid southeast of the Iberian Peninsula. Our study is framed within the context of habitat degradation and landscape disturbance effects, and the impact of managed honey bee introduction for agricultural purposes on wild insect floral visitor assemblages. Southeastern Spain is a particularly suitable area for this approach because it contains the largest extension of intensive greenhouse agriculture of the World. Greenhouse agriculture expansion caused that since the middle of XX century, this area suffered one of the highest rates of habitat degradation in Europe (Mota et al. 1996, Mendoza-Fernández et al. 2015). In addition, the use of managed honey bees for pollination into greenhouses is currently a widely extended practice. First, we describe the wild insect floral visitor assemblages, in terms of taxonomic and functional composition, of *Ziziphus lotus* (Rhmanaceae), a keystone species of the *Mediterranean arborescent scrubs with Ziziphus lotus* (Tirado 2009, *Habitat 5220** of European Directive 92/43/EEC, Anon 1992), and the number of visited flowers by these visitors (as a proxy of the pollinator service) in 21 populations distributed across an ample gradient of landscape degradation along its whole

Iberian distribution. Then, we assess (i) how the taxonomic and functional composition of the floral visitor assemblage and the number of visited flowers vary geographically among *Ziziphus* populations; (ii) how managed honey bee abundance influences wild pollinator abundance, composition and floral visits; and (iii) how population and landscape degradation-related features affect the number of flower visited and the composition and species richness of the floral visitor assemblage. We expect that (i) the variation across the region in wild visitor assemblages will be larger than the variation in honey bee abundance (by a generalized use of *Apis mellifera* in agricultural areas); (ii) the effects of managed honey bees will be higher on taxonomic than on functional composition of the wild insect visitor assemblage because of functional redundancy existence in a generalist system like ours (Tscharntke et al. 2005); and (iii) population and landscape features will affect differently the composition and richness of floral visitor assemblages and the pollination service (i.e. number of visited flowers).

MATERIALS AND METHODS

Study species and study area

Ziziphus lotus is a circumediterranean scrub distributed mostly across Northern Africa (though also appears in Sahara desert and the Arabic Peninsula). In Europe, it only grows in Spain, Cyprus and Sicily. In Spain it is a rare and relict species, abundant only in the semiarid southeast region (Sánchez-Gómez et al. 2002; Pérez-Latorre and Cabezudo 2009). It is a thorny, sclerophyllous, hemispheric shrub species with 1-3 m height and up to 30 m diameter (Rey et al. 2018) and intricate branches, diploid (2n = 20) and with numerous hermaphrodite flowers (Image S1, Suppl. Mat.). The perianth has five spatulate sepals, five very simple green-yellow petals, five free stamens, nectaries included in the disk of the receptacle and an ovary with 2-3 styles (Pérez-Latorre and Cabezudo 2009). As other *Ziziphus* species (Asatryan and Tel-Zur 2013), flowers show self-incompatibility and synchronous protandrous dicogamy. Thus, each individual matures its daily flowers in synchrony, first the anthers (during 5 h) and then the stigma (others 5 h), without (or little) overlap between both sexual stages. Opening flowers occur daily during the flowering period, but the flower lifetime is less that one day (around 10 h, from 8:00 h to 18:00 h, personal observation). Thus, each sexual organ is active/receptive (anthers/stigma) during 3-4 h (personal observation). Moreover, within a population, individuals also exhibit a particular pattern of synchrony, as two individual types can be noticed along the day: the first opens flowers in the morning and the second in the afternoon. Individuals that begin their floral maturity (male stage) in the afternoon, end it (female stage) the next morning. This permits that in the same time of the day some individuals are in male floral phase and others in female phase, enhancing the possibility of outcrossing within the populations (similar patterns described in other *Ziziphus* species, see Zietsman and Botha 1992). Individuals with male and female flowers are found in ca. 1:1 proportion per population at the same time (Nadia et al. 2007, personal observation). Flowering occurs from May to July, and fruit ripening is mainly in September. After pollination, a spherical red-brown fruit is produced (Pérez-Latorre and Cabezudo 2009).

This study was carried out from May to July 2015. We chose 399 *Ziziphus* individuals distributed in 21 populations differing in land-uses (2.4 to 92.6% of natural habitat cover) throughout the whole species range in the Iberian Peninsula (Almeria and Murcia provinces) (Fig.1). Based on the geographical distribution and the geographical structuring of population genetic diversity (see *Capítulo 3*), we defined 3 regions: *Core region*, with 11 populations. It is distributed along the Cabo de Gata-Níjar Natural Park (Almeria, Spain), a well-preserved area with some of the largest populations of this species, and where there is, apparently, a certain connection between populations. *West region*, with 4 populations. It is within the largest greenhouse agriculture nucleus of southern Europe, being the most degraded area and apparently disconnected from the *Core region* (ca. 36.5 km far and with no *Ziziphus* populations in between). *East* region, with 6 populations. They are smaller in size and density, and more isolated from each other than the populations from other regions (Fig.1).

In each population, 20-25 individuals were randomly selected (except in five populations where we chose 8-14 individuals; see details in Table 1) and geo-referenced. All these populations are located in a semiarid Mediterranean climate, with a mean annual temperature ranging from 17.4 to 19.6 °C and an annual precipitation from 180.7 to 269.8 mm (Table 1). Climatic values for each population were compiled from nearby climatic stations (data available at https://www.juntadeandalucia.es/agriculturaypesca/ifapa/ for Almeria populations and at http://siam.imida.es/ for Murcia populations).

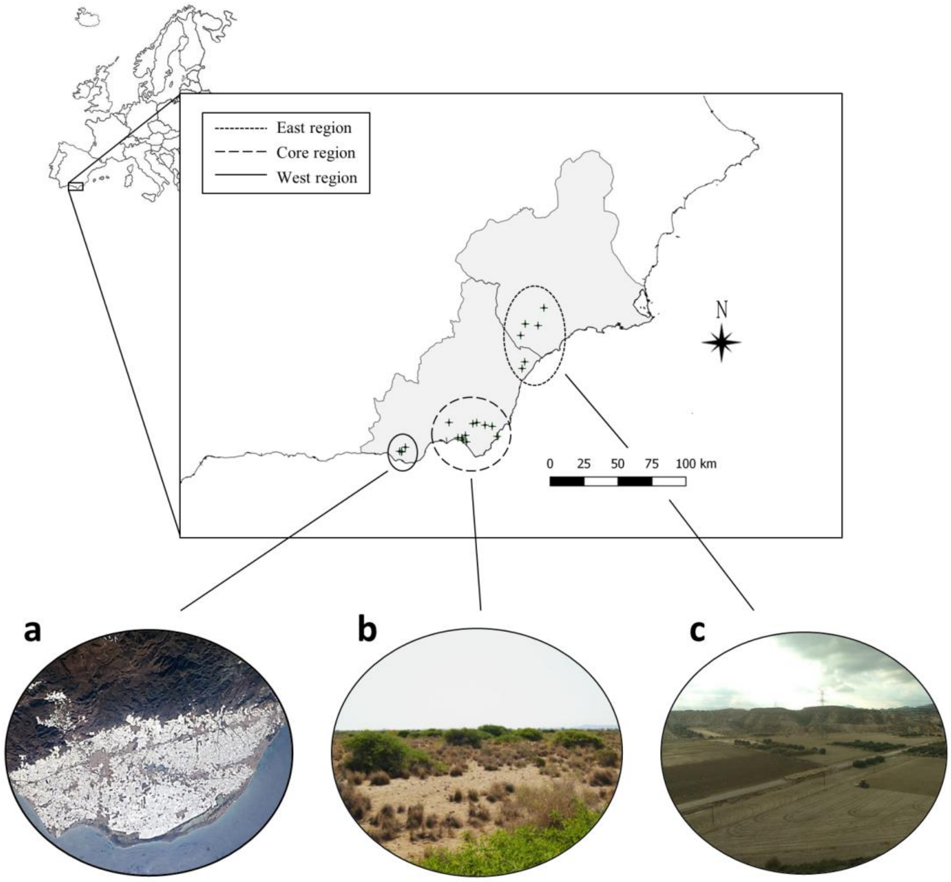


Fig. 1 Location of *Ziziphus lotus* studied populations in the southeast of the Iberian Peninsula. The 21 study populations were distributed in three regions: West region (4 populations, Almeria province), Core region (11 populations, Almeria province) and East region (6 populations, Almeria and Murcia provinces). Pictures below the map show the most representative habitat of each region: (***a***) satellite image from Google Earth showing the large white area of intensive agriculture in greenhouses; (***b***) well-preserved habitat in a protected area; (***c***) mosaic of intensive agriculture lands (without greenhouses) and abandoned areas where *Z. lotus* is usually growing in the limit of crops or in ramblas.

Pollinators and flower availability

We visited each population twice during June and July to perform pollinator censuses, conducting two 3-minute censuses on each geo-referenced individual plant. The field sampling included 798 censuses and 2394 minutes of observation. Preliminary sampling in 2014 showed that the foraging insect activity peaked between 9 00h and 16 00h. Therefore, our censuses were always performed within such timespan in 2015. Initially, observed floral visitors were taxonomically registered in the field at morphospecies level. We further took high quality photographs and collected insects after censuses for their taxonomic determination and functional classification. Taxonomic identification was done in collaboration with entomological specialists. Functional groups were further defined according to insect behavior on flowers and morphological and social features (Gómez et al. 2015, Martins et al. 2015, Otieno et al. 2015) (Table S1).

For each individual plant, we recorded the number of visitor insects per taxon (*pollinator abundance*) and the number of visited flowers per each insect *(count of visited flowers)* (see Table 1 for population mean values). We only considered legitimate visits when the insect clearly contacted a flower sexual organ. Simultaneously, we also monitored flower availability on all individual plants used for censuses. For this, we counted per plant the number of floral buds and open flowers in four sites (N, S, E and W orientations) with sampling squares (sized 225 cm^2) and estimated flower availability as the maximum mean number of flowers per square (flower density).

Characterization of landscape and habitat remnant degradation

Following Rey et al. (2017), we defined two levels of anthropogenic disturbance: local-scale (within *Ziziphus* habitat remnant) and large-scale (landscape) alteration.

In our study area, within-remnant degradation provokes heavy impacts on *Ziziphus* population spatial structure and size, and is mainly due to abandoned traditional agricultural uses, illegal rubbles and dumps, cattle overgrazing, and quarries. Briefly, to characterize the degradation (or quality) of each remnant, we used Quantum GIS 2.18.2 software (Quantum GIS Development Team 2016) and established aggregation patches of adult *Ziziphus* considering a 15 m-radius aggregation buffer around each adult individual. This provided nine metrics of the population structure that can inform about the remnant internal degradation and the habitat quality of each population (Table S2; Rey et al. 2017). We conducted a Principal Component Analysis (PCA) with the log-transformed metrics of each population that yielded three principal components (F1, F2, and F3, with eigenvalues accounting for 92.8% of total variance: 51.7%, 29.7%, and 11.4%, respectively). These defined habitat remnant quality-degradation and were used in subsequent analyses (Fig. S1 and Table S3). F1 defined a gradient with populations with

Table 1: Geographic location, acronym, number of individuals per population (N), climatic conditions, floral visitor abundance, number of visited flowers and insect species diversity of the 21 *Ziziphus lotus* study populations.

Population	Locality[a]	Region[b]	Acronym	N	Coordinates UTM (X)	Coordinates UTM (Y)	Tmin[c] (°C)	Tmax[c] (°C)	Pp[c] (mm)	Pollinator abundance[d]	Visited flowers[e]	Taxonomic diversity[f]	Functional diversity[f]
Béjar	S Murcia	East	BEJ	20	608753	4162689	17.55	30.25	180.70	316	1661	16.44	11.41
Campohemoso	E Almería	Core	CAMP	14	573207	4089516	19.65	30.27	258.60	295	520	30.12	16.64
Ctro. de Visitantes	E Almería	Core	CV	25	566902	4074470	20.73	29.53	244.60	155	504	27.27	10.67
Cuevas de Almazora	N Almería	East	CUE	20	606554	4129369	19.58	29.59	269.80	294	486	23.01	12.83
Dalías	W Almería	West	DALIAS	8	516503	4068393	19.61	28.93	253.40	129	340	34.37	12.00
El Ejido	W Almería	West	EJIDO	8	517838	4068287	19.61	28.93	253.40	169	354	24.27	10.00
Fernán Pérez	E Almería	Core	FP	20	584412	4086871	19.65	30.27	258.60	363	758	35.25	11.44
Guazamara	N Almería	East	GUAZ	25	608941	4134043	19.08	29.28	247.20	263	2134	36.41	14.22
Hospital de Poniente	W Almería	West	HOSPITAL	9	517810	4067793	19.61	28.93	253.40	175	561	29.36	14.84
Níjar	E Almería	Core	N	25	570227	4088796	19.65	30.27	258.60	427	1031	27.02	11.67
Playazo	E Almería	Core	PL	25	587885	4079328	20.73	29.53	244.60	721	2286	33.13	16.20
Puerto Lumbreras	S Murcia	East	LUM	20	605781	4154249	17.55	30.25	180.70	442	2055	29.97	10.00
Purias	S Murcia	East	PUR	14	618401	4161679	17.91	31.06	191.40	407	6181	30.66	11.91
Rambla Campohermoso	E Almería	Core	RCAMP	11	579257	4087669	19.65	30.27	258.60	157	331	24.91	11.61
Rambla Retamar	E Almería	Core	RR	25	562378	4077811	20.73	29.53	244.60	459	894	36.65	17.55
Retamar	E Almería	Core	AUT	20	564303	4079496	20.73	29.53	244.60	290	385	35.59	12.22
Sierra Alhamilla	Almería	Core	SALH	20	552531	4089557	19.65	30.27	258.60	180	262	31.98	10.41
Sierra de la Tercia	S Murcia	East	TER	20	624435	4175260	17.95	30.45	203.54	550	4703	26.38	11.00
Sta. María del Águila	W Almería	West	SMA	25	520643	4071120	19.61	28.93	253.40	908	2885	25.40	11.88
Torregarcía	E Almería	Core	TG	25	563288	4075416	20.73	29.53	244.60	231	387	25.19	12.88
El Toyo	E Almería	Core	TY	20	558505	4078249	20.73	29.53	244.60	189	425	36.20	12.23

[a] All populations are located in SE Spain; [b] Different sampling region (see details in Material and methods and Fig. 1); [c] Tmin and Tmax: average minimum and maximum temperature during the flowering season respectively; Pp: annual precipitation; [d] Total number of floral visitors per population; [e] Total number of visited flowers by floral visitors in each population; [f] Calculated from expected species accumulation curves (S_{40}) (see details in Materials and methods)

large continuous patches of regular area and many individuals at its upper extreme, and smaller populations dominated by isolated individuals or irregular patches at the lower endpoint. F2 differentiated between populations in small areas dominated by large *Ziziphus* patches at its upper endpoint, and populations extended throughout large areas dominated by small *Ziziphus* patches at its lower extreme. F3 defined populations with few dense but distant *Ziziphus* patches at the upper endpoint, and populations with nearby internally sparse *Ziziphus* patches at the lower end.

Landscape disturbance in our studied area is mainly caused by agricultural intensification (mostly greenhouses) and urban expansion, which have provoked a reduction of natural and semi-natural habitat cover and a relevant land-use transformation. Following Cancio et al. (2016) and Rey et al. (2017), we used the centroid of the convex hull delimiting the population as the center of a 1.5 km radius circular area around the population where we estimated the cover (%) of different land uses to obtain a Shannon-index of *land-use diversity* (as a measure of landscape complexity or heterogeneity; Fahrig et al. 2011), and the cover (%) of *Ziziphus* natural habitat (*natural habitat cover*). We used the land-use and vegetation cover cartography 2006 (1:10.000) of Junta de Andalucía (http://www.juntadeandalucia.es/medioambiente/site/rediam) for 17 Almeria populations and CORINE Land Cover 2006 (https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-corine) (1:100.000, 100 meters resolution) for four Murcia populations and Quantum GIS 2.18.2 software (Quantum GIS Development Team 2016). See Cancio et al. (2016) and Rey et al. (2017) for details.

Statistical analyses

Variation in the taxonomic and functional composition of floral visitor assemblages and their floral visit records were tested using PERMANOVA analysis, conducted on Bray-Curtis pairwise quantitative dissimilarity matrices (Clarke and Gorley 2006). These analyses were performed for each taxon, or each functional group, per plant. Factors in these analyses were region (*Core, West* and *East*) defined as fixed effect, and population as random effect nested within region. These analyses were conducted with PRIMER 6.1.15 software (Clarke and Gorley 2006) with 999 permutations.

Distance-based redundancy analyses (dbRDA and partial-dbRDA) were used to test the influence of honey bee relative abundance and its floral visits on abundance and floral visits of wild bees and non-bee taxa while accounting for geographic location. Separated analyses were conducted for taxonomical and functional composition. Population variation in honey bee abundance and its floral visits and in geography was expressed as quantitative pairwise between population dissimilarity matrices and summarized by a multidimensional scaling analysis (MDS or PCoA). PCoA scores of axis-1 and axis-2 were used as independent variables of dbRDA and partial-dbRDA. The same procedure was followed to test the contribution of different groups of floral visitors (honey bees, wild bees and non-bee taxa; taxonomical framework) on the number of visited flowers per population and to test the effects of population, remnant degradation and landscape features on abundance of visitors and flower visited. Mean flower density (per individual) of each population, distance to nearest population, latitude and longitude were used as population variables; F1, F2 and F3 components as habitat remnant variables (local-scale alteration); and natural habitat cover and land-use diversity as landscape variables (large-scale disturbance).

EstimateS 9.0 software (Colwell 2013) was used to estimate in each *Ziziphus* population taxonomical and functional insect visitor richness (S_x, being '*x*' the number of extrapolated samples), using sampled-based rarefaction curves (Gotelli and Colwell 2001, Colwell et al. 2004, 2012) with each adult *Ziziphus* being a sample. The expected species accumulation curves were computed using the floral visitor number per individual pooled per population and extrapolating it up to a common value of 40 samples (S_{40}). S_{40} was thus considered an adequate extrapolation point according to Colwell (2013), who recommended extrapolating the minimum number of observations by a maximum factor of 2 or 3, being 16 (8 individuals x 2 monthly censuses, considered independent) our minimum number of observations for a population. These estimates of richness were used as indicators of floral visitor diversity in our populations.

Finally, we tested the effects of population and landscape features on richness of floral visitors (S_{40}), using best-subset multiple regression models, fitted with maximum likelihood. Separated models were fitted for taxonomic and functional richness. Model selection was based on Akaike Information Criteria corrected for small sample sizes (AICc). Among all possible competing models, we chose models with the least AICc, used $\Delta_i < 2$ to define models equally valid statistically and selected among them the most parsimonious one (Burnham and Anderson 2002). Dependent variables of functional richness (S_{40} for each studied assemblage) were previously log-transformed to adjust a Gaussian distribution. All selected best models showed normality of their residuals.

To meet our aims, the taxonomic and functional groups of floral visitors were re-classified in no honey bees, non-bee taxa, wild bees, and honey bees for most of the analyses (details in Table S1).

DbRDA and partial-dbRDA analyses were performed with the R-package VEGAN (Oksanen et al. 2018) using the *capscale* function. MDS analyses were developed with R-package STATS (R Core Team 2017) from *cmdscale* function. Best subset multiple regression tests were conducted with R-package GLMULTI (Calcagno 2013) from *glmulti* function. All R software analyses were performed with R version 3.4.3 (R Core Team 2017).

Unless otherwise stated means and standard errors (SE) are reported in this study.

RESULTS

Overall composition of insect floral visitor assemblages of *Ziziphus lotus*

a) Description of the taxonomic assemblage

A total 7120 insect specimens belonging to 82 taxa were recorded visiting 29143 *Z. lotus* flowers in our 21 populations (Image S1 and Table S4). Averaged across populations, 8.36 ± 2.17% of the assemblage was represented by honey bees, 23.42 ± 3.07% by wild bees, and 68.22 ± 3.75% by non-bee taxa (see Fig. S2*a* for population details). Honey bees accounted on average for 38.77 ± 5.49% of all the flowers visited, whereas wild bees did 12.02 ± 3.16% and non-bees taxa 49.21 ± 5.27% (Fig.S2*b*). More details on the percentage of main taxonomic groups and visited flowers per population are shown in Fig. 2 (*a*) and (*b*), respectively. Complementary data can be found in Table S5 (for the whole assemblage). Specific information about bee visitor records (honey and wild bees) and of the other main nectar collectors (hoverflies and bee flies) can also be consulted in Table S6 and Fig S3 (the latter only for bees).

b) *Description of the functional assemblage*

Seventeen functional groups were defined to classify our 82 taxa (Table S1). Non-collecting flies and small collector bees were the most numerous groups, with 25.04 ± 4.22% and 22.70 ± 3.11% of counts, respectively. Diving ants represented 18.71 ± 3.82%, large collector bees 9.08 ± 2.25%, large collector wasps 6.54 ± 1.18%, diving beetles 4.27 ± 1.11%, small butterflies

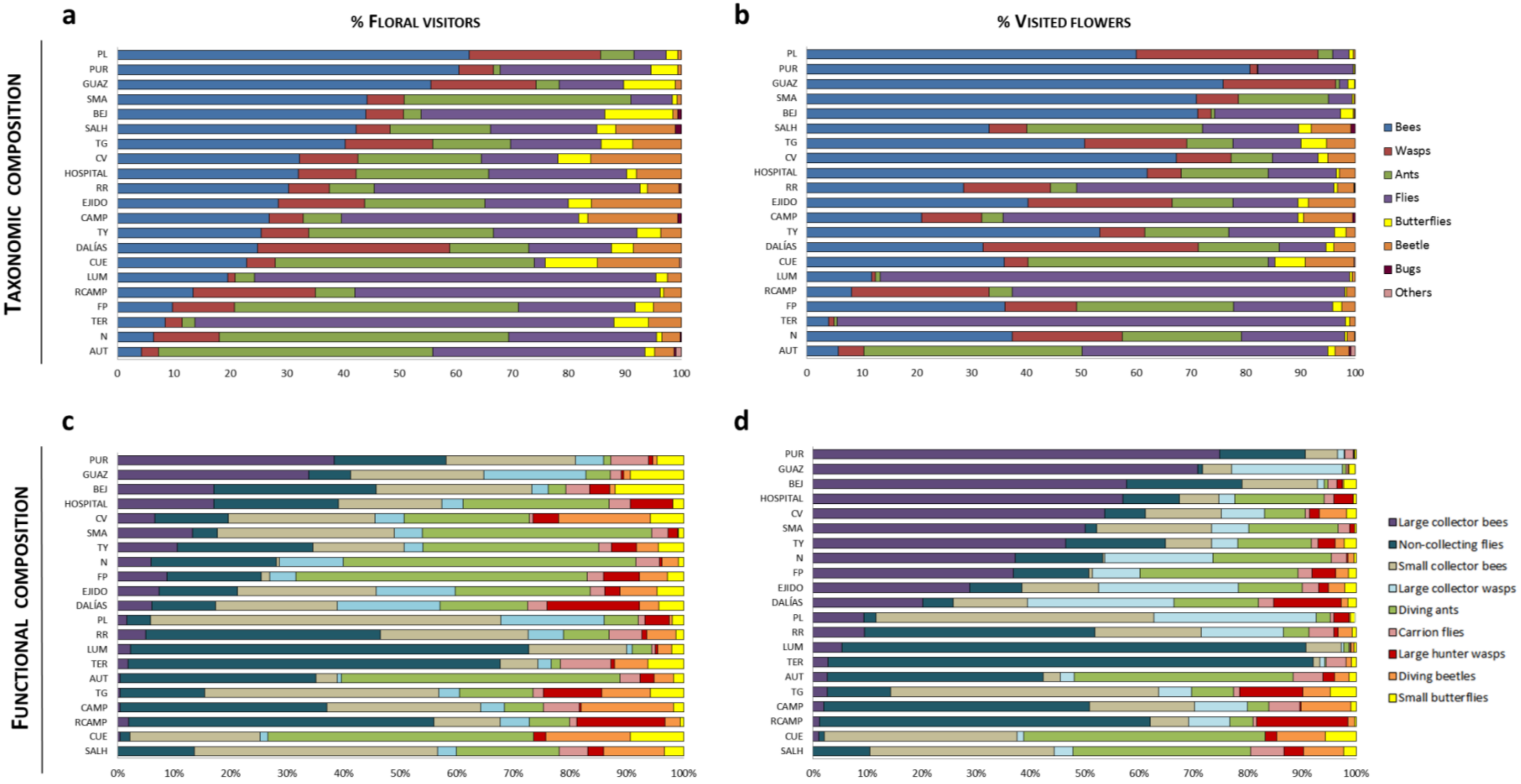


Fig. 2 Abundance and floral visits of the visitor assemblage in the 21 *Ziziphus lotus* study populations. Plots are drawn for taxonomic (***a*** and ***b***) and functional (***c*** and ***d***) assemblages. Data are referred only to the insect groups accounting for 96% of the total assemblage. Details of population acronyms can be consulted in Table 1.

3.64 ± 0.68%, carrion flies 3.55 ± 0.48% and large hunter wasps 3.45 ± 0.96%. The remaining functional groups pooled represented each 1% or less of total observations (Table S7). Further complementary information about the percentage of main functional groups per population (96% of total visitor assemblage) can be found in Fig. 2*c*.

According to the flowers visited per group, large collector bees and non-collecting flies were the groups visiting more flowers, with 39.56 ± 5.62% and 29.74 ± 5.79% of counts respectively. Small collector bees represented 11.23 ± 3.22%, large collector wasps 7.18 ± 2.00%, diving ants 5.92 ± 2.90%, carrion flies 2.00 ± 0.40%, large hunter wasps 1.48 ± 0.98%, and diving beetles 1.04 ± 0.63%. Each remaining functional group accounted for less than 1% of total floral visits (Table S7). Additional information on the percentage of flowers visited by the main functional groups in each population (96% of total visitor assemblage) is shown in Fig. 2*d*.

Regional and population variation in *Ziziphus lotus* floral visitor assemblages

a) Taxonomic assemblage

PERMANOVA analyses showed significant differences among populations and regions in the taxonomic composition of the whole visitor assemblage (Table 2, all floral visitors). Such differences still held after excluding bees (Table 2, non-bee taxa). However, analyzing only wild bees and honey bees resulted in differences among populations, but not among regions (Table 2, wild bees and honey bees).

Results on the number of flowers visited by the whole visitor assemblage followed a similar pattern since we found differences among populations and regions (Table 2, all floral visitors). This was also detected when bee taxa and only honey bees were excluded (Table 2, non-bee taxa and no honey bees, respectively). However, when wild bees and honey bees were analyzed independently, we only detected differences between populations (Table 2). These results indicated that non-bee taxa play a more relevant role than wild bees in the differences in taxonomic composition (in term of abundance and floral visits) among regions, showing differences among the three regions.

b) Functional assemblage

PERMANOVA analyses showed that the functional composition of the whole visitor assemblage varied significantly among populations but not among regions (Table 2, all floral

Table 2: Results of PERMANOVA analyses testing the variation among Populations (Po) and Regions (Re) in insect visitor assemblage composition and number of visited flowers. Tests were based on Bray-Curtis (dis)similarity index. Pairwise tests were performed only when differences among regions were detected and were based on Monte Carlo *p*-values.

		Abundance of visitor assemblage						Floral visits of visitor assemblage					
Assemblage	Source	*df*	MS	Pseudo-*F*	P(MC)	Pair-wise test		*df*	MS	Pseudo-*F*	P(MC)	Pair-wise test	
a) Taxonomic framework													
All floral visitors	Re	2	23146.0	1.675	0.029	C = E		2	32485.0	2.198	0.001	C ≠ E	**
	Po(Re)	18	16554.0	5.922	0.001	C ≠ W	.	18	17686.0	5.824	0.001	C ≠ W	*
	Res	369	2795.2			E ≠ W	*	369	3036.8			E ≠ W	*
	Total	389						389					
No honey bees	Re	2	21230.0	1.581	0.054	C = E		2	28716.0	1.940	0.005	C ≠ E	*
	Po(Re)	18	16068.0	5.759	0.001	C ≠ W	.	18	17745.0	6.067	0.001	C ≠ W	.
	Res	369	2790.2			E ≠ W	*	369	2925.1			E ≠ W	*
	Total	389						389					
Non-bee taxa	Re	2	25091.0	1.953	0.007	C ≠ E	.	2	32130.0	2.212	0.004	C ≠ E	*
	Po(Re)	18	15334.0	5.375	0.001	C ≠ W	*	18	17413.0	5.923	0.001	C ≠ W	*
	Res	368	2853.0			E ≠ W	*	368	2939.7			E ≠ W	*
	Total	388						388					
Wild bees	Re	2	7808.8	1.328	0.232			2	8927.2	1.290	0.240		
	Po(Re)	18	6684.2	3.526	0.001			1	7876.3	3.605	0.001		
	Res	189	1895.4					189	2185.0				
	Total	209						209					
Honey bees	Re	2	2013.3	1.186	0.316			2	2715.7	1.113	0.359		
	Po(Re)	16	2028.7	1.697	0.027			16	2820.4	1.514	0.030		
	Res	89	1195.8					89	1863.4				
	Total	107						107					

Note: df [degrees of freedom]; MS [mean square]; P(MC) [Monte Carlo p-values]; C [Core region]; E [East region]; W [West region]. For all analyses ‘Population’ was a nested factor within ‘Region’ [Po(Re)]. For pair-wise test: ** $p< 0.01$; * $p< 0.05$; $p< 0.1$

Table 2 (cont.): Results of PERMANOVA analyses testing the variation among Populations (Po) and Regions (Re) in insect visitor assemblage composition and number of visited flowers. Tests were based on Bray-Curtis (dis)similarity index. Pairwise tests were performed only when differences among regions were detected and were based on Monte Carlo *p*-values.

		Abundance of visitor assemblage						Floral visits of visitor assemblage					
Assemblage	Source	*df*	MS	Pseudo-*F*	P(MC)	Pair-wise test		*df*	MS	Pseudo-*F*	P(MC)	Pair-wise test	
b) Functional framework													
All floral visitors	Re	2	20343.0	1.568	0.105			2	35603.0	2.474	0.001	C ≠ E	**
	Po(Re)	18	15620.0	6.803	0.001			18	17302.0	6.507	0.001	C ≠ W	.
	Res	369	2296.2					369	2659.0			E ≠ W	*
	Total	389						389					
No honey bees	Re	2	17691.0	1.411	0.164			2	30181.0	2.124	0.008	C ≠ E	*
	Po(Re)	18	15085.0	6.628	0.001			18	17105.0	6.799	0.001	C = W	
	Res	369	2276.1					369	2516.0			E ≠ W	*
	Total	389						389					
Non-bee taxa	Re	2	20376.0	1.703	0.061	C = E		2	32603.0	2.334	0.006	C ≠ E	*
	Po(Re)	18	14372.0	6.276	0.001	C = W		18	16826.0	6.742	0.001	C ≠ W	.
	Res	368	2289.9			E ≠ W	.	368	2495.8			E ≠ W	*
	Total	388						388					
Wild bees	Re	2	5732.8	1.384	0.237			2	10444.0	1.923	0.095	C ≠ E	*
	Po(Re)	18	4689.7	3.289	0.001			18	6175.9	3.546	0.001	C = W	
	Res	189	1425.7					189	1741.6			E = W	
	Total	209						209					

Note: df [degrees of freedom]; MS [mean square]; P(MC) [Monte Carlo p-values]; C [Core region]; E [East region]; W [West region]. For all analyses 'Population' was a nested factor within 'Region' [Po(Re)]. For pair-wise test: ** $p< 0.01$; * $p< 0.05$; .$p< 0.1$

visitors). Non-bee taxa abundance differed significantly among populations within region and marginally among regions (Table 2, non-bee taxa). When honey-bees were removed from the analyses, differences were exclusively detected between populations (Table 2, no honey bees). Similarly, wild-bee abundance only differed between populations (Table 2, wild bees). Thus, apparently, the visitor assemblages of the three regions are functionally equivalent despite existing differences at taxonomic scale. Nonetheless, these functional similarities seem to be mainly due to bees (honey and wild).

Concerning the number of visited flowers, all comparisons of the functional composition of visitor assemblage rendered significant differences between populations and among regions (only marginally significant among regions for wild bees) (Table 2). Thus, these results are similar to the ones found when analyzing the taxonomic composition of visitor assemblage.

The influence of honey bees on floral visits and floral visitor assemblage variation

Between-population differences in the total number of visited flowers were mainly associated with honey bee abundance dissimilarities ($F_{1,12}$=107.901, *P*= 0.002) that explained 36.1% of floral visit variation, and secondarily to non-bee abundance differences (with a minor contribution of 9.2%; $F_{1,12}$= 30.266, *P*= 0.004). Differences in wild bee abundance and geographic location were not related to floral visit dissimilarities (details in Table S8). This indicates that honey bees more than any other visitor group influence the differences among populations in the pollinator service (i.e, total number of floral visits). It happens even if honey bees are not the most abundant floral visitors (Fig. 3).

Regarding the influence of honey bees in the rest of visitor assemblage, dbRDA and partial-dbRDA results showed that taxonomic differences among populations in abundance of non-bee taxa and in their number of flower visited were respectively linked with differences in honey bee abundance ($F_{1,16}$= 3.039, *P*= 0.002, *%var*= 9.8) and their floral visits ($F_{1.16}$= 2.774, *P*= 0.006, *%var*= 10.9), but they were also influenced by geographic location ($F_{1.16}$= 3.475, *P*= 0.001, *%var*=14.1 and $F_{1.16}$= 4.798, *P*= 0.002, *%var*= 18.3; respectively) (details in Table S9, taxonomic framework). Differences in wild bee abundance were only marginally associated with geographic location ($F_{1.16}$= 2.152, *P*= 0.053, *%var*=10.8) but not with variations in honey bee abundance; moreover, their dissimilarities in the number of visited flowers were not explained either by variations in honey bee floral visits or geographic location (Table S9, taxonomic framework). The relationship between honey bee and non-bee taxa exhibited a negative tendency, and the same occurred for their floral visits (details in Fig. S4).

Variations in honey bee abundance and in their floral visits did not explain differences in functional composition or number of visited flowers (respectively) either by non-bee taxa or wild bees (Table S9, functional framework). However, the geographic location was associated with differences in non-bee taxa abundance ($F_{1.16}$= 3.466, *P*= 0.010, *%var*=15.7) and in their floral visits ($F_{1.16}$= 5.005, *P*= 0.001, *%var*=20.7) but not with wild bee for any of both response variables (Table S9, functional framework). Considered together, these results suggest that non-bee taxa are more sensitive than wild bees to honey bee presence in taxonomical but not in functional terms.

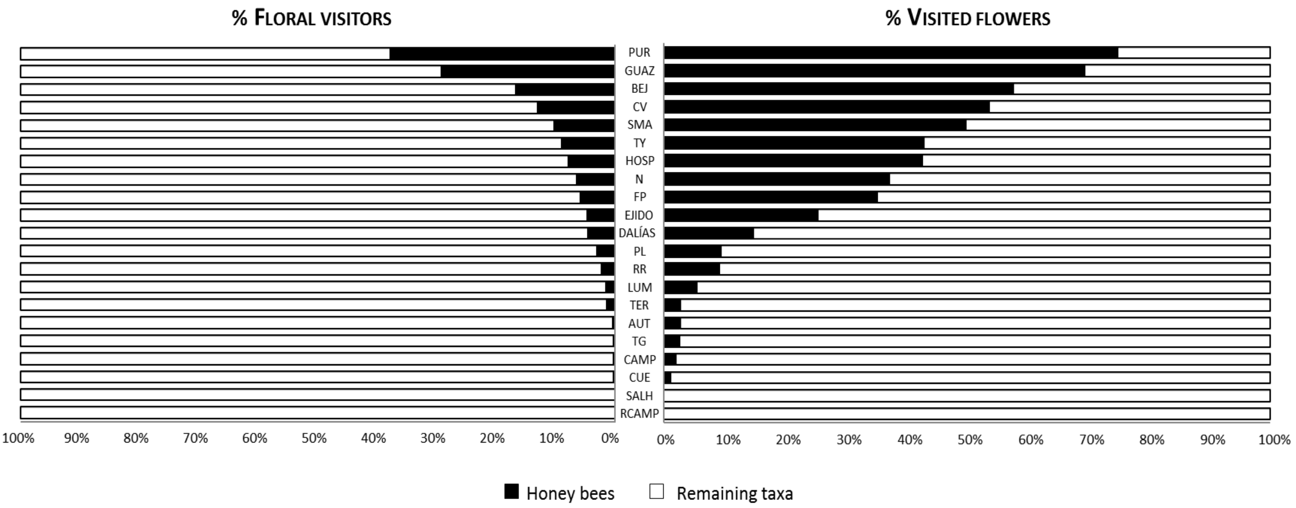


Fig. 3 Abundance (*left*) and floral visits (*right*) of honey bees (**black**) vs. remaining taxa (**white**) in 21 *Ziziphus lotus* populations. Honey bees show a high dominance in floral visits, despite not being dominant in terms of floral visitors (abundance). Details of population acronyms can be consulted in Table 1.

Variation of the visitor assemblage according to population and landscape features

a) Taxonomic and functional composition of the visitor assemblage

In terms of floral visitors, flower density was the most important variable to explain differences in taxonomic composition between populations ([Per assemblages] All floral visitors: *%var*= 7.9; No honey bees: *%var*= 8.9; Non-bee taxa: *%var*= 8.8; see Table S10 for details). Distance to the nearest population (*%var*= 3.8) and latitude (*%var*= 5.3) were significantly associated with differences in the non-bee taxa abundance among populations, but these effects were marginal when honey bee was excluded from the analysis (*%var*= 8.0, in each case). Land use diversity only influenced marginally non-bee taxa abundance (*%var*= 6.3). These results again suggest that non-bee taxa abundance seems to be more sensitive than wild bees to changes in population features (latitude and distance among populations) or landscape disturbance (land use diversity) (details in Table S10).

Differences in functional composition of floral visitors indicated quite similar effects to those found with the taxonomic composition (Table S11). Latitude showed no influence on the non-bee taxa, and flower density was the only variable associated with functional abundance when honey bees were excluded from the analysis (Table S11). Thus, the functional composition of the visitor assemblage apparently showed greater spatial consistency than the taxonomic composition. None of the studied variables was related with taxonomic or functional abundance of wild bees and with taxonomic abundance of honey bees when these assemblages were independently studied (details in Table S10 and S11, for taxonomic and functional framework, respectively).

Regarding floral visits (i.e. pollinator service), flower density ([Per assemblages] All floral visitors: *%var*= 4.3; No honey bees: *%var*= 8.1; Non-bee taxa: *%var*= 7.5) and distance to the nearest population (All floral visitors: *%var*= 4.7; No honey bees: *%var*= 5.0; Non-bee taxa: *%var*= 6.0) were the most important predictors of the taxonomic differences between populations (see Tabls S10 for details). However, latitude also influenced the floral visits of non-bee taxa (*%var*= 3.1). Likewise, the functional composition of the floral visits was influenced, though weaker, by these same predictors, although in this case latitude was not associated with floral visits of non-bee taxa (Table S11). Wild bee floral visits seemed to be unaffected by any studied variables (details in Table S10, taxonomic framework), but variation in their functional composition was associated with longitude (Table S11).

b) Visitor richness

Estimated visitor species richness (S_{40}) varied considerably among populations both taxonomically (range: 16.44 ± 2.70 (BEJ) and 36.65 ± 5.65 (RR)) and functionally (range: 10.00 ± 0.01 (LUM) and 17.55 ± 3.09 (RR)) (more details on diversity in Table 1).

Regarding taxonomic richness, the best model in the multiple regression showed that land-use diversity affected positively the visitor assemblage species richness (S_{40}), which was also negatively affected by F3 component of remnant degradation (mean distance among the nearest *Z. lotus* patches, so that the greater the distance between patches, the lower the richness) (Table 3). The effect of land use diversity (positive) and F3 (negative) on richness was significant in all analyses, except when wild bees were analyzed independently (Table 3, note that no variable was associated with wild bee taxonomic richness). This may indicate a special sensitivity to habitat alteration by the non-bee taxa, but not by wild bee species.

Land-use diversity was the only variable that showed positive effects on functional richness of the whole assemblage and this was also true when only non-bee taxa were considered (Table 3). Hence, the functional richness again seems to be less sensitive to habitat change than the taxonomic richness. No variable was associated to wild bee functional richness (null model selected as best model, Table 3).

DISCUSSION

The assemblage of floral visitors of *Z. lotus* comprises a wide variety of taxa (82 species, belonging to 35 families and 7 insect Orders) and functional groups (17) across the whole range of the species in southeastern Spain. We further detected a remarkable variety of floral visitors in most populations although the composition of visitor assemblage substantially varied among populations and, to lesser extent, geographically. Since all these visitors contacted sexual organs of the flower, they are probably relevant for pollination of this species. In consequence, the pollinator assemblage of *Z. lotus* may be qualified as highly generalist (*sensu* Gómez and Zamora 1999, Herrera 2005) both regionally and locally, as it would be expected from a mass-flowering species with a non-specialized flower structure. Studies on other species of *Ziziphus* from different continents also show generalist pollinator systems (e.g., *Z. celata* [Weekley and Race 2001], *Z. mauritiana* [Mishra et al. 2004], *Z. joazeiro* [Nadia et al. 2007], *Z. mistol* [Cerino et al. 2015]). Despite this generalist pollinator system, our study shows that the spillover of managed honey bees most likely from greenhouse agriculture (that accounted on average for 39% of floral visits) and the high landscape and within-habitat remnant degradation of this area are affecting wild *Z. lotus* insect pollinators and pollinator service. These effects seem however lessened in terms of functional composition and diversity of the assemblages. This suggests that functional redundancy in this generalist pollinator system may act to some extent as insurance for the pollination function of the species (Loreau et al. 2003) over the region.

Table 3: Model selection results by best-subset multiple regression and best-model regression tests for taxonomic and functional richness (S40).

Model selection					Regression test of best model					
Candidate models	AICc	*Δ* AICc	Weights		Variable	Estimate	Std. Error	t value	Pr(>|t|)	
a) Taxonomic frameworks										
S40 of total assemblage										
~ 1 + LandUse + F3	**123.324**		**1.29E-01**	†	(Intercept)	16.806	4.571	3.677	0.002	**
1 + Natcov + LandUse + F3	124.246	0.922	8.17E-02		LandUse	18.201	6.441	2.826	0.011	*
~ 1 + Natcov + LandUse + F1 + F3	124.765	1.441	6.30E-02		F3	-2.973	0.859	-3.460	0.003	**
S40 of assemblage without honey bees										
~ 1 + LandUse + F3	**123.944**		**1.34E-01**	†	(Intercept)	15.930	4.639	3.434	0.003	**
~ 1 + Natcov + LandUse + F3	124.375	-0.675	1.08E-01		LandUse	18.048	6.537	2.761	0.013	*
~ 1 + Natcov + LandUse + F1 + F3	125.137	0.086	7.36E-02		F3	-3.192	0.872	-3.660	0.002	**
S40 of non-bee taxa assemblage										
~ 1 + Natcov + LandUse + F1 + F2	125.050		8.94E-02		(Intercept)	14.200	4.885	2.907	0.009	**
~ 1 + Natcov + LandUse + F2	125.575	0.524	6.88E-02		LandUse	15.435	6.883	2.242	0.038	*
~ 1 + LandUse + F3	**126.110**	**1.060**	**5.26E-02**	†	F3	-2.063	0.918	-2.247	0.037	*
S40 of wild bee assemblage										
~ 1 + Lat	81.376		4.37E-02		(Intercept)	3.097	0.341	9.079	<0.001	***
~ 1 + F3	81.938	0.561	3.30E-02							
~ 1	**81.996**	**0.620**	**3.20E-02**	†						
b) Functional frameworks										
S40 of total assemblage										
~ 1 + LandUse + Lat + Lon	92.576		8.11E-02		(Intercept)	7.490	2.342	3.198	0.005	**
~ 1 + Lat + Lon	92.633	0.057	7.88E-02		LandUse	7.256	3.300	2.199	0.040	*
~ 1 + LandUse	**93.349**	**0.772**	**5.51E-02**	†						
S40 of assemblage without honey bees										
~ 1 + LandUse	**92.558**		**9.09E-02**	†	(Intercept)	5.492	2.298	2.390	0.027	*
~ 1 + LandUse + F1	92.680	0.123	8.55E-02		LandUse	9.238	3.238	2.853	0.010	*
~ 1 + LandUse + F1 + Lat + Lon	93.736	1.178	5.04E-02							
S40 of non-bee taxa assemblage										
~ 1 + LandUse + Lat + Lon	88.506		8.74E-02		(Intercept)	5.294	2.110	2.509	0.021	*
~ 1 + LandUse	**88.960**	**0.454**	**6.96E-02**	†	LandUse	7.371	2.972	2.480	0.023	*
~ 1 + LandUse + F1 + Lat + Lon	89.118	0.158	6.43E-02							
S40 of wild bee assemblage										
~ 1	**34.717**		**6.87E-02**	†	(Intercept)	1.429	0.111	12.910	<0.001	***
~ 1 + F3	34.898	0.181	6.28E-02							
~ 1 + F1	36.027	1.310	3.57E-02							

Note: Habitat natural cover [Natcov]; Land-use diversity [LandUse]; Distance to nearest population [Popdist]; Flower density [Flower]; Lat [Latitude]; Lon [Longitude]. † Best model (based in AICc and maximun parsimony); *** $p<$ 0.001; ** $p<$ 0.01; * $p<$ 0.05.

Managed honey bees

Honey bees (more abundant in the vicinity of greenhouses, personal observation) were important pollinators of *Z. lotus.* Although they accounted only for 12% of the floral visitors, honey bees exhibited disparate floral visit dominance (overall 39% in our censuses) with respect to the rest of visitors (Fig. 3). This underlines that honey bees are heavy floral resource consumers and strong competitors for the rest of wild visitors. The "*cross-habitat hypothesis*" stated by Tscharntke et al. (2012) warned that the spillover of organisms across managed and natural ecosystems is an important process that affects the structure and dynamics of wildlife populations and communities in human-dominated landscapes. Our results indicate that honey bees are likely modifying the composition of pollinator assemblages and, especially, the flower visitation rates by wild pollinators in *Z. lotus* populations in southeastern Spain (see González-Varo and Vila 2017, Magrach et al. 2017, and Geldmann and González-Varo 2018 for other cases in native plant communities). The high densities of managed honey bees in natural habitats are frequently due to spillovers from agrosystems (González-Varo and Vilà 2017). They represent a great competition for wild pollinators, particularly in search for feeding resources (Torné-Noguera et al. 2016, Magrach et al. 2017), since *A. mellifera* is known for its marked preference for dominant or mass-flowering plant species (Gross 2001, González-Varo and Vilá 2017). These features are worrisome to maintain wild pollinator diversity especially in habitats with limited resources or stressful conditions (Herbertsson et al. 2016, Magrach et al. 2017), as is the semiarid southeast of the Iberian Peninsula, particularly during the summer season. In this period, *Z. lotus* blooms massively and represents the main feeding resource for many insect species because the flowering of other plant species is scarce. Thus, the high honey bee values showed in this work are even more relevant.

Moreover, although *Apis mellifera* is the most useful managed pollinator (due to its floral visit rate) (Klein et al. 2012, Garibaldi et al. 2013), a high visitation rate of honey bees has also been associated with damage in floral reproductive structures (Sáez et al. 2014) or with the removal of pollen previously deposited (Gross and Mackay 1998), provoking losses in the fruit set. Thus, the continuous spillover of honey bees from crops to surrounding natural habitats entails competition for wild pollinators, but also may jeopardizes the reproductive performance of autochthonous plant species, being this effect probably greater in endemic or rare species (Valido et al. 2014, Torné-Noguera et al. 2016). By affecting wild pollinators, continuous spillovers from greenhouses (which are under crop production during the whole year) could have serious consequences for *Ziziphus* natural habitats in the long term.

Our results also suggest that non-bee taxa are the most sensitive group affected by the presence of honey bees. Honey bee abundance explained the variation in the abundance and floral visits of non-bee taxa but not of wild bees. This contrasts with recent findings by González-Varo and Vilà (2017) who showed that the increase of honey bee densities in natural habitat threats wild bees. Such discrepancy arises probably from specific features of our study area and plant species. *Z. lotus* is a highly generalist plant comprising a huge diversity of non-bee species, which are also responsible for regional differences in assemblage composition among our three study areas. However, despite the richness of non-bee taxa, no species appeared at high density in the censuses (neither in count of individuals nor in floral visits), which probably reflects low population sizes.

Contrastingly, the functional assemblage of floral visitors of *Z. lotus* seems less sensitive to managed honey bees since neither non-bee taxa nor wild bee composition correlated with the presence of honey bees. This agrees with the predictions of "*the insurance hypothesis of biodiversity*" (Loreau et al. 2003): biodiversity from habitats with a high taxonomic diversity usually exhibits an overlap or equivalence of functions (functional redundancy; Rosenfeld 2002). Thus, individuals of different taxa can play similar roles or perform a function similarly within the ecosystem, which makes the system more stable (Guillemot et al. 2011). After an environmental change, like intrusion by managed bees, it is expected that pollinator systems with great biodiversity, like a generalist pollinator system, maintain the pollination function better than others with lower diversity of pollinators. Thus, the high taxonomic diversity of *Z. lotus* pollinator assemblage should ensure functional stability and favor pollination service in the long run (Waser et al. 1996).

Habitat degradation and landscape disturbance

Variation in composition of pollinator assemblages may be as important for the pollination function as variation in abundance and richness of pollinators, and (although less studied) pollinator composition and richness may be also affected by land-use change (Winfree et al. 2011). Our results suggest that both *Z. lotus* floral visitor assemblage composition and their floral visits were influenced to a larger extent by population features, while taxonomic and functional richness were more influenced by landscape and habitat remnant degradation.

Population differences in visitor assemblage composition and floral visits were mainly explained by differences in population flower density (see also Steffan-Dewenter and

Tscharntke 2011) and secondarily by the distance to the nearest population. This was confirmed both for taxonomic and functional assemblages. Flower density effect is expected since pollinators rely on floral resources and typically track their abundance (Winfree et al. 2011, and references therein). Its effect on the floral visitor abundance and the visited flowers was consistently detected for the whole assemblage, but also for the assemblage without honey bees and for the non-bee taxa. The effect of distance between nearby *Z. lotus* populations is related to landscape disturbance (large-scale) in southeast of Spain, provoked by the loss of natural habitats and land conversion in the last 60 years (Mota et al. 1996, Puigdefábregas and Mendizábal 1998, Benito et al. 2009). Thus, it seems that the recent increase of population isolation is provoking changes in the *Z. lotus* visitor assemblage composition and function, especially in terms of floral visits (Table S10 and S11). It is expected to affect the fecundity of this species if they continue in the long run.

Local-scale disturbance causing isolation from natural patches has been linked to shifts in wild pollinator richness and abundance (Garibaldi et al. 2011, Rader et al. 2016), decreasing the pollination service as the isolation from natural patches increases (Lazaro and Tur 2018). Our results support this observation since wild visitor richness was negatively influenced by increased distance between neighbouring natural patches (F3 factor, intra-habitat degradation – local-scale disturbance) caused by the internal degradation (i.e., patch clearing) of the *Ziziphus* habitat remnants. However, the functional richness of the assemblage was not affected by internal degradation of the remnant, reinforcing the idea of functional stability conferred by the generalist pollinator system of *Z. lotus*. In contrast, alteration at the landscape level acted consistently both on the taxonomic and functional richness of visitor assemblage. Land-use diversity was positively associated with wild pollinator richness (see also Aizen and Harder 2009, Lazaro and Tur 2018). Tscharntke et al. (2005) emphasized that although agricultural landscapes are generally associated with loss of biodiversity, they can also contribute to ecosystem services. They underlined that low-intensity agricultural landscapes may harbor high diversity of land-use and greater variety of nesting niches that improve the biodiversity and ecosystem services of surrounding natural habitats.

In any case, honey bees, wild bees and non-bee taxa were affected differently by landscape and within-habitat remnant degradation. Few studies have investigated the response of different pollinator groups to anthropogenic alteration gradients, but results obtained so far do not suggest many differences (reviewed in Winfree et al. 2011). The relative abundance of honey bees in the visitor assemblage was not influenced by any landscape, habitat remnant or geographic features (see Garibaldi et al. 2011 for a similar pattern). This is probably because greenhouse agriculture is extended by most of the range of *Z. lotus* and it is also increasingly expanding towards protected areas. On the other hand, abundance and floral visits of non-bee taxa were again more sensitive to landscape and habitat alteration than wild bees, both in terms of composition and richness. This agrees with the already mentioned sensibility of non-bee taxa to anthropogenic disturbances, either by intrusion of managed honey bees or by landscape and habitat remnant degradation. A meta-analytical review by Winfree et al. (2011) showed that both bee abundance and richness are sensitive to extreme natural habitat loss but respond less clearly to moderate habitat loss. This makes difficult to detect a relationship between bee abundance or richness and anthropogenic disturbance throughout ample gradients of landscape and habitat alteration, like the one examined here.

Concluding remarks

Studies monitoring wild insect pollinators and flower visitation rates in generalist keystone plant species across many populations and ample gradients of anthropogenic land use are useful to evaluate the impact of different global change drivers on insect pollinators and pollinator service at the community level. Our study evidences that agricultural intensification and land conversion cause severe changes in the insect pollinator community of natural habitats. On the one hand, it supports the idea that the wild insect assemblage (and flower visitation rates) of native plants are negatively affected by elevated densities of managed honey bees. It is necessary to warn about the consequences that the eventual honey bee spillovers may have on pollination of many plant species, even conducting to regional extinctions of some wild pollinator species. On the other hand, we show that small (within-habitat remnant) and large scale (habitat loss and land conversion at the landscape level) anthropogenic degradation also impacts on pollinator assemblages. Maintenance of pollinator diversity and function in human-dominated landscapes requires therefore attention to different scales and types of anthropogenic impact.

Our results also highlight the importance of preserving a great taxonomic diversity of pollinator insects, as a mechanism to safeguard the functional diversity and pollination service of a system after an environmental change. At least in plant-pollinator generalist systems, taxonomically diverse insect communities tend to have species with overlapping functions. It makes the functionality of the system apparently more stable against anthropic changes like managed honey bee spillovers, habitat degradation and land conversion.

In the case of the *Z. lotus* habitat (and especially in the protected areas), it is essential to study and regulate the number of honey bee hives that this ecosystem can support in the surroundings, considering ecosystem features such as the plant community composition, the available floral density at each period of the year, and the wild insect community. This habitat will also require effective conservation practices for the still existing remnants, both in terms of impeding their internal deterioration and stopping the still ongoing process of land conversion to agricultural land.

ACKNOWLEDGMENTS

The authors thank J.M. Bastida for his help throughout the study; J.L. González-Rebollar, A.B. Robles, F. Robles, M. Robles, R. Tarifa, F.M. Camacho and Perruni for their advice and assistance during field samplings; the experts that generously contributed to insect species identification (including Fotografía y Biodiversidad Association [2015] with their Biodiversidad Virtual Project: http://www.biodiversidadvirtual.org); and Consejería de Medio Ambiente y Ordenación del Territorio (Junta de Andalucía) and M.A. Carrión (Dirección General de Medio Ambiente del Gobierno de Murcia) for providing permits for Almería and Murcia field samplings, respectively. This work was funded by a Junta de Andalucía Excelencia RNM-766 project and by funds from Fondo Europeo de Desarrollo Regional (FEDER). A. González-Robles and A.J. Manzaneda were supported by Universidad de Jaén fellowship (Plan Propio - Acción 16, 2013-2014) and a Ramón y Cajal contract (RYC-2010-06237), respectively.

REFERENCES

Aizen MA, Feinsinger P (2003) Bees not to be? Responses of insect pollinator faunas and flower pollination to habitat fragmentation. In: GA Bradshaw, P Marquet (eds.) *How landscapes change*. Springer, Berlin, Heidelberg, pp. 111–129.

Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2009) How much does agriculture depend on pollinators? Lessons from long- term trends in crop production. *Ann Bot*, 103, 1579–1588.

Aizen MA, Harder LD (2009) The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr Biol*, 19, 915–918.

Anon (1992) *Directive 92/43 of the council of the European community on the conservation of habitats and wild fauna and flora*. European Community, Brussels.

Asatryan A, Tel-Zur N (2013) Pollen tube growth and self-incompatibility in three *Ziziphus* species (Rhamnaceae). *Flora*, 208, 390–399.

Benito BM, Martínez-Ortega MM, Muñoz LM, Lorite J, Peñas J (2009) Assessing extinction–risk of endangered plants using species distribution models as: a case study of habitat depletion caused by the spread greenhouse. *Biodivers Conserv*, 18, 2509–2520.

Breeze TD, Vaissière BE, Bommarco R,Petanidou T, Seraphides N, Kozak L, et al. (2014) Agricultural policies exacerbate honeybee pollination service supply-demand mismatches across Europe. *PLoS One*, 9, e82996. doi: https://doi.org/10.1371/journal.pone.0082996

Burnham KP, Anderson DR (2002) *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach, second edition*. Springer, New York.

Calcagno V (2013) glmulti: Model selection and multimodel inference made easy. R package version 1.0.7. url: https://CRAN.R-project.org/package=glmulti

Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Salido T, Rey PJ (2016) Habitat loss exacerbates regional extinction risk of the keystone semiarid shrub *Ziziphus lotus* through collapsing the seed dispersal service by foxes (*Vulpes vulpes*). *Biodivers Conserv*, 25, 693–709.

Cerino MC, Richard GA, Torretta JP, Gutiérrez HF, Pensiero JF (2015) Reproductive biology of *Ziziphus mistol* Griseb. (Rhamnaceae), a wild fruit tree of saline environments. *Flora*, 211, 18–25.

Clarke KR, Gorley RN (2006) *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth-E Ltd.

Colwell RK 2013. *EstimateS: Statistical estimation of species richness and shared species from samples. Version 9*. User's Guide and application published at: http://purl.oclc.org/estimates

Colwell RK, Chao A, Gotelli NJ, Lin SY, Mao CX, Chazdon RL, Longino JT (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation, and comparison of assemblages. *J Plant Ecol*, 5, 3–21.

Colwell RK, Mao CX, Chang J (2004) Interpolating, extrapolating and comparing incidence-based species accumulation curves. *Ecology*, 85, 2717–2727.

Fahrig L, Baudry J, Brotons L, Burel FG, Crist TO, Fuller RJ, et al. (2011) Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. *Ecol Lett*, 14, 101–112.

Garibaldi LA, Steffan-Dewenter I, Kremen C, Morales JM, Bommarco R, Cunningham SA, et al. (2011) Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecol Lett*, 14, 1062–1072.

Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, et al. (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Sci*, 339, 1608–1611.

Geldmann J, González-Varo JP (2018) Conserving honey bees does not help wildlife. *Sci*, 359, 392–393.

Gill RJ, Baldock KCR, Brown MJF,Cresswell JE, Dicks LV, Fountain MT, et al. (2016) Protecting an ecosystem service: approaches to understanding and mitigating threats to wild insect pollinators. *Adv Ecol Res*, 54, 135–206.

Gómez JM, Perfectti F, Abdelaziz M, Lorite J, Muñoz-Pajares AJ, Valverde J (2015) Evolution of pollination niches in a generalist plant clade. *New Phytol*, 205, 440–453.

Gómez JM, Zamora R (1999). Generalization in the interaction between *Hormathophylla spinosa* (Cruciferae) and its pollinators. *Ecology*, 80, 796–805.

González-Varo JP, Arroyo J, Aparicio A (2009) Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (*Myrtus communis*). *Biol Conserv*, 142, 1058–1065

González-Varo JP, Biesmeijer JC, Bommarco R, Potts SG, Schweiger O, Smith HG, Steffan-Dewenter I, Szentgyörgyi H, Woyciechowski M, Vila M (2013) Combined effects of global change pressures on animal-mediated pollination. *Trends Ecol Evol*, 28, 524–530.

González-Varo JP, Geldmann J (2018) Response-“Bee conservation: Key role of managed bees” and “Bee conservation: Inclusive solutions”. *Sci*, 360, 390.

González-Varo JP, Vilà M (2017) Spillover of managed honeybees from mass-flowering crops into natural habitats. *Biol Conserv*, 212, 376–382.

Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett*, 4, 379–391.

Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. *Biol Conserv*, 102, 89–95.

Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub Melastoma affine (Melastomataceae). *Biol Conserv*, 86, 169–178.

Guillemot N, Kulbicki M, Chabanet P, Vigliola L (2011) Functional redundancy patterns reveal non-random assembly rules in a species-rich marine assemblage. *PLoS One*, 6, e26735. doi: https://doi.org/10.1371/journal.pone.0026735

Herbertsson L, Lindström SA, Rundlöf M, Dommarco R, Smith HG (2016) Competition between managed honeybees and wild bumblebees depends on landscape context. *Basic Appl Ecol*, 17, 609–616.

Herrera CM (2005). Plant generalization on pollinators: species property or local phenomenon? *Am J Bot*, 92, 13–20.

Holzschuh A, Dainese M, González-Varo JP, Mudri-Stojnić S, Riedinger V, Rundlöf M, et al. (2016) Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecol Lett*, 19, 1228–1236.

Kleijn D, Biesmeijer K, Dupont YL, Nielsen A, Potts SG, Settele J(2018) Bee conservation: Inclusive solutions. *Sci*, 360, 389–390.

Klein AM, Brittain C, Hendrix SD, Thorp R, Williams N, Kremen C (2012). Wild pollination services to California almond rely on semi-natural habitat. *J App Ecol*, 49, 723–732.

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc Lond B Biol Sci*, 274, 303–313.

Lázaro A, Tur C (2018) Los cambios de uso del suelo como responsables del declive de polinizadores. *Ecosistemas*, 27, 23–33.

Loreau M, Mouquet N, Gonzales A (2003). Biodiversity as spatial insurance in heterogeneous landscapes. *Proc Natl Acad Sci*, 100, 12765–12770.

Magrach A, González-Varo JP, Boiffier M, Vilà M, Bartomeus I (2017) Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. *Nat Ecol Evol*, 1, 1299–1307.

Martins KT, Gonzalez A, Lechowicz MJ (2015) Pollination services are mediated by bee functional diversity and landscape context. *Agric Ecosyst Environ*, 200, 12–20.

Mendoza-Fernández AJ, Martínez-Hernández F, Pérez-García FJ, Garrido-Becerra JA, Benito BM, Salmerón-Sánchez E, Guirado J; Merlo ME, Mota JF (2015). Extreme habitat loss in a Mediterranean habitat: *Maytenus senegalensis* subsp. *europaea*. *Plant Biosyst*, 149, 503–511.

Mishra RM, Gupta P, Yadav GP (2004) Intensity and diversity of flower-visiting insects in relation to plant density of *Zizyphus mauritiana* Lamk. *Trop Ecol*, 45, 263–270.

Morales CL, Sáez A, Garibaldi LA, Aizen MA (2017) Disruption of Pollination Services by Invasive Pollinator Species. In: M Vilà, PE Hulme (eds.) Impact of Biological Invasions on Ecosystem Services. Springer, Cham, pp 203–220. doi: http://10.1007/978-3-319-45121-3_13

Mota JF, Peñas J, Castro H, Cabello J, Guirado JS (1996) Agricultural development versus biodiversity conservation: the Mediterranean semiarid vegetation in El Ejido (Almería, southeastern Spain). *Biodivers Conserv*, 5, 1597–1617.

Nadia TDL, Machado IC, Lopes AV (2007) Fenologia reprodutiva e sistema de polinização de *Ziziphus joazeiro* Mart. (Rhamnaceae): atuação de *Apis mellifera* e de visitantes florais autóctones como polinizadores. *Acta Bot Brasilica*, 21, 835–845.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinnet D, et al. (2018) vegan: Community Ecology Package. R package version 2.4-6. url: https://CRAN.R-project.org/package=vegan

Otieno M, Sidhu CS, Woodcock BA, Wilby A, Vogiatzakis IN, Mauchline AL, Gikungu MW, Potts SG (2015) Local and landscape effects on bee functional guilds in pigeon pea crops in Kenya. *J Insect Conserv*, 19, 647–658.

Pérez Latorre AV, Cabezudo B (2009) Ziziphus Mill. In: Blanca G, Cabezudo B, Cueto M, Fernández López C, Morales Torres C (eds) *Flora Vascular de Andalucía Oriental, 3*. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, pp 46–50.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: Trends, impacts and drivers. *Trends Ecol Evol*, 25, 345–353.

Puigdefábregas J, Mendizábal T (1998) Perspectives on desertification: western Mediterranean. *J Arid Environ*, 39, 209–224.

Quantum GIS Development Team (2016) Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. url: https://www.R-project.org/

Rader R, Bartomeus I, Garibaldi LA, Garratt MPD, Howlett BD, Winfree R, et al. (2016) Non-bee insects are important contributors to global crop pollination. *Proc Natl Acad Sci*, 113: 146–151.

Rathcke BJ, Jules ES (1993) Habitat fragmentation and plant–pollinator interactions. *Current Sci*, 65, 273–277.

Ratto F, Simmons BI, Spake R, Zamora-Gutiérrez V, MacDonald MA, Merriman JC, Tremlett CJ, Poppy GM, Peh KSH, Dicks LV (2018). Global importance of vertebrate pollinators for plant reproductive success: a meta-analysis. *Front Ecol Environ*, 16, 82–90.

Rey PJ, Cancio I, González-Robles A, Bartida JM, Manzaneda AJ, Valera F, Salido T, Alcántara JM (2017) Local-scale and landscape disturbances impact through distinct pathways on the regional variation in seed dispersal by mammals in threatened semiarid habitats. *Perspect Plant Ecol Evol Syst*, 28, 10–18.

Rey PJ, Cancio I, Manzaneda AJ, González-Robles A, Valera F, Salido T, Alcántara JM (2018) Regeneration of a keystone semiarid shrub over its range in Spain: habitat degradation overrides the positive effects of plant – animal mutualisms. *Plant Biol*, 20, 1083–1092

Rosenfeld JS (2002) Functional redundancy in ecology and conservation. *Oikos*, 98, 156–162.

Rossi R (2017) European Commission: The EU's Beekeeping Sector (Inform of National Apiculture Programme 2016-2017). European Parliament. url: http://www.europarl.europa.eu/RegData/etudes/ATAG/2017/608786/EPRS_ATA(2017)608786_EN.pdf

Rucker RR, Thurman WN, Burgett M (2012) Honey bee pollination markets and the internalization of reciprocal benefits. *Am J Agric Econ*, 94, 956–977.

Sáez A, Morales CL, Ramos LY, Aizen MA (2014) Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *J Appl Ecol*, 51, 1603–1612.

Sánchez Gómez P, Carrión Vilches MA, Hernández González A, Guerra Montes J (2002) *Libro rojo de la flora silvestre protegida de la Región de Murcia*. Consejería de Agricultura, Agua y Medio Ambiente, D.G. del Medio Natural, Murcia.

Saunders ME, Smith TJ, Rader R (2018) Bee conservation: Key role of managed bees. *Sci*, 360, 389.

Stavert JR, Pattemore DE, Bartomeus I, Gaskett AC, Beggs JR (2018) Exotic flies maintain pollination services as native pollinators decline with agricultural expansion. *J Appl Ecol*, 55, 1737–1746.

Steffan-Dewenter I, Tscharntke T (2001) Succession of bee communities on fallows. *Ecography*, 24, 83–93.

Steffan-Dewenter I, Westphal C (2008) The interplay of pollinator diversity, pollination services and landscape change. *J Appl Ecol*, 45(3), 737–741.

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus* (*). In: *VV.AA., Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino.

Torné-Noguera A, Rodrigo A, Osorio S, Bosch J (2016) Collateral effects of beekeeping: Impacts on pollen-nectar resources and wild bee communities. *Basic Appl Ecol*, 17, 199–209.

Traveset A, Castro-Urgal R, Rotllàn-Puig X, Lázaro A (2017) Effects of habitat loss on the plant-flower visitor network structure of a dune community. *Oikos*, 127, 45–55.

Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity - Ecosystem service management. *Ecol Lett*, 8, 857–874.

Tscharntke T, Tylianakis JM, Rand TA, Didham RK, Fahrig L, Batáry P, et al. (2012) Landscape moderation of biodiversity patterns and processes - eight hypotheses. *Biol Rev*, 87, 661–685.

Tylianakis JM (2013) The global plight of pollinators. *Sci*, 339, 1532–1533.

Valido A, Rodríguez-Rodríguez MC, Jordano P (2014) Impacto de la introducción de la abeja doméstica (*Apis mellifera*, Apidae) en el Parque Nacional del Teide (Tenerife, Islas Canarias). *Ecosistemas*, 23, 58–59.

Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043–1060.

Weekley CW, Race T (2001) The breeding system of *Ziziphus celata* Judd and DW Hall (Rhamnaceae), a rare endemic plant of the Lake Wales Ridge, Florida, USA: implications for recovery. *Biol Conserv*, 100(2), 207–213.

Winfree R, Bartomeus I, Cariveau D (2011) Native Pollinators in Anthropogenic Habitats. *Annu Rev Ecol Evol Syst*, 42, 1–22.

Zietsman PC, Botha FC (1992) Flowering of *Ziziphus mucronata* subsp. *mucronata* (Rhamnaceae): anthesis, pollination and protein synthesis. *Bot Bull Acad Sinica*, 33, 33–42.

SUPPLEMENTARY MATERIAL

IMAGES

Image S1: Photographs of the study system showing: some species of the principal insect visitor groups of *Ziziphus lotus* flowers (two upper rows); open flowers with the typical rosette structure (central row, left); unripe fruit (central row, right); a well-preserved habitat remnant with the characteristic spatial structure of *Z. lotus* individuals (lower row).

FIGURES

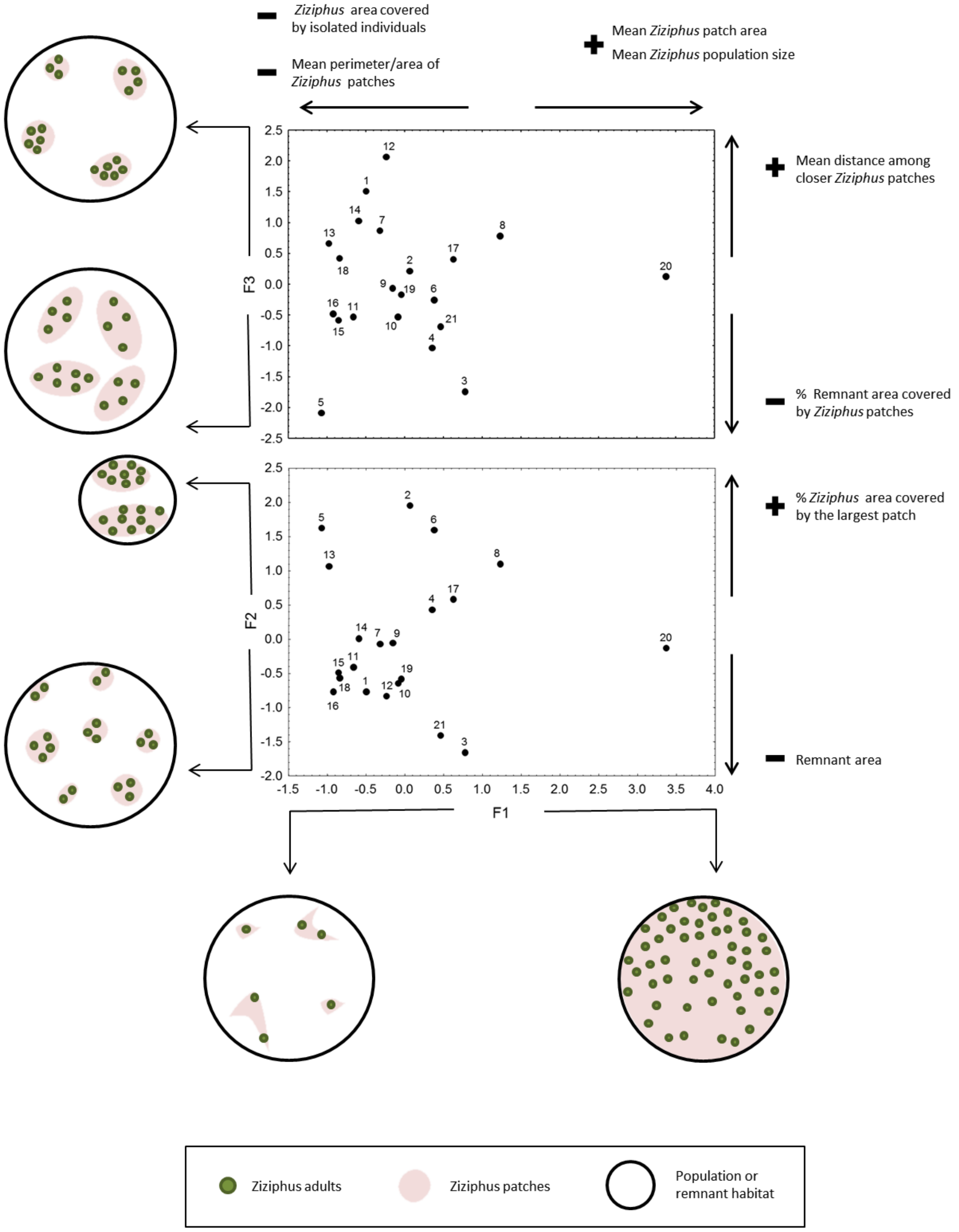


Fig. S1: Two-dimensional space representation of population factor scores obtained from the PCA of within-habitat remnant degradation variables. The lower panel represents the variation according to the first and second factors (F1 and F2), while the upper panel represents the variation according to first and third factors (F1 and F3). The variables best defining each factor and their correlation sign are shown in each case: F1 was mainly explained by the positive loading of mean *Ziziphus* patch area and the total number of adult plants, and the negative loadings of mean perimeter/area ratio and the proportion of *Ziziphus* patch area represented by isolated adults. F2 was positively explained by the proportion of *Ziziphus* patch area represented by the largest patch and negatively by the remnant area. F3 was mostly explained by the positive loading of mean distance to the nearest patches and the negative loading of the proportion of remnant area covered by *Ziziphus* patches (see more details in Table S3, Suppl. Mat.). Populations are identified by: (1) Béjar, (2) Campohermoso, (3) Ctr. Visitante, (4) Cuevas de Almanzora, (5) Dalías, (6) El Ejido, (7) Fernán Pérez, (8) Guazamara, (9) Hospital de Poniente, (10) Níjar, (11) Playazo, (12) Puerto Lumbreras, (13) Purias, (14) Rambla Campohermoso, (15) Rambla Retamar, (16) Retamar, (17) Sierra Alhamilla, (18) Sierra de la Tercia, (19) Sta. María del Águila, (20) Torregarcía, (21) El Toyo. In the upper and lower limits of each axis, the habitat spatial structure that defines their endpoints is represented in each case (according to their most important loadings).

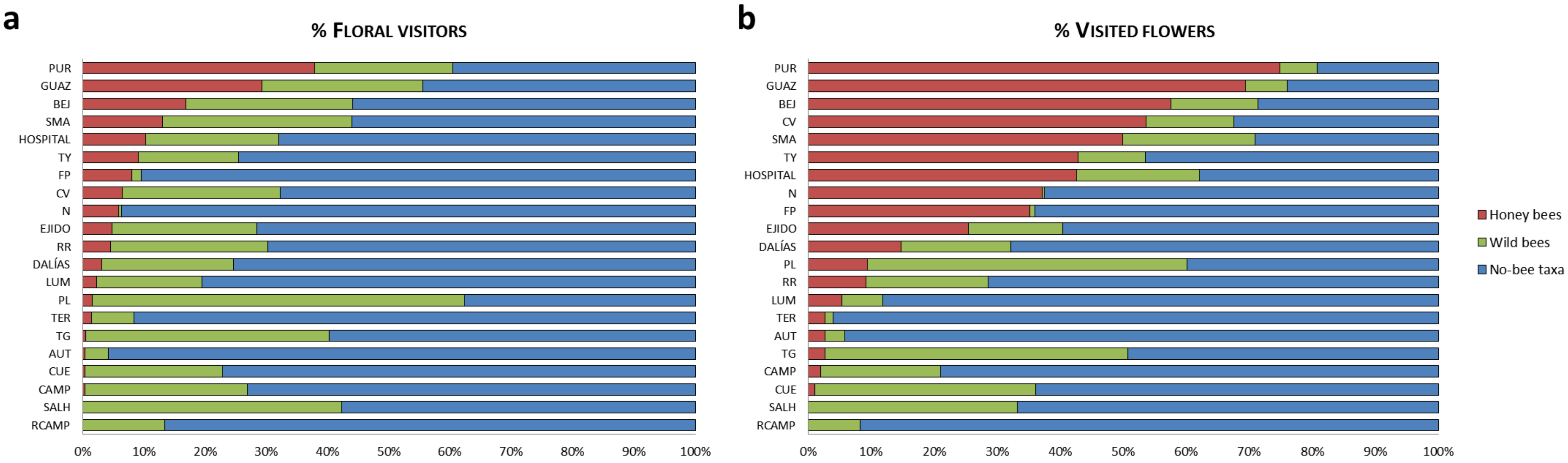


Fig. S2: Abundance of honey bees, wild bees and non-bee taxa (***a***) and percentage of floral visits by each of these groups (***b***) in 21 *Ziziphus lotus* populations. Details of population acronyms can be consulted in Table 1.

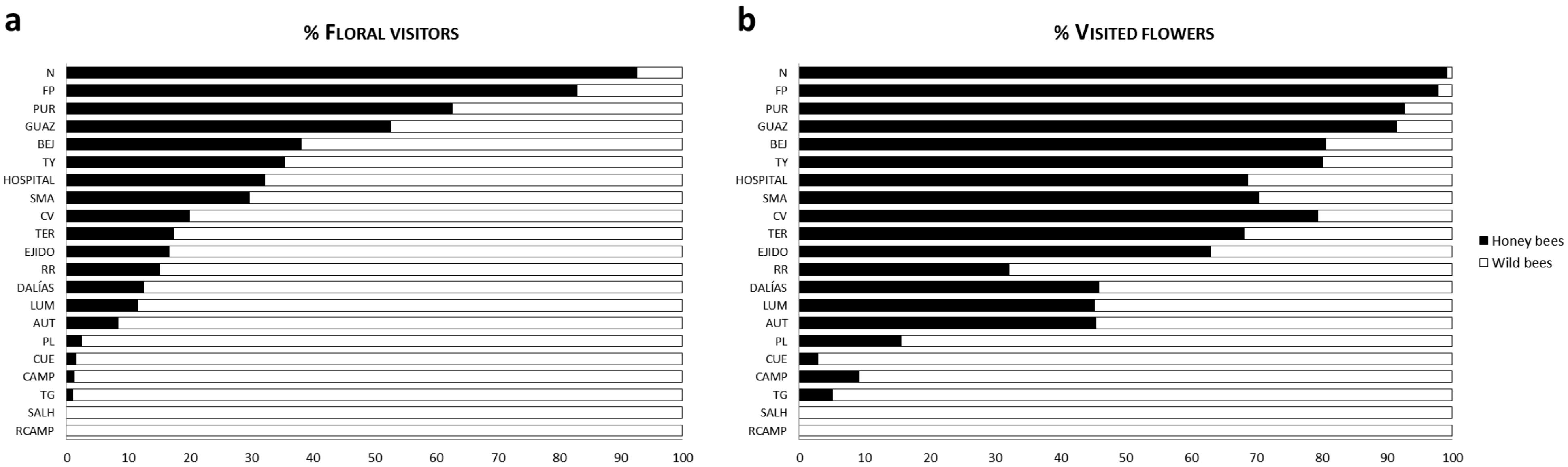
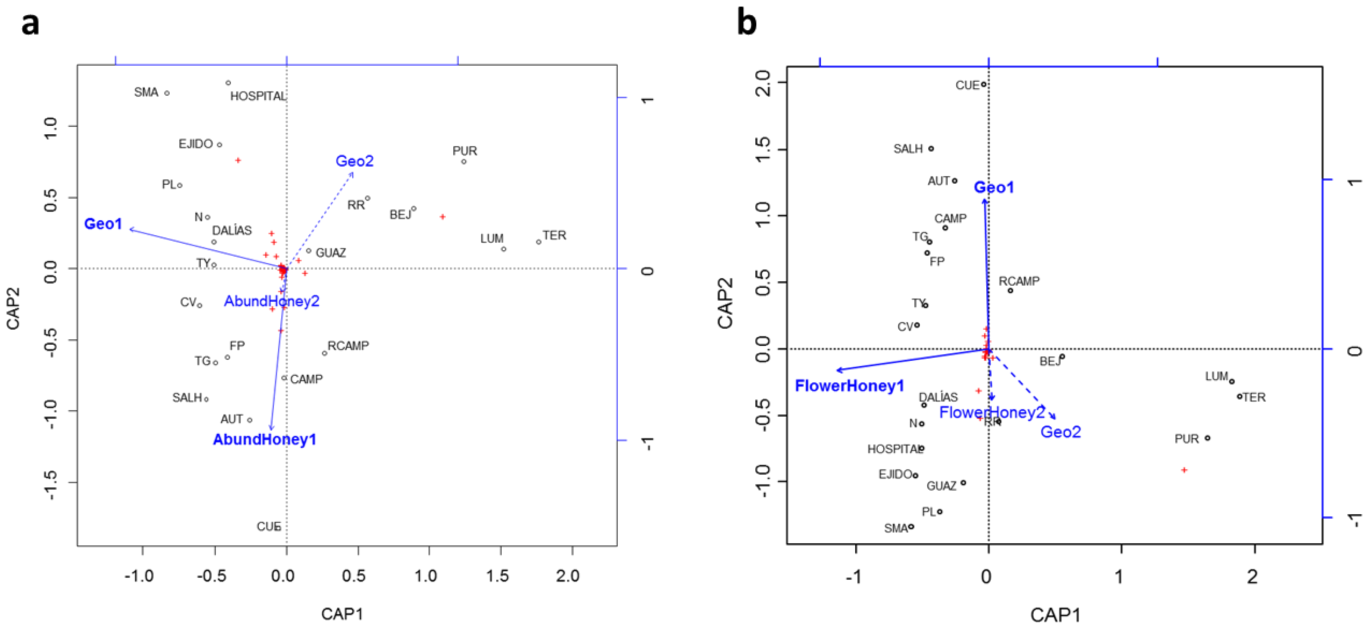


Fig. S3: Abundance (***a***) and floral visits (***b***) of honey bees (**black**) vs wild bees (**white**) in relation to the total number of bee visitors in 21 *Ziziphus lotus* populations. Details of population acronyms can be consulted in Table 1.

Fig. S4: Db-RDA plots showing how honey bee abundance (***a***) and their floral visits (***b***) are associated with non-bee taxa abundance (***a***) and non-bee taxa floral visits (***b***): in both cases a negative correlation is showed with honey bee variables. Significant variables in the model are indicated in bold by solid vectors, and non-significant variables are indicated by dashed vectors. Population acronyms can be consulted in Table 1.



TABLES

Table S1: Functional groups considered for the floral visitor assemblage of *Ziziphus lotus*.

General groups	Order	Funtional groups[a]	Body length	Sociability	Resource	Behavioural notes	Main families, genera and species
Bees	Hymenoptera	Large collector bees[bc]	20 mm <	Social	Pollen/Nectar	Looking for the resource, very mobile, responsible for many floral visits	Anthophoridae (*Anthophora*), Apidae *(Apis mellifera, Bombus terrestris*), Colletidae (*Colletes*)
		Small collector bees[c]	< 10 mm	Semi-social/Solitary	Pollen/Nectar	Looking for the resource, very mobile, responsible for many floral visits	Andrenidae (*Andrena*), Halictidae (*Halictus, Nomioides*), Megachilidae (*Osmia*)
Wasp	Hymenoptera	Large hunter wasps[d]	15 mm <	Solitary	Others	Exceptionally collecting pollen or nectar, usually using the plant to find prey	Cabronidae (*Tachysphex*), Pompilidae (*Cryptocheilus*), Scoliidae (*Scolia*), Sphecidae (*Ammophilinae, Bembix, Sceliphron*), Thiphiidae (*Poecilotiphia*)
		Small hunter wasps[d]	< 10 mm	Solitary	Others	Exceptionally collecting pollen or nectar, usually using the plant to find prey	Braconidae (*Glyptomorpha*), Cabronidae (*Philanthinae*), Chrysididae, Vespidae (*Eumenes*)
		Large collector wasps[d]	15 mm <	Solitary	Pollen/Nectar	Looking for the resource, relatively mobile, responsible for moderate floral visits	Vespidae (*Polistes*)
		Small collector wasps[d]	< 10 mm	Solitary	Pollen/Nectar	Looking for the resource, relatively mobile, responsible for moderate floral visits	Vespidae (*Vespula*)
Ants	Hymenoptera	Walker ants[d]	5 mm <	Semi-social/Solitary	Pollen/Nectar + Others	Randomly collecting the resource, very mobile, usually moving over the whole plant and flowers. Usually being reduced time on flowers	Formicidae (*Camponotus, Cataglyphis iberica*)
		Diving ants[d]	< 5 mm	Social	Pollen/Nectar	Looking for the resource, usually not very mobile, introducing the whole (or part of) body into the flower	Formicidae (*Camponotus, Crematogaster auberti, Crematogaster scutelaris, Plagiolepis schmitzii, Tapinoma nigerrimum*)

[a] Functional groups were based on the behavior of floral visitors within the flowers and on morphological and social features (following Gómez et al. 2015, Martins et al. 2015, Otieno et al. 2015). [b] Denote belonging to the group of “Honey bees” in analyses. [c] Denote belonging to the group of “Wild bees” in analyses. [d] Denote belonging to the group of “Non-bee taxa” in analyses.

Table S1 (cont.): Functional groups considered for the floral visitor assemblage of *Ziziphus lotus*.

General groups	Order	Funtional groups[a]	Body length	Sociability	Resource	Behavioural notes	Main families, genera and speci
Flies	Diptera	Carrion flies[d]	13 mm <	Solitary	Others	Collecting pollen or nectar attracted by the smell of flowers, but often using the plant for other purposes	Calliphoridae (*Chrysomya*), Sarcophagidae
		No-collecting flies[d]	< 13 mm	Solitary	Others	Collecting pollen or nectar attracted by the smell of flowers, but often using the plant for other purposes	Drosophilidae (*Drosophila*), Muscidae (*Musca*), Stratiomyidae (*Nemotelus*), Tephritidae (*Trupanea*)
		Nectar collecting flies[d]	Variable	Solitary	Pollen/Nectar	Looking for the resource, relatively mobile, responsible for moderate floral visits	Bombyliidae (*Bombylius, Exhyalanthrax, Petrorossia, Thyridanthrax, Villa*), Syrphidae (*Eristalinus, Eristalis, Eumerus, Riponnensia, Sphaerophoria*)
Beetles	Coleoptera	Walker beetles[d]	15 mm <	Solitary	Nectar	Looking for the resource, usually not very mobile, responsible for low floral visits	Coccinellidae (*Coccinella septempunctata*), Tenebrionidae (*Heliotaurus*)
		Diving beetles[d]	< 10 mm	Solitary	Nectar	Looking for the resource, usually low mobile, introducing the whole (or part of) body into the flower	Coccinellidae (*Chilocorus, Exochomus*), Curculionidae, Dermestidae (*Attagenus*), Mordellidae
Butterflies	Lepidoptera	Large butterflies[d]	60 mm <	Solitary	Pollen/Nectar	Looking for the resource, very mobile but not responsible for many floral visits	Nymphalidae (*Vanessa atalanta, Vanessa cardui*), Pieridae (Pieris)
		Small butterflies[d]	< 35 mm	Semi-social/Solitary	Pollen/Nectar	Looking for the resource, quite mobile, not responsible for many floral visits. Using the plant as a nutritious plant (*Tarucus theophrastus*)	Lycaenidae (*Tarucus theophrastus*)
Bugs	Hemiptera	Bugs[d]	Variable	Solitary	Pollen/Nectar + Others	Exceptionally collecting pollen or nectar, low mobile, responsible for very few floral visits	Pentatomidae (*Graphosoma semipunctatum*), Pyrrhocoridae (*Pyrrhocoris apterus*)
Others	Mainly, Thysanoptera, Neuroptera, Lepidoptera	Others (Month,Thrips, Lacewings)[d]	Variable	Solitary	Pollen/Nectar + Others	Exceptionally collecting pollen or nectar, low mobiles, resposible for very few floral visits. Minority groups.	Chrysopidae

[a] Functional groups were based on the behavior of floral visitors within the flowers and on morphological and social features (following Gómez et al. 2015, Martins et al. 2015, Otieno et al. 2015). [b] Denote belonging to the group of "Honey bees" in analyses. [c] Denote belonging to the group of "Wild bees" in analyses. [d] Denote belonging to the group of "Non-bee taxa" in analyses.

Table S2: Summary of the metric of within-habitat remnant quality (9 variables measured at the local scale of the habitat remnant) and landscape degradation (3 variables assessed at the large scale of the surrounding landscape) for the 21 *Z. lotus* study populations.

	Habitat remnant variables									Landscape variables		
Population (Habitat remnant)	Population size (Number of adults)	Population or remnant area (m^2)	Percentage of the remnant area covered by *Z. lotus* patches (%)	Mean *Z. lotus* patch area (m^2)	Percentage of the total area of *Z. lotus* patches represented by the largest patch (%)	Percentage of the total area of Z. lotus patches represented by isolated adults (%)	Mean perimeter/area of the *Z. lotus* patches	Mean distance to the nearest neighbor *Z. lotus* patch (m)	Aggregation index or index of nearest neighbors	Natural habitat cover (% in 1.5km radius)	Number of land uses	Land use diversity (H')
Béjar	89	10845067.08	0.55	882.94	4.41	66.57	0.1243	134.10	0.401	57.20	7	0.548
Campohermoso	20	72293.66	12.91	1166.82	23.50	22.60	0.1108	88.63	1.372	25.69	11	0.813
Ctro de Visitantes	1367	5691333.48	11.29	1623.05	2.03	21.56	0.1004	57.49	0.701	92.57	10	0.716
Cuevas de Almanzora	101	331310.14	12.72	1359.72	9.50	14.90	0.1102	58.35	0.932	36.75	19	0.713
Dalías	15	21824.04	38.17	925.53	18.48	50.66	0.1183	44.52	1.529	7.29	12	0.541
El Ejido	32	84920.10	19.01	1467.28	20.54	21.79	0.1046	73.50	1.139	8.74	15	0.712
Fernán Pérez	80	2469351.23	1.73	948.14	6.17	45.82	0.1204	116.71	0.657	15.90	15	0.800
Guazamara	81	503518.40	8.66	1895.94	18.33	12.90	0.1007	94.60	0.858	31.89	14	0.668
Hospital de Poniente	86	1353057.59	3.50	1100.64	7.19	39.84	0.1139	80.23	0.672	16.75	11	0.730
Níjar	255	3549060.87	3.90	1098.09	6.28	41.89	0.1158	69.99	0.645	40.37	13	0.822
Playazo	101	1870401.26	3.40	920.58	4.21	53.14	0.1220	80.94	0.845	10.93	15	0.625
Puerto Lumbreras	102	24988233.26	0.26	901.60	5.45	56.33	0.1236	143.20	0.374	27.10	10	0.633
Purias	16	248155.76	4.28	817.81	12.69	65.66	0.1272	106.88	0.718	2.43	7	0.515
Rambla Campohermoso	62	1580976.38	2.35	862.55	8.30	64.01	0.1248	116.26	0.406	4.78	11	0.471
Rambla Retamar	68	1655315.55	2.60	877.00	4.81	57.28	0.1239	70.42	0.594	56.91	17	0.990
Retamar	80	1881381.32	2.74	846.50	4.05	62.65	0.1255	74.01	0.426	83.96	12	0.631
Sierra Alhamilla	133	1097195.25	6.05	1383.15	15.72	28.60	0.1038	87.16	0.845	80.62	16	0.741
Sierra de la Tercia	32	2054608.79	0.95	846.92	7.13	53.74	0.1254	73.54	0.234	18.09	15	0.865
Sta Maria del Águila	117	2949633.22	2.17	1123.62	6.97	34.04	0.1139	67.00	0.438	6.42	12	0.523
Torregarcía	4598	7108890.07	21.51	3812.82	28.59	8.51	0.0742	62.93	0.722	64.76	13	0.760
El Toyo	1015	13772962.38	3.72	1243.40	3.70	31.71	0.1086	71.41	0.660	83.57	18	0.834

Table S3: Factor loadings of the Principal Component Analysis of the within-remnant habitat quality-degradation metrics. In bold, the variables with the highest loads in each component.

	F1	F2	F3
Population size (adults)	**0.7392**	-0.6249	-0.1596
Population or remnant area	0.2114	**-0.8967**	0.3668
Proportion of the remnant area covered by *Ziziphus* patches	0.4300	0.5654	**-0.6786**
Mean *Ziziphus* patch area	**0.9800**	0.0932	-0.1485
Proportion of the total area of *Ziziphus* patches represented by the largest patch	0.3911	**0.8594**	0.1122
Proportion of the total area of *Ziziphus* patches represented by isolated adults	**-0.8923**	-0.2446	0.2137
Mean perimeter/area ratio of the *Ziziphus* patches	**-0.9706**	-0.0915	0.1836
Mean distance to the nearest neighbor *Ziziphus* patch	-0.1904	-0.0486	**0.9271**
Aggregation index or index of nearest neighbors	0.2335	0.6824	-0.4921
Explained Variance	3.7184	2.7977	1.8362
Proportion of the Total variance explained	0.4132	0.3109	0.2040

Table S4: Species composing the floral visitor assemblage of *Ziziphus lotus* in the study area (the 21 studied populations are pooled).

Order	Family	Species
Hymenoptera	Andrenidae	*Andrena sp.*
	Anthophoridae	*Anthophora sp.*
	Apidae	*Apis mellifera*
		Bombus terrestris
	Braconidae	*Glyptomorpha sp.*
	Cabronidae	*Philanthinae sp.*
		Tachysphex sp.
	Chrysididae	*Crisidae sp.*
	Colletidae	*Colletes sp.*
	Formicidae	*Camponotus sp1*
		Camponotus sp2
		Cataglyphis iberica
		Crematogaster auberti
		Crematogaster scutelaris
		Plagiolepis schmitzii
		Tapinoma nigerrimum
	Halictidae	*Halictidae sp1*
		Halictidae sp2
		Halictidae sp3
		Halictidae sp4
		Nomioides sp.
	Megachilidae	*Osmia sp.*
	Pompilidae	*Cryptocheilus discolor*
		Popilidae sp.
	Scoliidae	*Scolia sp1*
		Scolia sp2
	Sphecidae	*Ammophilinae sp.*
		Bembix sp.
		Sceliphron sp.
	Thiphiidae	*Poecilotiphia rousselii*
	Vespidae	*Eumenes sp.*
		Polistes sp.
		Vespula sp.
Diptera	Bombyliidae	*Bombylius sp.*
		Exhyalanthrax afer
		Exhyalanthrax sp.
		Petrorossia sp.
		Thryridantrax sp.
		Villa sp.

Table S4 (cont.): Species composing the floral visitor assemblage of *Ziziphus lotus* in the study area (the 21 studied populations are pooled).

Order	Family	Species
Diptera	Calliphoridae	*Calliphoridae sp1*
		Calliphoridae sp2
		Calliphoridae sp3
		Calliphoridae sp4
		Calliphoridae sp5
		Chrysomya albiceps
		Chrysomya sp.
	Drosophilidae	*Drosophila sp1*
		Drosophila sp2
	Muscidae	*Musca domestica*
		Muscidae sp1
		Muscidae sp2
		Muscidae sp3
	Sarcophagidae	*Sarcophagidae sp1*
		Sarcophagidae sp2
	Stratiomydae	*Nemotelus sp.*
	Syrphidae	*Eristalinus aeneus*
		Eristalinus taeniops
		Eristalis sp.
		Eumerus sp.
		Riponnensia sp.
		Sphaerophoria sp.
	Tephritidae	*Trupanea sp.*
Coleoptera	Coccinellidae	*Chilocorus sp.*
		Coccinella septempunctata
		Exochomus sp.
	Curculionidae	*Curculionidae sp.*
	Dermestidae	*Attagenus sp.*
	Mordellidae	*Mordellidae sp.*
	Tenebrionidae	*Heliotaurus sp.*
Lepidoptera	Lycaenidae	*Tarucus theophrastus*
	Nymphalidae	*Vanessa atalanta*
		Vanessa cardui
	Pieridae	*Pieris sp.*
Hemiptera	Pentatomidae	*Graphosoma semipunctatum*
	Pyrrhocoridae	*Pyrrhocoris apterus*
	-	*Hemiptera sp1*
	-	*Hemiptera sp2*
	-	*Hemiptera sp3*
	-	*Hemiptera sp4*
Thysanoptera	Thysanoptera	*Thysanoptera sp.*
Neuroptera	Chrysopidae	*Chrysopidae sp.*

- : unknown family

Table S5: Across-populations mean values (in %) of the whole taxonomic groups of the insect visitor assemblage of *Ziziphus lotus* flowers and their respective floral visits (the 21 studied populations are pooled).

Visitor assemblage	% Floral visitors		% Visited flowers	
Taxonomic composition	Mean	SE	Mean	SE
Main groups				
Bees	31.78	3.75	50.79	5.27
Wasps	10.65	1.75	8.88	2.39
Ants	18.92	3.85	6.03	2.90
Flies	29.14	4.40	31.93	5.87
Beetles	5.65	1.18	1.42	0.66
Butterflies	3.67	0.67	0.91	0.30
Bugs	0.13	0.07	0.03	0.04
Others*	0.06	0.05	0.01	0.04

* Mainly Thysanoptera and Neuroptera

Table S6: Relative importance of bees and flies visiting *Ziziphus lotus* (the 21 studied populations are pooled). Figures in bold are referred to the whole insect assemblage while figures in normal font have been calculated within each group.

Visitor assemblage	% Floral visitors		% Visited flowers	
Taxonomic compositio	Mean	SE	Mean	SE
Bee group	**31.78**	**3.75**	**50.79**	**5.27**
Honey bees	24.74	5.93	73.55	7.65
Wild bees	75.26	5.93	26.45	7.65
Fly group	**29.14**	**4.4**	**31.93**	**5.87**
Hoverflies	1.03	0.64	0.37	0.61
Bee flies	1.42	1.17	0.46	1.23
Other fly families	97.55	1.37	99.17	1.42

Table S7: Across-populations mean values (in %) of the relative abundance of floral visitors and visited flowers in terms of the functional groups considered (the 21 studied populations are pooled).

Visitor assemblage	% Floral visitors		% Visited flowers	
Functional composition	Mean	SE	Mean	SE
Bees				
Large collector bees	9.08	2.25	39.56	5.62
Small collector bees	22.7	3.11	11.23	3.22
Wasps				
Large hunter wasps	3.45	0.96	1.48	0.98
Small hunter wasps	0.62	0.18	0.19	0.11
Large collector wasps	6.54	1.18	7.18	2
Small collector wasps	0.04	0.03	0.03	0.02
Ants				
Walker ants	0.21	0.16	0.11	0.11
Diving ants	18.71	3.82	5.92	2.9
Flies				
Carrion flies	3.55	0.48	2	0.4
No-collecing flies	25.04	4.22	29.74	5.79
Nectar collecting flies	0.55	0.14	0.19	0.12
Beetles				
Walker beetles	1.38	0.62	0.38	0.31
Diving beetles	4.27	1.11	1.04	0.63
Butterflies				
Large butterflies	0.03	0.03	0.01	0.02
Small butterflies	3.64	0.68	0.9	0.3
Bugs	0.13	0.07	0.03	0.04
Others	0.06	0.05	0.01	0.04

* Mainly Thysanoptera and Neuroptera

Table S8: Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects and contribution of the abundance of different groups of floral visitors and geographic location dissimilarities on the total count of visited flowers in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant predictors. Italic denotes conditional analysis results (partial-dbRDA).

Response	Predictors	*df*	*F*	*p-value*		% var	R^2
Visited flower per population						**45.3**	
	AbundHoney1	1	107.901	0.002	**	*36.1*	0.361
	AbundHoney2	1	0.099	0.746		*0.0*	2.35E-04
	AbundWildBees1	1	0.460	0.394		*2.4*	0.024
	AbundWildBees2	1	2.955	0.085	.	*0.0*	7.66E-06
	AbundNoBees1	1	30.266	0.004	**	*9.2*	0.092
	AbundNoBees2	1	2.630	0.130		*1.1*	0.011
	Geo1	1	0.003	0.951		*0.0*	1.76E-05
	Geo2	1	0.000	0.984		*0.0*	2.84E-06
	Residuals	12					

Note: 'AbundHoney1' and 'AbundHoney2', 'AbundWildBees1' and 'AbundWildBees2', 'AbundNoBees1' and 'AbundNoBees2', 'Geo1' and 'Geo2' predictors were defined by PCoA scores of axis-1 and axis-2, respectively, resulting from independent multidimensional scaling analyses (MDS) from population dissimilarity matrixes of honey bees, wild bees and non-bee taxa abundance, and geographic location (in each case) (see Methods for more details).

** $p < 0.01$; . $p < 0.1$

Table S9: Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of honey bee abundance and floral visits differences on non-bee taxa and wild bee abundance and floral visits differences (taxonomic and functional frameworks) in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant results. Italic denotes conditional analysis results (partial-dbRDA).

	Abundance of visitor assemblage							Account of visited flower by visitor assemblage						
Assemblage	Predictors	*df*	*F*	*p-value*		% var	R^2	Predictors	*df*	*F*	*p-value*		% var	R^2
a) Taxonomic framework														
Non-bee taxa						**23.8**							**29.2**	
	AbundHoney1	1	3.039	0.002	**	*9.8*	0.098	FlowerHoney1	1	2.774	0.006	**	*10.9*	0.109
	AbundHoney2	1	0.682	0.750		*2.9*	0.029	FlowerHoney2	1	0.635	0.751		*2.1*	0.021
	Geo1	1	3.475	0.001	***	*14.1*	0.141	Geo1	1	4.798	0.002	**	*18.3*	0.183
	Geo2	1	1.455	0.170		*5.9*	0.059	Geo2	1	1.708	0.105		*6.1*	0.061
	Residuals	16						Residuals	16					
Wild bees						**10.8**								
	AbundHoney1	1	0.704	0.641		*6.6*	0.066	FlowerHoney1	1	1.171	0.300		*7.4*	0.074
	AbundHoney2	1	0.824	0.563		*3.5*	0.035	FlowerHoney2	1	0.681	0.657		*2.5*	0.025
	Geo1	1	0.320	0.937		*1.6*	0.016	Geo1	1	0.461	0.855		*2.5*	0.025
	Geo2	1	2.152	0.053	.	*10.8*	0.108	Geo2	1	1.637	0.131		*8.5*	0.085
	Residuals	16	2.995					Residuals	16					
b) Functional framework														
Non-bee taxa						**15.7**							**20.7**	
	AbundHoney1	1	1.106	0.355		*4.3*	0.043	FlowerHoney1	1	0.962	0.402		*5.2*	0.052
	AbundHoney2	1	0.527	0.777		*2.9*	0.029	FlowerHoney2	1	0.196	0.973		*1.0*	0.010
	Geo1	1	3.466	0.010	**	*15.7*	0.157	Geo1	1	5.005	0.001	***	*20.7*	0.207
	Geo2	1	1.011	0.393		*4.6*	0.046	Geo2	1	1.619	0.148		*6.2*	0.062
	Residuals	16						Residuals	16					
Wild bees						**12.8**							**11.5**	
	AbundHoney1	1	0.502	0.701		*5.4*	0.054	FlowerHoney1	1	0.618	0.642		*5.6*	0.056
	AbundHoney2	1	0.497	0.724		*1.1*	0.011	FlowerHoney2	1	0.748	0.553		*1.8*	0.018
	Geo1	1	0.100	0.989		*0.5*	0.005	Geo1	1	0.362	0.841		*2.0*	0.020
	Geo2	1	2.510	0.071	.	*12.8*	0.128	Geo2	1	2.304	0.082	.	*11.5*	0.115
	Residuals	16						Residuals	16					

Note: 'AbundHoney1' and 'AbundHoney2', 'FlowerHoney1' and 'FlowerHoney2' 'Geo1' and 'Geo2' predictors were defined by PCoA scores of axis-1 and axis-2, respectively, resulting from independent multidimensional scaling analysis (MDS) from population dissimilarity matrix of honey bee abundance, their floral visits and geographic location (in each case) (see Methods for more details). *** $p< 0.05$; ** $p< 0.01$; * $p< 0.05$; . $p< 0.1$

Table S10: Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of flower density, distance at closer population, landscape disturbance (natural habitat cover and land-use diversity), local-scale habitat alteration (F1, F2 and F3 components) and geographic location (latitude and longitude) on abundance of floral visitors (taxonomic framework) and the number of flowers that they visited in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant predictors. Italic denotes conditional analysis results (partial-dbRDA).

		Abundance of visitor assemblage						Account of visited flower by visitor assemblage					
Assemblage	Predictors	*df*	*F*	*p-value*		% var	R^2	*df*	*F*	*p-value*		% var	R^2
All floral visitors						**7.9**						**9**	
	Flower	1	2.496	0.010	**	*7.9*	0.079	1	2.012	0.049	*	*4.3*	0.043
	Popdist	1	1.589	0.115		*2.9*	0.029	1	2.445	0.027	*	*4.7*	0.047
	Natcov	1	0.744	0.738		*4.2*	0.042	1	0.943	0.501		*3.3*	0.033
	LandUse	1	1.218	0.277		*5.7*	0.057	1	0.940	0.473		*4.5*	0.045
	F1	1	0.988	0.476		*4.5*	0.045	1	0.876	0.550		*4.1*	0.041
	F2	1	0.873	0.564		*4.2*	0.042	1	0.880	0.536		*4.3*	0.043
	F3	1	1.265	0.257		*4.4*	0.044	1	0.977	0.462		*4.4*	0.044
	Lat	1	1.501	0.142		*7.2*	0.072	1	1.290	0.272		*3.8*	0.038
	Long	1	1.251	0.263		*5.5*	0.055	1	0.667	0.760		*3.0*	0.030
	Residuals	11						11					
No honey bees						**19.6**						**13.1**	
	Flower	1	2.629	0.006	**	*8.9*	0.089	1	2.822	0.009	**	*8.1*	0.081
	Popdist	1	1.726	0.070	.	*2.7*	0.027	1	3.102	0.004	**	*5.0*	0.050
	Natcov	1	0.722	0.749		*4.5*	0.045	1	1.053	0.375		*4.9*	0.049
	LandUse	1	1.253	0.253		*5.5*	0.055	1	0.968	0.468		*3.5*	0.035
	F1	1	1.004	0.478		*4.4*	0.044	1	1.011	0.448		*4.3*	0.043
	F2	1	0.900	0.565		*4.2*	0.042	1	0.721	0.676		*3.3*	0.033
	F3	1	1.319	0.230		*4.2*	0.042	1	1.260	0.245		*4.6*	0.046
	Lat	1	1.683	0.097	.	*8*	0.080	1	1.690	0.110		*4.4*	0.044
	Long	1	1.393	0.177		*5.9*	0.059	1	1.073	0.300		*4.3*	0.043
	Residuals	11						11					

Note: Habitat natural cover [Natcov]; Land-use diversity [LandUse]; Distance to the nearest population [Popdist]; Flower density [Flower], Lartitude [Lat]; Longitude [Long].

*** *p*< 0.05; ** *p*< 0.01; * *p*< 0.05; . *p*< 0.1

Table S10 (cont.): Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of flower density, distance at closer population, landscape disturbance (natural habitat cover and land-use diversity), local-scale habitat alteration (F1, F2 and F3 components) and geographic location (latitude and longitude) on abundance of floral visitors (taxonomic framework) and the number of flowers that they visited in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant predictors. Italic denotes conditional analysis results (partial-dbRDA).

		Abundance of visitor assemblage						Account of visited flower by visitor assemblage					
Assemblage	Predictors	*df*	*F*	*p-value*		% var	R^2	*df*	*F*	*p-value*		% var	R^2
Non-bee taxa						**24.2**						**16.6**	
	Flower	1	3.633	0.001	***	*8.8*	0.088	1	3.385	0.001	***	*7.5*	0.075
	Popdist	1	1.950	0.029	*	*3.8*	0.038	1	3.418	0.002	**	*6.0*	0.060
	Natcov	1	1.028	0.436		*3.3*	0.033	1	1.127	0.390		*6.7*	0.067
	LandUse	1	1.696	0.087	.	*6.3*	0.063	1	1.212	0.275		*3.3*	0.033
	F1	1	1.235	0.271		*4.9*	0.049	1	1.477	0.158		*5.7*	0.057
	F2	1	1.067	0.409		*4.4*	0.044	1	0.690	0.691		*2.8*	0.028
	F3	1	1.356	0.195		*3.9*	0.039	1	1.417	0.216		*4.6*	0.046
	Lat	1	2.378	0.010	**	*5.3*	0.053	1	2.494	0.016	*	*3.1*	0.031
	Long	1	1.004	0.459		*3.8*	0.038	1	0.616	0.764		*2.3*	0.023
	Residuals	11						11					
Wild bees						**11.8**							
	Flower	1	0.644	0.630		*8.3*	0.083	1	0.648	0.690		*7.8*	0.078
	Popdist	1	0.329	0.885		*1.6*	0.016	1	0.499	0.759		*1.9*	0.019
	Natcov	1	0.922	0.497		*8.1*	0.081	1	0.940	0.453		*7.3*	0.073
	LandUse	1	0.281	0.924		*2.6*	0.026	1	0.336	0.893		*4.5*	0.045
	F1	1	0.457	0.742		*2*	0.020	1	0.429	0.773		*1.9*	0.019
	F2	1	0.892	0.480		*4.7*	0.047	1	0.922	0.440		*5.6*	0.056
	F3	1	1.425	0.235		*5.5*	0.055	1	1.278	0.270		*5.3*	0.053
	Lat	1	0.979	0.415		*14.8*	0.148	1	0.628	0.683		*12.0*	0.120
	Long	1	2.261	0.071	.	*11.8*	0.118	1	2.355	0.053	.	*12.4*	0.124
	Residuals	11						11					

Note: Habitat natural cover [Natcov]; Land-use diversity [LandUse]; Distance to the nearest population [Popdist]; Flower density [Flower], Lartitude [Lat]; Longitude [Long].

*** $p < 0.05$; ** $p < 0.01$; * $p < 0.05$; . $p < 0.1$

Table S10 (cont.): Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of flower density, distance at closer population, landscape disturbance (natural habitat cover and land-use diversity), local-scale habitat alteration (F1, F2 and F3 components) and geographic location (latitude and longitude) on abundance of floral visitors (taxonomic framework) and the number of flowers that they visited in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant predictors. Italic denotes conditional analysis results (partial-dbRDA).

		Abundance of visitor assemblage						Account of visited flower by visitor assemblage				
Assemblage	Predictors	*df*	*F*	*p-value*		% var	R^2	*df*	*F*	*p-value*	% var	R^2
Honey bees												
	Flower	1	3.256	0.089	.	*5.6*	0.056	1	1.615	0.163	*2.9*	0.029
	Popdist	1	0.273	0.592		*1.5*	0.015	1	0.827	0.338	*0.8*	0.008
	Natcov	1	1.333	0.260		*4.3*	0.043	1	1.468	0.254	*5.6*	0.056
	LandUse	1	0.384	0.529		*5.9*	0.059	1	0.286	0.611	*58.5*	0.585
	F1	1	0.041	0.778		*0*	0.000	1	0.014	0.844	*0.2*	0.002
	F2	1	1.031	0.341		*7.5*	0.075	1	1.046	0.335	*8.2*	0.082
	F3	1	1.084	0.323		*9.9*	0.099	1	1.790	0.225	*15.2*	0.152
	Lat	1	1.956	0.203		*0.6*	0.006	1	2.489	0.154	*0.4*	0.004
	Long	1	0.373	0.565		*1.8*	0.018	1	0.622	0.449	*2.9*	0.029
	Residuals	11						11				

Note: Habitat natural cover [Natcov]; Land-use diversity [LandUse]; Distance to the nearest population [Popdist]; Flower density [Flower], Lartitude [Lat]; Longitude [Long].

*** $p< 0.05$; ** $p< 0.01$; * $p< 0.05$; . $p< 0.1$

Table S11: Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of flower density, distance to nearest population, landscape disturbance (natural habitat cover and land-use diversity), local-scale habitat alteration (F1, F2 and F3 components) and geographic location (latitude and longitude) on abundance of floral visitors (functional framework) and the number of flower they visited in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant predictors. Italic denotes conditional analysis results (partial-dbRDA).

		Abundance of visitor assemblage						Account of visited flower by visitor assemblage					
Assemblage	Predictors	*df*	*F*	*p-value*		% var	R^2	*df*	*F*	*p-value*		% var	R^2
All floral visitors						**7.9**						**8.9**	
	Flower	1	2.232	0.044	*	*7.9*	0.079	1	1.904	0.078	.	*4.5*	0.045
	Popdist	1	1.612	0.152		*2.1*	0.021	1	2.563	0.015	*	*4.4*	0.044
	Natcov	1	1.038	0.440		*3.9*	0.039	1	1.376	0.233		*3.4*	0.034
	LandUse	1	1.529	0.175		*7.4*	0.074	1	1.023	0.453		*5.4*	0.054
	F1	1	1.092	0.384		*5.5*	0.055	1	0.987	0.458		*5.1*	0.051
	F2	1	0.518	0.835		*2.9*	0.029	1	0.726	0.684		*3.7*	0.037
	F3	1	0.888	0.532		*2.8*	0.028	1	0.914	0.513		*3.9*	0.039
	Lat	1	1.160	0.322		*7.0*	0.070	1	1.091	0.377		*4.1*	0.041
	Long	1	0.868	0.504		*4.0*	0.040	1	0.670	0.707		*3.0*	0.030
	Residuals	11						11					
No honey bees						**8.9**						**13.2**	
	Flower	1	2.217	0.043	*	*8.9*	0.089	1	2.620	0.023	*	*8.3*	0.083
	Popdist	1	1.783	0.106		*2.0*	0.020	1	3.366	0.006	**	*4.9*	0.049
	Natcov	1	0.961	0.457		*3.8*	0.038	1	1.483	0.196		*4.7*	0.047
	LandUse	1	1.627	0.160		*7.8*	0.078	1	1.131	0.350		*4.7*	0.047
	F1	1	1.126	0.338		*5.6*	0.056	1	1.169	0.342		*5.4*	0.054
	F2	1	0.508	0.829		*2.9*	0.029	1	0.421	0.878		*2.3*	0.023
	F3	1	0.962	0.433		*2.7*	0.027	1	1.103	0.362		*3.7*	0.037
	Lat	1	1.346	0.236		*7.8*	0.078	1	1.491	0.182		*4.3*	0.043
	Long	1	0.954	0.468		*4.2*	0.042	1	0.942	0.475		*3.8*	0.038
	Residuals	11						11					

Note: Habitat natural cover [Natcov]; Land-use diversity [LandUse]; Distance to the nearest population [Popdist]; Flower density [Flower], Lartitude [Lat]; Longitude [Long].

** $p < 0.01$; * $p < 0.05$; . $p > 0.1$

Table S11 (cont.): Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of flower density, distance to nearest population, landscape disturbance (natural habitat cover and land-use diversity), local-scale habitat alteration (F1, F2 and F3 components) and geographic location (latitude and longitude) on abundance of floral visitors (functional framework) and the number of flower they visited in 21 *Ziziphus lotus* populations in SE Iberian Peninsula. Bold highlights the cumulative variance explained by significant predictors. Italic denotes conditional analysis results (partial-dbRDA).

		Abundance of visitor assemblage						Account of visited flower by visitor assemblage					
Assemblage	Predictors	*df*	*F*	*p-value*		% var	R^2	*df*	*F*	*p-value*		% var	R^2
Non-bee taxa						**22.8**						**13.4**	
	Flower	1	3.370	0.008	**	*10.0*	0.100	1	2.943	0.015	*	*7.5*	0.075
	Popdist	1	2.199	0.059	.	*3.0*	0.030	1	3.654	0.009	**	*5.9*	0.059
	Natcov	1	1.443	0.226		*2.7*	0.027	1	1.797	0.131		*3.9*	0.039
	LandUse	1	2.464	0.042	*	*9.8*	0.098	1	1.526	0.176		*4.8*	0.048
	F1	1	1.316	0.264		*6.1*	0.061	1	1.653	0.153		*7.0*	0.070
	F2	1	0.810	0.560		*3.6*	0.036	1	0.393	0.875		*1.7*	0.017
	F3	1	1.129	0.347		*2.5*	0.025	1	1.267	0.261		*3.3*	0.033
	Lat	1	1.936	0.103		*5.2*	0.052	1	2.208	0.064	.	*3.0*	0.030
	Long	1	0.645	0.714		*2.5*	0.025	1	0.492	0.810		*1.8*	0.018
	Residuals	11						11					
Wild bees													
	Flower	1	0.600	0.611		*4.5*	0.045	1	0.784	0.563		*6.5*	0.065
	Popdist	1	0.074	0.991		*1.6*	0.016	1	0.281	0.904		*1.9*	0.019
	Natcov	1	0.610	0.602		*7.9*	0.079	1	0.578	0.673		*7.3*	0.073
	LandUse	1	0.191	0.932		*2.4*	0.024	1	0.355	0.834		*5.4*	0.054
	F1	1	0.415	0.727		*2.1*	0.021	1	0.596	0.645		*3.0*	0.030
	F2	1	0.253	0.897		*1.5*	0.015	1	0.502	0.756		*3.4*	0.034
	F3	1	1.409	0.271		*5.6*	0.056	1	1.365	0.257		*5.4*	0.054
	Lat	1	0.833	0.508		*14.6*	0.146	1	0.741	0.579		*14.9*	0.149
	Long	1	2.043	0.123		*11.7*	0.117	1	2.835	0.037	*	*14.9*	0.149
	Residuals	11						11					

Note: Habitat natural cover [Natcov]; Land-use diversity [LandUse]; Distance to the nearest population [Popdist]; Flower density [Flower], Lartitude [Lat]; Longitude [Long].

** $p< 0.01$; * $p< 0.05$; . $p> 0.1$

CAPÍTULO 2

DEVELOPMENT AND CHARACTERIZATION OF MICROSATELLITE PRIMERS IN THE ENDANGERED MEDITERRANEAN SHRUB *ZIZIPHUS LOTUS* (RHAMNACEAE)

Ana González-Robles[1], Antonio J. Manzaneda[1], Jesús M. Bastida[1], Nick Harvey[2], Rafael Jaime[1], Teresa Salido[1], Luisa M. Martínez[1], Ana Fernández-Ocaña[1], Julio M. Alcántara[1] and Pedro J. Rey[1]

[1]Departamento de Biología Animal, Vegetal y Ecología. Universidad de Jaén. E-23071 Jaén, Spain.

[2]Genetic Marker Services, Brighton, United Kingdom.

***Appl Plant Sci,* 4:1600092 (2016)**

ABSTRACT

Microsatellite primers were developed to characterize and evaluate patterns of genetic diversity and structure in the endangered Mediterranean shrub Ziziphus lotus (Rhamnaceae). Twenty microsatellite primers were developed for Z. lotus, of which 14 were polymorphic. We evaluated microsatellite polymorphism in 97 specimens from 18 Spanish and seven Moroccan populations. Between two and eight alleles were found per locus, and the average number of alleles was 5.54. Observed heterozygosity and expected heterozygosity ranged from 0.08 to 0.90 and from 0.08 to 0.82, respectively. Nine of these primers also amplified microsatellite loci in *Z. jujuba*. The microsatellite markers described here will be useful in studies on genetic variation, population genetic structure, and gene flow in the fragmented habitat of this species. These markers are a valuable resource for designing appropriate conservation measures for the species in the Mediterranean range.

INTRODUCTION

Ziziphus Mill. (Rhamnaceae) is a pantropical and paraphyletic genus comprising approximately 170 species, 150 of them native to tropical and subtropical regions of Europe, the Middle East, Africa, India, and Asia (Islam and Simmons 2006). The genus includes two economically important tree species, *Z. jujuba* Mill. (Chinese jujube) and *Z.* mauritiana Lam. (Indian jujube), that are cultivated for their fruit (Huang et al. 2015). *Z. lotus* (L.) Lam. is a diploid (2n = 20; Pérez-Latorre and Cabezudo 2009), hermaphrodite, sclerophyllous thorny shrub species occurring across the Mediterranean Basin, North Africa and the Sahara, and the Arabian Peninsula. In Europe, *Z. lotus* is restricted to some semiarid localities in the southeast of the Iberian Penin- sula (Pérez-Latorre and Cabezudo 2009) and the island of Sicily. *Z. lotus* blooms from May to July, and flowers are pollinated primarily by bees. The fruit ripening period occurs in September, and fruits (drupes) are dispersed by foxes and other mammals. It is a keystone species in those semiarid ecosystems (Tirado 2009). Since 1992, *Z. lotus* habitats have been included in the Habitats Directive of the European Commission (Council Directive 92/43/EEC 1992, namely Arborescent scrub with *Ziziphus*: habitat 5220*; Council of the European Union, 2007), which lists Europe's most endangered and vulnerable habitats. Population size ranges from 10 to thousands (typically less than 100) of individuals depending on the alteration status. In fact, European *Z. lotus* populations are seriously threatened by severe habitat destruction and fragmentation due to agriculture intensi- fication and land-use change (Mota et al. 1996, Tirado 2009, Mendoza-Fernández et al. 2015). Microsatellite (simple sequence repeat [SSR]) markers have been recently developed for *Z. jujuba* (Huang et al. 2015); however, transferability of jujube SSR primers to *Z. lotus* has not been shown. Here, we characterized 20 microsatellite markers (14 polymorphic) developed specifically for *Z. lotus*, which will be subsequently used to evaluate the impact of land-use change and fragmentation on the genetic diversity of the species. We also amplified polymorphic markers in 10 North African individuals of *Z. lotus* (from Morocco) to assess genetic variation, diversity levels, and population genetic structure across the region for conservation purposes. Finally, cross-amplification was tested in *Z. jujuba* samples, the other *Ziziphus* species with a presence in the Iberian Peninsula.

MATERIALS AND METHODS

Total genomic DNA was extracted from frozen young leaves following a slightly modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle 1987). We used 0.7 volumes of cold isopropanol to precipitate nucleic acid, a wash buffer with 70% EtOH (without ammonium acetate), and mixed RNase with distilled water to resuspend the nucleic acid pellet; the samples were then incubated for 60 min at 37°C. The last dilution step was removed from the protocol. Leaves were collected from seven individuals across seven distinct populations covering the range of distribution of *Z. lotus* in southern Spain. Microsatellite isolation was performed by Genetic Marker Services (Brighton, United Kingdom). Briefly, microsatellite isolation was based on the production of an enriched library, using a hybridization capture protocol. Enrichment involved incubating adapter-ligated, restricted DNA, with filter-bonded synthetic repeat motifs: $(AG)_{17}$, $(AC)_{17}$, $(AAC)_{10}$, $(CCG)_{10}$, $(CTG)_{10}$, and $(AAT)_{10}$. The library was transformed into *Escherichia coli* JM109 and plated onto Luria– Bertani agar/ampicillin plates. The motif-positive clones were screened, isolated, and sequenced. Primers were designed using the online primer design software Primer3 (Rozen and Skaletsky 1999). The amplifying products were 100–250 bp long, to help minimize later multiloading overlap ambiguities during sequencer genotyping. The GC content of the designed primers is given in Table 1. To test the effectiveness of primer amplification, we used a touchdown PCR protocol. PCR amplification was performed in a 25-µL reaction volume that contained 7 pmol of each primer, 1.5 mM of MgCl2, 0.2 mM of each dNTPs, 1× PCR buffer, 0.8 µg/µL bovine serum albumin (BSA), 0.5 units of Taq poly- merase (AmpliTaq Gold polymerase; Applied Biosystems, Carlsbad, California, USA), and 1.5 µL of DNA 1:20 diluted. PCR amplification of the template was performed according to the following protocol (32 cycles): 95°C for 60 s for initial denaturation; annealing for 60 s as two cycles each 64–59°C, 10 cycles 58°C, 10 cycles 57°C; elongation at 72°C for 60 s; and a final extension at 72°C for 5 min using a MyCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA). All PCR products were checked for specificity, active polymorphism, and null alleles on high-resolution agarose gels (4% MetaPhor; Lonza, Basel, Switzerland) in a TAE buffer system. Fourteen out of 20 loci showed clear and specific bands displaying size variation among the seven individuals assayed. These 14 loci were then selected for fluorescent labeling (Table 1). We tested the efficiency and functionality of the 14 selected microsatellites in 97 samples from 18 Iberian and seven Moroccan populations corresponding to the three main population centers of this species in the western Mediterranean (Table S1, Suppl. Mat.). The larger populations are located in

Almeria; the populations in Murcia and Morocco are basically relicts where scattered individuals occur, of- ten growing at the margin of cultivated fields. Multiplex PCRs were performed in 11.11-µL volumes containing 7 pmol of each primer (labeled with the fluorescent dye 5-HEX or 56-FAM; Table 1), 1.5 mM of MgCl2, 0.2 mM of dNTPs mix, 1× PCR buffer, 0.5 units AmpliTaq Gold polymerase (Applied Biosystems), and 10 ng/µL of DNA. Touchdown PCR conditions (32 cycles) comprised an initial heat step at 95°C for 4 min; followed by 10 cycles at 95°C for denaturation for 1 min, annealing at 64°C for 1 min (decreasing 1°C for each of two cycles), elongation at 72°C for 1 min; followed by 11 cycles with denaturation at 95°C for 1 min, annealing at 58°C for 1 min, elongation at 72°C for 1 min; followed by 11 cycles with denaturation at 95°C for 1 min, annealing at 57°C for 1 min, elongation at 72°C for 1 min; with a final extension of 5 min at 72°C. Mix A (Table 1) was best amplified and optimized with a common PCR protocol for SSR (Ghaffari and Hasnaoui 2013), which comprised an initial heat step at 95°C for 3 min, followed by 40 cycles with denaturation at 95°C for 20 s, annealing at 55°C for 1 min, elongation at 72°C for 1 min, and a final extension at 72°C for 6 min. Fluorescently labeled PCR products were analyzed on an ABI 3500 Genetic Analyzer sequencer (Applied Biosystems) using GeneScan 600 LIZ Size Standard (Applied Biosystems) in the automated genotyping. GeneMapper software version 4.1 (Applied Biosystems) was used for the assignment of alleles and fragment analysis. We used the package *pegas* (Paradis 2010) of R software version 3.2.2 (R Core Team 2015) to estimate the number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), and Hardy–Weinberg equilibrium (HWE). The presence of null alleles was checked using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004), and their statistical significance was assessed using Bonferroni corrected *P*-values. Linkage disequilibrium was estimated using GENEPOP software (Rousset 2008).

Table 1: Characteristics of 20 microsatellite loci developed in *Ziziphus lotus*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)	T_a (°C)	Fluorescent dye	Mix groups	GenBank accession no.
zlo60§	F: GTT CAT CAA TTT CAC CAT GC R: CCT ATG GAT TAC TCC TAT AAG CTG	$(CT)_{15}$	205-234	50	—	—	KU530111
zlo61§	F: GCA ACT TTC CCA ACC AAG AA R: CCA AAT TTT CGT TAC ACC ATG T	$(CT)_{24}$	364-411	53.5	—	—	KU255442
zlo64*	F: TTG GAT AAA AAG AGG TTA GCA CA R: CCA AAC AGC TTA GGG ACC AA	$(CT)_{15}$	61-90	52	5HEX	A	KU530112
zlo65*	F: ATT GTC CCA CCT TCC AAC AC R: AAC TCC ACC TGA AAC CTT GC	$(TC)_{10}$/actcc/$(CT)_5$	332-366	55	5HEX	A	KU255443
zlo66*	F: TAA AGA GCG TAA GCG GGA AA R: GCG CGC AGT AGA TAG ACA CC	$(AG)_{15}$	321-350	54	56-FAM	E	KU255444
zlo67*	F: GAT CTG AAC ACC GTG CGT TT R: AAA CCA ACG AAA CCA ACG AA	$(CT)_{19}$	139-176	53	5HEX	D	KU255445
zlo68*	F: TGA CGT TAG GAA AGA AGA TTC AAA R: GGC ATC CTT AAT GCA AGA GG	$(TG)_9$ $(AG)_{14}$	289-334	52.5	5HEX	E	KU255446
zlo69§	F: AGC TTG GTC TCT CCA ACG AA R: GCA AAA CCC TAG GTG GCT TA	$(CT)_6$/cc/$(CT)_{11}$	54-89	56	—	—	KU255447
zlo71*	F: CCG AGG TGA GGT ATG TCT TT R: TCC CCA AGA AGC AAA TAT CA	$(CT)_{20}$	57-96	51.5	56-FAM	C	KU255448
zlo73*	F: GGA CTG TGT ATT TCA GCA AGG R: AAA GTT GTG CCA ATC TTG TTT	$(TC)_{21}$	429-470	51	56-FAM	A	KU255449

Note: T_a = annealing temperature. * Tested for polymorphism. § Monomorphic markers

Table 1 (cont.): Characteristics of 20 microsatellite loci developed in *Ziziphus lotus*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)	T_a (°C)	Fluorescent dye	Mix groups	GenBank accession no.
zlo76*	F: ACA ACG TTT GCT GTC CTC TC	$(CT)_{16}$	108-139	55	56-FAM	C	KU255450
	R: TAA ACC CAC AAG AGG GAA TG						
zlo77*	F: CGC CAT GTA AAA TGG TGT TG	$(GA)_{12}$	217-240	52.5	56-FAM	D	KU235551
	R: CCA CTT CCC AGG AGA TCC AT						
zlo79*	F: TAT AAT CTC TTT CCA GAT TCC TAG AA	$(CT)_{19}$	311-348	51	56-FAM	B	KU235552
	R. CTT GTG TCA TCC CAA AAC CA						
zlo80*	F: TTT CAC GGT ATT GCA ACC AG	$(GT)_{10}$	377-396	53.5	5HEX	C	KU255451
	R: CAC CTG AGG CAC AAC AAA GTT						
zlo84§	F: CGT TTT CTG TTT GGA GAC CA	$(AG)_{18}$	216-251	53	—	—	KU255452
	R: AAT CAA TTC CTT CCC AAG CA						
zlo85§	F: TGG CAT TTA ATT GTG TCA TCA GA	$(AG)_{14}$	136-163	53	—	—	KU255453
	R: CCA TAC TGT CCC TTT CCA GTC						
zlo86*	F: TTT GAG ATT GAT GGC TCC TT	$(AC)_{10}$	233-252	51.5	56-FAM	E	KU255454
	R: GCA GTT TCC GTT GTT GTT TT						
zlo87*	F: CAG CTC GTC CTC TTC GAG AT	$(TC)_{16}$	134-164	56	56-FAM	B	KU255455
	R: GGA CAA TTT GGA ATT TCA GTT TC						
zlo88*	F: TTC ATA ACA GCA CGT CAA ACT	$(AG)_{21}$	409-450	52.5	56-FAM	B	KU255456
	R: TAT GGA GTG GGT TCA TTT CC						
zlo89§	F: TGG TCT TAA TTC ACC AAG CTC A	$(GA)_{14}/GG/(GA)_{10}$	158-207	54	—	—	KU255457
	R: GCT TGT CAG TTG TAT GTG AAG TGA						

Note: T_a = annealing temperature. * Tested for polymorphism. § Monomorphic markers

RESULTS

The number of alleles ranged from two to eight per locus, depending on the population (Table 2). H_o varied from 0.08 to 0.90, and H_e varied from 0.08 to 0.82. Overall, it is shown that H_o is lower than H_e in the three study areas (Almeria, Murcia, and Morocco; Table 2), likely as a result of nonrandom mating and/or genetic drift. Two loci (zlo71 and zlo86) showed a significant deviation from HWE in Murcia populations, while two other loci (zlo64 and zlo71) showed deviation from HWE in Moroccan populations. Null alleles were present in six loci (Table 2) concordant in some cases with deviation from HWE, which may be caused by the

Table 2: Genetic characterization of 14 newly developed microsatellites of *Ziziphus lotus*[a].

	Almeria, Spain (*n* = 50)				Murcia, Spain (*n* =37)				Morocco (*n* =10)			
Locus	*A*	H_o	H_e	HWE	*A*	H_o	H_e	HWE	*A*	H_o	H_e	HWE
zlo64[§]	8	0.72	0.79	0.011	8	0.62	0.78	0.006	5	0.20	0.72	0.000*
zlo65	8	0.74	0.74	0.005	6	0.62	0.72	0.015	7	0.80	0.80	0.346
zlo66	6	0.76	0.72	0.784	6	0.30	0.39	0.016	6	0.60	0.73	0.172
zlo67	6	0.58	0.67	0.231	4	0.43	0.44	0.199	8	0.70	0.80	0.021
zlo68	5	0.66	0.67	0.872	6	0.73	0.73	0.520	4	0.40	0.68	0.066
zlo71[§]	6	0.54	0.57	0.228	5	0.41	0.59	0.000*	7	0.20	0.70	0.000*
zlo73[§]	4	0.46	0.62	0.014	5	0.51	0.65	0.020	5	0.40	0.74	0.015
zlo76[§]	5	0.44	0.56	0.046	3	0.43	0.46	0.098	6	0.60	0.76	0.424
zlo77	3	0.18	0.17	1.000	2	0.08	0.08	1.000	3	0.60	0.55	0.587
zlo79[§]	6	0.74	0.72	0.249	5	0.49	0.65	0.046	7	0.70	0.82	0.079
zlo80[§]	6	0.68	0.72	0.203	6	0.59	0.74	0.020	4	0.50	0.66	0.131
zlo86	5	0.50	0.50	0.562	6	0.57	0.66	0.000*	8	0.60	0.71	0.011
zlo87	5	0.56	0.56	0.161	5	0.38	0.47	0.030	5	0.90	0.78	0.082
zlo88	7	0.50	0.55	0.078	6	0.46	0.55	0.015	5	0.50	0.72	0.238
Mean	5.71	0.58	0.61	—	5.21	0.47	0.57	—	5.71	0.55	0.73	—

Note: A = number of alleles*; Ho* = observed heterozygosity; *He* = expected heterozygosity*;* HWE = *P*-values of the exact test of Hardy–Weinberg equilibrium*; n* = number of individuals sampled.

[a] See Table S1 (Suppl. Mat.) for geographic locations of all populations sampled.

* Locus showed significant deviations from Hardy–Weinberg equilibrium, after Bonferroni correction ($P < 0.001$).

[§] Significant presence of null alleles (zlo73 and zlo76 on Almeria; zlo64, zlo71, zlo73, zlo79 and zlo80 on Murcia; zlo64, zlo71 and zlo73 on Morocco).

intensive fragmentation of *Z. lotus* habitat in those populations. The presence of microsatellite null alleles is reported frequently in PCR primer characterization, and it should be taken into account when estimating population differentiation (Chapuis and Estoup 2007). Significant linkage disequilibrium was detected only for zlo76/zlo80 loci after pairwise Bonferroni correction. Cross-amplification in *Z. jujuba* showed fragments of the expected size in nine of the 14 microsatellite loci (Table 3).

Table 3: Genetic properties of the microsatellite loci developed for *Ziziphus lotus* in single populations[a] of *Z. jujuba.*

	Ziziphus jujuba , Spain (*n* = 5)			
Locus	*A*	*Allele size range (bp)*	H_o	H_e
zlo64	—	—	—	—
zlo65	—	—	—	—
zlo66	3	172-178	0.4	0.62
zlo67	1	156	0	0
zlo68	5	158-176	1	0.74
zlo71	—	—	—	—
zlo73	—	—	—	—
zlo76	1	125	0	0
zlo77	1	136	0	0
zlo79	1	142	0	0
zlo80	2	142-155	1	0.5
zlo86	1	112	0	0
zlo87	1	114	0	0
zlo88	—	—	—	—

Note: — = not amplified; *A* = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; *n* = number of individuals sampled.

[a] See Table S1 (Suppl. Mat.) for geographic locations of all populations sampled.

CONCLUSIONS

These 20 microsatellite markers are the first markers devel-oped specifically for *Z. lotus*, and will be a useful tool for studies on genetic variation, diversity, population genetic structure, and gene flow in the fragmented habitat of this species. These markers are thus a valuable resource for designing appropriate conservation measures for the species in the Mediterranean range.

ACKNOWLEDGMENTS

The authors thank F. Amil for assistance with DNA extraction; J. Isla and F. Valera for help during Iberian sample collection; I. Cancio for help during Moroccan sample collection; C. Quesada, Universidad de Granada Herbarium, and A. B. Robles for their advice and assistance in the process of plant conservation; and M. A. Carrión (Dirección General de Medio Ambiente del Gobierno de Murcia) for facilitating permits for plant collection. This work was funded by a Junta de Andalucía Excelencia M766 project and by funds from Fondo Europeo de Desarrollo Regional (FEDER). A. González-Robles and A.J. Manzaneda were supported by Universidad de Jaén Plan Propio fellowship (Acción 16) and a Ramón y Cajal contract (RYC-2010-06237), respectively.

REFERENCES

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Mol Biol Evol*, 24, 621– 631.

Doyle JJ (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*, 19, 11–15.

Ghaffari S, Hasnaoui N (2013) Chapter 10: Microsatellite Amplification in Plants: Optimization Procedure of Major PCR Components. In: SK Kantartzi (ed.), *Microsatellites. Methods and Protocols*. 139–146. Springer, New York, New York, USA.

Huang J, Yang X, Zhang C, Yin X, Liu S, Li X (2015) Development of Chloroplast Microsatellite Markers and Analysis of Chloroplast Diversity in Chinese Jujube (*Ziziphus jujuba* Mill.) and Wild Jujube (*Ziziphus acidojujuba* Mill.). *PLoS ONE*, 10, e0134519. doi: https://doi.org/10.1371/journal.pone.0134519

Islam MB, Simmons MP (2006) A Thorny Dilemma: Testing Alternative Intrageneric Classifications within *Ziziphus* (Rhamnaceae). *Syst Bot*, 31, 826–842.

Mendoza-Fernández AJ, Martínez-Hernández F, Pérez-García F J, Garrido-Becerra JA, Benito BM, Salmerón-Sánchez E, Guirado JS, Merlo ME, Mota JF (2015) Extreme habitat loss in a Mediterranean habitat: *Maytenus senegalensis* subsp. *europaea*. *Plant Biosyst*, 149, 503–511.

Mota JF, Peñas J, Castro H, Cabello J, Guirado JS (1996) Agricultural development vs biodiversity conservation: the Mediterranean semiarid vegetation in El Ejido (Almeria, southeastern, Spain). *Biodivers Conserv,* 5, 1597–1617.

Paradis E (2010) *pegas*: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, 26, 419–420.

Pérez Latorre, A. V., and B. Cabezudo. 2009. *Ziziphus* Mill. In: G Blanca, B Cabezudo, M Cueto, C. Fernández-López, C Morales-Torres (eds.), *Flora Vascular de Andalucía Oriental, vol. 3*, 46–50. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, Spain.

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. url: https://www.R-project.org/.

Rousset F (2008). GENEPOP'007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour,* 8, 103–106.

Rozen S,Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: S Krawetz and S Misener (eds.) *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. 365–386. Humana Press, Totowa, NJ.

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus.* In: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid, Spain.

UE (1992) Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora [online]. url: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:01992L0043-20070101 [accessed 19 December 2015].

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Resour*, 4, 535–538.

SUPPLEMENTARY MATERIAL

Table S1: Voucher and location information for *Ziziphus lotus* populations and *Ziziphus jujuba* sampled used in this study.

Species	Voucher[a]	Population	Locality	Country of origin	Geographic coordinates[b]	n[c]
Ziziphus lotus (L.) Lam	GDA62533	El Ejido	Almería	Spain	36°45′40″N, 2°47′59″W	5
	GDA62534	Santa María del Águila	Almería	Spain	36°47′38″N, 2°46′16″W	5
	GDA62535	Sierra Alhamilla	Almería	Spain	36°56′55″N, 2°24′34″W	5
	GDA62541	El Toyo	Almería	Spain	36°50′51″N, 2°19′58″W	5
	GDA62540	Torregarcia	Almería	Spain	36°49′37″N, 2°17′22″W	5
	GDA62539	El Playazo	Almería	Spain	36°51′10″N, 2°01′05″W	5
	GDA62538	Campohermoso	Almería	Spain	36°56′44″N, 2°08′30″W	5
	GDA62537	Nijar	Almería	Spain	36°56′20″N, 2°13′10″W	5
	GDA62536	Autovia	Almería	Spain	36°52′11″N, 2°15′48″W	5
	GDA62542	Guazamara	Almería	Spain	37°20′53″N, 1°46′24″W	5
	GDA62543	Cantera Puerto Lumbreras	Murcia	Spain	37°31′51″N, 1°48′01″W	5
	GDA62544	Bejar-Torrecilla	Murcia	Spain	37°36′51″N, 1°45′17″W	5
	GDA62545	Purias	Murcia	Spain	37°35′28″N, 1°39′27″W	5
	GDA62546	Tercia	Murcia	Spain	37°42′57″N, 1°35′14″W	5
	GDA62548	Peñas Blancas	Murcia	Spain	37°40′52″N, 1°08′52″W	5
	GDA62549	Palas	Murcia	Spain	37°40′28″N, 1°13′48″W	5
	GDA62550	Los Garres	Murcia	Spain	37°56′14″N, 1°07′42″W	4
	GDA62547	Fuente Álamo	Murcia	Spain	37°42′12″N, 1°09′16″W	3
	GDA62551	Agadir	Agadir	Morocco	30°23′43″N, 9°34′52″W	2
	GDA62557	Ouazzane	Ouazzane	Morocco	34°34′48″N, 5°32′01″W	1
	GDA62556	Taza	Taza	Morocco	34°06′23″N, 4°27′13″W	2
	GDA62555	Fez	Fez	Morocco	33°56′21″N, 5°00′05″W	2
	GDA62552	Tiznit	Tiznit	Morocco	29°39′45″N, 9°51′53″W	1
	GDA62554	Zag	Assa-Zag	Morocco	27°58′47″N, 9°43′0″W	1
	GDA62553	Tistguezzemtz	Tistguezzemtz	Morocco	28°24′36″N, 9°10′48″W	1
Ziziphus jujuba Mill.	GDA62558	Pinos del Valle	Granada	Spain	36°53′29″N, 3°32′29″W	1
	GDA62559	Puente Tablas	Jaén	Spain	37°47′31″N, 3°45′11″W	1
	GDA62560	Las Yucas	Jaén	Spain	37°45′15″N, 3°41′02″W	2
	GDA62561	Algeciras	Cádiz	Spain	36°09′08″N, 5°27′20″W	1

Note: [a] Voucher specimens deposited at Universidad de Granada Herbarium (GDA), Universidad de Granada, Granada, España. [b] Datum: World Geodetic System 1984 (WGS84). [c] Number of individuals (samples) per locality.

CAPÍTULO 3

LONG-LIVED SEMIARID SCRUBS SHOW SIGNALS OF GENETIC EFFECTS BY HIGH RATES OF HABITAT LOSS OVER THE PAST CENTURY, BUT STILL MAINTAIN HIGH GENETIC DIVERSITY AND ITS HISTORICAL GEOGRAPHIC STRUCTURING

ABSTRACT

Understanding genetic factors that govern the population viability of threatened plant species is essential to design efficient conservation strategies. Land-use conversion and habitat loss and degradation are among the factors affecting populations and species genetic integrity. Here we analyze how environmental correlates, geographic location and anthropogenic disturbance (at local, landscape and regional scales) relate to population differentiation and genetic diversity and structuring of a keystone semiarid long-lived scrub, *Ziziphus lotus*. The European range of this species is restricted mainly to the southeast of the Iberian Peninsula but its area has shrunk considerably as a consequence of intensive agriculture and urban expansion since the 1950s. We characterize its genetic diversity and structure across its whole range in the Iberian Peninsula, considering 21 populations and an ample gradient of habitat and landscape disturbance. Three main genetic clusters were defined by structural analyses corresponding largely to west, central (core) and east of its distribution. Although all populations showed high and relatively similar values of genetic diversity, the ones in the core region ranked higher. The lowest genetic values were found in the western region. Molecular variance analysis revealed that 87.3% of genetic variation was grouped within populations, 5% among populations and 7% between regions. Geographic location has stronger correlation with population genetic differences than habitat or landscape characteristics (36.38% vs 5.82%, respectively). Natural habitat cover at the landscape level was the only environmental variable associated with these genetic differences. High values of genetic diversity and low genetic divergence among populations can be explained by features as great longevity, insect-mediated cross-pollination and mammalian seed dispersal. The detected population and geographic structure are related to historical gene flow and intensive land-use change of this area. Our results suggest that although the long-lived and resprouting strategy of the species is delaying the erosion of its genetic diversity at population and regional level, there are already genetic consequences of the habitat loss and degradation, which could be aggravated in the future.

INTRODUCTION

Isolation and population size are two key factors influencing genetic diversity in plant populations (Young et al. 1996). Small and isolated populations typically show genetic erosion features such as low heterozygosity, elevated inbreeding and/or reduced allelic richness, which in the long term may compromise population viability (Young et al. 1996, Lowe et al. 2005). Thus, smaller and isolated populations are expected to show lesser allelic diversity than larger and well-connected populations (Leimu et al. 2006, Aguilar et al. 2008). Across the range of a species, population isolation may arise naturally because of geography and elevation (i.e., isolation by distance, *IBD*) and/or as a result of human disturbance (Lowe et al. 2005, Wang and Bradburd 2014). In both cases, adaptive or non-adaptive genetic differentiation between populations may also occur in absence of gene flow among them (Hahn 2018). Therefore, understanding how genetic attributes of plant populations are being affected by landscape and habitat disturbance relative to historic and geographic factors is one of the main challenges of plant species conservation (Wang and Bradburd 2014, Kierepka and Latch 2015, Noguerales et al. 2016).

Anthropogenic disturbance (e.g., habitat loss, alteration and fragmentation) impacts directly on plant populations by reducing population size, which may entail the fixation of rare alleles or the accumulation of deleterious mutations, making populations more susceptible to differentiation due to genetic drift or to extinction after an alteration (Aparicio et al. 2012, Wereszczuk et al. 2017). Human disturbance may also affect plant populations indirectly by disrupting key mutualistic plant-animal interactions such as pollination or seed dispersal (Jordano 2010), which have usually important consequences on reproduction, demography and the gene flow of plant populations (Lowe et al. 2005, Browne and Karubian 2018). In fact, life-history traits influencing pollen and seed dispersal distances are thought to be the strongest determinants of spatial genetic structure in plant populations (Vekemans and Hardy 2004). It is known that reduction of pollinator and seed dispersers affects directly the plant reproductive success, which, in turn, has indirect consequences on the inbreeding and selfing rates of plant species (Lowe et al. 2005, Jordano et al. 2011). However, several studies have suggested that the impact of anthropogenic disturbance on population genetic structure depends on the nature of alteration, the timing of perturbation, the amount and type of the matrix surrounding habitat remnants, the mating system and seed dispersal modes and, especially, the species life-history (e.g., Young et al. 1996, Fahrig 2003, Lowe et al. 2005, Aguilar et al. 2008, Vranckx et al. 2011, Nora et al. 2015). For instance, for long-lived plant species, landscape alteration seems to affect in a larger extent the spatial genetic structure at intra-populational scale than the between-population differentiation and the geographic structuring of the genetic diversity at the short term (Wang et al. 2009, Browne et al. 2015). Nonetheless, when the environment alteration and habitat loss is very severe, these genetic changes may occur faster than expected (Aguilar et al. 2008, Browne al 2015). Likewise, genetic effects of habitat alteration at inter-populational scale are exacerbated when the abundance or occurrence of key seed dispersers are adversely affected (especially those that disperse seeds to long distances; Wang et al. 2011).

Here, we analyze the relative impact of intensive and continuous habitat alteration (local scale) and landscape disturbance (large scale) on the genetic diversity and genetic structure of a long-lived semiarid scrub (*Ziziphus lotus*) across its whole range in the Iberian Peninsula. *Ziziphus lotus* (L.) Lam (Rhamnaceae) is a relict shrub species in South Europe which mainly grows in semiarid habitats at the southeast of Iberian Peninsula, although it is also found in Cyprus and Sicily (European Environmental Agency https://eunis.eea.europa.eu/habitats/10099; Pérez Latorre and Cabezudo 2009, Sánchez Gómez et al. 2002). This study system is particularly adequate to test the effect of anthropogenic disturbance on genetic diversity and spatial structuring in semiarid habitats for several reasons: (1) This species is a keystone species of the *Habitat 5220** [*"Mediterranean arborescent scrubs with Ziziphus lotus* (L.)", a priority habitat of European Directive 92/43/EEC] (Anon 1992, Tirado 2009) and since the 50's of the last century, this habitat has suffered one of the highest rates of loss and degradation in Europe, mainly by the expansion of greenhouse agriculture and urban development (Mota et al. 1996, Mendoza-Fernández et al. 2015). (2) Recent studies have documented the impact of the intense anthropogenic alteration of *Ziziphus* habitat remnants on key stages of its life-cycle and on the viability of such populations. Thus, most *Z. lotus* populations are essentially constituted by adult individuals with few juvenile plants (Rey et al. 2018). (3) This is related with the disruption of critical processes like the alteration of the pollinator assemblages, which affects the pollination service (see *Capítulo 1*), the collapse of seed dispersal by mammalian carnivores (Cancio et al. 2016, 2017; Rey et al. 2017) and the subsequent lack of population regeneration (Rey et al. 2018). (4) Habitat degradation and loss is not regionally homogeneous, being more intense in the south-western and eastern areas of the species range in the Iberian Peninsula (Fig. 1), where degradation occurs both at the landscape level and in the remnant patches. The strongest disruptions of animal-plant associations (e.g., less diverse pollinator assemblages, high floral visit rate by managed bees, and/or reduced seed dispersal by animals) have been reported in these populations (Rey et al. 2017, see also *Capítulo 1*). In contrast, the

Z. lotus populations located in the well-preserved core of the range (Fig. 1) are much larger and efficient seed dispersal by animals has been reported (Rey et al. 2017).

No study has evaluated the effects of the anthropogenic modifications and loss of these natural habitats on genetic diversity and genetic structure of Iberian *Z. lotus* populations. This is essential to address adequate management plans that ensure the maintenance of this relict species in the region and in Europe. In particular, we aim at: i) estimating the overall genetic diversity of this species; ii) quantifying population inbreeding and selfing rates between areas with a different alteration status; iii) assessing the relative distribution of genetic variation within and between *Z. lotus* populations; iv) examining to what extent genetic differentiation is explained by geographic factors (isolation by distance) and/or habitat and landscape degradation. Provided the high rate of habitat destruction at landscape level, degradation at local-remnant level, and the documented disruption of plant-animal interactions with collapse of regeneration, we predict that: i) allelic diversity of small and degraded populations will be lower than the diversity of well-preserved populations; similarly, ii) inbreeding rates and selfing will be higher in altered populations; and iii) because of isolation resulting from human activity, genetic variability should be found mainly between populations (i.e., inter-populational genetic differentiation). Alternatively, we hypothesize that some life-history traits of this species such as its long life span and vegetative growth (with centenary resprouter individuals reaching up to 30m diameter, Rey et al. 2018) have delayed the erosion of the genetic diversity, allowing retention of much of the diversity within populations and preserving the historic geographic genetic structure of the species in the Iberian peninsula.

MATERIALS AND METHODS

Study species and system

Z. lotus (L.) Lam. is a diploid (2n = 20; Pérez-Latorre and Cabezudo, 2009) sclerophyllous thorny arborescent shrub species occurring across the Mediterranean Basin (mostly in North Africa, but also in Sahara and the Arabian Peninsula), being infrequent in the South of Europe (Spain, Cyprus and Sicily) (European Environmental Agency, https://eunis.eea.europa.eu/habitats/10099). In Spain this species is distributed in the southeastern most arid areas of the Iberian Peninsula, where it used to occupy large extensions in seasonal streams ('ramblas') and coastal plains. However, during the last 60 years, its original habitat has been reduced and fragmented by intensive greenhouse-based agriculture and urban expansion. In many sites less than 5% of the area occupied in the 1950s is left, which has led the habitat to a dramatic situation (Mota et al. 1996; Mendoza-Fernández et al. 2015). Based on the current cover of this habitat, its landscapes have been classified as relict, fragmented and variegated (sensu McIntire and Hobs 1999) (Cancio et al. 2016, Rey et al. 2017, 2018) and population sizes in the habitat remnants range from 10 to thousands of individuals (typically less than 100), depending on the habitat remnant alteration and landscape degradation.

Z. lotus has notable resprouter ability and individuals may reach huge sizes (up to 30 m diameter and 1-3 m height) and great longevity (Rey et al. 2018). Adults produce numerous hermaphrodite flowers pollinated by a wide assemblage of insects, although in more altered populations managed bees monopolize most of flower visits (see *Capítulo 1*). Flowers show self-incompatibility and synchronous protandrous dicogamy whereby male and female flowers are found in 1:1 proportion per population at the same time (Nadia et al. 2007, pers. obs.). The fruits are medium sized drupe-like diaspores and in southeastern Spain they are dispersed mainly by foxes and other mammals (Cancio et al. 2016, 2017).

Population sampling

From April to May 2015, we sampled a total of 397 individuals of *Z. lotus* distributed in 21 populations throughout the whole range of species in the Iberian Peninsula (Fig. 1; distance between populations located in the extremes of the distribution: 155 km; distance between the two nearest populations 415 m). In each population, 20-25 adult individuals were randomly selected and geo-referenced, with the exception of seven populations where only from 8 to 23 individuals were found (Table 1). Young fresh leaves were collected from each individual, dried with silica gel and stored at -50°C in the laboratory, until DNA extraction. A voucher specimen per population was deposited at the Universidad de Granada Herbarium (GDA) (Universidad de Granada, Granada, Spain) (see *Capítulo 2*, Table S1; González-Robles et al. 2016).

DNA extraction and PCR amplification

Total genomic DNA was extracted from the stored young leaves, previously unfrozen, following the cetyltrimethylammonium bromide (CTAB) protocol (Doyle 1987) with slight modifications (see full details in *Capítulo 2*; González-Robles et al. 2016). A total of 14 polymorphic microsatellites markers (SSRs), specifically developed for *Z. lotus* (zlo64, zlo65, zlo66, zlo67, zlo68, zlo71, zlo73, zlo76, zlo77, zlo79, zlo80, zlo86, zlo87 and zlo88; see *Capítulo 2*, González-Robles et al. 2016), were used in the genotyping of the 397 individuals. Four fluorescent dyes (5-HEX, 56-FAM, ATTO565 and ATTO550) were used to 5-end label the forward primer of each locus.

According to González-Robles et al. (2016) (see *Capítulo 2*), the amplification process was performed with multiplex PCRs in a final volume of 11.11-µL, containing 7 pmol of each primer (forward and reverse), 1.5 mM of MgCl2, 0.2 mM of dNTPs mix, 1× PCR buffer, 0.5 units AmpliTaq, GoTaq (Promega Biotech Ibérica, S.L.), and 10 ng/µL of DNA. Two different programs were used for polymerase chain reactions (PCR) depending on the recommendations for each primer: (i) a touchdown PCR which comprised an initial heat step at 95°C for 4 min; followed by 10 cycles at 95°C for denaturation for 1 min, annealing at 64°C for 1 min (decreasing 1°C for each of two cycles), elongation at 72°C for 1 min; followed by 11 cycles with denaturation at 95°C for 1 min, annealing at 58°C for 1 min, elongation at 72°C for 1 min; followed by 11 cycles with denaturation at 95°C for 1 min, annealing at 57°C for 1 min, elongation at 72°C for 1 min; with a final extension of 5 min at 72°C; (ii) a common PCR protocol for SSRs (Ghaffari and Hasnaoui 2013) which comprised an initial heat step at 95°C for 3 min; followed by 40 cycles with denaturation at 95°C for 20 s, annealing at 55°C for 1 min, elongation at 72°C for 1 min; and a final extension at 72°C for 6 min. An ABI 3500 Genetic Analyzer sequencer (Applied Biosystems) using GeneScan 600 LIZ Size Standard (Applied Biosystems) was used in the automated genotyping to analyze fluorescently labeled PCR products. GeneMapper software version 4.1 (Applied Biosystems) was used for the assignment of alleles and fragment analysis.

Statistical analyses

Genetic diversity, genetic divergence and population structure

For each population, we evaluated genetic polymorphism and diversity by calculating mean observed heterozygosity (H_o), mean expected heterozygosity ($\mathrm{H_e}$), allelic diversity as the total number of alleles (N_a), fixation index (F_{ST}) and inbreeding coefficient (F_{IS}) by R-package PEGAS (Paradis 2010). Rarefied allelic richness (A_R) was obtained by using R-package HIERFSTAT (Goudet and Jombart 2015), and selfing rate (s) was estimated from $s = 2F_{IS} /(1+ F_{IS})$ (Ritland

1990). Hardy-Weinberg Equilibrium (*HWE*) after pairwise Bonferroni correction was tested for each population (Table S1). Genetic diversity features of each locus can be consulted in Table S2. Linkage disequilibrium was tested based on 10000 permutations using GENEPOP v.4.2 software (Rousset 2008) and null allele frequencies were estimated in Micro-Checker v.2.2.0 (van Oosterhout et al. 2004).

Genetic divergence between populations was estimated from their fixation indexes (F_{ST}), which were used to build a neighbor-joining tree based on a F_{ST} genetics distance matrix (Table S3). It was performed with the R-package HIERFSTAT (Goudet and Jombart 2015). Mean values of F_{ST} per population are shown in Table 1.

Because we identified genetic differentiation at regional scale (see Results), we conducted analysis of molecular variance (AMOVA) to calculate the partition of genetic variance at two hierarchical levels: 'Region' (level defined by neighbor joining tree groups) and 'Population' (within 'Region'). Significance was obtained by Monte Carlo permutation tests. This analysis was performed with the R-package POPPR (v.2.6.1; Kamvar et al. 2014). ANOVA and Tukey tests were used to test differences among genetic regions for *Ho*, *He*, N_a, A_R, F_{IS} and *s* with the R-package STATS (R Core Team 2017). All these variables followed a normal distribution.

Individual assignment to determine population structure was inferred by performing a discriminant analysis of principal components (DAPC) with R-package ADEGENET (Jombart 2008). Following Jombart et al. (2010), we made the cluster selection using unambiguous group criteria and the lowest BIC value, taking into account the biological sense of the results. DAPC is an adequate approach to detect genetic structuring among populations, however it can infer erroneous discrete clusters when genetic diversity is distributed continuously and homogenously among populations. In this case, it is recommended to use an additional structuring analysis (e.g., STRUCTURE; Pritchard et al. 2000) for concordance (see Jombart et al. 2010 for a full explanation). Thus, we further conducted a Bayesian clustering analysis to estimate global ancestry with STRUCTURE software (v.2.3.4; Pritchard et al. 2000). The Bayesian clustering analysis assumed admixed model with alleles frequency correlated and used from 2 to 25 groups (*K*) to individual assignment, with 20 independent runs per *K* values. 50^6 Markov chain Monte Carlo (MDMD) repetitions were performed for each run, after 50^5 burn-in periods. The visualization of STRUCTURE results and the calculation of *K* optimal value (by the *ΔK* method; Evanno et al. 2005) were carried out in STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Testing isolation by distance (IBD) and anthropogenic disturbance impact

A distance-based redundancy analysis (dbRDA) was performed to examine the relative importance of geography (*IBD*) and habitat disturbance on the genetic differentiation of *Z. lotus* populations. Partial-dbRDAs were further performed to determine the percentage of variance explained by each variable.

To conduct these analyses, we previously characterized the study populations in terms of environmental disturbance of the populations at local and landscape scale (see procedures described in Cancio et al. 2016, Rey et al. 2017, 2018; see also *Capítulo 1*). In short, local-scale disturbance describes the remnant degradation due to the heavy anthropic impact (mainly abandoned agricultural uses, illegal rubbles and dumps, overgrazing and quarries). These actions entail huge changes in the spatial structure and size of *Ziziphus* populations. Large-scale disturbance defines the landscape alteration mainly by agricultural intensification and urban expansion. The later factors are responsible for a severe reduction of natural habitat cover and a drastic land-use transformation. Habitat remnant degradation (i.e., disturbance at local scale) was summarized by 3 principal components (‘*F1*’, ‘*F2*’ and ‘*F3*’), as a result of 9 metrics of internal degradation that were extracted from 15 m radius-buffer surrounding each adult plant of the populations (Tables S4 and S5). Large-scale disturbance (i.e., landscape features) was defined by the percentage of different land-use cover (land-use diversity, ‘*Divusos*’ hereafter) and by natural habitat cover (‘*Cobnat*’ hereafter) extracted from 1.5 km radius-buffer surrounding each population (Table S4).

Thus, to conduct dbRDA analyses, we used a pairwise-F_{ST} distance matrix as genetic data, the geographic coordinates from each population as geographic data, and the five habitat degradation variables (‘*F1*’, ‘*F2*’, ‘*F3*’, ‘*Divusos*’ and ‘*Cobnat*’) that depict habitat disturbance at local and large scale (see Kierepka and Latch 2015, see also Wang and Bradburd 2014, van Strien et al. 2015, Noguerales et al. 2016 for methodological details). DbRDA and partial-dbRDA analyses were performed with the R package VEGAN (Oksanen et al. 2018) from *capscale* function. Statistical significance and variance components were obtained from *anova.cca* and *RsquareAdj* functions within the same package.

All R software analyses were performed with R version 3.4.3 (R Core Team 2017).

RESULTS

Characterization of genetic diversity and genetic divergence between Z. lotus populations

Genetic diversity estimates from each population are given in Table 1. Observed and expected heterozygosity ranged from 0.44 (HOSPITAL) to 0.61 (CAMP, CV and PL) and from 0.46 (RCAMP) to 0.62 (TY), respectively. Four populations (CAMP, PL, RCAMP, and SALH) showed higher observed heterozygosity values than expected (Fig. S1). The total number of alleles (Na) ranged from 46 (RCAMP) to 71 (CV and TG). CV population harbored the highest allelic richness (A_R = 3.99), while RCAMP had the lowest one (A_R = 2.99).

Population inbreeding coefficients (F_{IS}) and selfing rates (*s*) were highest in HOSPITAL and lowest in RCAMP. Four populations (CAMP, PL, RCAMP and SALH) had negative values for both parameters (Table 1). The most relevant genetic indices per population are displayed in Fig.S2. No population deviated from *HWE* expectations (Table S1). Moreover, no evidences of linkage disequilibrium or null alleles were found for any loci.

F_{ST} mean values were low, ranging from 0.031 (RR) to 0.074 (HOSPITAL), suggesting overall low differentiation among populations (Table 1). However, neighbor-joining tree, based on F_{ST} genetic distances matrix (Table S3), showed three manifest genetic clusters, accordingly with three geographic regions: West, Core and East (Fig. 1). Partitioning of genetic variability by AMOVA revealed that 87.25% of the total genetic variation occurred within populations, 5.21% occurred among populations within region and 7.54% between regions. In all cases, genetic differentiation was significant ($P < 0.05$) (Table 2).

Table 1: Geographical location and main genetic diversity parameters obtained for 21 sampled *Ziziphus lotus* populations.

Population	Locality[a]	Region[b]	Acronym	N	Coordinates UTM (X)	Coordinates UTM (Y)	H_o	H_e	N_a	A_R	F_{ST}	F_{IS}	s^c
Béjar	Murcia	East	BEJ	20	608753	4162689	0.52	0.54	63	3.72	0.053	0.063	0.118
Campohermoso	Almería	Core	CAMP	14	573207	4089516	0.61	0.56	54	3.48	0.071	-0.024	-0.048
Ctro. de Visitantes	Almería	Core	CV	25	566902	4074470	0.61	0.61	71	3.99	0.035	0.009	0.018
Cuevas de Almazora	Almería	East	CUEV	20	606554	4129369	0.52	0.55	54	3.34	0.048	0.066	0.125
Dalías	Almería	West	DALIAS	8	516503	4068393	0.46	0.48	47	3.36	0.058	0.076	0.141
El Ejido	Almería	West	EJIDO	8	517838	4068287	0.52	0.55	47	3.36	0.071	0.117	0.210
Fernán Pérez	Almería	Core	FP	20	584412	4086871	0.53	0.57	66	3.85	0.041	0.074	0.138
Guazamara	Almería	East	GUAZ	25	608941	4134043	0.49	0.53	59	3.50	0.045	0.078	0.144
Hospital de Poniente	Almería	West	HOSPITAL	9	517810	4067793	0.44	0.48	47	3.27	0.074	0.187	0.315
Níjar	Almería	Core	N	25	570227	4088796	0.56	0.61	62	3.67	0.043	0.086	0.159
Playazo	Almería	Core	PL	25	587885	4079328	0.61	0.57	57	3.34	0.048	-0.024	-0.050
Puerto Lumbreras	Murcia	East	LUM	20	605781	4154249	0.55	0.58	63	3.75	0.044	0.060	0.113
Purias	Murcia	East	PUR	14	618401	4161679	0.48	0.53	61	3.83	0.055	0.095	0.174
Rambla Campohermoso	Almería	Core	RCAMP	11	579257	4087669	0.55	0.46	46	2.99	0.072	-0.093	-0.205
Rambla de Retamar	Almería	Core	RR	25	562378	4077811	0.58	0.60	70	3.95	0.031	0.045	0.086
Retamar	Almería	Core	AUT	20	564303	4079496	0.54	0.57	64	3.70	0.037	0.062	0.117
Sierra Alhamilla	Almería	Core	SALH	20	552531	4089557	0.59	0.57	58	3.54	0.043	-0.006	-0.011
Sierra de la Tercia	Murcia	East	TER	20	624435	4175260	0.50	0.53	64	3.66	0.050	0.077	0.143
Sta. María del Águila	Almería	West	SMA	23	520643	4071120	0.48	0.51	60	3.39	0.063	0.087	0.160
Torregarcía	Almería	Core	TG	25	563288	4075416	0.54	0.58	71	3.85	0.036	0.071	0.133
Toyo	Almería	Core	TY	20	558505	4078249	0.57	0.62	69	3.99	0.047	0.099	0.181
Mean across populations							0.54	0.55	59.7	3.59	0.051	0.058	0.103

Abbreviations: Number of individual used per population (N), observed heterozygosity (H_o), expected heterozygosity (H_e), total number of alleles (N_a), rarefied allelic richness (A_R), fixation index (F_{ST}), inbreeding coefficient (F_{IS}), and selfing rate (*s*). [a] All populations are located in SE Spain. Names correspond to provinces. [b] According to Fig.1. [c] Selfing rate [$s = 2F_{IS}/(1+F_{IS})$] (Ritland, 1990).

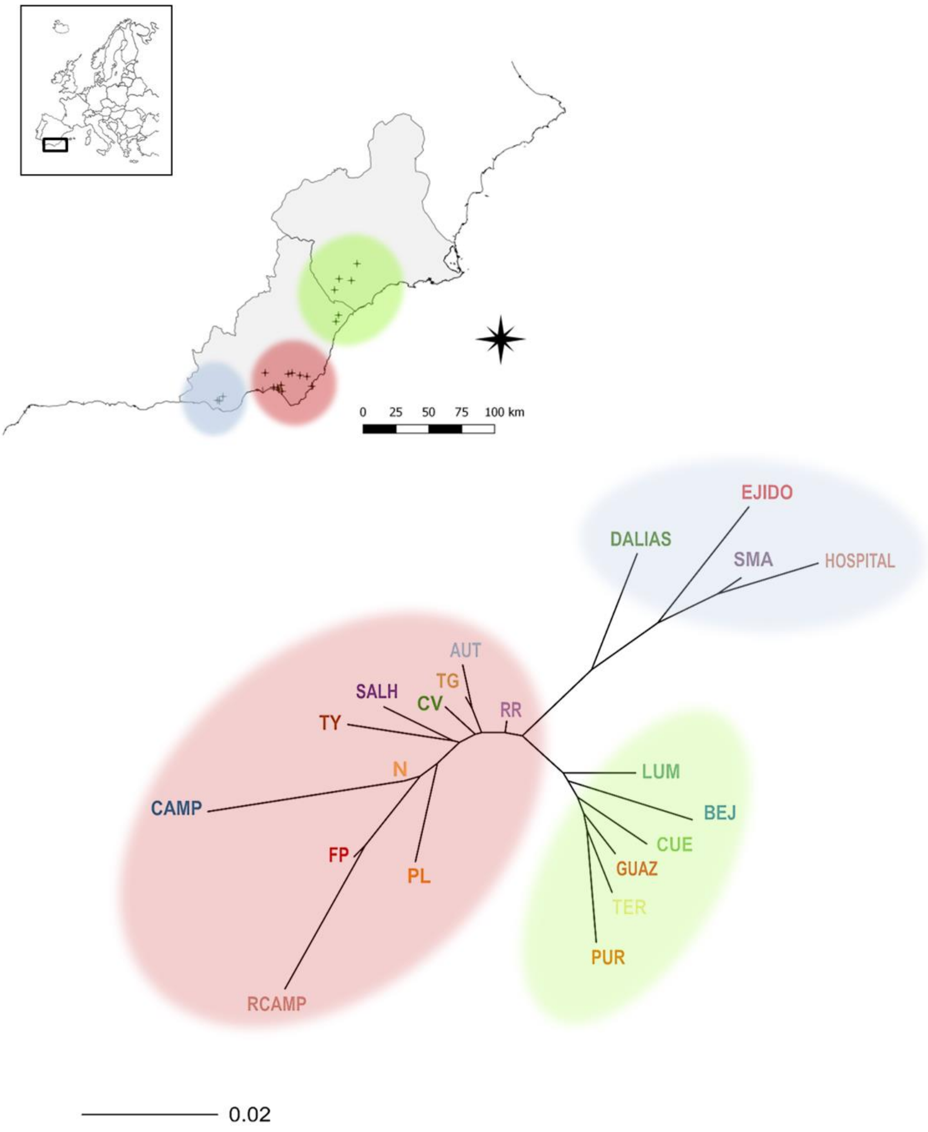


Fig. 1: Location of 21 *Ziziphus lotus* studied populations across the whole range of the species in the southeast of the Iberian Peninsula (above). Neighbor-joining tree based on a F_{ST} genetic distance matrix showing the clustering of 21 *Ziziphus lotus* populations (below). Branch length is proportional to F_{ST} genetic distance between populations. The location of each cluster (East in green, Core in red and West in blue) is shown in the upper map.

Table 2: Results of AMOVA explaining the amount of genetic variation observed in 21 *Z. lotus* populations at three hierarchical levels, based on 14 SSR markers. The percentage of variation explained by each level has been highlighted in bold.

	df	Sum Sq	Variance components (Sigma)	Percentage of variation	F-stadistics	P-value
Between regions	2	158.52	0.582	**7.54**	0.075	0.01
Between populations within region	18	259.63	0.402	**5.21**	0.056	0.01
Within populations	381	2566.49	6.736	**87.25**	0.127	0.01
Total	401	2984.64	7.72	100		

The Core region showed the highest mean values (±1SE) for H_o (0.57±0.01), H_e (0.57±0.01) and N_a (62.55±2.44). Mean A_R ranged from 3.34±0.03 (West) to 3.67±0.09 (Core) (Table 3). The East region showed mean values similar to those of Core for A_R (3.63±0.07). Mean F_{IS} and s were higher in the West region (0.117±0.025 and 0.206±0.039, respectively) than in the other two regions. Significant differences (P <0.05) were found between Core and West for H_e, N_a, F_{IS} and s, but not between Core and East or between East and West. Core also exhibited significant differences with West and East regions for H_o. A_R showed non-significant differences among the three regions (a summary of average values per geographical regions and ANOVA-Tukey test results are given in Table 3). The most remarkable average values per region are represented in Fig 2.

Table 3: Averages of each genetic diversity parameter per region defined by the neighbor-joining tree based on the pairwise F_{ST} genetics distance matrix of the 21 *Z. lotus* populations. Differences between populations are represented by different superscripts (ANOVAs + Tukey's range tests).

Region	H_o	H_e	N_a	A_R	F_{IS}	s
Core	0.57^{a}	0.57^{c}	62.55^{e}	3.67^{g}	0.027^{h}	0.047^{j}
East	0.51^{b}	0.54^{cd}	60.67^{ef}	3.63^{g}	0.073^{hi}	0.136^{jk}
West	0.48^{b}	0.51^{d}	50.25^{f}	3.34^{g}	0.117^{i}	0.206^{k}

Abbreviations: Observed heterozygosity (H_o), expected heterozygosity (H_e), total number of alleles (N_a), rarefied allelic richness (A_R), inbreeding coefficient (F_{IS}), and selfing rate (s).

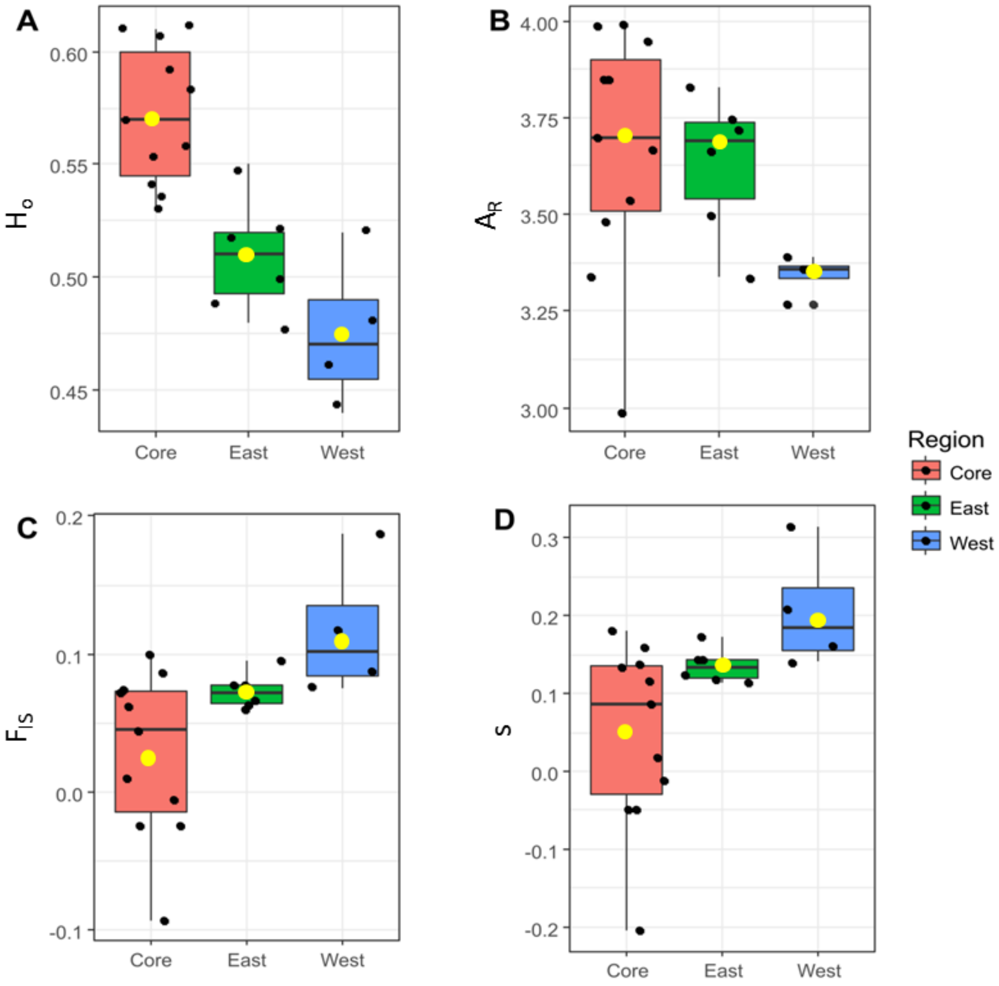


Fig. 2: Comparison of the most remarkable genetic parameters per region: (A) Observed heterozygosis (H_o); (B) allelic richness (A_R); (C) inbreeding coefficients (F_{IS}); (D) selfing rates (*s*). Mean values are represented with a yellow spot and medians with a black horizontal line. Each black spot defines a population.

Genetic population structure

DAPC showed an expected overlap between *K* = 4 and *K* = 5 clusters (see Fig S3) due to the high genetic variability found within populations (more than 87%, see AMOVA results, Table 2). However, the BIC value differences between *K* = 3 and the *K*-value with the lowest BIC was minimal (e.g., BIC values was 606 in *K* = 3, 602 in *K* = 4 and 598 in *K* = 5; see Fig. S4). Thus, we chose a biologically informative and unambiguous cluster number (*K* = 3) for the *K*-means, retaining 30 principal components, and only two available discriminant functions (eigenvalues). We obtained three differentiated, non-overlapping genetic groups (Fig.3).

The Bayesian clustering, that assigned individuals of each population on the basis on their genetic similarity, showed again that K = 3 was the most likely number of genetic groups for the 21 selected populations (Fig. S5). The individual assignment for K = 3 (Fig. 4) indicated that: the first genetic group (in blue, q = 0.896) is mainly represented by DALIAS, EJIDO, HOSPITAL and SMA populations (West region); the second group (in red, q = 0.748) is mostly integrated by AUT, CAMP, CV, FP, N, PL, RCAMP, RR, SALH, TG and TY (Core region); and the third group (in green, q = 0.873) is mainly composed by BEJ, LUM, CUEV, GUAZ, PUR and TER (East region). The Core region shows a greater genetic mixture, exhibiting coloration of the three genetic linages. In contrast, West and East regions show a clear dominance of a single lineage. These mixing patterns reveal the greater degree of isolation and genetic impoverishment of the outer regions (East and West), which are also the most degraded ones. STRUCTURE Bayesian cluster analyses are summarized in Table S6, where q-values of each cluster are shown for the 21 populations.

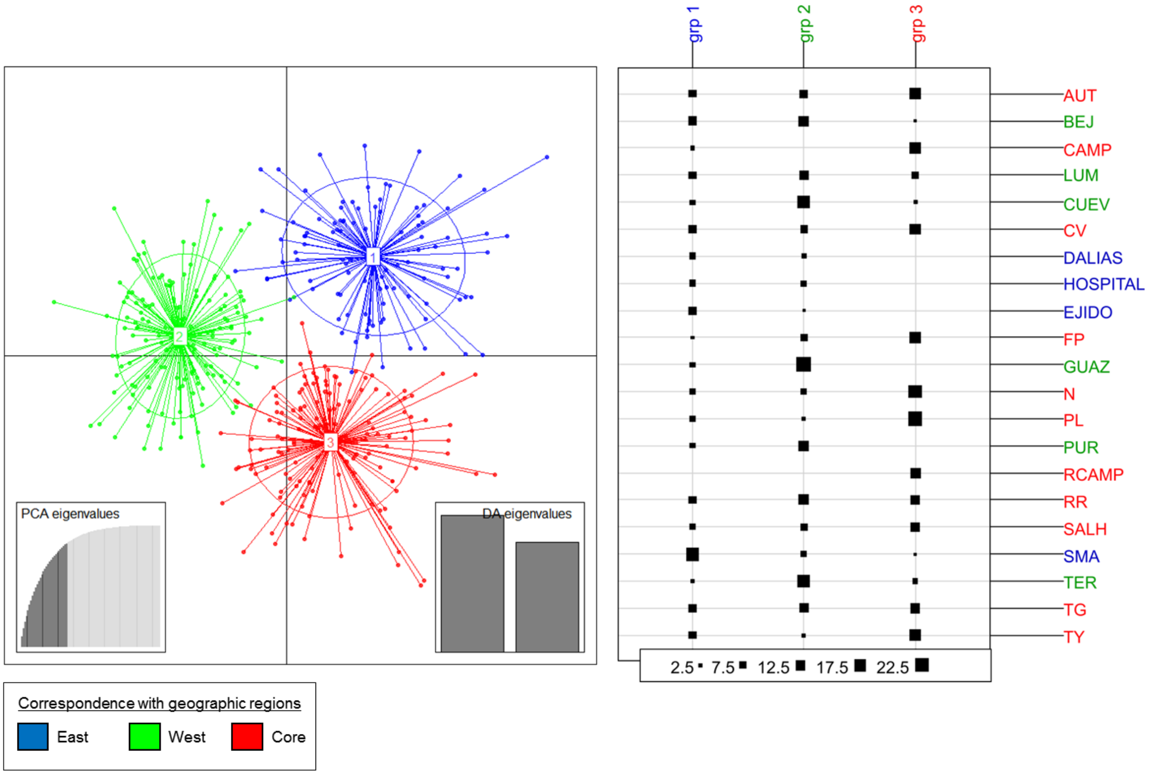


Fig. 3: DAPC (left) and K-means plots (right) showing the genetic structure of 21 *Z. lotus* populations according to 14 polymorphic microsatellites markers. Each population and group in the K-means plot was colored in accordance with its more likely membership group in DAPC plot. The colors of these classification groups are the ones used for the genetic regions showed in Fig. 1.

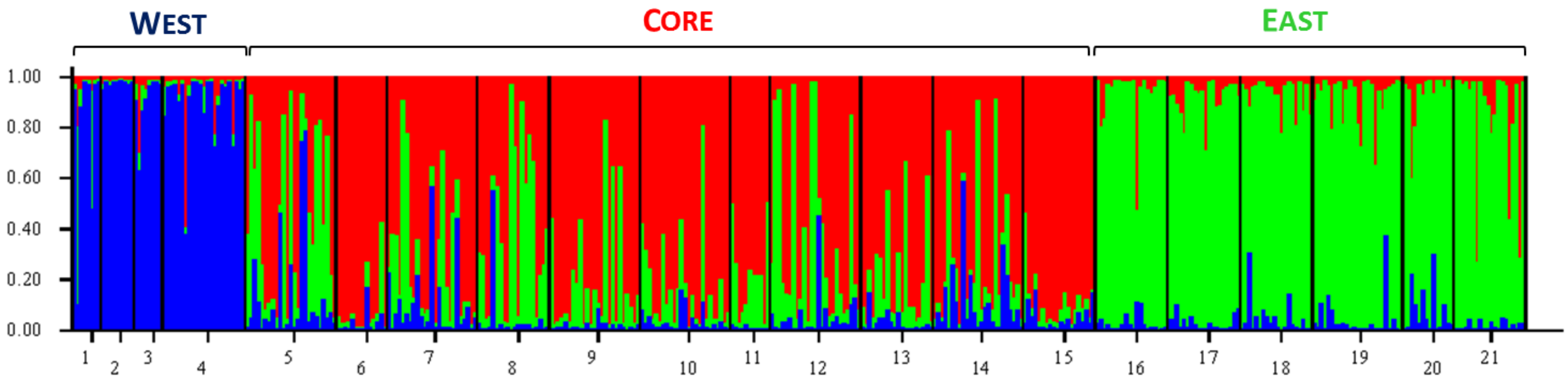


Fig. 4: Bar plot summarizing the individual assignment of 21 *Z. lotus* populations in the semiarid southeast of Iberian Peninsula by Structure Software. Assignation probabilities are presented in axis-y, and population in axis-x: (1) Dalías, (2) Hospital de Poniente, (3) El Ejido, (4) Sta. María del Águila, (5) Retamar, (6) Campohermoso, (7) Ctro. de Visitantes, (8) Fernán Pérez, (9) Níjar, (10) Playazo, (11) Rambla Campohermoso, (12) Rambla Retamar, (13) Sierra Alhamilla, (14) Torregarcía, (15) Toyo, (16) Béjar, (17) Puerto Lumbreras, (18) Cuevas de Almanzora, (19) Guazamara, (20) Purias, (21) Sierra de la Tercia. Visualization obtained by Structure Harvester Online (Earl and vonHoldt 2012).

Isolation by distance (IBD) and environmental disturbance

The dbRDA was significant ($F_{7,13}$ = 3.705, P = 0.001) and explained 66.61% of the F_{ST} variation of populations. The first three axes were statistically significant. The axis-1 and axis-2 accumulated 54.05% and 80.75% of the total variation ($F_{1,13}$ = 13.55, P = 0.001; and $F_{1,13}$ = 6.70, P = 0.001, respectively). Adding the axis-3 we obtained 95.70% of the fitted variation and 63.03% of the total variation ($F_{1,13}$ = 3.75, P = 0.005). The only significant variables, and therefore the most relevant ones for the model, were '*Latitude*' ($F_{1,13}$ = 6.562, P = 0.001), '*Longitude*' ($F_{1,13}$ = 10.134, P = 0.001) and '*Cobnat*' (*Natural habitat cover*; ($F_{1,13}$ = 5.530, P = 0.005) (Table 4). This strongly suggests that geographic location and the percentage of natural habitat cover were the parameters explaining most the genetic differentiation between populations (37.88% and 5.82%, respectively (Table 4; Fig. 5).

Table 4: Results of distance-based redundancy analysis (dbRDA and partial-dbRDA) testing the effects of geographic location and habitat and landscape disturbance on the genetic differentiation (F_{ST} population matrix) of 21 *Z. lotus* populations in the southeast of the Iberian Peninsula.

Variable	*df*	*F*	*P*	% Variation
Latitude	1	6.562	**0.001**	17.06
Longitude	1	10.134	**0.001**	20.82
Natural habitat cover	1	5.530	**0.005**	5.82
Land-use diversity	1	1.473	0.195	3.62
F1	1	0.062	0.998	0.69
F2	1	1.061	0.369	2.76
F3	1	1.113	0.362	2.86
Residuals	13			

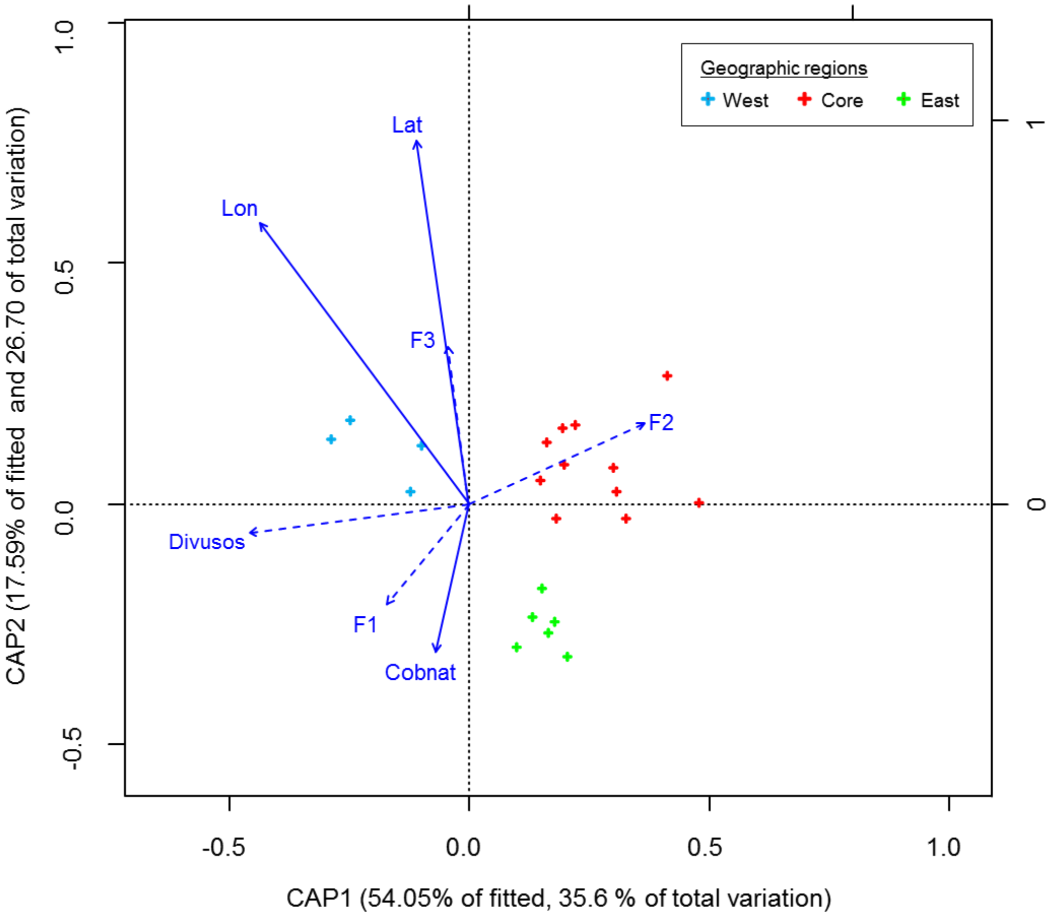


Fig. 5: Db-RDA plot representing the relative position of 21 *Z. lotus* populations (points) as explained by its geographic location and a set of habitat variables related with the degree of habitat and landscape disturbance. Points were colored according the geographic location of each population: blue (West), red (Core) and green (East). Significant variables in the model are indicated by solid vectors, and no-significant variables are indicated by dashed vector.

DISCUSSION

Understanding the processes underlying the origin and maintenance of genetic variation in natural plant populations is essential in population ecology, evolution and conservation (Lee and Mitchell-Olds 2011). In particular, how habitat and landscape alterations shape the genetic variation and structure of plant communities in natural habitats is one of the goals of the emerging landscape genetics, which integrates approaches from population genetics and landscape ecology (Balkenhol et al. 2016). Thus, while we have previously documented the effects of anthropogenic impact on key reproductive stages and demography on *Z. lotus* populations (Cancio et al. 2016, 2017, Rey et al. 2017, 2018, see also *Capítulo 1*), here we present the first population genetic study conducted on the keystone species *Z. lotus* in the Iberian Peninsula. We have found that despite overall low genetic differentiation among populations, there is three clear genetic groups across the species's range that neatly correspond with three relatively isolated geographic areas (West, Core and East) (Fig. 1). Within these regions, populations still harbor a significant amount of genetic diversity despite the negative impact of human disturbances on reproduction and natural regeneration of *Z. lotus* in the region (Rey et al. 2017, 2018). As predicted, genetic diversity was higher in larger and well-preserved areas (e.g., Core region) whereas inbreeding rates and selfing were much higher in altered populations (e.g., West region, Fig 2). In relative terms, variation within populations accounted for most of the genetic variance. Geographical factors explained largely genetic divergence between populations. However, large-scale environmental disturbances (i.e., at landscape scale) also influenced significantly genetic differentiation and the diversity within the population. Our results suggest that although the long-lived and resprouting strategy of the species is probably delaying the erosion of its genetic diversity at population and regional level, there is already some genetic consequences of habitat degradation and, especially, of habitat loss at landscape level which could be aggravated in the future.

Genetic diversity and structure of Iberian *Z. lotus* populations

Ziziphus lotus populations showed overall high values of genetic diversity regardless their size and alteration degree. Population heterozygosity values were very close to expected values and allelic richness was overall very similar. Our results concur with recent findings from *Z. jujuba* (Wang et al. 2014), for which high values of genetic diversity were also detected in seven populations from China. Contrastingly, our results differ from observations made in fragmented *Z. celata* populations in Florida, which seem to be genetically impoverished (Weekley et al. 2002). In any case, high genetic diversity is also common in other endangered long-lived trees and shrubs species (as *Cedrus libani* [Fady et al. 2008], *Juniperus excelsa* [Douaihy et al. 2011], *Cedrus brevifolia* [Eliades et al. 2011] and *Juniperus thurifera* [Teixeira et al. 2014] in the Mediterranean region; or *Quercus petraea* [Muir et al. 2004] and *Rhododendron protistum var. giganteum* [Wu et al. 2014] in other regions). In all these cases higher genetic variability than expected was found despite exhibiting small or reduced population size which is a consequence derived from their life history. Woody species are known to conserve their ancestry genetic history as consequence of their long-life (Vranckx et al. 2011). At the same time, other species features of *Z. lotus* as outcrossing breeding systems, animal-ingested seed dispersal and large distribution range commonly favor preservation of high genetic diversity (Hamrick et al. 1992, Hamrick and Godt 1996, Vranckx et al. 2011).

Theory predicts that small and isolated populations are prone to suffer genetic divergence and genetic erosion by genetic drift and inbreeding, among others, which in the long term may jeopardize the continuity of these populations (Young et al. 1996, Lowe et al. 2005). Our results partially support this expectation. Despite the high values of genetic diversity, populations in the most altered areas exhibited higher inbreeding and selfing rates and lower heterozygosis values than populations in the well-preserved areas (with larger population sizes). In our study, altered populations show significantly higher floral visitation rates by managed honey bees (*Apis mellifera*) than well-preserved populations (see *Capítulo 1*). Managed honey bees are known by their high floral visitation rates (within plant or between nearby individuals) and their competitive effect against other pollinators (Magrach et al. 2017). Honey bee foraging behavior often limits cross-pollination (González-Varo et al. 2010, Magrach et al. 2017), and consequently differences in occurrence and abundance of honey bees and in distance between *Z. lotus* individuals (more distant in altered populations) may be related with the variation in inbreeding and selfing rates between our different areas.

Despite the differences detected between areas, inbreeding and selfing rates are not particularly high in Iberian *Ziziphus* populations (ranges: 0.027 - 0.117 and 0.047 - 0.206, respectively, Table 3), and are close to the ones reported for other woody plants species of altered landscapes (Wrang et al. 2011, Nora et al. 2015; but see González-Varo et al. 2010). This likely indicates that endogamy is not yet an issue in our study populations. High levels of intra-population genetic variations and low selfing may result from effective gene flow and connectivity (Hamrick et al. 1992, Aguilar et al. 2008) that could be favored in our populations due to efficient cross-pollination (Vranckx et al. 2012) and long-distance seed dispersal. These mechanisms have probably been more successful in former times since collapse of mammalian seed dispersal (long-distance dispersal drivers for the species) has been reported in *Ziziphus* habitat relict landscapes (Cancio et al. 2016). Beyond population regeneration effects (Rey et al. 2018), the results of this study warn about the delayed genetic consequences of the collapse of seed dispersal by mammalian carnivores.

Genetic effects at inter-population scale due to habitat disturbance are commonly delayed in long-lived plants (Wang et al. 2009, Browne et al. 2015). When seed dispersal processes are affected, a faster interpopulation structuration than expected could be then detected (Wang et al. 2011). This would explain why low inter-population genetic differentiation (5% of variations occurred among populations within region and 7% between regions) were enough to establish three distinct genetic areas: the *West region* (the most degraded area by greenhouse agricultural intensification and with the smallest populations), the *Central* (the largest, most populated and less degraded nucleus that comprises Cabo de Gata-Níjar Natural Park; Core region), and the *East region* (furthest from the others, disturbed by agricultural intensification and with smaller and isolated populations, most of them with sparse individuals) (see Fig 1). These observations were supported by F_{ST} neighbor-joining tree, and dbRDA and STRUCTURE analyses. They reflect that the Central region is somewhat connected to the other two regions (Fig. 4) and, although singular in its predominant genetic group, shows an admixture of all genetic groups, while the East and West are more defined genetically and show a much lower genetic mix.

The connectivity and isolation degree among regions along with the variation in the alteration degree of this area could explain the detected divergence patterns. Nowadays, although the Core and West regions are close to each other, both are separated by a large-urbanized matrix and an immense greenhouse area. The greater genetic dissimilarity between the Core and the West region (less diverse) may result from the fact that their separation occurred before than the one between the Core and the East. Moreover, some isolated individuals immersed in a degraded semiarid plain matrix (authors pers. observ) remain between the latter regions. So, the most plausible explanation for such relationships is that historically there was a large and relatively continuous *Z. lotus* population spanning throughout the southeastern Iberian coastal plains that was progressively disturbed and fragmented giving rise to subdivided and isolated populations at both sides of the Core region (Tirado 2009, Mendoza-Fernández et al. 2015).

Support for this explanation comes from land photo flights in the 1950s that shown habitat continuity in the area (Caparrós-Lorenzo 2008).

Looking at the intra-population level, a slight intra-population genetic structure or genetic cline is apparent in some populations. That would explain the overlap between clusters for a same population according to DAPC results of K=4 or K=5 (Fig. S3 and S4). Slight intra-population variations of genetic structure are one of the first genetic responses detected in long-lived plants after landscape alteration (Wang et al. 2009, Browne et al. 2015).

Historic and environmental determinants of genetic differentiation between populations

Woody plants usually maintain more genetic variation within populations and less among populations compared to species with other life form (Hamrick et al. 1992). Our results support this idea: approximately 87% of genetic variation was grouped within *Z. lotus* populations, and 13% among them. We also detected that population F_{ST} were very low in all populations (range: 0.031 - 0.072), corroborating the low overall population genetic differentiation. A similar result has been reported in Wang et al. (2014) for the close relative *Z. jujuba* where 98.2% of genetic variation was accounted within-populations. Low values of F_{ST} have been found in other woody plant species in altered landscapes: *Pistacia lentiscus* (0.006-0.018), *Myrtus communis* (0.033-0.050) (Nora et al. 2015) or *Castanopsis sclerophylla* (0.109-0.124) (Wrang et al. 2011,). Again, these genetic patterns are likely linked to the long life span (several years or even centuries; see Aparicio et al. 2012) typical of woody plants and their resistance to ecological alterations due to specific features such as large geographic range, long distance seed/pollen dispersal, or outcrossing breeding system (Hamrick et al. 1992).

Strong habitat or climatic alterations are needed to provoke changes in the genetic characteristics of long-lived plants (Aguilar et al. 2008, Browne al 2015). The loss and degradation degree of *Z. lotus* habitat is really severe since the 1950’s, given the urbanization and, especially, the intensive agriculture boom and the poor management of waste disposal. However, the special characteristics of the study species and its life-history could delay the expected effects of continuous human alterations on their genetic diversity and population differentiation. *Ziziphus lotus* is a relict of the Messinian – more than 5 million years -, when the flora of North Africa and South Europe were connected (Blondel et al. 2010) (Cabo de Gata area, where the best preserved populations of the species currently occur, has been recently considered one possible point of connection between both continents, Booth-Rea et al. 2018).

This species has a very deep, pivoting root system capable of capturing groundwater and allowing its survival under the typical water stress conditions of semiarid systems. This, together with its slow growth rate and its elevated capacity for resprouting, are the factors responsible for its high longevity. Nonetheless, previous studies have recently reported the alarming low juvenile density and poor regeneration in the Iberian *Z. lotus* populations (due to disruption of plant-mammal seed dispersal among others; Rey et al. 2017, 2018). The expected positive effects of long-distance seed dispersal on population regeneration may thus have disappeared because of loss of habitat quality (Rey et al. 2018). So, adult individuals (some with 3 m height and 30 m diameter, [Rey et al. 2018]) are very likely very ancient ones. The detected genetic patterns are probably the result of historical gene flow rather than current one. Thus, the particular phytogeographical history of this species and its aforementioned life-history traits would explain the current high genetic diversity and low population differentiation detected in the Iberian *Z. lotus* populations.

Geographic range is also considered among the most important predictors of population genetic variation in woody species (Hamrick et al. 1992). However, very few studies have analyzed at which scale (local or landscape) human disturbances impact on these genetic patterns. Our dbRDA results show that both geographic and habitat-quality features determine the genetic differentiation between populations. Differences in geography (latitude and longitude variations) explained genetic differences among populations to a larger extent than habitat-quality features (37.88% vs 5.82%, respectively), highlighting the importance of isolation by distance in the genetic differentiation of this system. This would explain the strong correspondence between the three defined genetic clusters and their geographic location (west, center and east of the study area; Fig. 1).

We found that large-scale human disturbance was more important than local-scale one for the population genetic differentiation. Natural habitat cover (which characterizes the degree of landscape disturbance by land-use transformation) was the only variable associated with the genetic differences between populations. Severe habitat loss is related with pollen and gene flow limitation, especially in wild-pollinated woody plants (Vranckx et al. 2011) and, in the case of the Iberian *Z. lotus* populations, is related with the collapse of seed dispersal (Cancio et al. 2016, Rey et al. 2017). This would explain the relevance of natural habitat cover to explain part of the genetic variation of Iberian *Z. lotus* populations.

Conclusion

The long-life span of *Z. lotus* is probably delaying the erosion of its genetic diversity and the modification of its historic geographic structuring, and buffering the genetic effect of habitat loss, fragmentation and degradation. While *Z. lotus* populations still harbor a significant amount of genetic diversity and retain its historic geographic structuring our data show that anthropogenic disturbances, that will predictably go on in the near future, are already impacting on the genetic diversity of this species, especially in the fringes of the range, where two out of the three genetic groups are mainly confined. Because natural habitat coverage is closely linked to the size and isolation of *Z. lotus* populations, urgent appropriate conservation measures in those non-protected areas should be implemented to preserve the genetic diversity of the species. This would be fundamental also to maintain the integrity of its habitat, since *Z. lotus* is a keystone and ecological engineer species (Tirado 2009). If natural habitat cover continues to decrease, habitat loss will eventually severely affect the regional and population divergence of *Z. lotus*, and will decrease intra- and inter-population genetic diversity. In the long term, local extinctions of the smallest populations will likely occur as announced by their reported collapse of regeneration (Rey et la. 2018), and the remaining will be more exposed to genetic bottleneck processes.

ACKNOWLEDGEMENTS

Thanks J.M. Bastida for his help throughout the study; F. Valera, J. Isla, J.L. González-Rebollar and A.B. Robles for their advice and assistance during field samplings; C. García for the opportunity of collaboration and her help during analyses and result interpretation; A.M. Fernández-Ocaña for her useful guidance during the laboratory work; CICT of Universidad de Jaén for fragment sequencing work; Junta de Andalucía and M.A. Carrión (Dirección General de Medio Ambiente del Gobierno de Murcia) for facilitating permits for Almería and Murcia field samplings, respectively. This work was funded by a Junta de Andalucía Excelencia RNM-766 project and by funds from Fondo Europeo de Desarrollo Regional (FEDER). A. González-Robles and A.J. Manzaneda were supported by Universidad de Jaén fellowship (Plan Propio - Acción 16, 2013-2014) and a Ramón y Cajal contract (RYC-2010-06237), respectively. C. García was funded by Fundação para a Ciência e a Tecnologia (FCT) through the Independent Fellowship IF-*Investigador* program (IF/01375/2012).

REFERENCES

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches. *Mol Ecol*, 17, 5177–5188.

Anon (1992) Directive 92/43 of the council of the European community on the conservation of habitats and wild fauna and flora. European Community, Brussels.

Aparicio A, Hampe A, Fernández-Carrillo L, Albaladejo RG (2012) Fragmentation and comparative genetic structure of four mediterranean woody species: Complex interactions between life history traits and the landscape context. *Divers Distrib*, 18, 226–235.

Balkenhol N, Cushman S, Storfer A, Waits L (2016) *Landscape genetics: concepts, methods, applications*. John Wiley & Sons, New Jersey, USA, 288 pp.

Blondel J, Aronson J, Bodiou JY, Boeuf G (2010) *The Mediterranean Region: Biological Diversity in Space and Time* (2nd edition). Oxford, New York, 384pp.

Booth-Rea G, Ranero CR, Grevemeyer I (2018) The Alboran volcanic-arc modulated the Messinian faunal exchange and salinity crisis*. Sci Rep*, 8, 13015. doi: https://doi.org/10.1038/s41598-018-31307-7

Browne L, Karubian J (2018) Habitat loss and fragmentation reduce effective gene flow by disrupting seed dispersal in a neotropical palm. *Mol Ecol*, 27, 3055–3069.

Browne L, Ottewell K, Karubian J (2015) Short-term genetic consequences of habitat loss and fragmentation for the neotropical palm *Oenocarpus bataua*. *Heredity*, 115, 389–395.

Cancio I, González-Robles A, Bastida JM, Isla J, Manzaneda AJ, Salido T, Rey PJ (2017) Landscape degradation affects red fox (*Vulpes vulpes*) diet and its ecosystem services in the threatened *Ziziphus lotus* scrubland habitats of semiarid Spain. *J Arid Environ*, 145, 24–34.

Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Salido T, Rey PJ (2016) Habitat loss exacerbates regional extinction risk of the keystone semiarid shrub *Ziziphus lotus* through collapsing the seed dispersal service by foxes (*Vulpes vulpes*). *Biodivers Conserv*, 25, 693–709.

Caparrós-Lorenzo, R (2008*) 50 años de Transformaciones Territoriales en Almería: Aniversario del Vuelo Americano (1957-2006)*. Instituto de Estudios Almerienses, Almería, 84 pp.

Douaihy B, Vendramin GG, Boratyński A, Machon N, Dagher-Kharrat MB (2011) High genetic diversity with moderate differentiation in *Juniperus excelsa* from Lebanon and the eastern Mediterranean region. *AoB Plants*, plr003. doi: https://doi.org/10.1093/aobpla/plr003

Doyle JJ (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

Earl DA and vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. C*onserv Genet Resour,* 4, 359-361.

Eliades NGH, Gailing O, Leinemann L, Fady B, Finkeldey R (2011) High genetic diversity and significant population structure in *Cedrus brevifolia* Henry, a narrow endemic Mediterranean tree from Cyprus. *Plant Syst Evol*, 294, 185–198.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*, 14, 2611–2620.

Fady B, Lefèvre F, Vendramin GG, Ambert A, Régnier C, Bariteau M (2008) Genetic consequences of past climate and human impact on eastern Mediterranean *Cedrus libani* forests. Implications for their conservation. *Conserv Genet*, 9, 85–95.

Fahrig L (2003) Effects of Habitat Fragmentation on Biodiversity. *Annu Rev Ecol Evol Syst*, 34, 487–515.

Ghaffari S, Hasnaoui N (2013) Microsatellite amplification in plants: Optimization procedure of major PCR components. In: SK Kantartzi (ed.), *Methods in molecular biology, vol. 1006: Microsatellites: Methods and protocols*, 139–146. Springer, New York, New York, USA.

González-Robles A, Manzaneda AJ, Bastida JM, Harvey N, Jaime R, Salido T, Martínez LM, Fernández-Ocaña A, Alcántara JM, and Rey PJ (2016) Development and characterization of microsatellite primers in the endangered Mediterranean shrub *Ziziphus lotus* (Rhamnaceae). *Appl Plant Sci*, 4(12), 1600092. doi: https://dx.doi.org/10.3732%2Fapps.1600092

González-Varo JP, Albaladejo RG, Aparicio A, Arroyo J (2010) Linking genetic diversity, mating patterns and progeny performance in fragmented populations of a Mediterranean shrub. *J. Appl. Ecol.*, 47, 1242–1252.

Goudet J, Jombart T (2015) HIERFSTAT: Estimation and Tests of Hierarchical F-Statistics. R package version 0.04-22. Available at: https://CRAN.R-project.org/package=hierfstat

Hahn MW (2018) *Molecular Population Genetics*. Sinauer Associates, New York.

Hamrick JL, Godt MJW (1996) Effects of Life History Traits on Genetic Diversity in Plant Species. *Philos Trans R Soc Lond B Biol Sci*, 351, 1291–1298.

Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New For*, 6, 95–124.

Jombart T (2008) ADEGENET: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet*, 11, 94. doi: https://doi.org/10.1186/1471-2156-11-94

Jordano P (2010) Pollen, seeds and genes: The movement ecology of plants. *Heredity*, 105, 329–330.

Jordano P, Forget P, Lambert JE, Böhning-Gaese K, Traveset A, Wright SJ (2011) Frugivores and seed dispersal: mechanisms and consequences for biodiversity of a key ecological interaction. *Biol Lett*, 7, 321–323.

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281. doi: https://doi.org/10.7717/peerj.281

Kierepka EM, Latch EK (2015) Performance of partial statistics in individual-based landscape genetics. *Mol Ecol Resour*, 15, 512–525.

Lee CR, Mitchell-Olds T (2011) Quantifying effects of environmental and geographical factors on patterns of genetic differentiation. *Mol Ecol*, 20, 4631–4642.

Leimu R, Mutikainen P, Koricheva J, Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? *J Ecol*, 94, 942–952.

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005) Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity*, 95, 255–273.

Magrach A, González-Varo JP, Boiffier M, Vilà M, Bartomeus I (2017) Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. *Nat Ecol Evol*, 1, 1299–1307.

McIntire S, Hobbs R (1999) A framework for conceptualizing human effects on landscapes and its relevance to management and research models. *Conserv Biol*, 13, 1282–1292

Mendoza-Fernández AJ, Martínez-Hernández F, Pérez-García FJ, Garrido-Becerra JA, Benito BM, Salmerón-Sánchez E, Guirado J, Merlo ME, Mota JF (2015) Extreme habitat loss in a Mediterranean habitat: *Maytenus senegalensis* subsp. *europaea*. *Plant Biosyst,* 149, 503–511.

Mota JF, Peñas J, Castro H, Cabello J, Guirado J (1996) Agricultural development versus biodiversity conservation: the Mediterranean semiarid vegetation in El Ejido (Almería, southeastern Spain). *Biodivers Conserv*, 5, 1597–1617.

Muir G, Lowe AJ, Fleming CC, Vogl C (2004) High nuclear genetic diversity, high levels of outcrossing and low differentiation among remnant populations of *Quercus petraea* at the margin of its range in Ireland. *Ann Bot*, 93, 691–697.

Nadia TDL, Machado IC, Lopes AV (2007) Fenologia reprodutiva e sistema de polinização de *Ziziphus joazeiro* Mart. (Rhamnaceae): atuação de *Apis mellifera* e de visitantes florais autóctones como polinizadores. *Acta Bot Brasilica,* 21, 835-845.

Noguerales V, Cordero PJ, Ortego J (2016) Hierarchical genetic structure shaped by topography in a narrow-endemic montane grasshopper. *BMC Evol Biol*, 16, 1–15. doi: https://doi.org/10.1186/s12862-016-0663-7

Nora S, Albaladejo RG, Aparicio A (2015) Genetic variation and structure in the Mediterranean shrubs *Myrtus communis* and *Pistacia lentiscus* in different landscape contexts. *Plant Biol*, 17, 311–319.

Oksanen J, Guillaume-Blanchet F, Friendly M, Kindt R, Legendre P et al. (2018) VEGAN: Community Ecology Package. R package version 2.4-6. Available at: https://CRAN.R-project.org/package=vegan

Paradis E (2010) PEGAS: an R package for population genetics with an integrated-modular approach. *Bioinformatics,* 26, 419–420.

Pérez Latorre AV, Cabezudo B (2009) *Ziziphus* Mill. In: G Blanca, B Cabezudo, M Cueto, C Fernández López and C Morales Torres (eds.), *Flora Vascular de Andalucía Oriental, vol. 3*, 46–50. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/

Rey PJ, Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Valera F, Salido T, Alcántara JM (2017) Local-scale and landscape disturbances impact through distinct pathways on the regional variation in seed dispersal by mammals in threatened semiarid habitats. *Perspect Plant Ecol Evol Syst*, 28, 10–18.

Rey PJ, Cancio I, Manzaneda AJ, González-Robles A, Valera F, Salido T, Alcantara JM (2018) Regeneration of a keystone semiarid shrub over its range in Spain : habitat degradation overrides the positive effects of plant-animal mutualisms. *Plant Biol*, 20, 1083–1092.

Ritland K (1990) Inferences about inbreeding depression based on changes of the inbreeding coefficient. *Evolution*, 44, 1230–1241.

Rousset F (2008) GENEPOP’007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour,* 8, 103–106.

Sánchez Gómez P, Carrión Vilches MA, Hernández González A, Guerra Montes J (2002) *Libro rojo de la flora silvestre protegida de la Región de Murcia*. Consejería de Agricultura, Agua y Medio Ambiente, D.G. del Medio Natural, Murcia, 685 p.

Teixeira H, Rodríguez-Echeverría S, Nabais C (2014) Genetic diversity and differentiation of Juniperus thurifera in Spain and Morocco as determined by SSR. *PLoS One*, 9(2), e88996. doi: https://doi.org/10.1371/journal.pone.0088996

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus* (*). In: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, 68 p.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Resour*, 4, 535–538.

van Strien MJ, Holderegger R, Van Heck HJ (2015) Isolation-by-distance in landscapes: considerations for landscape genetics. *Heredity*, 114, 27–37.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol*, 13, 921–935.

Vranckx G, Jacquemyn H, Muys B, Honnay O (2011) Meta-Analysis of Susceptibility of Woody Plants to Loss of Genetic Diversity through Habitat Fragmentation. *Conserv Biol*, 26, 228–237.

Wang IJ, Bradburd GS (2014) Isolation by environment. *Mol Ecol*, 23, 5649–5662.

Wang R, Compton SG, Chen XY (2011) Fragmentation can increase spatial genetic structure without decreasing pollen-mediated gene flow in a wind-pollinated tree. *Mol Ecol*, 20, 4421–4432.

Wang R, Ai B, Gao BQ, Yu S, Li YY, Chen XY (2009) Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. *pumila* (Moraceae). *Popul Ecol*, 51, 307–315.

Wang S, Liu Y, Ma L, Liu H, Tang Y, Wu L, Wang Z, Li Y, Wu R, Pang X (2014) Isolation and characterization of microsatellite markers and analysis of genetic diversity in Chinese jujube (*Ziziphus jujuba* Mill.). *PLoS One*, 9, e99842. doi: https://doi.org/10.1371/journal.pone.0099842

Weekley CW, Kubisiak TL, Race TM (2002) Genetic impoverishment and cross-incompatibility in remnant genotypes of *Ziziphus celata* (Rhamnaceae), a rare shrub endemic to the Lake Wales Ridge, Florida. *Biodivers Conserv*, 11, 2027–2046.

Wereszczuk A, Leblois R, Zalewski A (2017) Genetic diversity and structure related to expansion history and habitat isolation: Stone marten populating rural-urban habitats. *BMC Ecol*, 17, 46. doi: https://dx.doi.org/10.1186%2Fs12898-017-0156-6

Wu FQ, Shen SK, Zhang XJ, Wang YH, Sun WB (2014) Genetic diversity and population structure of an extremely endangered species: the world's largest *Rhododendron*. *AoB Plants*, 7. doi: https://doi.org/10.1093/aobpla/plu082

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol Evol*, 11, 413–418.

SUPPLEMENTARY MATERIAL

FIGURES

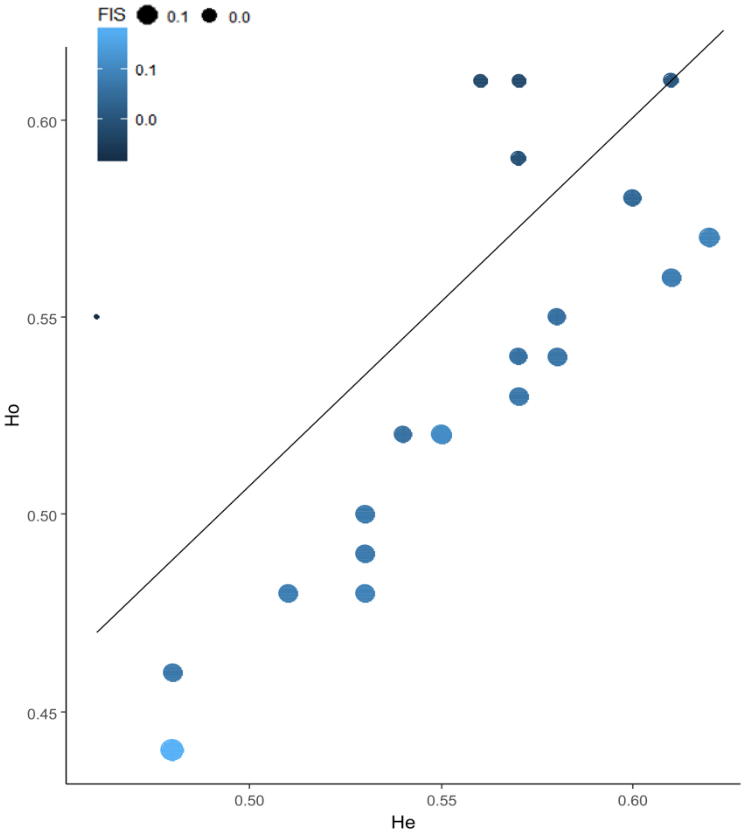


Fig. S1: Relation between observed (H_o) and expected (H_e) heterozygosity per population. All populations showed observed values close to the expectations and four populations had higher values of H_o. The size and color of spots is related to the inbreeding coefficient (F_{IS}) (see graph legend).

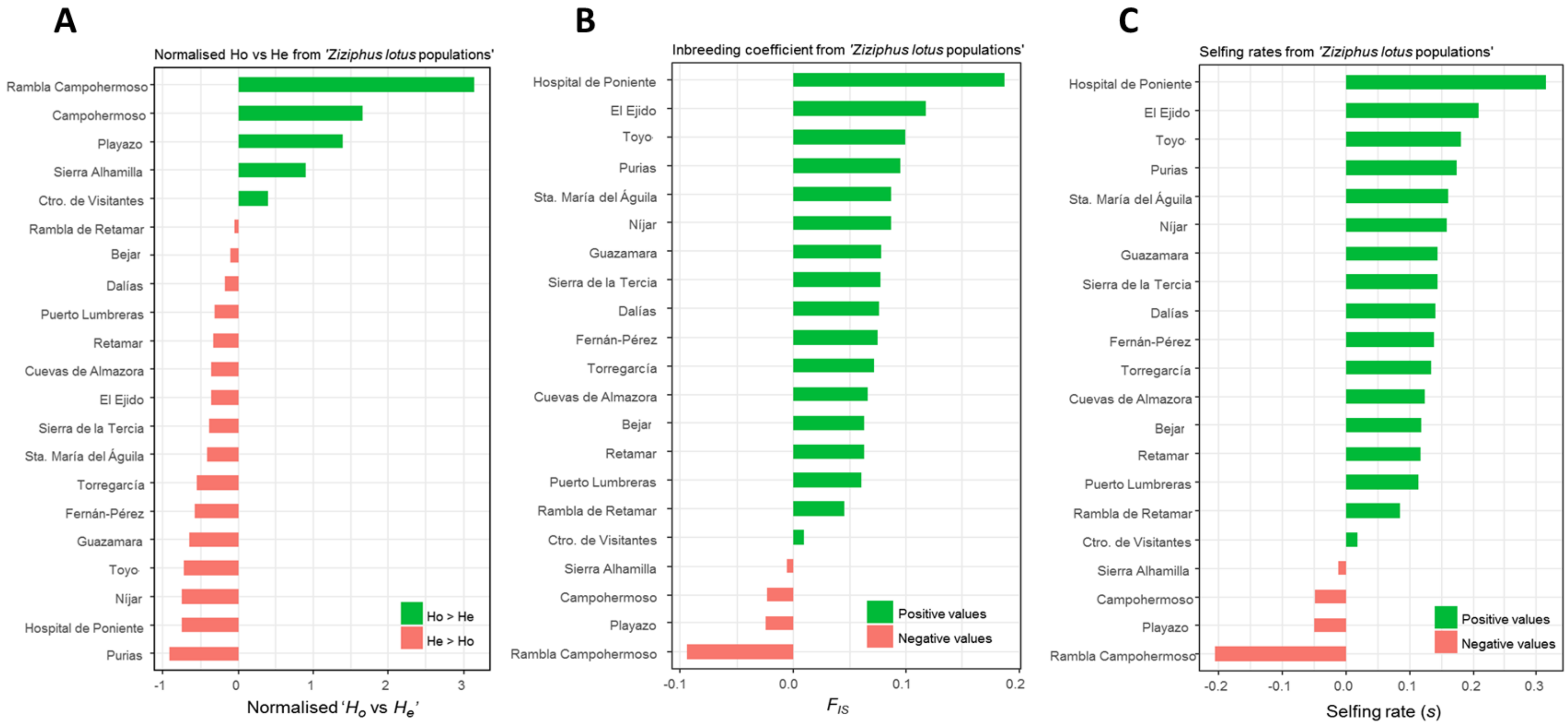


Fig. S2: Contrasting general values of genetic diversity per population. (A) Relationship between the observed heterozygosis (H_o) and expected heterozygosis (H_e). The gross values have been normalized to mark the increase or decrease in each case; (B) inbreeding coefficients (F_{IS}); (C) selfing rates (*s*). Positive values are highlighted in green and negative values in red.

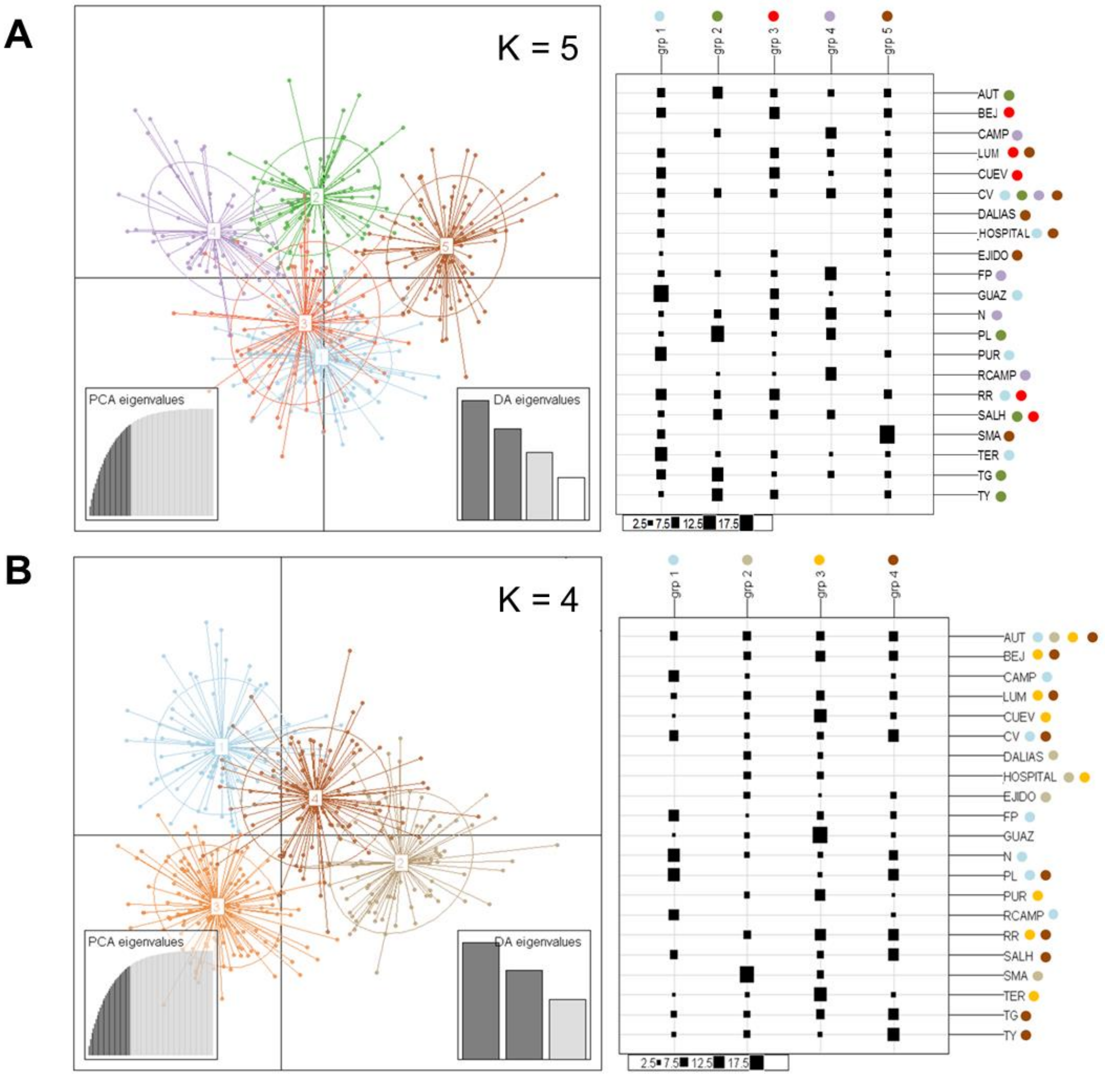


Fig. S3: Scatterplots of DAPC results for *K* = 5 (A) and *K* = 4 (B): overlaps between the k groups seem to be due to the great genetic variability detected within populations (more than 87%, based on the results of the AMOVA), perhaps reflecting an intra-population genetic cline. This is the reason why the same population is attributed to different clusters at the same time (see diagrams on the right in A and B: each cluster is marked with a color spot according to DAPC scatterplot, and each population is marked with the color of the cluster to which most probably belongs). Several colors indicate the same probability of belonging to several clusters. [Abbreviations: AUT, Retamar; BEJ, Béjar; CAMP, Campohermoso; CUE, Cuevas de Almanzora; CV, Ctro. de Visitantes; DALIAS, Dalías; EJIDO, El Ejido; FP, Fernán-Pérez; GUAZ, Guazamara; HOSP, Hospital de Poniente; LUM, Puerto Lumbreras; N, Níjar; PL, Playazo; PUR, Purias; RCAMP, Rambla Campohermoso; RR, Rambla de Retamar; SALH, Sierra Alhamilla; SMA, Sta. María del Águila; TER, Sierra de la Tercia; TG, Torregarcía; TY, Toyo].

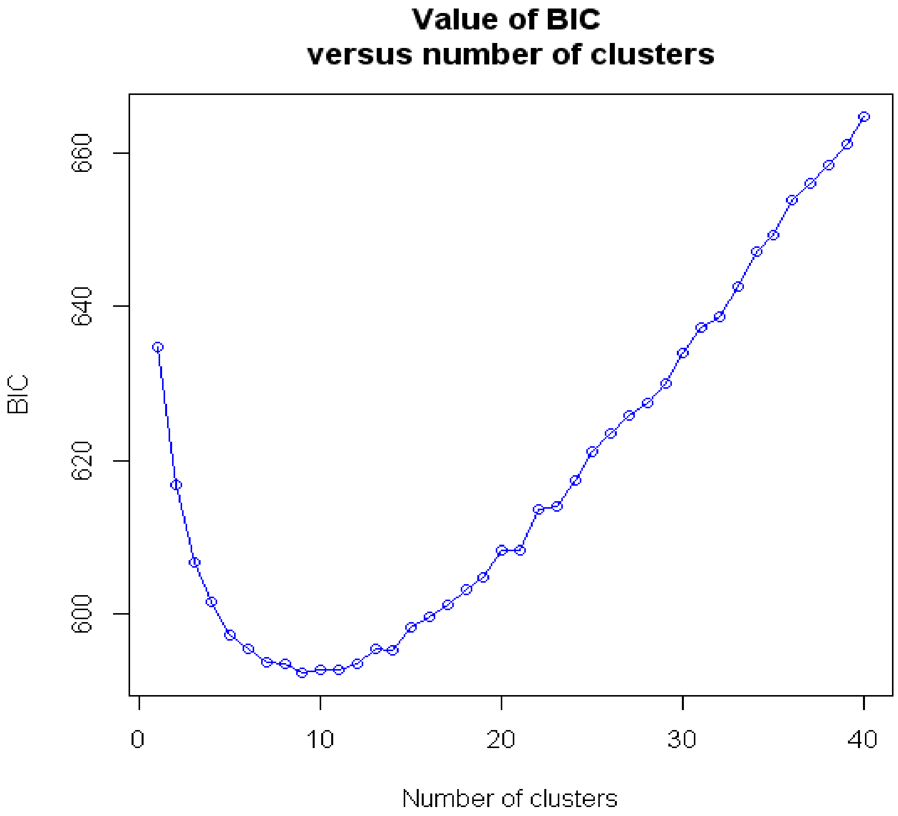


Fig. S4: Inference of the number of clusters for DAPC by Bayesian information criterion (BIC). Apparently, *K* = 5-7 seems to be the most likely cluster number. However, when genetic diversity is distributed continuously and homogenously among populations, it can infer erroneous discrete overlapping clusters (see Fig. S3). In this case, it is necessary to visualize and reinterpret scatterplot clusters to determine the real distribution of genetic variability (Jombart et al. 2010). In our case, *K* = 3 was the best number of clusters to explain genetic structuring (probably with slight intra-population cline according to this figure) of *Z. lotus* populations in the southeast of the Iberian Peninsula.

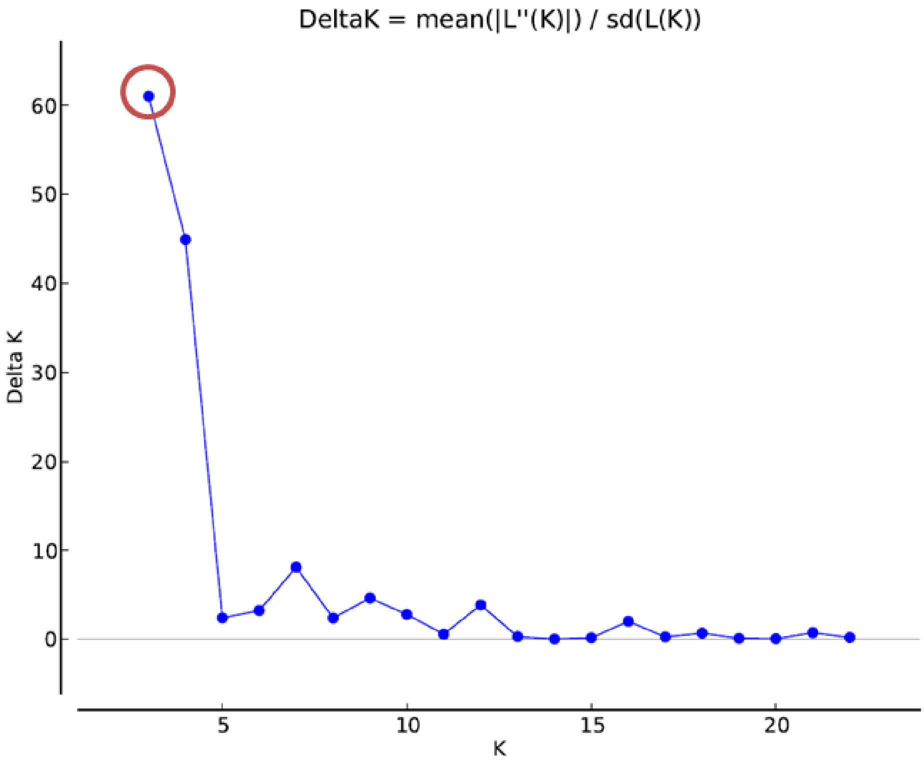


Fig. S5: Graph of Delta *K* values calculated by Evanno method of Structure Harvester Online. *K* = 3 (with a red circle) was the most probable number of genetic groups for the 21 *Z. lotus* studied populations.

TABLES

Table S1: Results of Hardy-Weinberg Equilibrium (HWE) test for 21 studied *Ziziphus lotus* populations.

Population	Code	*P*-value	S.E.
Béjar	BEJ	0.411	0.100
Campohermoso	CAMP	0.214	0.069
Ctro. de Visitantes	CV	0.332	0.079
Cuevas de Almazora	CUEV	0.322	0.071
Dalías	DALIAS	0.603	0.101
El Ejido	EJIDO	0.679	0.102
Fernán Pérez	FP	0.351	0.093
Guazamara	GUAZ	0.408	0.094
Hospital de Poniente	HOSPITAL	0.491	0.108
Níjar	N	0.437	0.100
Playazo	PL	0.408	0.078
Puerto Lumbreras	LUM	0.417	0.103
Purias	PUR	0.440	0.093
Rambla Campohermoso	RCAMP	0.364	0.104
Rambla de Retamar	RR	0.341	0.101
Retamar	AUT	0.470	0.089
Sierra Alhamilla	SALH	0.467	0.085
Sierra de la Tercia	TER	0.483	0.117
Sta. María del Águila	SMA	0.498	0.104
Torregarcía	TG	0.441	0.089
Toyo	TY	0.370	0.072

Note that all populations are in HWE ($p > 0.05$)

Table S2: Genetic diversity statistics for the nuclear microsatellites markers used in this Ziziphus lotus genetic study.

Locus	Repeat motif	N_a	H_o	H_e	F_{IS}
zlo64	$(CT)_{15}$	10	0.71	0.80	0.06
zlo65	$(TC)_{10}$/ACTCC/$(CT)_5$	8	0.71	0.74	0.02
zlo73	$(TC)_{21}$	9	0.45	0.67	0.28
zlo68	$(TG)_9(AG)_{14}$	7	0.61	0.73	0.07
zlo79	$(CT)_{19}$	8	0.64	0.75	0.09
zlo87	$(TC)_{16}$	6	0.58	0.58	-0.12
zlo88	$(AG)_{21}$	8	0.54	0.55	0.05
zlo77	$(GA)_{12}$	3	0.17	0.18	0.06
zlo67	$(CT)_{19}$	6	0.56	0.61	-0.07
zlo71	$(CT)_{20}$	9	0.45	0.57	0.14
zlo76	$(CT)_{16}$	10	0.48	0.60	0.14
zlo80	$(GT)_{10}$	7	0.68	0.73	-0.05
zlo86	$(AC)_{10}$	6	0.39	0.43	0.08
zlo66	$(AG)_{15}$	7	0.54	0.62	0.07
	Total	104			

Abbreviations: Total number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficient (F_{IS})

Table S3: Fixation index (F_{ST}) matrix for the 21 *Ziziphus lotus* populations.

Population[a]	AUT	BEJ	CAMP	CUE	CV	DALIAS	EJIDO	FP	GUAZ	HOSP	LUM	N	PL	PUR	RCAMP	RR	SALH	SMA	TER	TG
BEJ	0.039																			
CAMP	0.052	0.098																		
CUE	0.040	0.031	0.083																	
CV	0.017	0.044	0.035	0.039																
DALIAS	0.041	0.058	0.110	0.060	0.046															
EJIDO	0.052	0.064	0.111	0.060	0.055	0.067														
FP	0.039	0.056	0.046	0.043	0.029	0.071	0.092													
GUAZ	0.039	0.035	0.084	0.019	0.039	0.042	0.073	0.040												
HOSPITAL	0.053	0.080	0.127	0.077	0.057	0.048	0.045	0.099	0.076											
LUM	0.032	0.029	0.078	0.029	0.030	0.056	0.065	0.041	0.032	0.068										
N	0.028	0.052	0.030	0.045	0.019	0.065	0.067	0.028	0.046	0.080	0.035									
PL	0.035	0.070	0.040	0.055	0.029	0.053	0.066	0.039	0.047	0.064	0.053	0.029								
PUR	0.038	0.043	0.104	0.039	0.042	0.056	0.087	0.056	0.027	0.079	0.035	0.059	0.059							
RCAMP	0.058	0.080	0.055	0.073	0.046	0.117	0.156	0.026	0.060	0.155	0.070	0.044	0.049	0.090						
RR	0.019	0.028	0.051	0.026	0.013	0.037	0.051	0.026	0.022	0.055	0.024	0.024	0.028	0.032	0.047					
SALH	0.029	0.046	0.042	0.036	0.022	0.062	0.076	0.031	0.035	0.086	0.038	0.021	0.035	0.059	0.051	0.016				
SMA	0.049	0.067	0.094	0.071	0.052	0.020	0.037	0.087	0.072	0.020	0.061	0.083	0.073	0.060	0.101	0.053	0.074			
TER	0.039	0.039	0.086	0.029	0.039	0.061	0.086	0.034	0.018	0.088	0.030	0.045	0.062	0.026	0.051	0.030	0.043	0.080		
TG	0.009	0.044	0.048	0.044	0.013	0.041	0.052	0.039	0.042	0.050	0.032	0.024	0.028	0.047	0.059	0.015	0.022	0.049	0.048	
TY	0.030	0.050	0.043	0.057	0.026	0.057	0.065	0.048	0.054	0.072	0.045	0.027	0.038	0.070	0.058	0.026	0.027	0.063	0.062	0.021

[a] Abbreviations: AUT, Retamar; BEJ, Béjar; CAMP, Campohermoso; CUE, Cuevas de Almanzora; CV, Ctro. de Visitantes; DALIAS, Dalías; EJIDO, El Ejido; FP, Fernán-Pérez; GUAZ, Guazamara; HOSP, Hospital de Poniente; LUM, Puerto Lumbreras; N, Níjar; PL, Playazo; PUR, Purias; RCAMP, Rambla Campohermoso; RR, Rambla de Retamar; SALH, Sierra Alhamilla; SMA, Sta. María del Águila; TER, Sierra de la Tercia; TG, Torregarcía; TY, Toyo.

Table S4. Summary of the metrics of within-habitat remnant degradation (9 variables measured at the local scale of the habitat remnant) and landscape degradation (3 variables assessed at the large scale of the surrounding landscape) for the 21 *Z. lotus* study populations. See details on Cancio et al. 2016, Rey et al. 2017, 2018; see also *Capítulo 1*.

Habitat remnant variables										Landscape variables		
Population (Habitat remnant)	Population size (Number of adults)	Population or remnant area (m^2)	Percentage of the remnant area covered by *Z. lotus* patches (%)	Mean *Z. lotus* patch area (m^2)	Percentage of the total area of *Z. lotus* patches represented by the largest patch (%)	Percentage of the total area of Z. lotus patches represented by isolated adults (%)	Mean perimeter/area of the *Z. lotus* patches	Mean distance to the nearest neighbor *Z. lotus* patch (m)	Aggregation index or index of nearest neighbors	Natural habitat cover (% in 1.5km radius)	Number of land uses	Land use diversity (H')
Béjar	89	10845067.08	0.55	882.94	4.41	66.57	0.1243	134.10	0.401	57.20	7	0.548
Campohermoso	20	72293.66	12.91	1166.82	23.50	22.60	0.1108	88.63	1.372	25.69	11	0.813
Ctro de Visitantes	1367	5691333.48	11.29	1623.05	2.03	21.56	0.1004	57.49	0.701	92.57	10	0.716
Cuevas de Almanzora	101	331310.14	12.72	1359.72	9.50	14.90	0.1102	58.35	0.932	36.75	19	0.713
Dalías	15	21824.04	38.17	925.53	18.48	50.66	0.1183	44.52	1.529	7.29	12	0.541
El Ejido	32	84920.10	19.01	1467.28	20.54	21.79	0.1046	73.50	1.139	8.74	15	0.712
Fernán Pérez	80	2469351.23	1.73	948.14	6.17	45.82	0.1204	116.71	0.657	15.90	15	0.800
Guazamara	81	503518.40	8.66	1895.94	18.33	12.90	0.1007	94.60	0.858	31.89	14	0.668
Hospital de Poniente	86	1353057.59	3.50	1100.64	7.19	39.84	0.1139	80.23	0.672	16.75	11	0.730
Níjar	255	3549060.87	3.90	1098.09	6.28	41.89	0.1158	69.99	0.645	40.37	13	0.822
Playazo	101	1870401.26	3.40	920.58	4.21	53.14	0.1220	80.94	0.845	10.93	15	0.625
Puerto Lumbreras	102	24988233.26	0.26	901.60	5.45	56.33	0.1236	143.20	0.374	27.10	10	0.633
Purias	16	248155.76	4.28	817.81	12.69	65.66	0.1272	106.88	0.718	2.43	7	0.515
Rambla Campohermoso	62	1580976.38	2.35	862.55	8.30	64.01	0.1248	116.26	0.406	4.78	11	0.471
Rambla Retamar	68	1655315.55	2.60	877.00	4.81	57.28	0.1239	70.42	0.594	56.91	17	0.990
Retamar	80	1881381.32	2.74	846.50	4.05	62.65	0.1255	74.01	0.426	83.96	12	0.631
Sierra Alhamilla	133	1097195.25	6.05	1383.15	15.72	28.60	0.1038	87.16	0.845	80.62	16	0.741
Sierra de la Tercia	32	2054608.79	0.95	846.92	7.13	53.74	0.1254	73.54	0.234	18.09	15	0.865
Sta Maria del Águila	117	2949633.22	2.17	1123.62	6.97	34.04	0.1139	67.00	0.438	6.42	12	0.523
Torregarcía	4598	7108890.07	21.51	3812.82	28.59	8.51	0.0742	62.93	0.722	64.76	13	0.760
El Toyo	1015	13772962.38	3.72	1243.40	3.70	31.71	0.1086	71.41	0.660	83.57	18	0.834

Table S5: Factor loadings of the Principal Component Analyses on the within-remnant habitat quality metrics. In bold, the variables that loaded the highest weight in each component.

	F1	F2	F3
Population size (adults)	**0.7392**	-0.6249	-0.1596
Population or remnant area	0.2114	**-0.8967**	0.3668
Proportion of the remnant area covered by *Ziziphus* patches	0.4300	0.5654	**-0.6786**
Mean *Ziziphus* patch area	**0.9800**	0.0932	-0.1485
Proportion of the total area of *Ziziphus* patches represented by the largest patch	0.3911	**0.8594**	0.1122
Proportion of the total area of *Ziziphus* patches represented by isolated adults	**-0.8923**	-0.2446	0.2137
Mean perimeter/area ratio of the *Ziziphus* patches	**-0.9706**	-0.0915	0.1836
Mean distance to the nearest neighbor *Ziziphus* patch	-0.1904	-0.0486	**0.9271**
Aggregation index or index of nearest neighbors	0.2335	0.6824	-0.4921
Explained Variance	3.7184	2.7977	1.8362
Proportion of the Total variance explained	0.4132	0.3109	0.2040

Note: Ecological interpretation of the three components can be found in the Supplementary Material of *Capítulo 1*.

Table S6: Results of the assignment of individuals (*q* values) in the 21 *Z. lotus* populations by STRUCTURE software.

Populations	Acronym[a]	Assigned color for cluster[b]	Inferred clusters (*q* values)			Number of individuals
			1	2	3	
1	DALIAS	Blue	0.045	0.162	0.793	8
2	HOSPITAL		0.012	0.011	0.977	9
3	EJIDO		0.055	0.040	0.905	8
4	SMA		0.064	0.029	0.908	23
Mean			0.044	0.061	**0.896**	
5	AUT	Red	0.530	0.330	0.141	25
6	CAMP		0.917	0.051	0.032	14
7	CV		0.713	0.186	0.101	25
8	FP		0.603	0.352	0.046	20
9	N		0.811	0.168	0.021	25
10	PL		0.829	0.131	0.040	25
11	RCAMP		0.781	0.204	0.015	11
12	RR		0.633	0.311	0.056	25
13	SALH		0.800	0.165	0.035	20
14	TG		0.726	0.165	0.109	25
15	TY		0.889	0.060	0.051	20
Mean			**0.748**	0.193	0.059	
16	BEJ	Green	0.064	0.904	0.031	20
17	LUM		0.079	0.888	0.034	20
18	CUE		0.065	0.886	0.049	20
19	GUAZ		0.076	0.880	0.044	25
20	PURIAS		0.069	0.852	0.079	14
21	TER		0.149	0.828	0.023	20
Mean			0.084	**0.873**	0.043	

[a] Abbreviations: AUT, Retamar; BEJ, Béjar; CAMP, Campohermoso; CUE, Cuevas de Almanzora; CV, Ctro. de Visitantes; DALIAS, Dalías; EJIDO, El Ejido; FP, Fernán-Pérez; GUAZ, Guazamara; HOSPITAL, Hospital de Poniente; LUM, Puerto Lumbreras; N, Níjar; PL, Playazo; PUR, Purias; RCAMP, Rambla Campohermoso; RR, Rambla de Retamar; SALH, Sierra Alhamilla; SMA, Sta. María del Águila; TER, Sierra de la Tercia; TG, Torregarcía; TY, Toyo. In bold, the mean q-values of assigned cluster for each genetic group (color group).

[b] See Fig. 4.

CAPÍTULO 4

EXTENSIVE POLLEN-MEDIATED GENE FLOW ACROSS INTENSIVELY MANAGED LANDSCAPES IN AN INSECT-POLLINATED SHRUB NATIVE TO SEMIARID HABITATS

ABSTRACT

Anthropic activity has profoundly modified semiarid environments across the Mediterranean basin for centuries and increasing aridity is expected to hard-hit this highly diverse biogeographic region. Among other activities, agriculture has reduced formerly native plant communities to small, isolated patches embedded in a matrix of rural and urban landscape with disrupted connectivity and altered plant mating patterns. However, most empirical evidences that show the detrimental effects of forest fragmentation and degradation have been performed in temperate or tropical habitats, with arid and semi-arid environments overly underrepresented in the literature. Here we investigated the relative contribution of plant traits, pollinator activity and environmental factors to explain variation in mating patterns of an insect-pollinated semiarid shrub*, Ziziphus lotus,* across three populations that occupy a strongly altered landscape. We used 14 SSRs, seed paternity analyses, and individual Mixed Effect Mating Models (MEMMi) to jointly estimate the individual mating variables and the population pollen dispersal kernel. Individual spatial location within populations, plant height and flower density, as well as floral visitation rate explained most of the variation of the mating variables within and among populations. Mean pollen dispersal distance showed the highest values in more isolated populations with scattered individuals and a spatial linear distribution of their adult specimens (TER: 4693 m), while other spatial patterning or a higher densities favored shorter dispersal distances (N: 3314 m, EJ: 2055 m). Contrary to our expectations, the population surrounded by the most degraded matrix dominated by intense greenhouse agriculture activity exhibited an increased fraction of pollen immigration (EJ: 0.81 vs N: 0.55 and TER: 0.46), but the lowest immigration rate was detected in the most isolated population. Our results documented that an active assemblage of pollinators maintain increased connectivity levels, even in highly altered landscapes, potentially halting genetic isolation within and among populations. Assuring the connectivity among remnant patches of native flora is essential if we aim to maintain the biological diversity of highly diverse but exceedingly threatened habitats in arid and semiarid regions.

INTRODUCTION

Intensive human activities have caused massive landscape alterations that have derived in habitat loss, one of the main threats to worldwide ecosystems and the biodiversity they host (Foley et al. 2005, Brook et al. 2008). As a result, large and continuous natural habitats have become reduced to several isolated patches of variable sizes embedded in a matrix of agriculture and urban land-use (Fahrig 2003) that alter gene flow and mating patterns of natural populations (Slatkin 1985, Lowe et al. 2005). Many studies that investigate the negative effects of habitat loss and fragmentation on gene flow and mating patterns have been conducted in animal populations (Monteiro et al. 2019), e.g. fishes (DeWoody and Avise 2001), amphibians (Myers and Zamudio 2004), reptiles (Uller and Olsson 2008), birds (Harrison et al. 2012) or mammals (Janečka et al. 2007). However, plant populations are also prone to be impacted by habitat loss and fragmentation due to their reduced motility (Barrett and Harder 2017). The effects of habitat fragmentation on plant mating patterns have been mainly studied in tropical (e.g. Konuma et al. 2000, Ottewel et al. 2012, Guidugli et al. 2016) and template species (e.g. Gauzere et al. 2013, Moracho et 2016, Chybicki and Oleksa 2018) but they are poorly understood in semiarid and arid environments. Nowadays, these habitats cover ca. 30% of the Earth surface (Malagnoux 2007) and this proportion is expected to raise as environmental conditions become increasingly arid. For centuries, ecosystems that occupy arid and semiarid regions have been profoundly disturbed and converted to intensive agriculture lands where native plant communities have been reduced to isolated patches that occupy marginal sites unsuitable to agriculture, such as dry riverbeds (Galvin et al. 2008). Therefore, understanding the functioning of pollen flow and mating patterns of these remnant populations that occupy intensively managed agro-systems is of utmost importance if we aim to achieve food security challenges as we protect remnant biodiversity in a sustainable manner (Kremen and Merenlender 2018).

Animal-pollinated plants that inhabit agro-systems are reported to show increased level of genetic erosion, selfing rates and inbreeding depression (Rathcke and Jules 1993, , Breed et al. 2012b, Breed et al. 2015, Honnay and Jacquemyn 2017), partly because pollinators find it difficult to move across a highly intensified agricultural matrix (Ghazoul 2005, Dirzo et al. 2014). Further, recent studies has underlined that an active assemblage of pollinators dampen the negative outcomes of mating-pattern shifts across managed landscapes. For example, some agro-systems still host a diverse array of insect pollinators that proved to connect distant populations through long distance dispersal movements (Ottewell et al. 2012, Breed et al.

2015). Therefore, future studies should integrate variation on phenotypic traits (Vranckx et al. 2011), environmental heterogeneity and pollinator activity to evaluate the detrimental consequences of agricultural intensification on gene flow and mating patterns for native flora (Breed et al. 2015).

Due to their ecological and evolutionary outcomes, the study of mating patterns has gained prominence in recent years, partly due to the advent of highly polymorphic molecular markers, namely SSRs and SNPs (Ritland 2002, Kaiser et al. 2017, Garcia et al. 2018). This has advanced our knowledge on the impact of various environmental factors that shape mating and gene flow patterns at the population level and how they shifted across increasingly managed landscapes. Notably, highly polymorphic molecular markers also inform us about the intra-population variability in mating variables (such as male and female fecundity on an individual basis), as well as on the effect of individual phenotypic traits that determine them (García et al. 2005, Gauzere et al. 2013). This allow us to scale our findings on intra-population variation on mating patterns to the landscape level where ecological features that define agro-systems are taken into account (Klein et al. 2011, Moracho et al. 2016). However, to date most studies exploring the intra-population variation of mating and gene flow patterns in managed ecosystems has focused on a single population (Robledo-Arnuncio and Gil 2005, Breed et al. 2012a,b, Guidugli et al. 2016, Chybicki and Oleksa 2018). Therefore, we ignore whether the observed intra-population variation shifts across populations in highly altered landscapes (Gauzere et al. 2013).

Here, we specifically investigated how individual plant traits, pollinator activity and environmental variables affect the variation of mating patterns within and across three remnant populations of *Ziziphus lotus*. This is a semiarid Mediterranean shrub that frequently thrives in highly fragmented and isolated populations surrounded by intensively managed lands dominated by greenhouse agriculture. Overall, we expected that small, isolated populations would show a low frequency of immigrant pollen and reduced mean pollen dispersal distances, as well as a low number of pollen donors per mother tree due to a diminished pollinator activity. Within populations, we expected that large, more conspicuous trees growing in high density neighborhoods would attract more pollinators and, thus, exhibit an increased number of pollen donors. Our specific aims were: (i) characterizing the individual mating variables within the three *Ziziphus* populations; (ii) quantifying the average pollen dispersal distances and estimate the proportion of immigrant pollen at each population; and (iii) estimating the impact of individual plant traits, conspecific neighborhood density and pollinator visits on mating patterns within and among populations. To these goals, we used 14 specific microsatellites molecular markers (SSRs) (see *Capítulo 2*; González-Robles et al. 2016) to obtain the multilocus genotypes of fruiting trees and maternal progenies along with all nearby candidate pollen donors. Then, we applied paternity analyses with CERVUS (Kalinowski et al. 2007) and POLDISP software (Robledo-Arnuncio et al. 2007) to identify the pollen donors for sampled maternal progenies and to estimate male fecundities and the effective number of sires per mother tree. We further inferred mating variables on an individual basis jointly with the population pollen dispersal kernel applying a modified version of the Mixed Effect Mating Models (MEMM, Klein et al. 2008) referred as individual Mixed Effect Mating Models (MEMMi, Gauzere et al. 2013).

MATERIALS AND METHODS

Study species

The semiarid shrub *Ziziphus lotus* (L.) Lam (Rhamnaceae) is a sclerophyllous species distributed across the Mediterranean Basin from the Iberian Peninsula, Cyprus and Sicily to the Sahara and the Arabian Peninsula (European Commission 2013). It is a long-lived and diploid (2n = 20) species, with 1-3 m height and up to 30 m diameter (Rey et al. 2018). It is a monoecious species that produces numerous hermaphrodite and generalist flowers pollinated by a diverse assemblage of insects, namely bees, wasps, flies, ants, beetles and butterflies, among others (Pérez Latorre and Cabezudo 2009, see also *Capítulo 1*). Flowers show apparently self-incompatibility and synchronous protandrous dicogamy, i.e., each individual matures its flowers synchronically with anthers maturing in the first place followed by the stigma without (or little) overlapping between both sexual forms. The lifetime of each flower is less that one day (see more details in *Capítulo 1*) but trees open new flowers daily during the flowering season that spans May to July. This species is distributed in the most southeastern of the Iberian Peninsula namely across seasonal streams or dry riverbeds (‘ramblas’) and coastal plains. This is a semi-arid European priority habitat (Anon 1992; *Habitat 5220*: Arborescent shrub with Ziziphus,* Tirado 2009) highly degraded and fragmented where remnant scrubland fragments typically contain < 100 individuals (Rey et al. 2018). Anthropic activities have increased over the latest 60 years in the area mainly due to urban expansion and agricultural intensification dominated by extensive areas occupied by greenhouses (Mota et al. 1996, Mendoza-Fernández et al. 2015).

Study sites and sampling design

We chose three representative populations of the environmental conditions where this species thrives across the southeastern region of the Iberian Peninsula (Table S1 for further details): El Ejido (EJ, the most degraded study area surrounded by the largest greenhouse agriculture nucleus of southern Europe); Níjar (N, one of the largest and continuous remnant populations of this species, a relative well-preserved area) and La Tercia (TER, an area dominated by small, isolated and low-density populations) (Fig 1). During the flowering season 2015, we mapped (GARMIN GPSMAP-64s) all adult trees in the study populations (N= 342) and we collected leaf samples and kept them in individual envelops deposited in plastic zip bags at -50 °C until further molecular work. For subsequent paternity analysis, all adult trees were considered potential candidate pollen donors (fathers) and we randomly chose 20-25 adults per population as maternal trees where we collected 20-25 ripe fruits (maternal progenies) from their canopy during the fruiting season (August and September) (Table 1).

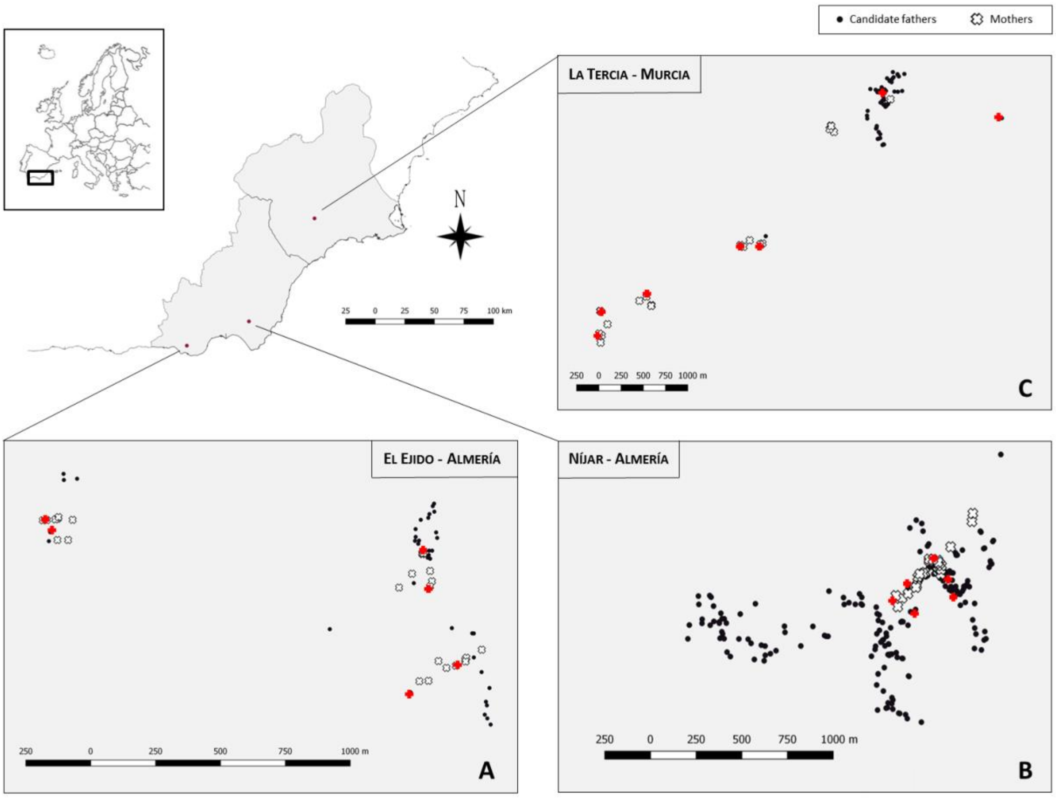


Fig. 1: Location of the study populations in the southeastern corner of the Iberian Peninsula. Insets (A, B, C) show the location of the maternal trees (with crosses) and candidate fathers (black dots) within each study population: El Ejido (A); Níjar (B); and La Tercia (C). Red crosses represent the important pollen donors in each population according our results.

Table 1: The overall sample sizes per population used in this study to characterize mating and gene flow patterns within and across populations.

	El Ejido	Níjar	La Tercia	Total
Maternal trees genotyped and phenotyped	25	25	20 [11]*	50
Candidate fathers genotyped (including mothers)	63	201	78	342
Total maternal progeny genotyped	240	221	31	492
Mean maternal progeny per individual	9.60	8.84	2.82	-
Ecological neighbourhood characterized	25	25	20	70

* Denote that a large number of seeds failed to germinate. It records the total maternal trees genotyped (offside bracket), and the final number of maternal trees with effective progeny (within bracket). Mean maternal progeny per individual was calculated from this last number.

Genotyping of maternal progenies and adult trees

Due to the small size of the embryos, we germinated them under control conditions in a Petri dish to ensure an amount of tissue that suffices for further DNA extraction. To that end we removed the fleshy tissue attached to each seed by hand under sterile conditions (depulpation) and then we removed the seed exocarp and mesocarp tissues and we opened the endocarp to retain the embryo (1 or 2 per seed). Each embryo was seeded in a sterile Petri dish with autoclaved distilled water and maintained in a germination chamber at 18-20°C for 4-10 days. Each Petri dish was sealed with parafilm to avoid possible contamination by exogenous fungus. We checked petri dishes every 2 days to check their moister conditions and the occurrence of any pathogen. When the radicle showed a length of ca. 1-2 cm, embryos were transplanted to soil in individual pots and grown in germination chambers (at 24°C with 14h of daylight and 50% of relative humidity) during 2-3 months. These germinated seedlings served as maternal progenies in our study and we collected 2-3 fresh leaves per seedling and kept them at -50°C until their further DNA extractions. We planted 20-25 seeds per mother tree to obtain, at least, 10 seedlings per mother (a total of 700 seedlings). Nonetheless, some seeds, mainly those collected from the TER population, failed to germinate. As a result, 492 germinated successfully and constitute our sample of maternal progenies (Table 1).

All adult trees and maternal progenies were genotyped with 14 species-specific microsatellites marker for *Z. lotus*: (zlo64, zlo65, zlo66, zlo67, zlo68, zlo71, zlo73, zlo76, zlo77, zlo79, zlo80, zlo86, zlo87 and zlo88; see *Capítulo 2* for details on SSRs and multiplex conditions; González-Robles et al. 2016). Four fluorescent dyes (5-HEX, 56-FAM, ATTO565 and ATTO550) were used to 5-end label the forward primer of each locus (see *Capítulo 2;* González-Robles et al. 2016). DNA extraction and PCR amplifications were performed using methods and conditions described in *Capítulo 2*; González-Robles et al. 2016). Automated genotyping to analyze fluorescent-labeled PCR products was performed by an ABI 3500 Genetic Analyzer sequencer (Applied Biosystems) using GeneScan 600 LIZ Size Standard (Applied Biosystems). GeneMapper software version 4.1 (Applied Biosystems) was used for the assignment of alleles and fragment analyses. Linkage disequilibrium per locus was tested based on 10000 permutations using GENEPOP v.4.2 software (Rousset 2008) and null allele frequencies were estimated in Micro-Checker v.2.2.0 (van Oosterhout et al. 2004) (Table S2, see also *Capítulo 2*; González-Robles et al. 2006). Additionally, two people checked independently the quality of the scoring to minimize genotyping errors.

Ecological variables: plant traits, neighborhood and pollinator variables

In each population, we characterized three types of variables per mother that depict their phenotype, ecological neighborhood, and the assemblage of pollinator that visit their canopy (mean values reported in Table S1). Phenotypic traits were assessed as the tree height (*HEI*, in m), canopy projection area (*CPA,* m^2) and flower density (*FD*, n/225 cm^2). To estimate *FD*, we averaged the number of open flowers counted within four replicated squares (225 cm^2 each) during the blooming period (from May to July 2015). To describe the ecological neighborhood of each mother tree, we defined a 25 and 50 m radius circle around each mother and we recorded the number of conspecific individuals within that area (*NEI25* and *NEI50*, respectively) by projecting the spatial location of all mapped adults using Quantum GIS 2.18.2 software (Quantum GIS Development Team 2016) (Fig. S2). Finally, we characterized the assemblage of pollinators visiting each maternal tree based on two 3-minute censuses during the blooming period (70 mother tree x 2 censuses/individual = 140 pollinator censuses). For each census, we recorded: (i) pollination visitation rate (PVR), as the mean number of insects visiting flowers; and (ii) flower visitation rate (FVR), as the mean number of flowers visited by insects per census (see *Capítulo 1* for further details on pollinator censuses).

Estimates of mating variables and dispersal kernel parameters

Following Gauzere et al. (2013), we used the multilocus genotypes obtained from maternal progenies, mother trees, and candidate fathers to estimate the proportion of selfing (s) and migration events (m) per mother tree, as well as the number of pollen donors within population that sired their progeny (*Np*) based on the Bayesian approach implemented in the individual Mixed Effect Mating Models (MEMMi). Similarly, we inferred the male fecundity for each candidate father (*Mf*) as the number of fertilized seeds by a given pollen donor and the number of pollen donors per mother within population. MEMMi additionally estimates the population mean pollen dispersal distance (δ) (meters) along with the kernel dispersal parameters (shape, b). We run MEMMi for each studied population using MCMC of 50 000 steps and burn-in of 5000 steps. We employed a Gamma distribution for male fecundity variable and uniform prior distributions on the intervals [0, 1], [0, 1], [0, 10000], [0, 10], [0, 1000] for s_{ij}, m_{ij}, δ, b, and σ^2 (total variance of quantity of pollen emitted by fathers), respectively (see Gauzere et al. 2013 for further details on the modeling approach and the parameters required). Additionally, we estimated the average effective number of fathers pollinating each mother (Nep_i) per population by applying POLDISP software (Robledo-Arnuncio et al. 2007) based on the within-sibship correlated paternity estimates (r_{gg}): $Nep = 1/r_{gg}$ (Gauzere et al. 2013).

Finally, we performed paternity analysis on maternal progenies with known mother trees to identify the most likely pollen donor that sired maternal progenies García et al. (2005). To that end, we applied CERVUS 3.0 software (Kalinowski et al. 2007) with a relaxed and strict confidence level (80% and 90%, respectively) for paternity assignment. Based on our previous knowledge, we set the following parameters per population (EJ, N and TER, respectively): *n* offsprings = 100 000; *n* candidate fathers = 100, 250, 110; proportion of candidate father sampled = 0.80, 0.60, 0.80; proportion of loci mistyped = 0.02, 0.02, 0.03; error rate in likelihood calculations = 0.02, 0.02, 0.03; minimum number of typed loci = 11. Simulations also considered the inbreeding level per population (EJ= 0.1171; N=0.1591; TER =0.1432) derived from previous genetic diversity analyses within our study populations (see *Capítulo 3*).

Statistical analyses

ANOVA and Tukey tests were used to test for differences among populations in mating system variables (s, m, *Np* and *Mf*). The values of all studied variables followed a normal distribution, but *Mf* was previously log-transformed to meet this requirement. Linear Mixed Models (LMMs) were used to evaluate the effect of the ecological correlates on the described individual mating variables (*s*, *m*, *Np* and *Mf*). Initially, we considered *HEI*, *CPA*, *FD*, *NEI25*, NEI50, *PVR* and *FVR* as continuous ecological covariates per individual. Prior to model construction, we assessed the non-collinearity of ecological variables by using the Pearson correlations (threshold: r ≤ |0.6|) and variance inflation factors (VIF). We removed *CPA* and *NEI25* because they showed high correlation values with *HEI* and *NEI50,* respectively. Equation 1 shows the final structure of LMMs (based on Zuur and Ieno 2016):

$$s_{ij} \text{ or } m_{ij} \text{ or } Np_{ij} \text{ or } log(Mf_{ij}) = HEI_{ij} + FD_{ij} + NEI50_{ij} + PVR_{ij} + FVR_{ij} + Population_i \tag{eqn 1}$$

where $variable_{ij}$ is the *j*th observation in population *i*, $i = 1,\ldots,3$, and $Population_i$ is the random intercept.

We used the package '*nmle'* (Pinheiro et al. 2017) and '*MuMin*' (Barton 2018) in the software R v3.4.3 (R Core Team 2017) to fit all LMMs with maximum likelihood. Model selection was based on Akaike Information Criteria corrected for small sample sizes (AICc). Among all possible competing models ($\Delta\mathrm{AICc}_i < 2$ defined models equally valid), we chose models with the least AICc and selected among them the most parsimonious one as suggested by Burnham and Anderson (2002). Additionally, we controlled for the spatial correlation of our dependent variables by adding a correlation function to the best model previously selected. Following Zuur et al. (2009), we tested for the effect of the spatial location by accommodating *x*, *y* (geographic coordinates) as independent variables within each LMM by applying the package '*lme4'* (Bates et al. 2015). Then, we compared the performance of models that accommodate spatial structure with the null model that lacks spatial structure and we selected the best fitted model following the same explained criteria above (less AICc and parsimony). Variograms were further used to check the spatial correlation and to assess the independence of residuals by *'Variogram'* function of R-package '*nlme*' (Pinheiro et al. 2017). All selected models were validated by plotting their standardized residuals against fitted values and by testing whether they meet normality and homoscedasticity (Fig S2).

RESULTS

Mating patterns within and across populations and ecological correlates

Maternal trees showed a reduced proportion of selfing events (*s*) across all study populations with values ranging from 0.00% to 0.71% (EJ: 0.00%-0.71%; N: 0.00%-0.68%; TER: 0.00%-0.44%; Table S3), and these values did not show significant differences among populations (Table 2, Fig 2A). The individual values of *s* were explained by the *HEI* (t-value = -2.4796, *p*= 0.0164) and *FVR* (t-value= 2.2701, *p*= 0.0274) suggesting that shorter trees with highly visited flowers tended to show increased values of *s.* At the intra-population level, only EJ showed significant differences in *s* among individuals (t-value = 4.1697, *p*= 0.0001; Table 3), being *HEI* the only variable responsible of these variations (t-value = -2.8217, *p*= 0.0069*;* Table 3). The trends of *s* values per population as a function of each explanatory variable are included in Fig. S3.

Table 2: Mean values estimated by MEMMi, POLDIST and CERVUS on different mating variables assessed at the individual (m_i, s_i, Mf_i, Np_i and Nep_i) and population level (δ, b) . All these estimates were based on individual data, except pollen dispersal distance and the shape of pollen kernel which were estimated per population. Differences between populations are represented by letters in bold (ANOVAs + Tukey's range tests).

Variables	El Ejido			Níjar sub.			La Tercia		
	[$\bar{X}$ ± SE]			[$\bar{X}$ ± SE]			[$\bar{X}$ ± SE]		
Migration rate (m_i)	0.8059	0.0203	***a***	0.5479	0.0374	***b***	0.4570	0.0324	***b***
Selfing rate (s_i)	0.0027	0.0004	***c***	0.0021	0.0004	***c***	0.0023	0.0005	***c***
Pollen dispersal distance (δ)‡	2055	10		3314	12		4693	868	
Shape of the pollen kernel (b)*	0.2746	0.0014		0.1693	0.0003		0.3166	0.0978	
Male fecundity§ (Mf_i)	0.9994	0.0489	***d***	1.0006	0.0575	***d***	0.9904	0.0989	***d***
Average number of pollen donors per mother within population (Np_i)	4.1600	0.3544	***e***	3.2800	0.3441	***e***	1.6364	0.3636	***f***
Average of effective number of pollen donors (Nep_i)$^{\Phi}$	7.81			6.64			6.93		

‡ Meters (m); * $b > 1$: 'thin-tailed' kernel; $b < 1$: 'fat-tailed' kernel; § denote log-transformation variable for ANOVA and Tukey's range tests; $^{\Phi}$ considering sampled and unsampled individuals.

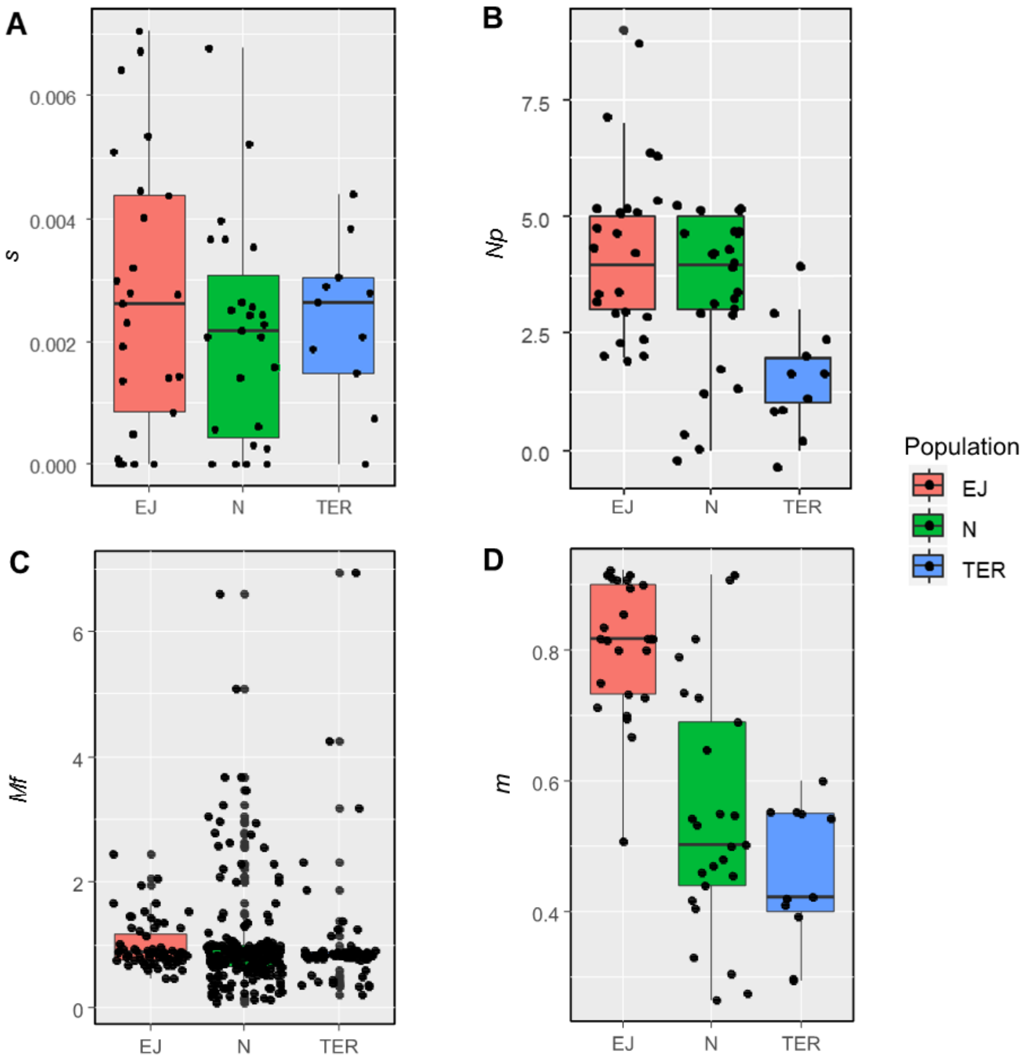


Fig. 2: Variation of main mating variables within and between *Z. lotus* populations: *s*, selfing rate (A); *Np*, the number of pollen donors per mother (B); *Mf*, male fecundity (C); *m*, migration rate (D)

Table 3: Linear mixed model (LMM) results: effects of plant traits, neighborhood and pollination variables on mating parameters in the three study population of *Z. lotus*. Best selected model by AICc is shown in each case. Population was included as a random factor in all models.

Variable	Spatial correlation structure	Range	Nugget	Value	Std.Error	df	t-value	*P*	Effects[a]		
									EJ	N	TER
(A) *m*	Gaussian	47.35	0.41								
Intercept				0.6211	0.1057	58	5.8766	0.0000	0.8059^{***}	0.5479^{***}	0.4570^{***}
(B) *s*	–	–	–								
Intercept				0.0042	0.0008	52	4.9693	0.0000	0.0052^{***}	0.0025^{ns}	0.0008^{ns}
HEI				-0.0012	0.0005	52	-2.4796	0.0164	-0.0020^{**}	-0.0005^{ns}	0.0003^{ns}
FVR				<0.0001	<0.0001	52	2.2701	0.0274	$<0.0001^{ns}$	$<0.0001^{ns}$	$<0.0001^{ns}$
(C) M_f	–	–	–								
Intercept				-0.6932	0.2055	65	-3.3733	0.0013	-0.3804^{ns}	-1.3420^{***}	-0.2566^{ns}
FD				0.0035	0.0012	65	3.0277	0.0035	0.0015^{ns}	0.0096^{***}	-0.0006^{ns}
FVR				-0.0017	0.0010	65	-1.7332	0.0878	0.0007^{ns}	-0.0189^{*}	-0.0010^{ns}
(D) Np	–	–	–								
Intercept				3.1308	0.6647	58	4.7103	0.0000	4.1600^{***}	3.2800^{***}	1.8182^{***}

[a] Show the effects per variable in each population (Estimated values); [b] denote log-transformed variable.

*** $p< 0.001$; ** $p< 0.01$; * $p< 0.05$; . $p< 0.1$; '*ns*' no significant

For the 90% confidence level, paternity tests assigned 27% (TER), 23% (EJ) and 22% (N) of maternal progenies. However, since we are more interested in depicting an overall picture of mating patterns within and across populations than in estimating individual fitness values, we focused on the results obtained when applied the 80 % confidence level. In this latter case, paternity test assigned a local candidate father for at least half of the maternal progenies surveyed, with the highest proportion found at TER (70%) followed by EJ (57%) and N (48%) (Table 4). The location of the candidate pollen donors that contributed the most to sire maternal progeny within each population is depicted at Fig.1. Notably, some mother trees had all their maternal progeny sired by unsampled pollen donors suggesting a high frequency of immigrant pollen (Table S3). The average *Np* within population was significantly higher at EJ and N compared to TER (ANOVA: $F_{2,58}$= 7.387, *p*-value= 0.001) (Table 2, Fig 2B, Table S3). Similarly, the *Nep* also showed increased values at EJ (7.8) but N and TER exhibited similar values (6.6 and 6.9, respectively, Table 2). None of the study variables showed any impact on *Np* (Table 3).

Table 4: Results of seed parentage analyses in the three studied population of *Z. lotus* by CERVUS software.

Level	Confidence (%)	Critical Delta	Assignments	
			Observed	Expected
El Ejido				
Strict	95	2.96	54 [23%]	139 [58%]
Relaxed	80	0.38	136 [57%]	209 [87%]
Unassigned			104 [43%]	31 [13%]
Total			240 [100%]	240 [100%]
Níjar				
Strict	95	4.49	48 [22%]	77 [35%]
Relaxed	80	1.88	104 [48%]	127 [59%]
Unassigned			112 [52%]	89 [41%]
Total			216 [100%]	216 [100%]
La Tercia				
Strict	95	3.17	8 [27%]	15 [49%]
Relaxed	80	0.73	21 [70%]	24 [79%]
Unassigned			9 [30%]	6 [21%]
Total			30 [100%]	30 [100%]

Average values of male fecundity (*Mf)* were very similar across populations (Table 2, Fig 2C) but N and TER showed wider variation ranges (0.48-2.46 EJ; 0.09-6.61 N; 0.20-6.94 TER). Attending only to the pollen donors with offspring within each population (51 in EJ, 50 in N and 12 in TER), we observed slight variation in the average number of sired embryos per pollen donor (EJ: 2.7, N: 2.1 and TER: 1.8). Notably, the maximum number of embryos sired by only one pollen donor was three times larger at EJ and N compared to TER (10, 11 and 3 embryos, respectively). The individual values of *Mf* was positively impacted by *FD* (t-value = 3.0277, *p*= 0.0035) and negatively by *FVR* (t-value = -1.7332, *p*= 0.0878) (Table 3). Thus, trees with increased values of flower density and /or lower number of floral visits by pollinators are those siring a higher number of embryos. At intra-population level, N was the only population that showed differences among its individuals due also to differences in both *FD* (t-value = 4.3021, *p*< 0.0001) and *FVR* (t-value = -2.5580, *p*= 0.0130) (Table 3). The trends of *Mf* values per population as a function of each explanatory variable are included in Fig. S3.

Migration patterns and ecological correlates

The proportion of migrant pollen donors per mother tree (*m*) showed a wide intra-population variation that ranged on average from 26.43% to 92.33% (EJ: 50.73%-92.33%; N: 26.43%-91.60%; TER: 29.33%-59.88%) (Table S3). EJ showed significant increased mean values of *m* compared to N and TER (ANOVA: $F_{2,58}$= 30.63; *p*-value <0.0001) (Table 2, Fig 2D). This high proportion of migrant pollen concurred with the fat-tailed pollen dispersal kernel (*b*<1) observed for all study populations (ranging from 0.1693 (N) to 0.3166 (TER)) and high average pollen dispersal distances (*δ*) that ranged from 2055 m (EJ) to 4693 m (TER)) (Table 2). The best fitted model for *m* accommodated a Gaussian spatial correlation structure (Table 3), which indicates that *m* was significantly impacted by the spatial location of the mother trees (t-value = 5.8766, *p*< 0.0001). Note that the range of the spatial structure reaches 47.35 m, which broadly coincides with the area covered by the *NEI50* and this spatial effect is stronger at EJ compared to N and TER (Table 3).

DISCUSSION

We found an extensive movement of pollen within and among populations in spite of the intense degradation of the landscape that surrounds all study *Z. lotus* populations. On the one hand, mother trees showed a diverse pool of pollen donors, a high frequency of migrant pollen and a low proportion of selfing events. On the other hand, populations showed moderate- to high- mean values of pollen dispersal distance and exhibited increased proportions of immigrant pollen, which suggests that populations of this insect-pollinated shrub retain high connectivity levels across the landscape. As expected for a highly managed agro-system, population size (*N*), spatial structure (density and spatial arrangement of the adults) and isolation level determined average pollen dispersal distances and the mean number of pollen donors that sired each mother tree. Besides key population features, individual phenotypic traits also influenced mating variables, most notably tree size and flower display, but also pollinator activity. Finally, the spatial location of each tree within population also shows a significant effect in determining mating variables at mother trees.

Overall, habitat loss and landscape changes typically shift mating patterns both within and between plant populations by decreasing gene flow among them (Young et al. 1996) which results in increased frequency of selfing events and a low proportion of migrant pollen (Aguilar et al. 2008). Yet, previous studies have found mixed trends on the effect of fragmentation on the frequency of selfing events. For example, selfing rates increased in other insect-pollinated species with increased landscape fragmentation, as *Gomortega keule* (Lander et al. 2010) or *Eucalyptus socialis* and *E. gracilis* (Breed et al. 2015), but other insect-pollinated trees showed low selfing rates, such as *Cariniana estrellensis* (Guidugli et al. 2016). *Z. lotus* shows a favored outcrossing system, as *C. estrellensis* does (Guidugli et al. 2016) and this would partly explain the consistent low values of selfing events across *Z. lotus* individuals.

According to our results, plant traits and pollinator visits explained individual selfing values. *HEI* had a negative effect on the proportion of selfing events and *FVR* had a positive impact. As we expected and it was previously shown, larger and more conspicuous trees showed a higher frequency of outcrossing events, including those from distant locations. For example, O'Connel (2003) positively related the likelihood of outcrossing with tree height and de-Lucas et al. (2008) evidenced that taller mother trees showed a competitive advantage to attract pollen from various nearby trees. Contrary to our expectations, *FVR* had a positive effect on the proportion of selfing events. Diverse and mobile insect pollinator assemblages usually increased the frequency of outcrossing events within and among populations (Breed et al. 2015). *Z. lotus* benefits from a diverse assemblage of pollinators that includes 82 taxa of insects (see *Capítulo 1*). Yet, it is also known that some abundant pollinators in the study area, such as *Apis mellifera*, are strongly attracted by the most abundant floral resource (Hung et al.

2019). During its blooming period, *Z. lotus* is the main flowering species in the study area, which might enhance that pollinators visits a large number of flowers within the same tree, thus promoting geitonogamous events (i.e, pollen transport among flowers within the same tree; García et al. 2005, Karron et al. 2004, Minnaar et al. 2018). Similarly, Valido et al. (2019) demonstrate that the introduction of beehives within a protected natural area significantly reduced the reproductive performance of endemic plants because honey bees displaced most native pollinators that provided complementary pollination services.

Overall, male fecundity showed similar values among populations (Table 2) although some individuals were clearly more successful in siring fruiting trees than others within each population (Fig. 1). This variance in male fecundity was explained by a positive effect of *FD* and a negative, but marginal effect, of *FVR*. Slight *Np* variations were also detected at intra-population level in the most isolated population (TER, Table 2). This result concurs with previous findings that demonstrate that a greater floral display increases the likelihood of siring progeny, although it may also increase the frequency of selfing events (Barret et al. 1994, Tani et al. 2009, Minnaar et al. 2018). One of the first effects of habitat loss and fragmentation in natural populations is the variation of individual fecundity. Analogous cases have been shown in other agro-system, e.g. Ismail et al. (2012) showed how changes on population density vary the likelihood of outcrossing among individuals, and Nason et al. (1998) associated these negative reproductive effects with pollinator movement limitations by habitat fragmentation.

Our results additionally showed an extensive extra-population pollen flow that resulted surprisingly high in the population inhabiting the most degraded area surrounded by a highly anthropic matrix dominated by greenhouses and scarce patches of native flora (EJ). We expected this matrix would act as a barrier to pollen flow thus preventing pollen immigration (Sork and Smouse 2006). However, altered and fragmented populations do not always exhibit a complete isolation (Lander et al. 2010), particularly for insect-pollinated species (Breed et al. 2015). Thus, in spite that EJ is a small-sized population its closest population is only ca. 2 km further apart (Santa María del Águila, see more details in *Capítulo 1*) and some unnoticed individuals might occur nearby these populations. Solitary individuals across agro-systems are documented to act as stepping-stone structures that favour pollen flow among nearby populations (Kwak et al. 1998, Lander et al. 2010). These solitary and scattered individuals frequently receive high values of long-distance pollen movement, since they act as food source and shelter for insect pollinators. Thus, their presence has been associated with great genetic connectivity levels between natural remnant-patches even within highly fragmented agro-forest landscapes (Ismail et al. 2012). Lander et al. (2010) underlined the fundamental role of these individuals to maintain the resilience of natural populations, reducing their risk of inbreeding and local extinction within altered landscape. Our models also show that the spatial location of individuals within population determines their migration rates, and that all study populations exhibited high pollen dispersal distances (from 2055 m to 4693 m). These favorable values are probably related with their distance to surrounding isolated individuals or nearby populations, the spatial structure of each population and the great mobility of insect pollinators, especially honey bees, that may be involved in long-distance pollen dispersal since they are able to move several km from their hives to foraging patches across disturbed landscapes (Steffan-Dewenter and Kuhn 2003). The lower pollen dispersal distance were showed for the more reduced population (EJ), where habitat remnant patches are surrounded by a highly altered landscape but where the *Z. lotus* population is internally dense, while the highest values were detected in the most scattered population but where adults specimens are linearly distributed within a large occupation area (ca. 3500 m in length; TER, see Fig 1).

Overall, our results showed similar trends to those previously observed for temperate and tropical species inhabiting agro-systems, with the level of population isolation as the most important factor determining mating patterns (see review of Sork and Smouse 2006, but also Nason et al. 1998, Gauzere et al. 2013, Garcia et al. 2005, Breed et al. 2012a,b). As in temperate and tropical species, factors that determined mating patterns in our study species include flower density, plant height and pollinator activity. However, we detected some interesting differences with other habitats. On the one hand, temperate and tropical forests usually show closed-canopy forests that might prevent the arrival of pollen from distant locations or isolated trees (e.g., *Prunus mahaleb* Garcia et al. 2005 or *Fagus sylvatica* Gauzere et al. 2013). But semiarid habitats show an open and homogenous canopy structure that might instead favor pollen movement which might explain why high-density areas show reduced pollen dispersal distances (Robledo-Arnuncio and Gil 2005, Sork and Smouse 2006). Our results also supported this trend, showing higher pollen dispersal distance and gene flow compared to other reduced or fragmented habitats from template and tropical areas (see Robledo-Arnuncio and Gil 2005, Garcia et al. 2007, Santos et al. 2016, Chybicki and Oleksa 2018, Kassa et al. 2018). These results suggest different functional response of mating traits to land-use changes in arid and semiarid compared to tropical and template species. Future studies, as meta-analyses, should investigate if these patterns are observed in other semi-arid agrosystems.

In spite of our thorough sampling and genotyping effort, some questions remained unclear. For example, none of the ecological variables considered in this study explained the number of pollen donors per mother tree. Barret and Harder (2017) underlined that the mating likelihood of plant species is conditioned by complex associations between variables, where biotic and abiotic factors could be also play an important role. So, future studies should be performed at the landscape level (Fahrig 2003) to evaluate the impact of features present in the matrix in addition to the effect of features measured at the population level. Additionally, note that we found a high proportion of seeds from one population failed to germinate (Table 1 and S3), which might suggest that fruiting trees from this population are subjected to particularly stressful growing conditions that hamper seed germination. This study only spans early demographic stages (effective pollination) but further studies should investigate the long-term consequences of observed mating patterns across successive demographic stages (fruiting, germination, survival and mortality). This will allow us to gain a comprehensive understanding of the impact of different agriculture practices on the persistence and viability of highly threaten species that will allow us to meet one of the most challenging sustainable goals: meeting food security needs while preserving remnant biodiversity (Kremen and Merenlender 2018).

ACKNOWLEDGEMENTS

Thanks to C. García for the opportunity of collaboration and her her help throughout all the development of this chapter; E.K. Klein for providing MEMMi software, and for his help and assistance during the analysis and interpretation of results; J.M. Bastida and F. Camacho for their help in field samplings and microsatellites genotyping; F. Valera, J.L. González-Rebollar and A.B. Robles for their advice and assistance during field samplings; A.M. Fernández-Ocaña for her useful guidance during the laboratory work; CICT of Universidad de Jaén for fragment sequencing work; Junta de Andalucía and M.A. Carrión (Dirección General de Medio Ambiente del Gobierno de Murcia) for facilitating permits for Almería and Murcia field samplings, respectively. This work was funded by a Junta de Andalucía Excelencia RNM-766 project and by funds from Fondo Europeo de Desarrollo Regional (FEDER). A. González-Robles and A.J. Manzaneda were supported by Universidad de Jaén fellowship (Plan Propio - Acción 16, 2013-2014) and a Ramón y Cajal contract (RYC-2010-06237), respectively. C. García was funded by

Fundação para a Ciência e a Tecnologia (FCT) through the Independent Fellowship IF-*Investigador* program (IF/01375/2012).

REFERENCES

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches. *Mol Ecol*, 17, 5177–5188.

Anon (1992) Directive 92/43 of the council of the European community on the conservation of habitats and wild fauna and flora. European Community, Brussels.

Barrett SCH, Harder LD (2017) The Ecology of Mating and Its Evolutionary Consequences in Seed Plants. *Annu Rev Ecol Evol Syst*, 48, 135–157.

Barrett SCH, Harder LD, Cole WW (1994) Effects of Flower Number and Position on Self-Fertilization in Experimental Populations of *Eichhornia paniculata* (Pontederiaceae). *Funct Ecol*, 8, 526–535.

Barton K (2018). MuMIn: Multi-Model Inference. R package version 1.42.1. Available at: https://CRAN.R-project.org/package=MuMIn

Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. *Trends Ecol. Evol*, 23, 453–460.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw*, 67, 1–48.

Breed MF, Gardner MG, Ottewell KM, Navarro CM, Lowe AJ (2012a) Shifts in reproductive assurance strategies and inbreeding costs associated with habitat fragmentation in Central American mahogany. *Ecol Lett*, 15, 444–452.

Breed MF, Marklund MHK, Ottewell KM, Gardner MG, Harris JB, Lowe AJ (2012b) Pollen diversity matters: revealing the neglected effect of pollen diversity on fitness in fragmented landscapes. *Mol Ecol*, 21, 5955–5968.

Breed MF, Ottewell KM, Gardner MG, Marklund MHK, Dormontt EE, Lowe AJ (2015) Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. *Heredity*, 115, 108–114.

Burnham KP, Anderson DR (2002) *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach, second edition*. Springer, New York.

Chybicki IJ, Oleksa A (2018) Seed and pollen gene dispersal in *Taxus baccata*, a dioecious conifer in the face of strong population fragmentation. *Ann Bot*, 122, 409–421.

DeWoody JA, Avise JC (2001) Genetic perspectives on the natural history of fish mating systems. *J Hered*, 92, 167-172.

Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B (2014) Defaunation in the Anthropocene. *Science*, 345, 401–406.

European Commission (2013) Interpretation Manual of European Union Habitats, version EUR 28. Available at: https://eunis.eea.europa.eu/habitats/10099

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annu Rev Ecol Evol Syst*, 34, 487–515.

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, et al. (2005) Global consequences of land use. *Sci*, 309, 570–574.

Galvin KA, Reid RS, Behnke RH, Hobbs NT (2008) *Fragmentation in semi-arid and arid landscapes. Consequences for Human and Natural Systems.* Dordrecht, The Netherlands, Springer.

García C, Arroyo JM, Godoy JA, Jordano P (2005) Mating patterns, pollen dispersal, and the ecological maternal neighbourhood in a *Prunus mahaleb* L. population. *Mol Ecol*, 14, 1821–1830.

García C, Guichoux E, Hampe A (2018) A comparative analysis between SNPs and SSRs to investigate genetic variation in a juniper species (*Juniperus phoenicea* ssp. *turbinata*). *Tree Genet Genomes*, 14,87. doi: https://doi.org/10.1007/s11295-018-1301-x

García C, Jordano P, Godoy JA (2007) Contemporary pollen and seed dispersal in a *Prunus mahaleb* population: Patterns in distance and direction. *Mol Ecol,* 16, 1947–1955.

Gauzere J, Klein EK, Oddou-Muratorio S (2013) Ecological determinants of mating system within and between three *Fagus sylvatica* populations along an elevational gradient. *Mol Ecol* 22, 5001–5015.

Ghazoul J (2005) Pollen and seed dispersal among dispersed plants. *Biol Rev*, 80, 413–443

González-Robles A, Manzaneda AJ, Bastida JM, Harvey N, Jaime R, Salido T, Martínez LM, Fenández-Ocaña A, Alcántara J, Rey PJ (2016) Development and characterization of microsatellite primers in the endangered Mediterranean shrub *Ziziphus lotus* (Rhamnaceae). *Appl Plant Sci*, 4, 1600092. doi: https://doi.org/10.3732/apps.1600092

Guidugli MC, Nazareno AG, Feres JM, Contel EPB, Mestriner MA, Alzate-Marin AL (2016) Small but not isolated: A population genetic survey of the tropical tree *Cariniana estrellensis* (Lecythidaceae) in a highly fragmented habitat. *Heredity*, 116, 339–347.

Harrisson KA, Pavlova A, Amos JN, Tkeuchi N, Lill A, Radford Q, Sunnucks P (2012) Fine-scale effects of habitat loss and fragmentation despite large-scale gene flow for some regionally declining woodland bird species. *Landsc Ecol*, 27, 813–827.

Honnay O, Jacquemyn H (2007) Susceptibility of Common and Rare Plant Species to the Genetic Consequences of Habitat Fragmentation. *Conserv Biol*, 21, 823–831.

Hung K-LJ, Kingston JM, Lee A, Holway DA, Kohn JR (2019) Non-native honey bees disproportionately dominate the most abundant floral resources in a biodiversity hotspot. *Proc R Soc B Biol Sci*, 286. doi: https://doi.org/10.1098/rspb.2018.2901

Ismail SA, Ghazoul J, Ravikanth G, Uma Shaanker R, Kushalappa CG, Kettle CJ (2012) Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of *Dysoxylum malabaricum* in an agro-forest landscape. *Mol Ecol*, 21, 5484–5496.

Janečka JE, Blankenship TL, Hirth DH, William Kilpatrick C, Tewes ME, Grassman LI (2007) Evidence for male-biased dispersal in bobcats *Lynx rufus* using relatedness analysis. *Wildlife Biol*, 13, 38–47.

Kaiser SA, Taylor SA, Chen N, Sillett TS, Bondra ER, Webster MS (2017) A comparative assessment of SNP and microsatellite markers for assigning parentage in a socially monogamous bird. *Mol Ecol Resour*, 17, 183–193.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol*, 16, 1099–1106.

Karron JD, Mitchell RJ, Holmquist KG, Bell JM, Funk B (2004) The influence of floral display size on selfing rates in *Mimulus ringens*. *Heredity*, 92, 242–248.

Kassa A, Konrad H, Geburek T (2018) Mating pattern and pollen dispersal in the wild olive tree (*Olea europaea* subsp. *cuspidata*). *Tree Genet Genomes*, 14,3. doi: https://doi.org/10.1007/s11295-017-1215-z

Klein EK, Carpentier FH, Oddou-Muratorio S (2011) Estimating the variance of male fecundity from genotypes of progeny arrays: evaluation of the Bayesian forward approach. *Methods Ecol Evol*, 2, 349–361.

Klein EK, Desassis N, Oddou-Muratorio S (2008) Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. IV. Whole interindividual variance of male fecundity estimated jointly with the dispersal kernel. *Mol Ecol*, 17, 3323–3336.

Konuma A, Tsumura Y, Lee CT, Lee SL, Okuda T (2000) Estimation of gene flow in the tropical-rainforest tree *Neobalanocarpus heimii* (Dipterocarpaceae), inferred from paternity analysis. *Mol Ecol*, 9, 1843–1852.

Kremen C, Merenlender AM (2018) Landscapes that work for biodiversity and people. *Sci*, 362, eaau6020. doi: https://doi.org/10.1126/science.aau6020

Kwak MM, Velterop O, van Andel J (1998) Pollen and gene flow in fragmented habitats. *Appl Veg Sci*, 1, 37–54.

Lander TA, Boshier DH, Harris SA (2010) Fragmented but not isolated: Contribution of single trees , small patches and long-distance pollen flow to genetic connectivity for *Gomortega keule*, an endangered Chilean tree. *Biol Conserv*, 143, 2583–2590.

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005) Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity*, 95, 255–273.

de-Lucas AI, Robledo-Arnuncio JJ, Hidalgo E, González-Martínez SC (2008) Mating system and pollen gene flow in Mediterranean maritime pine. *Heredity*, 100, 390–399.

Malagnoux M (2007) Arid Land Forest of the World: Global Environmental Perspectives. Forestry Department (FAO). In *International Conference on Afforestation and Sustainable Forests as a Means to Combat Desertification.* Jerusalem, Israel, 14 pp

Mendoza-Fernández AJ, Martínez-Hernández F, Pérez-García FJ, Garrido-Becerra JA, Benito BM, Salmerón-Sánchez E, Guirado J, Merlo ME, Mota JF (2015) Extreme habitat loss in a Mediterranean habitat: *Maytenus senegalensis* subsp. *europaea*. *Plant Biosyst,* 149, 503–511.

Minnaar C, Anderson B, de Jager ML, Karron JD (2018) Plant-pollinator interactions along the pathway to paternity. *Ann Bot*, 123, 225–245.

Monteiro WP, Veiga JC, Silva AR, Carvalho CS, Lanes ECM, Rico Y, Jaffé R (2019) Everything you always wanted to know about gene flow in tropical landscapes (but were afraid to ask). PeerJ, 7, e6446. doi: https://doi.org/10.7717/peerj.6446

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

Mota JF, Peñas J, Castro H, Cabello J (1996) Agricultural development vs biodiversity conservation: the Mediterranean semiarid vegetation in El Ejido (Almeria, southeastern Spain). *Biodivers Conserv*, 5, 1597–1617.

Myers EM, Zamudio, KR (2004) Multiple paternity in an aggregate breeding amphibian: the effect of reproductive skew on estimates of male reproductive success. *Mol Ecol*, 13, 1951–1963.

Nason JD, Herre EA, Hamrick JL (1998). Breeding structure of a tropical keystone plant resource. *Nature*, 391, 385–387

O'Connell LM (2003) *The evolution of inbreeding in western redcedar (Thuja plicata: Cupressaceae)*. PhD Thesis, University of British Columbia. 162 pp.

Ottewell K, Grey E, Castillo F, Karubian J (2012) The pollen dispersal kernel and mating system of an insect-pollinated tropical palm, *Oenocarpus bataua*. *Heredity*, 109, 332–339.

Pérez Latorre AV, Cabezudo B (2009) *Ziziphus* Mill. In: G Blanca, B Cabezudo, M Cueto, C Fernández López and C Morales Torres (eds.), *Flora Vascular de Andalucía Oriental,* 3, 46–50. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2017) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131. Available at: https://CRAN.R-project.org/package=nlme.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Quantum GIS Development Team (2016) Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. Available at: http://qgis.osgeo.org

Rathcke BJ and Jules ES (1993) Habitat fragmentation and plant-pollinator interactions. *Curr Sci*, 65, 273–277.

Rey PJ, Cancio I, Manzaneda AJ, González-Robles A, Valera F, Salido T, Alcántara JM (2018) Regeneration of a keystone semiarid shrub over its range in Spain: habitat degradation overrides the positive effects of plant – animal mutualisms. *Plant Biol*, 20, 1083–1092.

Ritland K (2002) Extensions of models for the estimation of mating systems using *n* independent loci. *Heredity*, 88, 221–228.

Robledo-Arnuncio JJ, Austerlitz F, Smouse PE (2007) Poldisp: a software package for indirect estimation of contemporary pollen dispersal. *Mol Ecol Notes*, 7, 763–766.

Robledo-Arnuncio JJ, Gil L (2005) Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. *Heredity*, 94, 13–22.

Rousset F (2008) GENEPOP’007: A complete reimplementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour*, 8, 103–106.

Santos AS, Cazetta E, Dodonov P, Faria D, Gaiotto FA (2016) Landscape-scale deforestation decreases gene flow distance of a keystone tropical palm, *Euterpe edulis* Mart (Arecaceae). *Ecol Evol* 6, 6586–6598.

Slatkin M (1985) Gene flow in natural populations. *Annu Rev Ecol Evol Syst*, 393-430.

Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landsc Ecol*, 21, 821–836.

Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. *Proc R Soc Lond B Biol Sci*, 270, 569–575.

Tani N, Tsumura Y, Kado T, Taguchi Y, Lee SL, Muhammad N, Ng KKS, Numata S, Nishimura S, Konuma A, Okuda T (2009) Paternity analysis-based inference of pollen dispersal patterns, male fecundity variation, and influence of flowering tree density and general flowering magnitude in two dipterocarp species. *Ann Bot*, 104, 1421–1434.

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus* (*). In: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, 68 p.

Uller T, Olsson M (2008) Multiple paternity in reptiles: patterns and processes. *Mol Ecol*, 17, 2566–2580.

Valido A, Rodríguez-Rodríguez MC, Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Sci Reports*, 91, 9, 4711. doi: https://doi.org/10.1038/s41598-019-41271-5

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Resour*, 4, 535–538.

Vranckx G, Jacquemyn H, Muys B, Honnay O (2011) Meta-Analysis of Susceptibility of Woody Plants to Loss of Genetic Diversity through Habitat Fragmentation. *Conserv Biol*, 26, 228–237.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol Evol*, 11, 413–418.

Zuur AF, Ieno EN (2016) A protocol for conducting and presenting results of regression-type analyses. *Methods Ecol Evol,* 7, 636–645.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

SUPPLEMENTARY MATERIAL

FIGURES

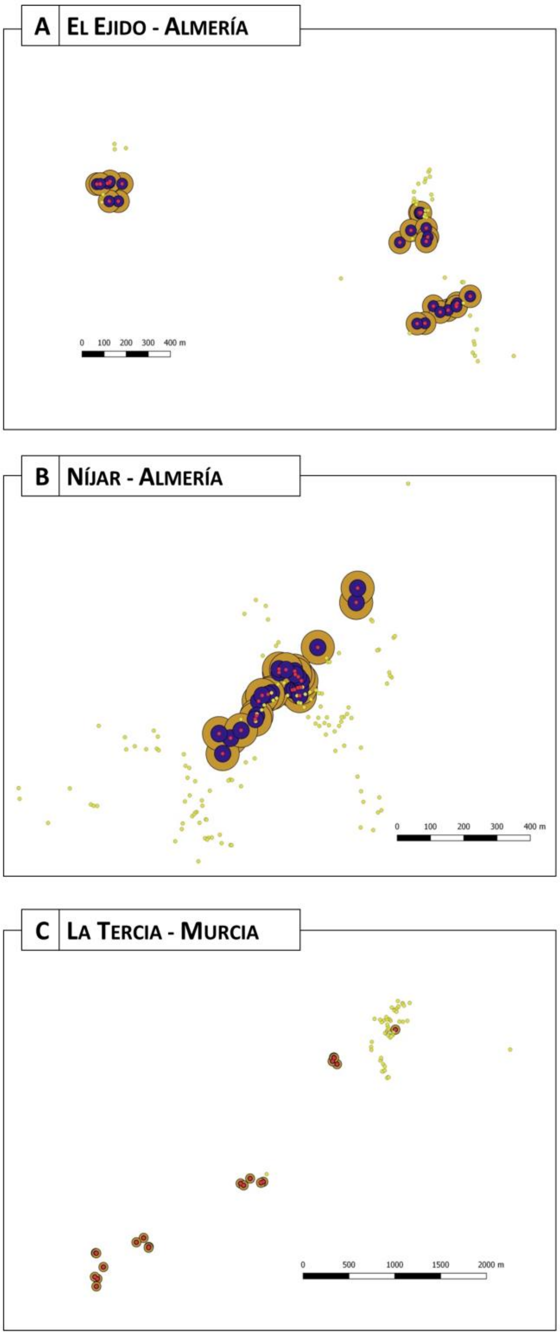


Fig. S1: Green and pink dots denote the spatial distribution of mapped and sampled trees per population: EJ (A), N (B) and TER(C). Green dots indicate pollen donors whereas pink dots show trees sampled as maternal trees. For the latter, a 25 m and 50 m radius circle (purple and brown area, respectively) were defined centered at each tree to characterize their ecological neighborhood.

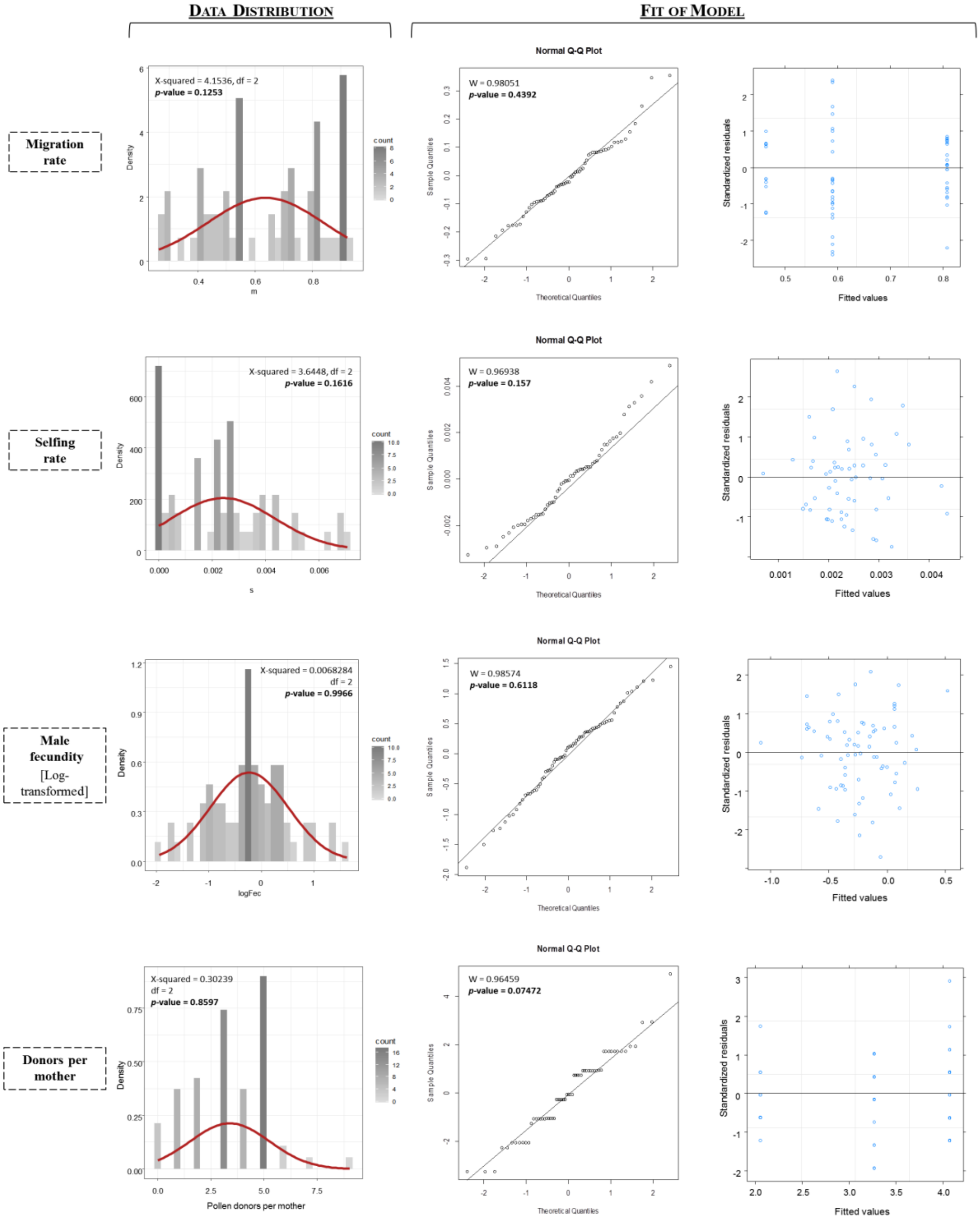


Fig. S2: Normal distribution fit of dependent variables (left column) and LMMs (central and right columns). *Male fecundity* was previously log-transformed to adjust a normal distribution. All models were validated plotting normal Q-Q plots (central column) and their standardized residuals against fitted values (right column).Normality test result for dependent variables and model residuals are underlined in each case. *R*-packages: '*ggplot2*' (Wickham 2016)[1] and '*stats*'[2] (R Core Team 2017).

---

[1] Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer, New York.

[2] R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

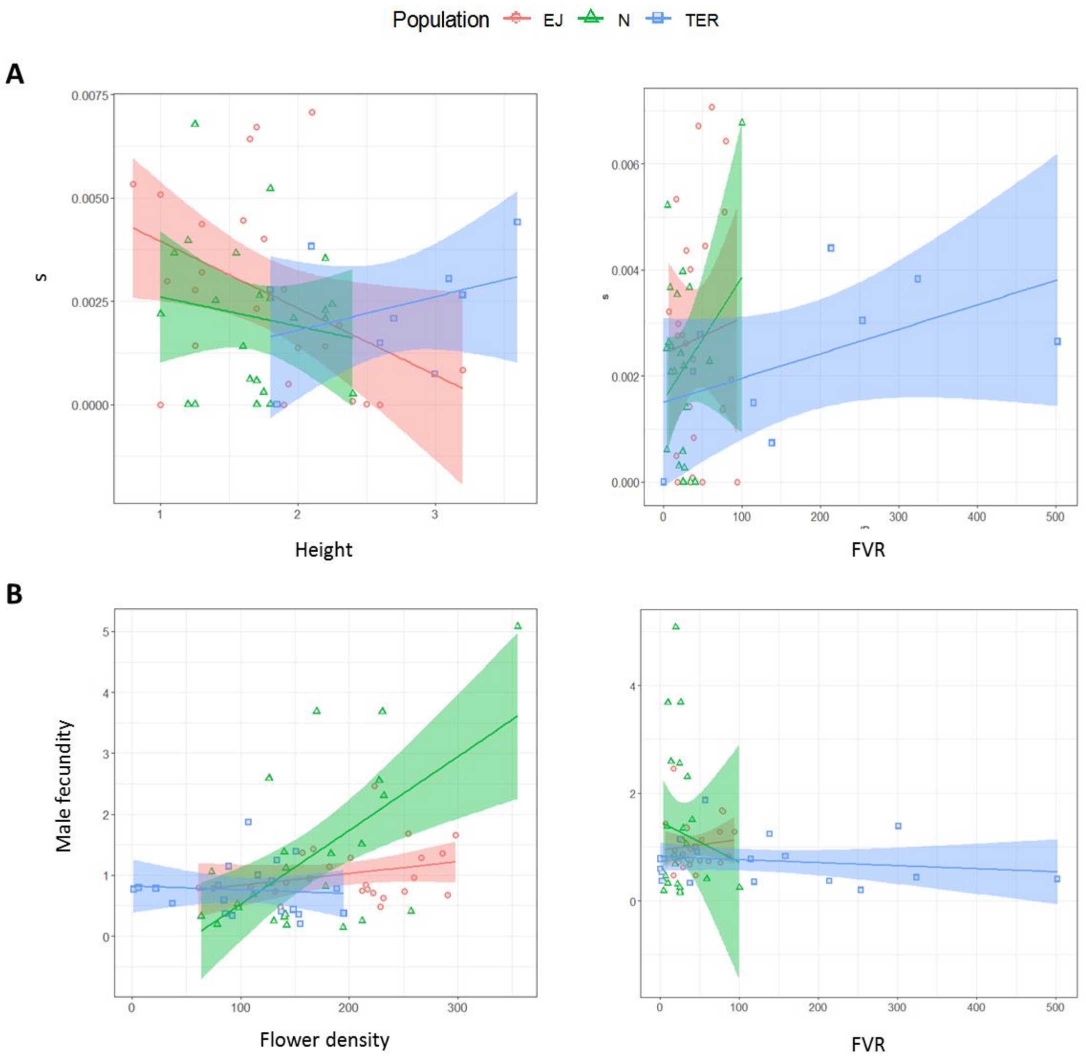


Fig. S3: Model results for selfing rate (A) and male fecundity (B). Effect variation is shown at intra-population level in each case: red (El Ejido), green (Nijar) and blue (La Tercia).

TABLES

Table S1: Details of climatic, geographical and ecological features of studied populations.

	El Ejido	Níjar	La Tercia
Altitude (*m*)	50-80	250-290	250-290
Anual mean temperature (°*C*)	22.5	20.0	20.5
Anual precipitation (*mm*)	253.40	258.60	203.54
Population size (*N adults*)	63	201	78
Natural habitat cover *(% in 1.5 km buffer*)	8.74	40.37	18.09
Distance to nearest population (*m*)	1850	2390	13110
Mean height* (*m*)	1.77	1.68	2.80
Canopy projection area* (m^2)	23.85	33.61	109.66
Flower density[a] *	199.15	162.52	105.85
Pollinator visitation rate[b] (PVR)*	15.00	7.97	18.59
Flower visitation rate[c] (FVR)*	43.58	24.78	161.68
Number of conspecifics within 25 m radius buffer*	1.08	2.68	0.18
Number of conspecifics within 50 m radius buffer*	2.88	7.84	0.63

*Mean values per individual within each population; [a] mean number of flowers/225 cm^2; [b] mean number of insects visiting flowers/3-minute census; [c] mean number of flowers visited by insects per census.

Table S2: Details null allele frequencies and linkage disequilibrium test per locus.

Locus	Frequency of null alleles per population
zlo64	-0.0277
zlo65	0.0081
zlo66	0.0460
zlo67	-0.0179
zlo68	0.1222
zlo71	0.1582
zlo73	0.1365
zlo76[§]	-0.0426
zlo77	-0.0290
zlo79	0.0025
zlo80[§]	-0.0563
zlo86	-0.0092
zlo87	-0.0528
zlo88	-0.0171

[§] Denote significant linkage disequilibrium after pairwise Bonferroni correction

Table S3: Migration rates (*m*), selfing rates (*s*) and number of pollen donor per mother (*Np*) in the three *Z. lotus* study population using MEMMi and CERVUS software.

EJ				N				TER			
Mother	*s* (%)	*m* (%)	*Np*	Mother	*s* (%)	*m* (%)	*Np*	Mother	*s* (%)	*m* (%)	*Np*
EJ1	0.01	89.53	6	N1	0.24	43.85	1	TER1	0.15	54.19	0
EJ2	0.08	69.91	4	N2	0.18	50.27	5	TER2	0.27	40.86	0
EJ3	0.26	91.58	3	N3	0.35	73.50	4	TER3	0.26	42.20	3
EJ4	0.00	90.99	5	N4	0.03	90.84	1	TER4	-	-	-
EJ5	0.14	71.37	2	N5	0.24	91.60	3	TER5	-	-	-
EJ6	0.45	90.90	5	N6	0.26	81.75	5	TER6	0.38	29.33	2
EJ7	0.64	81.85	2	N7	0.21	54.94	5	TER7	0.44	39.16	4
EJ8	0.14	73.20	3	N8	0.06	45.41	5	TER8	0.19	29.75	1
EJ9	0.00	81.89	3	N9	0.52	33.00	3	TER9	0.21	55.28	2
EJ10	0.71	91.67	7	N10	0.21	53.12	3	TER10	0.30	55.10	2
EJ11	0.51	80.01	5	N11	0.26	72.75	4	TER12	-	-	-
EJ12	0.32	50.73	2	N12	0.37	45.84	3	TER13	-	-	-
EJ13	0.30	81.66	3	N13	0.00	30.32	0	TER14	0.00	59.88	2
EJ14	0.53	81.68	3	N14	0.06	26.43	5	TER15	0.07	41.97	1
EJ15	0.40	92.33	3	N15	0.37	27.50	3	TER16	-	-	-
EJ16	0.67	81.74	5	N16	0.68	50.04	4	TER17	-	-	-
EJ17	0.14	74.98	9	N17	0.25	40.34	3	TER18	-	-	-
EJ18	0.28	83.55	4	N18	0.22	46.94	0	TER20	-	-	-
EJ19	0.44	66.74	2	N19	0.00	41.60	5	TER21	-	-	-
EJ20	0.23	69.46	5	N20	0.00	68.89	4	TER22	0.28	55.02	1
EJ21	0.19	80.07	2	N21	0.00	48.01	4				
EJ22	0.05	72.67	5	N22	0.14	54.19	2				
EJ23	0.00	90.73	6	N23	0.03	54.59	5				
EJ24	0.00	85.54	5	N24	0.23	79.05	0				
EJ25	0.28	90.04	5	N25	0.40	64.83	5				

- Denote insufficient offspring for these analyses due to problems with seed fertility

Note: *Np = 0* indicates that all maternal progeny was sired by unsampled pollen donors (from outside the population)

DISCUSIÓN GENERAL

Aunque los ecosistemas mediterráneos han estado históricamente ligados a las actividades humanas, el crecimiento urbano y agrícola desde la última mitad del siglo XX ha provocado grandes pérdidas en la calidad y cobertura de los mismos (Valladares 2014). El sureste semiárido de la Península Ibérica ha sido una de las zonas más afectadas por estos cambios (Puigdefábregas y Mendizábal 1998), mostrando actualmente un paisaje dominado por cultivos agrícolas (en uso o abandono) e importantes núcleos turísticos en sus proximidades a la costa (Mota et al. 1996, Mendoza-Fernández et al. 2015). Lejos de detenerse, el impacto antropogénico en estos hábitats semiáridos sigue creciendo y ha alcanzado no solo una dimensión local sino también regional, incrementando el riesgo de extinción de especies (Benito et al. 2009). Estos hechos ponen de manifiesto la especial importancia que tiene entender cómo la acelerada degradación de los hábitats del sureste semiárido peninsular está afectando a la viabilidad de sus poblaciones vegetales, de tal manera que urge la aplicación de medidas basadas en datos sobre el estado de estos hábitats (Rey et al. 2018).

Los matorrales arborescentes con *Z. lotus* han sido elegidos como objeto de este trabajo no solo porque se encuentran en un serio estado de retroceso y degradación (Tirado 2009, Rey et al. 2018), sino porque definen además uno de los ecosistemas más característicos de estas zonas semiáridas con una flora e historia biogeográfica singular dentro de Europa. La preocupación sobre el estado de conservación de este hábitat es manifiesta y está catalogado de interés prioritario dentro de los hábitats de interés comunitario de la Unión Europea, *Habitat 5220*: Matorrales arborescentes con Ziziphus*. Actualmente, se están llevando a cabo diversas iniciativas de restauración de los mismos dentro de proyectos del programa LIFE de la Comisión Europea (proyecto LIFE Conhabit, LIFE+13 NAT/ES/000586; proyecto LIFE Adaptamed, LIFE14 CCA/ES/000612) y de la Fundación Biodiversidad (proyecto Co-Adapta, CAESCG, Universidad de Almería), los cuales contemplan entre sus actuaciones la retirada de especies exóticas (*Agave sisalana* y *A. fourcroydes*), que fueron durante un tiempo cultivadas y se naturalizaron, o campañas de repoblación con *Z. lotus*. Sin embargo, dichas actuaciones alcanzan solo áreas muy restringidas y dentro de espacios protegidos, fuera de las cuales se constata que el ecosistema sigue desmantelándose por el levantamiento de nuevos invernaderos y otras actividades antrópicas en muchas áreas de las provincias de Almería y Murcia. Entre el elenco florístico de este hábitat, *Z. lotus* destaca por ejercer una función clave y facilitadora para el resto de especies del medio, permitiendo de esta forma la viabilidad de estos ecosistemas (Tirado y Pugnaire 2003, 2005, Tirado 2009). Todos estos aspectos justifican la elección de *Z. lotus* como especie de estudio, al mismo tiempo que la idoneidad del sureste semiárido de la Península Ibérica para llevar a cabo investigaciones sobre el impacto que la intensificación en los usos del suelo tiene sobre las comunidades naturales de plantas en las zonas semiáridas de Europa.

En referente a ello, nuestro trabajo muestra resultados muy relevantes sobre los efectos directos que la alteración antrópica del sureste peninsular tiene sobre las poblaciones remanentes de *Z. lotus* y sobre su viabilidad. Aunque es cierto que tal y como esperábamos las áreas más alteradas por la intensificación agrícola y urbana (especialmente el poniente almeriense) acogen los valores más bajos de diversidad genética, en general, las poblaciones de *Z. lotus* del sureste peninsular muestran altos valores de diversidad, no manifestando así efectos graves de erosión genética como consecuencia de la alteración y pérdida de sus hábitats. Este trabajo detecta que el 87.3% de la variación genética queda explicada dentro de población, dejando sólo un 12.7% fuera de ellas. Sin embargo, esta pequeña variación existente entre poblaciones muestra una gran estructuración regional, que divide nuestra área de estudio en tres zonas bien diferenciadas: poniente almeriense, levante almeriense y norte-sur almeriense-murciano. Aunque el aislamiento geográfico es el principal factor de explicación de esta estructuración, la degradación a escala de paisaje (en cuanto a cobertura de hábitat natural) empieza a mostrar efectos directos sobre la misma.

Teniendo presente los problemas de dispersión de semillas y regeneración natural constatados en estas poblaciones (ver Cancio et al. 2016, 2017 y Rey et al. 2017, 2018), la gran longevidad y capacidad de rebrote de los individuos de *Z. lotus* parecen ser los principales responsables de estos altos valores de diversidad genética intrapoblacional. Se conoce que las especies de árboles y arbustos tienden a conservar valores de diversidad genética elevados, incluso en ambientes profundamente fragmentados, como consecuencia de su larga vida ("paradoja de los bosques fragmentados", ver Kramer et al. 2008; y también Vranckx et al. 2011), de tal forma que la diversidad genética que presentan actualmente no es más que un reflejo de una situación pasada, en la que probablemente, como debió ser también el caso de *Z. lotus*, la conectividad entre poblaciones e individuos sería muy superior a la actual. Sin embargo, nuestros resultados han subrayado que la pérdida de hábitat natural explica ya, parte de las diferencias genéticas encontradas entre poblaciones, y por tanto de esa estructuración genética-espacial actual, lo que alerta sobre el posible agravamiento del estado genético de las poblaciones de cara a escenarios futuros. Si la degradación de las poblaciones de *Z. lotus* continúan a este ritmo, es previsible que las consecuencias genéticas de la pérdida hábitat aumenten, retroalimentando los problemas de persistencia a largo plazo de sus poblaciones que anuncia el colapso de la regeneración natural por semillas (Rey et al. 2018). En esta especie la dispersión de semillas a larga distancia es promovida por mamíferos carnívoros generalistas de mediano tamaño (Cancio et al. 2016), especialmente el zorro, que además ejerce un control sobre herbívoros (particularmente el conejo) que dificultan la regeneración de la especie (Cancio et al. 2017). Sin embargo, ambos servicios colapsan en situaciones de elevada pérdida del hábitat (Rey et al. 2018). De esta forma, mantener la integridad y mejorar la conectividad de las poblaciones de *Z. lotus* deberían ser uno de los principales objetivos de los planes de gestión y conservación de esta especie. Para ello, es indispensable que dichos planes atiendan no solo a la escala local (evitando la pérdida de individuos longevos y el fallo en regeneración por semillas), sino también a la escala de paisaje para favorecer el flujo génico por dispersión de semillas a larga distancia.

Los resultados referentes a los patrones de cruzamiento y flujo génico de las poblaciones de *Z. lotus*, han sido otro de los aspectos relevantes de este trabajo. Conociendo la disrupción existente en cuanto a interacciones mutualistas planta-dispersores de semillas en las poblaciones ibéricas de *Z. lotus*, y considerando la gran pérdida de hábitat de los últimos 60 años, era esperable que los movimientos de polen entre poblaciones y la fecundidad de los individuos de *Z. lotus* pudieran estar viéndose también afectados como consecuencia de mayor aislamiento inter-poblacional. Sin embargo, nuestros resultados muestran una alta distancia de dispersión de polen, junto con unas elevadas tasas de migración y cruzamiento, en las tres poblaciones estudiadas a pesar del diferente grado de alteración de sus hábitats. Poseer un ensamblaje diverso de insectos polinizadores parece haber supuesto un seguro para el mantenimiento de altos patrones de cruzamiento y flujo génico, los cuales subrayan el papel fundamental que tienen los insectos como conectores de poblaciones de plantas, incluso en sistemas altamente degradados y fragmentados (Breed et al. 2015), especialmente los insectos silvestres. Aunque *Apis mellifera* puede contribuir en parte a estos valores de dispersión a larga distancias, por definir sus movimientos dentro de áreas amplias (Steffan-Dewenter y Kuhn 2003), distintos autores constatan que una vez son atraídas por una fuente determinada, las abejas melíferas tienden a concentrar sus movimientos sobre ese recurso (a veces incluso un solo arbusto o árbol), restringiendo mucho su área de movimiento durante el forrajeo (Aizen and Feinsinger 2003). En cambio, los taxones de insectos silvestres muestran una gran diversidad en los tiempos de persistencia y forrajeo sobre una planta dada, además de en las distancias de vuelo, que a su vez diversifican las distancias de flujo de polen (Herrera 1987, Aizen and Feinsinger 2003). Abejas pequeñas o solitarias (Gathmann and Tscharntke 2002, Ottowell et al. 2012, Brownwe et al. 2015), avispas (Nason et al. 1998), sírfidos (Lander et al. 2010) o incluso coleópteros (Ottowell et al. 2012, Brownwe et al. 2015) han sido reconocidos en distintos trabajos como principales responsables de dispersión de polen a larga distancia en distintas especies de plantas (generalmente hasta 1 km, pero en algunos casos incluso mayores; ver especialmente Nason et al. 1998, y Lander et al. 2010 y sus referencias). Todos estos trabajos subrayan el importante papel que los insectos nativos tienen sobre el mantenimiento de los patrones de flujo génico entre poblaciones de plantas silvestres, los cuales no pueden ser atribuidos únicamente a la presencia *de A. mellifera* (Aizen and Feinsinger 2003). Por ello, entender en qué medida la intensificación agrícola y urbana del sureste peninsular puede estar afectando a la comunidad de insectos polinizadores de *Z. lotu*s, es indispensable a la hora de asegurar la continuidad y conservación de la su poblaciones ibéricas. A tenor de nuestros resultados, y aunque el sistema de polinización de *Z. lotus* es muy generalista, nuestro trabajo ya advierte sobre los efectos negativos de la intensificación agraria y urbana sobre el ensamblaje de visitantes florales de *Z. lotus*. Este trabajo sugiere que, aunque *A. mellifera* pueda contribuir a los movimientos de polen a larga distancia, la sobrecarga de abejas melíferas (*A. mellifera*) en hábitats naturales (presumiblemente por su uso excesivo como polinizador en agricultura intensiva) supone una amenaza para los visitantes florales silvestres de *Z. lotus* (tanto en abundancia como en número visitas florales). En cuanto a las escalas de degradación antrópica del hábitat, mientras que la escala local (principalmente por su influencia sobre la densidad de flores de los individuos de *Z. lotus* y la distancia entre poblaciones cercanas) muestra efectos adversos sobre la abundancia y visitas florales de polinizadores silvestres de *Z. lotus,* la degradación a escala de paisaje (en términos de diversidad de usos del suelo) exhibe un impacto negativo sobre la riqueza de insectos visitantes. Dado que todos estos efectos fueron mayores a nivel taxonómico que a nivel funcional, el alto número de especies en los grupos funcionales parece estar actuando como seguro para el cumplimiento de la función de polinización. Por otro lado, nuestros resultados correlacionales muestran que, en general, los insectos silvestres no pertenecientes al grupo de las abejas destacaron por ser los taxones más sensibles a todos estos cambios, mientras que las abejas silvestres (en el caso de *Z. lotus*, abejas solitarias típicamente de mediano-pequeño tamaño) resultaron menos afectadas. Estos resultados son un tanto inesperados puesto que algunos estudios han mostrado que otros grupos de insectos dependen menos de los hábitats naturales que las abejas silvestres (Rader et al. 2016). Así, diferentes grupos de dípteros como las moscas y los bombílidos utilizan con frecuencia hábitats muy alterados, incluyendo paisajes con alta intensificación agrícola (Winfree et al. 2011, Raymond et al. 2014, Trillo et al. 2018). Por el contrario, las abejas silvestres son consideradas organismos muy sensibles a cambios de hábitat y paisaje, o a desplazamientos competitivos o de otro tipo (por ejemplo, transmisión de patógenos) por parte de abejas comerciales (abejas domésticas de la miel o abejorros) (Kleijn et al. 2012, Holzschuh et al. 2016). A pesar de ello, trabajos recientes también han mostrado que determinadas especies de abejas silvestres aunque muestran preferencias por hábitats naturales son capaces aprovechar recursos de hábitat urbanos o alterados (Collado et al. 2019). Nuestros resultados son probablemente debidos a que los taxones que nos pertenecen al grupo de las abejas son especies que presentan inherentemente menores números poblacionales en nuestros hábitats de estudio, por lo que cualquier tipo de alteración que afecte a su presencia, puede hacerlos desaparecer de ellos. Dado que nuestra área de estudio se encuentra inmersa dentro de una gran matriz antrópica con distintas gradaciones de perturbación según la zona geográfica, quizás algunas de las abejas silvestres detectadas en los censos son especies capaces de sobrevivir en medios antrópicamente alterados, aprovechando recursos de ellos, y de esa manera vean atenuados los efectos negativos de la degradación de sus hábitats naturales. Así, el conjunto de estos resultados deja patente, por un lado, la necesidad de regulación de los usos de *A. mellifera* como polinizador de cultivos comerciales en las provincias de Almería y Murcia debido a sus efectos adversos sobre los insectos silvestres, uniéndonos de esta forma a las llamadas de atención hechas por distintas investigaciones en otras zonas agrarias por los mismos motivos (ver Holzschuh et al. 2016, Torné-Noguera et al. 2016, González-Varo y Vilà 2017, Magrach et al. 2017, Valido et al. 2019); y por otro lado, de nuevo, la importancia de establecer medidas de conservación y gestión de este hábitat no solo a escala local, sino también a escala de paisaje, la cual de ser ignorada puede llevar a ineficientes resultados de las actuaciones de manejo.

En otro orden de ideas, otro aspecto interesante de nuestro resultados es que la altura y densidad de flores de los individuos reproductores de *Z. lotus* son las principales características fenotípicas que inciden positivamente sobre la probabilidad de cruzamiento de sus individuos. Normalmente, hábitats mejor conservados muestran individuos más longevos, con mayores dimensiones y, por tanto, con mayor disponibilidad floral. Sin embargo, sorprendentemente, un número elevado de visitas florales en los individuos de *Z. lotus* parece asociarse negativamente con los eventos de polinización cruzada. A *priori*, resulta contradictorio pensar que una mayor visita de insectos, se asocie inversamente con la probabilidad de cruzamientos entre plantas distintas. Sin embargo, en nuestro caso, la presencia de abejas melíferas podría ser la responsable de estos efectos, ya que nuestros censos poblacionales muestran un gran número de visitas florales por parte de ellas. Teniendo en cuenta que cada individuo de *Z. lotus*, durante el periodo de floración, cuenta con un número muy elevado de flores disponibles (frecuentemente miles), la gran atracción de *A. mellifera* hacia recursos florales dominantes del medio podría estar causando un gran número de visitas dentro del mismo individuo (Aizen and Feinsinger 2003, Hung et al. 2019), las cuales, al acabar generalmente en cruzamientos fallidos, por la obligada polinización cruzada de la especie (ver Zietsman y Botha 1992, Asatryan and Tel-Zur 2013, para otras especies del mismo género), disminuyen las posibilidades de esos individuos de formar descendencia. En cualquier caso, las limitaciones de polinización de la especie parecen menores que las limitaciones a la dispersión de semillas reflejadas en las mismas poblaciones por Cancio et al. (2016) y Rey et al. (2017). Datos propios no reflejados en esta memoria (González-Robles et al. 2016) sugieren fructificaciones significativas en la mayoría de las poblaciones, con tasas fructificación cuyo promedio poblacional varía entre 950 y más de 130 000 frutos/individuo adulto (n=18 poblaciones). Estos valores indican que la formación de fruto, y por tanto la función de polinización, parece no ser el factor limitante de la falta de regeneración y envejecimiento de estas poblaciones, aunque diferencias detectadas entre poblaciones indicaron que este proceso también está siendo afectado por la alteración del paisaje y degradación interna de las poblaciones. Futuros trabajos analizarán con más detalle estos efectos sobre la fructificación de las poblaciones de *Z. lotus*.

En el contexto de todos los resultados expuestos, para el mantenimiento de los flujos de polen de las poblaciones de *Z. lotus* es necesario prestar atención a las comunidades de polinizadores silvestres y a todas aquellos factores que suponen una amenaza para los mismos, tales como la sobrecarga de abejas melíferas en el medio, los cambios de calidad de los hábitats de *Ziziphus* a escala local y de cobertura del hábitat a nivel de paisaje. Además, y a pesar de los eficientes patrones de dispersión de polen mostrados en nuestras poblaciones, un buen sistema de cruzamiento por muy eficiente que sea carece de utilidad si la dispersión de semillas del sistema es ineficiente (Sork and Smouse 2006). Por tanto, estos resultados matizan que aunque nuestra especie de estudio cuenta con características propias que están ayudando a paliar los efectos negativos de la degradación y alteración de sus poblaciones, es urgente que las medidas de conservación prioricen la mejora de conectividad e integridad de sus hábitats (ej. aprovechando la estructura de ramblas como conectores naturales de hábitats). Esto favorecerá no solo a los procesos dispersivos de semillas a larga distancia y a la regeneración natural de las poblaciones, sino que además contribuirá a la conservación de los insectos polinizadores silvestres y facilitará sus movimientos entre parches. De lo contrario, si la pérdida de hábitat continúa, es esperable que las poblaciones actuales acaben sufriendo problemas de erosión genética y aquellas poblaciones más pequeñas y aisladas terminen por desaparecer.

En cuanto a futuros marcos de trabajo interesantes dentro de estos hábitats destaca que, aunque *Z. lotus* es definida como una especie fundamental del sistema su rol ecológico de especie clave aún está por demostrar. Así, futuros estudios deberían abordar la identificación operacional de *Z. lotus* como especie clave en este ecosistema bajo la hipótesis de que efectivamente cumple tal rol. Ello podría abordarse mediante análisis de topología de la red, junto con la caracterización del grado y centralidad de las especies. Redes de interacciones planta-polinizador y de reclutamiento planta-planta son candidatas idóneas para tal identificación. De verificarse dicho rol para *Z. lotus*, podría demandarse con mayor rigor una gestión del hábitat centrada en esta especie.

Esperamos que los resultados sobre diversidad genética, flujo génico y polinización de *Z. lotus* de este trabajo de tesis, unidos a la información previa sobre dispersión y estado de regeneración de la especie a lo largo de su rango de distribución en la Península Ibérica, sirvan de apoyo y herramienta para establecer medidas de gestión y conservación adecuadas de estos hábitat prioritarios del sureste peninsular y de su especie 'clave', *Z. lotus*, además de para mejorar la calidad de los mismos. Desde un contexto más general, esperamos que contribuyan a comprender la magnitud de los efectos que la intensificación de los usos del suelo tiene sobre las poblaciones de plantas naturales y sus hábitats, especialmente las prácticas agrícolas bajo invernadero, que están teniendo una gran expansión por todo el mundo; asimismo que a cubrir el hueco de conocimiento existente sobre todos estos temas en los hábitats áridos/semiáridos.

REFERENCIAS

Aizen MA, Feinsinger P (2003) Bees not to be? Responses of insect pollinator faunas and flower pollination to habitat fragmentation. En: GA Bradshaw, P Marquet (eds) *How landscapes change*. Springer, Berlin, Heidelberg, pp. 111–129.

Asatryan A, Tel-Zur N (2013) Pollen tube growth and self-incompatibility in three *Ziziphus* species (Rhamnaceae). *Flora*, 208, 390-399.

Benito BM, Martínez-Ortega MM, Muñoz LM, Lorite J, Peñas J (2009) Assessing extinction–risk of endangered plants using species distribution models as: a case study of habitat depletion caused by the spread greenhouse. *Biodivers Conserv*, 18, 2509-2520.

Breed MF, Ottewell KM, Gardner MG, Marklund MHK, Dormontt EE, Lowe AJ (2015) Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. *Heredity*, 115, 108–114.

Browne L, Ottewell K, Karubian J (2015) Short-term genetic consequences of habitat loss and fragmentation for the neotropical palm *Oenocarpus bataua*. *Heredity*, 115, 389–395.

Cancio I, González-Robles A, Bastida JM, Isla J, Manzaneda AJ, Salidos T, Rey PJ (2017) Landscape degradation affects red fox (*Vulpes vulpes*) diet and its ecosystem services in the threatened *Ziziphus lotus* scrubland habitats of semiarid Spain. *J Arid Environ*, 145, 24–34.

Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Salido T, Rey PJ (2016) Habitat loss exacerbates regional extinction risk of the keystone semiarid shrub *Ziziphus lotus* through collapsing the seed dispersal service by foxes (*Vulpes vulpes*). *Biodivers Conserv*, 25, 693–709.

Gathmann A, Tscharntke T (2002) Foraging ranges of solitary bees. *J Anim Ecol*, 71, 757–764.

González-Robles A, Rey PJ, Manzaneda AJ, Salidos T (2016) Effects of landscape and intra-habitat degradation in plant-pollinator associations in *Ziziphus lotus*, a keystone scrub from threatened European semiarid habitats. En: Annual Meeting British Ecological Society (BES), 11-14 December. ACC, Liverpool, UK.

González-Varo JP, Vilà M (2017) Spillover of managed honeybees from mass-flowering crops into natural habitats. *Biol Conserv*, 212, 376–382.

Herrera CM (1987) Components of pollinator “quality”: comparative analysis of a diverse insect assemblage. *Oikos*, 50, 79–90.

Holzschuh A, Dainese M, González-Varo JP, Mudri-Stojnic S, Riedinger V, et al. (2016) Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecol Lett*, 19, 1228–1236.

Hung K-LJ, Kingston JM, Lee A, Holway DA, Kohn JR (2019) Non-native honey bees disproportionately dominate the most abundant floral resources in a biodiversity hotspot. *Proc R Soc B Biol Sci*, 286.

Klein AM, Brittain C, Hendrix SD, Thorp R, Williams N, Kremen C (2012) Wild pollination services to California almond rely on semi-natural habitat. *J Appl Ecol*, 49, 723–732.

Kramer AT, Ison JL, Ashley MV, Howe HF (2008) The paradox of forest fragmentation genetics. *Conserv Biol*, 22, 878–885.

Lander TA, Boshier DH, Harris SA (2010) Fragmented but not isolated: Contribution of single trees , small patches and long-distance pollen flow to genetic connectivity for Gomortega keule , an endangered Chilean tree. *Biol Conserv*, 143, 2583–2590.

Magrach A, González-Varo JP, Boiffier M, Vilà M, Bartomeus I (2017) Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. *Nat Ecol Evol*, 1, 1299–1307.

Mendoza-Fernández AJ, Martínez-Hernández F, Pérez-García FJ, et al. (2015). Extreme habitat loss in a Mediterranean habitat: *Maytenus senegalensis* subsp. *europaea*. *Plant Biosyst,* 149, 503–511.

Mota JF, Peñas J, Castro H, Cabello J, Guirado J (1996) Agricultural development versus biodiversity conservation: the Mediterranean semiarid vegetation in El Ejido (Almería, southeastern Spain). *Biodivers Conserv*, 5, 1597–1617.

Nason JD, Herre EA, Hamrick JL (1998) The breeding structure of a tropical keystone plant resource. *Nature*, 391, 685–687.

Ottewell K, Grey E, Castillo F, Karubian J (2012) The pollen dispersal kernel and mating system of an insect-pollinated tropical palm, *Oenocarpus bataua*. *Heredity*, 109, 332–339.

Puigdefábregas J, Mendizábal T (1998) Perspectives on desertification: western Mediterranean. *J Arid Environ*, 39, 209–224.

Rader R, Batomeus I, Garibaldi L, Garratt MPD, Howlett B, Cunningham S A, et al. (2016) Non-bee insects are important contributors to global crop pollination. *Proc Natl Acad Sci*, 113, 146–151.

Raymond L, Sarthou JP, Plantegenest M, Gauffre B, Ladet S, Vialatte A (2014) Immature hoverflies overwinter in cultivated fields and may significantly control aphid populations in autumn. *Agric, Ecosyst Environ*, 185, 99–105.

Rey PJ, Alcantara JM (2014) Effects of habitat alteration on the effectiveness of plant-avian seed dispersal mutualisms: Consequences for plant regeneration. *Perspect Plant Ecol Evol Syst*, 16, 21–31.

Rey PJ, Cancio I, González-Robles A, Bastida JM, Manzaneda AJ, Valera F, Salido T, Alcántara JM (2017) Local-scale and landscape disturbances impact through distinct pathways on the regional variation in seed dispersal by mammals in threatened semiarid habitats. *Perspect Plant Ecol Evol Syst*, 28, 10–18.

Rey PJ, Cancio I, Manzaneda AJ, González-Robles A, Valera F, Salido T, Alcántara JM (2018) Regeneration of a keystone semiarid shrub over its range in Spain: habitat degradation overrides the positive effects of plant – animal mutualisms. *Plant Biol*, 20, 1083–1092.

Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landsc Ecol*, 21, 821–836.

Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. *Proc R Soc Lond B Biol Sci*, 270, 569–575.

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus* (*). En: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, 68 p.

Tirado R (2009) 5220 Matorrales arborescentes con *Ziziphus* (*). En: VV.AA., *Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, 68 p.

Tirado R, Pugnaire FI (2003) Shrub spatial aggregation and consequences for reproductive success. *Oecol*, 136, 296–301.

Tirado R, Pugnaire FI (2005) Community structure and positive interactions in constraining environments. *Oikos*, 111, 437–444.

Torné-Noguera A, Rodrigo A, Osorio S, Bosch J (2016) Collateral effects of beekeeping: Impacts on pollen-nectar resources and wild bee communities. *Basic Appl Ecol*, 17, 199–209.

Trillo A, Herrera JM, Vilà M (2018) Managed bumble bees increase flower visitation but not fruit weight in polytunnel strawberry crops. *Basic Appl Ecol*, 30, 32–40.

Winfree R, Williams NM, Dushoff J, Kremen C (2007) Native bees provide insurance against ongoing honey bee losses. *Ecol Lett*, 10, 1105–1113.

Valido A, Rodríguez-Rodríguez MC, Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Sci Reports*, 91, 9, 4711. doi: https://doi.org/10.1038 /s41598-019-41271-5 1

Valladares F, Benavides R, Rabasa SG, Díaz M, Pausas JG, Paula S, Simonson WD (2014) Global change and Mediterranean forests: current impacts and potential responses. En: DA Coomes, DFRP Burslem, WD. Simonson, *Forests and global change*, pp 47–75.

Vranckx G, Jacquemyn H, Muys B, Honnay O (2011) Meta-Analysis of Susceptibility of Woody Plants to Loss of Genetic Diversity through Habitat Fragmentation. *Conserv Biol,* 26, 228–237.

Zietsman PC, Botha FC (1992) Flowering of *Ziziphus mucronata* subsp. *mucronata* (Rhamnaceae): anthesis, pollination and protein synthesis. *Bot Bull Acad Sinica*, 33, 33-42.

CONCLUSIONS

The main results of this study could be summarized in the following conclusions:

1. Iberian *Z. lotus* populations have maintained historically high levels of genetic diversity, exhibiting most of genetic variation within populations.

2. Three main genetic clusters define the genetic structure of *Z. lotus* populations in the semiarid southeast of Iberian Peninsula (West, Core and East regions), which are closely associated to the geographic location of *Z. lotus* populations. This genetic geographic structuring seems to have mainly arisen due to isolation by distance.

3. Some life-history traits of this species, as its long-life span, sprouting ability and pollination system, are probably buffering the erosion of the genetic diversity of Iberian *Z. lotus* populations. However, the intense habitat loss and degradation of its habitats, mainly through natural habitat cover shifts (landscape degradation scale), are already impacting on the genetic component (diversity and structure) of *Z. lotus* populations.

4. The increase of managed honey bee densities (mainly *Apis mellifera*) within natural ecosystems constitutes a huge threat for the wild pollinator assemblages of *Z. lotus*, impacting on their abundance, composition and floral visits.

5. Anthropogenic disturbance at the habitat and landscape scales show different effects on the insect visitor assemblage of *Z. lotus*. While shifts of individual flower density and distance between nearby populations (habitat or local degradation scale) affect the abundance and floral visits of wild insect visitors; changes of land-use diversity (landscape degradation scale) impact on the floral visitor richness of *Z. lotus*. Therefore, it is important that the conservation plans for this habitat consider acting simultaneously on both scales.

6. Overall, the functional insect visitor assemblage exhibits more spatial stability to anthropic alterations than the taxonomic assemblage, which suggests that the high taxonomic diversity of the visitor assemblage may be acting as an insurance for

pollinator service of this species. Nevertheless, non-bee taxa seem to be more sensitive wild pollinator groups than wild bees to anthropic alterations (managed honey bee intrusions, habitat degradation and landscape disturbance).

7. Despite the intensive anthropic degradation of its habitats, *Z. lotus* populations exhibit important long-distance pollen movements and high pollen immigration rates which are mainly favored by its diverse pollinator assemblage, including some long-distance mobile pollinators (honey bee among them) but also by the characteristic open structure of arid and semiarid habitats.

8. Although male fecundity is non-homogenous among individuals within *Z. lotus* populations, their mating pattern variables show great spatial stability to anthropogenic disturbance. Individual height, flower density and floral visitation rates by insects are the main influencing factors on *Z. lotus* mating patterns. Nonetheless, other population abiotic factors could be impacting on *Z. lotus* seed fertility.

General conclusion: Besides stopping habitat and landscape degradation, ensuring the connectivity among *Z. lotus* habitat remnants is one of the main management measures to improve the quality of its natural habitats within the highly-altered landscapes of the semiarid southeast of Iberian Peninsula. This will contribute to the conservation of wild insect pollinators, and will favor long-distance pollen movements among *Z. lotus* habitats. The conservation of active and diverse *Z. lot*us pollinator assemblage will help to counteract the deleterious effects of habitat fragmentation and intense agriculture management, providing also high resilience levels.

A pesar de que las comunidades de *Ziziphus lotus* (L) Lam definen uno de los hábitats europeos de interés prioritario (HIC 5220*), el conocimiento existente sobre las mismas es notablemente inferior al que se tiene sobre otros hábitats de interés comunitario. Estas circunstancias, junto a la acusada regresión y alteración de sus hábitats, justifican el interés y la oportunidad de esta Tesis Doctoral.

Universidad
de Jaén