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Interacción entre polinizadores y la planta exótica *Hedysarum coronarium* a distintas escalas espaciales

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CERTIFICA:

Que Ana Montero Castaño, Licenciada en Biología por la Universidad Complutense de Madrid, ha realizado bajo su dirección el trabajo titulado “Interacción entre polinizadores y la planta exótica *Hedysarum coronarium* a distintas escalas espaciales” y que a su juicio reúne los méritos suficientes para optar al grado de Doctor en Biología.

Y para que así conste, firma el presente documento en Sevilla a 10 de junio de 2014.

Fdo: Montserrat Vilà Planella

A mis padres

A María y Carlos, mis hermanos

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Resumen

Interacción entre polinizadores y la planta exótica *Hedysarum coronarium* a distintas escalas espaciales

Las invasiones biológicas son un componente del Cambio Global que en algunas regiones constituyen la segunda mayor amenaza para la biodiversidad y el funcionamiento de los ecosistemas, tan sólo por detrás de la pérdida y fragmentación del hábitat.

Las actividades humanas han permitido, ya sea de forma intencionada o accidental, la llegada de especies a lugares fuera de su área de distribución natural. Sobre todo en los últimos 200 años, con la intensificación del comercio internacional y el desarrollo de grandes infraestructuras.

De estas especies exóticas que aparecen fuera de su área de distribución nativa debido a la acción humana, algunas consiguen superar una serie de barreras bióticas y abióticas convirtiéndose en invasoras. El éxito o fracaso de un proceso de invasión, depende entre otras cosas de las interacciones mutualistas que las especies exóticas establezcan con las especies residentes en su nueva área de distribución. Las interacciones mutualistas más influyentes en los procesos de invasión de plantas exóticas son la simbiosis con micorrizas y con bacterias fijadoras de nitrógeno, la polinización y la dispersión de frutos y semillas mediadas por animales. La importancia de estos mutualismos varía a lo largo de las distintas fases del proceso de invasión. Esta Tesis se enmarca en el papel de las interacciones de polinización entre una planta exótica y los insectos polinizadores de la comunidad receptora. Interacciones que serán determinantes en la superación de las barreras reproductivas.

Las plantas exóticas entomófilas suelen integrarse rápidamente en las redes planta-polinizador, recibiendo la visita de polinizadores residentes generalistas. Al mismo tiempo, las plantas en flor de la comunidad receptora se verán afectadas por la compartición de polinizadores con la exótica. El efecto puede ser positivo si, por ejemplo, la presencia de la exótica, al aumentar la oferta floral, atrae a una mayor cantidad de polinizadores a la zona (efecto magnético). Por el contrario, puede ser negativo si monopoliza las visitas de los polinizadores residentes o si implica la deposición de polen heteroespecífico en los estigmas de las plantas residentes. Tanto el signo como la magnitud de estos efectos dependen de factores que actúan a distintas escalas espaciales y que son tanto intrínsecos de las especies implicadas como propios del medio donde se desarrollan. Consecuentemente, el efecto de una especie exótica sobre la polinización de las plantas de la comunidad receptora, también dependerá de la escala espacial de estudio.

El objetivo general de esta Tesis es comprender la relación bidireccional entre los procesos de invasión de plantas exóticas entomófilas y las relaciones mutualistas de polinización. Este objetivo se divide en dos aspectos. En primer lugar, investigar el papel de la polinización como facilitadora de la capacidad reproductiva de una especie entomófila en su área de introducción. En segundo lugar, estudiar el efecto de esta introducción sobre la polinización de las plantas residentes en la comunidad receptora a distintas escalas espaciales. Este objetivo general se divide en los siguientes objetivos específicos, cada uno de los cuales es abordado en un capítulo de la Tesis:

1. Comparar las distintas etapas del proceso de polinización de plantas entomófilas en sus áreas de distribución nativa y de introducción (**Capítulo 1**).
2. Estudiar el efecto directo e indirecto de las plantas exóticas entomófilas en la polinización y éxito reproductivo de plantas nativas a una escala de vecindario (**Capítulo 2**).
3. Comprender el efecto de las plantas exóticas entomófilas en la estructura y funcionamiento de las redes planta-polinizador nativas a una escala local (**Capítulo 3**).
4. Analizar si los cultivos de floración masiva de especies exóticas entomófilas afectan a la polinización de los hábitats naturales adyacentes a través del vertido de polinizadores (*spill-over*) a escala de paisaje (**Capítulo 4**).
5. Cuantificar a escala global el efecto de las especies exóticas en los patrones de polinización de plantas nativas y compararlo con el efecto que tiene la alteración del paisaje (**Capítulo 5**).

Para abordar estos objetivos, elegimos como especie de estudio *Hedysarum coronarium*, una leguminosa bianual o perenne de corta vida, con inflorescencias con hasta 30 flores ricas en polen y néctar. Es una especie auto compatible pero necesita polinizadores, principalmente abejas, para su fecundación. Es Nativa de la Cuenca Mediterránea occidental pero no de las Islas Baleares, y se introdujo en Menorca alrededor de 1860 como planta forrajera. Desde entonces sigue siendo cultivada y además se ha naturalizado en cunetas, bordes de caminos y áreas con cierto grado de perturbación. La existencia de poblaciones naturalizadas y cultivadas de *Hedysarum* la convierten en una buena especie de estudio para explorar su efecto a distintas escalas espaciales, ya que sus cultivos pueden considerarse parches homogéneos de invasión a gran escala.

En el **Capítulo 1**, se analizaron las distintas etapas del proceso de polinización (tasa de visitas, cargas de polen en insectos y en estigmas y producción de frutos y semillas) en poblaciones de *Hedysarum* nativas e introducidas. Se observó que *Hedysarum* se integra en la dieta de los polinizadores de las comunidades receptoras. Al igual que en su área nativa, se comporta como generalista, recibiendo la visita de 20 especies de polinizadores (aunque se estima que el número sea mayor), en su mayoría dípteros, coleópteros y sobre todo abejas. La abeja de la miel realiza más del 80% de sus visitas.

A pesar de integrarse en la dieta de los polinizadores en Menorca, *Hedysarum* presenta limitación polínica debido a que la abundancia de polinizadores es insuficiente. Por lo que otros factores, como por ejemplo la presión de propágulos proveniente de los cultivos, deben de ser más determinantes que la polinización para su persistencia y expansión en Menorca.

En el **Capítulo 2**, se exploró el efecto de *Hedysarum* en la polinización y éxito reproductivo de individuos de la especie nativa *Muscari comosum* a una escala de vecindario (1 m de radio alrededor de las plantas nativas). En concreto, se exploró si este efecto es directo, a través de los polinizadores compartidos, y/o indirecto, a través de la modificación de la comunidad floral receptora. A través de su despliegue floral *Hedysarum* ejerce un efecto magnético directo sobre *Muscari* atrayéndole polinizadores, principalmente la abeja de la miel. Simultáneamente, a través de su parte vegetativa, *Hedysarum* interacciona con otras plantas por el uso de otros recursos (nutrientes, agua, luz, herbívoros, etc.), disminuyendo la diversidad floral en su vecindario. Esta menor diversidad se asocia con mayores tasas de visitas y menor fructificación de *Muscari*. De este modo, *Hedysarum* también ejerce un efecto indirecto sobre la polinización y éxito reproductivo de *Muscari*. Los efectos directos e indirectos son aditivos para la tasa de visitas, pero se contrarrestan para la fructificación. Por tanto, la mayor tasa de visitas no se traduce en un mayor éxito reproductivo de *Muscari* en los vecindarios invadidos.

En el **Capítulo 3**, a una escala local (parcelas de 20 x 20 m), se exploró el efecto de *Hedysarum* a nivel de toda la red planta-polinizador y si éste está influenciado por la similitud en morfología floral de *Hedysarum* con las plantas residentes. A esta escala, el efecto magnético de *Hedysarum* desaparece. De hecho, desde un punto de vista cuantitativo, la presencia de *Hedysarum* no altera ni la tasa de visitas, ni el grado de generalización, ni el solapamiento de nicho, ni cuán de dependientes son los polinizadores de las plantas residentes (*species strength*). Esta falta de efecto es independiente de la similitud en morfología floral entre *Hedysarum* y las plantas residentes; es decir, *Hedysarum* no compite por los polinizadores con mayor intensidad con otras leguminosas que con plantas que no lo son.

Sin embargo, a esta misma escala local, la polinización de las plantas en flor acompañantes sí se ve alterada en términos cualitativos por la presencia de *Hedysarum*, ya que la identidad de las interacciones planta-polinizador cambia en las comunidades invadidas. *Hedysarum* monopoliza las visitas de la abeja de la miel de modo que los recursos florales de las plantas acompañantes quedan accesibles para otros polinizadores. El cambio en la identidad de las interacciones planta-polinizador tiene implicaciones en la estructura de las redes planta-polinizador invadidas, que aumentan de forma moderada pero generalizada su modularidad y asimetría, mientras que se mantienen anidadas. Parece que, independientemente de la identidad de las interacciones, y por ende de la presencia de *Hedysarum*, las redes planta-polinizador tienden a estructurarse de forma anidada, lo cual probablemente minimice la competencia interespecífica y maximice la coexistencia de especies y biodiversidad en estas comunidades.

En el **Capítulo 4** se analizó si la presencia de cultivos de *Hedysarum* en el paisaje (radio de 500 m) afecta a la polinización de los hábitats naturales adyacentes a través del vertido de polinizadores (*spill-over*), concretamente de abejas. Los cultivos monopolizan a la abeja de la miel, que es atraída no sólo desde zonas adyacentes sino desde distancias superiores a los 500 m, mientras que las otras abejas parecen compartirlas con las comunidades adyacentes. Así, existe un vertido de abejas desde el paisaje circundante hacia los cultivos. Después de la siega de los cultivos, el vertido de abejas en el otro sentido debe de estar espacialmente diluido ya que no se observa un aumento en la abundancia de abejas en las zonas adyacentes. Por todo ello, en paisajes agrícolas como el menorquín, que son heterogéneos, parcheados y dominados por la abeja de la miel, los cultivos de floración masiva no parecen favorecer las comunidades de abejas de las áreas adyacentes.

Finalmente, para poner en un contexto global los efectos de las invasiones biológicas sobre los polinizadores y enmarcar en él nuestros resultados, en el **Capítulo 5** se llevó a cabo una revisión bibliográfica, que incluyó un total de 143 estudios que exploran el efecto sobre las comunidades de polinizadores de las invasiones biológicas o de la alteración del hábitat, dos de los principales componentes del Cambio Global. A partir de estos datos, que se analizaron con técnicas de meta-análisis, se observa que las invasiones biológicas tienen un efecto negativo sobre las comunidades de polinizadores de similar magnitud que el de la alteración del hábitat. El efecto se debe principalmente a la disminución de las tasas de visitas de insectos pertenecientes a grupos distintos de las abejas. Cabe destacar también que este efecto negativo en las tasas de visitas se debe más a la invasión por parte de animales que por parte de plantas.

De los resultados de esta Tesis se concluye que el efecto de una planta exótica entomófila en la polinización de las plantas residentes en la comunidad invadida varía en función de las escalas espacial y temporal de estudio. Además, el efecto también difiere, e incluso llega a ser contradictorio, en función del grupo de polinizadores en el que se centre el estudio, así como del nivel de organización al que sea evaluado el efecto (especie o comunidad). Queda así patente la complejidad de mecanismos e

impactos de una planta invasora en la polinización de plantas nativas en el área de introducción y la importancia de las aproximaciones multiescala para su estudio.

Abstract

Interacción entre polinizadores y la planta exótica *Hedysarum coronarium* a distintas escalas espaciales

Biological Invasions are a Global Change component that in some areas represent the second threat to biodiversity and ecosystems functioning, only behind habitat loss and fragmentation.

Human activities, intentionally or not, have assisted the arrival of non-native species to areas outside their natural ranges of distribution. This phenomenon has increased in the last two centuries, mainly due to the intensification of international trade and the expansion of infrastructures.

Some non-native species are able to overcome the biotic and abiotic barriers they encounter in the introduced region and become invasive. The success or failure of an invasion process depends, among other factors, on the mutualistic relationships that the non-native species establish with the resident species in their new area. The main influential mutualistic relationships in the invasion process of non-native plants are those with N-fixing bacteria, mycorrhizal fungi, pollinators and fruit and seed dispersers. The importance of these mutualistic relationships differs along the stages of the invasion process. This Thesis is focused in the role of pollination interactions between a non-native plant and the resident pollinators; which are decisive in overcoming reproductive barriers.

Non-native plants usually become well integrated into resident plant-pollinator networks by generalist pollinators, affecting co-flowering native plants through shared pollinators. The effect of non-native plants on the pollination of native plants varies from facilitative to competitive. Native plants can benefit from non-native plants if the latter attract more shared pollinators to the community (i.e. magnet effect). However, native plants can be negatively affected if the non-native plants monopolize pollinator visits or increase heterospecific pollen deposition on native stigmas. Both the sign and magnitude of these effects depend on a wide variety of factors acting at different spatial

scales, whether these factors are intrinsic to the species involved or environmental factors. Subsequently, the effect of a non-native species on the pollination of co-flowering natives will also depend on the spatial scale of study.

This Thesis aims at understanding the bidirectional relationship between plant invasions and pollination mutualistic relationships. This general objective embraces two main aspects. First, to investigate the role of pollination in facilitating or constraining the reproductive success of an entomophilous non-native plant in an introduced area. Second, to study the effect of such introduction on the pollination of co-flowering resident species at different spatial scales. The general objective is divided into the following specific objectives, each one addressed in one chapter of the Thesis:

1. To compare different stages of the pollination process of an entomophilous plant species in its native and introduced areas (**Chapter 1**).
2. To study the direct and indirect effects of an entomophilous non-native plant on the pollination and reproductive success of co-flowering native plants at the neighbourhood scale (**Chapter 2**).
3. To understand the effect of an entomophilous non-native plant on the structure and functioning of plant-pollinator networks in the recipient community at the local scale (**Chapter 3**).
4. To analyze whether mass flowering crops of an entomophilous non-native species affect the pollination of enthomophilous plants in adjacent natural habitats through a pollinator spill-over at the landscape scale (**Chapter 4**).
5. To quantify the effect of non-native species on the pollination patterns of native plants at the global scale and to compare this effect with that of landscape alteration (**Chapter 5**).

In order to approach these objectives, *Hedysarum coronariun* was selected as the study species. This biannual or short-lived legume species has inflorescences with up to 30 flowers rich in pollen and nectar that are self-compatible but require pollinators, mainly bees, to set fruits. Native of the western Mediterranean basin but absent in the Balearic Islands, it was introduced in Menorca as a forage plant around 1860. Since then, it has been cultivated but has also escaped from crops and

naturalized in roadsides and disturbed areas. Its naturalized and cultivated populations make *Hedysarum* a good study species to explore effects at different spatial scales as crops can be considered as monospecific invaded patches at the large scale.

In **Chapter 1**, different stages of the pollination process were analyzed (visitation rates, pollen loads on insects and stigmas, and fruit and seed sets) in *Hedysarum* native and introduced populations. We found that *Hedysarum* is integrated in the diet of resident pollinators in the introduced area. In a similar manner as in its native area, it behaves as a generalist, been visited by 20 pollinator species (though the estimated number is even higher), mainly dipterans, coleopterans and mostly bees. The honeybee achieves more than the 80% of the visits.

Despite *Hedysarum* been integrated in the diet of resident pollinators, it is pollen limited due to an insufficient abundance of pollinators. Thus, other factors, as for instance the propagule pressure from crops, might be more influential than pollination on the survival and expansion of *Hedysarum* in Menorca.

In **Chapter 2**, we explored the effect of *Hedysarum* on the pollination and reproductive success of the native species *Muscari comosum* at the neighbourhood scale (1 m radius around the target native plants). Specifically, we explored whether the effect was direct, i.e. through shared pollinators, and/or indirect, i.e. through the alteration of the recipient floral community. Due to its floral display, *Hedysarum* exerted a direct magnet effect on *Muscari* by attracting pollinators, mainly the honeybee. Simultaneously, through its vegetative parts, *Hedysarum* interacted with resident plants for the use of other resources (nutrients, water, light, herbivores, etc.), decreasing the floral diversity in its neighbourhood. Lower floral diversity is associated with higher visitation rates and lower fructification of *Muscari* target plants. Therefore, *Hedysarum* has also an indirect effect on the pollination and reproductive success of *Muscari*. Direct and indirect effects are additive for visitation rates but counteract each other in the case of fructification. That is, a higher visitation rate does not necessarily translate into higher reproductive success of *Muscari* target plants in invaded neighbourhoods.

In **Chapter 3**, we explored the effect of *Hedysarum* on the entire plant-pollinator networks at a local scale (20 x 20 m plots); and whether this effect was influenced by the similarity in flower morphology between *Hedysarum* and resident plants. At this scale, the magnet effect of *Hedysarum* disappears. Quantitatively, neither visitation rate, linkage level, niche overlap, nor species strength of co-flowering resident plants are modified by *Hedysarum* presence, irrespectively to their similarity in flower morphology with *Hedysarum*. Thus, *Hedysarum* does not compete for pollinators more strongly with other legume species.

However, qualitatively, the pollination of co-flowering plants is affected at the local scale, as the identity of plant-pollinator interactions shifts (i.e. interaction rewiring) when *Hedysarum* is present*.* The non-native monopolizes the visits of the honeybee, so that native floral resources are available to other pollinators. The interaction rewiring implies changes in the structure of invaded plant-pollinator networks, which marginally but consistently increase their modularity and asymmetry, while remain nested. It seems that, irrespectively to the identity of the interactions, and therefore to the presence of *Hedysarum*, plant-pollinator networks tend to be nested. This pattern might minimize competition and maximize species coexistence and biodiversity in communities.

In **Chapter 4**, we analyzed whether the presence of *Hedysarum* mass flowering crops in the landscape (500 m radius) affects the pollination of natural adjacent habitats through a pollinator spill-over of bees. Mass flowering crops monopolize the honeybee, which is attracted not only from adjacent areas, but also from distances larger than 500 m. Meanwhile, the other bee species are shared between mass flowering crops and adjacent natural habitats. That is, there is a spill-over of bees from the surrounding landscape to mass flowering crops. Then, after harvesting, the spill-over of bees from mass flowering crops to the surrounding landscape might be spatially diluted as we did not observe an increase of bee abundance in adjacent areas. We conclude that, in agricultural landscapes which are heterogeneous, patchy and dominated by the honeybee like our study site, mass flowering crops do not favor bee communities in adjacent areas.

Finally, in order to detect general trends on the effect of invasions on pollinators and their pollination service and to frame our results in such a global context, in **Chapter 5** we conducted a literature search. We included 143 study cases about the effect on pollinator communities of invasions and habitat alteration, two of the main components of Global Change. These data, analyzed through a meta-analysis, showed that invasions have a similar negative effect on pollinators than habitat alteration. Such negative effect is mainly due to a decrease in the visitation rates achieved by insects different from bees. Moreover, the negative effect is mainly due pollinator invasions rather than to plant invasions.

Overall, we conclude that the effect of an entomophilous non-native plant on the pollination of co-flowering native plants differs within spatial and temporal scales. In addition, the effects differ, and are even contradictory, depending on the pollinator taxa and depending on the level of ecological organization focus (i.e. species or community). Therefore, the results of this Thesis highlight the complexity of mechanisms and impacts that a non-native entomophilous plant can have on the pollination of resident co-flowering plants, as well as the importance of conducting multiscale approaches.

Introducción General

Las invasiones biológicas

Las invasiones biológicas son un componente del Cambio Global y en algunas áreas constituyen la segunda mayor amenaza para la biodiversidad y el funcionamiento de los ecosistemas, por detrás de la pérdida y fragmentación del hábitat (Mack *et al.* 2000; McNeely *et al.* 2001).

Las actividades humanas han permitido, ya sea de forma intencionada o accidental, la llegada de especies a lugares fuera de su área de distribución natural. Fenómeno que ha ido en aumento en los últimos 200 años debido a la intensificación del comercio internacional y el desarrollo de infraestructuras (Mooney & Cleland 2001). De estas especies exóticas que aparecen fuera de su área de distribución nativa debido a la acción humana, algunas consiguen superar una serie de barreras bióticas y abióticas convirtiéndose en invasoras. Centrándonos en el caso de las plantas, y siguiendo la clasificación establecida por Pyšek *et al.* (2004), las especies exóticas que superan barreras ambientales y son capaces de sobrevivir pero necesitan de la acción del ser humano para el aporte de nuevos propágulos o individuos, son llamadas subespontáneas. Cuando superan las barreras reproductivas y son capaces de auto mantener sus poblaciones, son llamadas naturalizadas. Finalmente, dentro de las especies naturalizadas, aquellas que superan barreras dispersivas y se expanden rápidamente son las que llamamos invasoras (Richardson *et al.* 2000b) (Fig. 1).

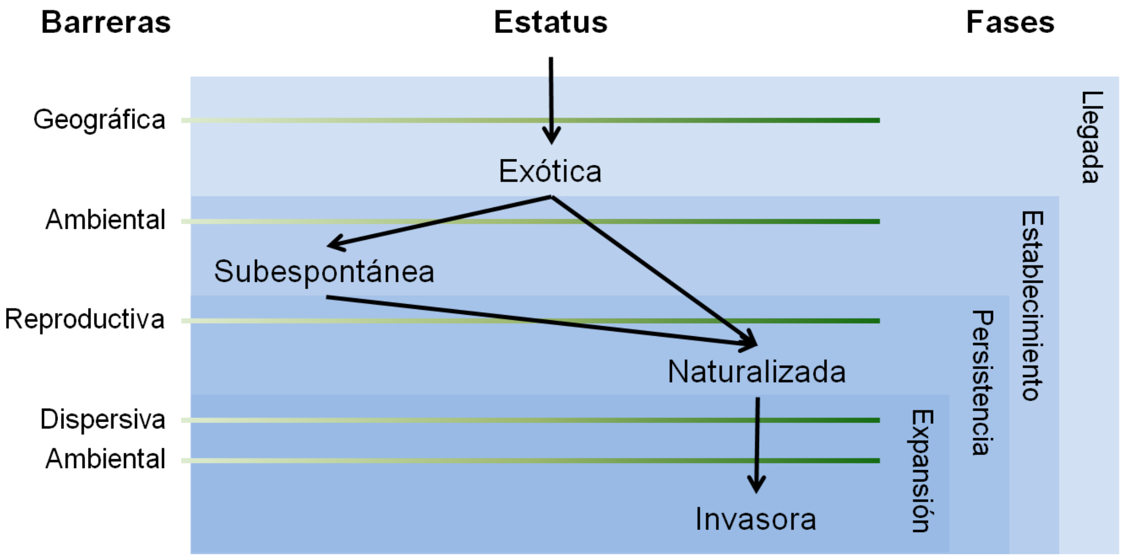

Figura 1. Estatus de las especies exóticas y las fases del proceso de invasión en el que se encuentran en función de las barreras superadas en sus nuevas áreas de distribución. Basado en Richardson *et al.* (2000b), Pyšek *et al.* (2004) y Davis (2009).

La presencia de especies fuera de su área de distribución nativa ya fue advertida por investigadores y naturalistas del siglo XVIII (Davis 2009), pero la aparición de la Biología de las Invasiones como una nueva disciplina científica surge en 1958 con la obra de Elton *The ecology of invasions by animals and plants*. Sin embargo, no fue hasta la década de los 80 del siglo XX cuando las invasiones biológicas empezaron a acaparar mayor atención por parte de la comunidad científica y de la sociedad en general (Richardson & Pyšek 2008). Gran parte de los estudios se han centrado en detectar rasgos comunes a las especies exóticas que puedan explicar su capacidad de invasión (Pyšek & Richardson 2007), así como las características comunes de los hábitats que puedan explicar su mayor susceptibilidad a ser invadidos o invasibilidad (Lonsdale 1999). En los últimos 10 años muchos estudios se han centrado en detectar los impactos de las especies invasoras, que pueden ser de muy diversa naturaleza. Desde impactos económicos (pérdidas de producción agrícola, daños en infraestructuras, etc.) hasta impactos en la salud pública (vectores de enfermedades, agentes alergénicos, etc.) y socio-culturales (interferencia en actividades de ocio y usos tradicionales, modificación de valores estéticos, etc.). En cuanto a los impactos ecológicos, éstos abarcan todos los niveles de organización, desde la hibridación con organismos nativos (Mack *et al.* 2000), hasta la alteración de la estructura y funcionamiento de los ecosistemas (Dukes & Mooney 1999), por ejemplo alterando los ciclos de nutrientes y regímenes de perturbación (Mack *et al.* 2000; Ehrenfeld 2003; Brooks *et al.* 2004).

La idea subyacente en muchos estudios en Biología de las Invasiones es que las relaciones de competencia son las que determinan el éxito o el fracaso de una especie invasora; ya sea competencia por el uso de recursos abióticos como el espacio, la luz, los nutrientes o el agua, o competencia directa con otros organismos (herbívoros, patógenos). De esta idea han surgido algunas de las hipótesis más exploradas en Biología de las Invasiones. Por ejemplo, la Hipótesis del Escape de los Enemigos Naturales (*Enemy Release Hypothesis*), según la cual las plantas exóticas, al dejar atrás, en sus áreas de origen, a sus enemigos especialistas, tienen una ventaja competitiva frente a las nativas, que se enfrentan tanto a enemigos generalistas como a sus especialistas (Richardson & Rejmánek 2011). Otro ejemplo es la Hipótesis de la Resistencia Biótica (*Biotic Resistance Hypothesis*), según la cual los hábitats con mayor diversidad de especies son los menos susceptibles a ser invadidos, ya que las especies exóticas tienen más probabilidad de encontrar su nicho ecológico ocupado por las especies nativas (Levine & Rejmanek 1999).

Sin embargo, el éxito o fracaso de los procesos de invasión también depende de las interacciones mutualistas que las especies exóticas establecen con las especies residentes en su nueva área de distribución (Richardson *et al.* 2000a). Las interacciones mutualistas más influyentes en los procesos de invasión de plantas exóticas son la simbiosis con micorrizas y con bacterias fijadoras de nitrógeno, la polinización y la dispersión de semillas mediadas por animales (Richardson *et al.* 2000a). La importancia de estos mutualismos varía a lo largo de las distintas fases del proceso de invasión (Fig. 1). Así, las interacciones mutualistas con micorrizas serán mucho más determinantes en la fase de establecimiento, mientras que las interacciones con polinizadores y dispersores de semillas serán mucho más determinantes en la persistencia y expansión de las especies exóticas (Pyšek *et al.*

2011). Esta Tesis se enmarca en el papel de las interacciones de polinización entre una planta exótica y los insectos polinizadores de la comunidad receptora.

Polinización de plantas exóticas

Aproximadamente el 90% de las 300000 especies de angiospermas estimadas dependen de forma obligatoria o facultativa de animales para su reproducción sexual (Kearns, Inouye & Waser 1998; Ollerton, Winfree & Tarrant 2011). Estos animales son en su mayoría insectos, aunque algunas lagartijas, aves, murciélagos y otros pequeños mamíferos también pueden desarrollar esta función (Olesen & Valido 2003; Quesada *et al.* 2003; Rodríguez-Rodríguez & Valido 2008).

Clásicamente, la dependencia de polinizadores para la reproducción, se ha relacionado negativamente con la capacidad invasora de las plantas exóticas. Las plantas exóticas requieren encontrar polinizadores adecuados en las áreas de introducción, mientras que las especies de plantas autocompatibles y autógamas no tienen esta limitación (Baker 1955; Kleunen *et al.* 2008). Sin embargo, no faltan ejemplos de plantas invasoras entomófilas obligadas o facultativas como *Senecio inaequidens,* invasora en Europa; *Cytisus scoparius,* invasora en Australia y América o *Lythrum salicaria,* invasora en Norte América (Mal *et al.* 1992; Simpson, Gross & Silberbauer 2005; Vanparys, Meerts & Jacquemart 2008). Para estas plantas exóticas entomófilas, las interacciones que establezcan con los polinizadores residentes serán determinantes para producir semillas y por tanto, para su éxito reproductivo e invasor.

Muchos trabajos de campo muestran que el establecimiento de interacciones con los polinizadores residentes no supone una barrera importante para la invasión por parte de plantas exóticas entomófilas. Las interacciones planta-polinizador son en su gran mayoría generalistas (Jordano 1987), de modo que la mayoría de las especies de plantas son visitadas por varias especies de polinizadores, y viceversa. Es decir, estas interacciones no ocurren de forma aislada, sino inmersas en redes complejas de interacción planta-polinizador. Como todos los sistemas complejos de interacción, las redes de polinización no son sólo la suma de sus componentes, sino que poseen una serie de propiedades emergentes resultado de las interacciones entre éstos (Barabási & Oltvai 2004). De ahí la importancia de los estudios a nivel de comunidad para alcanzar una visión más completa de cómo las plantas exóticas afectan a la polinización de las nativas.

Las plantas exóticas entomófilas suelen integrarse rápidamente en las redes planta-polinizador recibiendo la visita de polinizadores residentes generalistas (Memmott & Waser 2002; Vilà *et al.* 2009), algunos de los cuales pueden ser a su vez exóticos en esa área (Olesen *et al.* 2007). El éxito reproductivo de las plantas exóticas también depende del componente cualitativo de estas interacciones (Aizen & Harder 2007) (Fig. 2). En el caso de polinizadores generalistas, uno de los factores que más puede comprometer la calidad del polen que depositan en los estigmas es la presencia de polen de otras especies distintas a la visitada, lo que se conoce como polen heteroespecífico (Mitchell *et al.* 2009).

De igual manera, las plantas residentes pueden verse afectadas por la compartición de polinizadores con las plantas exóticas (Bjerknes *et al.* 2007). El efecto puede ser positivo si, por ejemplo, la presencia de la exótica, al aumentar la oferta floral, atrae a una mayor cantidad de polinizadores a la zona (Molina-Montenegro, Badano & Cavieres 2008). Por el contrario, puede ser negativo si monopoliza las visitas de los polinizadores residentes (Chittka & Schürkens 2001) o si implica la deposición de polen heteroespecífico en los estigmas de las plantas residentes (Brown & Mitchell 2001).

Las interacciones de polinización vienen determinadas tanto por los factores intrínsecos a las especies implicadas como por los factores del medio en el que se desarrollan (Morales & Aizen 2006) (Fig. 2). De modo que el efecto que una planta exótica tenga en la polinización de las plantas residentes dependerá de cómo afecte a estos factores.

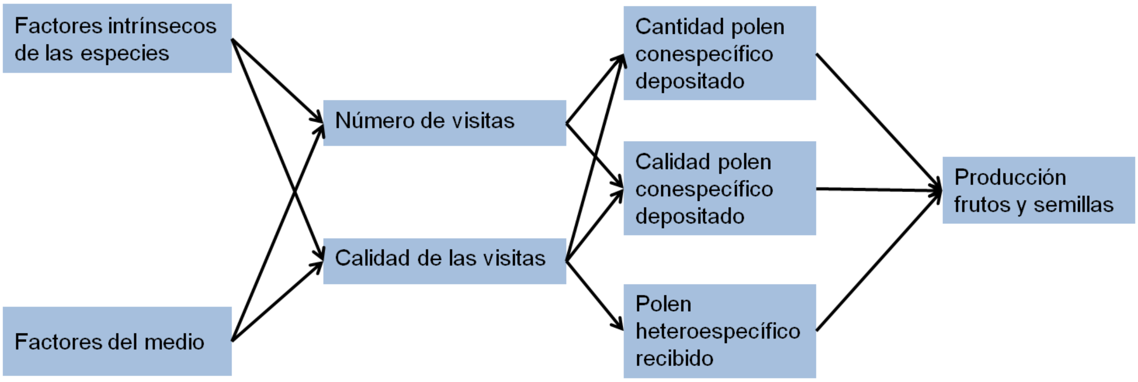

Figura 2. Factores que afectan a los componentes cuantitativo y cualitativo de las interacciones planta-polinizador y los mecanismos subyacentes. Este marco conceptual se centra en el éxito reproductivo desde el punto de vista de la función sexual femenina de las plantas, independiente del origen nativo o exótico de las mismas. Adaptado de Mitchell *et al.* (2009).

Factores intrínsecos como el color y la simetría de la corola definen en gran medida la comunidad de polinizadores que una planta recibe. Por tanto, la similitud en estos rasgos florales entre plantas exóticas y residentes puede determinar la identidad de los polinizadores compartidos, así como el efecto de las plantas exóticas en la polinización de las residentes (Morales & Traveset 2009). La similitud floral podría ser más influyente en el caso de plantas con morfologías florales restrictivas, como es el caso de las leguminosas, a cuyos órganos sexuales no todos los polinizadores son capaces de acceder (Córdoba & Cocucci 2011). Factores del medio, como las abundancias relativas de las especies, también influyen en el comportamiento de los polinizadores (Dietzsch, Stanley & Stout 2011), que buscan maximizar la ingesta de alimento durante su actividad de forrajeo (Armbruster & Herzig 1984). Abundancias relativas altas de plantas exóticas, pueden resultarles beneficiosas en detrimento de las plantas residentes, que serían menos visitadas. Estos son temas que se tratarán en esta Tesis.

Los distintos factores que influyen en los procesos de polinización, ya sean intrínsecos de las especies o del medio, actúan a distintas escalas espaciales; a lo largo de de las cuales varía su importancia relativa (Dorrough *et al.* 2007; Cariveau & Norton 2009). Consecuentemente, el efecto de las plantas exóticas en la polinización de las plantas residentes también variará en función de la escala espacial de estudio.

Los polinizadores perciben y explotan su medio con finalidades diferentes según la escala espacial. A escalas de vecindad o locales (Fig. 3a, b) los polinizadores buscan optimizar su actividad de forrajeo (Burkle & Alarcón 2011), de modo que factores como los rasgos florales de las plantas, el tipo de recurso que ofrecen (polen o néctar), sus abundancias relativas o las condiciones microclimáticas (insolación, humedad) serán los más determinantes del comportamiento de los polinizadores. Sin embargo, a escalas mayores como la escala de paisaje (Fig. 3c), los polinizadores no sólo deben cubrir sus necesidades alimenticias sino también encontrar lugares adecuados donde anidar y cubrir todas sus fases vitales, como períodos larvarios (Cane & Tepedino 2001; Winfree, Bartomeus & Cariveau 2011). Por ello, otros factores como sus distancias máximas de vuelo o los usos del suelo y coberturas vegetales pasan a ser más determinantes en el comportamiento de los polinizadores (Dorrough *et al.* 2007; Burkle & Alarcón 2011).

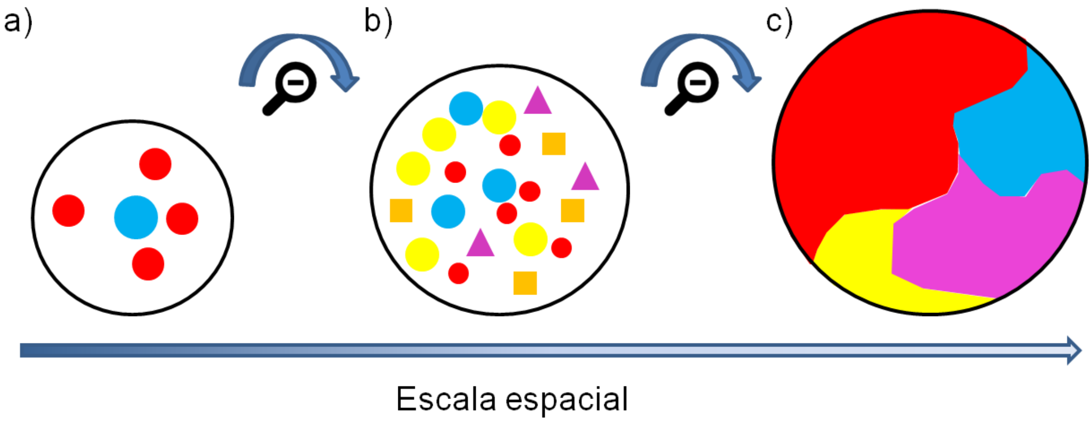

Figura 3. Esquema de cómo los polinizadores perciben su medio y cuáles son los factores potencialmente más influyentes en su comportamiento a distintas escalas espaciales. A escalas pequeñas de vecindad (a) o local (b), factores como la abundancia, color y forma de la corola de las plantas son factores potencialmente importantes mientras que a escala de paisaje (c) la cobertura de cada tipo de formación vegetal puede ser el factor más determinante.

Objetivos de la Tesis Doctoral

El objetivo general de esta Tesis es comprender la relación bidireccional entre los procesos de invasión de plantas exóticas entomófilas y las relaciones mutualistas de polinización. Este objetivo se divide en dos aspectos. En primer lugar, investigar el papel facilitador o limitante de la polinización sobre la capacidad reproductiva de una especie entomófila en su área de introducción. En segundo lugar, estudiar el efecto de esta introducción sobre la polinización de las plantas residentes en la comunidad receptora a distintas escalas espaciales.

Los objetivos específicos son los siguientes:

1. Comparar las distintas etapas del proceso de polinización de plantas entomófilas en sus áreas de distribución nativa y de introducción.
2. Estudiar el efecto directo e indirecto de las plantas exóticas entomófilas en la polinización y éxito reproductivo de plantas nativas a una escala de vecindario.
3. Comprender el efecto de las plantas exóticas entomófilas en la estructura y funcionamiento de las redes planta-polinizador nativas a una escala local.
4. Analizar si los cultivos de floración masiva de especies exóticas entomófilas afectan a la polinización de los hábitats naturales adyacentes a través del vertido de polinizadores (*spill-over*) a escala de paisaje.
5. Cuantificar a escala global el efecto de las especies exóticas en los patrones de polinización de plantas nativas y compararlo con el efecto que tiene la alteración del paisaje.

Para abordar estos objetivos se ha realizado trabajo de campo observacional y experimental y también se ha llevado a cabo una revisión bibliográfica.

Sistema de estudio

La especie exótica de estudio es *Hedysarum coronarium* L. (*Hedysarum* en adelante), conocida comúnmente como zulla, sulla o anclover. Se trata de una leguminosa bianual o perenne de corta vida (Bullitta, Saba & Bullitta 2000; Sulas *et al.* 2000) que puede alcanzar desde los 2 m de altura hasta tener un porte rastrero (Montes Pérez 1993/94, Bustamante *et al.* 1998), en función del ambiente en el que crezca. Sus inflorescencias son racimos con hasta 30 flores zigomorfas ricas en néctar y polen que se abren normalmente entre los meses de abril y mayo (Fig. 4a, b). Es una especie autocompatible pero necesita de insectos para su polinización (Louati-Namouchi *et al.* 2000, Yagoubi y Chriki 2000), principalmente abejas (Louati-Namouchi *et al.* 2000; Satta *et al.* 2000).

Hedysarum es nativa del suroeste de la Península Ibérica y norte de África (Guitiérrez Más 1982; Talavera *et al.* 1988). Se ha introducido a lo largo de la Cuenca Mediterránea como planta forrajera, para control de la erosión, revegetación y para la producción melífera (Flores *et al.* 1997; Satta *et al.* 2000). En la actualidad, ya sea de forma natural o porque ha sido introducida, crece en muchos países de la Cuenca Mediterránea, desde España hasta Turquía (Flores *et al.* 1997).

En Menorca, la más septentrional de las Islas Baleares, *Hedysarum* se introdujo entre finales del siglo XVIII y principios del XIX (Ortells & Campos 1983). Desde 1860 se cultiva como planta forrajera dentro del sistema tradicional agro-ganadero de la isla (Bustamante, Allés & Espadas 2007) (Fig. 4c). Este sistema consiste en cultivar *Hedysarum* durante dos años. Al año siguiente, aprovechando la fijación de nitrógeno y aireación del suelo llevada a cabo por *Hedysarum*, se siembra cereal y el cuarto año se deja en barbecho (Bustamante *et al.* 2007). Este sistema tradicional se ha mantenido con mayores o menores modificaciones, pero no así la superficie dedicada a él, que ha disminuido considerablemente con la intensificación e introducción de nuevos cultivos.

Hedysarum ha escapado de los cultivos y en la actualidad también aparece en cunetas, bordes de caminos, campos abandonados y zonas ruderales. Normalmente se trata de estados sucesionales tempranos de formaciones vegetales dominadas por acebuche (*Olea europaea* ssp. *sylvestris*) y lentisco (*Pistacea lentiscus*), similares a las que ocupa en su área nativa (Fig. 4d).

Al tratarse de una especie cultivada, es difícil discernir si su expansión hacia las áreas naturales o seminaturales se debe a la superación de barreras dispersivas y ambientales o al aporte periódico de propágulos desde las zonas de cultivo. Por lo tanto, es difícil establecer si su estatus es de especie naturalizada o invasora (Fig. 1). En esta Tesis tomamos una posición conservadora y, de acuerdo con el Catálogo de la floral vascular de Menorca (Fraga *et al.* 2004), consideramos a *Hedysarum* naturalizada en Menorca. Los estudios en invasiones están sesgados hacia especies altamente invasoras. Sin embargo, el estudio de exóticas con otros estatus, incluso el estudio de introducciones fallidas, es también necesario para arrojar luz acerca de cuáles son los procesos que facilitan o limitan los procesos de invasión, así como sus efectos. Esto convierte a *Hedysarum* en una especie de estudio interesante.

Respecto al área nativa seleccionada para esta Tesis, fue el sur de la provincia de Cádiz. La elección se basó en que este área, además de pertenecer a la misma región biogeográfica, comparte características climatológicas, paisajísticas y de tipo de vegetación con el área de introducción estudiada. Esto, a diferencia de lo que ocurre en la mayoría de los casos, en los que las plantas son introducidas en regiones biogeográficas muy distantes, permite comparar el comportamiento de una especie entre el área nativa y de introducción de forma más fidedigna.

Finalmente, el que se trate de una especie que sigue siendo cultivada en el área de introducción, convierte a *Hedysarum* en una especie de estudio idónea para estudiar su efecto a distintas escalas espaciales, ya que podemos considerar sus campos de cultivo como parches de invasión homogéneos a una gran escala espacial.

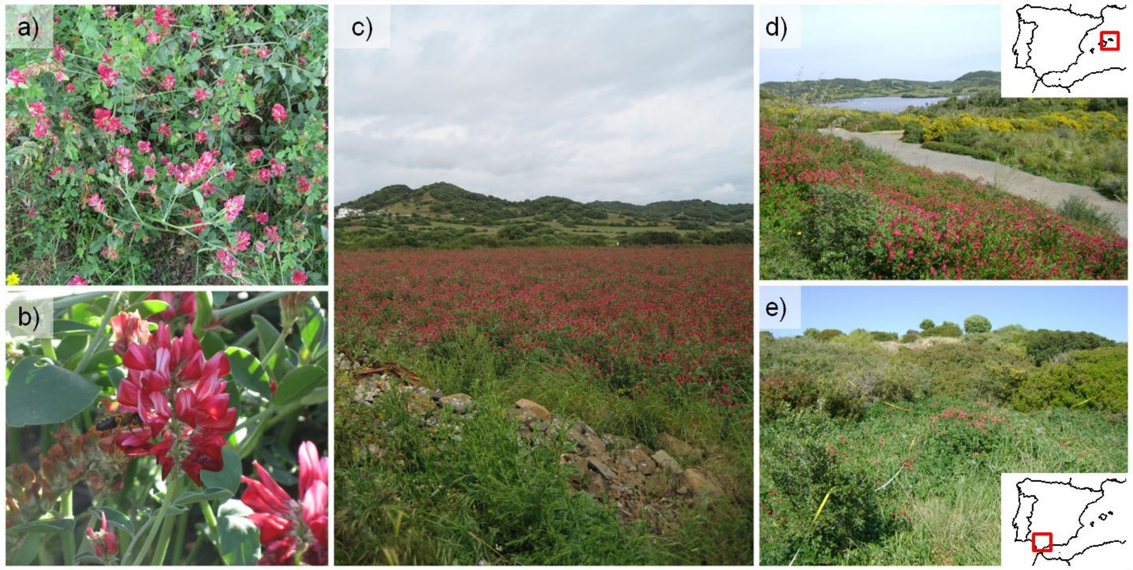

Figura 4. Sistema de estudio**:** (a) planta de *Hedysarum,* (b) detalle de las inflorescencias de *Hedysarum* siendo visitada por una abeja de la miel, (c) campo de cultivo de *Hedysarum* en Menorca, (d) y (e) matorral dominado por acebuches y lentiscos con *Hedysarum* naturalizada en Menorca y como nativa en el sur de Cádiz, respectivamente.

Estructura de la Tesis Doctoral

Además de esta Introducción General, esta Tesis consta de cinco capítulos que responden a los 5 objetivos específicos planteados anteriormente. Estos capítulos corresponden a trabajos publicados o en vías de publicación.

En el **Capítulo 1** (*Pollination ecology of a plant in its native and introduced areas*) se analizan distintas etapas del proceso de polinización (tasa de visitas, cargas de polen en insectos y en estigmas y producción de frutos y semillas) en poblaciones de *Hedysarum* nativas e introducidas para evaluar el papel que tienen las interacciones de polinización como facilitadores del proceso de invasión. Dado el éxito de las poblaciones introducidas, y al tratarse de una especie entomófila obligada, se espera que la eficiencia de cada una de las etapas analizadas sea similar entre las áreas nativas y de introducción

Una vez se observa que *Hedysarum* está integrada en la dieta de los polinizadores residentes en el área de introducción, en los tres siguientes capítulos se estudia en campo el efecto que dicha integración tiene en la polinización de las plantas nativas a distintas escalas espaciales (vecindad, local y paisaje). Además, en cada capítulo se explora cómo el efecto de *Hedysarum* puede verse influido por otros factores, ya sean intrínsecos de las especies implicadas o del medio. Como estos factores actúan a distintas escalas espaciales, en cada capítulo se exploran aquel o aquellos factores potencialmente más influyentes para la escala de estudio en cuestión.

En el **Capítulo 2** (*Direct and indirect influence of non-native neighbours on pollination and fruit production of a native plant species*), se explora el efecto de *Hedysarum* en la polinización y éxito reproductivo de individuos de la especie nativa *Muscari comosum*. En concreto se explora si este efecto es directo, a través de los polinizadores compartidos y/o indirecto, a través de la modificación de la comunidad floral receptora. El estudio se lleva a cabo a una escala de vecindario (a 1 m de radio alrededor de las plantas nativas), que es donde ambos tipos de efectos confluyen.

En el área de introducción *Hedysarum* coexiste con otras plantas entomófilas, no sólo *Muscari*. Además, se espera que su efecto en la polinización de las nativas sea especie dependiente. Por ello, en el **Capítulo 3** (*Interaction rewiring in plant-pollinator networks invaded by a non-native plant*) a una escala local, que para este estudio se considera una parcela de 20 x 20 m, se explora el efecto de *Hedysarum* a nivel de toda la red planta-polinizador (*network analysis*). Como factor de influencia se explora la similitud en morfología floral de las plantas residentes con *Hedysarum*; es decir, el si son leguminosas y por tanto con flores papilionáceas y poco accesibles igual que *Hedysarum*, o si por el contrario tienen flores accesibles.

El hecho de que *Hedysarum* sea una especie de floración masiva cultivada en su área de introducción permite considerar estos cultivos como zonas homogéneas de invasión y explorar su efecto a gran escala. En el **Capítulo 4** (*Mass flowering crops in agricultural landscapes reduce bee abundance and visitation rates in adjacent shrublands*) se analiza si la presencia de cultivos de *Hedysarum* en el paisaje (que para este estudio se establece hasta una distancia máxima de 500 m) afecta a la polinización de los hábitats naturales adyacentes a través del vertido de polinizadores (*spill-over*), concretamente de abejas. Se estudia el vertido de polinizadores tanto en el tiempo (antes y después de la siega de los cultivos) como en el espacio (paisajes con y paisajes sin cultivos de *Hedysarum*).

Posteriormente, en el **Capítulo 5** (*Impact of landscape alteration and invasions on pollinators: a meta-analysis*) se cuantifica el efecto de las especies exóticas en los patrones de polinización de las plantas nativas a escala global. Para este estudio, a diferencia de los capítulos anteriores, se parte de datos bibliográficos que son analizados con técnicas de meta-análisis para contestar a las siguientes preguntas: ¿Existe un efecto neto y en ese caso, cuál es su signo y magnitud?; ¿depende ese efecto de si los estudios son observacionales o experimentales?; ¿varía el efecto entre los distintos taxones de especies exóticas? Para contextualizar los efectos provocados por las especies exóticas dentro del actual escenario de Cambio Global, la magnitud de este efecto se compara con el efecto del principal componente del Cambio Global, la alteración del hábitat.

Finalmente, una vez expuestos estos capítulos, sus resultados son discutidos de forma integrada en la **Discusión General**, donde también se plantean futuras líneas de investigación, así como las principales fortalezas y limitaciones de esta Tesis. La Tesis se cierra con un apartado de **Conclusiones Generales** donde se enumeran las más relevantes, tanto desde un punto de vista teórico como metodológico.

Capítulo 1

Pollination ecology of a plant in its native and introduced areas

Resumen

Las plantas exóticas entomófilas que además requieren polinización cruzada para su fecundación, necesitan integrarse en las redes planta-polinizador residentes para producir semillas y establecerse en su nueva área. Sin embargo, se desconoce cómo los patrones de polinización difieren entre las áreas nativa y de introducción.

Comparamos la identidad y abundancia de polinizadores, las cargas de polen en insectos y en estigmas y la producción de frutos y semillas de *Hedysarum* en poblaciones de sus áreas de distribución nativa y de introdución en España.

En ambas áreas, *Hedysarum* fue visitada por un número similar de especies, principalmente himenópteros, siendo siete de ellas comunes entre el área nativa y de introducción. A pesar de ello, la riqueza, abundancia y tasa de visitas de polinizadores, así como la producción de frutos y semillas, fueron mayores en el área nativa. Las cargas de polen en los estigmas de *Hedysarum* y en la abeja de la miel (*Apis mellifera*), su polinizador más común, no difirieron entre áreas. La menor abundancia de polinizadores podría explicar la menor tasa de visitas a *Hedysarum*, lo que en último término se traduce en la reducción de su producción de frutos y semillas en el área de introducción.

La aproximación biogeográfica llevada a cabo muestra que la integración de una planta exótica en la red planta-polinizador residente no evita que presente limitación polínica en el área de introducción. Por tanto, aunque necesarias, las interacciones mutualistas de polinización no parecen ser limitantes en el éxito invasor de las plantas exóticas entomófilas.

Abstract

Entomophilous and obligate out-crossing non-native plants need to become well integrated in the resident plant-pollinator networks to set seeds and become established. However, it is largely unknown how pollination patterns differ between native ranges and those where plants have been introduced.

We compared the identity and abundance of pollinators, insect pollen loads, pollen deposition on stigmas, and fruit and seed sets of *Hedysarum* in populations from native and introduced ranges in Spain.

In both areas, *Hedysarum* was visited by a similar number of species, mainly hymenopterans; seven species were common between native and introduced areas. However, pollinator richness, abundance, and visits per flower were greater in the native than in the introduced range, as were fruit and seed sets. *Hedysarum* pollen loads on stigmas and on the honeybee (*Apis mellifera*), the most common pollinator, did not differ between areas. Lower abundance of pollinators might be causing lower visitation rates, and to some extent reducing *Hedysarum* fruit and seed sets in the introduced area.

Our biogeographical approach shows that integration of a non-native plant in a resident plant-pollinator network does not prevent pollen limitation in the introduced area. Therefore, despite being necessary, pollination mutualistic relationships might not be the key for non-native plant species invasion success in the introduced area.

Introduction

The invasion success of many non-native plants depends on the mutualistic relationships they establish in the range where introduced (Richardson *et al.* 2000a). For instance, entomophilous and obligate out-crossing non-native plant species require resident pollinators in order to reproduce and to invade (Parker 1997; Chittka & Schürkens 2001; Vanparys *et al.* 2008; Goodell, McKinney & Lin 2010; Gross *et al.* 2010; Rodger, van Kleunen & Johnson 2010). However, most research on the pollination of non-native plant species has focussed on their impact on the pollination and subsequent reproductive success of co-flowering native species (Traveset & Richardson 2006; Bjerknes *et al.* 2007), rather than on the role of pollination in facilitating or constraining their invasion (but see Parker 1997; Parker & Haubensak 2002; Stout, Kells & Goulson 2002; Simpson *et al.* 2005; Gross *et al.* 2010; Rodger *et al.* 2010).

Generalization in pollination is more often the rule than the exception (Jordano 1987; Waser *et al.* 1996), enabling non-native plants quick integration into resident plant-pollinator networks (Memmott & Waser 2002; Vilà *et al.* 2009). In many cases, super-generalist pollinators such as honeybee and bumblebees *Bombus* spp., which have been introduced worldwide and often massively, play a key role in such integration (Stout *et al.* 2002; Simpson *et al.* 2005; Jesse, Moloney & Obrycki 2006; Gross *et al.* 2010).

Not only non-native plants with generalist pollination systems integrate into the resident plant-pollinator communities, but specialist species can also be integrated in different ways. Some may find specialist pollinators if these have wide distribution ranges or have also been introduced there (i.e. “invader complexes” *sensu* Olesen, Eskildsen & Venkatasamy 2002). Other non-native plants may generalize their specialist pollination behaviour, as in the case of *Fuchsia magellanica*, which in its native range in South America is mainly visited by a hummingbird (*Sephanoides galeritus*) (Traveset, Willson & Sabag 1998) while in its area of introduction in Britain is visited by several generalist insects (Valentine 1977). Even self-pollinated plant species may be included in resident plant-pollinator communities, promoting their invasion through increased out-crossing and seed sets, if autonomous self-pollination does not result in the fertilization of all ovules (Aizen & Harder 2007).

In addition to non-native plants being integrated into the resident plant-pollinator community, their pollination success requires pollinator visits to be efficient in terms of quantity and quality of pollen loads transported among conspecific plant individuals (Feinsinger 1987; Aizen & Harder 2007; Mitchell *et al.* 2009). Pollinator species differ in their pollination efficiency. Moreover, subsequent reproductive success of non-native plants also depends on plant variables (availability of abiotic resources, requirement of a minimum threshold of pollen deposition for fruit and seed production, etc.) (Ne'eman *et al.* 2010). Therefore, in some cases, as it has been observed for the invasive *Lonicera maackii,* high visitation rates do not prevent pollen limitation (Goodell *et al.* 2010); while in other cases visitation rates can constitute a good surrogate of reproductive success (Parker 1997; Vázquez, Morris & Jordano 2005). Furthermore, pollinator communities show high inter-annual variability (Roubik 2001; Petanidou *et al.* 2008). Therefore, although this is rarely done (but see Parker 1997; Brown, Mitchell & Graham 2002; Moragues & Traveset 2005; Jesse *et al.* 2006; Dietzsch *et al.* 2011), studies should contemplate more than one season in order to attribute the invasion process of a plant species with the relationships it establishes with the resident pollinator community (Petanidou *et al.* 2008).

Most studies on the role of pollination on invasions have been conducted solely in the introduced range, often with little knowledge of the pollination ecology in its native range. A biogeographical approach comparing native and invaded areas would help to disentangle the processes that enable non-natives to succeed in their new ranges (Hierro, Maron & Callaway 2005; van Kleunen, Weber & Fischer 2010). But, to our knowledge, only the pollination ecology of *Rhododendron ponticum* has been studied from this biogeographical perspective by following a standard field sampling protocols both in the native and introduced areas (Stout *et al.* 2006).

In this study we apply this biogeographical approach to the pollination ecology and reproductive success of an entomophilous plant species whose native and introduced areas have a close regional proximity. Our main questions are: a) Do the identity of pollinators and generalization degree of plant populations differ between native and introduced areas? b) Do pollinator richness, abundance, and visitation rates differ between the two areas? c) How efficient are the visits of the most common pollinators in terms of conspecific pollen loads?, and finally d) Do fruit and seed sets differ between areas? Our hypothesis is that an entomophilous non-native plant species which has become naturalized in a new area might have similar pollination patterns than in the native area. We expect resident pollinators to provide non-native plants a pollination service preventing pollen limitation and allowing for similar seed sets than in their native area.

Materials and methods

Study sites

The study was conducted in two areas of Spain. The native area was located in the province of Cádiz, S Spain, while the introduced area was the NE of Menorca, the northernmost of the Balearic Islands (Fig. 1). These areas have a close regional proximity and share a Mediterranean climate with similar average monthly temperatures around 17ºC, and an average annual precipitation of 600 mm (“AEMET”). We are aware that, as the introduced area is an island, description of patterns of pollination between native mainland areas and introduced insular areas cannot disentangle nativity from insular differences. However, invasions in insular areas by mainland species are highly common phenomena (Kueffer *et al.* 2010) that deserve exploration even if causality cannot be inferred.

In each study area, we selected four 400 m^2 plots of early successional shrublands dominated by *Olea europaea* ssp. *sylvestris* and *Pistacea lentiscus* with a rich herbaceous understorey located in similar landscape types (i.e. dispersed human settlements close to coastal areas). Managed honeybee hives were absent within the 500 m radius around all study plots.

Hedysarum cover (mean ± SD) was similar in both areas (Table A1.1): 49.53 ± 14.92% in Cádiz (native area) and 47.69 ± 26.81% in Menorca (introduced area) plots (Wilcoxon test statistic = 10, P = 0.69).

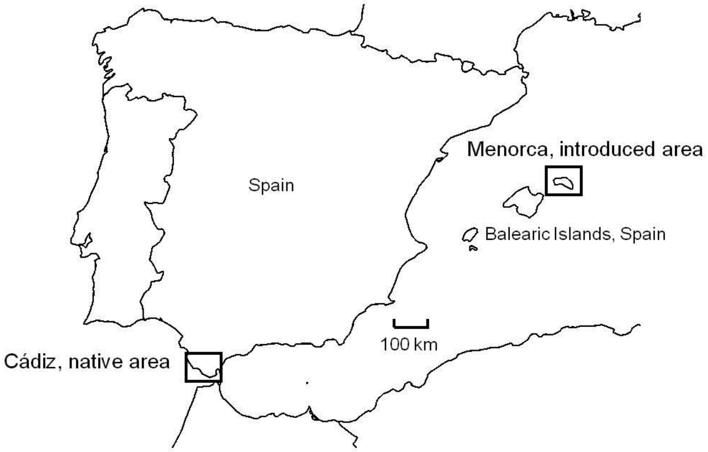

Figure 1. Location of the study areas.

In the introduced area the average (±SD) distance between plots was 9247.00 ± 7875.89 m, but distances were shorter in the native area: 632.55 ± 224.28 m (Table A1.1). In the native area in S Spain, many communities with *Hedysarum* were not suitable for the study because they were grazed by cattle, were located in forbidden military precincts, were located inland at a distance to the coast farther than selected plots in Menorca, or were smaller than the established 400 m^2. Considering the mentioned limitations, we sought to maximize plot distances but also to have similar *Hedysarum* population sizes, vegetation and landscape structure as in the introduced area.

Despite other pollinator studies having also used distances between plant populations similar to ours (see Dohzono *et al.* 2008; Yang, Ferrari & Shea 2011; King & Sargent 2012), we are aware that honeybees and some bumblebees *Bombus* ssp. can embrace larger foraging ranges (Osborne *et al.* 2008; Bommarco *et al.* 2010). However, they often limit their foraging distances when diverse and abundant flower resources are available at the local scale (Johnson *et al.* 2003; Greenleaf *et al.* 2007). In our plots, more than 15 plant species (Montero-Castaño *personal observation*) were in bloom simultaneously with *Hedysarum*, providing abundant and diverse floral resources. In addition, the maximum foraging distances of solitary bees range from 150 to 600 m (Gathmann & Tscharntke 2002), and coleopterans in general are highly sedentary (Mawdsley & Sithole 2009). Therefore we are confident that study plots were independent from each other.

Pollination censuses

We conducted pollination censuses on *Hedysarum* simultaneously in both study areas and during two consecutive seasons: spring 2009 and 2010. Weather conditions in both study years fell into the average ranges for the study areas (“AEMET”).

Pollination censuses were performed during sunny, warm (≥ 17 ºC) and non-windy days, from 10 a.m. to 6 p.m. Within each plot, we randomly selected patches of *Hedysarum,* with different sizes and distances to closest conspecifics, and alternatively surveyed the plots within each study area.

We observed pollinators visiting plants for 15 min periods. During each observation period, we counted the number of flowers observed, the number and identity of pollinators, and the number of visits of each pollinator species. A visitor was considered a pollinator when it entered a flower and touched its sexual parts. The species that could not be identified in the field were sorted into distinct morphospecies and caught for later identification by specialists. Voucher specimens are deposited at EBD-CSIC.

As abundance and evenness of pollinators was not expected to be the same in each plot, the number of censuses differed in each plot, establishing a compromise between sampling effort and quality of the data collected. We considered a plot to be properly surveyed when, according to its rarefaction curve (Appendix 2), we found no new visitor species after three or more observation periods. Nevertheless, to overcome any difference in sampling effort, in each plot we extrapolated the expected *Hedysarum* pollinator generalization degree with the first-order Jackknife species-richness estimator. We considered this estimator to be the most suitable one for our data because non-parametric estimators are less sensitive to unevenness of species incidence, and in general perform better than the ones based on species-accumulation curves or on species-area relationships (Brose, Martínez & Williams 2003; Hortal, Borges & Gaspar 2006). In addition, the first-order Jackknife estimator has been demonstrated to perform adequately for non-biased, precise, and accurate estimations when sampling coverages differ, and the grain of the measures is small and constant among all the plots (Hortal *et al.* 2006), as was the case in this study.

We estimated *Hedysarum* degree of generalization (i.e. visitor-species richness) by the first-order Jackknife for each study area and year separately and for both years pooled. We compared *Hedysarum* generalization degree between native and introduced areas by looking at the 95% confidence intervals of the estimates.

We compared the number of pollinator species, number of individuals and number of visits (hereafter richness, abundance, and visitation rates, respectively) between native and introduced areas after controlling for the number of observed flowers in each observation period. We explored the differences in these response variables within the R statistical computing environment (R Development Core Team 2001) by building generalized mixed models (*lme4* library). Area (native/introduced) was included in the model as a fixed effect, the logarithm of the number of observed flowers as offset, plot and year as random effects and *log* as link function of the Poisson family. We also explored differences in the number of visits per individual pollinator for the total pool of pollinator species and for the most common pollinator species (honeybee) by building linear models with area (native/introduced) as fixed effect.

Pollen loads on honeybee

As the honeybee was the most common *Hedysarum* visitor species, we assessed its efficiency in carrying *Hedysarum* pollen and whether pollen dispersal differed between areas. Pollinators, when foraging, seek to optimise floral rewards (Armbruster & Herzig 1984), their success depending on the relative abundance and quality of available floral resources (Dietzsch *et al.* 2011). Flowering communities were not exactly the same between the native and the introduced areas, either in terms of plant species identity or their relative abundances. In addition, pollinator visits are not equally efficient in terms of pollen removal and transport, depending on the plant species visited, on their spatial distribution (Ne'eman *et al.* 2010), etc. Therefore, we could expect honeybees to carry different percentages of heterospecific pollen between areas.

During the flowering peak of 2009 field season (mid-April), 10-15 specimens per plot were captured just after a visit to a *Hedysarum* flower. We preserved each captured specimen individually in a paper bag inside a plastic vial with a piece of cotton soaked with ethyl acetate (Forup & Memmott 2005; Gibson *et al.* 2006; Lopezaraiza-Mikel *et al.* 2007). We obtained two pollen samples per specimen by rubbing two small cubes (3 x 3 mm^2) of fuxine-stained glycerine jelly (Beattie 1971) on the ventral and dorsal parts, respectively, of each bee body. The samples were mounted on microscope slides for examination. Pollen identification was based on a reference pollen collection of the flowering plant species in the study areas. However, as the frequency of pollen from other species was very low, we distinguished only two categories: *Hedysarum* and heterospecific pollen. Percentages of these two pollen categories were quantified by combing the entire slide and counting all the pollen grains in every two 200x magnification microscope fields (Bartomeus, Bosch & Vilà 2008a).

We explored differences in the percentages of *Hedysarum* and heterospecific pollen loads by linear mixed models. The response variables were logit-transformed according to Warton & Hui (2011). The area (native/introduced) and body part (dorsal/ventral) were included in the models as fixed effects, and individual nested in plot as a random effect.

Pollen deposition on stigmas

We also explored the efficiency of the honeybee by examining the pollen deposited on *Hedysarum* stigmas after a visit by this species. In each plot, and simultaneously to the capture of honeybee individuals for the analysis of body pollen loads, we collected 15 *Hedysarum* flowers immediately after them being visited by a honeybee individual. We kept flowers in separate paper bags and later in the laboratory, using forceps, we extracted styles and immersed them in a drop of melted fuxine-stained glycerine jelly on a microscope slide. As before, for the identification and quantification of pollen loads, slides were examined at 200x magnification. We considered only the pollen adhering to the stigma hairs. As for pollen loads, all heterospecific pollen was grouped in a single category because of the low incidence. Accurate pollen counts were not always feasible because pollen grains were sometimes clumped or masked by stigma tissue. Therefore, as in Bartomeus *et al.* (2008a), our analysis of pollen abundance on stigmas was semi-quantitative. We established six abundance categories: absent, present (only one grain), low (≤25% of the grains), medium (25%<>75%), high (≥75%), and sole (100%). For each collected stigma, one abundance category was assigned for *Hedysarum* pollen and another for heterospecific pollen. For both *Hedysarum* and heterospecific pollen depositions, we performed Chi-square tests for each category in order to assess any differences between the native and the introduced areas.

Fruit and seed sets

In each plot, during the flowering peak (mid-April) we randomly singled out 18-20 *Hedysarum* plants and, in each, marked 3 flowers from 3 different inflorescences to which we randomly assigned one of the following treatments: a) open pollination: flowers were not manipulated; b) autonomous self pollination: inflorescences were bagged with a teabag to avoid any pollen transfer mediated by pollinators; and c) out-cross pollination: flowers were hand-pollinated with a mixture of pollen from neighbouring conspecific plants. In total, we selected 151 *Hedysarum* plants and marked 453 flowers. Approximately one month after the treatment, we collected ripe fruits, and counted the number of seeds. Reproductive success was calculated as fruit and seed production per flower.

Differences in fruit production between native and introduced areas and between treatments were tested by Chi-square analysis. Differences in the number of seeds per fruit were analysed by a linear mixed model with the response variable log-transformed, area (native/introduced), treatment (open pollination/out-cross pollination) and their interaction as fixed effects, and individual nested in plot as random effect. *Post hoc* multiple comparisons were made with the function *ghlt* (library *multcomp*), by previously building our contrast matrices with the function *contrast* (library *contrast*).

Results

Pollinator identity and degree of generalization of Hedysarum

We conducted a total of 248 censuses ranging from 7 (105 min) to 29 (435 min) censuses per plot. During these censuses, 21 pollinator species in the native area and 20 in the introduced area were observed to legitimately visit *Hedysarum*. No nectar robbery was detected. The native area shared seven species with the introduced area (Appendix 3).

The species belonged to 20 different genera of 11 families and three orders: Coleoptera, Diptera and Hymenoptera, with Hymenoptera being by far the most represented order (79.41%) with 27 species. We sporadically observed lepidopterans but we did not considered them because during their visits they did not touch the reproductive parts of the flowers.

The shared species were five hymenopterans and two coleopterans, representing similar percentages of the pollinator species pool in the native (33.33%) and in the introduced (35.00%) area and achieving the 93.69% and the 85.76% of the visits in each area, respectively. Dipterans were detected only in the native area.

In 2009, the expected degree of generalization (i.e. according to the first-order Jackknife) of *Hedysarum* was higher in the native area (19.84 ± 2.58 visitor species) than in the introduced area (9.91 ± 1.68). However, in 2010 the opposite trend was found with a lower number of pollinator species visiting *Hedysarum* in the native (18.94 ± 1.97) than in the introduced (30.89 ± 3.43) area. With both years pooled, differences between areas were not significant (native: 30.92 ± 3.29, introduced: 31.92 ± 3.29).

Pollinator richness, abundance, and visitation rates

Pollinator richness (n = 248, *df* = 4, Z = -5.38, P < 0.001), abundance (n = 248, *df* = 4, Z = -7.05, P < 0.001), and visitation rates (n = 248, *df* = 4, Z = -8.88, P < 0.001) were higher in the native than in the introduced area (Fig. 2).

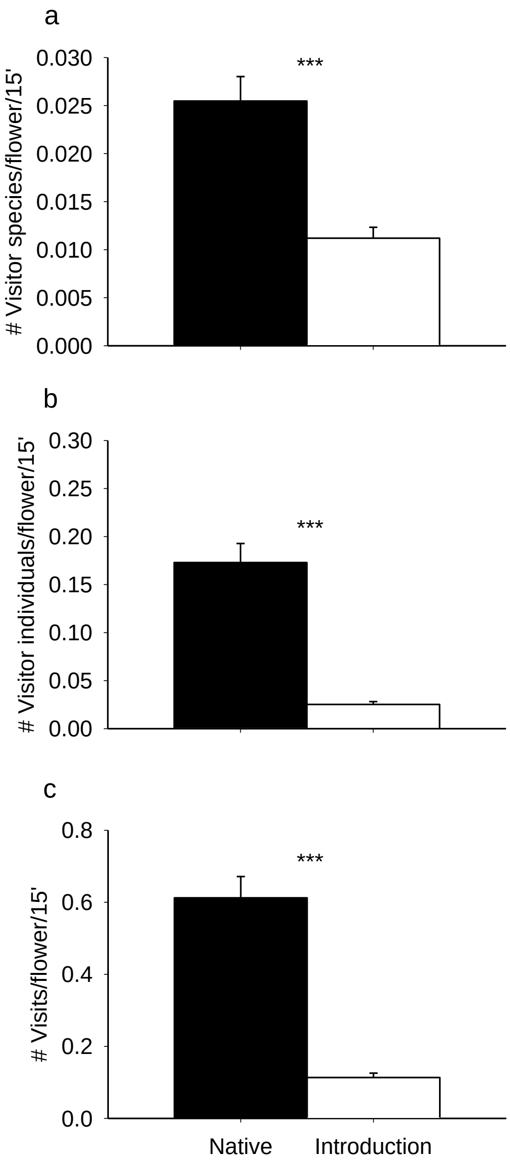

Figure 2. Mean (+ SD) richness (a), abundance (b) and visitation rates (c) of *Hedysarum* pollinators in the native (filled bars) and introduction (open bars) areas pooled for the two sampling years.

For all variables, year explained much of the variance, pointing to a high inter-annual variability in pollinator assemblages. In fact, the general trend of higher pollinator richness, abundance, and visitation rates in the native area was found mainly in 2010.

The higher visitation rates in the native area were due to the higher pollinator abundance, as the number of visits per individual pollinator did not differ between study areas (native = 4.68 ± 3.44, introduced = 5.76 ± 6.52, n = 206, t = 1.48, *df* = 156.77, P = 0.14).

Honeybee, appearing in 169 out of the 248 censuses (68.15%) and being present in both areas, was the most abundant pollinator and the one that made the most visits both in the native and in the introduced area during the two study years (Table 1, Fig. 3). The contribution of this pollinator species to *Hedysarum* pollination matched the trend found for the total pollinator pool in terms of abundance (native = 0.184 ± 0.204, introduced = 0.030 ± 0.031, Z = -5.26, *df* = 4, P < 0.001) and visitation rates (native = 0.677 ± 0.600, introduced = 0.154 ± 0.153, Z = -6.00, *df* = 4, P < 0.001), which were also significantly higher in the native than in the introduced area. Meanwhile, the number of visits achieved per each honeybee individual did not differ between areas (native = 5.39 ± 4.44, introduced = 6.85 ± 7.40, t = 1.58, *df* = 167, P = 0.12).

Table 1. Total number and percentage (mean ± SD) of individuals and visits of the honeybee to *Hedysarum* in native and introduced areas.

Area	Year	# honeybee individuals	% honeybee individuals	# honeybee visits	% honeybee visits
Native	2009	75	76.48 ± 15.16	511	89.48 ± 12.05
	2010	965	87.47 ± 9.26	3733	91.95 ± 6.58
Introduced	2009	101	77.27 ± 21.51	645	93.00 ± 9.75
	2010	165	69.78 ± 23.62	1066	80.79 ± 20.53

Figure 3. Honeybees visiting *Hedysarum*.

Honeybee pollen loads

Hedysarum pollen grains represented high and not significantly different percentages of the pollen loads on honeybees in both native (99.34 ± 0.02%) and introduced (99.18 ± 0.02%) areas (n = 198, F = 1.83, *df* = 6, P = 0.23), as well as in both ventral (99.38 ± 0.02%) and dorsal (99.10 ± 0.02%) parts of their bodies (n = 198, F = 2.07, *df* = 97, P= 0.15). The interaction of these factors was not significant, either (n = 198, F = 0.15, *df* = 97, P= 0.70).

Pollen loads on stigmas

Of the 119 stigmas analysed, only five had no pollen grains adhering (two collected in the native area and three in the introduced area). In the remaining 114 stigmas *Hedysarum* dominated pollen loads. In 111 cases (97.37%), only *Hedysarum* grains were found (Fig. 4), while in the other three, heterospecific pollen was just present (i.e. one grain) or in low quantity (< 25%). The frequency of the observed pollen loads did not vary between native and introduced areas (Chi-square test, P > 0.1 in all cases) (Table 2).

Figure 4. Stigma of *Hedysarum* with only conspecific pollen grains adhered.

Table 2. Number of *Hedysarum* stigmas in the native (n = 65) and introduced (n = 51) areas carrying different percentages of conspecific and heterospecific pollen grains classified in the following categories: absent, present (only one grain), low (≤25%), medium (25%<>75%), high (≥75%) or sole. Chi-square statistics and P-values of the contingency tables for each category and type of pollen are given.

	Conspecific				**Heterospecific**			
	N native	**N introduced**	χ^2	**P**	**N native**	**N introduced**	χ^2	**P**
Absent	2	3	0.05	0.83	65	51	1.79	0.18
Present	0	0	−	−	0	2	0.72	0.40
Low	0	0	−	−	0	1	0.01	0.93
Medium	0	0	−	−	0	0	−	−
High	0	3	1.79	0.18	0	0	−	−
Sole	63	48	1.89	0.17	0	0	−	−

Fruit and seed sets

None of the bagged flowers (i.e. pollinator exclusion) produced fruits autonomously, either in the native or in the introduced area (Fig. 5a). Fruit production of the other two treatments was higher in the native than in the introduced area (Chi-square = 29.28, *df* = 1, P < 0.001). In the native area, fruit production in open pollinated flowers did not differ from out-cross pollinated flowers (Chi-square = 2.02, *df* = 1, P =

0.16). However, in the introduced area out-cross pollinated flowers set more fruits than did open pollinated flowers (Chi-square = 4.10, *df* = 1, P = 0.04).

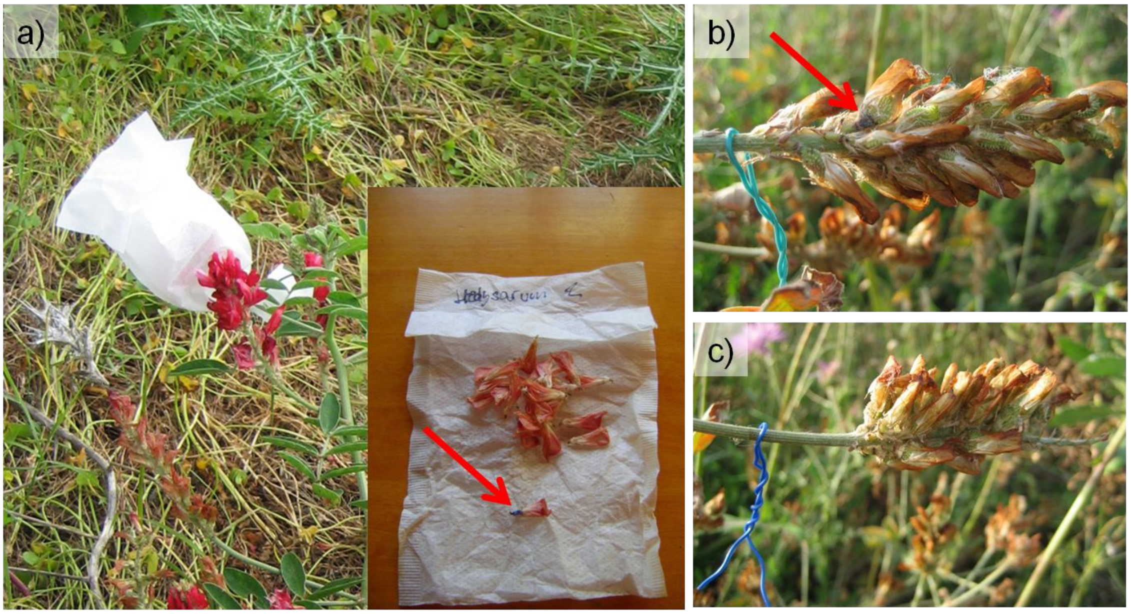

Figure 5. Fruit set of (a) autonomous self-pollination, (b) out-cross pollination and (c) open pollination treatments. The three flowers per inflorescence that were treated were marked in the sepals with a permanent pen, as point the red arrows.

We found the same pattern for seed number per fruit (excluding the treatment autonomous self pollination as no fruits were set), which was higher in the native area than in the introduced area (*t* = -5.11, *df* = 6, P < 0.002). Comparisons among treatments differed between study areas. In the native area, differences in number of seeds per fruit among treatments did not differ (Z = -1.16, P = 0.24) and therefore no pollen limitation was found whereas, in the introduced area, out-cross pollinated flowers set 40.98% more seeds than did open pollinated flowers (Z = -2.42, P = 0.02), indicating pollen limitation (Fig. 6).

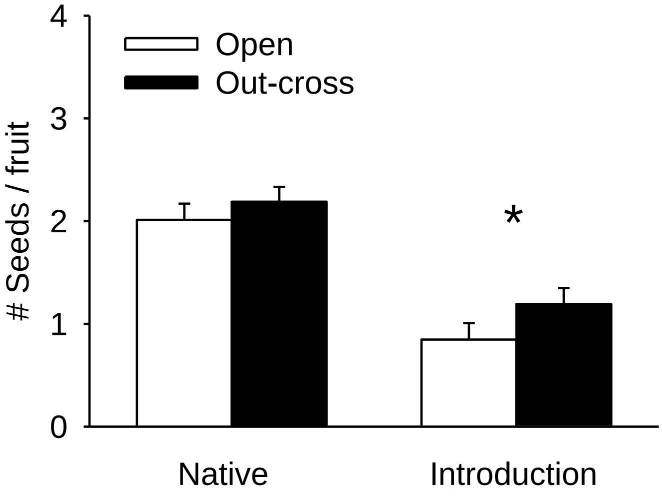

Figure 6. Mean (+ SD) number of seeds per fruit in the native (n = 78) and introduction (n = 72) areas for open (open bars) and out-cross (filled bars) pollination flowers.

Discussion

The degree of generalization of *Hedysarum* populations did not differ between the native and introduced areas even when only 20.59% of the pollinator species were shared between the two areas. The presence of shared pollinators was not due to introduced pollinators establishing alien complexes (*sensu* Olesen *et al.* 2002) since all of them were native in both areas. Stout *et al.* (2006) found a similar pattern for *Rhododendron ponticum*, as invasive populations of this species in Ireland showed similar generalization levels compared to native populations in southern Spain, though pollinator identities differed. Despite the high level of generalization, in both areas the honeybee was the most common pollinator of *Hedysarum* and the one that made most of the visits, as found in other areas (Satta *et al.* 2000; Galloni *et al.* 2008). This finding points to the important role that domestic social pollinators play in the integration of non-native plants into new regions (Grabas & Laverty 1999; Barthell *et al.* 2001; Parker & Haubensak 2002; Jesse *et al.* 2006). In *Hedysarum*, native and introduced areas belong to the same biogeographical region, and both are included in the native distribution range of the honeybee (Goulson 2003). The honeybee is a super-generalist pollinator (Huryn 1997) that can broaden its diet to include new food resources, including non-native plants (Stout *et al.* 2002; Simpson *et al.* 2005; Gross *et al.* 2010). Moreover, it has being widely introduced around the world being also well integrated (Moritz, Hartel & Neumann 2005). Therefore, a non-native plant pollinated by the honeybee in its native area and finding it in its introduced area might be more frequent than previously expected (e.g. *Cytisus scoparius*; Simpson *et al.* 2005).

Despite that *Hedysarum* was well integrated into the diet of resident pollinators in the introduced area, its reproductive success in terms of fruit and seed sets proved lower than in the native range. Differences were not due to contrasting reproductive strategies among study areas but rather to pollen limitation. In the introduced area, fruit and seed sets increased when pollen was added. Pollen limitation can result from reduced quantity and/or quality of pollen deposited on stigmas (Aizen & Harder 2007). In *Hedysarum*, pollen limitation seems to be more related to low pollen quantity than to low pollen quality reaching the stigmas. Since in both areas the honeybee made more than the 80% of the visits, pollen limitation in the introduced populations cannot be attributed to a shift in pollinator identity (Larson, Fowler & Walker 2002; Bartomeus & Vilà 2009) and subsequent differences in pollinator effectiveness (Lau & Galloway 2004; Dohzono & Yokoyama 2010; Ne'eman *et al.* 2010). In addition, the foraging behaviour of the honeybee appeared to be the same in both areas, making a similar number of visits per individual per patch and carrying similarly high percentages of *Hedysarum* pollen loads. Individual honeybees are constant in the specificity of the floral resources they visit (Grüeter *et al.* 2011) to increase foraging efficiency as long as this resource is abundant (Armbruster & Herzig 1984).

We could not estimate whether the total quantity of pollen depositions on stigmas was lower in the introduced area than in the native area because our methodology was semi-quantitative (i.e. we explored relative abundances of pollen depositions, but not absolute values). However, pollen transfer is a function of visitation rates (Wilcock & Neiland 2002), and in the introduced area visitation rates were lower than in the native areas. Thus, pollen limitation in the introduced area might be related to lower pollen deposition due to lower visitation rates, which in many cases is a good predictor of reproductive success (Vázquez *et al.* 2005; Dauber *et al.* 2010; but see Dietzsch *et al.* 2011).

The lower visitation rates might be a direct consequence of the lower pollinator abundance found in the introduced area. The lower pollinator abundance in introduced *Hedysarum* populations could have various non-mutually exclusive explanations. First, the lower observed pollinator richness in the introduced area could indicate that resident pollinators are not yet intensively exploiting this resource (Armbruster & Herzig 1984). Competition for pollinators between introduced and native plant species might occur and might depend on the relative abundance of floral resources within the community (Rathcke 1988; Burns *et al.* 2011; Dietzsch *et al.* 2011). Furthermore, there might be a lag time to achieve the same pollination levels as in the native range (Crooks 2005).

Second, baseline pollinator abundance could be lower in the introduced area than in the native area, moreover being the introduced area an island and being the main pollinator a managed species. Baseline data on the pollinator abundance is usually lacking (Winfree 2010). Pollination analysis of three plant species present and native to the two study areas, and that share pollinators with *Hedysarum*, showed that pollinator richness, abundance, and visitation rates did not differ between native and introduced areas (Appendix 4), suggesting that pollinators with potential to visit *Hedysarum* may not be less abundant in the introduced compared to the native area, despite the former being and insular ecosystem. The impoverished biota in comparison with corresponding continental areas typifies oceanic islands (Wardle 2002) and Menorca is a continental island that was connected to mainland during the Messinian period (between 5.70 and 5.35 million years ago) (Alcover 2010). Though in this study case a single pollinator species is the responsible of more than the 80% of the visits in both areas, and therefore we do not expect insularity to be affecting the results, we cannot disregard the fact that the study introduced area is an insular ecosystem where pollinator fauna might be depauperated (MacArthur & Wilson 1967). We can neither rule out the possibility that the different honeybee abundances found between study areas were not due to management reasons and not to the invasion process.

Overall, by comparing the pollination ecology of an abundant plant species in native and introduced areas, we found that despite showing the same high degree of pollination generalization in both areas, and being mainly pollinated by a generalist, pervasive and managed species like the honeybee, plants are pollen limited and have lower seed set in the introduced than in the native area. We cannot infer the demographic consequences of these differences (Feinsinger 1987). The establishment and spread of non-native species are long-term processes that do not depend on the success of a single reproductive season (e.g. Downey & Brown 2000). In short-lived perennials such as our study species, one-year seed production might be more related to population demography (Parker 1997) than in long-lived non-native species. However, the lower seed set of non-native species can be counteracted by other biotic and abiotic factors acting in other stages of the plant-life cycle (Blackburn *et al.* 2011; Carrillo-Gavilan *et al.* 2012). Lloret *et al.* (2005), for instance, found that for 350 naturalized plant species across the Mediterranean region, seed dispersal correlated better with non-native species abundance than did pollination. Moreover, constant propagule pressure can also counterbalance pollinator and pollen deficiencies. *Hedysarum* annual seeding in traditional agro-systems intensifies propagule pressure and might to some extent offset the lower seed set of already naturalized populations.

Despite that our biogeographical approach cannot conclude that pollination differences are due to the nativity status of the studied populations, our experimental design has allowed for describing mutualistic patterns of pollination that are possibly very common because many introduced species in islands have a mainland origin. Furthermore, the close geographic proximity between the native and the introduced range has allowed for comparing highly similar ecological settings (i.e. climate, vegetation type, species assemblages, landscape configuration), reducing the influence of major confounding factors that preclude any causality. However, extrapolation of these results, both for other plant species or even for *Hedysarum* in other introduced areas, should be made with caution. First of all, studied introduced populations are established and spreading (according to Blackburn *et al.* 2011) and influential factors differ over the different stages of the invasion process (Lloret *et al.* 2005; Aizen, Morales & Morales 2008b). Second, study native and introduced areas belong to the same biogeographical region and results might differ from cases in which plants are introduced in completely distant and dissimilar biogeographical regions.

Further research applying this biogeographical approach is needed in other case studies to overcome these limitations and reach stronger generalizations.

Conclusions

This study adds evidence to the integration of non-native plants into resident plant-pollinator networks reported in other systems. However, and contrary to our hypothesis, our biogeographical approach has shown that such integration does not prevent pollen limitation in the introduced area. Therefore, integration of non-native plants into the native plant-pollinator community, despite being necessary, might not be the key for their persistence and spread in introduced areas.

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Appendix 1. Location and characteristics of study plots

Table A1.1. Location and main characteristics of the eight study plots.

Area	**Plot name**	**Latitude**	**Longitude**	**Distance closest plot (m)**	***Hedysarum* cover (%)**
Native	*Anfiteatro*	36° 5.468'N	5° 46.575'W	385.96	37.64
Native	*Parking*	36° 5.516'N	5° 46.154'W	638.36	51.18
Native	*Pinos*	36° 5.500'N	5° 46.829'W	385.96	39.30
Native	*Pradera*	36° 5.726'N	5° 46.671'W	480.72	70.00
Introduced	*Recepción*	39° 56.395'N	4° 15.052'E	571.92	85.71
Introduced	*Itinerario*	39° 56.677'N	4° 14.892'E	571.92	47.11
Introduced	*Sa Mola*	39° 56.191'N	4° 13.000'E	2853.43	30.58
Introduced	*Tirant*	40° 2.429'N	4° 5.908'E	15399.89	27.27

Appendix 2. Rarefaction curves of *Hedysarum* pollinator species richness

Rarefaction curves of *Hedysarum* pollinator species richness for each study plot and for spring 2009 and 2010. Curves were calculated and plotted within the R statistical computing environment (R Development Core Team 2001). Curves are based on 100 randomizations without replacement and boxplots are represented for each level of randomization. Shaded areas represent 95% confidence intervals. Dashed lines enclose the number of censuses needed to observe a new pollinator species visiting *Hedysarum* at the end of the study period.

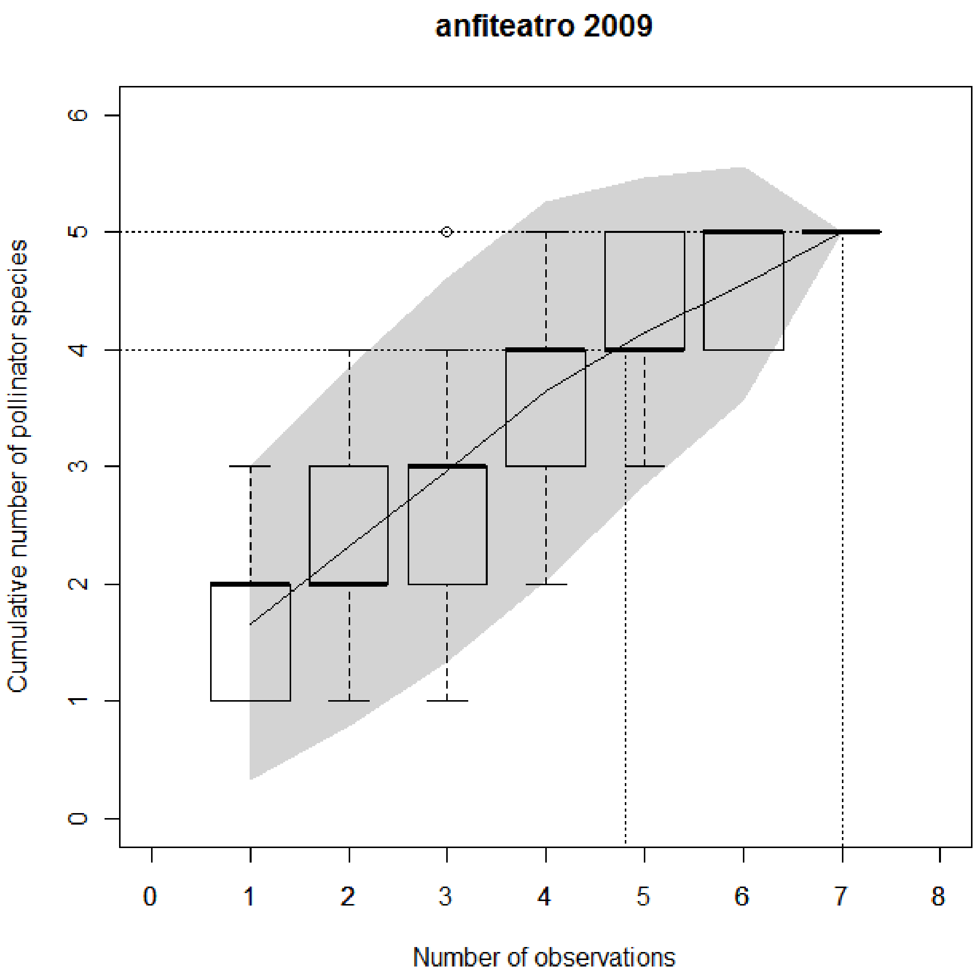


Figure. A2.1. Rarefaction curve for plot *anfiteatro* (native area) in 2009.

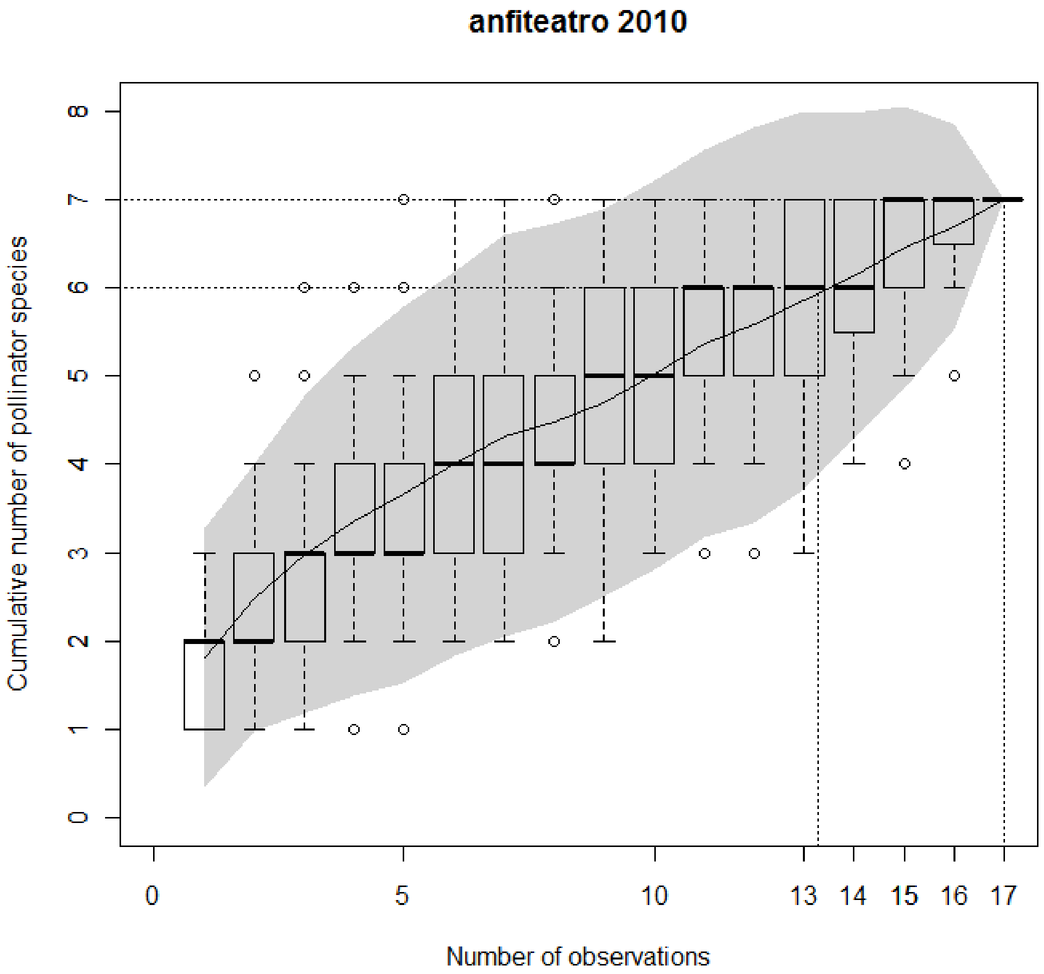


Figure. A2.2. Rarefaction curve for plot *anfiteatro* (native area) in 2010.

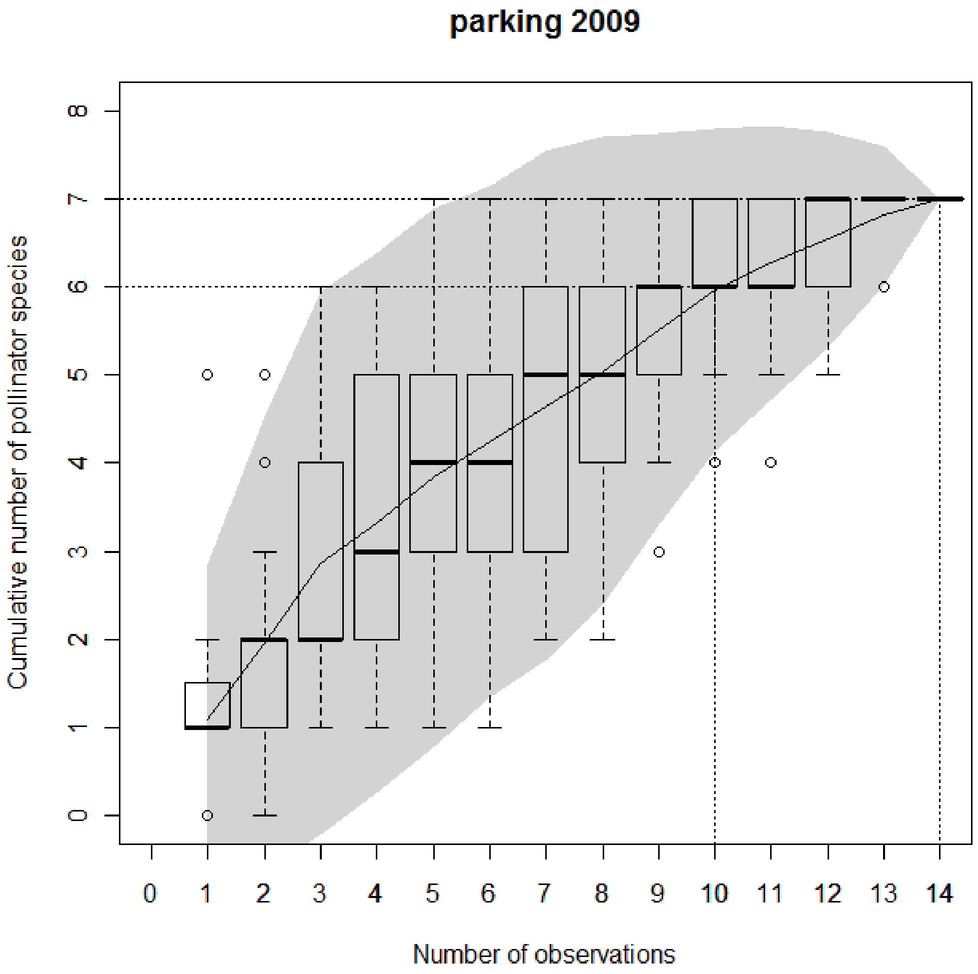


Figure A2.3. Rarefaction curve for plot *parking* (native area) in 2009.

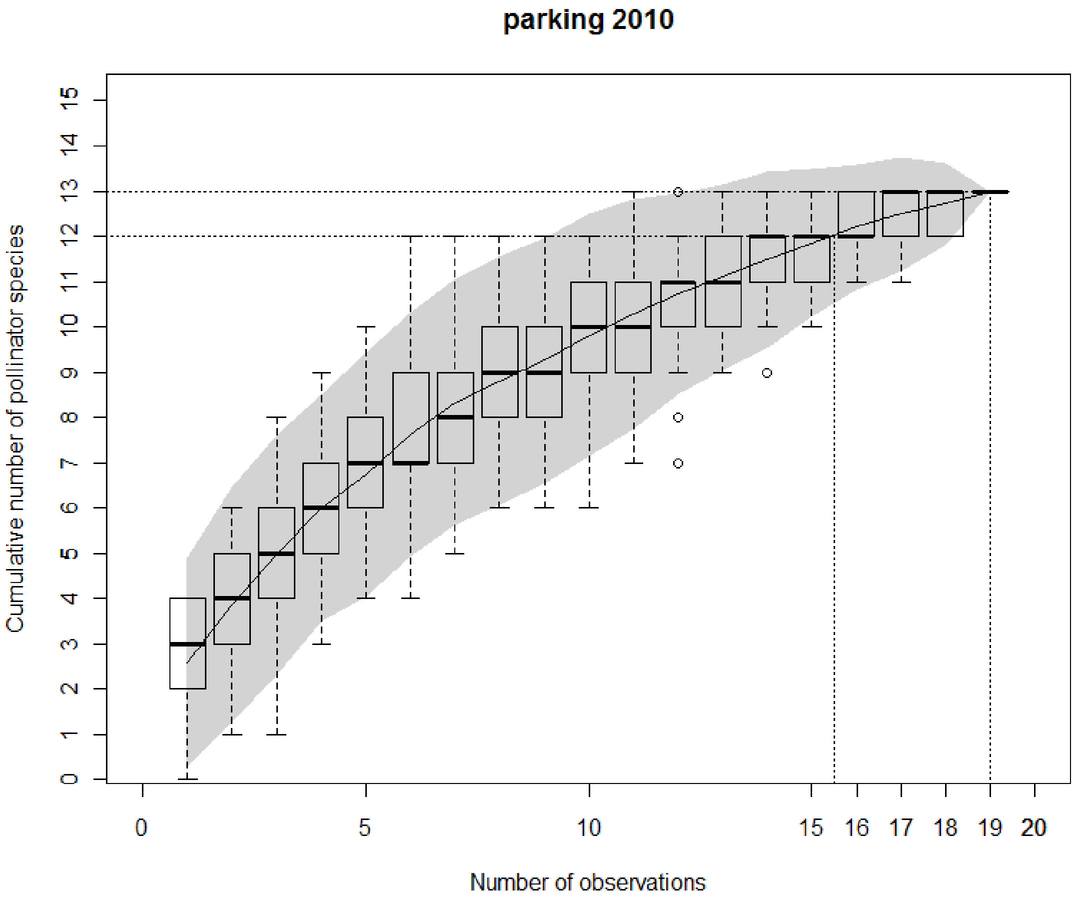


Figure A2.4. Rarefaction curve for plot *parking* (native area) in 2010.

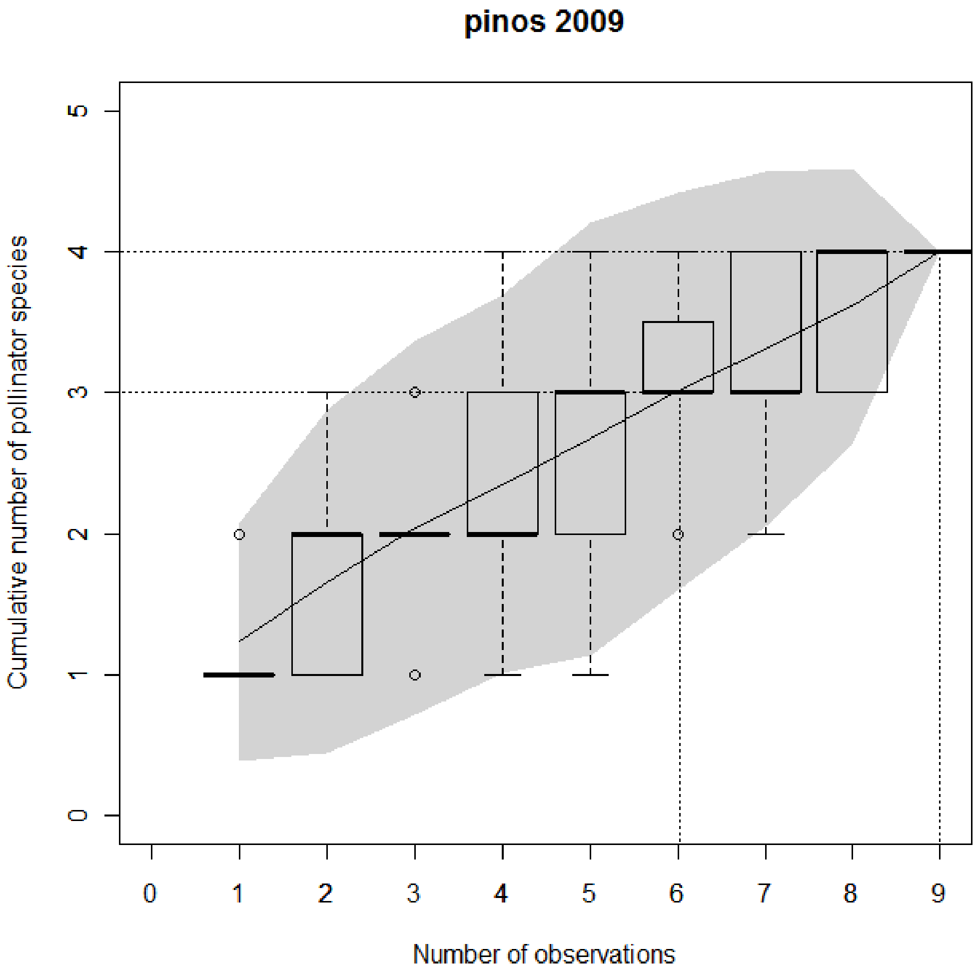


Figure A2.5. Rarefaction curve for plot *pinos* (native area) in 2009.

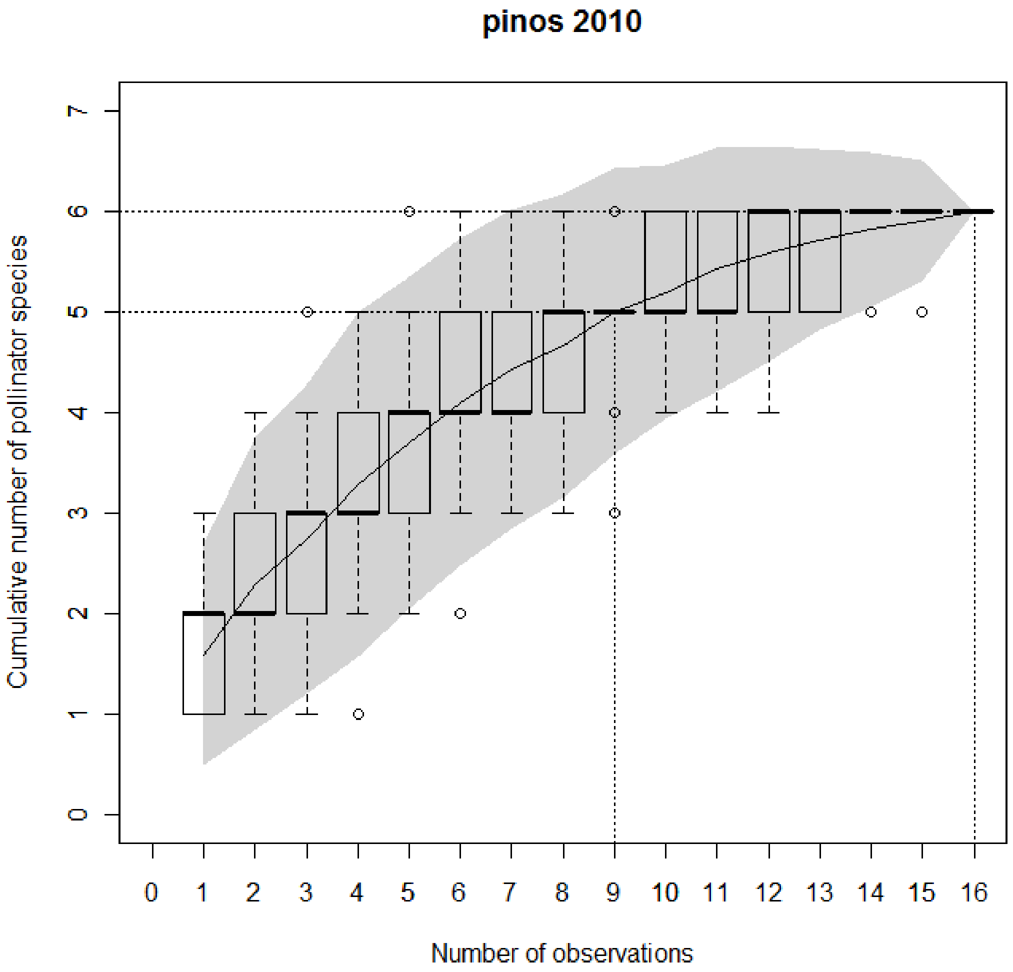


Figure A2.6. Rarefaction curve for plot *pinos* (native area) in 2010.

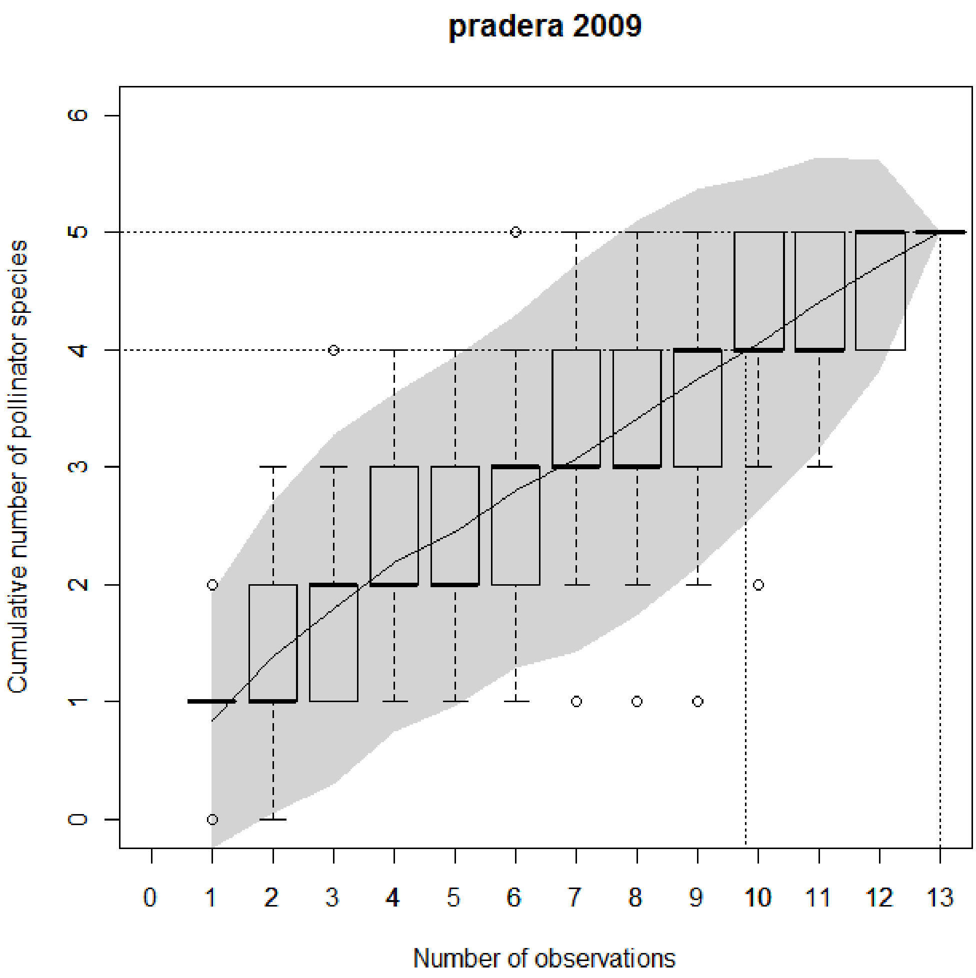


Figure A2.7. Rarefaction curve for plot *pradera* (native area) in 2009.

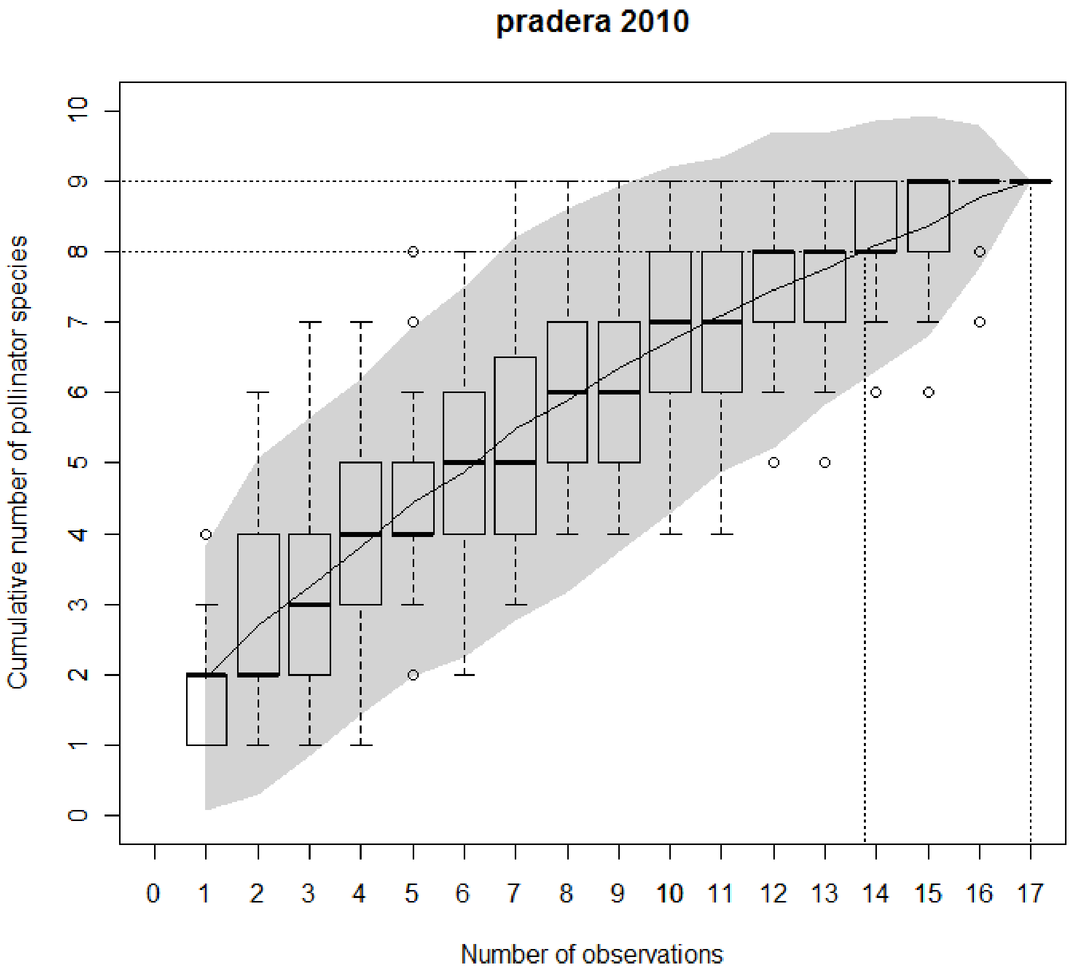


Figure A2.8. Rarefaction curve for plot *pradera* (native area) in 2010.

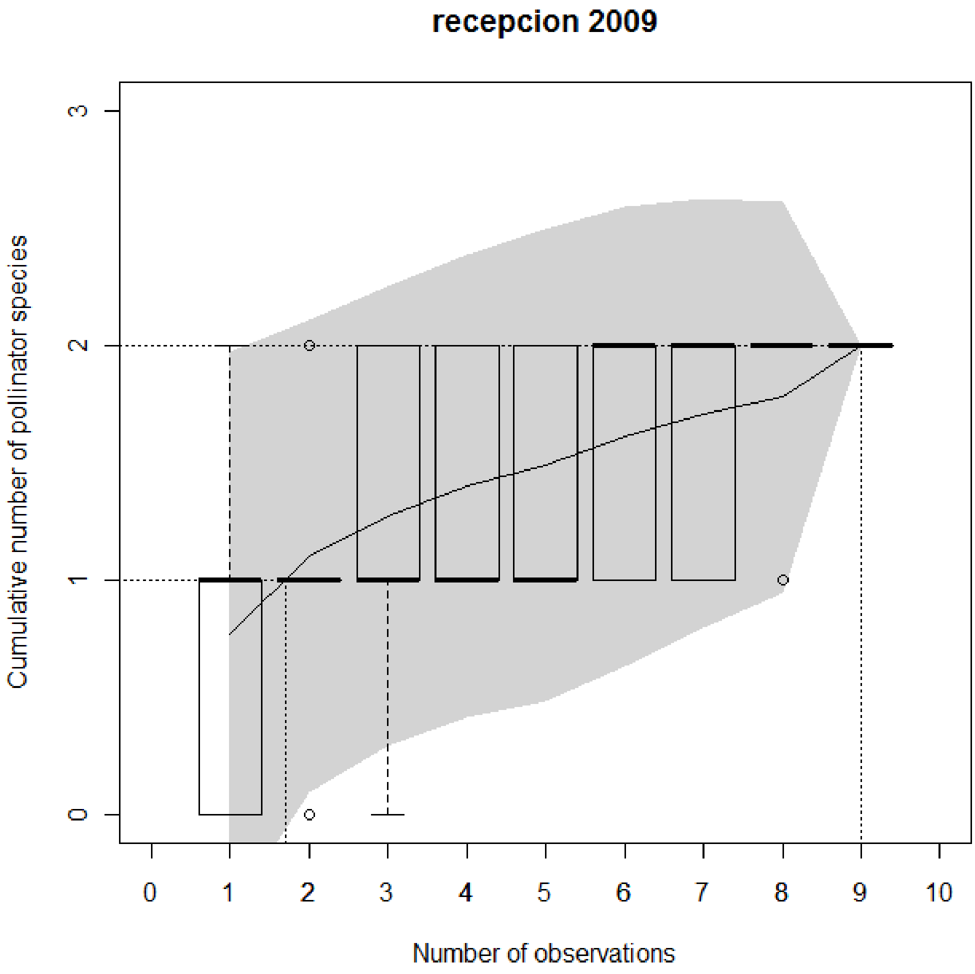


Figure A2.9. Rarefaction curve for plot *recepcion* (introduced area) in 2009.

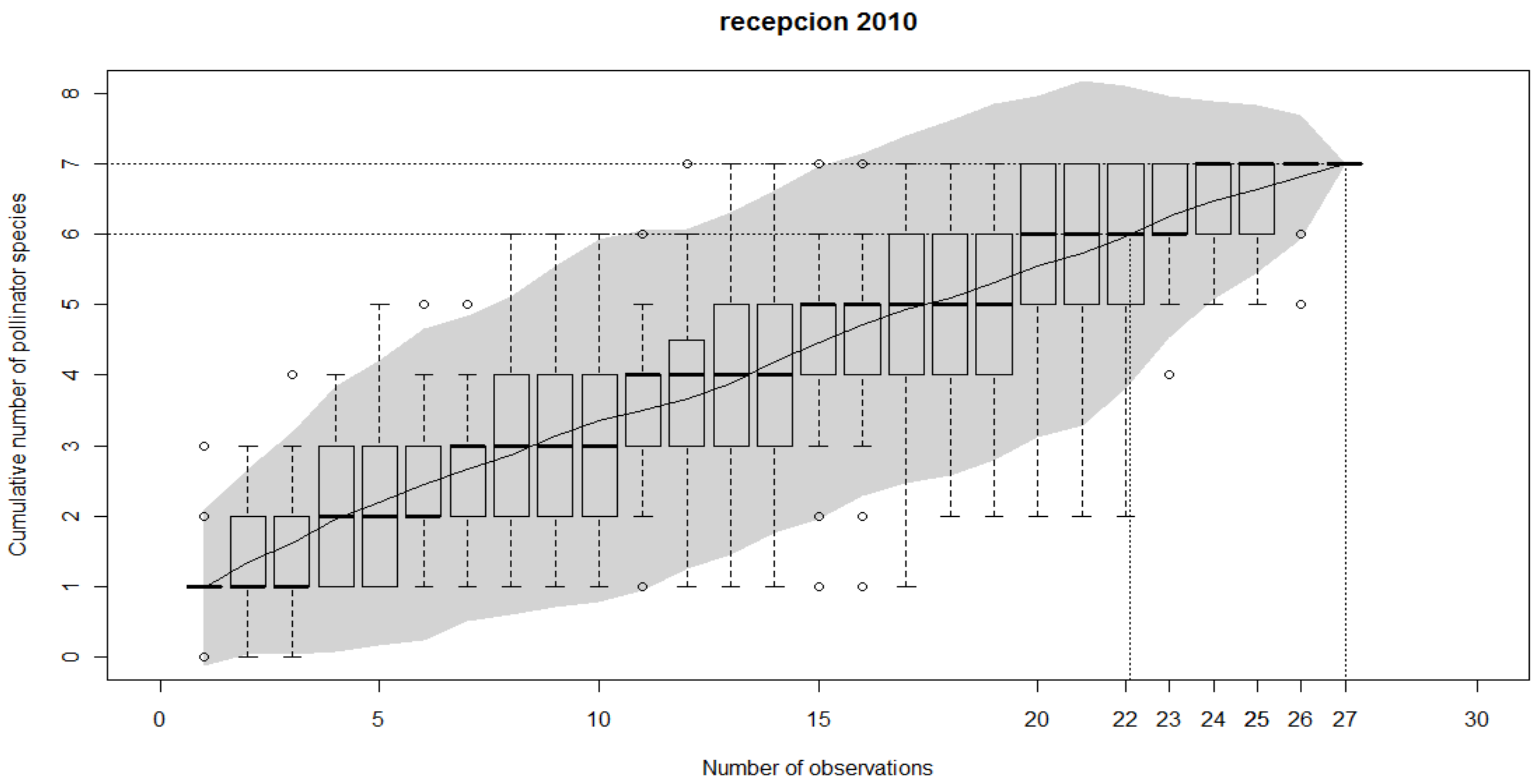


Figure A2.10. Rarefaction curve for plot *recepcion* (introduced area) in 2010.

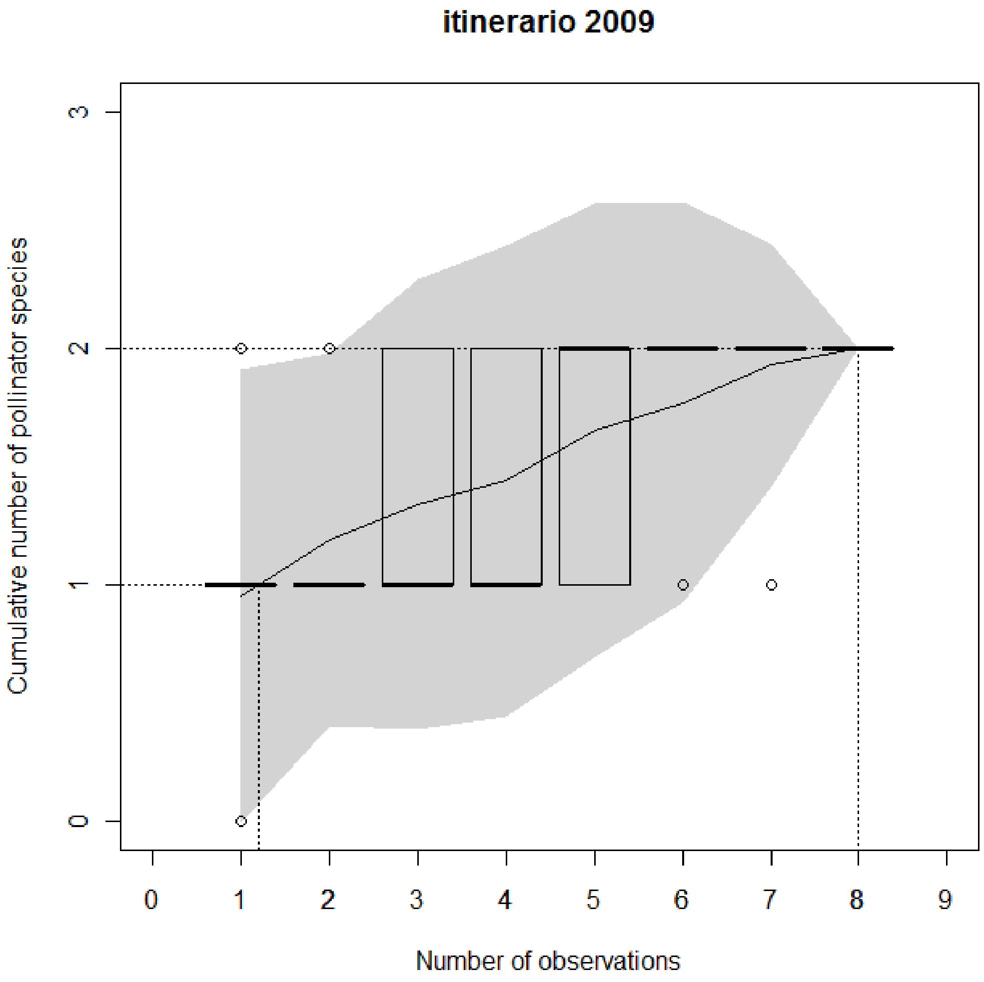


Figure A2.11. Rarefaction curve for plot *itinerario* (introduced area) in 2009.

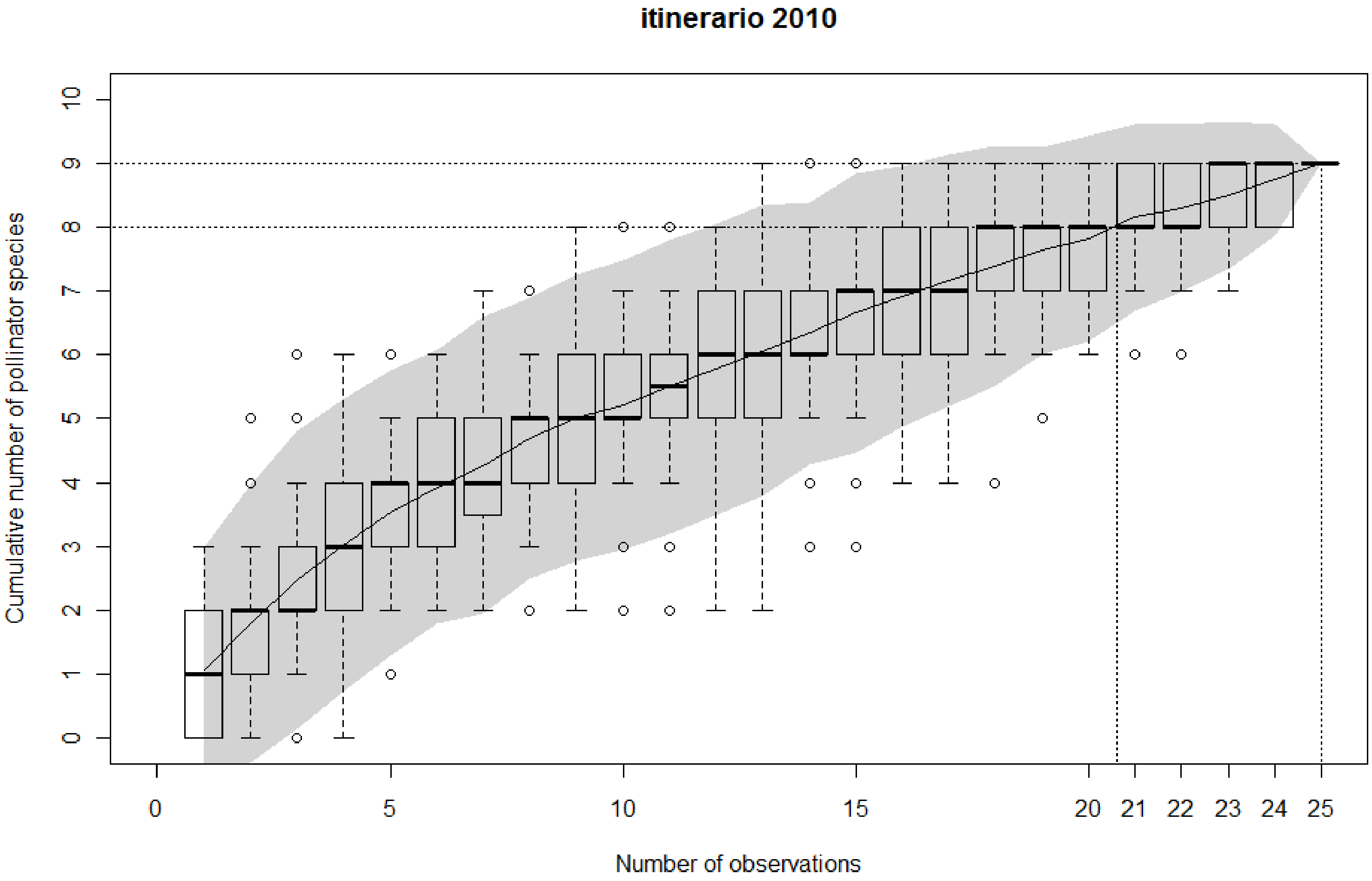


Figure A2.12. Rarefaction curve for plot *itinerario* (introduced area) in 2010.

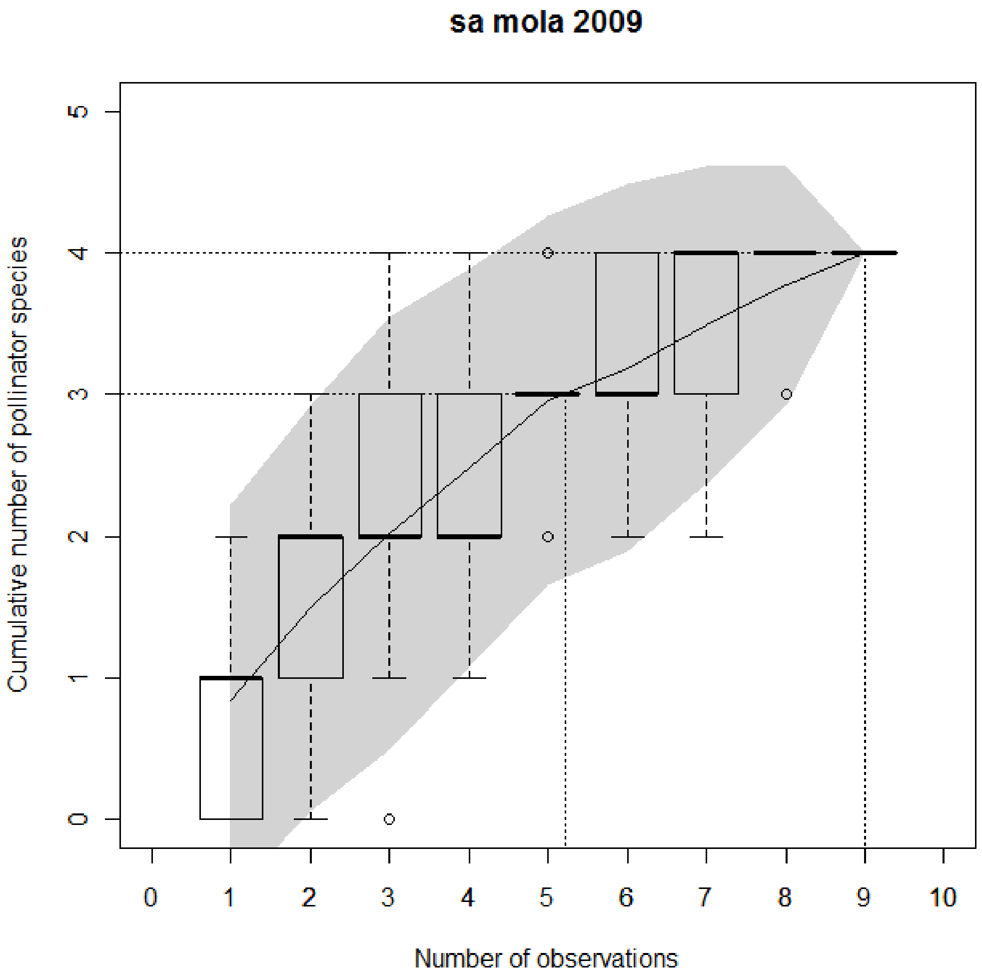


Figure A2.13. Rarefaction curve for plot *sa mola* (introduced area) in 2009.

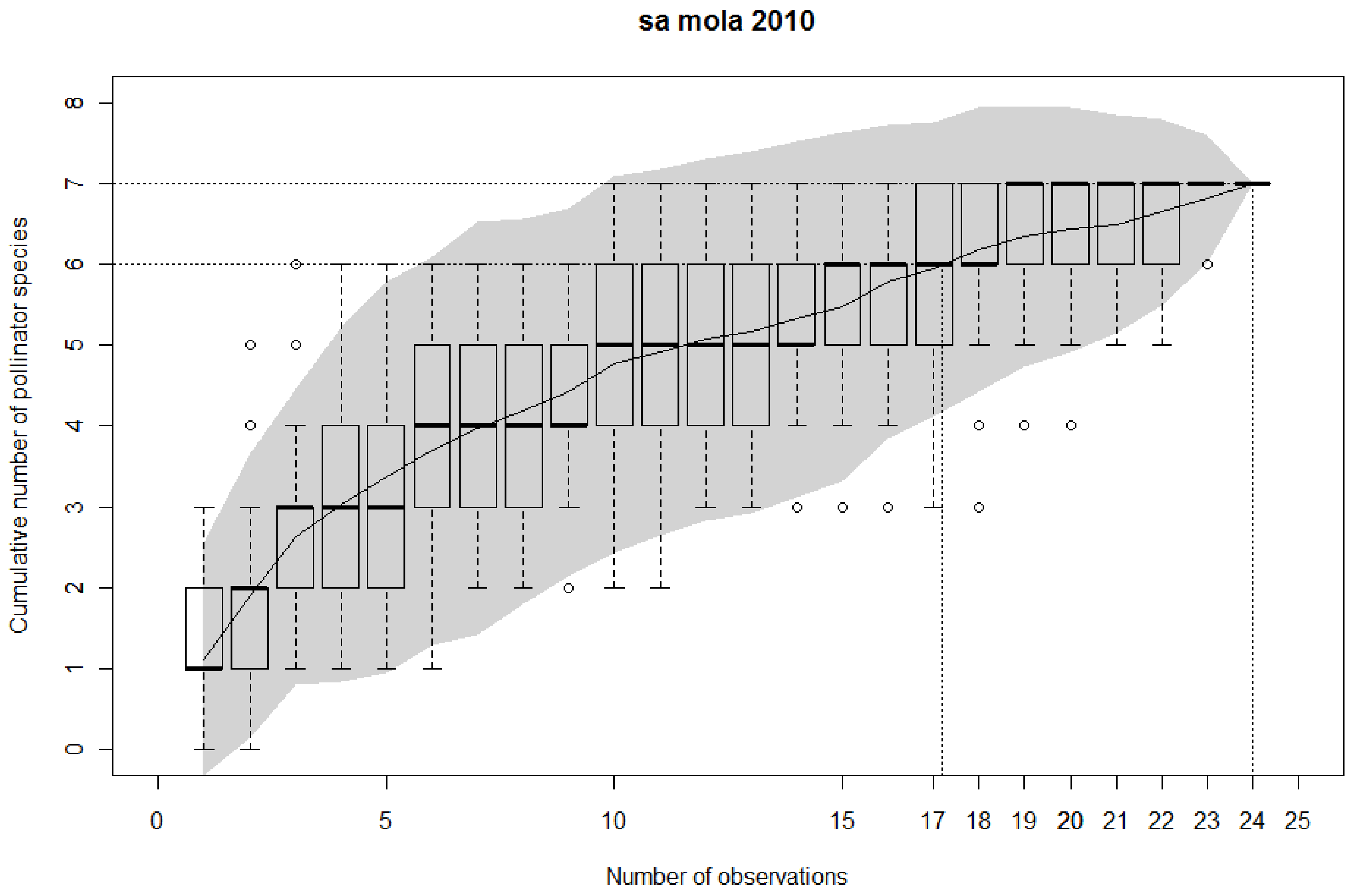
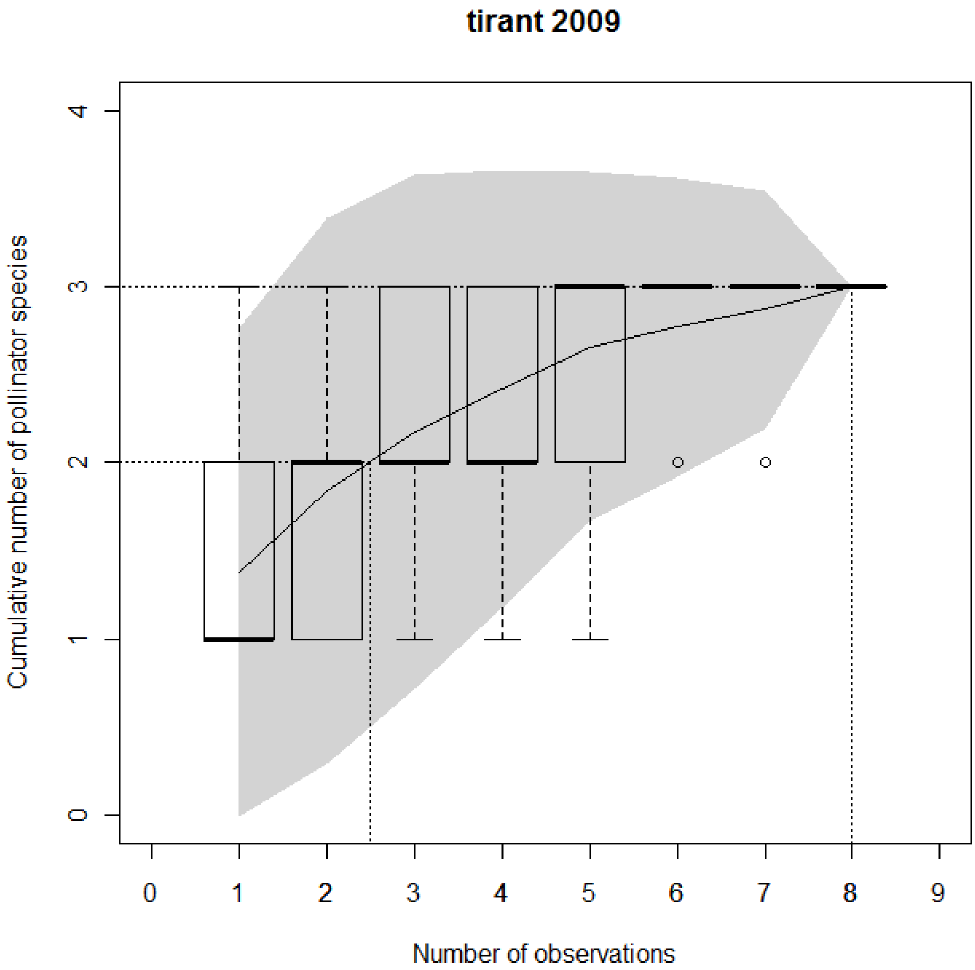
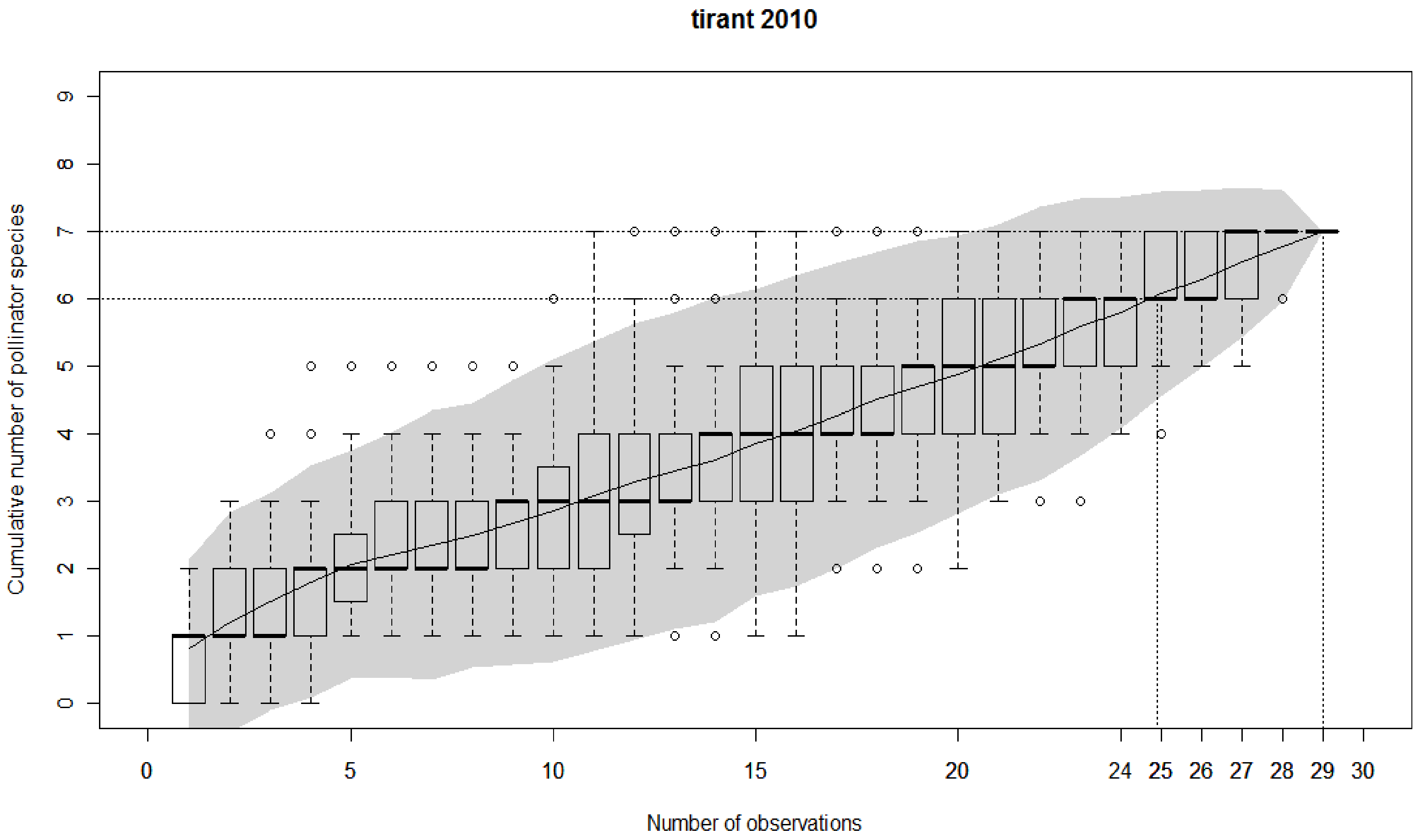


Figure A2.14. Rarefaction curve for plot s*a mola* (introduced area) in 2010.



Figre A2.15. Rarefaction curve for plot *tirant* (introduced area) in 2009.



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igure A2.16. Rarefaction curve for plot *tirant* (introduced area) in 2010.

Appendix 3. *Hedysarum* pollinator species

Table A3.1. List of pollinator species observed visiting *Hedysarum*. When identification to the species level was not possible, morphospecies were named with the acutest taxonomical level reached.

Species	**Family**	**Order**
Exclusive species of the native area		
Andrena labialis	Andrenidae	Hymenoptera
Bombus ruderatus	Apidae	Hymenoptera
Bombylius sp.	Bombyliidae	Diptera
Cardiophorus melampus	Elateridae	Coleoptera
Chalicodoma albonotata	Megachilidae	Hymenoptera
Eucera clypeata	Apidae	Hymenoptera
Eucera codinai	Apidae	Hymenoptera
Eucera eucnemidea	Apidae	Hymenoptera
Eupeodes corollae	Syrphidae	Diptera
Hoplitis ravouxi	Megachilidae	Hymenoptera
Lasioglossum sp.	Halictidae	Hymenoptera
Oedemera flavipes	Cucujidae	Coleoptera
Osmia versicolor	Megachilidae	Hymenoptera
Rhodanthidium sticticum	Megachilidae	Hymenoptera
Shared species between native and introduced areas		
Andrena ovatula	Andrenidae	Hymenoptera
Apis mellifera	Apidae	Hymenoptera
Bombus terrestris	Apidae	Hymenoptera
Eucera numida	Apidae	Hymenoptera
Osmia caerulescens	Megachilidae	Hymenoptera
Oxythyrea funesta	Cetoniidae	Coleoptera
Psilothrix viridicoerulea	Melyridae	Coleoptera

Exclusive species of the introduced area		
Anthophora balearica	Apidae	Hymenoptera
Anthophora plumipes	Apidae	Hymenoptera
Anthophora subterranea	Apidae	Hymenoptera
Anthophora sp.	Apidae	Hymenoptera
Ceratina curcubitina	Apidae	Hymenoptera
Chalicodoma sicula	Megachilidae	Hymenoptera
Eucera hungarica	Apidae	Hymenoptera
Eucera nigrilabris	Apidae	Hymenoptera
Eucera oraniensis	Apidae	Hymenoptera
Meligethes nigritus	Nitidulidae	Coleoptera
Rhodanthidium septemdentatum	Megachilidae	Hymenoptera
Tropinota hirta	Cetoniidae	Coleoptera
Xylocopa violacea	Apidae	Hymenoptera

Appendix 4. Exploration of baseline pollinator abundance in the native area

Menorca and the rest of the Balearic archipelago are considered continental islands; i.e. they have not always being islands as they were connected to the continent during the Messinian period (between 5.70 and 5.35 million years ago) (Alcover 2010). Besides, they are only 200 km from the continent. The impoverished biota in comparison with corresponding continental areas, usually refer to oceanic islands (Wardle 2002; Kueffer *et al.* 2010). Likewise, continental islands rarely include a significant neoendemic element in their biota (Fernández-Palacios 2010) nor show higher invasion degree than their mainland counterparts (Vilá *et al.* 2010); characteristics usually assigned to oceanic islands. Therefore, we did not expect significant differences in pollinator biota between our study areas.

However, in order to fell more confident about our sampling design, during the spring of 2008 we carried out pollination censuses in the native and the introduced areas to explore potential intrinsic differences between the pollinator communities of both areas. We observed three plant species native in both areas following the same methodology than with *Hedysarum*. The studied species *Daucus carota* (Umbeliferae), *Galactites tomentosa* (Compositae) and *Trifolium campestre* (Leguminosae) co-flower with *Hedysarum* and represent a wide variety of pollination syndromes or forms to offer their floral rewards.

We compared the number of pollinator species, individuals and visits, standardized by the number of observed flowers in each observation period (hereafter richness, abundance and visitation rates, respectively), between native and introduced areas with the non-parametrical Wilcoxon test. We did not find differences between native and introduced areas for any of the response variables for any of the three studied species (Table A4.1).

Table A4.1. Mean ± SD pollinator richness, abundance and visitation rates observed for *D. carota*, *G. tomentosa* and *T. campestre* in native and introduced areas. Number of observation periods (n) and Wilcoxon tests comparing the native and introduced areas are given.

		Richness				**Abundance**				**Visitation rates**			
Species	**Area**	**Mean ± SD**	**N**	**W**	**P**	**Mean ± SD**	**N**	**W**	**P**	**Mean ± SD**	**N**	**W**	**P**
Daucus carota	Native	2.98 ± 1.95	9	35.50	1.00	8.89 ± 4.74	9	37.00	0.96	8.89 ± 4.74	9	37.00	0.96
	Introduced	2.94 ± 1.43	8			12.73 ± 14.01	8			12.75 ± 14.00	8		
Galactites tomentosa	Native	0.11 ± 0.06	9	12.00	0.09	0.24 ± 0.13	9	14.50	0.16	0.66 ± 0.35	9	35.50	0.34
	Introduced	0.29 ± 0.23	6			0.47 ± 0.40	6			0.50 ± 0.48	6		
Trifolium campestre	Native	0.02 ± 0.06	9	40.00	0.41	0.02 ± 0.06	9	40.00	0.41	0.02 ± 0.06	9	40.00	0.41
	Introduced	0.00 ± 0.00	8			0.00 ± 0.00	8			0.00 ± 0.00	8		

Capítulo 2

Direct and indirect influence of non-native neighbours on pollination and fruit production of a native plant species

Resumen

Las plantas exóticas entomófilas se integran en las comunidades planta-polinizador residentes. De este modo, afectan a la polinización y al éxito reproductivo de las plantas nativas de forma directa, a través de los polinizadores compartidos; y de forma indirecta, alterando la composición y abundancia floral de la comunidad invadida. Diferenciar sus efectos directos e indirectos es crucial para conocer el signo y la magnitud de los impactos de las especies exóticas. En el caso de organismos sésiles como las plantas, la mayoría de estos efectos ocurren en su contexto espacial más inmediato (vecindario). Sin embargo, la influencia del vecindario en las interacciones planta-polinizador ha sido poco explorada.

Llevamos a cabo un trabajo de campo observacional y experimental a escala de vecindario en una comunidad de matorral mediterráneo para explorar los efectos de la planta exótica entomófila *Hedysarum coronarium* en la tasa de visitas, presencia de abeja de la miel y producción de frutos en la planta nativa *Muscari comosum*.

Hedysarum, de forma directa y positiva afectó a la tasa de visitas y a la fructificación de las plantas nativas focales a través de su despliegue floral. De forma indirecta, al reducir la diversidad floral en su vecindario, *Hedysarum* afectó positivamente a la tasa de visitas y negativamente a la fructificación de las plantas nativas focales. Así pues, aunque los efectos directos e indirectos fueron aditivos para la tasa de visitas, se anularon mutuamente en el caso de la fructificación; de modo que el efecto neto de *Hedysarum* en el éxito reproductivo de las plantas focales de *Muscari* no fue significativo.

La combinación de estudios observacionales y manipulativos como los llevados a cabo en este trabajo, permite abordar la complejidad de los efectos directos e indirectos que las plantas exóticas pueden tener en el éxito reproductivo de las nativas a escala de vecindario.

Abstract

Entomophilous non-native plants become integrated into the recipient plant-pollinator communities. They thereby affect the pollination and reproductive success of native plant species directly through shared pollinators and indirectly by altering the composition and abundance of floral resources in the invaded community. Separating direct from indirect effects is critical for understanding the magnitude and direction of impacts of non-native species. For sessile organisms such as plants, most of these effects occur within their most immediate spatial context (i.e. neighbourhood). However, the influence of the neighbourhood on plant-pollinator interactions remains largely unexplored.

We conducted field observations and a flower removal experiment at a neighbourhood scale in a Mediterranean shrubland to explore the effects of the non-native entomophilous plant *Hedysarum coronarium* on pollinator visitation rates, the presence of the honeybee and fruit production of the native and co-flowering species *Muscari comosum*.

The non-native species directly and positively affected the visitation rate and fructification of native target plants through its floral display. Indirectly, by reducing the diversity of floral resources in recipient communities, the non-native species positively affected visitation rates of native target plants and negatively affected their fructification. Although direct and indirect effects were additive for visitation rate, these effects offset each other for fructification rate, resulting in an overall non-significant effect of the non-native species on the reproductive success of native target plants.

By combining field observations with a manipulative experiment our study illustrates the complexity of direct and indirect effects that non-native species can exert on the reproductive output of native species at the neighbourhood scale.

Introduction

Non-native species alter the biodiversity and functioning of native communities (Simberloff 2005). However, in many cases we are unaware of the underlying ecological mechanisms of such impacts (Levine *et al.* 2003) and whether they are directly or indirectly (i.e. through the modification of other species' presence, abundance or behaviour in the community) caused by the non-natives (McKinney & Goodell 2010).

Entomophilous non-native plants require the pollination service of the area in which they are introduced and they usually become well integrated into resident plant-pollinator communities, affecting native plants through shared pollinators (Memmott & Waser 2002; Vilà *et al.* 2009) (continuous black arrow in Fig. 1). Their effect on the pollination of native plants varies from facilitative to competitive (Bjerknes *et al.* 2007). Native plants can benefit from non-native plants if the latter attract more shared pollinators to the community (i.e. magnet effect) (Molina-Montenegro *et al.* 2008). Entomophilous plant species differ in their attractiveness to pollinators depending on the relative abundance, likelihood of being detected and quality of their floral resources (Cresswell & Osborne 2004; Dietzsch *et al.* 2011). Thus, less attractive species growing in close proximity to high rewarding ones may receive more visits because either attracted pollinators are now able to detect them or their floral rewards are worth the effort to travel short distances. However, native plants can be negatively affected if the non-native plants monopolize pollinator visits (Chittka & Schürkens 2001) or increase heterospecific pollen deposition (Brown & Mitchell 2001).

Moreover, even if entomophilous non-native plants do not share pollinators with native plants, they can indirectly affect the pollination and reproductive success of native plants by altering the composition and abundance of floral resources in the recipient community (Lázaro & Totland 2010a) and, consequently, by altering the foraging behaviour of pollinators (Pollnac, Maxwell & Menalled 2009) (dashed arrows in Fig.1).

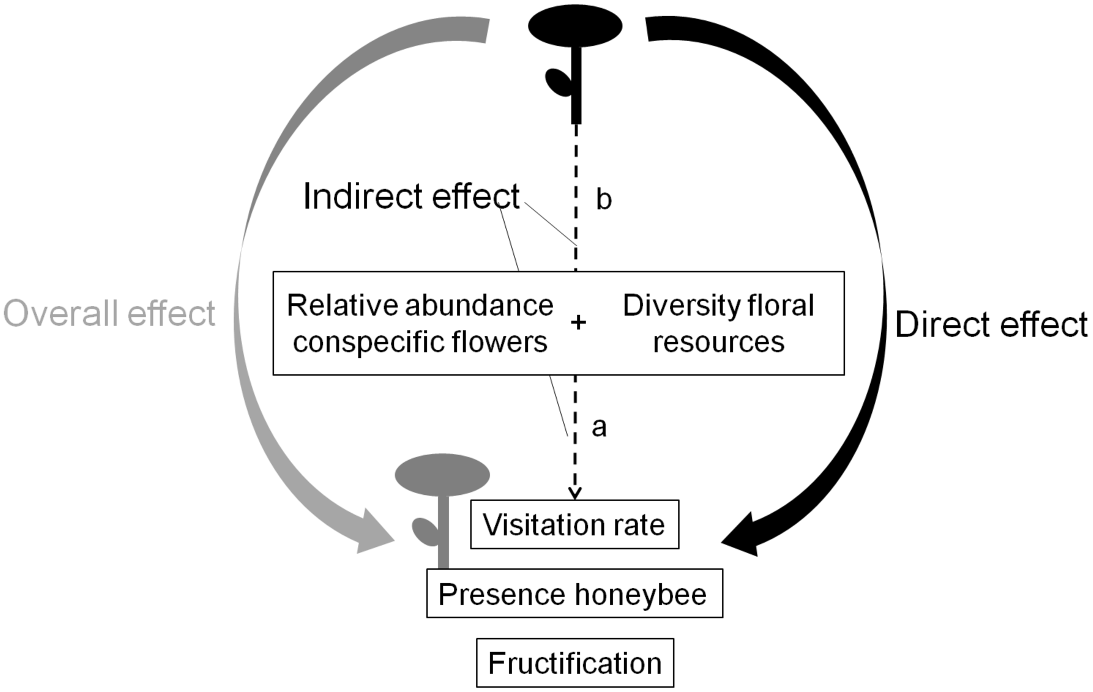

Figure 1. Schematic diagram on the effect of a non-native plant on the pollination and reproductive success of its neighbours. The overall effect (continuous grey arrow) can be the result of direct (continuous black arrow) and indirect (dashed arrows) effects. Letters beside dashed arrows indicate the two relationships analyzed to explore indirect effects.

It is known that the relative abundance of conspecific flowers and the diversity of floral resources highly influence the pollination and subsequent reproductive success of plants (Rathcke 1983; Ghazoul 2006). A high relative abundance of conspecifics is likely to increase attractiveness to pollinators, resulting in a positive effect on visitation rates (Muñoz & Cavieres 2008), although the relationship may be asymptotic (Courchamp, Clutton-Brock & Grenfell 1999; Cheptou & Avendano 2006). Pollinators, such as the honeybee, with intensive foraging behaviour, high flower constancy (Grüeter *et al.* 2011) and short flying distances between consecutive visits (Gross 2001) would benefit the most from high conspecific abundances. High relative abundances of conspecifics can also have a negative effect by increasing the deposition of low quality pollen coming from the individual plants themselves (Liao *et al.* 2011) or from closely related ones (Bosch & Waser 2001).

The diversity of floral resources can also have a positive effect on visitation rates. A diverse floral display may provide mutual benefits to plants if pollinators do not discriminate between floral types or if they are generalists. Thus, they would be attracted to a highly diverse patch in a manner similar to that of a patch with high floral density. A diverse floral display may also attract pollinators that seek multiple resources (i.e. pollen, nectar, mates) provided by different plant species (Ghazoul 2006). Its effect can also be negative if it implies an increase in heterospecific pollen deposition.

Non-native plants can produce such alterations in their recipient communities at short adjacent areas of influence (hereafter neighbourhood scale) (Silander & Pacala 1985; Tilman 1994; Schnurr *et al.* 2004; Jones & Comita 2008) by either competing with natives for the use of soil resources and light (e.g. Dyer & Rice 1999), producing allelopathic compounds (e.g. Gómez-Aparicio & Canham 2008) or interacting with their seed predators, herbivores or pathogens (e.g. Schnurr *et al.* 2004; Agrawal, Lau & Hamback 2006; Gómez-Aparicio *et al.* 2012, respectively). Plant-pollinator interactions respond to the characteristics of the community at different spatial scales, including the neighbourhood scale (Roll *et al.* 1997; Spigler & Chang 2008; Jakobsson, Lázaro & Totland 2009a; Lázaro & Totland 2010a), according to the mobility, dietary breath and foraging behaviour of pollinators (Ghazoul 2005). Therefore, the neighbourhood is the ideal spatial scale at which to explore both direct and indirect effects of non-native plants on plant-pollinator interactions. Nonetheless, the influence of this small spatial context on plant-pollinator interactions remains largely unexplored (Mitchell *et al.* 2009), but see (Jakobsson *et al.* 2009a; Lázaro & Totland 2010a; Waters, Fisher & Hille Ris Lambers 2014).

Here we present a study in which we combined field observations and flower removal experiments to explore the direct and indirect effects of a non-native, generalist and high-rewarding entomophilous N-fixing species on the pollination and reproductive success of a native species at the neighbourhood scale. We address the following questions: (i) Does the non-native plant directly affect the visitation rate, presence of the honeybee and fructification of a native plant? (continuous black arrow in Fig. 1); (ii) Is the direct effect of the non-native plant on a native plant mediated by its floral display, or is it more closely related to the vegetative parts of the plant?; and (iii)

Does the non-native plant indirectly affect the visitation rate, presence of the honeybee and fructification of a native plant by altering the relative abundance of conspecific flowers and/or the diversity of flowers in the neighbourhood? (dashed arrows in Fig. 1).

Materials and Methods

Study native species and site

As the native study species we selected *Muscari comosum* (L.) Miller (hereafter *Muscari*), a geophyte native to regions of the Mediterranean Basin. It is a 30 cm tall herb with prostrate leaves and a raceme inflorescence of up to 20 fertile greenish flowers with the floral pieces completely united in 2-3 mm wide actinomorphic cylinders. At the top of the inflorescence there is a group of sterile violet flowers (Valdés, Talavera & Fernández-Galiano 1987) (Fig. 2). *Muscari* was chosen as the target native species because it met the following requirements: (i) it grows in communities in which *Hedysarum* has become naturalized; (ii) its flowering phenology overlaps with that of *Hedysarum*; (iii) its reproduction is sexual (Garrido-Ramos *et al.* 1998), although it is self-compatible, it highly depends on out-crossing (Rejón *et al.* 1985; Alonso & Reguera 1989) (Table 1); and (iv) it shares some pollinator species with *Hedysarum* (Table 2 and Table A1.2).

Table 1. Differences in fruit production for *Muscari* flowers assigned to different treatments: a) control: flowers not manipulated; b) autogamy: flowers bagged with a tea bag to avoid any pollen transfer mediated by insects. Each treatment was randomly assigned to one of the two flowers marked in 30 individuals of *Muscari* selected in the study site in spring 2009. Differences in fruit production between treatments were analysed with Chi-square tests.

Treatment	N Flowers	N Fruits	% Fructification	$\chi 2$	df	P
Autogamy	29	0	0.00	21.16	1	< 0.001
Control	23	14	60.87			

Table 2. Pollinator species of the native *Muscari* observed in the study area during 147 censuses (36.75 h). Species in bold letters are the ones shared with *Hedysarum* (see Table A1.2). Percentages of total number of visits achieved for each pollinator species in each neighbourhood treatment are also given.

Species	Family	Order	% Visits		
			Control	Invaded	Removal
Dasytes virens	Melyridae	Coleoptera	0.00	0.00	3.28
Oedemera sp.	Cucujidae	Coleoptera	0.00	3.03	0.00
Psilothrix viridicoerulea	Melyridae	Coleoptera	60.61	39.39	80.33
Apis mellifera	Apidae	Hymenoptera	30.30	56.06	0.00
Chalicodoma sicula	Megachilidae	Hymenoptera	6.06	0.00	0.00
Lasioglossum sp.	Halictidae	Hymenoptera	3.03	0.00	0.00
Osmia niveata	Megachilidae	Hymenoptera	0.00	0.00	4.92
Plagiolephis pygmaea	Formicidae	Hymenoptera	0.00	1.52	8.20
Platygastridae sp.	Platygastridae	Hymenoptera	0.00	0.00	3.28

Figure 2. Study native plant *Muscari.*

The study site comprised a 3 ha shrubland (40º2.468'N, 4º5.845'E) dominated by *Olea europaea* ssp. *sylvestris* and *Pistacia lentiscus* with a rich herbaceous understory in which both *Muscari* and *Hedysarum* were present together with 18 additional flowering plant species belonging to seven different families (Table A1.1).

Experimental design and neighbourhood characterization

In spring 2010 we selected 43 *Muscari* target plants, with a minimum distance of 2 m between individuals, and established a 1 m radius neighbourhood around each target plant. The size of the neighbourhood, though smaller than in other pollination neighbourhood studies (e.g. Jakobsson *et al.* 2009; Lázaro & Totland 2010a; Waters *et al.* 2014), was established on the basis of previous census results (**Chapter 3**). Briefly, we conducted a total of 185 pollinator censuses on all native co-flowering plant species in a 20 x 20 m^2 invaded plot. We found that for the pool of native plants, the visitation rate was three fold higher for individuals in which the closest *Hedysarum* flower was ≤ 1 m , than for those located > 1 m from *Hedysarum* flowers (1.15 ± 0.31 and 0.34 ± 0.13 visits/flower/hour, respectively; N = 185, Z = -3.677, P < 0.001; Fig. A2.1). In addition, we considered a 1 m radius to be a suitable distance to define the area of influence of *Hedysarum* vegetative parts on surrounding plants. *Hedysarum* might compete for soil resources at distances of only a few centimetres, like most non-tree species (Vilà & Weiner 2004), but competition for light and space may occur up to 1 m due to either its height when growing erect or its prostrate growth (Bustamante *et al.* 1998).

We established three non-native neighbourhood treatments within the 1 m radius around *Muscari* target plants: (i) Control, *Hedysarum* plants absent; (ii) Invaded, *Hedysarum* flowering plants present; and (iii) Removal, *Hedysarum* plants with clipped inflorescences but intact vegetative parts present (Fig. 3). There were 14 *Muscari* target plants without non-native neighbourhoods (Control treatment). The rest of the *Muscari* target plants had *Hedysarum* individuals in their neighbourhoods. We manually clipped all *Hedysarum* inflorescences in 18 randomly selected neighbourhoods (Removal treatment). The remaining 11 target plants were assigned to the Invaded treatment. *Hedysarum* cover did not differ between Invaded and Removal treatments (N = 29, t = -0.171, P = 0.866).

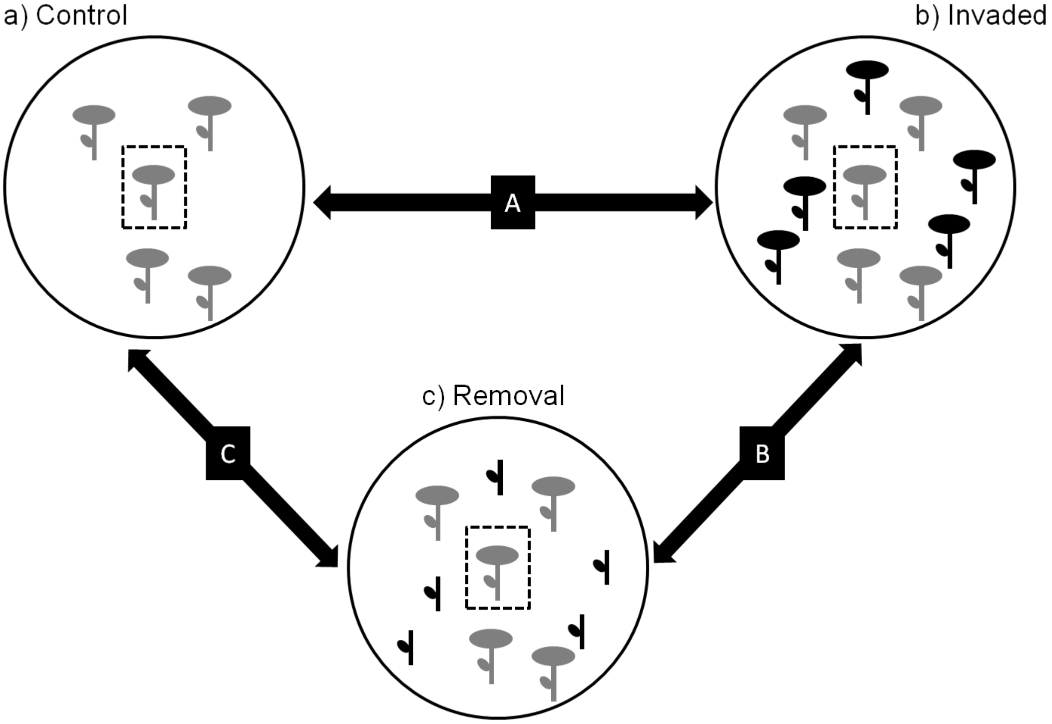

Figure 3. Non-native neighbourhood treatments. The non-native *Hedysarum* is represented in black while the native species, whether *Muscari* or others, are represented in grey. Target *Muscari* plants appear into the dashed lined squares. Arrows represent the different comparisons done to asses the effect overall effect of *Hedysarum* (A); the effects mediated by its floral display (B); and the effects related with vegetative interactions (C).

These three treatments allowed us to explore the overall effect of the non-native species (Control *vs.* Invaded treatments; arrow *A* in Fig. 3) and to isolate the effect mediated by the floral display of the non-native species (Invaded *vs.* Removal; arrow *B* in Fig. 3) from other effects associated with the vegetative parts of the plants (Control *vs*. Removal; arrow *C* in Fig. 3). By way of their vegetative parts, non-native plants might not only compete for abiotic resources (light, soil nutrients, water) but also produce allelopathic compounds and interact with other biotic resources such as herbivores or pathogens. Given that identifying which of these mechanisms were taking place was beyond the scope of this study, we use the term vegetative interaction to embrace all of them.

In each neighbourhood we established eight 0.4 x 0.4 m^2 quadrants, two located at each of the four cardinal directions. In each quadrant we counted all open flowers, excluding the target plants, and noted the species they belonged to. We then extrapolated these values to the total neighbourhood area.

Pollination censuses and fruit production

Pollination censuses were conducted on sunny, warm (≥ 17 ºC), non-windy days, from 10 a.m. to 6 p.m. Each census lasted 15 min during which we noted the number of visits of each pollinator species. A visitor was considered a pollinator if it entered a flower and touched the sexual parts of the plant. After each observation period we counted and marked all open flowers of the target plant (Mitchell 1994). As the flowers of this species do not last more than one day (Montero-Castaño, *personal observation*), estimates derived from our censuses are highly accurate. Each target plant was observed a minimum of three times randomly distributed throughout the day and the study period. In total we conducted 147 censuses (36.75 h). For each target plant, we estimated the visitation rate (i.e. visits/flower/hour) and the presence/absence of the honeybee as response variables. Approximately one month after the pollination censuses, we collected ripe fruit from observed flowers. The percentage of observed flowers that set fruit (hereafter, fructification) was also explored as a response variable.

Similarity between pollinator communities among the neighbourhood treatments was tested using the Sørensen similarity index: $QS = 2C / (A + B)$; where A and B are the number of species in neighbourhoods A and B, respectively, and C is the number of shared species. QS values range from 0 (no overlap in species composition) to 1 (complete overlap).

Statistical analyses

a) Effect of neighbourhood characteristics on the pollination and reproductive success of Muscari

Firstly, in order to explore both direct and indirect effects of *Hedysarum* on *Muscari*, we analyzed the characteristics of the neighbourhood that affected the visitation rate, presence of the honeybee and fructification in *Muscari* target plants (dashed arrow *a* in Fig. 1). We built a generalized linear model for each response variable with the relative abundance of *Muscari* conspecific flowers and floral diversity as fixed factors. Values of conspecific flowers and floral diversity were not correlated (Pearson's correlation coefficient = 0.067, P = 0.67). For visitation rate, the logarithm of the number of observed flowers and the logarithm of the hours of observation were included as offsets and the error distribution family was quasi-Poisson to deal with overdispersion. For presence of the honeybee and fructification, the error distribution family was binomial. Fructification was converted into a two column variable with the number of fruits and the number of flowers that did not set fruit representing the two columns.

b) Direct effect of Hedysarum on the pollination and reproductive success of Muscari

To analyze the direct effect of *Hedysarum* on the pollination and reproductive success of *Muscari* target plants (continuous black arrow in Fig.1) we built generalized linear models with treatment (Control, Invaded and Removal) as the fixed factor. For visitation rate, the logarithm of the number of observed flowers and the logarithm of the hours of observation were included as offsets and the error distribution family was quasi-Poisson. For presence of the honeybee and fructification, the error distribution family was binomial. Then, to control for the potential indirect effects as a result of the alteration of the neighbourhood, in each model we included as a covariate the characteristic of the neighbourhood (i.e. the relative abundance of conspecific flowers or the diversity of floral resources) that was found to affect each response variable (dashed arrows *a* in Fig. 1) according to the analyses described in the previous section.

Differences among the three neighbourhood treatments were analyzed through *post hoc* Tukey tests.

c) *Indirect effect of Hedysarum on the pollination and reproductive success of Muscari through the alteration of neighbourhood characteristics*

To analyze the influence of *Hedysarum* on its neighbourhood (dashed arrow *b* in Fig. 1), we built two generalized linear models with treatment (Control, Invaded and Removal) as the fixed factor. For the response variable relative abundance of conspecific flowers, the error distribution family was binomial. Relative abundance was converted into a two column variable with the number of *Muscari* conspecific flowers and the number of flowers of the remaining species representing the two columns. For the response variable diversity of flowers +1, Gamma was the error distribution family. Differences among the three neighbourhood treatments were analyzed through *post hoc* Tukey tests.

These analyses, together with aforementioned described models, allowed us to explore the indirect effect of *Hedysarum* on the pollination and reproductive success of *Muscari* target plants (dashed arrows in Fig. 1).

Analyses were conducted in the R statistical computing environment R (Development Core Team 2001). *Post hoc* tests were conducted with the library *multcomp*. Library *arn* was used to build the generalized linear models for the presence of the honeybee as response variable in order to deal with separation (i.e. when one or more explicative variables perfectly predict the outcome of interest; in our case, the honeybee was never present in the treatment Removal).

Results

We observed nine species visiting *Muscari* plants, including six bees and three beetles; two of the former taxa and one of the latter were shared with *Hedysarum* (Table 2 and Table A1.2). The Sørensen similarity index for pollinator species visiting *Muscari* plants was 0.60 between Control and Invaded, 0.22 between Control and Removal and 0.44 between Invaded and Removal treatments.

Effect of neighbourhood characteristics on the pollination and reproductive success of Muscari

The two characteristics of the neighbourhood considered in this study, namely relative abundance of conspecific flowers and diversity of floral resources, differed in their effect on visitation rate, presence of the honeybee and fructification (Table 4) in *Muscari* target plants. The relative abundance of conspecific flowers enhanced the presence of the honeybee, while it did not influence either visitation rate or fructification. The diversity of floral resources positively affected visitation rate and negatively affected fructification.

Table 3. Ranges (min and max values) of the independent variables estimated for the characterization of 1 m radius neighbourhoods around 43 *Muscari* target individuals depending on the treatment they were assigned to.

Treatment	# Target plant flowers*	*Hedysarum* cover (%)	# *Hedysarum* flowers	# *Muscari* flowers	# Total flowers	# Flowering species	Relative abundance *Muscari* flowers	Diversity flowers (Shannon index)
Control	6 - 26	-	-	0 - 215.6	12.3 - 215.6	1 - 6	0 - 1.00	0 - 1.52
Invaded	8 - 24	15.6 - 65.6	4.9 - 102.9	0 - 245.0	24.5 - 279.3	1 - 3	0 - 0.94	0 - 1.03
Removal	4 - 22	3.1 - 81.3	-	0 - 58.8	0 - 78.4	0 - 5	0 - 0.88	0 - 1.04

* Total number of flowers observed during the study

Table 4. Effect of the floral neighbourhoods on visitation rate, presence of the honeybee and fructification of *Muscari* target individuals. Significance levels: ˙ $P = 0.05$, * $P < 0.05$, ** $P < 0.01$.

Response variable	Floral Neighbourhood	Estimate	SE	Z/t	P		R^2
Visitation rate	Relative abundance conspecific flowers	0.375	0.376	0.998	0.324		0.33
	Diversity floral resources	-0.753	0.412	-1.829	0.075	·	
Presence *Apis*	Relative abundance conspecific flowers	3.947	1.418	2.784	0.005	**	0.41
	Diversity floral resources	-1.548	1.484	-1.044	0.297		
% Fructification	Relative abundance conspecific flowers	-0.150	0.282	-0.530	0.596		0.10
	Diversity floral resources	0.516	0.258	2.000	0.046	*	

Direct effect of Hedysarum on the pollination and reproductive success of Muscari

When controlling for the diversity of floral resources in the neighbourhood, we still observed different visitation rates in *Muscari* plants among treatments, indicating that *Hedysarum* had a direct effect on this response variable (continuous black arrow in Fig. 4a). Visitation rate was higher for the Invaded than for the Removal treatment, while the Control treatment showed intermediate values, which were not significantly different from those of the other two treatments (Fig. 5a). Therefore, *Muscari* visitation rate was positively related to the floral display of *Hedysarum*.

When controlling for the relative abundance of conspecifics, the differences in the presence of the honeybee among treatments (continuous grey arrow in Fig. 4b) disappeared (Fig. 5b). Thus, we found no evidence of a direct effect of *Hedysarum* on the presence of the honeybee (continuous black arrow in Fig. 4b).

Finally, when controlling for the diversity of floral resources, we found different fructification rates for *Muscari* plants among treatments (Fig. 5c), indicating that *Hedysarum* had a direct effect on this response variable (continuous black arrow in Fig. 4c). Fructification was higher for Invaded than for Removal treatments, while the Control treatment showed intermediate values, which were not significantly different from those observed for the other two treatments. Therefore, *Muscari* fructification was positively related to the floral display of *Hedysarum*.

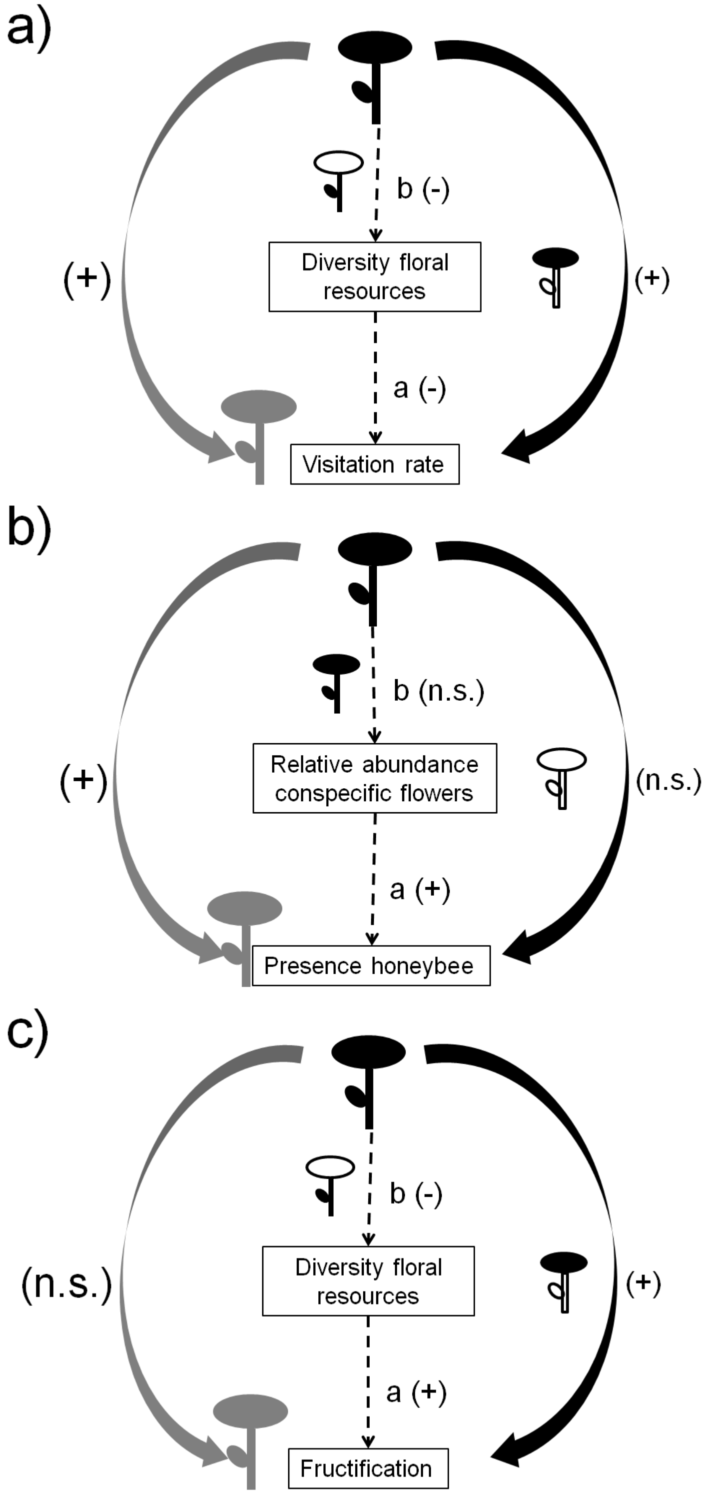

Figure 4. Schematic diagram resuming the results obtained for visitation rate (a), presence of the honeybee (b) and fructification (c) of *Muscari* target plants. The non-native *Hedysarum* is represented in black while the native *Muscari* is represented in grey. Grey continuous arrows represent the overall effect of *Hedysarum* on *Muscari* target plants while black continuous and black dashed arrows represent direct and indirect effects, respectively. The sign of the effect is given in brackets next to each arrow. Whether the effect is mediated by the vegetative part or by the floral display of *Hedysarum*, is indicated by coloring the part involved in the effect and leaving the not involved in bold.

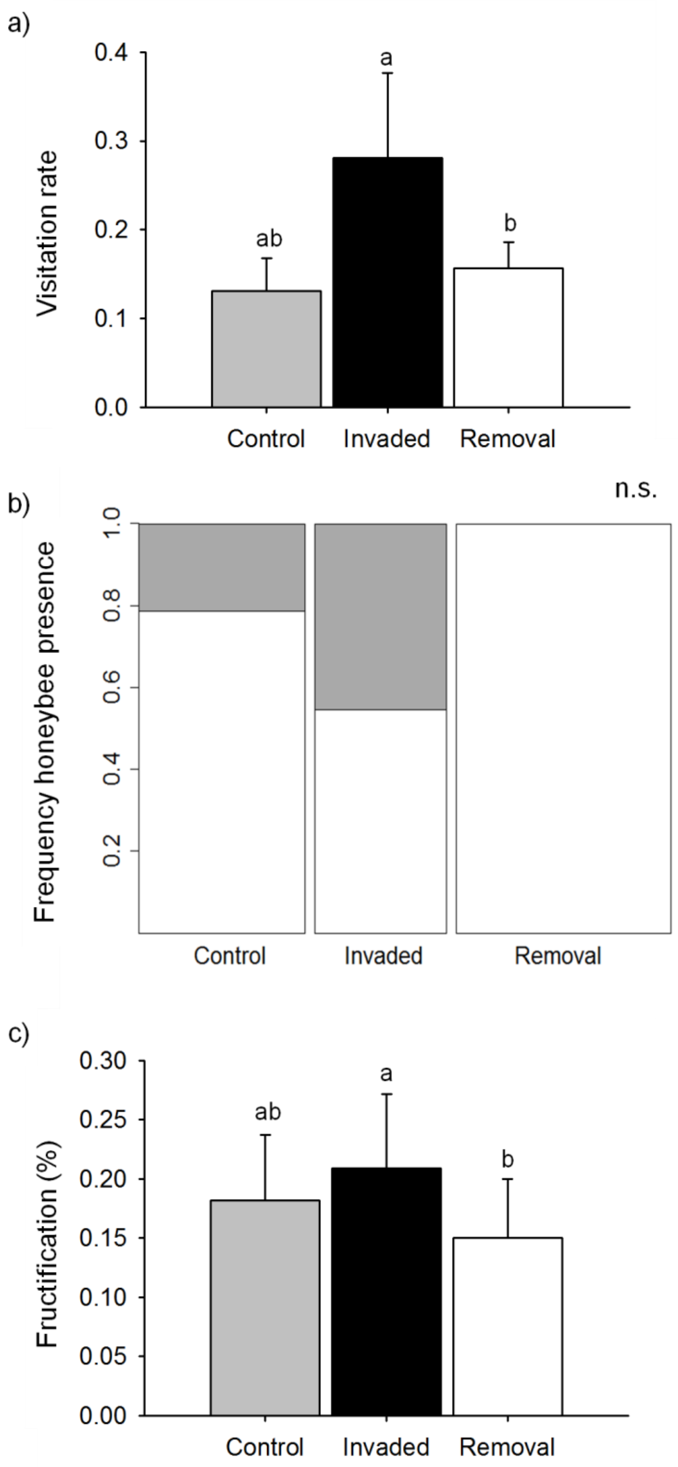

Figure 5. Mean + SE (a) visitation rate (i.e. visits/flower/hour) and (c) fructification (i.e. percentage of observed flowers that set fruits) in *Muscari* target plants with Control (grey bar), Invaded (black) and Removal (bold) neighbourhoods. Significant differences are represented by different letters above bars according to Tukey *post hoc* tests conducted for the models including the diversity of floral resources as a covariate. (b) Represents the frequency of the honeybee presence (grey) *vs*. absence (white) in *Muscari* target plants with Control, Invaded and Removal neighbourhoods. The width of the columns is proportional to the sample size of each neighbourhood treatment. Non-significant differences (P > 0.05) were found according to Tukey *post hoc* tests conducted for the model including the relative abundance of conspecifics flowers as a covariate.

Indirect effect of Hedysarum on the pollination and reproductive success of Muscari through the alteration of neighbourhood characteristics

The relative abundance of conspecific flowers was lowest for the Removal treatment, while no differences were found between Control and Invaded treatments (Fig. 6a). The relative abundance of *Muscari* conspecific flowers was positively related to the floral display of *Hedysarum,* but negatively related to the vegetative part of *Hedysarum*. These contrasting trends resulted in the similarity, or lack of difference, between the Control and the Invaded treatment. Therefore, *Hedysarum* did not indirectly affect the presence of the honeybee at *Muscari* target plants through the alteration of the relative abundance of *Muscari* conspecifics (dashed black arrows in Fig. 4b).

The diversity of floral resources in the neighbourhood was higher for the Control than for the Removal treatment, while for the Invaded treatment diversity was intermediate and non-significantly different from the other two treatments (Fig. 6b). This result suggests that the effect of *Hedysarum* on the diversity of floral resources was primarily mediated by vegetative interactions. Therefore, by reducing the diversity of floral resources in the neighbourhood, *Hedysarum* indirectly increased visitation rate but decreased fructification of *Muscari* target plants (dashed black arrows in Fig. 4a and Fig. 4c, respectively).

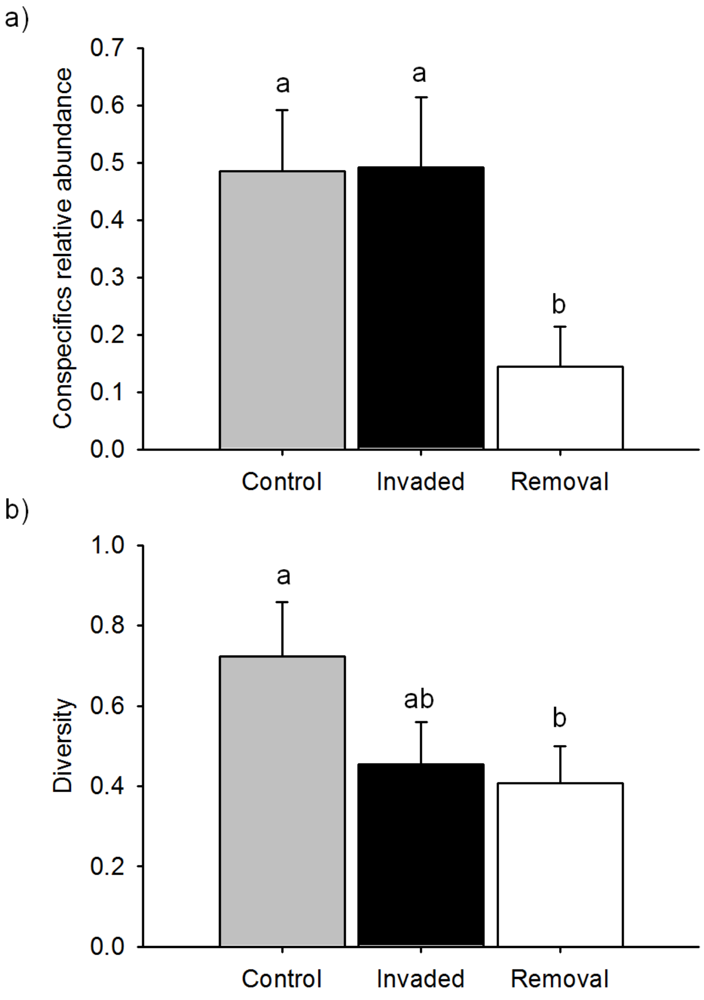

Figure 6. Mean + SE *Muscari* (a) relative abundance of conspecific flowers (i.e. conspecific flowers/total flowers) and (b) diversity of flowers (Shannon index) in Control (grey bar), Invaded (black) and Removal (bold) neighbourhoods. Significant differences are represented by different letters above bars.

Discussion

We found that non-native entomophilous plant species can affect the pollination and reproductive success of their neighbours both directly and indirectly by altering the species composition and abundance of their neighbourhoods. Those effects do not always act in the same direction and they can even nullify each other.

Aditive direct and indirect effects of the non-native on visitation rates

Hedysarum had an overall positive effect on the visitation rate to *Muscari* target plants, which was the result of direct and indirect effects acting in the same direction.

Hedysarum decreased the diversity of floral resources in the neighbourhood and thus indirectly affected the visitation rate of *Muscari* target plants. Such a decrease in diversity was related to the vegetative part of *Hedysarum* plants. Although we cannot identify the mechanisms involved in the observed vegetative interaction, competition for abiotic resources may occur. Non-native plants that are able to persist and invade a community usually outcompete natives for the use of abiotic resources (Vilà & Weiner 2004). As a result, the diversity of species, and subsequently the diversity of floral resources in the recipient community, might decrease as observed in this study.

The visitation rate to *Muscari* target plants was negatively affected by the diversity of floral resources in the neighbourhood. Pollinators seek to optimize their foraging behaviour, which it is partially determined by the relative attractiveness of co-flowering floral rewards. Thus, in diverse floral neighbourhoods, pollinators that visit plant species with low showy flowers like *Muscari* (Morales, Traveset & Harder 2013) might have more opportunities, and it may be beneficial for them to switch to another more attractive and rewarding plant species (Raine & Chittka 2005).

Simultaneously, due to its floral display, *Hedysarum* directly and positively affected the visitation rate to *Muscari* plants by attracting pollinators, thus exerting a magnet effect. Low showy plant species like *Muscari*, which is also usually hidden in the herb vegetation layer (Montero-Castaño, *personal observation*), might benefit greatly from associating with attractive and generalist plant species like *Hedysarum*.

The magnet effect was mainly mediated by the honeybee, which achieved most of *Muscari* visits when *Hedysarum* was in its neighbourhood. This species is the main pollinator of *Hedysarum* (Satta *et al.* 2000) and shows high flower constancy (Grüeter *et al.* 2011) but also an intensive foraging behaviour with short flying distances between two consecutive visits (Gross 2001). Therefore, it might be beneficial for honeybees to make consecutive interspecific visits as far as flowers are at short flying distances. Though the presence of the honeybee was not associated with *Hedysarum* floral display, as the honeybee is a very active pollinator that achieves several visits within the same plant individuals (Gross 2001), small differences in its presence can have significant effects in terms of visitation rates. On its behalf, the presence of the honeybee was not associated either with *Hedysarum* floral display or with the relative abundance of *Muscari* conspecific flowers. Subsequently, other indirect effects of *Hedysarum* through the alteration of its neighbourhood, different from the ones studied here, might explain the lowest presence of the honeybee in Removal treatment.

Opposed direct and indirect effects of the non-native on fructification

The overall positive effect of *Hedysarum* on the visitation rate to *Muscari* target plants did not translate into higher fructification. This was due to contrasting direct and indirect effects counteracting each other.

Despite the fact that the diversity of floral rewards was negatively associated with the visitation rate to *Muscari* target plants, its relationship with fructification was positive. Therefore, by decreasing the diversity of floral resources, *Hedysarum* indirectly and negatively affected fructification. The overall result is that in high diversity neighbourhoods *Muscari* target plants exhibited higher percentages of fructification even though they had lower visitation rates. The decoupling of visitation rates and fructification is a common phenomenon (e.g. Goodell *et al.* 2010). Fructification is not only determined by the availability of pollen for the fertilization of ovules, but also by the resources available for fruit and seed production (Zimmerman & Pyke 1988). Thus, in high diversity neighbourhoods, resource partitioning could inhibit competition and enhance fructification of coexisting plants (Fridley 2003).

Nevertheless, in the case of the direct magnet effect exerted by *Hedysarum* floral displays, visitation rate proved to be a good surrogate for fructification in *Muscari* target plants (Vázquez *et al.* 2005). Therefore, the direct effect of *Hedysarum* on *Muscari* fructification was positive, contrary to its indirect effect described above. This result also indicates that *Muscari* target plants were pollen limited and that pollinator visits were effective and, as a result, the deposition of *Hedysarum* heterospecific pollen was avoided. Pollinators, once they were attracted to the floral display of neighbourhoods invaded by *Hedysarum*, could directly visit *Muscari* instead of *Hedysarum*, avoiding the deposition of heterospecific pollen on the stigmas of *Muscari* target plants. The deposition of *Hedysarum* pollen on *Muscari* stigmas could also be avoided if it were collected on body parts of an insect different than those in touch with *Muscari* stigmas (Wolfe & Barrett 1989).

Conclusions

For a comprehensive understanding of the impacts of non-native plants on the pollination of native plants, both direct and indirect effects must be taken into account. Not doing so could lead to misestimating the effect of non-native species, depending on the species and the context. Manipulative experiments allow the assessment of whether non-native plants compete for abiotic resources (i.e. light, nutrients, water), the most studied interaction of plant invasions, or their effect is mediated by their floral display and therefore, through shared pollinators. Consequently, field experiments provide a means to better clarify the mechanisms underlying the impact of entomophilous non-native species.

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Appendix 1. Native flowering plant community at the study site and pollinator community of the study species.

Table A1.1. Flowering plant species and family found in the pool of the 43 study neighbourhoods, including the two study species *Hedysarum* and *Muscari* highlighted in bold letters.

Species	**Family**
Reichardia picroides	Compositae
Hypochoeris achyrophorus	Compositae
Centaurium maritimum	Gentaniaceae
Geranium molle	Geraniaceae
Hedysarum coronarium	Leguminosae
Lotus edulis	Leguminosae
Scorpiurus sulcatus	Leguminosae
Medicago murex	Leguminosae
Lotus ornithopodioides	Leguminosae
Melilotus indicus	Leguminosae
Trifolium campestre	Leguminosae
Anthyllis tetraphylla	Leguminosae
Trifolium stellatum	Leguminosae
Calicotome infesta	Leguminosae
Medicago polymorpha	Leguminosae
Muscari comosum	Liliaceae
Asphodelus aestivus	Liliaceae
Ophrys vermixia	Orchidaceae
Serapias lingua	Orchidaceae
Anagallis arvensis	Primulaceae

Table A1.2. Pollinator species of *Hedysarum* observed during 138 censuses (34.5 h). Censuses were carried out on *Hedysarum* individuals distributed among four 20 x 20 m^2 independent plots were *Hedysarum* was naturalized (*sensu* Pyšek *et al.* 2004) in Menorca (Site 1: 39° 56.395'N, 4° 15.052'E; Site 2: 39° 56.677'N, 4° 14.892'E; Site 3: 39° 56.191'N, 4° 13.000'E; Site 4: 40° 2.429'N, 4° 5.908'E). Percentages of total number of visits achieved for each pollinator species are also given.

Species	**Family**	**Order**	**% Visits**
Oxythyrea funesta	Cetoniidae	Coleoptera	0.29
Tropinota hirta	Cetoniidae	Coleoptera	0.05
Psilothrix viridicoerulea	Melyridae	Coleoptera	0.10
Meligethes nigritus	Nitidulidae	Coleoptera	0.10
Andrena ovatula	Andrenidae	Hymenoptera	1.27
Anthophora balearica	Apidae	Hymenoptera	0.10
Anthophora plumipes	Apidae	Hymenoptera	0.34
Anthophora sp.	Apidae	Hymenoptera	0.10
Anthophora subterranea	Apidae	Hymenoptera	0.44
Apis mellifera	Apidae	Hymenoptera	83.34
Bombus terrestris	Apidae	Hymenoptera	0.15
Ceratina curcubitina	Apidae	Hymenoptera	0.05
Eucera hungarica	Apidae	Hymenoptera	0.15
Eucera nigrilabris	Apidae	Hymenoptera	0.05
Eucera numida	Apidae	Hymenoptera	0.10
Eucera oraniensis	Apidae	Hymenoptera	0.15
Xylocopa violacea	Apidae	Hymenoptera	2.14
Chalicodoma sicula	Megachilidae	Hymenoptera	11.01
Osmia caerulescens	Megachilidae	Hymenoptera	0.05
Rhodanthidium septemdentatum	Megachilidae	Hymenoptera	0.05

Appendix 2. Justification of neighbourhood size

As part of a complementary study, we conducted a total of 185 censuses on the entire flowering plant community in a 20 x 20 m^2 plot located within the study site and following the same protocol than in this study (**Chapter 3**).

We found that the visitation rates observed in those individuals whose closest *Hedysarum* flower was <1 m apart, were more than threefold higher than in individuals whose closest *Hedysarum* flowers were more distant (1.15 ± 0.31 and 0.34 ± 0.13 visits/flower/hour, respectively; Fig. A2.1), when considering the whole pool of native co-flowering plants.

We statistically analyzed such differences in visitation rates by building a generalized linear model with distance to the closest *Hedysarum* flower (≤ 1 m *vs.* > 1 m) as fixed factor and plant species as random factor. The logarithm of the number of observed flowers was included as an offset and the error distribution family was quasi-Poisson to deal with overdispersion. The analysis was conducted with the library *lme4* of the R statistical computing environment (Development Core Team 2001).

We found differences to be statistically significant ($N = 185$, $Z = -3.677$, $P < 0.001$).

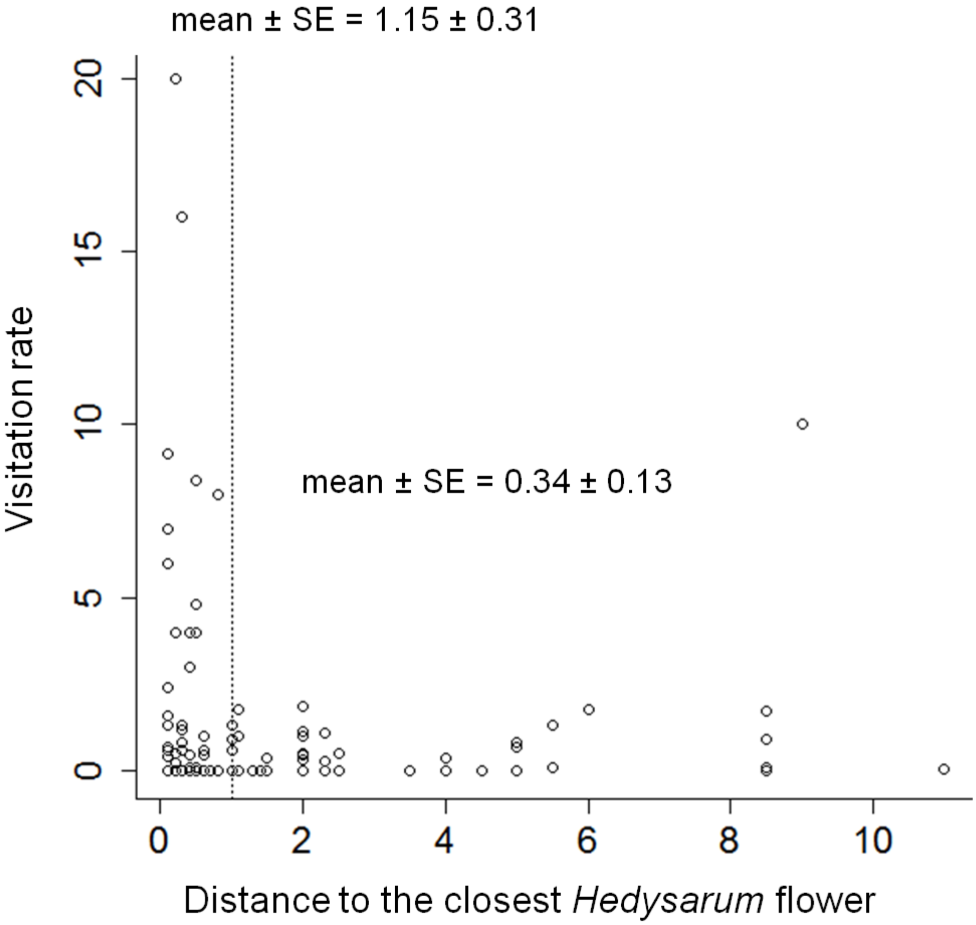
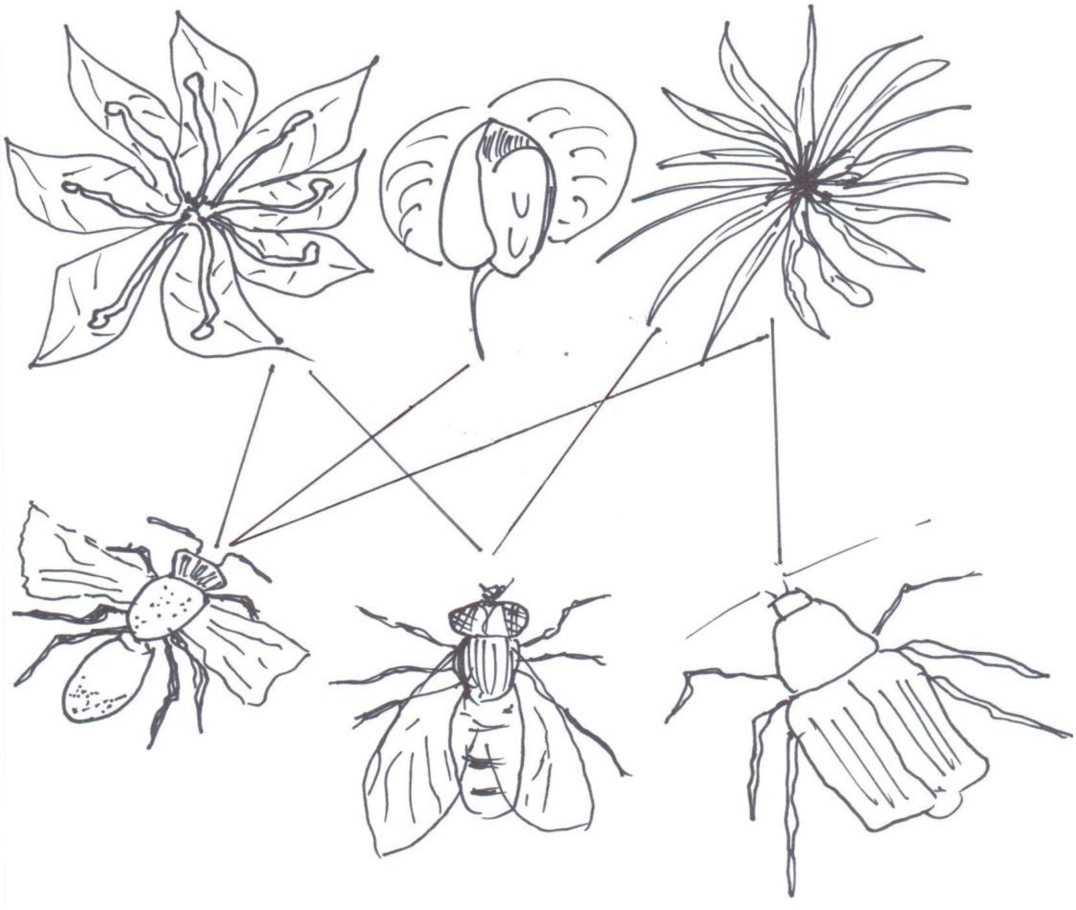


Figure A2.1. Relationship between visitation rate (visits/flower/hour) and the distance to the closest *Hedysarum* flower for the pool of co-flowering native plants in the study site. On the left of the dashed line, values for distances <1 m and on the right, values for distances >1 m. Mean ± SE of visitation rate for both cases are given.

Capítulo 3

Interaction rewiring in plant-pollinator networks invaded by a non-native plant



Resumen

Las plantas exóticas entomófilas se integran en las comunidades planta-polinizador, afectando a las plantas nativas a través de los polinizadores generalistas compartidos. Su efecto en las nativas puede ser de facilitación o de competencia, dependiendo, entre otras cosas, de las características de las especies implicadas (por ejemplo, sus rasgos florales). Estos efectos no ocurren de forma asilada, sino inmersos en redes complejas de interacción, de modo que las plantas exóticas también pueden afectar a las propiedades de las redes.

Se llevó a cabo un experimento de eliminación para explorar el efecto de *Hedysarum* en las redes plata-polinizador de un matorral mediterráneo y si éste está influenciado por la similitud en morfología floral de la exótica con las nativas (papilionáceas *vs*. no papilionáceas). Se analizó toda la comunidad de polinizadores y la abeja de la miel por separado, por ser el principal polinizador de *Hedysarum*.

Se observó que *Hedysarum* se integra en las redes planta-polinizador residentes a través de polinizadores generalistas. A pesar de ello, la tasa de visitas, el grado de generalización, el solapamiento de nicho y cuán dependientes de ellas son los polinizadores (*species strength*), no se vieron afectadas en las plantas nativas, siendo siempre menores para nativas con flores papilionáceas. La conectancia de la red tampoco se vio afectada. Sin embargo, la tasa de visitas de la abeja de la miel en las nativas fue menor en las comunidades invadidas, independientemente de su morfología floral, mientras que se observaron otras interacciones protagonizadas por polinizadores silvestres. Estos cambios en la identidad de las interacciones afectaron a la modularidad pero no al anidamiento de las redes.

El efecto de las plantas exóticas con morfología floral restrictiva en la estructura de las redes no parece estar relacionado con su similitud con las nativas en morfología floral. Con la presencia de la exótica, algunas propiedades de las redes se conservan (anidamiento) mientras que otras se ven alteradas (modularidad), lo cual puede deberse no a cambios en el número, sino en la identidad de las interacciones. Estos cambios en la identidad de las interacciones deben tenerse en cuenta a la hora de predecir la respuesta de las comunidades ante la llegada de especies exóticas.

Abstract

Entomophilous non-native plants become well integrated into plant-pollinator communities, affecting native plants through generalist shared pollinators. The effect on native plant pollination varies from facilitative to competitive, and might depend on species traits (e.g. flower morphology). The effects of non-native plants on native plant-pollinator interactions do not occur in isolation but within complex interacting networks so that non-native plants can also alter properties of the entire network.

We conducted a flower removal experiment to explore the effect of *Hedysarum* on Mediterranean shrubland plant-pollinator networks and whether its effect is influenced by its similarity to the native species in flower morphology (papilionate *vs*. non-papilionate). We conducted the analysis for both the entire pollinator community and the honeybee exclusively, as it is the main pollinator of *Hedysarum.*

Hedysarum was well integrated into the resident plant-pollinator networks by generalist pollinators. Nevertheless, visitation rate, linkage level, niche overlap and strength of native plant species were not overall affected, and were always lower in natives with restrictive papilionate flowers. Network connectance was not affected either. However, honeybee visitation rate to native plants decreased in invaded communities, irrespective of flower morphology, while other interactions involving wild pollinators appeared. These changes in the identity of interactions (i.e. interaction rewiring) altered modularity, while nestedness was maintained.

The effect of a non-native plant species with restrictive flower morphology on the network topology does not appear to be related to its similarity to natives in flower morphology. With the presence of non-native flowers some topological patterns might be maintained (e.g. nestedness), whereas others might be altered (e.g. modularity) though they are not necessarily accompanied by changes in the number of interactions, but rather in their identity. Therefore, interaction rewiring must be taken into account for predicting community responses to the arrival of non-native plants.

Introduction

Many entomophilous and obligate out-crossing non-native plants can become well integrated into the resident plant-pollinator networks in which they have been introduced (Vilà *et al.* 2009; Traveset *et al.* 2013). Super-generalist and abundant pollinator species like the honeybee or bumblebees (*Bombus* spp.) usually play an important role in such integration (Olesen *et al.* 2002; Stout *et al.* 2002; Gross *et al.* 2010). Consequently, non-native plants can affect native plant species in the resident networks through shared pollinators. The effect on the pollination of a particular native plant species varies from facilitative to competitive (Traveset & Richardson 2006; Bjerknes *et al.* 2007). Whether the non-native plant has a neutral, negative or positive effect on coexisting native plants, might depend, among other things, on floral traits of both the native and non-native species involved (e.g. corolla colour, flower symmetry) (Morales & Aizen 2006; Mitchell *et al.* 2009). In general, similar flower symmetry between pairs of co-flowering non-native and native species reduces pollinator visitation rates to native plants (Morales & Traveset 2009). However, this trend does not hold when considering similarity among all native plant species in the invaded plant-pollinator community (Morales & Aizen 2006; Vilà *et al.* 2009). The influence of flower similarity might be due to close phylogenetic relationships among species. Non-natives, which belong to families well represented in the native flora, tend to be visited by more pollinator species (Memmott & Waser 2002). Therefore, we expect non-natives to compete more strongly for pollinators with taxonomically close resident native species than with less related species, even more so if they share restrictive flower morphologies not accessible to all pollinators such as papilionate flowers (Córdoba & Cocucci 2011).

The effects of non-native plant species on pair-wise interactions do not occur in isolation but within complex plant-pollinator interacting networks (Montoya, Pimm & Solé 2006). Therefore, the influence of similarity in flower morphology on each pair–wise interaction between the non-native and natives, can affect the emergent properties of the entire plant-pollinator network (Morales & Aizen 2002; Olesen *et al.* 2002; Lopezaraiza-Mikel *et al.* 2007; Bartomeus, Vilà & Santamaría 2008b; Vázquez *et* *al.* 2009). The theory and analytical tools developed for network analysis provide a useful framework for approaching such community level studies (Rezende *et al.* 2007; Tylianakis *et al.* 2010). Plant-pollinator networks are usually heterogeneous (i.e. few highly connected species interact with many poorly connected ones), asymmetric (i.e. if a plant species highly depends on a pollinator species, the pollinator does not depend on that plant to the same extent, and vice versa), nested (i.e. specialist species interact with subsets of the species that generalists interact with) and modular (i.e. composed of clusters of highly interconnected species) (Vázquez & Aizen 2004; Jordano, Bascompte & Olesen 2006; Olesen *et al.* 2007; Bascompte 2009). However, pollinator species are constantly optimizing their foraging behavior by shifting the plant species they interact with, so that interactions experience a constant turnover over time and space (i.e. interaction rewiring) (Burkle & Alarcon 2011, Poisot *et al.* 2012). Thus, the prevalence of topological patterns despite such interaction rewiring might have important ecological and evolutionary implications, though the underlying mechanisms responsible for this topology are still poorly understood (Vázquez *et al.* 2009).

Empirical studies on the effect of non-native plants on plant-pollinator networks are scarce due to the high sampling effort required (but see Memmott & Waser 2002; Olesen *et al.* 2002; Morales & Aizen 2006; Bartomeus *et al.* 2008; Padrón *et al.* 2009; Vilà *et al.* 2009; Ibanez 2012; Traveset *et al.* 2013) and most studies are based on model simulations. Some of these studies, have already explored the importance of mechanisms in the structuring of networks such as the phenology, morphology, phylogenetic distance and abundance of the species in the studied communities (Ibanez 2012; Russo *et al.* 2014; Vizentin-Bugoni *et al.* 2014; Perazzo *et al.* 2014; Stout & Casey 2014). However, the results do not exhibit a general trend. Manipulative experiments would help to isolate the effect of non-native species from other confounding factors and to disentangle the underlying mechanisms as well as to validate models. To our knowledge, only Lopezaraiza-Mikel *et al.* (2007) and Ferrero *et al.* (2013) have conducted manipulative removal experiments to explore the effect of invasive plant species on plant-pollinator networks. However, in any of them both the effect on networks structure and the underlying mechanisms are simultaneously explored.

Here we present a manipulative field experiment, conducted during two consecutive years to deal with the inter-annual variability of pollinator communities (Alarcón, Waser & Ollerton 2008), to (a) investigate the integration of a non-native entomophilous plant with restrictive flower morphology into resident plant-pollinator networks and to (b) investigate whether its effect on network topology was dependent on the similarity in flower morphology between the non-native and native species. Our hypothesis is that the non-native species becomes integrated into resident plant-pollinator networks through generalist pollinators, such as the honeybee, which are able to access its flowers. We also predict that the non-native competes more strongly for pollinators with taxonomically closely related native species, i.e. those with similar flower morphology. Therefore, we expect that, by monopolizing the visits of its pollinators, and thus reducing the number of visits and plant-pollinator interactions involving closely related natives, the non-native increases network asymmetry. In addition, as monopolized pollinators are only those species able to access the restrictive flowers of the non-native, we expect nestedness to decrease and modularity to increase. Overall, the non-native plant will play a central role within its module composed of taxonomically closely related native plant species.

Materials and methods

Experimental design and pollination censuses

We established three pairs of invaded 20 x 20 m^2 plots in early successional shrublands (Carreras, Pons & Canals 2007) (Fig. 1). Paired plots were located a maximum of 500 m apart, while the minimum distance between pairs was 600 m.

Despite *Hedysarum* being one of the most dominant species in the shrublands (cover ranging from 26.44% to 48.64% across plots), in each plot it coexisted with 8.33 ± 0.33 (mean ± SE, hereafter) native co-flowering species. Overall, ten native plant species also belonged to Leguminosae and represented on average 36.39 ± 7.34% of the plant species in each plot. The rest of the native plant species (17) had open and accessible flowers (Appendix 1).

To investigate the effect of *Hedysarum* on recipient plant-pollinator networks, we manually clipped all *Hedysarum* inflorescences from one randomly selected plot of each pair (removal plot, hereafter), while the other plot was not manipulated (invaded plot, hereafter) (Fig. 2).

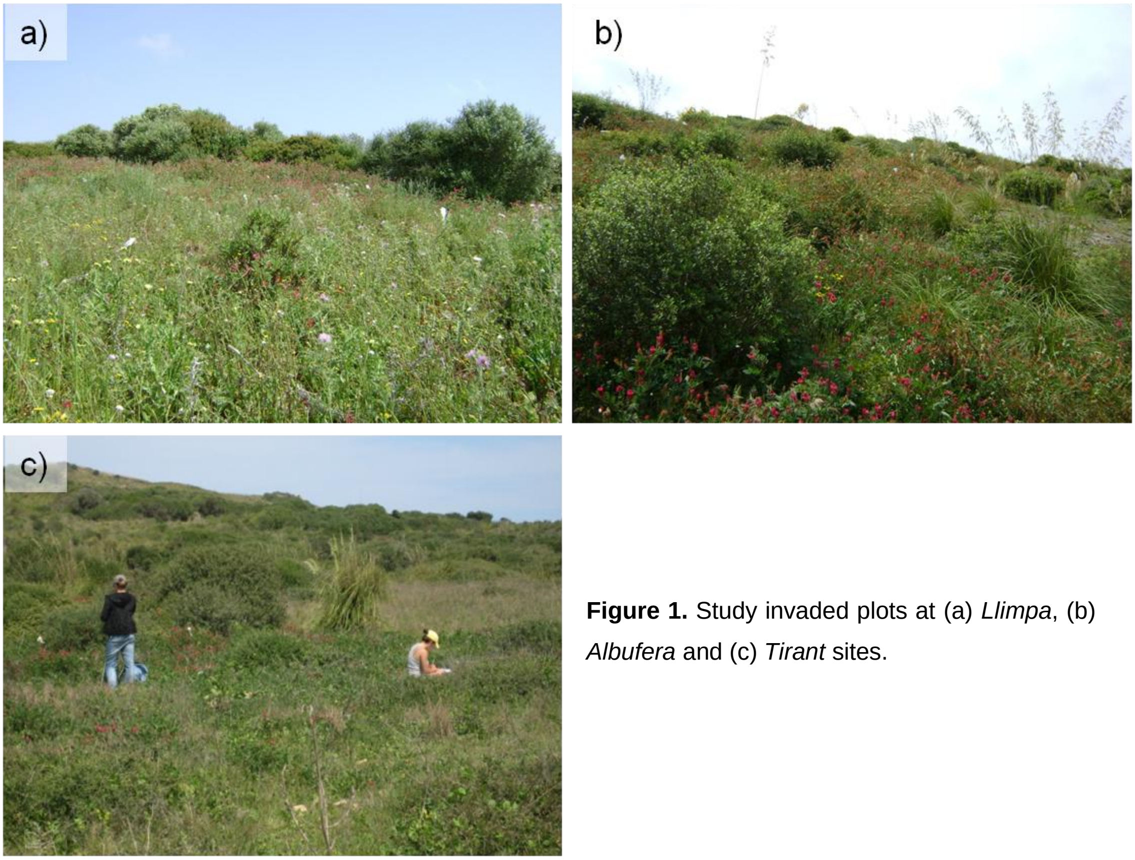


Figure 1. Study invaded plots at (a) *Llimpa*, (b) *Albufera* and (c) *Tirant* sites.

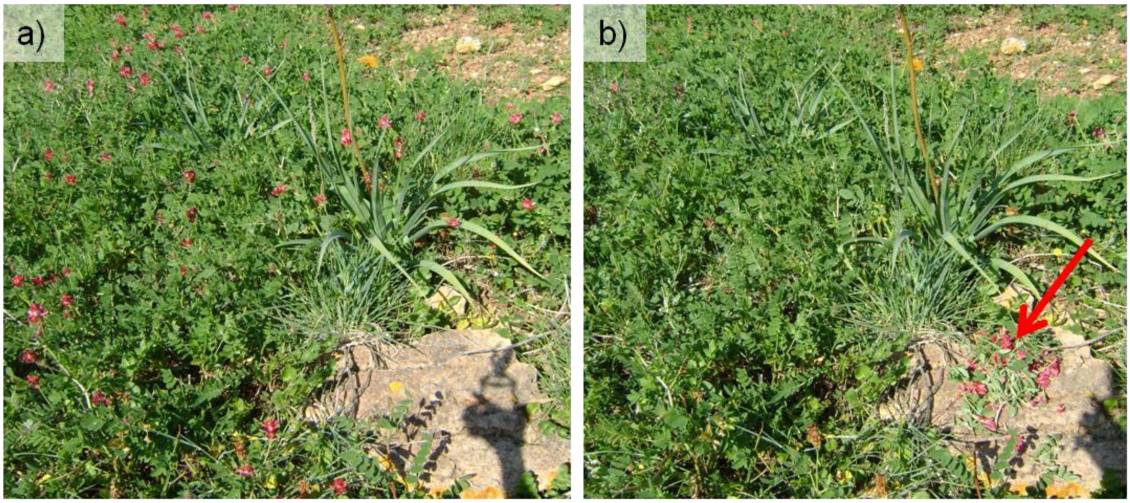


Figure 2. Removal study plot (a) before and (b) after manual clipping of *Hedysarum* inflorescences. The red arrow points to the pilled clipped inflorescences.

We conducted pollination censuses in the springs of 2009 and 2010 throughout the entire flowering period of *Hedysarum* (April-May). In both years weather conditions fell within the average ranges for these months in the study area (“AEMET”).

Pollination censuses were conducted on sunny, warm (≥ 17 ºC) and non-windy days, from 10 a.m. to 6 p.m. During each observation period (15 min), we counted the number of floral units (hereafter flowers, according to Dicks *et al.* 2002) under observation, the number and identity of pollinators and the number of visits of each pollinator species. A visitor was considered a pollinator if it entered a flower and touched its sexual parts. The pollinator species that could not be identified in the field were sorted into distinct morphospecies and caught for later identification by specialists. Voucher specimens are deposited at EBD-CSIC.

The observation schedule for each plant species and individual under observation was randomly established. We considered a plot to be properly surveyed when, according to its rarefaction curve, we found no new plant-pollinator interaction after six or more censuses (Appendix 2). In total, we conducted 1252 censuses (313 h). On average each plant species was observed for 5.79 ± 0.60 h per plot, ranging from 1 h to 23.25 h.

Network analysis

We built qualitative plant-pollinator networks with the data gathered during the two study years for each study plot (i.e. six networks: three invaded and three removal, hereafter “plot networks”) and for the pooled data (i.e. two networks: one invaded and one removal, hereafter “island networks”).

A network is defined as a two dimensional matrix (i*j) describing the interaction between the flowering plant species (i) and the pollinator species (j) in the community. Each cell in the matrix (a_{ij}) can be 1 or 0 indicating, respectively, whether the interaction between the plant species i and the visitor species j is observed or not. Quantitative networks were built following the same criteria, except that each a_{ij} value is the weight of the interaction between the plant species i and the pollinator j measured as the visitation rate (nº visits/flower/hour) (Jordano, Bascompte & Olesen 2003).

We calculated 14 network topological parameters (Table 1). Ten parameters were calculated at the network level and four at the plant species level. Some parameters were based on qualitative networks (i.e. presence/absence data) while other parameters were based on quantitative networks (i.e. weight of each plant-pollinator interaction measured as the visitation rate).

We used the *bipartite* library in R (R Development Core Team 2001) to calculate most of parameters except asymmetry, nestedness and modularity (see Appendix 3 for calculation details).

Table 1. Network parameters calculated with indication of the type of data and the level at which they were calculated.

Parameter	Symbol	Definition	Data	Level
Pollinator richness	*A*	Number of pollinator species	Qualitative	Network
Plant richness	*P*	Number of plant species	Qualitative	Network
Network size	*S*	A + P	Qualitative	Network
Pollinator-plant ratio	*R*	A / P	Qualitative	Network
Interaction richness	*I*	Number of interactions between pollinators and plants	Qualitative	Network
Connectance	*C*	Proportion of all potential interactions that are observed (I / (A * P))	Qualitative	Network
Exclusive interactions	*Ie*	Percentage of exclusive interactions in each plot considering only common species with its paired plot	Qualitative	Network
Asymetry	*Asy*	A measure of how different are mutual dependences of interacting species. Calculated according to Bascompte *et al.* 2006 (see Appendix 2 for calculation details)	Quantitative	Network
Nestedness	*N*	The degree to which species interacting with specialists are proper subset of the species interacting with generalists. Calculated with the algorithm NODF according to Almeida-Neto *et al.* (2008) (see Appendix 2 for calculation details)	Qualitative	Network
Modularity	*M*	The degree to which nodes are organized into distinct groups of nodes within the network. Calculated with the software BIPMOD according to Thébautl (2013). N_M is the number of modules that maximizes M (see Appendix 2 for calculation details)	Qualitative	Network
Visitation rate	*V*	Number of visits a plant species receives per flower and hour	Quantitative	Species
Pollinator linkage	*La*	Proportion of the total number of plant species a particular pollinator species interacts with	Qualitative	Species
Plant linkage	*Lp*	Proportion of the total number of pollinator species a particular plant species is visited by	Qualitative	Species
Pollinator niche overlap	*NOa*	Proportion of the total number of pollinator species that a particular pollinator species shares host plants with	Qualitative	Species
Plant niche overlap	*NOp*	Proportion of the total number of plant species that a particular plant species shares pollinators with	Qualitative	Species
Pollinator strength	*Sta*	Sum of the proportion of visits received by each plant species that are achieved by a particular pollinator species	Quantitative	Species
Plant strength	*Stp*	Sum of the proportion of visits realized by each pollinator species that are achieved in a particular plant species	Quantitative	Species

Statistical analyses

For those network parameters calculated at the species level, we explored the effect of *Hedysarum* on native plant species and whether their flower morphology influenced such an effect. We built generalized mixed models with the effect of treatment (invaded *vs*. removal), flower morphology (papilionate *vs*. non-papilionate) and their interaction on visitation rate (V), plant linkage level (L_p), plant niche overlap (NO_p) and plant strength (St_p) as response variables; plot was included as a random factor. The error distribution family was Gamma for V and St_p and binomial for L_p and NO_p.

We repeated the analyses for the honeybee, as it was the pollinator that visited *Hedysarum* the most (**Chapter 1**). First, we tested the effect of treatment, flower morphology and their interaction on the honeybee presence with binomial as the error distribution family. When the honeybee was present, we tested if the number of visits achieved per individual differed between invaded and removal plots. For that purpose we built a generalized mixed model with treatment as the fixed factor, plant species and plot as random factors and Gamma as the error distribution family.

Comparisons of the topological parameters calculated at the species level between invaded and removal networks were conducted through generalized models. Treatment (invaded *vs.* removal) was the fixed factor and the error distribution families were Gamma for V and St and binomial for L and NO.

Analyses were conducted with the libraries *lme4* and *glmmADMB* in R (R Development Core Team 2001).

Results

We observed a total of 28 flowering plant species from eight different families that were visited by 93 pollinator species belonging to 38 families of Coleoptera (19.36%), Diptera (38.71%) and Hymenoptera (41.94%) (Fig. 3, Appendix 3). All pollinator species are considered native to Menorca.

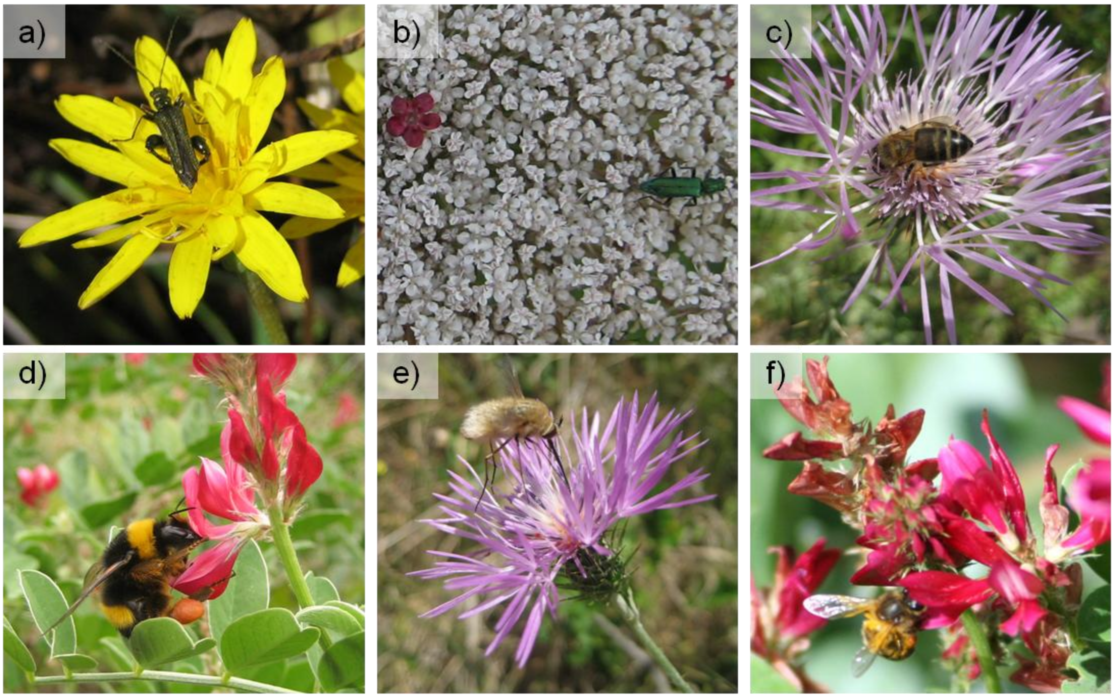


Figure 3. Examples of plant-pollinator interactions observed during censuses: (a) *Hyoseris radiata – Oedemera sp.*, (b) *Daucus carota – Psilothrix viridicoerulea*, (c) *Galactites tomentosa – Apis mellifera*, (d) *Hedysarum coronarium – Bombus terrestris*, (e) *Galactites tomentosa – Bombylidus sp.* and (f) *Hedysarum coronarium – Apis mellifera*.

Integration of Hedysarum into the plant-pollinator network

Hedysarum was visited by a total of 15 pollinator species: 11 hymenopterans (including seven Apidae) and four coleopterans. These pollinators represented 16.13% of the total, whereas native plant species were visited on average by only 9.66 ± 1.55% of the pollinator species. *Hedysarum* was primarily visited by the honeybee (91.38% of the visits).

Although *Hedysarum* received 54.04% of the visits observed in invaded plots, when standardized by the number of flowers, the *Hedysarum* visitation rate was below the average of the native plants (Fig. 4a).

In the invaded island network, the *Hedysarum* linkage level was larger than the average of the native species (Fig. 4b). Pollinators visiting *Hedysarum* were on average more generalized than pollinators visiting only natives ($L_a = 0.24 \pm 0.07$ and 0.09 ± 0.01, respectively; $Z = -5.081$, $P < 0.001$). Due to this high generalization, *Hedysarum* generally overlapped its niche with more plant species than did the other native plant species (Fig. 4c). Its strength was also higher than the average of the natives (Fig. 4d), mainly due to six pollinators visiting *Hedysarum* exclusively.

The three invaded plot networks showed the abovementioned trends for visitation rate and plant linkage level. There were differences, however, among sites for plant niche overlap and strength, which were below the average of the natives at the *Llimpa* site.

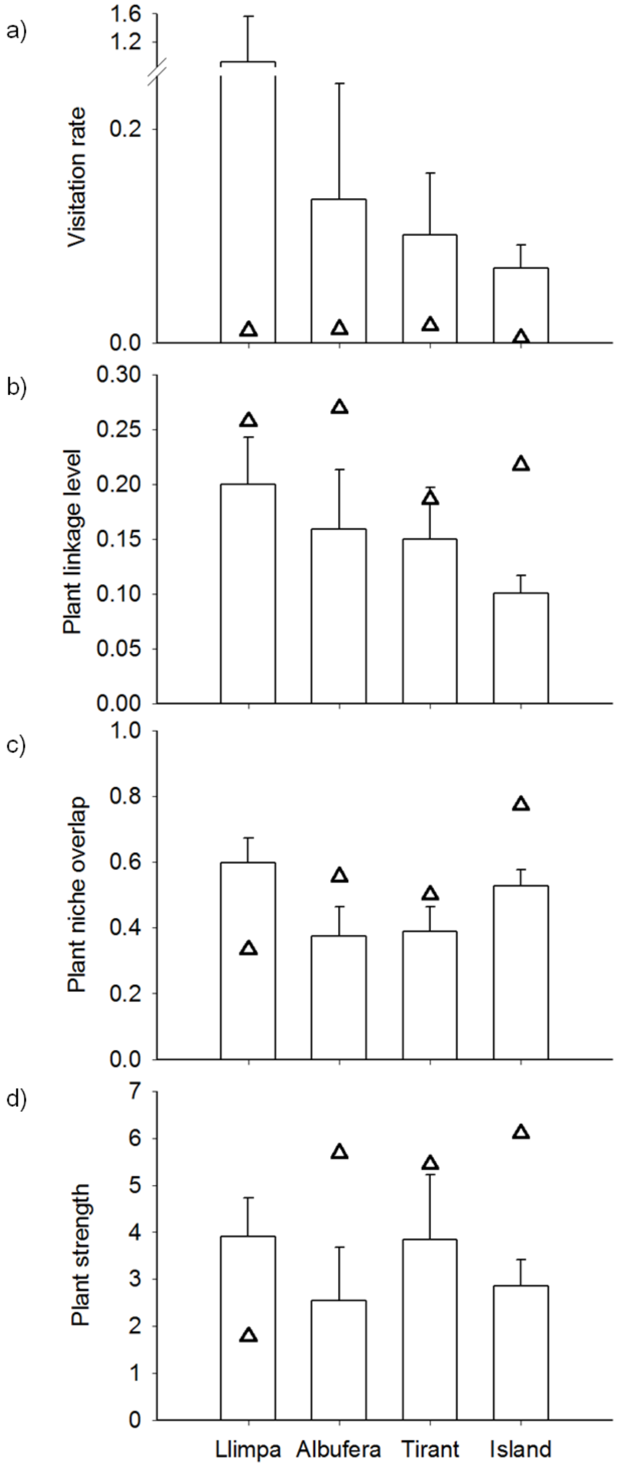


Figure 4. Mean + SE of (a) visitation rate, (b) linkage level, (c) niche overlap and (d) strength of *Hedysarum* (triangles) compared to average values for native plant species (bars) in each invaded plot and island networks.

Effect of Hedysarum on network topology and the influence of flower morphology

When comparing invaded *versus* removal plots, the integration of *Hedysarum* into resident plant-pollinator networks did not affect the visitation rate, linkage level, niche overlap or strength of native plant species (Table 2). However, flower morphology strongly influenced these four variables, which were lower for species with papilionate flowers than for species with other flower morphologies, regardless of whether *Hedysarum* flowers were removed or not (Table 2, Fig. 5).

Table 2. Analyses of the combined effect of treatment (invaded *vs.* removal) and native species flower morphology (papilionate *vs.* non-papilionate) on network parameters at the plant species level: visitation rate (V), linkage level (L_p), niche overlap (NO_p), strength (St_p) and presence of the honeybee. Estimates given are calculated with invaded and papilionate as reference levels of the factors treatment and flower morphology, respectively.

Response variable	**Fixed factors**	**N**	**Estimate**	**Z**	**P**	
V	Treatment	54	-0.28	-0.44	0.660	
	Morphology		4.78	8.30	<0.001	***
	Treatment*Morphology		-0.25	-0.31	0.760	
L_p	Treatment	54	0.05	0.15	0.881	
	Morphology		1.78	6.86	<0.001	***
	Treatment*Morphology		0.01	0.03	0.973	
NO_p	Treatment	54	0.17	0.54	0.586	
	Morphology		0.96	3.36	0.001	***
	Treatment*Morphology		0.23	0.58	0.560	
St_p	Treatment	54	-0.67	-1.90	0.058	.
	Morphology		1.63	5.03	<0.001	***
	Treatment*Morphology		0.49	1.08	0.279	
Honeybee presence	Treatment	54	2.93	2.26	0.024	*
	Morphology		1.34	1.06	0.289	
	Treatment*Morphology		-1.07	-0.71	0.481	

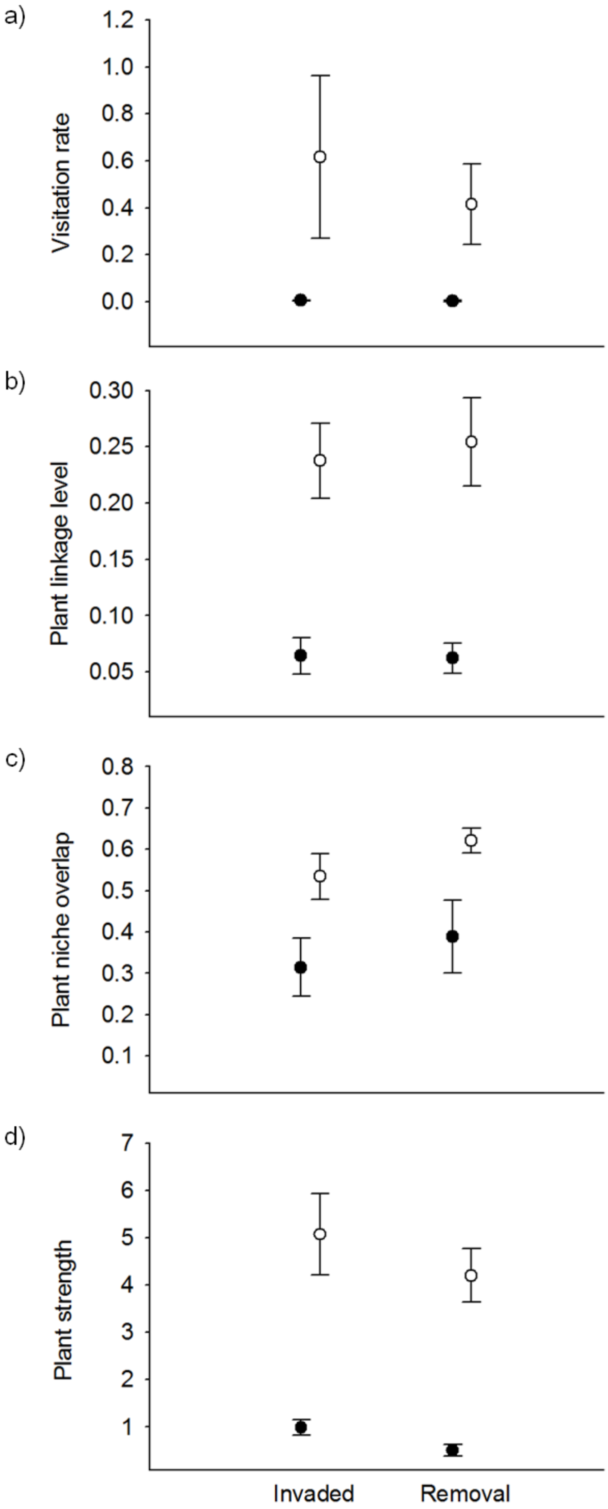


Figure 5. Mean ± SE of (a) visitation rate, (b) linkage level, (c) niche overlap and (d) strength of native plants for each treatment (invaded *vs.* removal) and flower morphology (papilionate; black *vs.* non-papilionate; white).

The abovementioned results obtained for the entire pool of pollinators that visited native plants, contrasted with the results obtained for the honeybee*.* The presence of the honeybee on native plants was higher when *Hedysarum* flowers were removed, irrespective of their flower morphology (Table 2). The honeybee visited 19.91 ± 3.79% and 62.77 ± 18.63% of the native plant species in invaded and removal plots, respectively.

Focusing on island networks, the size (*S*) of the invaded network was slightly larger than that of the removal network due to a greater number of pollinator species (*A*); while the number of plant species (*P*) remained the same. Therefore, the pollinator-plant species ratio (*R*) was also larger in the invaded network. The number of interactions (*I*) was greater in the invaded network as a result of its larger network size. However, connectance (*C*) did not differ between invaded and removal networks (Table 3, Fig. 6).

Despite the similar *C*, the identity and weight of plant-pollinator interactions differed between invaded and removal networks. The similarity in plant species composition between invaded and removal networks was 0.73 (Sørensen index). This similarity index fell to 0.58 for pollinator species and to 0.38 for interactions between plant and pollinator species present in both invaded and removal networks. Yet, 20.50% and 32.89% of the interactions between shared plant and pollinator species were exclusive to the invaded or removal networks, respectively.

Despite this interaction rewiring, both invaded and removal island networks showed a nested pattern. However, asymmetry (*Asy*) and modularity (*M*) were slightly greater in the invaded network than in the removal network. Although these differences in *Asy* and *M* were slight, they were consistent along the three paired invaded and removal plot networks (Table 3, Table A4.1).

Table 3. Network parameters calculated for removal and invaded plot networks (*Llimpa*, *Albufera* and *Tirant*) and for the island network. Mean ± SE values are indicated for parameters at the species level. Significance levels: ˙ P = 0.05, * P < 0.05, ** P < 0.01, *** P < 0.001. For modularity (*M*) significance tests see Table A4.1. See Table 1 for parameter definitions.

Plot	Treatment	A	P	S	R	I	C	Ie	Asy	N	M	N_M
Llimpa	Removal	36	11	47	3.27	67	0.17	23.88	0.55 ± 0.04	24.12 *	0.45	-
	Invaded	35	9	44	3.89	68	0.22	11.76	0.56 ± 0.04	26.49	0.47	4
Albufera	Removal	24	7	31	3.43	43	0.26	13.95	0.61 ± 0.05	32.66 ˙	0.39	-
	Invaded	26	9	35	2.89	42	0.18	11.90	0.66 ± 0.05	22.96	0.55	4
Tirant	Removal	33	11	44	3.00	60	0.17	16.67	0.61 ± 0.04	20.47	0.51	-
	Invaded	43	10	53	4.30	69	0.16	20.29	0.64 ± 0.04	20.20	0.53	6
Island	Removal	62	22	84	2.82	152	0.11	32.89	0.53 ± 0.02	16.35 ***	0.44	-
	Invaded	69	22	91	3.14	161	0.11	20.50	0.56 ± 0.03	14.83 ***	0.48	7

Table 3 (continuation).

Plot	Treatment	V	La	NOa	Sta	Lp	NOp	Stp
Llimpa	Removal	0.08±0.04	0.17±0.02	0.49±0.04	0.34±0.10	0.17±0.05	0.58±0.07	2.91±0.89
	Invaded	0.82±0.57	0.22±0.02	˙ 0.39±0.03	0.27±0.06	0.21±0.04	0.57±0.07	3.67±0.76
Albufera	Removal	0.05±0.03	0.26±0.03	0.58±0.05	0.33±0.16	0.26±0.09	0.57±0.06	3.00±1.31
	Invaded	0.12±0.10	˙ 0.18±0.02	*** 0.34±0.03	0.35±0.13	0.17±0.05	0.40±0.08	2.89±1.07
Tirant	Removal	0.62±0.29	0.17±0.02	0.31±0.03	0.34±0.11	0.17±0.03	0.50±0.08	2.91±0.62
	Invaded	0.09±0.05	0.16±0.01	0.34±0.03	0.25±0.08	0.15±0.04	0.40±0.07	4.00±1.24
Island	Removal	0.06±0.02	0.11±0.02	0.32±0.03	0.40±0.10	0.11±0.02	0.55±0.05	2.50±0.45
	Invaded	0.07±0.02	0.11±0.01	˙ 0.25±0.02	0.33±0.08	0.11±0.02	0.54±0.05	3.00±0.56

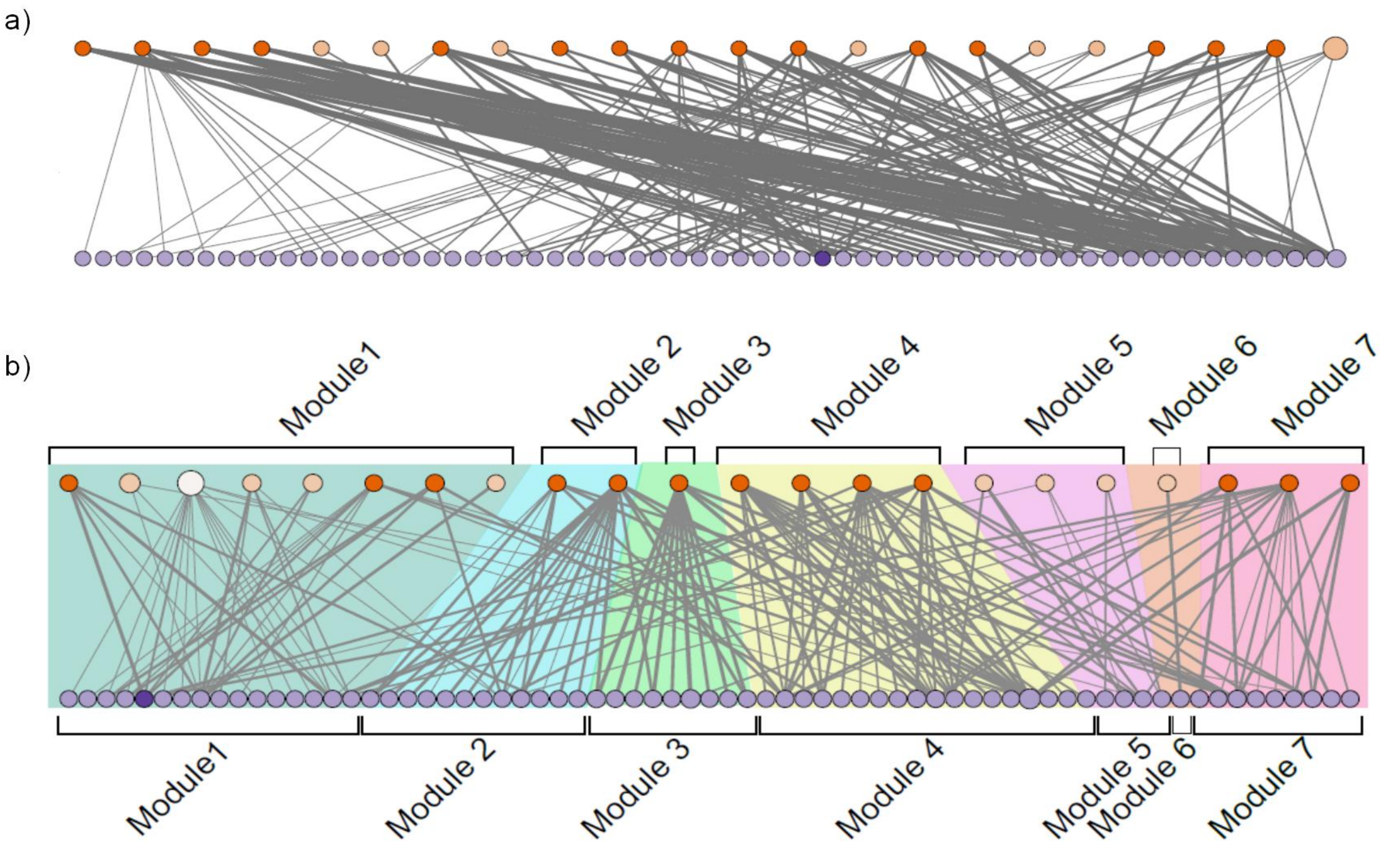


Figure 6. Removal (a) and invaded (b) island networks. Upper nodes represent plant species and lower nodes represent pollinator species. Lines between nodes represent the observed interactions. The size of the nodes is proportional to species abundance and the thickness of the lines is proportional to interaction weight (i.e. visitation rate). Dark orange nodes are non-papilionate plant species, light orange nodes are papilionate plant species, the white node in the invaded network represents *Hedysarum* and dark purple nodes represent the honeybee. In the invaded network, species belonging to the same module are within brackets and shadowed with the same color.

The invaded island network had a modular pattern composed of seven modules within which 52.61% of the interactions occurred. *Hedysarum* belonged to a module together with seven other plant species (four of them also from the family Leguminosae), 14 hymenopterans, one coleopteran and one dipteran (Table A4.5). *Hedysarum* belonged to the same module as 12 of its 15 pollinator species; thus, it had most of its links within its own module; i.e. it acted as a module hub (Table 4, Table A4.5). The honeybee acted as a connector for the same module (Table 4, Table A4.5); i.e. it linked several modules. The remaining Leguminosae species were distributed in two modules, both only containing Leguminosae plant species (Table A4.5).

From the abovementioned trends observed for island networks, only those for *Asy* and *M* were also consistently observed in plot networks throughout the three study sites (Table 3).

Table 4. Role played by *Hedysarum* and the honeybee in the modular networks according to their among-module connectivity (*C*) and the within-module degree (*z*) values calculated for each species (see Appendix 2 for calculation details).

		Hedysarum			**Honeybee**		
Site	**Treatment**	**Role**	**c**	**z**	**Role**	**c**	**z**
Llimpa	Invaded	Module hub	0.320	2.720	Peripheral	0.444	-0.331
Albufera	Invaded	Module hub	0.219	2.789	Peripheral	0.000	0.119
Tirant	Invaded	Module hub	0.198	2.615	Peripheral	0.444	-0.131
Island	Invaded	Module hub	0.338	3.493	Connector	0.612	0.445

Discussion

The honeybee as the main species responsible for the integration of Hedysarum into the recipient plant-pollinator network

The honeybee played an important role in *Hedysarum* integration by achieving most of its visits. The honeybee, like other generalized, abundant and ubiquitous pollinators (Goulson 2003), is able to include many plant species in its diet, even non-natives (Memmott & Waser 2002; Olesen *et al.* 2002; Morales & Aizen 2006; Padrón *et* *al.* 2009). Besides the honeybee, *Hedysarum* was visited by more species than the average of the natives, some of them exclusively or almost exclusively visiting *Hedysarum*, as shown by the high linkage level and strength values, respectively. Pollinators vary their linkage level in time and space in order to optimize their foraging behaviour (Olesen *et al.* 2008; Petanidou *et al.* 2008; Lázaro & Totland 2010b). Thus, it might be advantageous for pollinators to include abundant and high-rewarding species like *Hedysarum* in their diet.

Despite *Hedysarum* integration, pollinator species visiting *Hedysarum* represented a lower percentage of the total pool of pollinator species (16.13%) compared to other non-native species in other systems. For instance, Vilà *et al.* (2009) studied five non-native plant species and found that they were visited by 31 to 50% of the pollinator species in the community. However, these non-native species had open and non-restrictive flower morphologies, allowing a wider range of resident pollinators to visit them. Meanwhile, non-natives with more restrictive flower morphologies like legumes, filter pollinators according to their ability to access rewards (Córdoba & Cocucci 2011). Non-native plants with restrictive flower morphologies might face similar limitations in introduced areas and native ranges. In fact, *Hedysarum* has a linkage level in its native range comparable to that in the introduced communities on Menorca (**Chapter 1**).

Flower morphology determines network topology irrespective of Hedysarum invasion

The integration of *Hedysarum* in resident plant-pollinator networks did not affect the pollination patterns of native plants in terms of visitation rate, plant linkage level, niche overlap or strength. Conversely, flower morphology strongly influenced network topology, irrespective of the presence or absence of *Hedysarum*. Thus, contrary to our expectation, the non-native species did not compete more strongly for pollinators with plants exhibiting similar flower morphology.

In our study system, similarity in flower morphology refers to papilionate flowers, which are not accessible to all types of pollinators (Córdoba & Cocucci 2011). In fact, natives with papilionate flowers had low linkage levels and visitation rates in both invaded and removal plots, obscuring the detection of an influence of *Hedysarum* presence. Similarity in flower morphology between native and non-native species might be more influential in plant species with minimally restrictive flower morphologies such as Composites, as observed by Morales & Traveset (2009).

Though *Hedysarum* did not have an overall effect on the average frequency, number or strength of interactions involving natives, it affected the identity of some of those interactions. That is, *Hedysarum* modified the behavior of some pollinator species. For instance, the honeybee actively selected *Hedysarum*, probably owing to both its abundant and rewarding floral offer. Thus, the presence of the honeybee on native plants decreased, irrespective of their flower morphology. Other wild pollinator species might take advantage of the low presence of the honeybee on natives in invaded plots. Conversely, in removal plots, where honeybees were not monopolized by *Hedysarum*, honeybees might outcompete other pollinator species (Roubik 1983; Paini 2004) due to its generalized diet and systematic foraging behaviour (Huryn 1997; Gathmann & Tscharntke 2002; Steffan-Dewenter *et al.* 2002, Steffan-Dewenter & Kuhn 2003). Thus, in invaded plots more native floral resources are available to other pollinator species resulting in new interactions. Such interaction turnover is known as interaction rewiring (Ramos-Jiliberto *et al.* 2012).

Plant-pollinator networks invaded by Hedysarum are more modular but remain nested

As the integration of *Hedysarum* into resident plant-pollinator networks did not affect the number but rather the identity of interactions involving native plants (i.e. interaction rewiring), some topological parameters of the networks were altered. The most noticeable topological difference between invaded and removal networks was modularity. Although only marginally significant, all invaded networks were modular while removal networks were not. *Hedysarum* mainly interacted with hymenopterans. Such a functional specialization implies that some interactions are forbidden (Olesen *et* *al.* 2011) and that some species are more highly interconnected than others, which could explain the modular pattern observed in the invaded network.

Invaded networks were composed of seven modules, a number which falls within the average number observed in previously analyzed plant-pollinator networks (Olesen *et al.* 2007). The modules showed clear taxonomical and location signals. First, closely related plant species, mainly those belonging to the families Leguminosae and Compositae, belonged to the same modules since they shared more pollinators, which might be the result of coevolutionary processes. Second, some modules appeared to be simply the result of the spatial segregation of species into the different plots.

Hedysarum and the honeybee played central roles in the invaded island network. *Hedysarum*, acted as a hub, similar to what has been observed in other non-native species that behave as super-generalists (Bjerknes *et al.* 2007; Aizen *et al.* 2008b), while the honeybee acted as a connector.

Both invaded and removal island networks were nested. However, nestedness did not show a consistent pattern among the three study sites. A nested pattern has important ecological and evolutionary implications because it reduces interspecific competition which facilitates species coexistence and biodiversity maintenance (Bascompte, Jordano & Olesen 2006; Bastolla *et al.* 2009). In addition, a nested pattern seems to be robust to the integration of non-native species (Vilà *et al.* 2009). However, high levels of invasion (i.e. high proportion of non-native species) could alter nestedness, probably increasing network invasibility and presenting native species with novel ecological and evolutionary dynamics (Aizen *et al.* 2008b).

Conclusions

Plant-pollinator networks appear to be permeable to the arrival of non-native plants. In the case of a non-native with restrictive flower morphology, the effect of its integration on resident networks did not depend on its similarity in flower morphology to natives. Its influence on native plants might be related to other species traits such as its relative quantity and/or the quality of the floral resources. Even though the integration of the non-native altered the identity of interactions and, therefore, some topological patterns (e.g. modularity), the connectance and the nested pattern were maintained.

Both modularity and nestedness have been related to network robustness (i.e. network resistance to secondary extinction following species loss) (Tylianakis *et al.* 2010; Traveset *et al.* 2013). Nested networks are expected to be robust to the extinction of rare and specialized species, given that the remaining species will still have other species as interacting partners (Tylianakis *et al.* 2010), but vulnerable to the removal of highly generalized species (Barabási & Bonabeau 2003). In the case of modular networks, secondary extinctions are expected to be restrained. For instance, the removal of a module hub may cause its module to fragment with little or no cascading effect on other modules, whereas the extinction of a connector may cause the entire network to fragment into isolated modules without major impacts on the internal structure of individual modules (Traveset *et al.* 2013).

Thus, in the short term, the interaction rewiring due to the presence of *Hedysarum* might contribute to network robustness. However, in the long term, as pollinator species are not equivalent in their pollination efficiency (Ne'eman *et al.* 2010), the interaction rewiring might affect the reproductive success of native plants and secondary extinctions could eventually occur.

To obtain a more realistic projection of the long-term response of networks to the arrival or removal of species, both interaction efficiency and rewiring are now being included in static (Kaiser-Bunbury *et al.* 2010) and dynamic (Ramos-Jiliberto *et al.* 2012; Valdovinos *et al.* 2013) models (i.e. considering the bidirectional causality between topology and dynamics).

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Appendix 1. Plant-pollinator networks

Table A1.1. Flowering plant species present in the study plots. The study non-native species *Hedysarum* is highlighted in bold letters. Nomenclature according to Fraga *et al*. (2004).

Species	Family
Galactites tomentosa	Compositae
Hyoseris radiata	Compositae
Hypochoeris achyrophorus	Compositae
Reichardia picroides	Compositae
Sonchus oleraceus	Compositae
Sonchus tenerrimus	Compositae
Urospermum dalechampii	Compositae
Alyssum maritimum	Cruciferae
Euphorbia terracina	Euphorbiaceae
Geranium dissectum	Geraniaceae
Geranium molle	Geraniaceae
Calicotome infesta	Leguminosae
Hedysarum coronarium	**Leguminosae**
Lathyrus clymenum	Leguminosae
Lotus cytisoides	Leguminosae
Lotus edulis	Leguminosae
Lotus ornithopodioides	Leguminosae
Lupinus micranthus	Leguminosae
Melilotus indicus	Leguminosae
Scorpiurus sulcatus	Leguminosae
Trifolium nigrescens	Leguminosae
Vicia benghalensis	Leguminosae
Allium roseum	Liliaceae
Asphodelus aestivus	Liliaceae
Muscari comosum	Liliaceae
Oxalis pes-caprae(*)	Oxalidaceae
Daucus carota	Umbelliferae
Kundmania sicula	Umbelliferae

(*) This species is also non-native in the study area (Fraga *et al*. 2004). It overlaps with *Hedysarum* at the very end of its flowering period.

Table A1.2. Pollinator species observed visiting flowering plants (Table S3.1) in the study plots. Species highlighted in bold letters were observed visiting *Hedysarum.*

Species	Family	Order
Coleoptera *sp.1*	-	Coleoptera
Aspidapion radiolus	Brentidae	Coleoptera
Anthaxia sp.1	Buprestidae	Coleoptera
Rhagonycha fulva	Cantharidae	Coleoptera
Stenopterus rufus	Cerambycidae	Coleoptera
Bruchidius seminarius	Chrysomelidae	Coleoptera
Bruchidius sp.1	Chrysomelidae	Coleoptera
Coccinellidae sp.1	Cucujidae	Coleoptera
Dasytes virens	Dasytidae	Coleoptera
Psilothrix viridicoerulea	Dasytidae	Coleoptera
Psylliodes sp.1	Dasytidae	Coleoptera
Caenocoris nerii	Lygaeidae	Coleoptera
Machiinae *sp.1*	Machiinae	Coleoptera
Mordella holomelaena	Mordellidae	Coleoptera
Meligethes nigritus	Nitidulidae	Coleoptera
Oedemera sp.1	Oedemeridae	Coleoptera
Oxythyrea funesta	Scarabaeidae	Coleoptera
Tropinota hirta	Scarabaeidae	Coleoptera
Diptera *sp.1*	-	Diptera
Diptera *sp.2*	-	Diptera
Anthomyia quinquemaculata	Anthomyiidae	Diptera
Delia platura	Anthomyiidae	Diptera
Dilophus febrilis	Bibionidae	Diptera
Oscinellinae sp.1	Chloropidae	Diptera
Oscinimorpha longirrostris	Chloropidae	Diptera
Ortochile nigrocoerulea	Dolichopodidae	Diptera
Scaptomyza pallida	Drosophilidae	Diptera
Empis tessellatta	Empididae	Diptera
Hilara sp.1	Empididae	Diptera
Tetrastichinae sp.1	Eulophidae	Diptera
Antlemon halidayi	Keroplatidae	Diptera
Neomyia cornicina	Muscidae	Diptera
Platygastridae *sp.1*	Platygastridae	Diptera
Rhinophoridae *sp.1*	Rhinophoridae	Diptera
Sarcophaga africa	Sarcophagidae	Diptera
Sarcophaga amita	Sarcophagidae	Diptera
Sarcophaga sp.1	Sarcophagidae	Diptera
Scathophaga stercoraria	Sarcophagidae	Diptera
Bradysia nitidicollis	Sciaridae	Diptera
Saltella sphondylii	Sepsidae	Diptera
Sepsis sp1	Sepsidae	Diptera
Odontomyia discolor	Stratiomyidae	Diptera

Chrysotoxum intermedium	Syrphidae	Diptera
Eristalis arbustorum	Syrphidae	Diptera
Eristalis sepulchralis	Syrphidae	Diptera
Eristalis tenax	Syrphidae	Diptera
Eumerus pulchellus	Syrphidae	Diptera
Eumerus sp.1	Syrphidae	Diptera
Eupeodes corollae	Syrphidae	Diptera
Melanostoma mellium	Syrphidae	Diptera
Paragus bicolor	Syrphidae	Diptera
Paragus tibialis	Syrphidae	Diptera
Shaerophoria scripta	Syrphidae	Diptera
Syritta pipiens	Syrphidae	Diptera
Hymenoptera *sp.1*	-	Hymenoptera
Andrena agilissima	Andrenidae	Hymenoptera
Andrena flavipes	Andrenidae	Hymenoptera
Andrena nigroolivacea	Andrenidae	Hymenoptera
Andrena ovatula	Andrenidae	Hymenoptera
Andrena tenuistriata	Andrenidae	Hymenoptera
Anthophora balearica	Apidae	Hymenoptera
Anthophora plumipes	Apidae	Hymenoptera
Anthophora subterranea	Apidae	Hymenoptera
Apis mellifera	Apidae	Hymenoptera
Bombus terrestris	Apidae	Hymenoptera
Ceratina curcubitina	Apidae	Hymenoptera
Ceratina dallatorreana	Apidae	Hymenoptera
Eucera numida	Apidae	Hymenoptera
Eucera oraniensis	Apidae	Hymenoptera
Synhalonia hungarica	Apidae	Hymenoptera
Xylocopa violacea	Apidae	Hymenoptera
Braconidae *sp.1*	Braconidae	Hymenoptera
Braconidae *sp.2*	Braconidae	Hymenoptera
Halictus gemmeus	Halictidae	Hymenoptera
Halictus scabiosae	Halictidae	Hymenoptera
Halictus sp.1	Halictidae	Hymenoptera
Lasioglossum angusticeps	Halictidae	Hymenoptera
Lasioglossum malachurum	Halictidae	Hymenoptera
Lasioglossum sp.1	Halictidae	Hymenoptera
Lasioglossum sp.2	Halictidae	Hymenoptera
Ichneumonidae *sp.1*	Ichneumonidae	Hymenoptera
Chalicodoma sicula	Megachilidae	Hymenoptera
Hoplitis andrenoides	Megachilidae	Hymenoptera
Osmia caerulescens	Megachilidae	Hymenoptera
Osmia niveata	Megachilidae	Hymenoptera
Osmia versicolor	Megachilidae	Hymenoptera
Rhodanthidium septemdentatum	Megachilidae	Hymenoptera

Megascolia bidens	Scoliidae	Hymenoptera
Scoliidae *sp.1*	Scoliidae	Hymenoptera
Vespidae *sp.1*	Vespidae	Hymenoptera
Vespidae *sp.2*	Vespidae	Hymenoptera
Vespidae *sp.3*	Vespidae	Hymenoptera
Vespidae *sp.4*	Vespidae	Hymenoptera

Appendix 2. Rarefaction curves

Rarefaction curves of plant- pollinator interaction richness for each study plot (2009 and 2010 pooled data) and for all invaded and all removal plots pooled together. Curves were based on 100 randomizations without replacement. Shaded areas represent 95% confidence intervals. Dashed lines enclose the number of censuses needed to observe a new interaction at the end of the study period. Curves were calculated and plotted within the R statistical computing environment (R Development Core Team 2001).

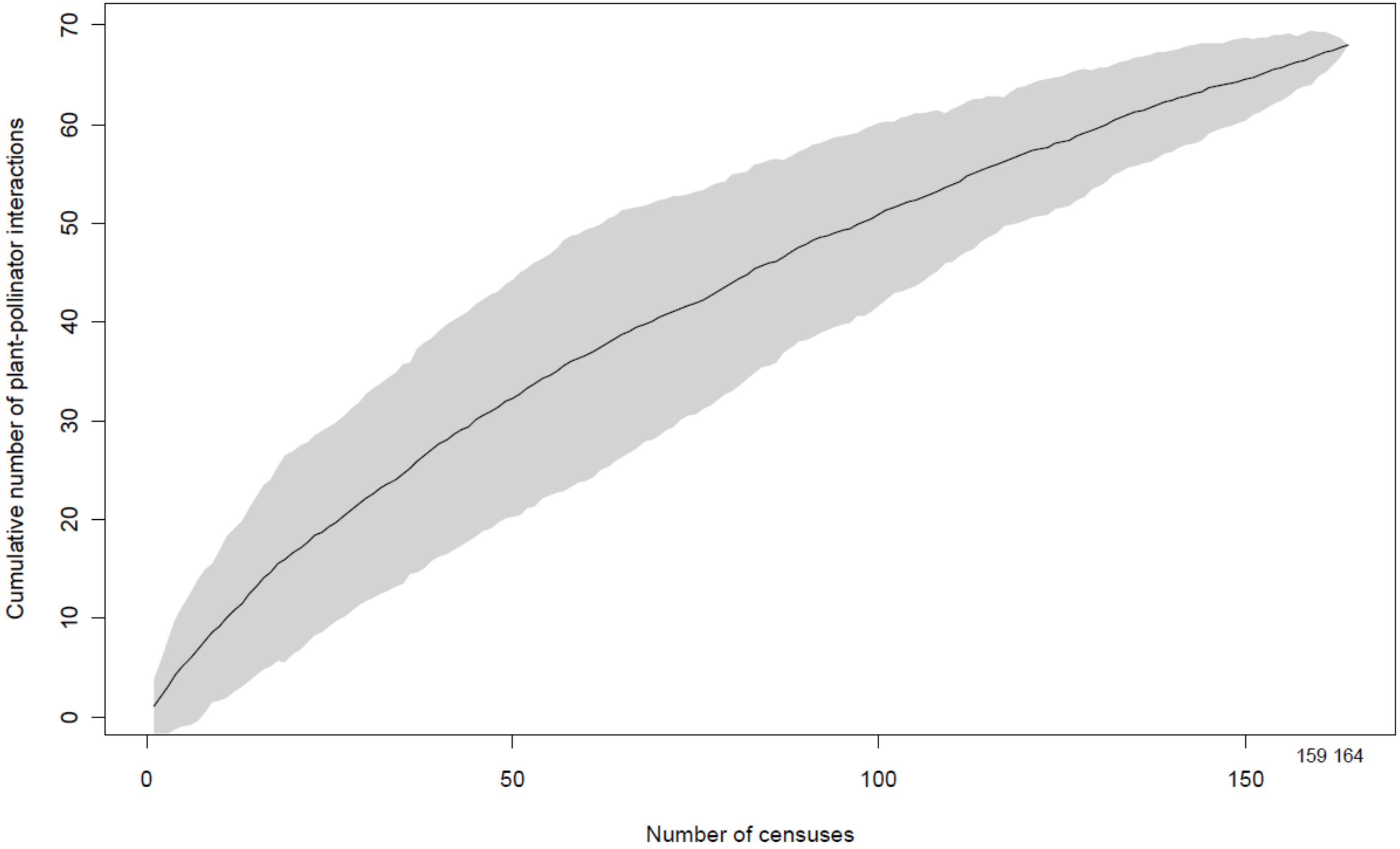

Figure A2.1 Rarefaction curve for invaded plot at *Llimpa*.

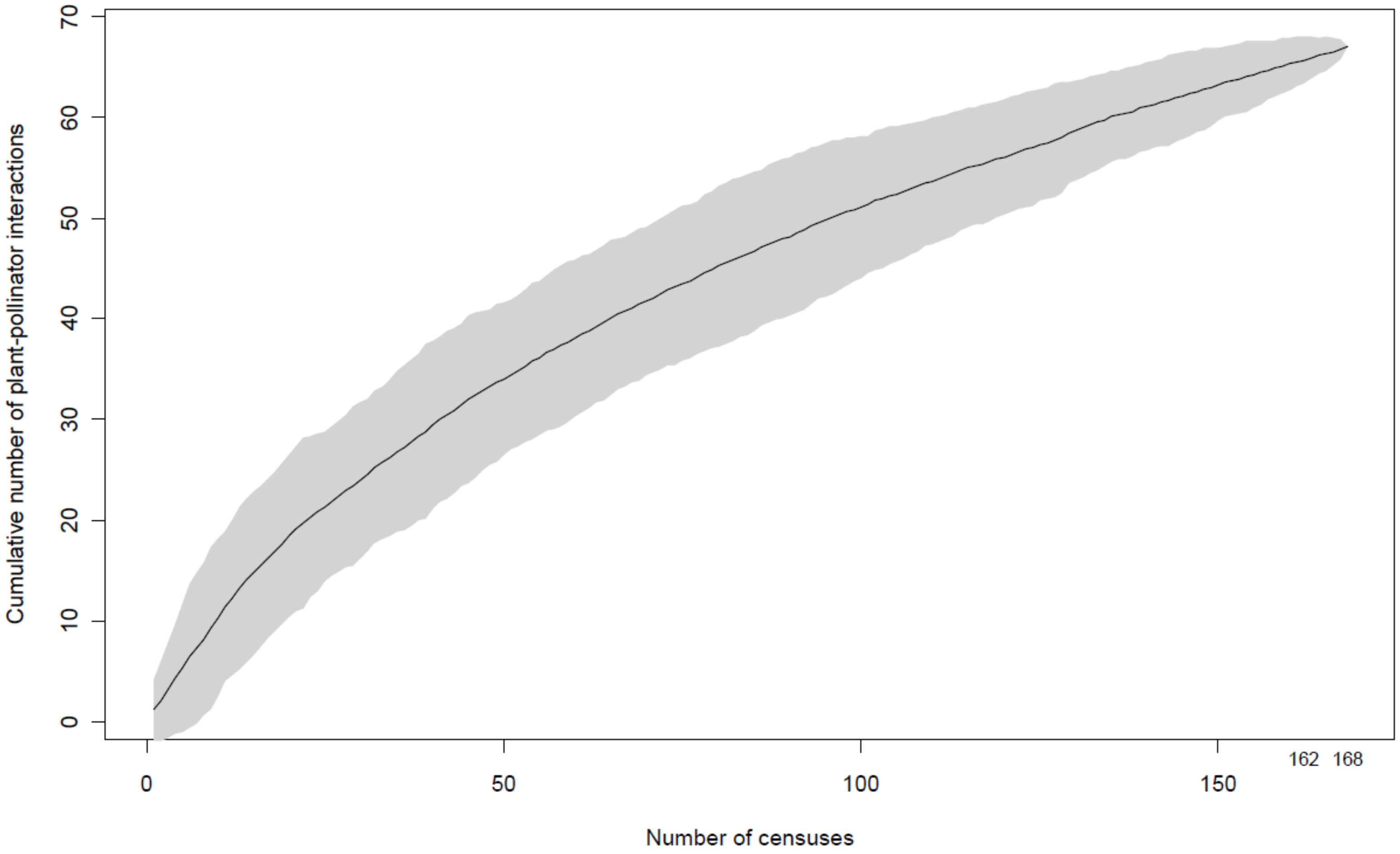

Figure A2.2 Rarefaction curve for removal plot at *Llimpa*.

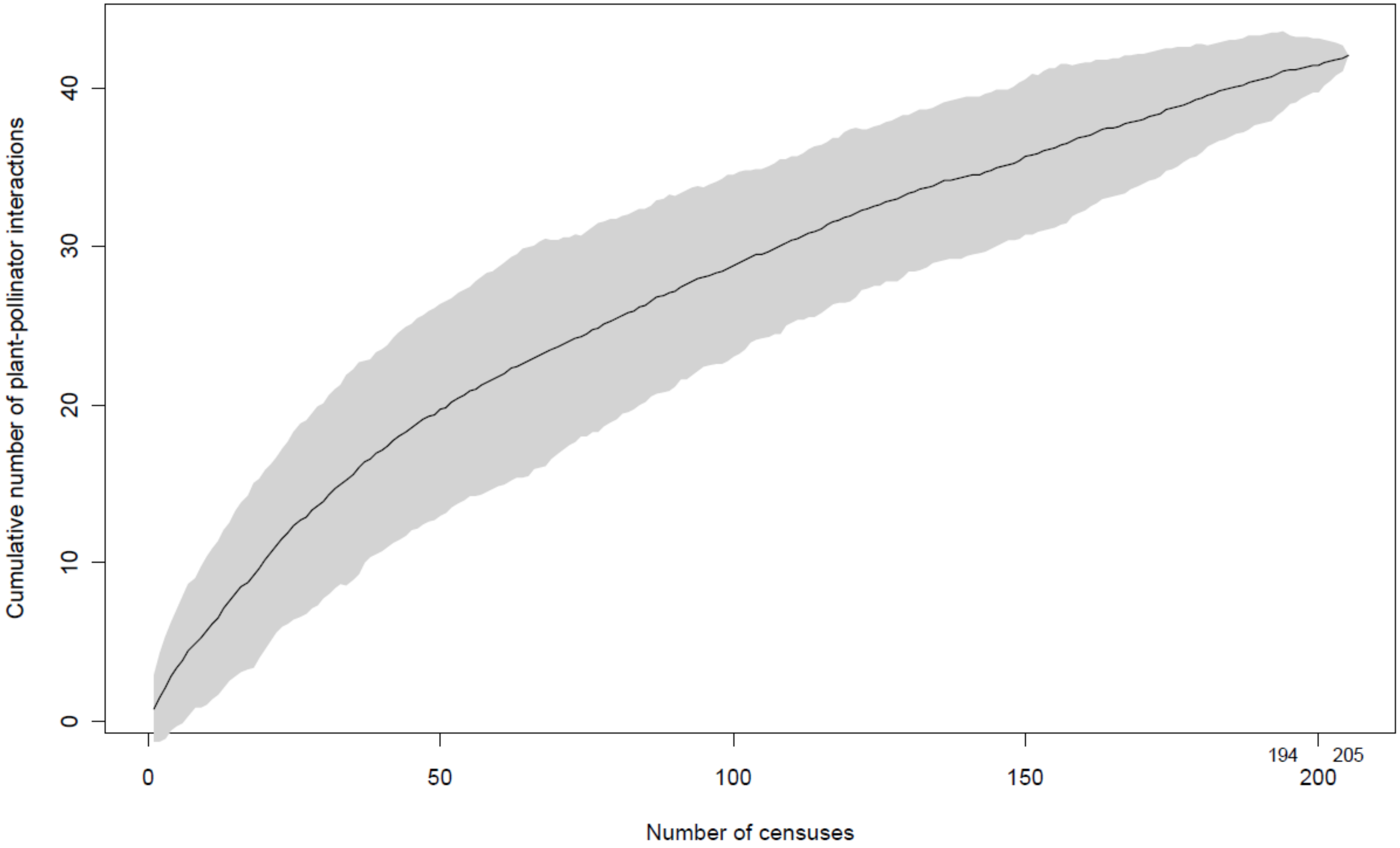

Figure A2.3 Rarefaction curve for invaded plot at *Albufera*.

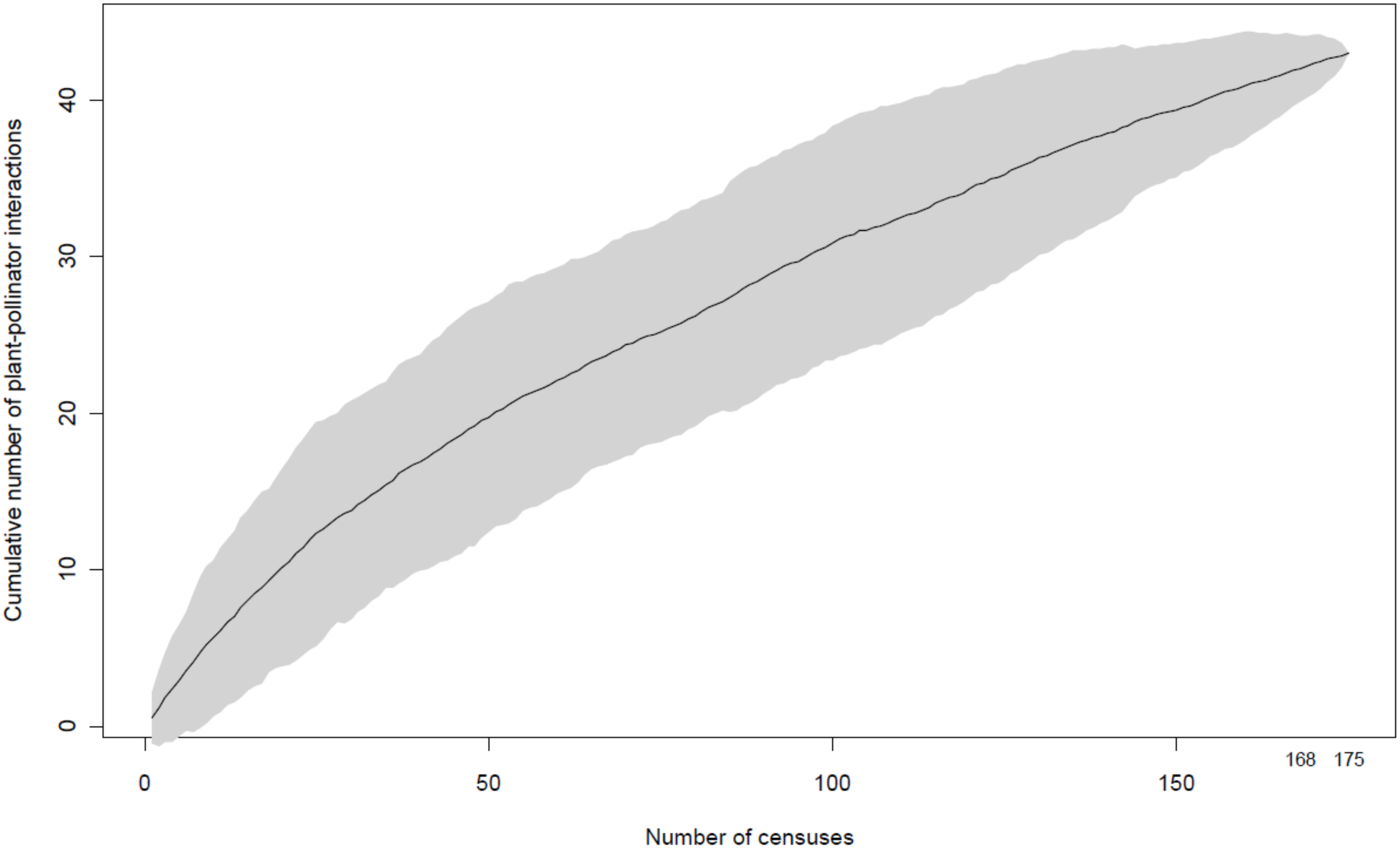

Figure A2.4 Rarefaction curve for removal plot at *Albufera*.

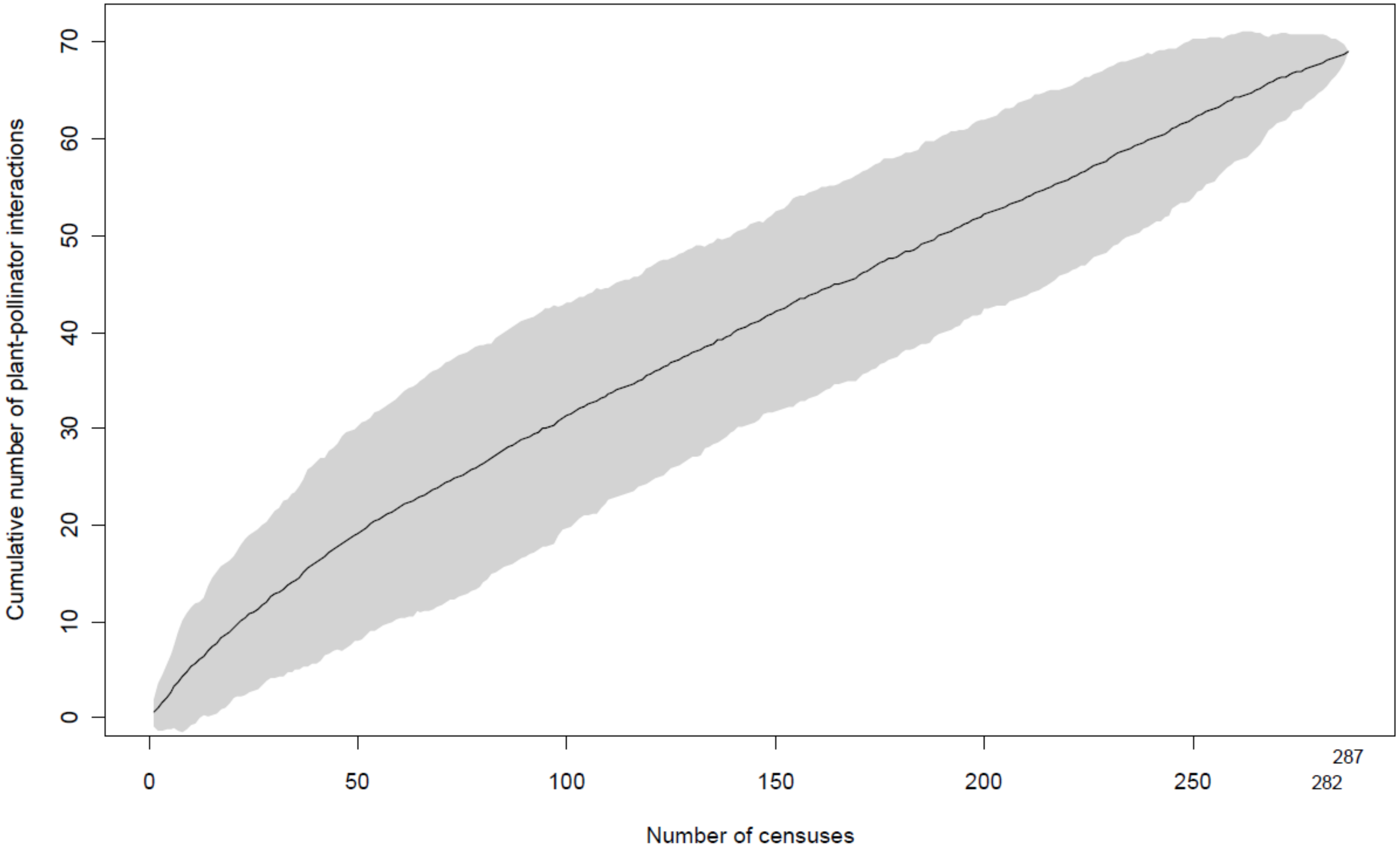

Figure A2.5 Rarefaction curve for invaded plot at *Tirant.*

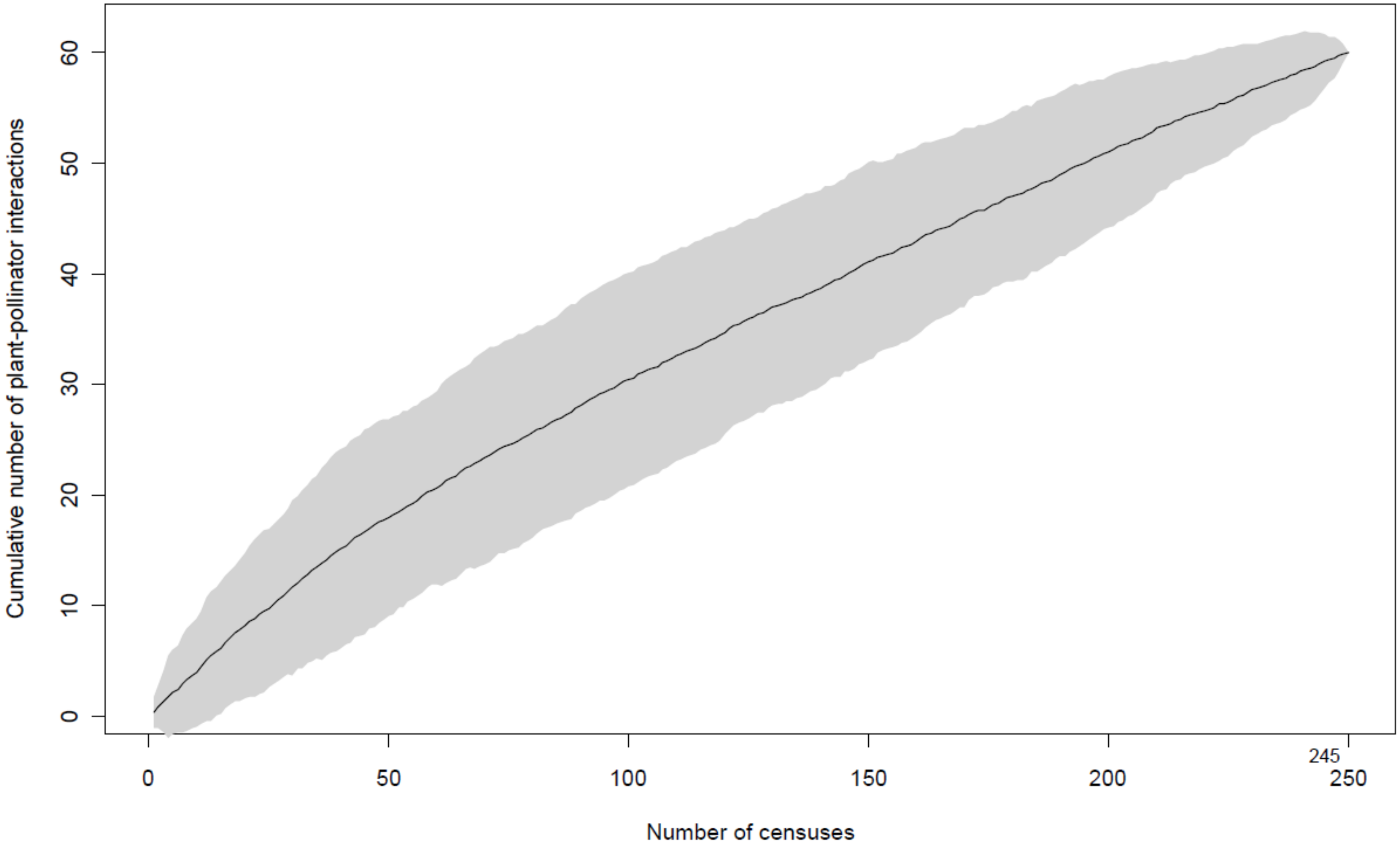

Figure A2.6 Rarefaction curve for removal plot at *Tirant.*

Appendix 3. Asymmetry, nestedness and modularity calculations

Asymmetry

We calculated the asymmetry of mutual dependences of each interacting plant and pollinator species according to Bascompte, Jordano & Olesen (2006). From the quantitative matrices (i.e. those whose cell values represented the weight of each interaction measured as visitation rate) we calculated two dependence values for each plant-pollinator interaction: dependence of plant *i* on pollinator *j* (d_{ij}) as the proportion of visits received by plant *i* that come from pollinator *j*; and dependence of pollinator *j* on plant *i* (d_{ji}) as the proportion of visits by pollinator *j* going to plant *i*.

Then, the asymmetry of each pair-wise interaction was estimated as follows:

$$\mathrm{Asy}_{ij} = |d_{ij} - d_{ji}| \Big/ \max\ (d_{ij}, d_{ji})$$

Nestedness

The nested structure of the matrices was tested with the software Aninhado (Guimarães & Guimarães 2006) using the algorithm NODF (Almeida-Neto *et al.* 2008) as the nestedness index and the null model CE. Null model CE is a conservative model that sets the probability of a matrix cell showing a presence as $\left(\frac{P_i}{C} + \frac{P_j}{R}\right) \Big/ 2$, in which P_i is the number of presences in the row *i*, P_j is the number of presences in the column *j*, *C* is the number of columns and *R* is the number of rows.

Modularity

The existence of modules (i.e. groups of highly interconnected species with relative few or no interactions with other groups) was analyzed with the software NETCARTO (Guimerà & Amaral 2005). Modularity ranges between 0 and 1-1/n, where n is number of modules. Modules were identified and counted by maximizing modularity using a simulated annealing algorithm (for a detailed description of the calculation see Guimerà & Amaral, 2005). Then, statistical significance was evaluated against a distribution obtained from 100 random networks of same size and connectance than the empirical one. When the modularity value calculated for the empirical network lies above the 95% confidence interval for the modularity of randomized networks, the empirical network is significantly modular.

We then assigned a role (peripheral, connector or module or network hub) to *Hedysarum* and *Apis* in each modular network on the base of the among- module connectivity (*c*) and the within module degree (*z*) values calculated for each species, following Olesen *et al.* (2007).

Appendix 4. Detected modules

Table A4.1. Modularity (*M*) values obtained for each empirical network and 100 randomized networks of same size and connectance. The upper limit of the 95% CI of the M of randomized networks is given to test the significance of the M obtained for empirical ones. When M lies above this limit, networks are considered modular.

		Empirical	**Randomized**	
Site	**Treatment**	**M**	**M**	**95% CI upper limit**
Llimpa	Removal	0.450	0.453	0.480
	Invaded	0.466	0.445	0.469
Albufera	Removal	0.390	0.403	0.437
	Invaded	0.552	0.510	0.548
Tirant	Removal	0.512	0.501	0.535
	Invaded	0.530	0.510	0.537
Island	Removal	0.438	0.444	0.464
	Invaded	0.475	0.461	0.479

Table A4.2. Modules detected in the invaded plot network of the site *Llimpa*. Family and order of pollinator species and family of plant species are given. Plant species are highlighted in grey and the non-native study species *Hedysarum* in bold letters.

Module	Species	Family	Order
1	*Daucus carota*	Umbelliferae	
1	*Anthaxia sp.1*	Buprestidae	Coleoptera
1	*Rhagonycha fulva*	Cantharidae	Coleoptera
1	*Anthomyia quinquemaculata*	Anthomyiidae	Diptera
1	*Sarcophaga africa*	Sarcophagidae	Diptera
1	*Sarcophaga sp.1*	Sarcophagidae	Diptera
1	*Bradysia nitidicollis*	Sciaridae	Diptera
1	*Odontomyia discolor*	Stratiomyidae	Diptera
1	*Syritta pipiens*	Syrphidae	Diptera
1	*Andrena tenuistriata*	Andrenidae	Hymenoptera
2	*Galactites tomentosa*	Compositae	
2	*Sonchus oleraceus*	Compositae	
2	*Sonchus tenerrimus*	Compositae	
2	Coleoptera *sp.1*	-	Coleoptera
2	*Stenopterus rufus*	Cerambycidae	Coleoptera
2	*Psilothrix viridicoerulea*	Dasytidae	Coleoptera

2	*Oedemera sp.1*	Oedemeridae	Coleoptera
2	*Tropinota hirta*	Scarabaeidae	Coleoptera
2	*Eristalis arbustorum*	Syrphidae	Diptera
2	*Eristalis tenax*	Syrphidae	Diptera
2	*Apis mellifera*	Apidae	Hymenoptera
2	*Eucera oraniensis*	Apidae	Hymenoptera
2	*Halictus scabiosae*	Halictidae	Hymenoptera
2	*Osmia niveata*	Megachilidae	Hymenoptera
3	***Hedysarum coronarium***	Leguminosae	
3	*Oxalis pes-caprae*	Oxalidaceae	
3	*Meligethes nigritus*	Nitidulidae	Coleoptera
3	*Oxythyrea funesta*	Scarabaeidae	Coleoptera
3	*Anthophora plumipes*	Apidae	Hymenoptera
3	*Anthophora subterranea*	Apidae	Hymenoptera
3	*Bombus terrestris*	Apidae	Hymenoptera
3	*Xylocopa violacea*	Apidae	Hymenoptera
3	*Chalicodoma sicula*	Megachilidae	Hymenoptera
3	*Rhodanthidium septemdentatum*	Megachilidae	Hymenoptera
4	*Reichardia picroides*	Compositae	
4	*Alyssum maritimum*	Cruciferae	
4	*Lotus edulis*	Leguminosae	
4	*Bruchidius seminarius*	Chrysomelidae	Coleoptera
4	*Coccinellidae sp.1*	Cucujidae	Coleoptera
4	*Dasytes virens*	Dasytidae	Coleoptera
4	*Mordella holomelaena*	Mordellidae	Coleoptera
4	*Ortochile nigrocoerulea*	Dolichopodidae	Diptera
4	*Antlemon halidayi*	Keroplatidae	Diptera
4	*Lasioglossum angusticeps*	Halictidae	Hymenoptera

Table A4.3. Modules detected in the invaded plot network of the site *Albufera*. Family and order of pollinator species and family of plant species are given. Plant species are highlighted in grey and the non-native study species *Hedysarum* in bold letters.

Module	Species	Family	Order
1	***Hedysarum coronarium***	Leguminosae	
1	*Lotus cytisoides*	Leguminosae	
1	*Oxythyrea funesta*	Scarabaeidae	Coleoptera
1	*Andrena ovatula*	Andrenidae	Hymenoptera
1	*Anthophora plumipes*	Apidae	Hymenoptera
1	*Anthophora subterranea*	Apidae	Hymenoptera
1	*Apis mellifera*	Apidae	Hymenoptera
1	*Bombus terrestris*	Apidae	Hymenoptera
1	*Xylocopa violacea*	Apidae	Hymenoptera
2	*Calicotome infesta*	Leguminosae	
2	*Lathyrus clymenum*	Leguminosae	
2	*Vicia benghalensis*	Leguminosae	
2	*Asphodelus aestivus*	Liliaceae	
2	*Mordella holomelaena*	Mordellidae	Coleoptera
2	*Tropinota hirta*	Scarabaeidae	Coleoptera
2	Diptera *sp.1*	-	Diptera
2	*Chalicodoma sicula*	Megachilidae	Hymenoptera
3	*Hyoseris radiata*	Compositae	
3	*Hypochoeris achyrophorus*	Compositae	
3	*Aspidapion radiolus*	Brentidae	Coleoptera
3	*Bruchidius seminarius*	Chrysomelidae	Coleoptera
3	*Coccinellidae sp.1*	Cucujidae	Coleoptera
3	*Dasytes virens*	Dasytidae	Coleoptera
3	*Psilothrix viridicoerulea*	Dasytidae	Coleoptera
3	*Caenocoris nerii*	Lygaeidae	Coleoptera
3	*Meligethes nigritus*	Nitidulidae	Coleoptera
3	*Oedemera sp.1*	Oedemeridae	Coleoptera
3	*Oscinellinae sp.1*	Chloropidae	Diptera
3	*Oscinimorpha longirrostris*	Chloropidae	Diptera
3	*Ortochile nigrocoerulea*	Dolichopodidae	Diptera
3	*Scaptomyza pallida*	Drosophilidae	Diptera
3	*Tetrastichinae sp.1*	Eulophidae	Diptera
3	*Osmia niveata*	Megachilidae	Hymenoptera
4	*Melilotus indicus*	Leguminosae	
4	*Paragus tibialis*	Syrphidae	Diptera

Table A4.4. Modules detected in the invaded plot network of the site *Tirant*. Family and order of pollinator species and family of plant species are given. Plant species are highlighted in grey and the non-native study species *Hedysarum* in bold letters.

Module	Species	Family	Order
1	*Reichardia picroides*	Compositae	
1	*Coccinellidae sp.1*	Cucujidae	Coleoptera
1	Machiinae *sp.1*	Machiinae	Coleoptera
1	*Oedemera sp.1*	Oedemeridae	Coleoptera
1	Platygastridae *sp.1*	Platygastridae	Diptera
1	*Scathophaga stercoraria*	Sarcophagidae	Diptera
1	Hymenoptera *sp.1*	-	Hymenoptera
1	*Halictus scabiosae*	Halictidae	Hymenoptera
1	*Lasioglossum malachurum*	Halictidae	Hymenoptera
1	*Lasioglossum sp.1*	Halictidae	Hymenoptera
2	*Asphodelus aestivus*	Liliaceae	
2	*Tropinota hirta*	Scarabaeidae	Coleoptera
2	*Megascolia bidens*	Scoliidae	Hymenoptera
2	Vespidae *sp.2*	Vespidae	Hymenoptera
3	*Allium roseum*	Liliaceae	
3	*Bruchidius seminarius*	Chrysomelidae	Coleoptera
3	*Dasytes virens*	Dasytidae	Coleoptera
3	*Oxythyrea funesta*	Scarabaeidae	Coleoptera
3	Braconidae *sp.1*	Braconidae	Hymenoptera
3	*Halictus sp.1*	Halictidae	Hymenoptera
3	Vespidae *sp.1*	Vespidae	Hymenoptera
4	*Lotus edulis*	Leguminosae	
4	*Lotus ornithopodioides*	Leguminosae	
4	*Scorpiurus sulcatus*	Leguminosae	
4	*Lasioglossum angusticeps*	Halictidae	Hymenoptera
4	*Hoplitis andrenoides*	Megachilidae	Hymenoptera
4	*Osmia niveata*	Megachilidae	Hymenoptera
4	*Osmia versicolor*	Megachilidae	Hymenoptera
5	*Kundmania sicula*	Umbelliferae	
5	*Delia platura*	Anthomyiidae	Diptera
5	*Neomyia cornicina*	Muscidae	Diptera
5	*Bradysia nitidicollis*	Sciaridae	Diptera
5	*Eupeodes corollae*	Syrphidae	Diptera
5	*Melanostoma mellium*	Syrphidae	Diptera
5	*Syritta pipiens*	Syrphidae	Diptera
5	*Andrena tenuistriata*	Andrenidae	Hymenoptera
5	Ichneumonidae *sp.1*	Ichneumonidae	Hymenoptera
5	Vespidae *sp.3*	Vespidae	Hymenoptera
5	Vespidae *sp.4*	Vespidae	Hymenoptera
6	*Calicotome infesta*	Leguminosae	
6	***Hedysarum coronarium***	Leguminosae	

6	*Muscari comosum*	Liliaceae	
6	*Psilothrix viridicoerulea*	Dasytidae	Coleoptera
6	*Andrena ovatula*	Andrenidae	Hymenoptera
6	*Anthophora balearica*	Apidae	Hymenoptera
6	*Anthophora plumipes*	Apidae	Hymenoptera
6	*Anthophora subterranea*	Apidae	Hymenoptera
6	*Apis mellifera*	Apidae	Hymenoptera
6	*Ceratina curcubitina*	Apidae	Hymenoptera
6	*Synhalonia hungarica*	Apidae	Hymenoptera
6	*Lasioglossum sp.2*	Halictidae	Hymenoptera
6	*Chalicodoma sicula*	Megachilidae	Hymenoptera
6	*Osmia caerulescens*	Megachilidae	Hymenoptera

Table A4.5. Modules detected in the invaded island network. Family and order of pollinator species and family of plant species are given. Plant species are highlighted in grey and the non-native study species *Hedysarum* in bold letters.

Module	Species	Family	Order
1	*Calicotome infesta*	Leguminosae	
1	***Hedysarum coronarium***	Leguminosae	
1	*Lathyrus clymenum*	Leguminosae	
1	*Lotus cytisoides*	Leguminosae	
1	*Vicia benghalensis*	Leguminosae	
1	*Asphodelus aestivus*	Liliaceae	
1	*Muscari comosum*	Liliaceae	
1	*Oxalis pes-caprae*	Oxalidaceae	
1	*Tropinota hirta*	Scarabaeidae	Coleoptera
1	Diptera *sp.1*	-	Diptera
1	*Andrena ovatula*	Andrenidae	Hymenoptera
1	*Anthophora balearica*	Apidae	Hymenoptera
1	*Anthophora plumipes*	Apidae	Hymenoptera
1	*Anthophora subterranea*	Apidae	Hymenoptera
1	*Apis mellifera*	Apidae	Hymenoptera
1	*Bombus terrestris*	Apidae	Hymenoptera
1	*Ceratina curcubitina*	Apidae	Hymenoptera
1	*Synhalonia hungarica*	Apidae	Hymenoptera
1	*Xylocopa violacea*	Apidae	Hymenoptera
1	*Lasioglossum sp.2*	Halictidae	Hymenoptera
1	*Chalicodoma sicula*	Megachilidae	Hymenoptera
1	*Osmia caerulescens*	Megachilidae	Hymenoptera
1	*Rhodanthidium septemdentatum*	Megachilidae	Hymenoptera
1	*Megascolia bidens*	Scoliidae	Hymenoptera

2	*Allium roseum*	Liliaceae	
2	*Kundmania sicula*	Umbelliferae	
2	*Oxythyrea funesta*	Scarabaeidae	Coleoptera
2	*Delia platura*	Anthomyiidae	Diptera
2	*Neomyia cornicina*	Muscidae	Diptera
2	*Eupeodes corollae*	Syrphidae	Diptera
2	*Melanostoma mellium*	Syrphidae	Diptera
2	Braconidae *sp.1*	Braconidae	Hymenoptera
2	*Halictus sp.1*	Halictidae	Hymenoptera
2	Ichneumonidae *sp.1*	Ichneumonidae	Hymenoptera
2	Vespidae *sp.1*	Vespidae	Hymenoptera
2	Vespidae *sp.2*	Vespidae	Hymenoptera
2	Vespidae *sp.3*	Vespidae	Hymenoptera
2	Vespidae *sp.4*	Vespidae	Hymenoptera
3	*Daucus carota*	Umbelliferae	
3	*Anthaxia sp.1*	Buprestidae	Coleoptera
3	*Rhagonycha fulva*	Cantharidae	Coleoptera
3	*Anthomyia quinquemaculata*	Anthomyiidae	Diptera
3	*Sarcophaga africa*	Sarcophagidae	Diptera
3	*Sarcophaga sp.1*	Sarcophagidae	Diptera
3	*Bradysia nitidicollis*	Sciaridae	Diptera
3	*Odontomyia discolor*	Stratiomyidae	Diptera
3	*Syritta pipiens*	Syrphidae	Diptera
3	*Andrena tenuistriata*	Andrenidae	Hymenoptera
4	*Hyoseris radiata*	Compositae	
4	*Hypochoeris achyrophorus*	Compositae	
4	*Reichardia picroides*	Compositae	
4	*Sonchus tenerrimus*	Compositae	
4	*Aspidapion radiolus*	Brentidae	Coleoptera
4	*Bruchidius seminarius*	Chrysomelidae	Coleoptera
4	*Coccinellidae sp.1*	Cucujidae	Coleoptera
4	*Psilothrix viridicoerulea*	Dasytidae	Coleoptera
4	*Caenocoris nerii*	Lygaeidae	Coleoptera
4	Machiinae *sp.1*	Machiinae	Coleoptera
4	*Meligethes nigritus*	Nitidulidae	Coleoptera
4	*Oedemera sp.1*	Oedemeridae	Coleoptera
4	*Oscinellinae sp.1*	Chloropidae	Diptera
4	*Oscinimorpha longirrostris*	Chloropidae	Diptera
4	*Ortochile nigrocoerulea*	Dolichopodidae	Diptera
4	*Scaptomyza pallida*	Drosophilidae	Diptera
4	*Tetrastichinae sp.1*	Eulophidae	Diptera
4	Platygastridae *sp.1*	Platygastridae	Diptera
4	*Scathophaga stercoraria*	Sarcophagidae	Diptera
4	Hymenoptera *sp.1*	-	Hymenoptera
4	*Lasioglossum malachurum*	Halictidae	Hymenoptera
4	*Lasioglossum sp.1*	Halictidae	Hymenoptera

5	*Lotus edulis*	Leguminosae	
5	*Lotus ornithopodioides*	Leguminosae	
5	*Scorpiurus sulcatus*	Leguminosae	
5	*Lasioglossum angusticeps*	Halictidae	Hymenoptera
5	*Hoplitis andrenoides*	Megachilidae	Hymenoptera
5	*Osmia niveata*	Megachilidae	Hymenoptera
5	*Osmia versicolor*	Megachilidae	Hymenoptera
6	*Melilotus indicus*	Leguminosae	
6	*Paragus tibialis*	Syrphidae	Diptera
7	*Galactites tomentosa*	Compositae	
7	*Sonchus oleraceus*	Compositae	
7	*Alyssum maritimum*	Cruciferae	
7	Coleoptera *sp.1*	-	Coleoptera
7	*Stenopterus rufus*	Cerambycidae	Coleoptera
7	*Dasytes virens*	Dasytidae	Coleoptera
7	*Mordella holomelaena*	Mordellidae	Coleoptera
7	*Antlemon halidayi*	Keroplatidae	Diptera
7	*Eristalis arbustorum*	Syrphidae	Diptera
7	*Eristalis tenax*	Syrphidae	Diptera
7	*Eucera oraniensis*	Apidae	Hymenoptera
7	*Halictus scabiosae*	Halictidae	Hymenoptera

Capítulo 4

Mass flowering crops in agricultural landscapes reduce bee abundance and visitation rates in adjacent shrublands

Resumen

El vertido de polinizadores (*pollinator spill-over*) entre hábitats puede ocurrir cuando la oferta floral difiere entre ellos, ya sea en el tiempo o en el espacio. La puntual pero abundante oferta floral de los cultivos de floración masiva puede promover el vertido de polinizadores entre los campos de cultivo y las zonas naturales adyacentes.

Exploramos los patrones de polinización de los cultivos de floración masiva de la leguminosa *Hedysarum coronarium* y su influencia en las comunidades de polinizadores del matorral mediterráneo adyacente en un paisaje agrícola heterogéneo y parcheado. Estudiamos el vertido temporal (durante *vs.* después de la floración masiva) y espacial (matorral adyacente *vs.* distante) de polinizadores.

La abeja de la miel resultó ser el principal polinizador de *Hedysarum*, pero en el matorral adyacente su abundancia y la de otras abejas no varió significativamente entre durante y después de la floración masiva. Sin embargo, a escala de paisaje, tanto la abeja de la miel como las otras abejas resultaron ser menos abundantes en el matorral adyacente a los cultivos de *Hedysarum* que en matorrales distantes a éstos, al igual que sus tasas de visitas.

Estos resultados muestran que los cultivos de floración masiva pueden afectar a los patrones de polinización del paisaje circundante compitiendo por polinizadores generalistas con las plantas nativas. Por tanto, el papel de los cultivos de floración masiva como sustento y fuente de polinizadores para los hábitats naturales adyacentes debe ser reconsiderado, al menos en paisajes agrícolas heterogéneos y parcheados, y en particular para el caso de las abejas silvestres.

Abstract

Pollinator spill-over among habitats can arise whenever differences in floral resource offer change over time or space. The pulsed and abundant floral offer of mass flowering crops might promote pollinator spill-over between cultivated and adjacent natural areas.

We explored pollinator patterns in the mass flowering legume crop *Hedysarum coronarium* and its influence on the pollinator communities of adjacent shrublands in a heterogeneous and patchy agricultural landscape. We studied the temporal (i.e. during *vs.* after mass flowering) and spatial (i.e. adjacent *vs.* distant shrublands) pollinator spill-over.

The honeybee was the main pollinator visiting *Hedysarum,* yet its abundance and that of other bee species visiting native plants in adjacent shrublands during and after *Hedysarum* mass flowering was not significantly different. However, at the landscape scale, both the honeybee and the other bee species were less abundant in shrublands adjacent to *Hedysarum* crops compared to distant ones, as well as their visitation rates.

These results show that mass flowering crops can influence pollinator patterns in the surrounding landscape by competing for generalist pollinators with native plants. Therefore, the role of mass flowering crops as supporters and sources of pollinators for adjacent natural areas has to be reconsidered, at least in patchy and heterogeneous agricultural landscapes, and particularly for wild bees.

Introduction

There is growing concern about local and regional declines in pollinator species and the pollination services they provide (Bartomeus *et al.* 2013; Potts *et al.* 2010). Moreover, plant-pollinator interactions may be even more sensitive than the species themselves (Tylianakis *et al.* 2008), and factors driving the decline of pollinators might interact in non-additive ways (González-Varo *et al.* 2013).

More than 75% of the cultivated species depend on, or benefit from, animal mediated pollination (Klein *et al.* 2007), and the area devoted to pollinator-dependent crops is disproportionately growing (Aizen *et al.* 2008a). In this context, during the last two decades, scientists have explored the role of remaining natural areas within agricultural landscapes as reservoirs of pollinators to provide pollination service to pollinator-dependent crops. Maintaining and restoring these areas in agricultural landscapes is one of the most commonly implemented agri-environment schemes. The underlying rationale is that remaining natural areas offer pollinators feeding resources and/or nesting sites not provided by the crop or not stable over time due to the inherent disturbance frequency (Westphal, Steffan-Dewenter & Tscharntke 2003).

The movement of pollinators from one area to another where they can meet their feeding and/or nesting requirements is called pollinator spill-over (Blitzer *et al.* 2012). Spill-over can occur whenever the offer of a required resource differs between habitats; therefore, it can occur in both directions. However, only recently has the spill-over of pollinators from entomophilous mass flowering crops (MFC, hereafter) to natural habitats received the attention of scientists and managers (Blitzer *et al.* 2012; Holzschuh *et al.* 2011). MFCs, despite offering only pulsed floral rewards, could compensate for food resource limitation during periodic intervals, and help maintain pollinator communities in agricultural landscapes (Westphal *et al.* 2003), as long as nesting sites and other feeding areas are also available within the foraging ranges of pollinators. In fact, spill-over would be more likely in heterogeneous agricultural areas (Blitzer *et al.* 2012).

In addition to spill-over between habitats with different resource offer at a given period of time (i.e. spatial spill-over), differences in resource offer between habitats can also arise at different moments in time (i.e. temporal spill-over). For instance, the high floral rewards of a MFC compared to its surrounding habitats can be reverted after the MFC flowering peak (Hanley *et al.* 2011).

Here we study the effect of a high rewarding bee-pollinated MFC on the bee community in surrounding shrublands of a patchy and heterogeneous Mediterranean agricultural landscape (Fig. 1).

Figure 1. Patchy and heterogeneous Mediterranean agricultural landscape in Menorca.

We address the following questions: (a) Does the MFC affect the bee community of plant species in adjacent shrublands through a temporal bee spill-over during and after mass flowering? (b) Is there a spatial bee spill-over detected when comparing shrublands adjacent and distant to the crop? (c) Is the role of the honeybee (the main pollinator of the MFC) different from that of the remaining bee species, for both the temporal and spatial spill-over?

We expect the MFC to attract a large number of bees during its mass flowering; i.e., a bee spill-over from natural areas to the MFC. After mass flowering, bees would spill-over from the MFC to adjacent natural areas (i.e. temporal spill-over). During mass flowering, the bees attracted and spilled-over to the MFC would come from adjacent natural areas rather than from distant ones (i.e. spatial spill-over). We expect both temporal and spatial spill-over to be largely mediated by the honeybee, as it is the main pollinator of the mass flowering species observed in this study. Furthermore, the honeybee has larger maximum foraging areas, broader diets and higher communication capacities than most wild bees (Gathmann & Tscharntke 2002; Steffan-Dewenter *et al.* 2002; Steffan-Dewenter & Kuhn, 2003). These characteristics allow honeybees to locate and efficiently utilize resources at large landscapes, probably outcompeting other pollinators in agricultural landscapes.

Materials and Methods

Study sites

In 2009, to explore whether there was a temporal bee spill-over between *Hedysarum* crops and adjacent shrublands, we selected four Mediterranean shrublands adjacent to *Hedysarum* crops (Fig. 2a) that were studied during and after mass flowering (i.e. after crops were harvested during the flowering peak; Fig. 2b and c). Each shrubland was located a minimum distance of 500 m from the others. Despite the fact that honeybees and bumblebee species can fly great distances (Greenleaf *et al.* 2007; Osborne *et al.* 2008), pollinators do not usually travel very far when rewards are available in the vicinity (Greenleaf *et al.* 2007; Johnson *et al.* 2003; Wolf & Moritz, 2008). Moreover, due to the heterogeneity of the Menorcan agricultural landscape we considered 500 m to be a sufficient distance to assure study shrubland independence.

In 2010, in order to investigate whether there was a spatial bee spill-over at the landscape scale, we selected four *Hedysarum* crops (inside, hereafter) and six Mediterranean shrublands, including four adjacent to *Hedysarum* crops (adjacent, hereafter) (Fig. 2a) and two without *Hedysarum* crops in the surrounding landscape (distant, hereafter). For the reasons presented above, we again established 500 m radius landscapes.

The area of the studied MFCs ranged from 3380 to 21066 m^2 with a mean flower density of 9100 ± 940 flowers/m^2. There were no honeybee hives in the 500 m radius of the surrounding landscapes (Montero-Castaño, *personal observation*). Study shrublands had an area that ranged from 133 to 29743 m^2. Adjacent and distant shrublands of our 2010 study had similar flowering plant species richness values (0.46 ± 0.04 and 0.43 ± 0.03 species/m^2, respectively; t = -0.067, P = 0.950) and similar floral unit densities (hereafter flowers, according to Dicks *et al.*, 2002) (100.12 ± 24.49 and 163.24 ± 30.95 flowers/m^2, respectively; t = 0.616, P = 0.571). See Fig. 2 and Table 1 for location and characterization of study shrublands and their surrounding landscapes.

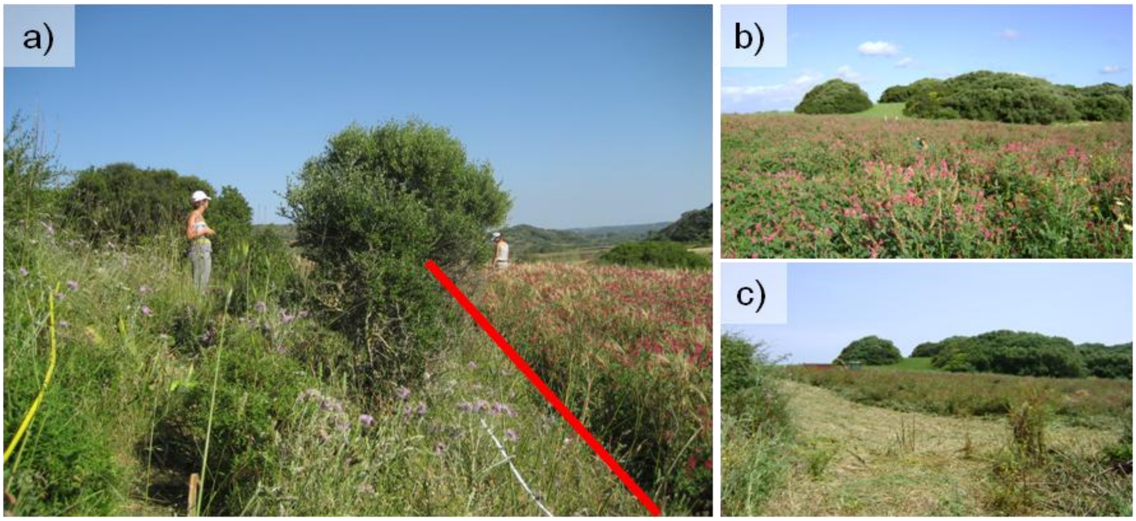

Figure 2. Study sites: (a) Mediterranean shrubland (right to the red line) adjacent to a *Hedysarum* mass flowering crop (on the left hand of the red line). *Hedysarum* mass flowering crop (b) during flowering peak and (c) after harvesting.

Table 1. Location, area and characterization of the 500 m radius surrounding landscape of each study shrubland in Menorca. Landscape characterization was based on the land-use cover map (Carreras *et al.* 2013).

Site	**Year**	**Latitude**	**Longitude**	**Area (m^2)**		**% Surrounding landscape (500 m radius)**			
				Study shrubland	**Adjacent MFC**	**MFC**	**Other crops**	**Natural areas**	**Artificial areas**
Binicalaf	2009	39°52'14.81"N	4°10'2.49"E	2940.30	3844.45	0.49	34.82	55.17	9.14
Binixabó	2009	39°56'12.04"N	4° 6'57.23"E	873.54	3379.52	0.43	47.03	47.95	4.48
Mila1	2009	39°55'29.35"N	4°15'12.05"E	151.53	15542.47	4.47	58.60	34.46	2.45
Mila2	2009	39°55'40.88"N	4°15'21.39"E	15837.37	20522.74	4.59	55.36	35.89	2.14
Albufera	2010	39°56'27.50"N	4°15'21.11"E	29742.80	-	0.00	4.37	82.03	9.81
Binigurdó	2010	39°59'56.09"N	4° 6'2.40"E	2707.70	2240.15	0.29	60.54	36.48	2.35
Favaraix	2010	39°58'26.19"N	4°13'39.69"E	13745.07	-	0.00	61.86	34.14	2.25
Molí	2010	39°59'50.42"N	4° 5'34.13"E	455.82	11487.12	1.46	79.30	13.65	5.52
Mongofre	2010	39°59'3.85"N	4°13'18.29"E	3090.83	21065.59	2.68	63.94	32.98	0.00
Palafanguer	2010	39°55'35.74"N	4°14'15.21"E	132.95	6110.35	0.78	44.23	54.09	0.88

Pollination censuses

We conducted pollination censuses during the peak flowering period of *Hedysarum* (from 30th April to 25th May and from 28th April to 24th May in 2009 and 2010, respectively) on sunny, warm (≥ 17 ºC) and non-windy days, from 10 am to 6 pm. In both years weather conditions fell within the average ranges for these months in the study area (“AEMET”).

The pollinator species that could not be identified in the field were sorted into distinct morphospecies and caught for later identification by specialists. Voucher specimens are deposited at EBD-CSIC.

a) Temporal bee spill-over

In 2009, in each adjacent shrubland we surveyed two or three target plant species (seven in total, Appendix 1) that shared pollinators with *Hedysarum* and were in bloom during and after *Hedysarum* mass flowering. Moreover, we selected target species with low-restrictive flower morphologies because we expected them to attain higher visitation rates than those with more restrictive flower morphologies (**Chapter 3**) so that the effect would be easily observed.

For each target species we conducted focal censuses that lasted 15 min during which we noted the number and identity of bee pollinators; we then counted the number of flowers under observation. A visitor was considered a pollinator when it entered a flower and touched its sexual parts.

Censuses lasted 13 days and on average were conducted 3.50 ± 1.35 days before and after crop harvesting. The observation schedule for each site, plant species and individual was randomly established. We conducted a total of 134 focal censuses (33.5 h), including 66 during and 68 after *Hedysarum* mass flowering, with each plant species observed an average of 1.84 ± 0.09 h and 1.89 ± 0.08 h during and after mass flowering, respectively. For statistical analyses, data were pooled for each species.

b) Spatial bee spill-over

In 2010, we conducted bee censuses in the shrublands by walking along 20 m long transects, randomly established parallel to *Hedysarum* crops, for a duration of 10 min. During those 10 min, we noted the number and identity of the bees and the identity of the plant for each plant-bee interaction that was observed.

In each shrubland we established between three and 17 transects. In total, we established 52 transects (36 and 16 in adjacent and distant areas, respectively). Study shrublands and transects were alternatively sampled. We sampled shrublands until we found no new plant-bee interactions after six or more transect walks according to rarefaction curves (Appendix 2), which we considered a good compromise between sampling effort and data accuracy. Overall, we conducted a total of 278 transect walks (46.33 h) along the 52 transects: 164 (27.33 h) in adjacent and 114 (19.00 h) in distant shrublands. Each transect was walked an average of 5.35 ± 0.35 times (0.89 ± 0.06 h), ranging from two to 11 times. For statistical analyses data were pooled for each transect.

At every meter along each transect we placed 0.4 x 0.4 m^2 quadrants in which we counted all open flowers as well as identified the plant species present. In total we observed 46 plant species belonging to 34 genera and 17 families: 38 species in adjacent shrublands and 24 in distant ones (Sørensen similarity index = 0.52; see below calculation details).

Simultaneously, we conducted censuses in the four *Hedysarum* crops following the same methodology as in the shrublands. We established a total of 21 transects (three to seven transects per crop). Each transect was walked an average of 5.24 ± 0.39 times (0.87 ± 0.07 h) accounting for a total of 18.33 h of crop sampling.

Data analyses

The response variables analyzed in both study years were bee composition, richness and abundance. In 2010 we also analyzed bee visitation rate and plant-bee interaction richness. In 2009, values were standardized per flower and per hour while in

2010 they were standardized per transect and per 10 min. Mean ± SE values are given through the text.

To account for differences in bee species composition, we calculated the Sørensen similarity index: $QS = 2C / (A + B)$; where A is the number of species at a specified time (i.e. during or after mass flowering) or at distance A (i.e. inside, adjacent or distant to *Hedysarum* crops) and B is the number of species at a specified time or at distance B; and C is the number of shared species. *QS* values range from 0 (no overlap in species composition) to 1 (complete overlap).

a) *Temporal bee spill-over*

In 2009, we explored whether *Hedysarum* crops affected bee pollinator richness and abundance in target plant species in adjacent shrublands by comparing the abovementioned variables during and after mass flowering.

For the response variable richness + 1, we built a generalized mixed model with time (during *vs.* after mass flowering) as a fixed factor. Target plant species nested in site was included as a random factor. Because the response variable was continuous and non-normal, we used Gamma as the error distribution family and log as the link function. For abundance + 1, we built a similar model with pollinator group (honeybee *vs.* other bees) and its interaction with time included as fixed factors. Moreover, we explored whether time and pollinator group in these response variables differed for each target plant species and site by conducting Wilcoxon tests.

b) *Spatial bee spill-over*

In 2010, we assessed whether bee pollinator richness and abundance differed among *Hedysarum* crops, and shrublands adjacent and distant to them. We also compared whether the response was different for the honeybee than for other bee species. Furthermore, we compared differences in visitation rates and richness of plant-bee interactions between adjacent and distant shrublands.

Differences in species richness and interaction richness were tested by building linear mixed models with distance to *Hedysarum* crops as the fixed factor and site as a random factor. In the case of species richness, the fixed factor had three levels (i.e.

inside, adjacent, distant), and Tukey *post hoc* comparisons among them were conducted. In the case of interaction richness, it had only two levels (adjacent and distant).

For abundance +1 and visitation rate +1, we built generalized instead of linear mixed models with distance to *Hedysarum* crops, pollinator group and their interaction included as fixed factors. Site was included as a random factor. Gamma was the error distribution family and log was the link function. Similar to the analysis above, the fixed factor distance to *Hedysarum* crops for abundance had three levels (inside, adjacent, distant), while for visitation rate it had only two levels (adjacent and distant). In both cases, *post hoc* comparisons among treatments and within pollinator group were conducted by building our contrast matrices.

We are aware that the number of replicates is unbalanced among treatments with distant sites underrepresented. To account for this limitation we repeated the analysis by excluding the two most distant shrublands (*Molí* and *Binigurdó*). The results obtained did not significantly differ from those including all study sites (Table A4.2).

All analyses were conducted in R (R Development Core Team 2001) with the libraries *nlme* and *glmmADMB* for linear and generalized mixed models, respectively. The library *multcomp* was used for *post hoc* comparisons and the library *contrast* to build the contrast matrices.

Results

Pooling the 2009 and 2010 data, we observed a total of 25 bee species belonging to 16 genera. Nine species visited *Hedysarum* crops while 23 species visited plants in shrublands. All bee species that visited *Hedysarum* were shared with shrubland plants except two, *Bombus terrestris* and *Eucera numida*, which were exclusive to *Hedysarum* (Table 2).

Table 2. Bee pollinator species observed during 2009 and 2010 with indication of whether they were observed inside *Hedysarum* mass flowering crops and/or in natural shrublands.

Species	**Family**	***Hedysarum***	**Shrublands**
Andrena flavipes	Andrenidae		X
Andrena nigroolivacea	Andrenidae		X
Andrena ovatula	Andrenidae	X	X
Andrena parviceps	Andrenidae		X
Andrena tenuistriata	Andrenidae		X
Anthophora plumipes	Apidae		X
Apis mellifera	Apidae	X	X
Bombus terrestris	Apidae	X	
Ceratina cucurbitina	Anthophoridae		X
Ceratina dallatorreana	Anthophoridae		X
Chalicodoma sicula	Megachilidae	X	X
Eucera numida	Apidae	X	
Eucera oraniensis	Apidae	X	X
Halictus gemmeus	Halictidae		X
Halictus scabiosae	Halictidae		X
Hoplitis praestans	Megachilidae		X
Hoplosmia ligurica	Megachilidae		X
Hylaeus clypearis	Colletidae		X
Hymenoptera *sp.1*	-		X
Lasioglossum sp.1	Halictidae		X
Lasioglossum sp.2	Halictidae		X
Megachile pilidens	Megachilidae	X	X
Osmia caerulescens	Megachilidae	X	X
Osmia niveata	Megachilidae		X
Rhodanthidium septemdentatum	Megachilidae	X	X

Temporal bee spill-over

Hedysarum mass flowering did not affect bee richness in target plants of adjacent shrublands (during = 0.028 ± 0.008 and after = 0.029 ± 0.014 species/flower/h, Table 3). However, despite having similar richness, species identity and their relative abundances partially differed during and after mass flowering as indicated by the Sørensen similarity index *QS* = 0.53.

Both during and after mass flowering the honeybee was less abundant than the pool of other bee species (Fig. 3). Nevertheless, the abundance of both pollinator groups was not affected by *Hedysarum* mass flowering (Table 3, Fig. 3).

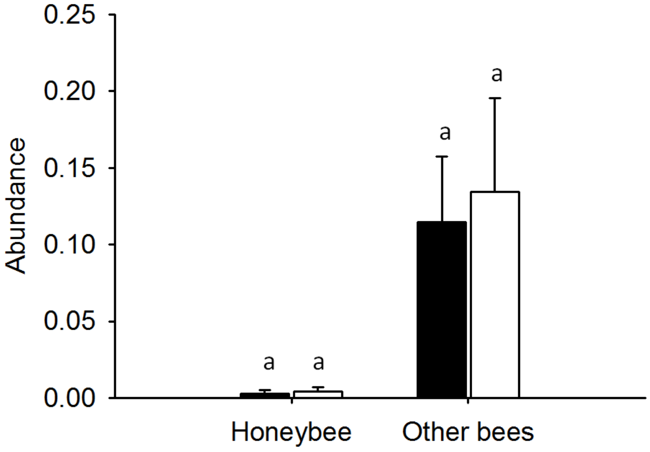

Figure 3. Mean + SE abundance (i.e. individuals/flower/h) of the honeybee and other bee species in plants adjacent to *Hedysarum* crops during (black) and after (bold) mass flowering. Letters above bars represent whether differences within pollinator groups are significant (different letters) or not (same letters).

When analyzing each target plant species in each site separately, we found that after mass flowering the honeybee abundance marginally decreased in one target plant species (*Asphodelus aestivus*) while in two other target plant species the abundance of other bees was higher after mass flowering (*Cistus albidus* and *Galactites tomentosa* in the *Binicalaf* site) (Fig. A3.2). Mass flowering also affected pollinator richness in three target plant species. In two of them (*Cistus albidus* and *Galactites tomentosa* in the *Mila1* site), bee species richness increased after mass flowering while in the other one (*Galactites tomentosa* in the *Binicalaf* site), we found the opposite trend. Thus, *Galactites tomentosa* showed significant but opposite trends in the two sites in which it was sampled (Fig. A3.2).

Spatial bee spill-over

Bee richness did not differ with distance to *Hedysarum* crops (0.421 ± 0.018, 0.345 ± 0.048 and 0.542 ± 0.098 species/transect/10', for inside, adjacent and distant sites, respectively; N = 73, F = 1.430, P = 0.247). Conversely, distance to *Hedysarum* crops affected bee abundance, and the effect differed between pollinator groups (Fig. 4 and Table A4.1 for complete analyses output). The abundance of honeybees and the other bees was higher in distant shrublands compared to adjacent ones. However, inside crops the two pollinator groups behaved differently. The honeybee abundance was the highest inside crops, while the abundance of other bees was lower inside crops, not differing from that in adjacent shrublands (Fig. 4).

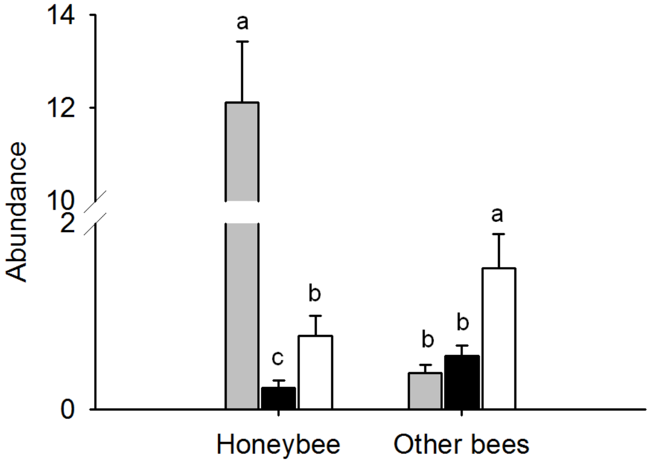

Figure 4. Mean + SE abundance (i.e. individuals/transect/10') of the honeybee and other bee species inside (grey), adjacent (black) and distant (bold) to *Hedysarum* crops. Letters above bars represent whether differences within pollinator groups are significant (different letters) or not (same letters).

The similarity in bee composition was higher between adjacent and distant shrublands (*QS* = 0.52) than between inside the crop and shrublands. In shrublands, the honeybee and the wild bee *Eucera oraniensis* were the most abundant species (Fig. 5), while the bee community in *Hedysarum* crops was largely dominated by the honeybee but lacked *E. oraniensis*; and its similarity with adjacent (*QS* = 0.30) and distant (*QS* = 0.38) shrublands was low. When pooling together the bee pollinator communities of crops and adjacent shrublands, its similarity with distant shrublands was *QS* = 0.62.

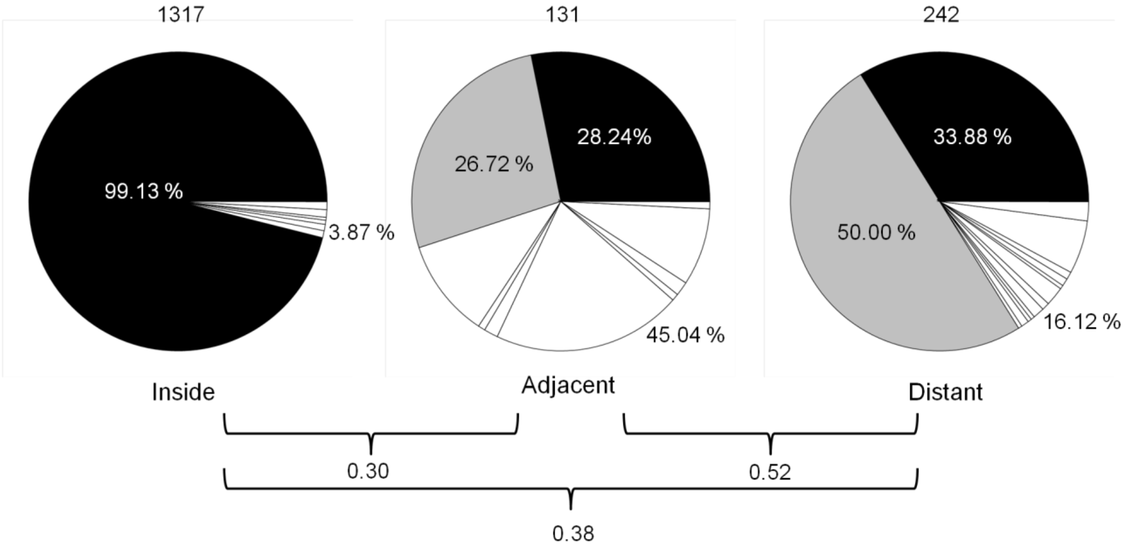

Figure 5. Relative abundance (%) of bee species inside, adjacent and distant to *Hedysarum* crops. The honeybee is represented in black, the wild bee *Eucera oraniensis* in grey and the rest of species in white. Total number of individuals observed is given above each pie chart. Bellow brackets the values for the Sørensen similarity index are given.

Excluding the honeybee and *E. oraniensis* from the analysis, differences in abundance between inside crops (0.395 ± 0.086) and distant shrublands (0.333 ± 0.070) disappeared (N = 73, Z = -0.45, P = 0.650).

There were no significant differences in interaction richness between adjacent and distant shrublands (0.345 ± 0.048 and 0.542 ± 0.098 interactions/transect/10’, respectively; N = 52, t = 0.597, P = 0.583). Visitation rates for the honeybee and other bees were higher in distant shrublands compared to adjacent areas (Fig. 6).

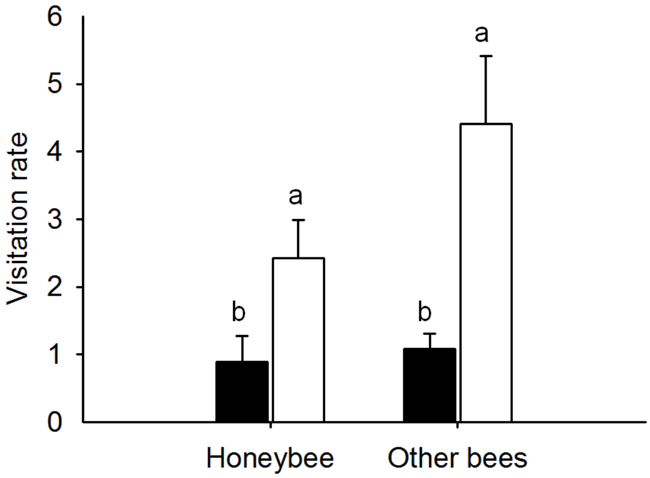

Figure 6. Mean + SE visitation rate (i.e. visits/transect/10') of the honeybee and the other bees in shrublands adjacent (black) and distant (bold) to *Hedysarum* crops. Significant differences within pollinator group are represented by different letters above bars.

Discussion

No temporal bee spill-over from MFCs to adjacent shrublands

Contrary to what we expected, we did not observe a temporal spill-over of bees from the MFC to adjacent areas after mass flowering. During mass flowering, the floral offer of MFCs seems to cover all the requirements of the honeybee so that the crops monopolize their visits. Then, after mass flowering, due to the capacity of honeybees to locate high rewarding resources at great distances (Cresswell & Osborne 2004), individuals might move to other still unharvested MFCs or to other highly rewarding natural areas within their foraging ranges, such as old-fields with high floral diversity. Therefore, the temporal spill-over effect in patchy and heterogeneous agricultural landscapes might be spatially diluted.

A temporal spill-over of other bees in adjacent areas was also not observed. We did not expect the other bee species to be strongly attracted to *Hedysarum* crops as prior studies have shown that most *Hedysarum* visits in cultivated and naturalized populations are made by the honeybee (**Chapter 1**; Satta *et al.*, 2000).The pollinator survey conducted in 2010 inside *Hedysarum* crops also supports this observation, as the other bee species represented only 3.87% of the visitors.

The predominance of the honeybee in MFCs is not exclusive to *Hedysarum* crops. In many parts of the world crop pollination relies on this single species (Winfree *et al.* 2007). Thus, as in our case study, other bee species may also not be very abundant in MFCs, so that an overall temporal spill-over would not be observed, even if spill-over of particular species occurred. In our study system, this was the case for *Megachile pilidens* and *Osmia caerulescens*, which are pollinators of *Hedysarum* crops (according to 2010 data) that were only observed in adjacent shrublands after mass flowering. However, due to their low abundance compared to the honeybee, we did not observe an overall significant spill-over of other bee species from *Hedysarum* crops to adjacent shrublands. In addition, similar to the honeybee, if the other bee species able to access the restrictive flowers of *Hedysarum* are medium to large-sized ones (Córdoba & Cocucci, 2011), and therefore with medium-large foraging ranges (Greenleaf *et al.* 2007), their potential temporal spill-over could also be spatially diluted. Nonetheless, we would expect the spatial dilution of other bees to occur at shorter distances than in the case of the honeybee. Maximum foraging distances from nesting sites for wild bees, which are mostly solitary central place foragers, fall below the ones described for honeybees (Gathmann & Tscharntke 2002; Steffan-Dewenter & Kuhn 2003).

When analyzing each target plant species separately, we reach a similar conclusion. The lack of an overall temporal spill-over is due to both non-significant trends in most target plant species and opposite signs in the few species with significant trends (Fig. A3.1).

Different spatial spill-over of the honeybee *compared to other bee species*

The honeybee preferentially selected *Hedysarum* crops, as its abundance was one order of magnitude higher inside than outside crops. Pollinators, when foraging, seek to optimise their floral rewards intake (Armbruster & Herzig 1984), and thus their success depends on the relative abundance and quality of available floral resources

(Dietzsch *et al.* 2011), both of which are usually high in MFCs. Furthermore, honeybees have an intensive foraging behavior with short flying distances between consecutive visits (Gross 2001) and might benefit greatly from MFCs.

The spill-over of the honeybee from shrublands to MFCs seems to occur not only from adjacent ones, but also from more distant ones, as even in distant shrublands the abundance of the honeybee was much lower than inside crops. Thus, shrublands without MFCs in their surrounding 500 m radius landscape might also be sources of honeybees attracted to MFCs. The large foraging range of the honeybee (Greenleaf *et al.* 2007; Osborne *et al.* 2008), together with its communication skills (Steffan-Dewenter & Kuhn 2003), allows the honeybee to perceive and exploit landscapes with more than a 500 m radius even if they are patchy and heterogeneous.

The pool of other bee species did not highly select the MFC; however, they did not avoid *Hedysarum* either, as indicated by the similar abundances inside crops and in adjacent shrublands. The lack of a general tendency in the abundance of other bee species between the MFC and adjacent shrublands, might be due to the contrasting responses of single species. However, due to the low abundance of other bee species apart from the honeybee, we could not conduct analyses for particular species separately to elucidate specific responses.

When pooling the bee communities of the MFC and adjacent shrublands, the Sørensen similarity index with distant shrublands was relatively high (QS = 0.62). Thus, on the one hand, our results suggest that the other bee species that are attracted to MFCs spill-over from adjacent areas. Most wild bees are central placed foragers (Cresswell, Osborne & Goulson 2000) and due to their more restrictive foraging ranges and communication skills compared to honeybees, their spill-over occurs at smaller spatial scales.

On the other hand, we suggest three potential non-exclusive explanations for the limited selection and exploitation of MFCs by other bee species. First, flower constancy at the individual level might be more highly associated with social pollinators like the honeybee (Leonhardt & Blüthgen 2012) than with solitary bees. Therefore, monospecific areas like MFCs do not fulfill the individual requirements of solitary bees. Second, other bee species could be excluded from MFCs due to competition with the honeybee (Paini 2004; Roubik 1983). Interspecific competition, which depends on the relative abundance of interacting species (Steffan-Dewenter & Tscharntke 2000), could arise with the high abundance of honeybees inside crops. And third, other factors co-varying with the presence of MFCs (for instance, some agricultural practices like the use of pesticides), could lead to the avoidance of MFCs by bee species.

In addition, some bee species not only avoid MFCs, but also seem to prefer landscapes without them. In our study system, this was the case for *E. oraniensis*, whose relative abundance was twofold in distant shrublands, compared to adjacent ones. In general, medium to large-sized bees (Greenleaf *et al.* 2007), regardless of the reason for their not exploiting MFCs, are able to perceive their landscapes at larger spatial scales (Steffan-Dewenter *et al.* 2002). Therefore, these bees can chose landscapes without MFCs and but with larger concentrations of their required resources (e.g. nesting sites, food) (Tscharntke *et al.* 2012).

Conclusions and implications for plant reproduction

In the studied agricultural landscape on Menorca, the presence of *Hedysarum* MFCs decreased pollinator abundance in adjacent shrublands by monopolizing the visits of the honeybee, and by sharing the visits of the other bee species. The proposed role of MFCs as supporters and sources of pollinators for surrounding natural areas, therefore, has to be reconsidered, at least in the case of wild bees in patchy and heterogeneous agricultural landscapes such the one studied here.

Our approach allowed us to detect a spatial bee spill-over, not from the MFC to adjacent natural habitats, but rather the reverse. The decrease in pollinator abundance in adjacent shrublands can subsequently affect the reproductive success of wild plants. *Hedysarum* crops are part of a cyclical agro-farming system (Bustamante *et al.* 2007) and are grown a maximum of two consecutive years in the same field so that the MFC effects on pollinator communities in adjacent areas could be buffered in the long term. It would therefore be interesting to study if the observed pattern is maintained in the long-term or if *Hedysarum* crops support and enhance the abundance of generalist pollinators and provide a benefit through greater pollinator service overall (Mitchell *et al.* 2009; Holzschuh *et al.* 2011).

Furthermore, *Hedysarum* has restrictive flower morphology so that competition for pollinators with natural areas is only mediated by bees. However, other MFCs with non-restrictive flower morphologies, such as sunflower (*Helianthus annuus*) and oilseed rape (*Brassica napus*), might compete with wild plants for a broader array of pollinators including bees, flies and beetles (Bommarco, Marini & Vaissière 2012).

Acknowledgements

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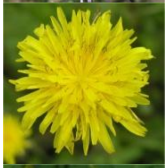
Appendix 1. Focal study plants

Table A1.1. Plant species studied in focal censuses in 2009 with indication of the site where they were sampled.

Species		Family	Site
	*Asphodelus aestivus*	Liliaceae	Mila2
	*Cistus albidus*	Cistaceae	Binixabó
	*Daucus carota*	Umbelliferae	Binicalaf and Mila2
	*Galactites tomentosa*	Compositae	Binicalaf and Mila1
	*Hypochoeris achyrophorus*	Compositae	Binicalaf
	*Oxalis pes-caprae*	Oxalidaceae	Mila1
	*Urospermum dalechampii*	Compositae	Binixabó

Appendix 2. Rarefaction curves for 2010 field sampling

Rarefaction curves for plant-bee interaction richness for each study site in 2010. Curves were calculated and plotted within the *bipartite* library in R (R Development Core Team 2001). Curves are based on 100 randomizations without replacement and boxplots are represented for each level of randomization. Shaded areas represent 95% confidence intervals. Dashed lines enclose the number of transect walks needed to observe a new plant-bee interaction in study shrublands.

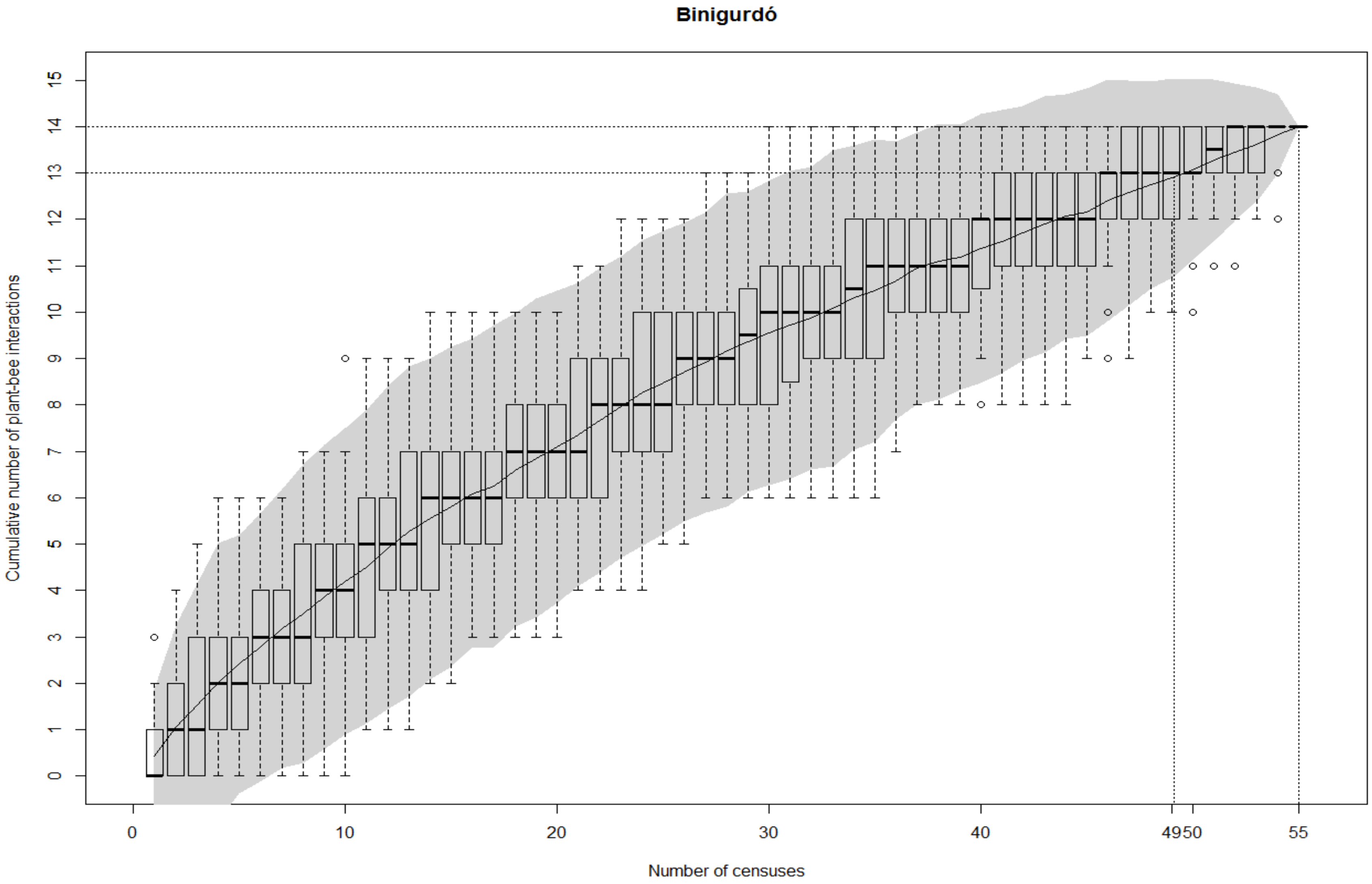


Figure A2.1. Rarefaction curve in site *Binigurdó*..

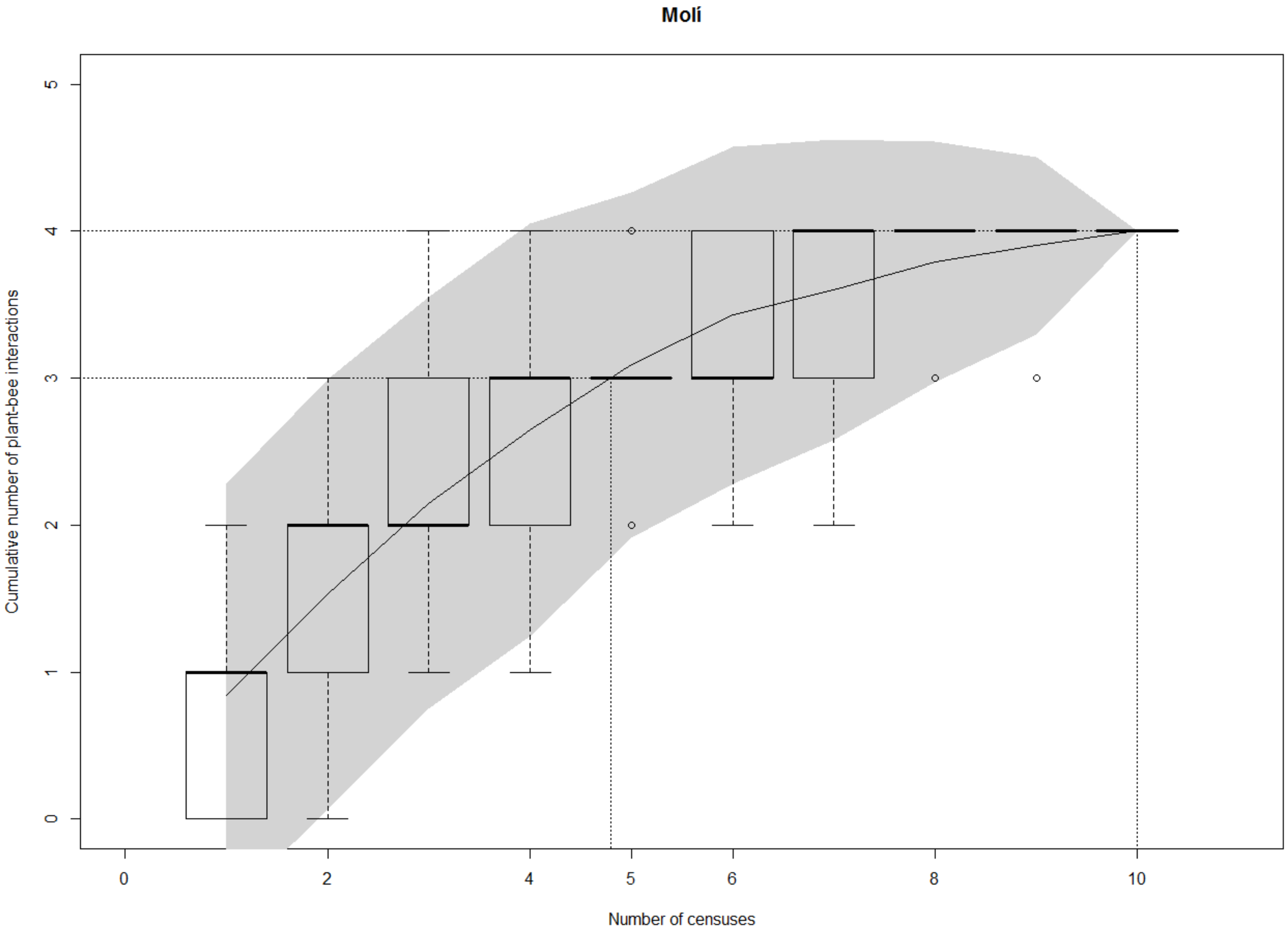


Figure A2.2. Rarefaction curve in site *Molí.*

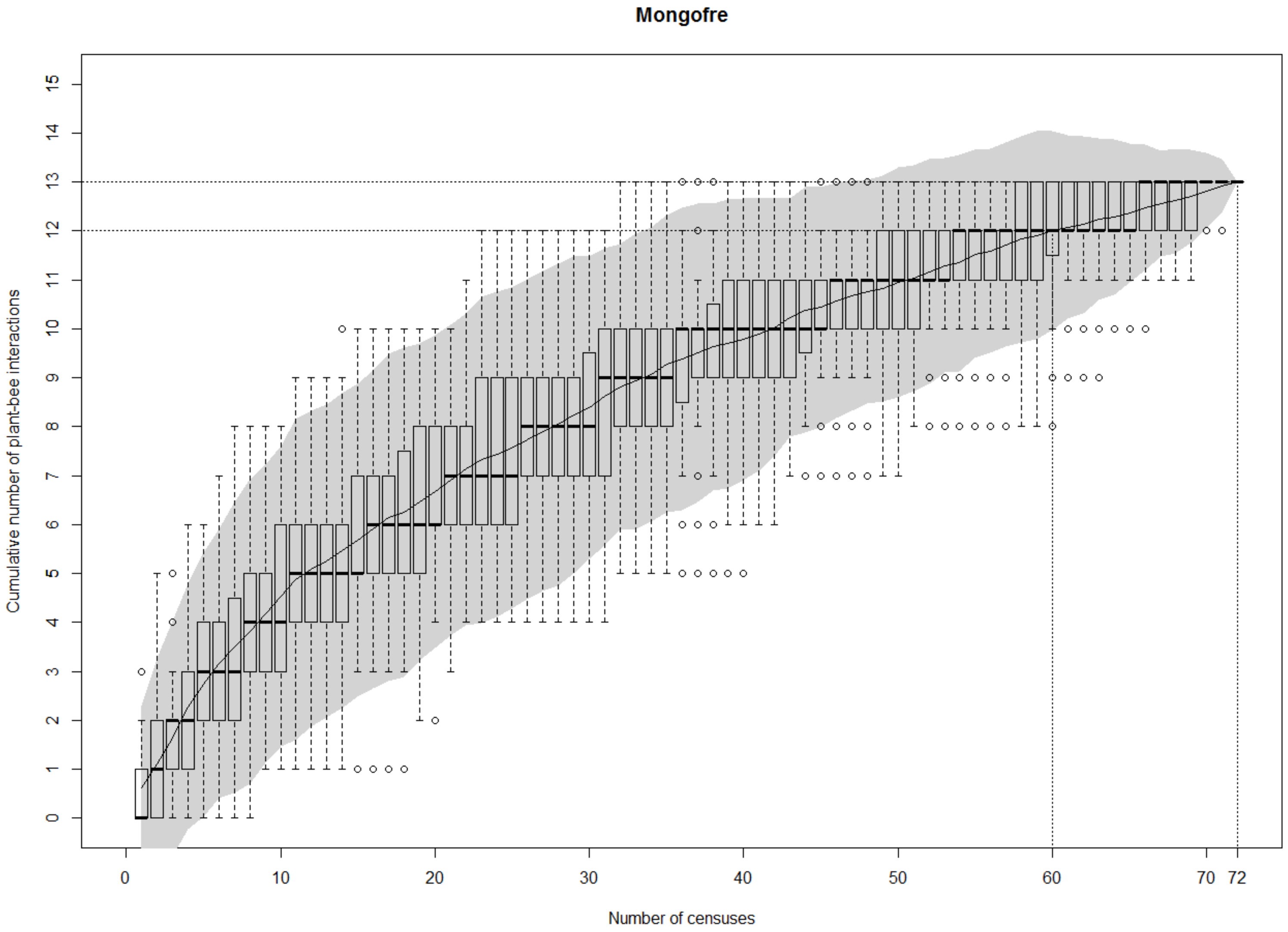


Figure A2.3. Rarefaction curve in site *Mongofre.*

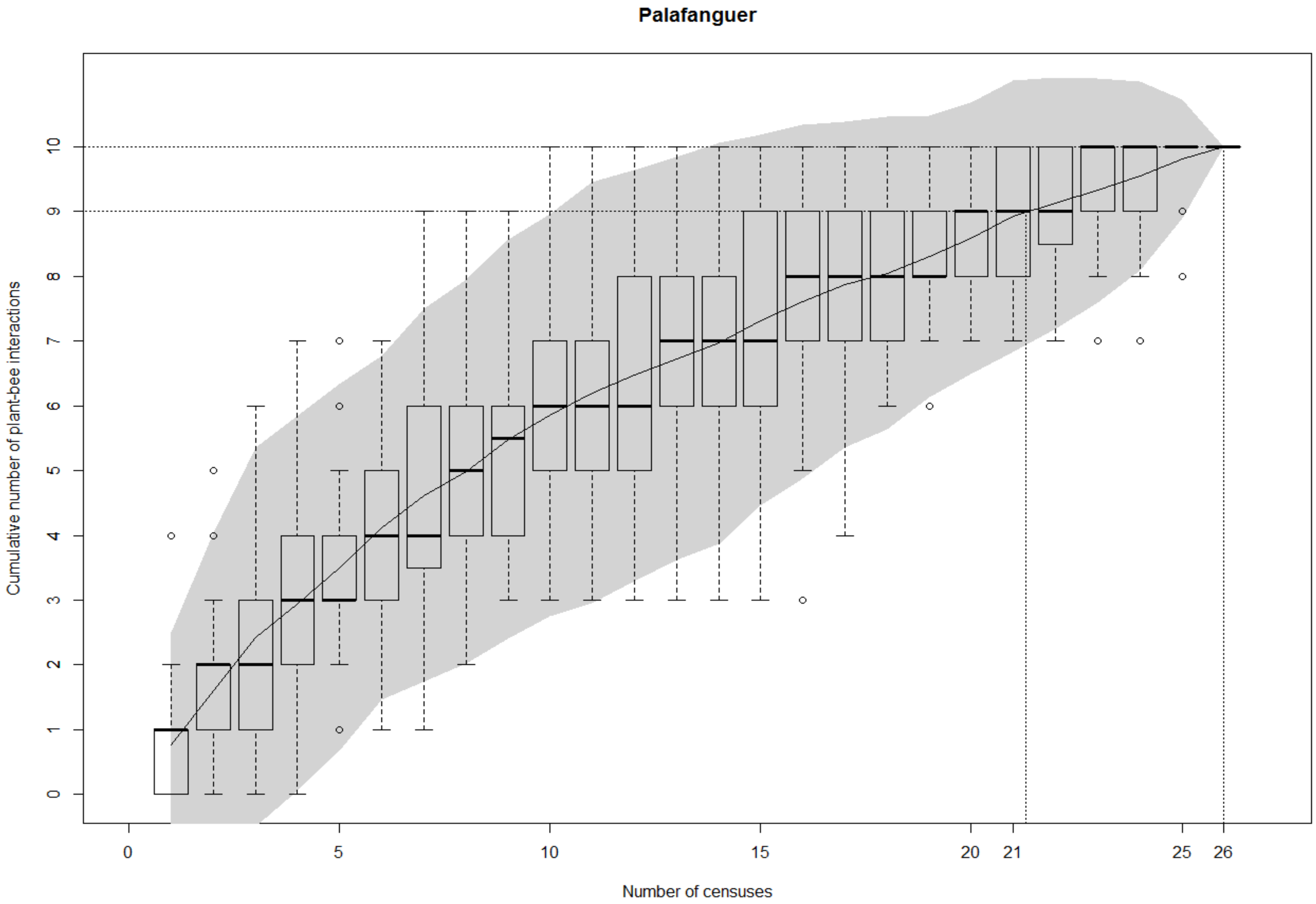


Figure A2.4. Rarefaction curve in site *Palafanguer.*

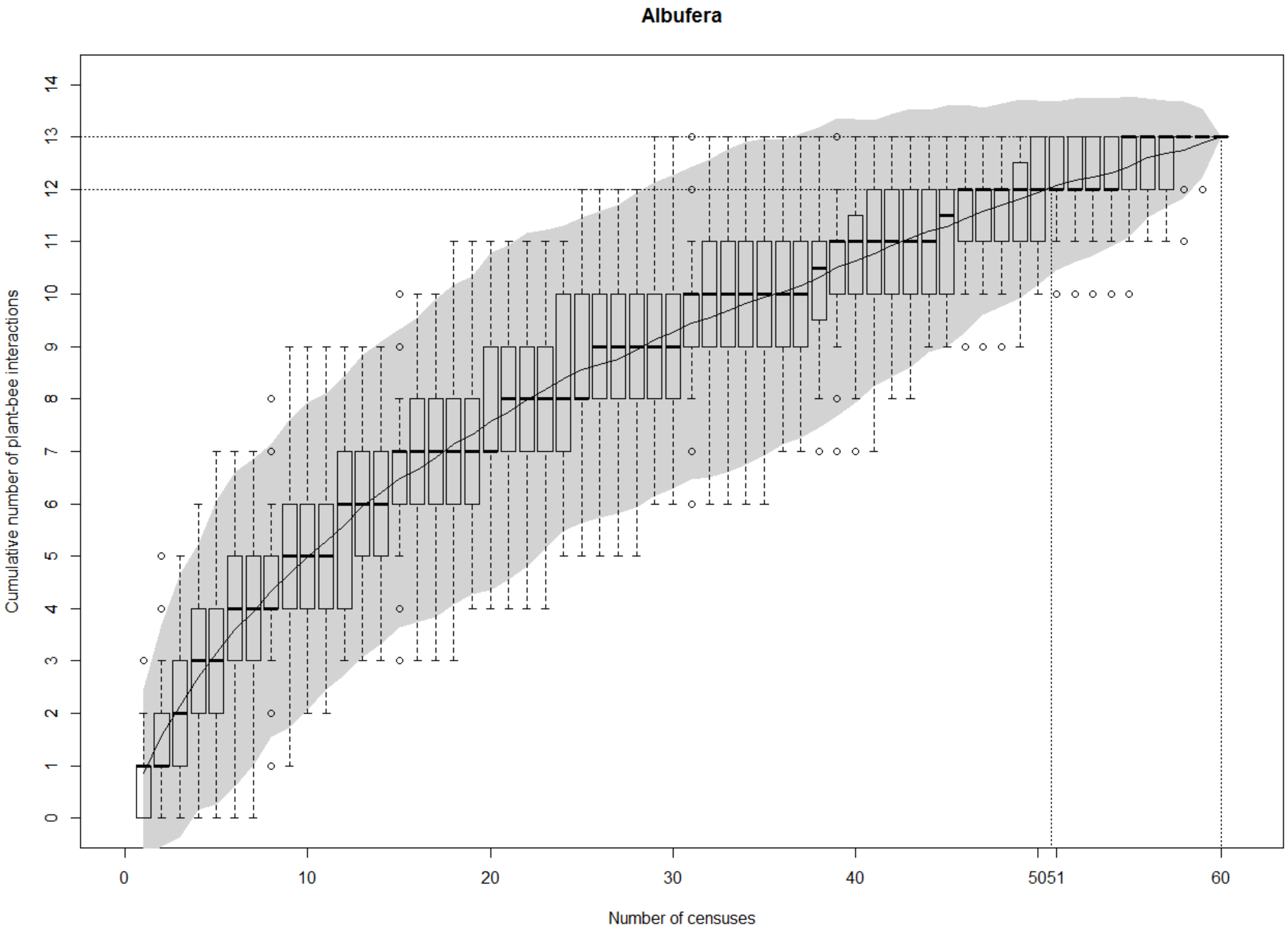


Figure A2.5. Rarefaction curve in site *Albufera.*

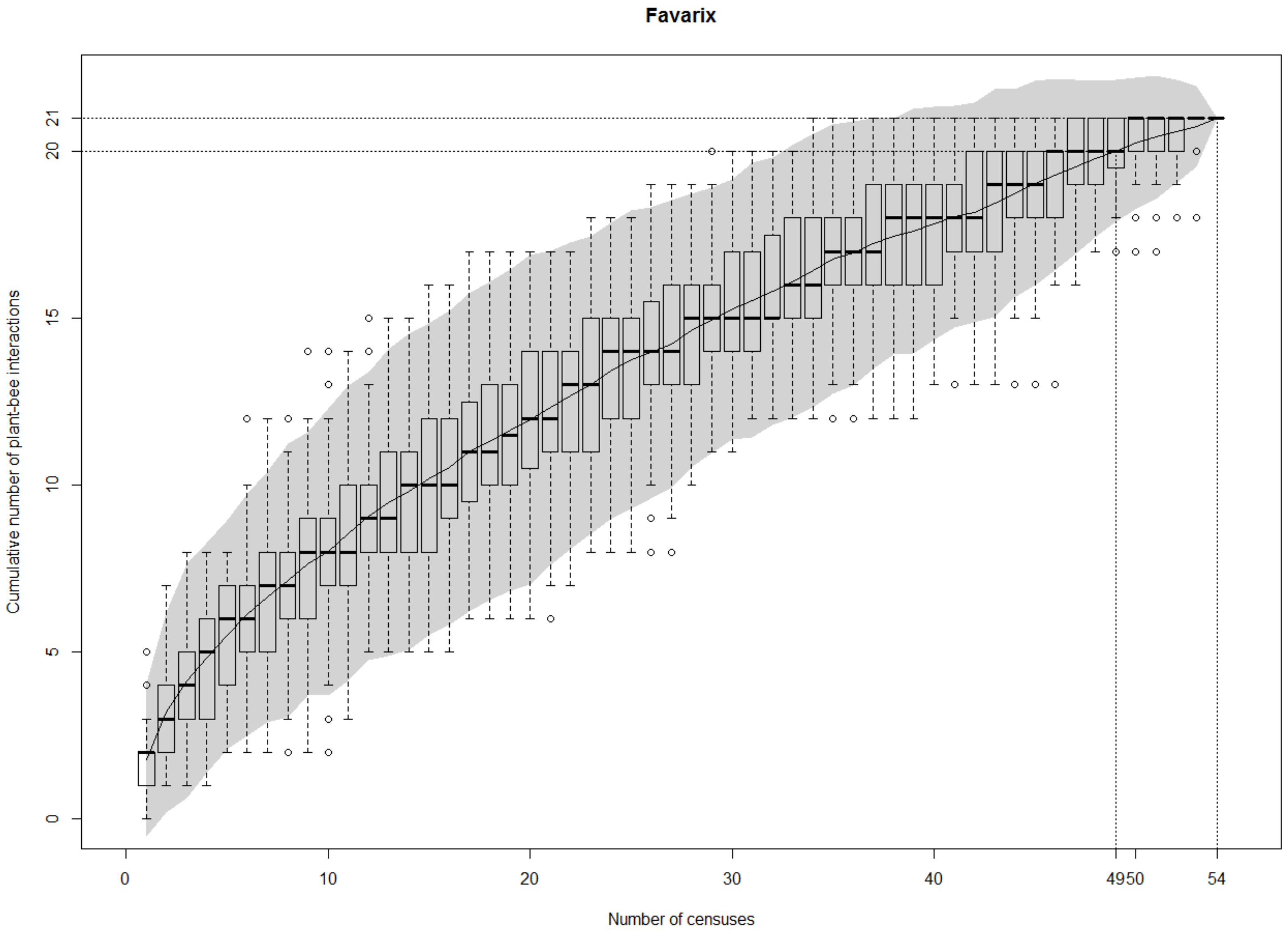


Figure A2.6. Rarefaction curve in site *Favarix.*

Appendix 3. Temporal spill-over per species and site in 2009

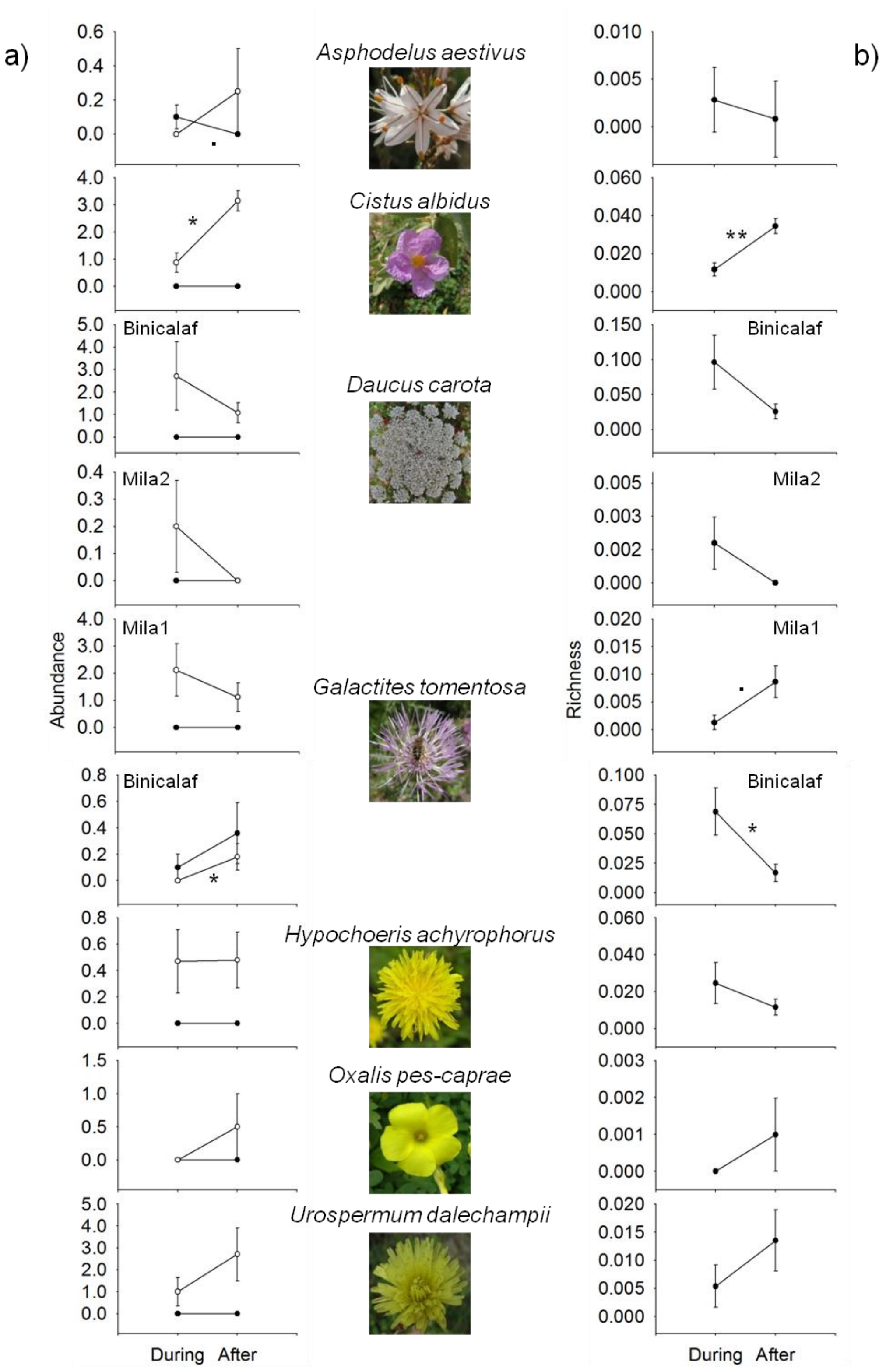


Figure A3.1. Mean ± SE (a) abundance (individuals/flower/hour) of the honeybee (black dots) and the other bee species (bold dots) and (b) bee pollinator richness (species/flower/hour) in 2009 during and after mass-flowering in each target plant species and shrubland adjacent to *Hedysarum* crops. The name of the study site is given for those target plant species sampled in more than one site. Significance levels: ˙ $p \approx 0.05$, * $p < 0.05$.

Appendix 4. Statistical analyses

Table A4.1. Output of the generalized mixed model to test differences in pollinator abundance among *Hedysarum* crops (inside MFC), adjacent and distant shrublands in 2010. Analysis was conducted in R with the library *glmmADMB.*

Formula	glmmadmb(abundance+1~ treatment*functional group+(1|site), data=data2010, family="Gamma", link="log", zeroInflation=F)
AIC	347.5
N	146

	Estimate	SE	Z	P	
Intercept	0.230	0.080	2.89	0.004	**
Treatment Distant	0.366	0.139	2.64	0.008	**
Treatment Inside MFC	2.353	0.102	23.10	<2e-16	***
Functional group Other bees	0.253	0.087	2.91	0.004	**
Treatment Distant : Functional group Other bees	0.044	0.162	0.27	0.788	
Treatment inside MFC : Functional group Other bees	-2.482	0.143	-17.32	<2e-16	***

Random effect variance: Site	Variance	SD
Intercept	0.009	0.095

Gamma shape parameter	7.373 ± 0.869 (mean ± SE)
Log-likelihood	-165.75

Table A4.2. Output of the generalized mixed model to test differences in pollinator abundance among *Hedysarum* crops (inside MFC), adjacent and distant shrublands excluding the sites *Molí* and *Binigurdó*. Analysis was conducted in R with the library *glmmADMB.*

Formula	glmmadmb(abundance+1~ treatment*functional group+(1|site), data=data2010, family="Gamma", link="log", zeroInflation=FALSE)
AIC	233.6
N	92

	Estimate	SE	Z	P	
Intercept	0.285	0.092	3.08	0.002	**
Treatment Distant	0.296	0.135	2.20	0.028	*
Treatment Inside MFC	2.147	0.146	14.72	<2e-16	***
Functional group Other bees	0.221	0.131	1.69	0.090	.
Treatment Distant : Functional group Other bees	0.122	0.190	0.64	0.520	
Treatment inside MFC : Functional group Other bees	-2.486	0.206	-12.05	<2e-16	***

Random effect variance: Site	Variance	SD
Intercept	5.68E-08	2.42E-04

Gamma shape parameter	6.539 ± 0.940 (mean ± SE)
Log-likelihood	-108.82

Capítulo 5

Impact of landscape alteration and invasions on pollinators: A meta-analysis

Resumen

La alteración del hábitat y las invasiones biológicas son dos de los principales componentes del Cambio Global que amenazan a la biodiversidad. Existe una gran preocupación acerca de su impacto en los polinizadores y el servicio de polinización que realizan. Sin embargo, la abundante bibliografía al respecto muestra resultados diferentes e incluso contradictorios.

Presentamos un meta-análisis global de 58 publicaciones con 143 casos de estudio (37 sobre alteración del hábitat y 21 sobre invasiones biológicas) para evaluar cuál es el efecto de estos componentes del Cambio Global en los polinizadores y si existen diferencias taxonómicas o en función del tipo de ecosistema en la respuesta de los polinizadores. También se cuantificó qué componente de la alteración del hábitat tiene un efecto mayor sobre los polinizadores y se evaluó si la respuesta de los polinizadores nativos difiere en función de si las invasiones son por parte de animales o por parte de plantas.

La alteración del hábitat y las invasiones afectaron a los polinizadores disminuyendo sus tasas de visitas en igual magnitud. Los vertebrados en hábitats alterados y los insectos (excluyendo las abejas) en hábitats invadidos fueron los taxones de polinizadores más afectados. Su abundancia se redujo más en ecosistemas forestales alterados que en praderas alteradas, mientras que se encontró el patrón opuesto para su riqueza. Sin embargo, la respuesta de los polinizadores a las invasiones fue independiente del tipo de ecosistema invadido. La perturbación de la matriz circundante mostró tener un efecto mayor en la reducción de las tasas de visitas de polinizadores que la reducción del tamaño del parche.

Los animales invasores mostraron un efecto negativo en las tasas de visitas de los polinizadores más consistente que el de las plantas invasoras.

Nuestro estudio subraya que estos componentes de Cambio Global tienen efectos negativos similares en los patrones de polinización, pero que las respuestas de los polinizadores varían entre taxones y ecosistemas, así como en función del componente de alteración del hábitat considerado y de si el organismo invasor es animal o planta.

Abstract

Alterations in land use and biological invasions are two major components of Global Change that threaten biodiversity. There is high concern about their impact on pollinators and the pollination services they provide. However, the growing literature shows different, even contradictory results.

We present a global meta-analysis of 58 publications reporting 143 studies (37 on landscape alteration and 21 on biological invasions) to assess the extent to which these components affect pollinators, and whether taxonomic and ecosystem-type differences in pollinator responses occur. We also quantified which component of landscape alteration had the largest effect on pollinators, and assessed whether animal invasions differ from plant invasions in their effect on native pollinators.

Habitat alteration and invasions affected pollinators to the same magnitude by decreasing visitation rates. Vertebrates in altered landscapes and insects in invaded areas (excluding bees), were the most affected pollinator taxa. Pollinator abundance was more reduced in altered forest ecosystems than in altered grasslands; while the reverse pattern was found for pollinator richness. However, the response of pollinators to invasions was independent of ecosystem type. Disturbance of the surrounding matrix was more important in decreasing pollinator visitation rates than fragment size.

Invasive animals seemed to have a more consistent negative effect on visitation rates than invasive plants.

Our study highlights that different components of Global Change have similar negative outcomes on pollination patterns, but that responses of pollinators vary among taxa and ecosystem types, as well as the attributes of landscape alteration considered and whether the invader is an animal or a plant.

Introduction

Among the different components of Global Change, landscape alteration and biological invasions are, in certain regions, the major causes of the biodiversity crisis (Wilcove *et al.* 1998; Satta *et al.* 2000) (Fig. 1). These components of Global Change decrease species diversity and disrupt biotic interactions (Tylianakis *et al.* 2008). For example, there is high concern regarding the impact these factors pose on pollinators and on the ecosystem service they provide (Buchmann & Nabhan 1996; Biesmeijer *et al.* 2006; Goulson, Lye & Darvill 2008; Carvalheiro *et al.* 2010; Potts *et al.* 2010). Changes in pollinator abundance, richness and behaviour might also have important consequences for the persistence of many flowering plants. Approximately 300000 plant species around the world rely on pollinators for their sexual reproduction (Kearns *et al.* 1998; Ollerton *et al.* 2011), including different crops that as a whole constitute 35% of global food production (Klein *et al.* 2007).

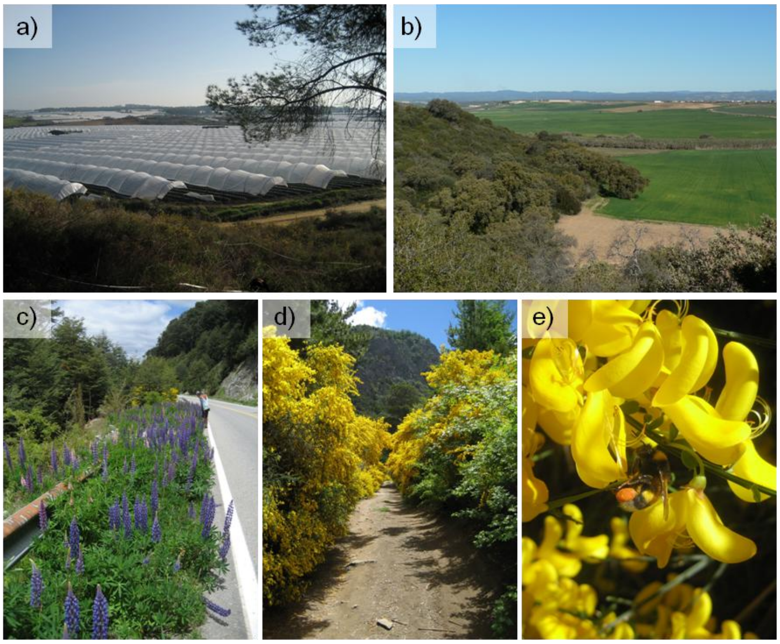


Figure 1. Habitat alteration due to agricultural practices in southern Iberian Peninsula (a) and (b). *Lupinus polyphylus* (c) and *Cytisus scoparius* (d) invading roadsides and trails in northen Argentine Patagonia. Invasion liaision between the pollinator *Bombus terrestris* and the plant *Cytisus scoparius*, both invaders in northen Argentine Patagonia (d).

Landscape alteration implies habitat fragmentation, habitat loss (i.e. reduction of the total amount of the habitat), habitat isolation and modification of the structure of the landscape. Landscape alteration can affect pollinators in several different ways. First, it can change the availability of food resources. The response of pollinators to the reduction and isolation of their food resources depends on their diet breadth, foraging range, longevity and migration capability (Rathcke & Jules 1993; Winfree *et al.* 2011). Second, the availability of nesting sites and larval host plants (Cane 2001; Winfree *et al.* 2011) can be modified. Finally, the response of pollinators to landscape alteration might be influenced indirectly by changes in the presence, abundance or behaviour of parasites and predators (Rathcke & Jules 1993).

Non-native species can become invasive and cause impacts on native species, communities and ecosystem processes (Levine *et al.* 2003). Their presence can affect pollinators in different ways depending on the type of non-native organism, which can be another pollinator, an herbivore, a pollinator’s predator or parasite, or an animal-pollinated plant (Traveset & Richardson 2006). For example, an invasive plant species can increase the floral resources offered and be included in the diet of generalist pollinators (Memmott & Waser 2002; Vilà *et al.* 2009). In contrast, an invasive pollinator can compete for the same resources as the native pollinators to the detriment of the latter (Bjerknes *et al.* 2007).

Therefore, studies that address the effects of landscape alteration and invasions on pollinators show mixed and even contradictory results. Moreover, these Global Change components are not independent and their effects can be additive, synergic or even antagonist (Sala *et al.* 2000; Didham *et al.* 2005, 2007). The growing literature addressing their effect on pollination suggests it is an opportune time to synthesize the available information to establish whether there is a clear pattern of global pollination decline. Meta-analysis techniques provide a quantitative tool for conducting such a synthesis (Rosenberg, Adams & Gurevitch 2000). Some recent reviews have already relied on meta-analysis to evaluate whether the effect of fragmentation on the pollination and reproduction of plants differs depending on plant reproductive traits (Aguilar *et al.* 2006); the effect of non-native plants on pollination and seed set of neighbouring native plants (Morales & Traveset 2009); and the influence of landscape disturbances on bees (Winfree *et al.* 2009). Apart from the latter, most effort has focused on the impact on plants and not on pollinators. Moreover, Winfree *et al.* (2009) explored only bees, and while they are the most studied pollinators, they are not the only ones. Other insect taxa and vertebrates, such as birds, bats, small mammals and even lizards, are reported to be efficient pollinators as well (Olesen & Valido 2003; Quesada *et al.* 2003; Rodríguez-Rodríguez & Valido 2008).

A review of the overall effect of the different components of landscape alteration and biological invasions from the pollinators' perspective is lacking. In this study we address the following questions: 1) Do landscape alteration and biological invasions affect pollinators? 2) Are there taxonomic differences in pollinator responses to these Global Change components? 3) Do the effects differ among ecosystem types? 4) Do results differ between observational and experimental studies? 5) Which component of landscape alteration affects pollinators the most? 6) Do animal invasions differ from plant invasions in their effect on native pollinators?

Materials and Methods

Literature search and data extraction

To survey the published literature on the effect of landscape alteration and non-native species on pollinators we conducted a search using the ISI Web of Science database up to August 2010 using the keyword combinations (pollinat* AND invas*), (pollinat* AND alien), (pollinat* AND fragm*) and (pollinat* AND habitat loss). In the literature it is common to find the term "habitat fragmentation" referring to the wider concept of landscape alteration instead of exclusively the breaking apart of the habitat (Fahrig 2003; Holzschuh, Steffan-Dewenter & Tscharntke 2010). Therefore, many of the matches for the keyword combination (pollinat* AND fragm*) referred to other landscape alteration components and not habitat fragmentation *per se*. We also screened the reference lists from all retrieved papers for other relevant publications. Only field observational or experimental studies were considered, excluding predictive modelling and anecdotal observations. As response variables we considered pollinator abundance, pollinator species richness and visitation rates to flowers, regardless of whether they referred to the whole pollinator community or to specific pollinator taxa.

For studies on landscape alteration, we classified them depending on the landscape characteristic explored: fragment and/or plant population size (small vs. large) and nature of the surrounding matrix (disturbed vs. non-disturbed or continuous). In studies on biological invasions, we distinguished whether the invader was a plant or an animal. Only the presence/absence of the invasive species was considered. Therefore, the independent variables were categorical with two levels: control and treatment groups. For studies on landscape alteration, control groups were those with the largest fragments, largest population sizes, closest to other fragments of the same cover type or to continuous habitats and fragments with the least disturbed surrounding matrices. For studies on invasions, control groups were those with the absence of the invasive species.

In total, we retrieved 207 publications for which the following criteria for data inclusion were adopted:

(1) When the effect of invasion or landscape alteration was measured on the same pollinator taxa or community, but in different and independent sites, we included all of them as independent records. When multiple taxa were simultaneously studied, we included all in the dataset. An exception to the latter was when the same response variable was measured simultaneously for both the whole pollinator community and specific taxa, in which case the values of the whole community were discarded as they were not independent of those of the specific taxa. A possible criticism of this criterion is the inclusion of pseudo-replicated entries in the dataset. This is a problem that meta-analysts continuously face and in this work we aimed at reaching a compromise between avoiding pseudo-replication and not sacrificing too much information, as has been done in previous meta-analyses (Liao *et al.* 2008; Rey-Benayas, Galvan & Carrascal 2010; Vilà *et al.* 2011). We considered that different taxonomic pollinator groups might not necessarily respond similarly to landscape alteration (Klein *et al.* 2002; Brosi *et al.* 2008; Tscheulin *et al.* 2011), or to invasion (Bartomeus *et al.* 2008b), and that the response of one single taxonomic group to these components (Jennersten

& Nilsson 1993), or the impact of a single invasive species (see Lopezaraiza-Mikel *et al.* 2007; Nienhuis, Dietzsch & Stout 2009) might be context dependent and therefore not necessarily the same in different sites. Moreover, two of the questions that we address in this work are whether there are taxonomic differences in the response to these Global Change components, and whether the responses differ depending on the type of ecosystem altered or invaded, which justifies the inclusion of this kind of entry.

(2) In studies on invasions, we only included those in which the response variables were measured on native pollinator communities (i.e. excluding pollinators visiting the non-native plant species).

(3) When a study was repeated over several years, we took the mean value of the response variable for the whole study period. However, if results were given for each year separately, we only included the data for one year in order to avoid pseudo-replication, despite the high temporal variability described for pollinator communities (Alarcón *et al.* 2008; Olesen *et al.* 2008; Petanidou *et al.* 2008). We considered that data gathering would improve and be more accurate with increasing sampling experience. Therefore, unless authors explicitly indicated otherwise, we chose the last year for analysis. If the study was repeated at different time periods separately, we included only the results for the time range with the highest value of the response variable in the control group. Such a time range would be the closest to pollinators' activity optimum and potential differences with treatment groups would be easier to detect.

(4) When a study considered the independent variable as a gradient instead of a two-level factor, and replicates of each level existed, only the lowest and highest extreme levels of the gradient were considered. On the other hand, for gradients with numerous and un-replicated levels, we averaged the response variable values for the lowest and highest levels of the gradient.

(5) In some studies of landscape alteration the two independent variables were simultaneously tested (i.e. fragment size and matrix disturbance). We included only the effect of one of the variables keeping constant the other one, and choosing the one with fewer entries in the dataset to equilibrate the number of entries per component of landscape alteration.

In total, 58 publications (37 on landscape alteration and 21 on biological invasions) met our primary criteria (see Appendix 1). Our dataset had 143 entries, 87 on landscape alteration and 56 on invasions (45 on plant invasions and 11 on animal invasions). Sixty refer to impacts on the whole pollinator community, while the remainder (83) refer to specific taxonomic groups, mostly insects, but also birds and bats. In order to have enough entries of each pollinator taxonomic group, we grouped them as: bees (32), bumblebees (16), other insects (23) and vertebrates (12). For the same reason, ecosystem types were also grouped into three general types: forests (67), shrublands (23) and grasslands (53). Regarding the type of study, 46 were experimental and 97 observational.

Only one of the 58 publications (Bartomeus, Vila & Steffan-Dewenter 2010, see Appendix 1) simultaneously studied the effect of both Global Change components and their interaction. Therefore, we could not explore how these Global Change components act together as we did not have enough entries to include the interaction in the analysis.

Meta-analysis

For each response variable we recorded sample size (N), mean and standard deviation (SD) for the control and experimental groups. If standard error (SE) or 95% confidence intervals (CI) were given, we transformed them to SD. When only figures were given, the software DATATHIEF III (Tummers 2006) was used to extract these parameters from the graphs. When necessary, we asked authors for the missing data. In a couple of papers, the exact N values were not given, but rather ranges of values. In those cases, we opted for the more conservative solution and chose the lowest N values.

For each entry of the dataset, we calculated *Hedges'd* as a measure of effect size. *Hedges'd* is an estimate of the standardized mean difference between control and experimental groups that is not biased by small sample sizes and unequal sampling variances (Rosenberg *et al.* 2000).

Hedges'd is a unit-free index which ranges from - ∞ to + ∞ and estimates the magnitude of the effect and its direction. As in classical statistical analysis, the highest effect sizes are from those studies showing large differences between control and treatment groups. Zero *d* values signify no difference in the response variable between non-altered and altered plots or between non-invaded and invaded plots. Positive and negative *d* values denote a general trend following landscape alteration or invasion for an increase or a decrease, respectively. *Hedges'd* calculations and statistical analysis were conducted with the MetaWin v2.1 Software (Rosenberg *et al.* 2000).

We first tested whether effect sizes across studies were homogeneous, using the Q_{total} statistic. A significant Q_{total} indicates that the variance among effect sizes is greater than that expected by sampling error alone (i.e. effect sizes are not equal across studies). On the other hand, a non-significant Q_{total} does not preclude the possibility of heterogeneity among studies. In order to assess the effect of different grouping variables (invasion versus landscape alteration, differences between ecosystem types, pollinator taxonomic groups, etc.), we developed categorical random-effects meta-analysis. These models, unlike fixed-effects models, incorporate a random component in the effect size variation apart from the sampling error; i.e. they do not assume a real effect size shared by all studies, which is not likely to be satisfied in ecological studies (Gurevitch & Hedges 1999). For each grouping category, a cumulative effect size (d_{++}) and a 95% confidence interval were calculated. A cumulative effect size is considered significant when its 95% confidence interval does not overlap zero. Confidence intervals were calculated using bias-corrected bootstrap resampling procedures with 3000 iterations from the effect sizes and their non-parametric variances according to Adams, Gurevitch & Rosenberg (1997) for groups of 10 or more entries. For groups with small sample sizes (less than 10 entries), bootstrap procedures were not used because they are biased due to resampling from the same small set of values (Bancroft, Baker & Blaustein 2007), and the more conservative parametric 95% confidence interval were used.

For categorical comparisons we examined the p_{random} values associated with the $Q_{between}$ statistic, which describes the variation in effect sizes that can be attributed to differences between categories. We also tested whether the remaining within-group heterogeneity (Q_{within}) was significant using a chi-square test (Rosenberg *et al.* 2000).

Non-independence of effect sizes and publication bias, two of the more important and frequent problems that arise when doing a meta-analysis (Gurevitch & Hedges 1999), were tested by MetaWin v2.1 Software (Rosenberg *et al.* 2000) (see detailed analysis and results in Appendix 2).

Results

Pollinator abundance was negatively affected by landscape alteration as indicated by d_{++} = -0.95, and a CI of mean effect sizes that did not overlap zero. A similar trend was not observed for invasions as CI overlapped zero. Neither landscape alteration nor invasions had a significant effect on pollinator richness. In contrast, both factors reduced significantly visitation rates (d_{++} = -0.61 and -0.62 for landscape alteration and invasions, respectively) (Fig. 2).

The overall effect of landscape alteration was not significantly different from the effect of biological invasions on pollinators' abundance ($Q_{between}$ = 2.04, p_{random} = 0.19), pollinators' richness ($Q_{between}$ = 0.83, p_{random} = 0.56) or visitation rates ($Q_{between}$ = 0.00, p_{random} = 0.98). This lack of difference is possibly a result of considerable variability in the effect sizes among studies (Table 1).

Table 1. Q_{total} and associated *P* values on the effect of landscape alteration and invasions to pollinator abundance, richness and visitation rates.

Global Change component	**Abundance**		**Richness**		**Visitation rates**	
	Q_{total}	**P**	Q_{total}	**P**	Q_{total}	**P**
Landscape alteration	15.07	0.82	29.24	0.01	41.06	0.78
Invasions	15.91	0.14	6.40	0.38	62.59	<0.01

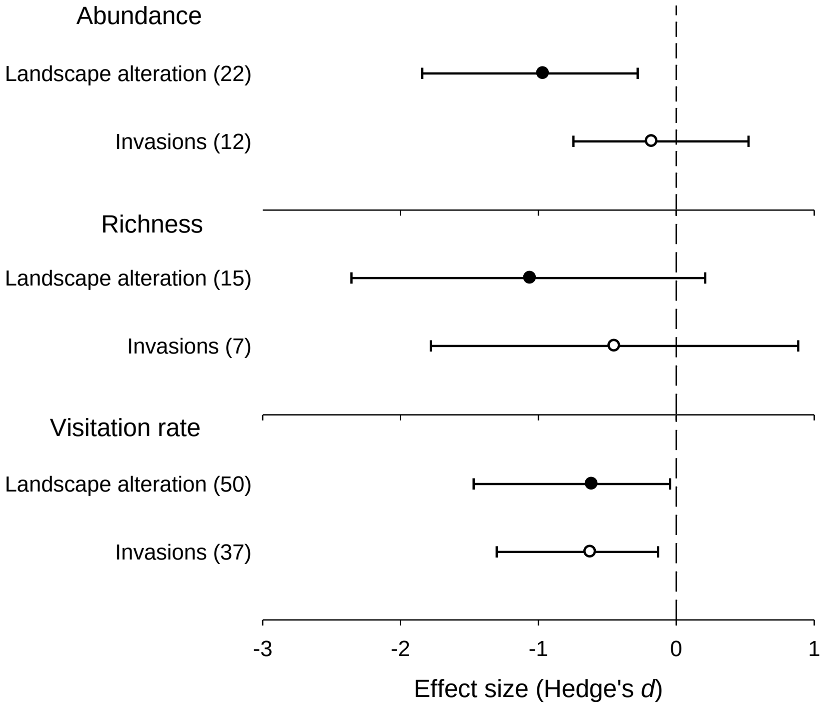


Figure 2. Overall cumulative effect sizes of landscape alteration (black circles) and invasions (open circles) on pollinators' abundance, richness and visitation rates. The bars around the means denote 95% CI (bias-corrected bootstrap for grouping variables with ≥ 10 entries, and parametric ones for grouping variables with < 10 entries). A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than altered and invaded habitats. In brackets the number of studies is indicated.

Landscape alteration

The effect of landscape alteration was not significantly different depending on whether it was measured for the whole pollinator community or for specific taxa for abundance ($Q_{between}$ = 1.60, p_{random} = 0.15), richness ($Q_{between}$ = 0.12, p_{random} = 0.82) or visitation rates ($Q_{between}$ = 0.22, p_{random} = 0.67). Focusing on specific pollinator taxa there were only marginally significant differences in visitation rates with vertebrates being most negatively affected (Table 2, Fig. 3).

Except for visitation rates, the effect of landscape alteration on pollinators was dependent on the type of ecosystem (Table 2, Fig. 4). Pollinator abundance decreased significantly in altered forests, but not in altered grasslands. The opposite trend was found for pollinator richness: it was negatively affected in grasslands but not in forests. Visitation rates were negatively affected in both ecosystems.

Pollinator abundance and visitation rates were not significantly different between experimental and observational studies. However, despite this lack of significance, abundance was found to be negatively affected in observational but not in experimental studies (Table 2). We could not compare pollinator richness because only one study was experimental.

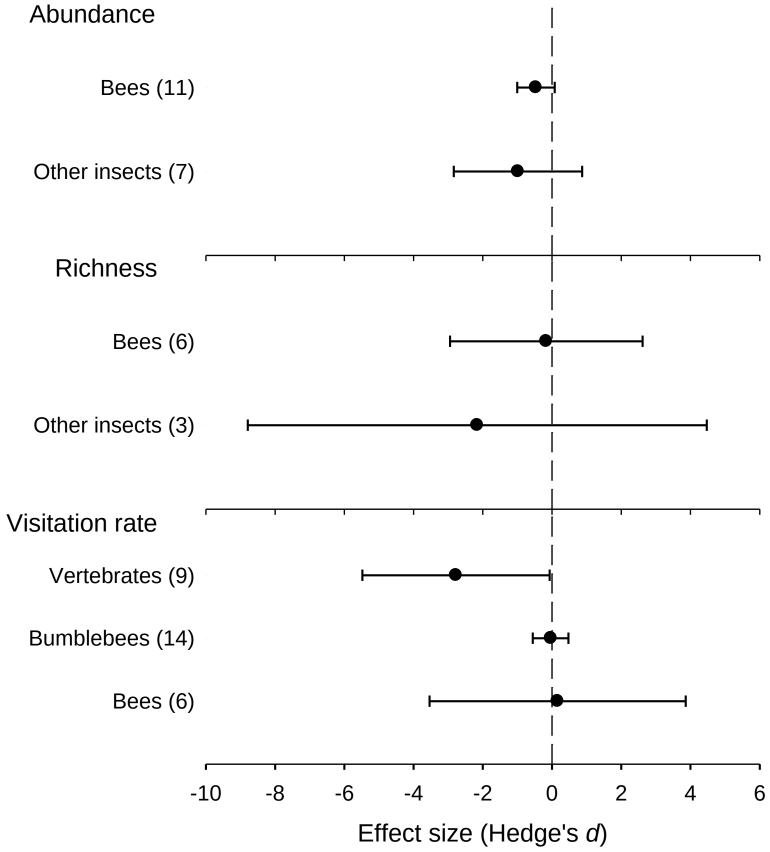


Figure 3. Overall cumulative effect sizes of landscape alteration on pollinators' abundance, richness and visitation rates depending on the taxonomical group they belong to. The bars around the means denote 95% CI (bias-corrected bootstrap for grouping variables with ≥ 10 entries, and parametric ones for grouping variables with < 10 entries). A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than altered habitats. In brackets the number of studies for each grouping variable is indicated.

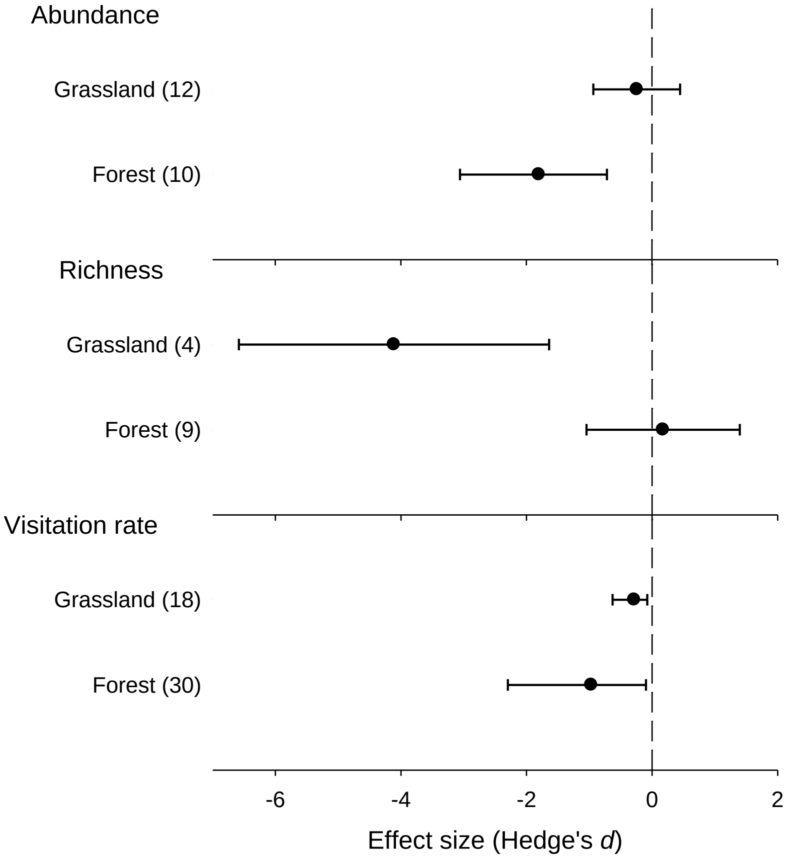


Figure 4. Overall cumulative effect sizes of landscape alteration on pollinators' abundance, richness and visitation rates depending on the type of ecosystem altered. The bars around the means denote 95% CI (bias-corrected bootstrap for grouping variables with ≥ 10 entries, and parametric ones for grouping variables with < 10 entries). A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than altered habitats. In brackets the number of studies for each grouping variable is indicated.

Pollinator visitation rates were affected differently depending on the component of landscape alteration considered. Matrix disturbance had a significant negative effect on visitation rates while the reduction of fragment size did not (Table 2, Fig. 5).

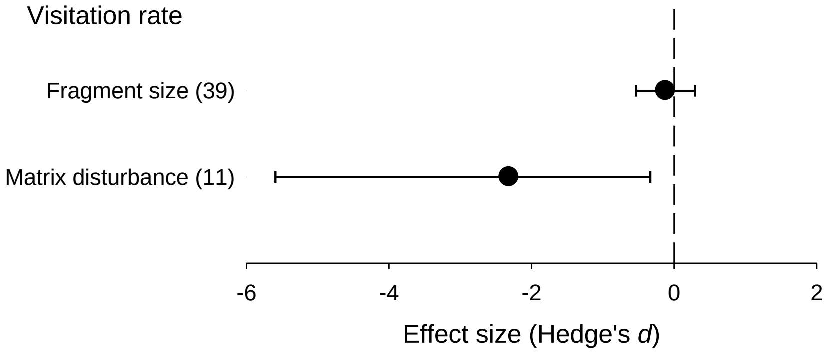


Figure 5. Overall cumulative effect sizes of different components of landscape alteration on pollinators' visitation rates. The bars around the means denote 95% CI (bias-corrected bootstrap). A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than altered habitats. In brackets the number of studies for each grouping variable is indicated.

Table 2. Meta-analysis of the effect of landscape alteration and invasions on pollinator abundance, richness and visitation rates for each grouping variable considered. For each grouping variable $Q_{between}$ and p_{random} are given. Some analysis could not be conducted due to small sample sizes.

	Categorical variable	**Levels**	**Abundance**		**Richness**		**Visitation rates**	
			$Q_{between}$	p_{random}	$Q_{between}$	p_{random}	$Q_{between}$	p_{random}
Landscape alteration	Pollinator taxa	Bees Other insects Bumblebees Vertebrates	1.05	0.20	1.12	0.47	4.12	0.05
	Type of study	Observational Experimental	2.26	0.09	-	-	0.21	0.67
	Type of ecosystem	Forest Grassland	5.74	0.01	22.82	0.01	1.95	0.19
	Component of landscape alteration	Fragment size Matrix disturbance	-	-	-	-	7.12	0.02
Invasions	Pollinator taxa	Bumblebees Bees Other insects	-	-	-	-	15.65	0.01
	Type of study	Observational Experimental	7.36	0.04	-	-	0.29	0.69
	Type of ecosystem	Forest Grassland Shrubland	0.96	0.70	-	-	3.97	0.34
	Type of invasor	Plant Animal	-	-	-	-	5.66	0.06

Biological invasions

The effect of biological invasions on pollinator abundance did not differ whether it was measured on the whole pollinator community or on specific taxonomic groups ($Q_{between}$ = 3.67, p_{random} = 0.13). However, there were significant differences in pollinator richness ($Q_{between}$ = 31.88, p_{random} = 0.03). Pollinator richness of particular taxa was negatively affected by invasions (d_{++} = -1.60, CI = -2.69 to -0.51, N = 3) while no effect was found on the whole community (d_{++} = 0.42, CI = -0.38 to 1.23, N = 4). Visitation rates were not differently affected whether they were measured for the whole pollinator community or only for specific taxa ($Q_{between}$ = 2.43, p_{random} = 0.25) possibly because there was large variation in the effect sizes within each grouping category (Q_{within} = 60.69, P = 0.01). While visitation rates in bees were not affected consistently, visitation rates of other insects decreased with invasion (Fig. 6).

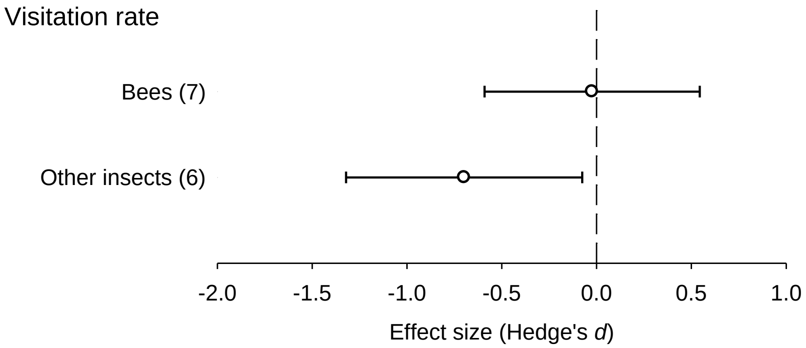


Figure 6. Cumulative effect sizes of invasions on pollinators' visitation rates depending on the taxonomical group they belong to. The bars around the means denote 95% parametric CI. A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than invaded habitats. In brackets the number of studies for each grouping variable is indicated.

The effect of invasions was not ecosystem dependent (i.e. forest, grassland or shrubland) for either abundance or visitation rates (Table 2, Fig. 7), but for the latter, heterogeneity within studies was significant (Q_{within} = 59.49, P < 0.01). Pollinator abundance decreased with invasion in observational studies but did not in experimental studies (Table 2). As with landscape alteration, these analyses could not be conducted for pollinator richness due to the small sample size.

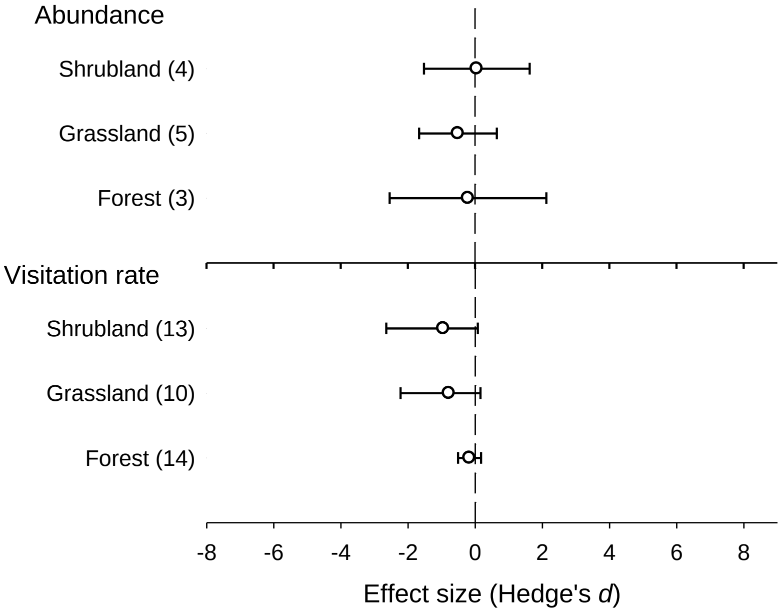


Figure 7. Cumulative effect sizes of invasions on pollinators' abundance and visitation rates depending on the type of invaded ecosystem (forests or grasslands). The bars around the means denote 95% CI (bias-corrected bootstrap for grouping variables with ≥ 10 entries, and parametric ones for grouping variables with < 10 entries). A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than invaded habitats. In brackets the number of studies for each grouping variable is indicated.

Whether the invasive organism was an animal or a plant did not affect differently pollinator visitation rates (Table 2, Fig. 8) possibly due to the large variation in the effect sizes within each grouping category (Q_{within} = 57.32, P = 0.01). While visitation rates decreased in habitats invaded by animals, a non-consistent effect for invasive plants was found. Differences between plant and animal invasions could not be compared for pollinator abundance and richness because of the scarcity of studies on animal invaders.

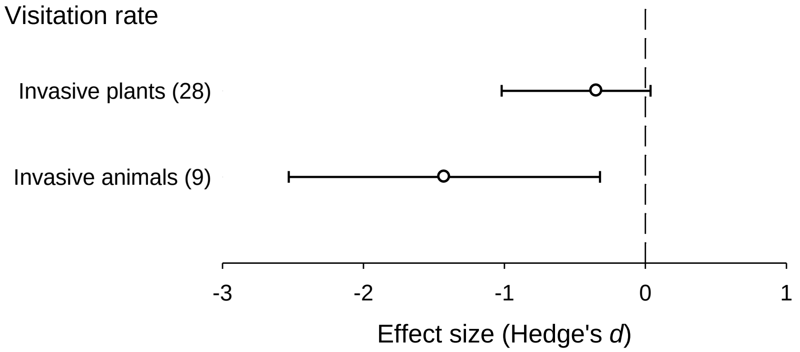


Figure 8. Cumulative effect sizes of plant and animal invasions on pollinator visitation rates. The bars around the means denote 95% CI (bias-corrected bootstrap for grouping variables with ≥ 10 entries, and parametric ones for grouping variables with < 10 entries). A cumulative effect size is significantly different from zero when its CI do not bracket zero. Negative cumulative effect sizes indicate that the control plots had on average greater values than invaded habitats. In brackets the number of studies for each grouping variable is indicated.

Discussion

The magnitude of the impact of landscape alteration and biological invasions on pollinators was similar. Across studies, there was a consistent negative effect of both components of Global Change on visitation rates. Landscape alteration also decreased pollinator abundance.

However, we could not explore how these Global Change components interact due to the limited number of publications which simultaneously examine both components. Didham *et al.* (2007) reviewed the literature available on the effect of invasions and habitat modification on biodiversity and also found that only 1.2% of these publications considered both Global Change components (and not necessarily their interaction). Despite the scarcity of studies, there is direct (see Bartomeus *et al.* 2010) and indirect (e.g. Aizen & Feinsinger 1994; Brosi *et al.* 2008) evidence for interactions between landscape alteration and invasions. These interactions are not necessarily additive but rather synergistic or antagonistic (Sala *et al.* 2000; Didham *et al.* 2005, 2007). Realistic and robust projections of the future of pollinator communities will require improved understanding about interactions among these (and others) Global Change components (Sala *et al.* 2000).

Taxonomical differences

Visitation rates by vertebrate pollinators (birds and bats) were the factors most affected by landscape alteration. Response differences between vertebrates and insects might be due to differences in their foraging distances. Foraging distances up to 2 km have been described for medium sized bees (Bommarco *et al.* 2010) and up to 5 km for bumblebees (Osborne *et al.* 2008), while flower-visiting birds and bats have larger foraging areas compared to most insect pollinators, given that they have greater energy requirements due to their endothermic metabolism (Fleming, Geiselman & Kress 2009). Bats and birds are recognised as excellent promoters of outcrossing (Fleming *et al.* 2009; Phillips, Hopper & Dixon 2010). Hadley & Betts (2009) tracked hummingbirds and found larger movement ranges in fragmented landscapes than in continuous forested areas. Therefore, different remaining patches of non-altered habitat can be included in the foraging areas of bats and birds but they are less intensively exploited than non-altered landscapes. Conversely, the smaller foraging areas of insects might be disrupted to a lower extent than in vertebrates.

However, if landscape alteration is extreme, a larger decrease of visitation rates will be expected in insects. Our results agree with Winfree *et al.* (2009) who also did not find a significant decrease of bee abundance and richness when moderate habitat loss was considered, although this effect became significant when habitat loss was extreme (defined as remaining fragments ≤ 1 ha or ≤ 5% natural habitat remaining in the surrounding matrix). The 66.67% and 61.54% of the entries included in our analysis reporting the effects on pollinator abundance and richness, respectively, refer to moderate (percentages calculated when possible according to the former definition) habitat fragmentation scenarios, which might be more representative of the global situation than the extreme ones (Winfree *et al.* 2009).

Another, and not mutually exclusive, explanation for the higher decrease of visitation rates of vertebrate pollinators compared to insect pollinators is the difference on diet breadth. Landscape alteration is more likely to have an effect in specialist pollinators, which depend exclusively on one or a few plant taxa as food sources, than on generalist pollinators, which are able to feed on a wider array of flower species (Kunin 1993; Winfree *et al.* 2011). Although it is not universal that vertebrate pollinators are more specialized than insect pollinators, in our review the insect pollinators included both bumblebees and other bees. From the six entries of other bees but bumblebees, three reported the effect of landscape alteration on the honeybee, which is known for its generalized diet (Huryn 1997). In the case of the bumblebees, we do not know the species identity of most of the entries; therefore, we cannot consider the diet breadth of the particular species studied. However, there are bumblebees that have been reported as generalists (excepting long-tongued bumblebees) and it is these species which are less affected by habitat loss, fragmentation, and degradation (Goulson *et al.* 2008). Moreover, both the honeybee and some species of *Bombus* have the capacity to broaden their diets including new food resources like non-native plants (Stout *et al.* 2002; Simpson *et al.* 2005; Gross *et al.* 2010) or mass-flowering crops (Goulson *et al.* 2002). Therefore, we can assume that the vertebrate pollinators in our review (bats and birds) could have narrower diet breadths than these insect groups. Flowers visited by bats satisfy the criteria for a set of syndromes (nocturnal anthesis, large nectar reward, cauliflory, etc.; Fleming *et al.* 2009), as do bird-visited ones (red-orange-yellow corolla colours, abundant dilute nectar, diurnal anthesis, absent scent and landing platform and long corollas; Valido, Dupont & Olesen 2004; Curti & Ortega-Baes 2011). These vertebrate pollinators would not be expected to significantly broaden their diet, but rather to forage for food resources in other sites. In contrast, the generalized pollinator systems of most of the insects included in this analysis might buffer the effects of landscape alteration by including the most abundant and new floral resources in their diet.

In invaded sites, pollinator abundance and richness were not affected, while pollinator foraging behaviour was modified, thereby reducing their visitation rates. Visitation rates of insect species, not including bees, were the most negatively affected. Compared to bees, the other insects might be more specialized (Winfree *et al.* 2011), and thus might not include new and non-native plant species in their diets. If non-native plants displace their food resources, they should look for them further afield and exploit resources which are still available nearby less intensively. In the case of animal invasions, the narrower foraging areas and periods of insects apart from bees, might easily overlap with the non-native species that usually have generalist diets, large foraging areas and periods, both during the day and throughout the year (Stout *et al.* 2002; Gross *et al.* 2010).

In general, although the number of studies suitable for meta-analysis was low for invasive animals, we found a trend towards a more negative effect of invasive animals than invasive plant species. Invasive plant species become well integrated into the native plant–pollinator networks (Vilà *et al.* 2009) through repeated visits by native pollinators and large effects are expected to occur on native plants rather than on native pollinators (Bjerknes *et al.* 2007). In contrast, non-native pollinators can directly compete with native pollinators, for nest sites and floral resources because their niches can overlap (Gross & Mackay 1998; Kato *et al.* 1999; Goulson 2003). In the case of honeybees and bumblebees, which are the most studied non-native pollinators (6 out of 9 entries of this meta-analysis), feeding niche overlap can be due to their polylectic diet, relatively large flight and foraging distances, relatively large vital cycles, capacity to start foraging earlier and their capacity to find and exploit food resources more rapidly (Goulson 2003). Non-native insects can also interfere with native insects by transmitting parasites or pathogens (Goulson 2003) or by direct exclusion. For example, invasive ants (3 studies in this meta-analysis) can exclude native pollinators in the plants they host, reducing their abundance and diversity (Cole *et al.* 1992).

Ecosystem type differences

The decrease in pollinator abundance was mainly found in altered forest, even though 85.71% of the entries belonged to moderate landscape alterations according to Winfree *et al.* (2009), but not in altered grasslands. Mid-successional habitats and more open areas with intermediate disturbance regimes seem to offer more nesting and feeding resources to most pollinator communities than forests (Steffan-Dewenter & Tscharntke 2002; Steffan-Dewenter & Westphal 2008). Forest pollinators might be more specialized and therefore vulnerable to landscape alteration (Steffan-Dewenter & Westphal 2008). Despite this, moderate and non-extreme landscape alteration might prevent species from totally disappearing, which may be the reason why we did not find a negative effect for richness; although changes in community composition might occur (Brosi *et al.* 2008). In grasslands the opposite trends were observed, i.e. while pollinator abundance was not affected, pollinator richness significantly decreased. The loss of pollinator species in altered grassland ecosystems could be replaced by generalist pollinators like managed honeybees (Aizen & Feinsinger 1994), thereby maintaining abundance levels.

On the contrary, the effect of invasions on pollinators was not influenced by ecosystem type. However, many aspects in the patterns of invasion and their ecological impacts are ecosystem-dependent (Vilà *et al.* 2006; Chytrý *et al.* 2009). It is possible that pollination patterns are more community context-dependent than ecosystem-dependent (Lonsdale 1999; Vilà *et al.* 2009), as there was a large variation in the effect sizes between studies (i.e. significant Q_{within} for visitation rates).

Observational vs. experimental studies

Only pollinator abundance after invasions differed between observational and experimental studies. In observational studies invasion reduced pollinator abundance, whereas this was not the case in experimental studies. This is perhaps due to the short duration of most field manipulative studies compared to the long-term dynamics associated with natural communities. Pollinators might change their foraging behaviour quickly, just after the introduction of the non-native species, but the effects on pollinator populations might have a lag-time before being detected (Kuussaari *et al.* 2009). The existence of such a lag-time could also explain the lack of a significant effect of invasion on pollinator abundance and richness.

Furthermore, experimental studies isolate the effect of the Global Change component of interest whereas in observational studies other components could be simultaneously acting, producing additive or synergistic effects with invasions (Sala *et al.* 2000).

Differences among landscape alteration components

The component of landscape alteration that primarily influenced pollinator patterns was disturbance of the surrounding matrix. The characteristics of the matrix could possibly lessen the effects of patch size reduction of non-altered habitat on visitation rates, thereby allowing the pollinators to persist in the area to reach other foraging areas or even exploit the matrix. As the nature of the matrix becomes more hostile, pollinators are more vulnerable and achieve fewer visits, probably due to a decrease in their abundance. In our study, 63.63% of the entries relating to the effect of matrix disturbance refer to matrices converted on agricultural land. In intensively managed agricultural lands, pollinator abundances have been reported to decrease within a 150-m zone outside remaining natural patches (Kohler *et al.* 2008), while more extensive croplands, such as those with semi-natural vegetation in fields margins, can offer foraging areas for pollinators (Pywell *et al.* 2005) generating less hostile landscapes and buffering the effect of the reduction of fragment size.

Conclusions

Our review found that pollinator communities are affected by landscape alteration and biological invasions similarly. Responses of pollinators to these two Global Change components vary among taxa and ecosystem types. Despite the fairly large amount of literature concerning the effects of landscape alteration and invasions on different steps of the plant reproduction process, from pollinator abundance to fruit and seed set (Aizen & Feinsinger 1994), there are few studies exploring the impact on plant–pollinator networks (Aizen *et al.* 2008b; Vilà *et al.* 2009), and on plant and pollinator progeny performance (Gonzalez-Varo *et al.* 2010; Potts *et al.* 2011).

Our results point to larger effects due to invasive animals than invasive plants. Many studies have investigated the effect of domestic honeybees and bumblebees on native pollinators (e.g. Dupont *et al.* 2004; Ings, Ward & Chittka 2006; Nagamitsu *et al.*

2010), however, a large number of these studies lack reference plots which prevented us from including many entries in the meta-analysis.

Bees and bumblebees are also the main studied species responding to invasion and landscape alteration. Although the growing concern on the pollination crisis focuses on these two groups of pollinators, more attention should be paid to vertebrate pollinators as they are the most affected by landscape alteration.

Acknowledgements

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Appendix 1. List of studies included in the meta-analysis

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Appendix 2. Exploration bias of response variables

Exploration of publication bias for the response of pollinator abundance, richness and visitation rates to habitat fragmentation and biological invasions. We followed three methods: funnel plots, Spearman rank correlations and fail-safe numbers.

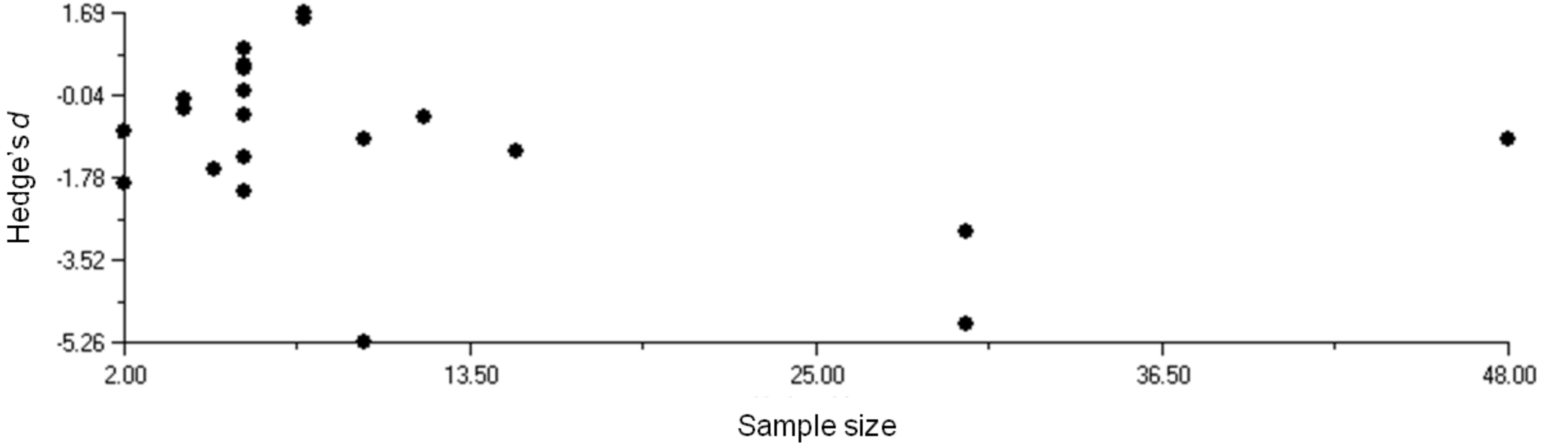


Figure A2.1. Funnel plot of effect sizes (*Hedges'd*) versus sample sizes for the response of pollinator abundance to landscape alteration.

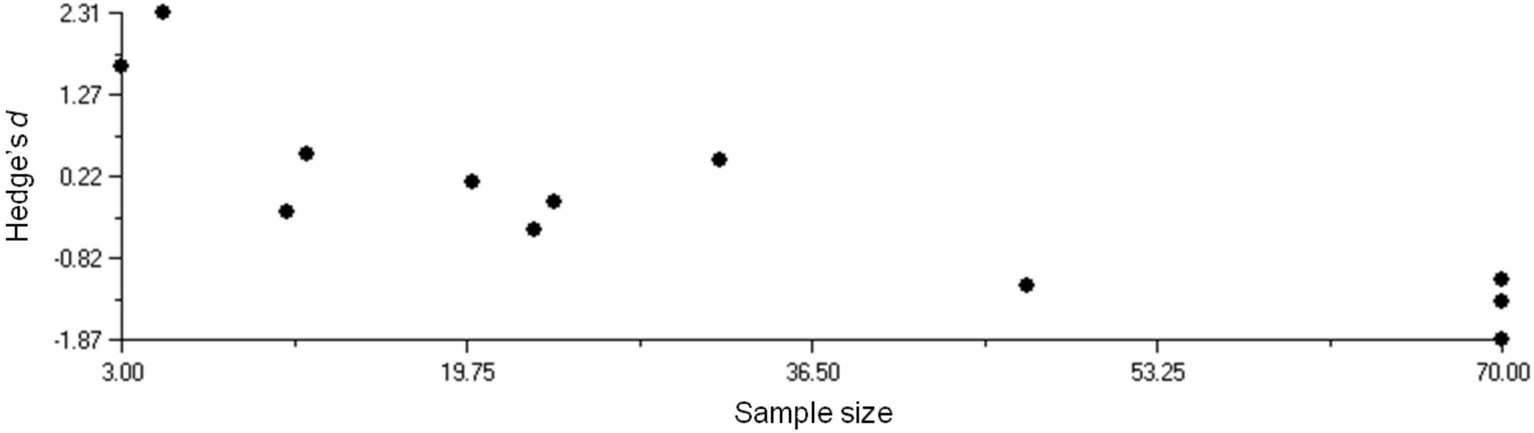


Figure A2.2. Funnel plot of effect sizes (*Hedges'd*) versus sample sizes for the response of pollinator abundance to biological invasions.

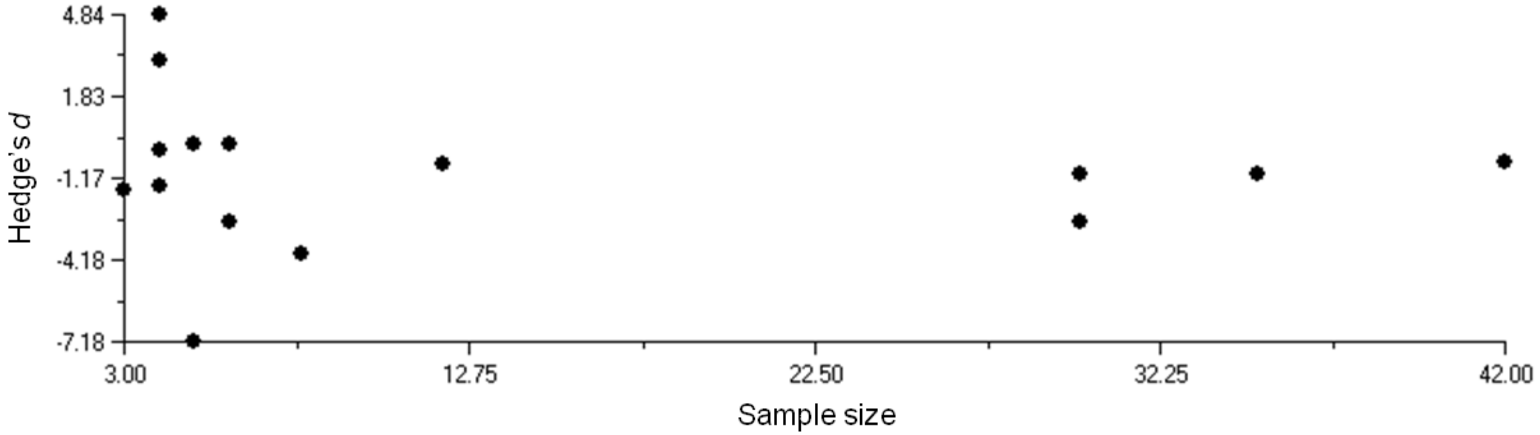


Figure A2.3. Funnel plot of effect sizes (*Hedges'd*) versus sample sizes for the response of pollinator richness to landscape alteration.

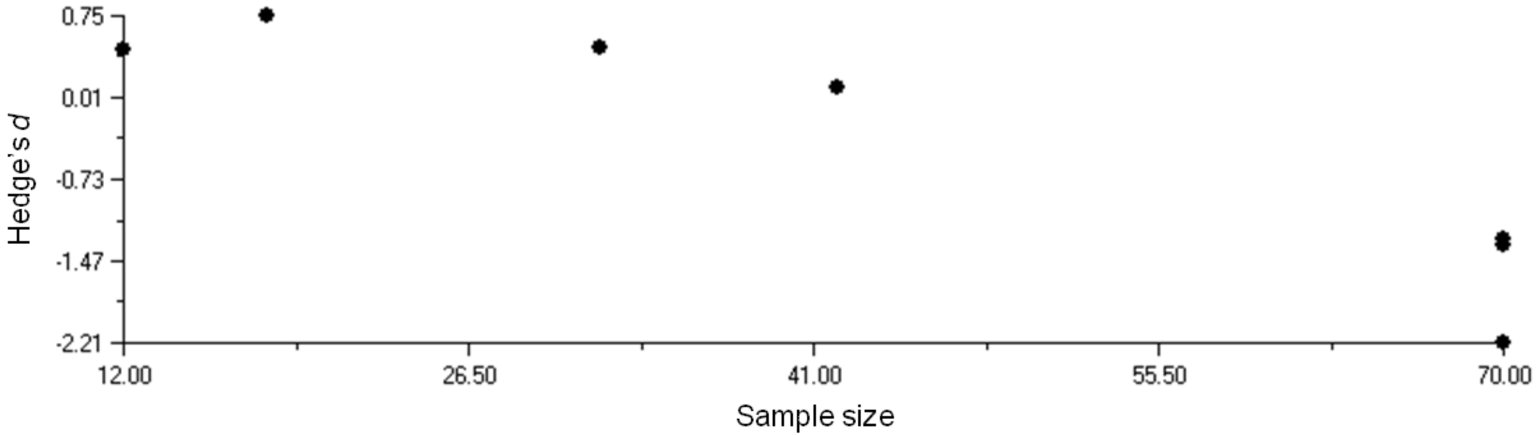


Figure A2.4. Funnel plot of effect sizes (*Hedges'd*) versus sample sizes for the response of pollinator richness to biological invasions.

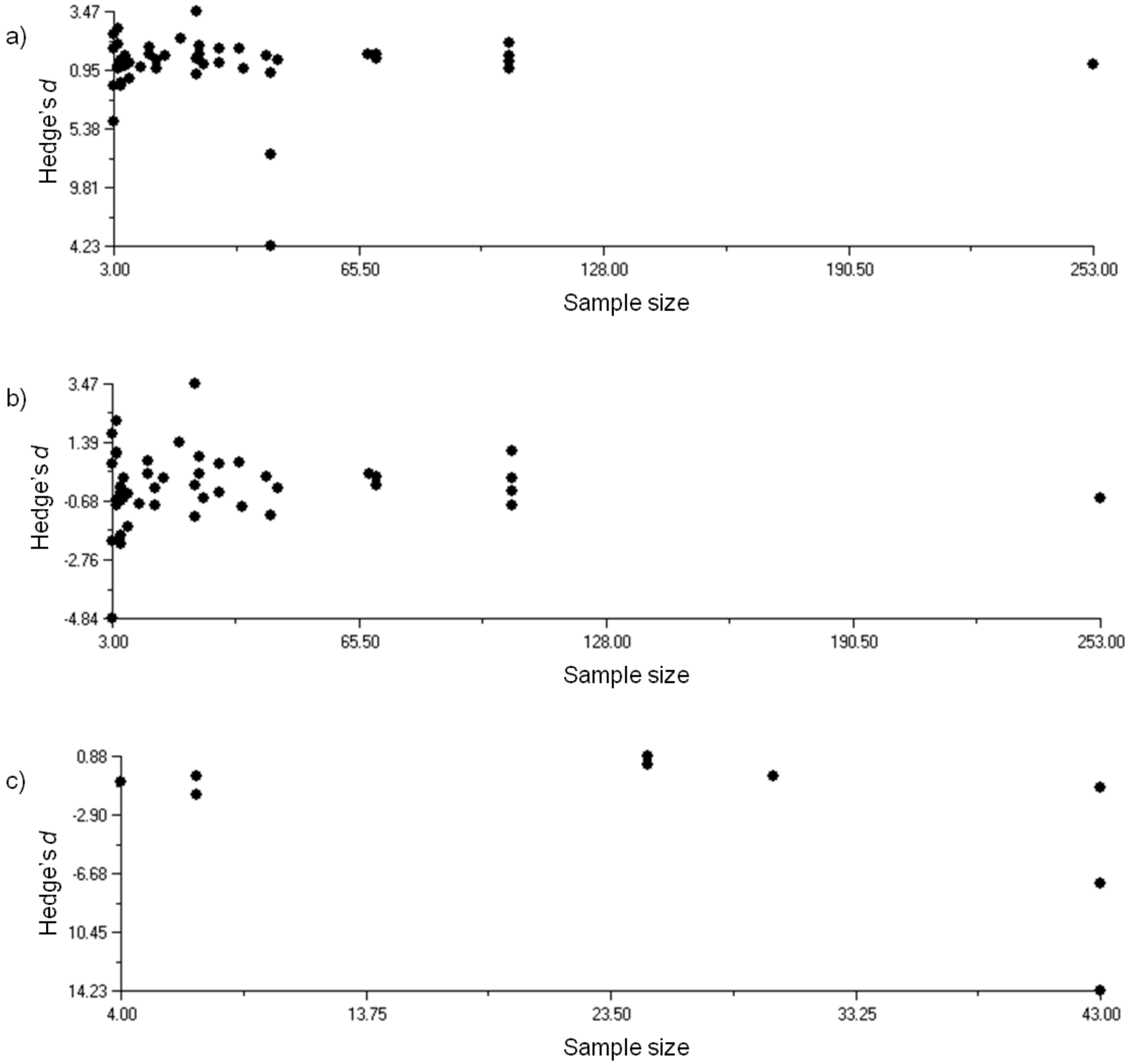


Figure A2.5. Funnel plots of effect sizes (*Hedges'd*) versus sample sizes for the response of pollinator visitation rates to landscape alteration for the whole raw of data (a); excluding two outlier entries on the effect of landscape alteration on vertebrate visitation rates (b); and for the total of entries on the effect of landscape alteration on vertebrate visitation rates (c).

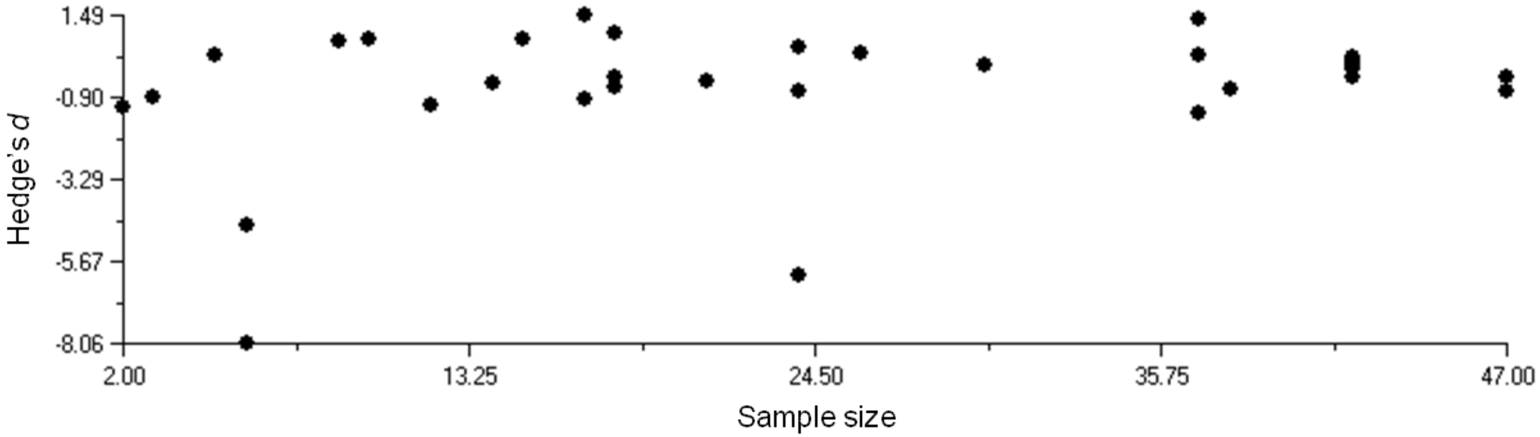


Figure A2.6. Funnel plot of effect sizes (*Hedges'd*) versus sample sizes for the response of pollinator visitation rates to biological invasions.

Table A2.1. Spearman rank correlation test and fail-safe numbers calculated for effect sizes (*Hedges'd*) for the response of pollinator abundance, richness and visitation rates to habitat fragmentation and biological invasions. For visitation rates in response to habitat fragmentation three analysis were done: for the whole raw of data (a); excluding two outlier entries on the effect of fragmentation on vertebrate visitation rates (b); and for the total of entries on the effect of fragmentation on vertebrate visitation rates (c).

		Spearman rank correlation		**Fail-safe number (Rosenthal method)**
		Rs	**P**	
Habitat fragmentation	Abundance	0.01	0.97	1395.20
	Richness	-0.10	0.73	617.30
	Visitation rates (a)	0.29	0.05	4541.60
	Visitation rates (b)	0.05	0.73	55.60
	Visitation rates (c)	-0.12	0.76	3849.90
Invasions	Abundance	-0.68	0.01	207.30
	Richness	-0.70	0.08	181.70
	Visitation rates	0.21	0.21	1174.50

Discusión General

Acts in what Hutchinson (1965) has called the "ecological theatre" are played out in various scales of space and time. To understand the drama, we must view it in the appropriate scale (Wiens 1989).

El objetivo general de esta Tesis ha sido avanzar en el conocimiento de la relación entre las invasiones biológicas por parte de plantas entomófilas y las interacciones mutualistas de polinización. Dado que se trata de una relación bidireccional, este objetivo ha implicado mirar las dos caras de una misma moneda. En primer lugar, investigar cómo la polinización puede influir el éxito reproductivo de una especie exótica. En segundo lugar, investigar cómo esa especie exótica puede afectar a las plantas residentes en el área de introducción. Este segundo aspecto se ha explorado a distintas escalas espaciales (vecindario, local y paisaje), lo que ha permitido alcanzar un conocimiento más completo e integrado de dicho efecto, así como identificar preguntas interesantes de abordar en el futuro.

En esta Tesis se han explorado no sólo los patrones, sino también algunos de los potenciales mecanismos subyacentes a los efectos observados. Para todo ello se ha llevado a cabo un trabajo de campo muy intenso y exhaustivo, acomentiendo experimentos manipulativos, que son muy ventajosos ya que permiten analizar relaciones causales. Además, esta Tesis ha supuesto la aplicación de distintas aproximaciones analíticas, desde la estadística probabilística clásica hasta el meta-análisis, pasando por el análisis de redes. Todos estos aspectos pueden considerarse fortalezas ya sea por su relevancia y novedad en el campo de las Invasiones Biológicas como desde un punto de vista metodológico.

La especie de estudio eligida fue la planta leguminosa *Hedysarum coronarium,* por reunir una serie de características que la hacen adecuada para abordar estos objetivos. Por una parte, su área de distribución nativa y parte de su área de introducción pertenecen a la misma región biogeográfica, lo cual permite controlar más factores del medio y hacer más legítima la comparación de los patrones de polinización entre ambas áreas (**Capítulo 1**). Por otra parte, desde un punto de vista logístico, la cercanía entre estas áreas facilitó llevar a cabo un estudio comparativo tomando el sur de la provincia de Cádiz como representativo del área nativa, y la isla de Menorca como representantiva del área de introducción. Además, al tratarse de una especie naturalizada (Fraga *et al.* 2004) y que sigue cultivándose en Menorca, ha permitido explorar su efecto a distintas escalas espaciales: vecindario, local y paisaje (**Capítulos 2, 3** y **4**, respectivamente).

Interacciones con polinizadores: necesarias pero no limitantes

Tal como se ha corroborado en el **Capítulo 1**, las interacciones con polinizadores, aunque necesarias, no necesariamente son un factor limitante para el establecimiento y persistencia de las plantas exóticas entomófilas en sus áreas de introducción. La limitación polínica en el área de introducción puede verse compensada en otras etapas del ciclo de vida. Por ejemplo, en el caso de *Hedysarum*, el aporte anual de propágulos en los campos de cultivo puede compensar el mayor número de semillas producidas en el área nativa. Menores tasas de depredación de semillas, o de herbivoría de plántulas (Wolfe 2002), como postula la Hipótesis del Escape de los Enemigos Naturales (*Enemy Release Hypothesis*), también podrían ser mecanismos que compensaran la limitación polínica en las áreas de introducción. Extender la aproximación biogeográfica que se ha llevado a cabo en el **Capítulo 1** a las distintas etapas del ciclo de vida de las especies exóticas, ayudaría a elucidar estas cuestiones. En el caso de *Hedysarum*, también permitiría establecer si su estatus en Menorca podría considerarse invasor.

Integrando escalas y planteando nuevas preguntas

A pesar de la limitación polínica, las especies exóticas entomófilas pueden integrarse bien en la dieta de los polinizadores residentes en su área de introducción. Con *Hedysarum* hemos observado que es visitada por un número similar de polinizadores en Menorca que en Cádiz (**Capítulo 1**) y superior al de la mayoría de las especies nativas (**Capítulo 3**). Esta integración tiene consecuencias en la polinización de las plantas residentes cuya fenología de floración solapa con la de las exóticas, lo cual ha sido explorado en los **Capítulos 2, 3** y **4**.

Para avanzar no sólo en los patrones de interacción planta-polinizador, sino también en los mecanismos subyacentes, en estos capítulos se explora la influencia de distintos factores en el efecto de *Hedysarum* sobre la polinización de las plantas residentes a distintas escalas espaciales. Los polinizadores perciben y explotan su medio con finalidades diferentes según la escala espacial, de modo que el efecto de las plantas exóticas entomófilas, así como los factores influyentes, también variarán con la escala espacial. A escalas pequeñas los polinizadores persiguen optimizar su actividad de forrajeo (Burkle & Alarcón 2011), mientras que a escalas mayores, no sólo deben cubrir sus necesidades alimenticias, sino también encontrar lugares adecuados donde anidar y completar todas sus fases vitales (Cane 2001; Winfree *et al.* 2011).

En esta Tesis, también se presta especial atención al comportamiento de la abeja de la miel, ya que ésta realiza más del 80% de las visitas que recibe *Hedysarum* y además, gracias a su abundancia y forrajeo intensivo y sistemático, compite fuertemente con los otros polinizadores por el uso de los recursos florales (Steffan-Dewenter & Tscharntke 2000; Roubik & Wolda 2001). Por lo tanto, el efecto de *Hedysarum* en la polinización de las plantas de la comunidad receptora está principalmente mediado por cambios en el comportamiento de esta especie. A escala de vecindario o local, los cambios en el comportamiento de forrajeo de la abeja de la miel pueden traducirse, por ejemplo, en cambios en su constancia floral o en su distancia de vuelo entre visitas consecutivas. A escala de paisaje, pueden traducirse en cambios en el tamaño del área de forrajeo, o en los patrones de comunicación. El protagonismo de la abeja de la miel no debe de ser exclusivo de nuestro sistema de estudio por tratarse de una abeja cosmopolita y manejada en todo el mundo (Stout *et al.* 2002), que además posee una dieta generalista (Huryn 1997) en la que incluye a especies exóticas (Gross *et al.* 2010).

Para integrar los resultados obtenidos en los **Capítulos 2**, **3** y **4**, resumidos en el Tabla 1, nos centramos en la variable respuesta visitas (ya sea su tasa o su presencia/ausencia), como hilo conductor de la discusión.

A escala de vecindario, las especies exóticas pueden ejercer un efecto magnético a través de su despliegue floral sobre otras plantas relativamente menos atractivas, como es el caso de *Hedysarum* con la nativa *Muscari*, respectivamente (**Capítulo 2**). La diferente atracción de los polinizadores hacia plantas entomófilas puede deberse a diferencias en la visibilidad, accesibilidad, cantidad y/o calidad de su oferta floral (Dietzsch *et al.* 2011; Bartomeus 2013). La visita a plantas menos atractivas sólo resulta rentable para los polinizadores cuando éstas se encuentran a cortas distancias de vuelo (Molina-Montenegro *et al.* 2008). Aún en el caso de que el efecto magnético de polinizadores desde las especies más atractivas hacia las menos atractivas sea un fenómeno poco frecuente, puede suponer cambios significativos en las tasas de visitas de estas últimas, como se ha observado para *Muscari*.

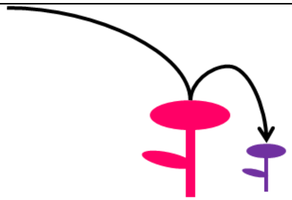
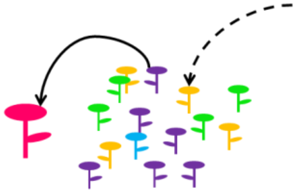
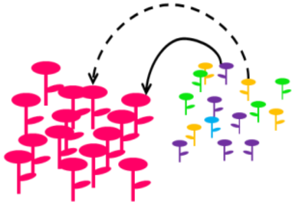
Simultáneamente al efecto magnético mediado por su despliegue floral, las especies exóticas también interactúan con otras plantas en su vecindario a través de su parte vegetativa, ya sea por el uso de recursos abióticos (nutrientes, agua, luz) o mediante la compartición de herbívoros, patógenos, etc. Las plantas exóticas suelen ser grandes competidoras de las nativas (Vilà & Weiner 2004). Así, la disminución en la diversidad floral observada en los vecindarios invadidos por *Hedysarum* (**Capítulo 2)**, podría esperarse en vecindarios invadidos por otras exóticas.

Sería interesante repetir este estudio para especies nativas cuya atracción para los polinizadores no difiera tanto de la de la exótica, para las que no esperaríamos encontrar un efecto magnético. Además, con un tamaño de muestra mayor se podrían explorar simultáneamente efectos directos e indirectos mediante modelos de ecuaciones estructurales (SEMs).

A escala local, el efecto de la exótica varía de unas especies a otras de la comunidad receptora. Para algunas especies, la presencia de la exótica tiene un efecto positivo (magnético) en sus tasas de visitas, mientras que para otras el efecto es negativo o neutro (Bjerknes *et al.* 2007; Vilà *et al.* 2009). De modo que, como se observa en el **Capítulo 3**, cuando se tienen en cuenta todas las plantas en flor de la comunidad receptora, los distintos efectos se contrarrestan y no se observan diferencias en las tasas de visitas a nativas. Una pregunta para abordar en el futuro sería explorar si el efecto observado para cada especie residente es el resultado del sumatorio de efectos a escala de vecindario y/o el efecto ejercido propiamente a escala local. Esta segunda opción será factible siempre y cuando los polinizadores residentes tengan capacidades de vuelo que abarquen áreas mayores a las de las parcelas de estudio, lo cual será el caso de la mayoría de los taxones, a excepción de algún coleóptero (Gathmann & Tscharntke 2002; Osborne *et al.* 2008; Mawdsley & Sithole 2009). De esta manera, los polinizadores tendrán la capacidad de responder a la presencia de la exótica a escala local, ya sea siendo atraídos o evitando las parcelas invadidas. Para dilucidar si el efecto es el sumatorio de efectos a escala de vecindario y/o el efecto ejercido propiamente a escala local, sería necesario un diseño experimental en el que se manipulara la presencia de la exótica tanto a escala local como de vecindario, haciendo todas las combinaciones posibles: presente a ambas escalas, presente sólo a una de ellas y ausente a ambas; similar a lo llevado a cabo por Jakobsson, Padrón & Traveset (2009).

En cualquier caso, el signo de la respuesta dependerá de las características de las especies implicadas y de factores propios del medio. En el **Capítulo 3** se ha explorado la influencia de la similitud en morfología floral entre la exótica y las plantas residentes sin encontrar evidencias de que sea significativa. De hecho, las especies con flores papilonáceas como *Hedysarum* reciben tasas de visitas significativamente menores a las de las demás especies en flor. Las flores papilionáceas no son accesibles a todo tipo de polinizadores (Córdoba & Cocucci 2011) y presentan tasas de visitas muy bajas. Para encontrar potenciales diferencias entre ambas morfologías florales, habría que aumentar considerablemente el esfuerzo de muestreo, pero cabría preguntarse si diferencias tan pequeñas, aunque significativas, tendrían significado biológico para las especies implicadas (Martínez-Abrain 2008). Quizás la similitud en morfología floral entre las plantas exóticas y las de la comunidad receptora debe de ser más influyente en el caso de especies con morfologías florales menos restrictivas. Morales & Traveset (2009) revisando distintos estudios que comparaban parejas de plantas nativas y exóticas mediante un meta-análisis sí encontraron que el efecto de las exóticas en la polinización y éxito reproductivo de las nativas era más negativo cuando ambas especies compartían rasgos florales.

Tabla 1. Efecto de *Hedysarum* en las visitas (tasa o presencia) de los distintos grupos de polinizadores estudiados sobre las plantas en flor de la comunidad receptora. También se indican la escala espacial, el nivel de organización sobre el que se estudió el efecto, así como los factores explorados en los Capítulos experimentales 2, 3 y 4. En los patrones observados *Hedysarum* está representado en rojo, *Muscari* en morado y los demás colores representan el resto de las plantas en flor. Las flechas continuas representan las visitas de la abeja de la miel, las punteadas las visitas de las otras abejas y las discontinuas las visitas de los otros polinizadores.

Escala espacial	**Plantas en flor de la comunidad receptora**				**Patrones observados**	**Factores explorados: el efecto de *Hedysarum* es…**	
	Nivel organización	**Visitas Total**	**Abeja de la miel**	**Otros**			
Vecindad	Especie	+	+	n.s.		Directo debido a su despliegue floral	SÍ
						Indirecto debido a la alteración de su vecindario	SÍ
Local	Comunidad	n.s.	-	-		Mayor en las plantas acompañantes leguminosas	NO
Paisaje	Comunidad	- (abejas)	-	- (abejas)		Mediado por el vertido temporal de polinizadores	NO
						Mediado por el vertido espacial de polinizadores	SÍ

Aún cuando las comunidades invadidas no presentan cambios cuantitativos, la polinización de las plantas en flor acompañantes sí puede verse alterada en términos cualitativos; es decir, por cambios en la identidad de las interacciones planta-polinizador. Estos cambios en la identidad de las interacciones planta-polinizador se han observado en otros procesos de invasión (Bartomeus *et al.* 2008b; Kaiser-Bunbury *et al.* 2011), así como en redes sometidas a otros tipos de perturbación, como la pérdida de hábitat y la fragmentación (Campos-Navarrete *et al.* 2013). A escala local, ante cualquier perturbación que altere la identidad, abundancia y distribución de los recursos florales los polinizadores adaptan su comportamiento de forrajeo modificando la intensidad e identidad de las relaciones que establecen (Ghazoul 2004; Valdovinos *et al.* 2013). La abeja de la miel, lidera estos cambios, como hemos observado en el **Capítulo 3** y en otros sistemas donde esta abeja ha sido introducida (Kaiser-Bunbury *et al.* 2011). En este sentido, sería interesante llevar a cabo una aproximación experimental similar a la del **Capítulo 3** pero en un sistema donde la abeja de la miel no fuera el polinizador dominante para explorar la respuesta de los polinizadores no condicionada a la de esta especie dominante.

Finalmente a escala de paisaje, como se ha observado en el **Capítulo 4**, los cultivos de floración masiva de especies exóticas, considerados como parches de invasión homogéneos, también afectan a la polinización de las comunidades seminaturales adyacentes atrayendo a sus polinizadores. No sólo la gran extensión, sino también la alta densidad de la especie exótica en estos cultivos, aspectos intrínseca y positivamente correlacionados, ejercen atracción sobre los polinizadores (Kunin 1997; Bernhardt, Mitchell & Michaels 2008). Esta atracción está en concordancia con las capacidades de vuelo y de comunicación de cada taxón de polinizador, mayores para la abeja de la miel (Steffan-Dewenter & Kuhn 2003; Greenleaf *et al.* 2007), que para las otras abejas (Gathmann & Tscharntke 2002). Es decir, al contrario de lo observado a escalas menores en las que la exótica se encuentra mezclada con otras plantas nativas, a escala de paisaje los cultivos de floración masiva pueden monopolizar también las visitas de las abejas silvestres. Claramente, en estos cultivos el recurso floral no es limitante. Por tanto, la competencia con la abeja de la miel (Morales & Aizen 2002) debe de rebajarse y otras abejas visitarán los campos de cultivo disminuyendo su presencia en las comunidades vegetales adyacentes. Esto se traduce en un vertido de polinizadores desde áreas naturales hacia cultivos de floración masiva. Sin embargo, después de la floración masiva, el vertido de abejas en el otro sentido debe de diluirse en el espacio a lo largo de las áreas de forrajeo que los polinizadores implicados son capaces de cubrir. Por todo ello, en paisajes agrícolas heterogéneos, parcheados y dominados por la abeja de la miel, como nuestro sistema de estudio, los cultivos de floración masiva no parecen favorecer las comunidades de abejas de las áreas adyacentes.

Según todos estos resultados, sería a la escala de paisaje a la que *Hedysarum* tendría un efecto negativo mayor (tanto en intensidad como en área de influencia) sobre la polinización de las plantas en flor en Menorca. No obstante, y aunque se espera que el efecto de *Hedysarum* esté principalmente mediado por abejas, para corroborar la prevalencia del efecto negativo, habría que explorar la respuesta de los otros taxones de polinizadores no contemplados en este estudio.

En conclusión, gracias a la aproximación experimental llevada a cabo en los **Capítulos 3, 4** y **5**, se ha observado que el efecto de *Hedysarum* en la polinización de las plantas residentes de la comunidad receptora varía en función de la escala espacial de estudio. Además, el efecto también difiere, e incluso llega a ser contradictorio, en función del grupo de polinizadores en el que se centre el estudio, así como del nivel de organización al que sea evaluado el efecto (especie o comunidad). Queda así patente la complejidad de mecanismos e impactos de una planta invasora en la polinización de plantas nativas en el área de introducción y la importancia de las aproximaciones multiescala para su estudio.

Enmarcando nuestros resultados en el contexto global

En el **Capítulo 5** se llevó a cabo una revisión bibliográfica y posterior meta-análisis para poner en un contexto global los efectos de las invasiones biológicas sobre los polinizadores y enmarcar en él nuestros resultados. Según esta revisión, las tasas de visitas a plantas nativas en áreas invadidas son generalmente más bajas que en áreas no invadidas. La revisión incluye principalmente casos de estudio llevados a cabo a escalas de vecindario y locales. Sin embargo, en nuestro sistema de estudio sólo se encontró un efecto negativo para las visitas de la abeja de la miel a escala local, el resto de efectos negativos se encontraron a escala de paisaje.

La falta de concordancia de los resultados de los **Capítulos 2** y **3** con la tendencia general puede tener dos explicaciones no mutuamente excluyentes. En primer lugar, al ser *Hedysarum* una leguminosa, su morfología floral es muy restrictiva y su efecto directo vía polinizadores compartidos con las plantas residentes estará acotado a un número más reducido de especies que en el caso de plantas exóticas con morfologías florales más accesibles, como pueden ser las compuestas y las umbelíferas. De hecho, los 55 casos de estudio incluidos en el meta-análisis sobre invasiones por parte de plantas, se refieren a especies con morfologías florales muy accesibles (ej. *Carpobrotus spp*., *Impatiens glandulifera*, *Oxalis pes-caprae*, *Lythrum salicaria*) y con potencial de alterar el comportamiento de un abanico de polinizadores más amplio. En segundo lugar, el que a escala local y de vecindario *Hedysarum* atraiga principalmente a la abeja de la miel, puede resultar beneficioso para los otros polinizadores, que verán reducida su competencia por recursos florales con ésta (Roubik 1983; Paini 2004). En sistemas dominados por la abeja de la miel, la presencia de una planta exótica melífera podría suponer el aumento de las visitas de otros polinizadores a las plantas de la comunidad receptora, desviando los resultados de la tendencia general a disminuir las tasas de visitas en zonas invadidas. Aunque la abeja de la miel se considera nativa del área de estudio (Garnery, Cornuet & Solignac 1992; Whitfield *et al.* 2006), al tratarse de una especie manejada y favorecida por el ser humano, desde el punto de vista de su efecto en otros polinizadores, podría considerarse como invasora. De hecho, según el meta-análisis, los polinizadores invasores tienen un efecto negativo mayor en las tasas de visitas a las plantas residentes que la plantas invasoras. La asociación entre *Hedysarum* y la abeja de la miel podría estar amortiguando el efecto de ambas especies en las interacciones planta-polinizador de la comunidad receptora silvestre.

El meta-análisis reveló que los polinizadores pertenecientes a otros grupos distintos de las abejas son aquellos cuyas tasas de visitas se ven más negativamente afectadas por las invasiones. Aunque en esta Tesis no se han analizado por separado los polinizadores que no fueran abejas, una vez más, cabría esperar que nuestros resultados difirieran de la tendencia general por el tipo de morfología floral de *Hedysarum*, que al menos de forma general no interactúa con polinizadores distintos a las abejas salvo en el caso de algún coleóptero.

Implicaciones para las comunidades invadidas

Como se ha observado en el **Capítulo 2**, el aumento en la tasa de visitas no necesariamente se traduce en un mayor porcentaje de fructificación, incluso en especies como *Muscari*, que depende de estas visitas para su fecundación. De forma inversa, tasas de visitas similares podrían ir acompañadas de cambios en los porcentajes de fructificación si cambia la identidad de los polinizadores que realizan esas visitas, como se ha observado a escala local (**Capítulo 3**). Para una misma especie vegetal, no todos los polinizadores que la visitan serán igual de eficientes ni en la captación de polen, ni en la deposición de éste (Ne'eman *et al.* 2010). Explorar el éxito reproductivo de toda la comunidad de plantas en flor receptora hubiera sido lo deseable, pero inabarcable desde el punto de vista logístico dada la replicación temporal (dos años de estudio) y espacial (tres pares de parcelas) de nuestros datos. Hasta donde sabemos, sólo Kaiser-Bunbury *et al.* (2011) y Ferrero *et al.* (2013) han explorado no sólo las visitas, sino también la producción de frutos de varias especies nativas de la comunidad en un gradiente de invasión y en presencia y ausencia de la exótica *Oxalis pes-caprae*, respectivamente. Estos autores encuentran que el efecto de la/s exótica/s en la producción de frutos de las plantas residentes es especie-dependiente, encontrando efectos neutros o positivos, pero en ningún caso negativos. Además, al igual que se ha observado aquí para *Muscari*, la producción de frutos y semillas no necesariamente están relacionadas con las tasas de visitas.

Así pues, los datos de tasas de visitas no permiten avanzar cuáles son las consecuencias de la presencia de exóticas en el éxito reproductivo de las plantas en flor de las comunidades receptoras. Aunque la tasa de visitas pueden ser un buen estimador del éxito reproductivo de las especies vegetales (Vázquez *et al.* 2005), en otros casos (e.g. Goodell *et al.* 2010) se observa un desacople entre ambos parámetros, ya que otros muchos factores están implicados. Para empezar, el cambio en la composición de los polinizadores implicará distintas estrategias de forrajeo en lo que se refiere a constancia floral, área abarcada y distancia de vuelo entre visitas consecutivas (Greenleaf *et al.* 2007; Leonhardt & Blüthgen 2012); así como cambios en el acople entre los estigmas y la zona del cuerpo del polinizador donde se deposita el polen (Wolfe & Barrett 1989). Todo ello conllevará cambios en el componente cualitativo de la polinización, ya sea porque alteran la cantidad de polen heteroespecífico o el grado de parentesco con el donador de polen intraespecífico (Souto, Aizen & Premoli 2002). Por otra parte, la biología reproductiva de cada especie, así como la existencia de limitación polínica o no, también influirán en el éxito reproductivo de las plantas de las comunidades receptoras (Ferrero *et al.* 2013).

Dado que la presencia de cultivos de floración masiva disminuye las visitas de las abejas en los parches de vegetación seminatural adyacentes (**Capítulo 4**), se esperarían cambios en el éxito reproductivo de esas comunidades vegetales (que una vez más, serán especie y contexto-dependientes). Sin embargo, en el caso de cultivos rotativos, como los de *Hedysarum*, este efecto quedará diluido a largo plazo y esperaríamos que no tuviera consecuencias en la demografía de las poblaciones de plantas implicadas.

En lo que se refiere al efecto de las invasiones por parte de plantas entomófilas en los polinizadores, al menos a escala local y de vecindario, debe de estar relacionado con cambios en el comportamiento más que en la demografía de éstos (Ghazoul 2004). Esto debido a que a escala local y de vecindario, la mayoría de polinizadores podrán abarcar en sus áreas de forrajeo zonas invadidas y no invadidas (Gathmann & Tscharntke 2002; Osborne *et al.* 2008; Mawdsley & Sithole 2009). Los resultados del meta-análisis (**Capítulo 5**) también apuntan en esta dirección ya que a pesar de la disminución generalizada de las tasas de visitas en áreas invadidas respecto a las no invadidas, ni la abundancia ni la riqueza de polinizadores se ven alteradas. A escala de paisaje, algunos polinizadores podrían no ser capaces de acceder a otras áreas por sus distancias de vuelo más restringidas. Pero de nuevo, en el caso de cultivos rotativos como los de *Hedysarum*, tampoco esperaríamos cambios demográficos en las poblaciones de polinizadores a esta escala.

Por último, el éxito reproductivo de las especies implicadas tanto de plantas como de polinizadores, junto con otros parámetros demográficos de las etapas del ciclo vital (tasas de supervivencia, etc.), determinarán su persistencia en las comunidades invadidas. A nivel de comunidad o de red, la supervivencia o robustez ante perturbaciones como la extinción de especies, se ha relacionado positivamente con la existencia de patrones anidados y modulares en las redes de interacción (Tylianakis *et al.* 2010; Traveset *et al.* 2013). Sin embargo, estas predicciones están basadas en asunciones que en el **Capítulo 3** se ha visto que son violadas en las redes empíricas. Este es el caso del cambio de identidad de las interacciones, aun cuando las mismas especies están presentes, que amortigua el efecto de la eliminación de especies. Fenómeno que debe tenerse en cuenta para no subestimar la robustez de las redes de interacción ante la extinción de especies, aun cuando éstas están altamente interconectadas en la red, como *Hedysarum*. Aunque existen todavía muchas limitaciones metodológicas, algunos modelos dinámicos predictivos están empezando a incluir estos aspectos para una proyección más realista a largo plazo de la robustez de las redes de interacción (Kaiser-Bunbury *et al.* 2010; Ramos-Jiliberto *et al.* 2012).

Conclusiones Generales

1. *Hedysarum coronarium* es una planta leguminosa exótica que se introdujo como planta forrajera en Menorca (Islas Baleares) hace unos 150 años y donde actualmente se ha naturalizado. *Hedysarum* se integra en la dieta de los polinizadores de las comunidades receptoras donde, al igual que en su área nativa, se comporta como generalista, recibiendo la visita de 20 especies de polinizadores (aunque se estima que el número sea mayor). En su mayoría son dípteros, coleópteros y sobre todo abejas, que a su vez son generalistas; pero es la abeja de la miel la que realiza más del 80% de sus visitas.

2. A pesar de integrarse en la dieta de los polinizadores en su área de introducción, *Hedysarum* presenta limitación polínica debido a que la abundancia de polinizadores es insuficiente. Por lo tanto, otros factores, como por ejemplo la presión de propágulos para su cultivo como planta forrajera de ciclo anual o bianual, deben de ser más determinantes que la polinización para la persistencia y expansión de *Hedysarum* en Menorca.

3. *Hedysarum*, a través de su despliegue floral ejerce un efecto magnético directo sobre la planta nativa *Muscari comosum* atrayéndole polinizadores, principalmente la abeja de la miel, cuando ésta crece en su vecindario. Simultáneamente, a través de su parte vegetativa, *Hedysarum* interacciona con otras plantas por el uso de otros recursos, disminuyendo la diversidad floral en su vecindario. Esta disminución en la diversidad de recursos florales afecta positivamente a la tasa de visitas y negativamente a la fructificación de *Muscari*. De este modo, *Hedysarum* también ejerce un efecto indirecto sobre la polinización y éxito reproductivo de *Muscari*. Estos efectos directos e indirectos son aditivos para la tasa de visitas, pero se contrarrestan para el porcentaje de fructificación. Por tanto, la mayor tasa de visitas no se traduce en un mayor éxito reproductivo de *Muscari* en los vecindarios invadidos.

4. A escala local, si se tiene en cuenta toda la comunidad de plantas en flor acompañantes, el efecto magnético de *Hedysarum* desaparece. De hecho, desde un punto de vista cuantitativo, la presencia de *Hedysarum* no altera ni la tasa de visitas, ni el grado de generalización, ni el solapamiento de nicho, ni cuán de dependientes son los polinizadores de las plantas residentes (*species strength*). Esta falta de efecto es independiente de la similitud en morfología floral entre *Hedysarum* y las plantas residentes; es decir, *Hedysarum* no compite por los polinizadores con mayor intensidad con otras leguminosas que con plantas que no lo son.

5. Sin embargo, a esta misma escala local, la polinización de las plantas en flor acompañantes sí se ve alterada en términos cualitativos por la presencia de *Hedysarum*, ya que la identidad de las interacciones planta-polinizador cambia en las comunidades invadidas*. Hedysarum* monopoliza las visitas de la abeja de la miel de modo que los recursos florales de las plantas acompañantes quedan accesibles para otros polinizadores, principalmente abejas.

6. El cambio en la identidad de las interacciones planta-polinizador tiene implicaciones en la estructura de las redes planta-polinizador invadidas, que aumentan de forma moderada pero generalizada su modularidad y asimetría. Sin embargo, otras propiedades como el anidamiento, se mantienen. Parece que independientemente de la identidad de las interacciones, y por tanto de la presencia de *Hedysarum*, las redes planta-polinizador tienden a estructurarse de forma anidada, lo cual probablemente minimice la competencia interespecífica y maximice la coexistencia de especies y biodiversidad en estas comunidades.

7. El cultivo de *Hedysarum* como planta forrajera en Menorca supone un cultivo de floración masiva que afecta a las comunidades de polinizadores del paisaje circundante dominado por comunidades herbáceas y arbustivas propias del encinar-acebuchal. Estos cultivos monopolizan a la abeja de la miel, que es atraída no sólo desde zonas adyacentes sino desde distancias superiores a los 500 m, mientras que las otras abejas parecen compartirlas con las comunidades adyacentes. Así, existe un vertido de abejas desde el paisaje circundante hacia los cultivos. Después de la siega de los cultivos, el vertido de abejas en el otro sentido debe de estar espacialmente diluido ya que no se observa un aumento en la abundancia de abejas en las zonas adyacentes. Por todo ello, en paisajes agrícolas como el menorquín que son, heterogéneos, parcheados y dominados por la abeja de la miel, los cultivos de floración masiva no parecen favorecer las comunidades de abejas de las áreas adyacentes.

8. La menor abundancia de abejas en zonas seminaturales adyacentes a cultivos de floración masiva de *Hedysarum* comparada con la de zonas alejadas conlleva una tasa de visitas menor a estas comunidades vegetales. A pesar de que esto pudiera afectar al éxito reproductivo de las comunidades vegetales adyacentes, al tratarse de cultivos rotativos, el efecto a largo plazo podría diluirse.

9. Gracias a esta aproximación experimental se ha observado que el efecto de una planta exótica entomófila en la polinización de las plantas residentes en la comunidad invadida varía en función de las escalas espacial y temporal de estudio. Además, el efecto también difiere, e incluso llega a ser contradictorio, en función del grupo de polinizadores en el que se centre el estudio, así como del nivel de organización al que sea evaluado el efecto (especie o comunidad). Queda así patente la complejidad de mecanismos e impactos de una planta invasora en la polinización de plantas nativas en el área de introducción y la importancia de las aproximaciones multiescala para su estudio.

10. Finalmente, para poner en un contexto global los efectos de las invasiones biológicas sobre los polinizadores y enmarcar en él nuestros resultados, se llevó a cabo una revisión bibliográfica, que incluyó un total de 143 estudios que exploraban el efecto sobre las comunidades de polinizadores de las invasiones biológicas o de la alteración del hábitat, dos de los principales componentes del Cambio Global. A partir de estos datos, que se analizaron con técnicas de meta-análisis, se observó que las invasiones biológicas tienen un efecto negativo sobre las comunidades de polinizadores de similar magnitud que el de la alteración del hábitat. El efecto se debe principalmente a la disminución de las tasas de visitas de insectos pertenecientes a grupos distintos de las abejas. Cabe destacar también que este efecto negativo en las tasas de visitas se debe más a la invasión por parte de animales que por parte de plantas.

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¡MIRA A LOS POLÍTICOS!.. ¡EL QUE NO ES ABOGADO ES INGENIERO. O MÉDICO
..O ARQUITECTO!...¡Y NO POR ESO SE VAN AL EXTRANJERO!
¡QUÉ LÁSTIMA!...
QUINO