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Implicaciones ecológicas y evolutivas del robo de néctar

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Implicaciones ecológicas y evolutivas del robo de néctar

Ecological and evolutionary implications of nectar robbery

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INFORMA que la presente Tesis Doctoral, titulada "Implicaciones ecológicas y evolutivas del robo de néctar", que presenta la Licenciada en Biología Sandra Victoria Rojas Nossa para la obtención del título de Doctor por la Universidad de Vigo con Mención de Doctor Internacional, ha sido realizada bajo mi dirección en el Departamento de Biología Vegetal y Ciencia del Suelo de esta Universidad, y que cumple los requisitos para poder optar a dicha Mención.

Vigo, 7 de mayo de 2015

Abstract

Plants interact simultaneously with a great variety of organisms that modify their biological success and act as selective agents. In the mutualistic interactions of pollination plants receive the benefit of pollen carryover between flowers, while animals obtain resources as reward. However, rewards and other parts of the plant are also consumed by exploiters which apparently do not provide any benefit in return. Nectar robbers are insects, birds and mammals that use a hole made in the corolla to extract the nectar accumulated at the base of the flowers or spurs without entering "legitimately" through the opening of the flower. Nectar robbery has consequences that differ in direction and magnitude for the reproduction of plants through direct and indirect mechanisms, according to the characteristics of the participants in the interactions. As a result, nectar robbers may act as selective agents, modify population dynamics and constitute important pieces for the maintenance of mutualisms. However, few biological systems have been thoroughly studied making the information about this phenomenon still too scarce, fragmented and limited to specific geographic areas, hindering generalizations. The main goal of this dissertation is to study the ecological and evolutionary implications of nectar robbery from a multiscale perspective. To achieve this we first studied the spatial and temporal variability in the robbery frequency of a vulnerable plant species. We analyzed the consequences of nectar robbery for male and female components of the reproductive success in two plant species of the Iberian Peninsula with different life histories: *Petrocoptis grandiflora* a perennial herb, self-compatible and restricted distribution, and *Lonicera etrusca* a scandent shrub, pollinator-dependent and common in Mediterranean ecosystems. The direction and magnitude of the effects of two types of exploiters (nectar robbers and sap-sucking herbivores) for the pollination of *L. etrusca* were studied. We developed a model to calculate and compare the pollination effectiveness of floral visitors for pollinator-dependent plants that include aspects of foraging behaviour and pollen carryover. Then, the frequency of nectar robbery by insects and birds in plant communities from temperate and tropical regions was studied and compared. Finally, we assessed the association between the frequencies of nectar robbery with several plant traits related with floral visitors. The results show that the percentage of robbed flowers in *P. grandiflora* was highly variable over 12 years, but not among populations. Strong changes in climatic conditions that affected population abundances of the main primary nectar robbers (*Xylocopa violacea* and *Bombus terrestris*) are the most

likely cause of this variation. The nectar robbery increased fruit set and distance of pollen dispersal in *P. grandiflora*. Unlike, in *L. etrusca* nectar robbery did not modify nor male neither female components of reproductive success. These robbers promote cross-pollination as they move among flowers, pierce the base of the perianth and extract nectar. In both plants robbers have positive effects for pollination, but differences in the reproductive system and the morphological match between insect and flower determine the changes in the reproductive success of the plants. On an opposite direction, aphids extract sap from branches and petioles of *L. etrusca* before blooming causing morphological and physiological changes driving a reduction in subsequent pollination and post-pollination processes. Despite the flowers of the plant have characteristics associated with a specialized hawkmoth pollination system, pollination is commonly performed by a wide arrange of insects. Hawkmoths are in fact high qualitative pollinators in terms of one single visit to one flower. However, at the population level, hymenopterans behaving as legitimate visitors and primary robbers are the most effective pollinators of *L. etrusca*. This result is related to their behaviour, their morphology and the high frequency of floral visits. In contrast, the beetles that behave as primary robbers are the less effective pollinators and cause considerable damages to the corolla and the reproductive structures. These results demonstrate that two groups of floral visitors which apparently behave similarly (i.e. behave as primary nectar robbers) have opposite consequences for the reproduction of the same plant species. These consequences are related to their morphology, behaviour and visit frequency. This reflects the importance of detailed studies of pollination systems that analyze and compare objectively the role of nectar robbers in plant-animal interactions. The results demonstrate that nectar robbery is a very common phenomena in plant communities of temperate and tropical regions. However, within communities a high variation of the frequency of robbery among species occurs. Such variation depends on the presence of animals with the capacity (morphological and behavioural) to rob nectar, but it is also related with mechanisms that operate at different scales. At a fine-scale the floral morphology, the nectar properties and the presence of physical barriers are the most relevant characters to determine the frequency of this behaviour. On a larger ecological scale, nectar robbery is a dense-dependent phenomenon which occurs more frequently in those species that represent abundant energetic resources. Thus, nectar robbery is a frequent phenomenon in angiosperms with long and narrow flowers with high nectar production and high density of flowers. In cases when the levels of robbery are lower than expected based on plants' traits, the evolution of defensive mechanisms against robbery (such as mechanical or barriers or chemical deterrents) is highly likely. The study of selective scenarios involving the role of robbers and legitimate visitors in the evolution of plants' traits is a promising study field that requires further attention. Also, researches regarding the role of these floral visitors in the stability of trophic networks are needed to obtain a more complete picture on this complex phenomenon and its consequences for the functioning of mutualisms.

A Dieter y Amber, a mis padres, hermanas y sobrinos

A mi gran familia colombiana, austríaca y sudafricana

Abejas,
trabajadoras puras,
ojivales
obreras,
finas, relampagueantes
proletarias,
perfectas,
temerarias milicias
que en el combate atacan
con aguijón suicida,
zumbad,
zumbad sobre
los dones de la tierra,
familia de oro,
multitud del viento,
sacudid el incendio
de las flores,
la sed de los estambres,
el agudo
hilo
de olor
que reúne los días,
y propagad
la miel
sobrepasando
los continentes húmedos, las islas
más lejanas del cielo
del Oeste.

Pablo Neruda, 1957

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Cielo, aire, luz, fuerza, motivo. Compañeros de viaje, campo, y copas. Dieter y Amber, de ustedes también es este paso.

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"I could give many facts, showing how anxious bees are to save time; for instance, their habit of cutting holes and sucking the nectar at the bases of certain flowers, which they can, with a very little more trouble, enter by the mouth."

Charles Darwin, 1859

The Origin of Species

Capítulo I

Introducción general

Contexto

Las plantas con flor o Angiospermas reúnen cerca de 300 000 especies y representan el segundo mayor grupo de organismos que ha existido después de los artrópodos (Glover, 2007). Esta extraordinaria cantidad de especies ha sido el resultado de un acelerado proceso de diversificación que tomó aproximadamente 40 ma. y diferentes hipótesis se han planteado para explicar el enorme éxito biológico de este grupo de plantas (Hughes, 1982; Crepet y Niklas, 2009; Friis et al., 2011). Algunas hipótesis sugieren que este proceso podría tener relación con la diversificación paralela de grupos de animales consumidores de partes florales, polen y néctar que potenciaron los procesos evolutivos (Raven, 1977; Frame, 2003). Así, las primeras interacciones mutualistas de polinización se remontan al Cretácico con la aparición de la flor como estructura clave, responsable de desencadenar muchos de los procesos que han dado forma a la vida que observamos hoy sobre nuestro planeta (Ehrlich y Raven, 1964; Wikström et al., 2001).

En las interacciones planta-polinizador ambos grupos de organismos obtienen beneficios para su éxito reproductivo (Waser y Price, 1983; Bronstein, 1994). Los polinizadores obtienen recursos nutritivos y no nutritivos en la flor (tales como néctar, polen, carbohidratos, resinas, aceites y fragancias), mientras que las plantas reciben el servicio de transporte de polen entre las estructuras reproductivas de las flores (Faegri y van der Pijl, 1979; Simpson y Neff, 1981; Proctor et al., 1996; Dellinger et al., 2014). Este proceso reviste tal importancia que la reproducción sexual del 87.5 % de las angiospermas depende de los animales como polinizadores (Ollerton et al., 2011).

Explotación de las interacciones de polinización

Las recompensas ofrecidas por las plantas a sus polinizadores son aprovechadas por animales “explotadores” que aparentemente no proveen ningún servicio o recompensa a cambio y por ello largamente se les consideró antagonistas, engañadores o parásitos de los mutualismos planta-polinizador (Bronstein, 2001). No obstante, los resultados de los explotadores no siempre son negativos para los mutualistas. El tipo de recurso, el comportamiento particular para acceder al mismo y una serie de atributos de los participantes de la interacción pueden tener consecuencias contrastantes sobre el comportamiento de otros visitantes florales y finalmente contribuir al proceso reproductivo de las plantas (Maloof e Inouye, 2000; Genini et al., 2010).

Las interacciones mutualistas de polinización también están sometidas a la explotación por diversos organismos. Algunos consumen directamente partes de la flor (florívoros), o las recompensas (en el caso de los robadores, los ladrones de néctar o los ladrones de polen) reduciendo el éxito reproductivo de las plantas (Maloof e Inouye, 2000; McCall e Irwin, 2006). También, hay explotadores que consumen tejidos fotosintéticos o savia y que perjudican la reproducción de la planta hospedera a través de rutas fisiológicas que no son tan obvias. Por ejemplo, el forrajeo por herbívoros masticadores genera una reducción en el número de flores estaminadas, el número y el tamaño de polen y óvulos y la reducción en el tamaño de las flores afectando así el proceso de polinización (Strauss, 1997; Krupnick y Weis, 1999; Lehtilä y Strauss, 1999; Mothershead y Marquis, 2000). También el forrajeo de savia por herbívoros chupadores, como los áfidos, causa una disminución en la producción de flores y frutos en especies nativas y cultivadas (Blackman e Eastop, 1984, 2006; Snow y Stanton, 1988; Van Emden y Harrington, 2007; Alford, 2012).

Sin embargo, bajo determinadas circunstancias los explotadores pueden ejercer efectos positivos generando un incremento en el éxito reproductivo de las plantas e incluso pueden ser importantes para la estabilidad de las redes mutualistas y la diversidad genotípica y fenotípica asociada a ellas (Irwin et al., 2003; Wilson et al., 2003). La dirección y magnitud de estos efectos depende de una compleja red de factores y mecanismos que actúan simultáneamente (Ferrière et al., 2007; Irwin et al., 2010). Por este motivo, los estudios dedicados a entender las causas y las consecuencias ecológicas y evolutivas de las interacciones planta-animal deben involucrar una visión multiescala de los posibles factores y mecanismos involucrados.

Los robadores de néctar y su comportamiento

La explotación de néctar puede dividirse en tres categorías dependiendo de la forma en la cual los animales acceden a este recurso (Inouye, 1980, 1983). El **robo de néctar** implica que el animal abre una perforación en el perianto con sus estructuras bucales (robador primario), o hace uso de una perforación hecha por otro robador

(robador secundario) para introducir su lengua y acceder al néctar (Fig. 1.1). En el **ladroneo de néctar** no se producen daños en la corola, pero diferencias en la morfología del animal con respecto a la morfología floral hacen poco probable que éste contacte las estructuras reproductivas de la flor y la polinice. Y finalmente, en el **trabajo de base** los visitantes florales acceden al néctar entre los pétalos de una flor simpétala.

Dentro de éstas categorías el robo de néctar ha sido mejor documentado, en parte, gracias a las visibles marcas que dejan los robadores en las flores (Irwin et al., 2010). Tales marcas son evidencias informativas que permiten hacer inferencias sobre el comportamiento de estos organismos en la flor incluso mucho después de que se produjo la visita (ver Barrows, 1980; Navarro, 2000; Utelli y Roy, 2001; Irwin, 2006; Rojas-Nossa, 2007; Teppner, 2011; entre otros). Gracias a ello, las interacciones entre plantas, polinizadores y robadores de néctar son ideales para entender las consecuencias de diferentes tipos de visitantes florales para la ecología y evolución de los sistemas de polinización.

Figura 1.1. Insectos y aves son los robadores de néctar más comunes. **a)** *Diglossa humeralis* realizando robo primario de néctar en *Cavendishia nitida* (La Calera, Colombia. Foto: Rojas-Nossa, 2007). **b)** Abeja usando una perforación hecha previamente por *Diglossa bruneiventris* (robo secundario de néctar) en flor de *Nicotiana glauca* (Huacarpay, Perú).

Una gran diversidad de insectos, aves y mamíferos nectarívoros se comportan como robadores primarios o secundarios de néctar (Maloof e Inouye, 2000; Irwin et al., 2010). Sin embargo, éstos visitantes florales no se comportan únicamente como robadores, sino que con mayor o menor frecuencia suelen también realizar visitas legítimas (i.e. a través de la boca de la flor *sensu* Inouye, 1980) a las flores de la misma u otras especies de plantas de acuerdo a su morfología y a la de la flor. Con frecuencia estos animales tienen probóscides cortas y estructuras bucales fuertes y cortantes que les permiten perforar las corolas, pero también las capacidades cognitivas para cambiar sus estrategias de extracción de néctar de acuerdo a la morfología y la posición de la flor (Rojas-Nossa, 2007).

Varias especies de insectos de los órdenes Hymenoptera y Coleoptera se comportan como robadores primarios de néctar en diversas regiones geográficas alrededor del mundo (Fig. 1.2). Dentro de Hymenoptera, los géneros *Xylocopa*, *Bombus, Megachile* y *Trigona* son conocidos por perforar las corolas de una amplia diversidad de plantas nativas y cultivadas (ver Barrows, 1980; Maloof e Inouye, 2000; Goulson, 2003; Castro et al., 2009; Zhang et al., 2009a, 2009b, 2011; Zhu et al., 2014; Irwin et al., 2010, entre otros). Las abejas del género *Xylocopa* son de gran tamaño y poseen una galea maxilar en forma de cuchillo usada por el animal para abrir huecos en material vegetal para hacer su nido (Gerling et al., 1989). Estas estructuras bucales también son usadas para abrir incisiones alargadas en el perianto para robar el néctar. Otras abejas usan las mandíbulas para morder los tejidos florales y así abrir las perforaciones (Inouye, 1983; Teppner, 2011). Aunque aún se desconocen las características que facilitan el robo por parte de ciertas especies y de otras no, algunos abejorros y abejas tienen la capacidad de aprender el comportamiento de forrajeo por observación de otros miembros de su especie e incluso de especies diferentes (Chittka y Leadbeater, 2005; Leadbeater y Chittka, 2008; Sherry, 2008; Goulson et al., 2013).

Diversos grupos de aves en regiones templadas y tropicales han sido observadas comportándose como robadoras de néctar (Askins et al., 1987; Traveset et al., 1998, Lasso y Naranjo, 2003; Vogt, 2006; Geerts y Pauw, 2009; Anderson et al., 2011). No obstante, los casos mejor documentados ocurren en ecosistemas Neotropicales (Fig. 1.2). En zonas de tierras bajas la Reinita mielera (*Coereba flaveola*) y otras paseriformes son robadores frecuentes en flores polinizadas por otras aves (Gill et al., 1998; Fumero-Cabán y Meléndez-Ackerman, 2007; Aximoff y Freitas, 2009). En áreas montañosas de Centro y Sudamérica los pinchaflores del género *Diglossa* son aves predominantemente nectarívoras con características particulares que las sitúan como las aves paseriformes robadoras de néctar más especializadas (Schondube y Martínez Del Rio, 2004). La maxila termina en un gancho usado por el ave para asir la flor mientras realiza un hueco en el perianto con la mandíbula puntiaguda e inserta repetidamente la lengua para extraer el néctar (Fig. 1.1a). La morfología particular y las características fisiológicas del sistema digestivo les permiten alimentarse eficientemente de néctar floral (Moller, 1932; Skutch, 1954; Villeumier, 1969; Schondube y Martínez Del Rio, 2003). Estas aves forrajean mayormente en flores polinizadas por colibríes y se considera que son actores importantes en las interacciones de polinización de los ambientes montañosos Neotropicales gracias su diversidad, a sus grandes abundancias poblacionales en determinados ecosistemas y a los efectos que tienen sobre la reproducción de las plantas que visitan (Arizmendi et al., 1996; Navarro, 1999, 2001; Arizmendi 2001; Rojas-Nossa, 2007, 2013; Navarro et al., 2008; Pelayo et al., 2011). No obstante, aún existen grandes vacíos en el conocimiento de las implicaciones ecológicas y evolutivas de estos robadores de néctar sobre los sistemas de polinización en estas regiones megadiversas.

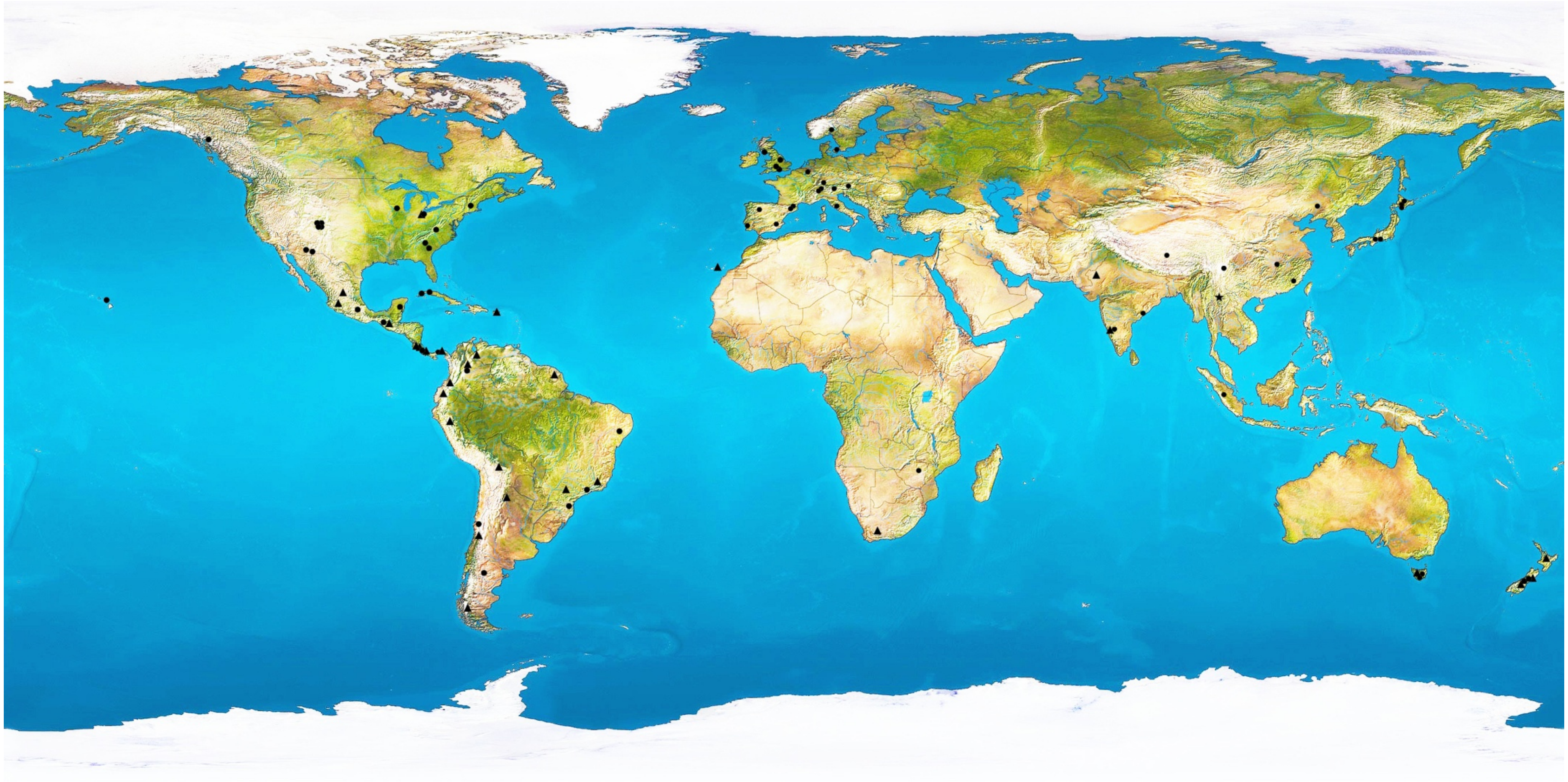


Figura 1.2. Casos de robo primario de néctar por insectos (puntos), aves (triángulos) y mamíferos (estrellas) alrededor del mundo. Se representan las localidades para 142 publicaciones que reportan este comportamiento.

A pesar de los procesos coevolutivos que caracterizan las estrechas interacciones mutualistas entre los colibríes (familia Trochilidae) y sus plantas alimenticias (Feinsinger y Colwell, 1978; Stiles, 1981, 1985; Kodric-Brown et al., 1984; Cotton 1998), las evidencias revelan que algunas especies se comportan ocasional o regularmente como robadores primarios y/o secundarios de néctar (McDade y Kinsman, 1980; Feinsinger et al., 1987; Navarro, 1999; Lara y Ornelas, 2001; Kjonaas y Rengifo, 2006; Maruyama et al., 2015). Ornelas (1994) propuso que las estructuras en forma de sierra presentes en el tomio de algunas especies podría facilitar el robo de néctar. Aunque no se ha puesto a prueba la validez de esta hipótesis, recientes evidencias sugieren que esas estructuras también son útiles para el proceso de ingestión de néctar (Rico-Guevara y Rubega, 2011). Para lograr un mejor entendimiento de los cambios en las estrategias de forrajeo de estas aves y sus consecuencias es relevante documentar mejor los casos en los cuales los colibríes actúan como robadores y evaluar su impacto sobre el servicio de polinización.

Aunque se han documentado casos de mamíferos robadores, como la ardilla *Tamiops swinhoes hainanus* en los bosques tropicales del sur de China (Deng et al., 2004), este es un grupo en donde el robo de néctar ha sido poco observado y estudiado (Fig. 1.2).

Causas del robo de néctar

Se ha observado robo de néctar en más de 240 especies de angiospermas pertenecientes a 59 familias diferentes alrededor del globo excepto en la Antártica (Fig. 1.2; Maloof e Inouye, 2000; Irwin y Maloof, 2002). Las limitaciones morfológicas que poseen los animales de lenguas cortas para acceder al néctar acumulado en flores de corola larga o con espolones son la causa más evidente de este fenómeno. No obstante, no todos los individuos ni todas las especies de plantas con corolas largas presentan similares frecuencias de robo de néctar. Por el contrario, se ha documentado una gran variabilidad en los niveles de robo experimentado por diferentes individuos, parches, o poblaciones de la misma especie, o entre especies de una misma comunidad (Morris, 1996; Irwin et al., 2001, 2010; Rojas-Nossa, 2007). Mientras que en algunas plantas se observa robo de néctar ocasionalmente, en otros casos prácticamente todas las flores de una población están perforadas (Roubik et al., 1985; Guitián et al., 1994; Maloof e Inouye, 2000; Utelly y Roy, 2001). Se han planteado diferentes hipótesis para explicar esta variación, tales como cambios temporales o espaciales en la disponibilidad de recursos alternativos, cambios temporales en las abundancias poblacionales de los robadores, o diferencias en las características de las plantas que las hacen más apetecibles para los robadores (Navarro, 2000; Irwin y Maloof, 2002; Rojas-Nossa, 2013). Sin embargo, a pesar de la importancia que esta variabilidad puede representar para la reproducción de las especies, la dinámica de las comunidades y la estabilidad de las redes tróficas, los factores que determinan las diferencias en los niveles de robo de néctar en las plantas han sido prácticamente inexplorados.

Consecuencias del robo de néctar para la reproducción de las plantas

Aunque el comportamiento de robo de néctar ha llamado la atención de los científicos por siglos (ver por ejemplo Sprengel, 1793; Darwin, 1859, 1876), solo hasta después de 1972 se comenzaron a publicar regularmente trabajos relacionados con este tipo de interacción (Fig. 1.3). Aunque le mayor parte de ellos ha evaluado el efecto del robo de néctar sobre la reproducción de las plantas (ver la última revisión de Irwin et al., 2010), solo recientemente se les ha comenzado a considerar participantes importantes en las interacciones de polinización, agentes de la evolución floral y piezas clave en el mantenimiento y estabilidad de los sistemas mutualistas (Navarro y Medel, 2009; Genini et al., 2010; Wang et al., 2015).

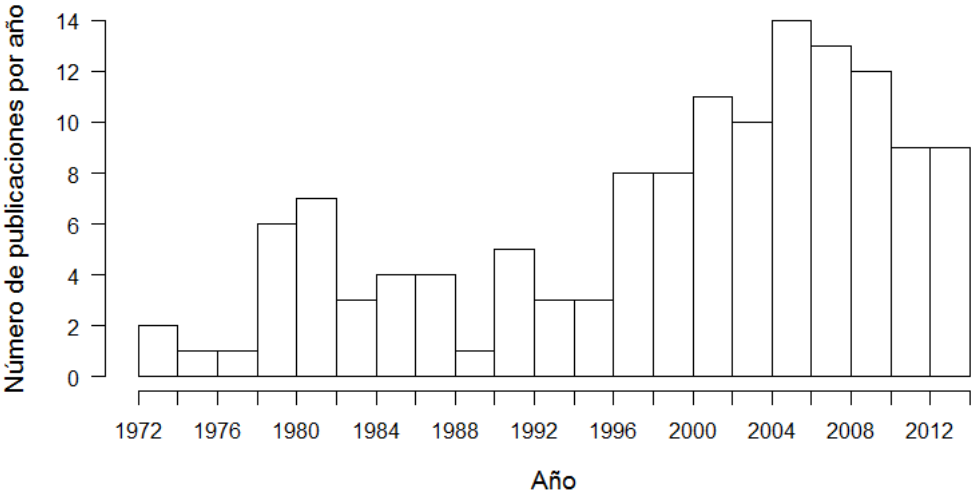


Figura 1.3. Número de publicaciones científicas relacionadas con robo de néctar desde 1972 hasta el presente.

Además de su ubicuidad, la relevancia de los robadores de néctar se relaciona con el hecho de que comúnmente modifican directa o indirectamente el éxito reproductivo de las plantas de las cuales se alimentan. Las consecuencias del robo de néctar dependen de diferentes factores relacionados con la identidad de los participantes (plantas, polinizadores y robadores), y el ambiente en el cual ocurren las interacciones. Esos factores incluyen por ejemplo el nivel de limitación por polen y el sistema reproductivo de la planta, o detalles del comportamiento, la morfología y la fisiología de los visitantes legítimos y los robadores (Burkle et al., 2007; Zhang et al., 2009a; Irwin et al 2010). En muchos casos la influencia de los robadores sobre la reproducción de la planta ocurre de forma directa. No obstante, en muchos casos los efectos son indirectos, de manera que el forrajeo de los robadores afecta el comportamiento de otros visitantes florales, afectando los servicios de polinización y producíendo así cambios en el éxito reproductivo de la planta (Maloof e Inouye, 2000). Tales cambios pueden involucrar una reducción o un aumento en diferentes variables que miden el éxito reproductivo y comúnmente han sido estudiados en uno de los dos componentes de la reproducción (i.e. masculino o femenino) y en sistemas aislados. En pocas ocasiones el resultado del robo de néctar es neutro, es decir que no se observan cambios significativos, ni positivos ni negativos, sobre alguno de los componentes del éxito reproductivo de la planta o sobre ambos (Zimmerman y Cook, 1985; Morris, 1996; Maloof, 2001; Richardson, 2004a).

La mayoría de los estudios ha encontrado que el robo de néctar tiene consecuencias negativas sobre el componente femenino del éxito reproductivo de las plantas, es decir, causa una reducción en la cantidad y/o calidad de frutos y semillas (ver Irwin et al., 2001, 2010; Burkle et al., 2007; Wang et al., 2013). Esto ocurre en casos en los cuales los robadores ocasionan daños a las estructuras reproductivas de la flor o cuando disminuyen su longevidad (McDade y Kinsman, 1980; Traveset et al., 1998; Zhang et al., 2007, 2009b; Milet-Pinheiro y Schlindwein, 2009). También cuando hay una reducción en los recursos para la formación de frutos y semillas debida al exceso de producción extra de néctar que la planta debe realizar para compensar el néctar consumido por los robadores (Navarro, 2001). Otros estudios han encontrado que el robo de néctar puede ser negativo para el componente masculino de la reproducción, es decir sobre la donación de polen. En algunas ocasiones se producen pérdidas considerables de polen durante el forrajeo de los robadores (Navarro, 1999; Navarro et al., 2008; Irwin et al., 2010). En otros casos, los daños a los tejidos florales pueden generar un acortamiento en la duración de la fase masculina causando una reducción en la donación de polen, aunque no se producen cambios significativos en el tiempo de vida total de la flor (Temeles y Pan, 2002).

El robo de néctar también puede tener consecuencias indirectas negativas sobre la reproducción de las plantas. Estas pueden involucrar defensa de territorios de alimentación por parte de los robadores que reducen las visitas de los polinizadores disminuyendo la cantidad semillas producidas (Roubik, 1982), o cambios en la estrategia de extracción de néctar de los polinizadores cuando éstos comienzan a comportarse como robadores secundarios (Inouye, 1983; Roubik et al., 1985). Sin embargo, una de las formas más comunes en las cuales los robadores afectan indirectamente la reproducción de las plantas consiste en la reducción de la cantidad o la calidad del néctar disponible para otros visitantes florales (McDade y Kinsman, 1980; McDade y Weeks, 2004; Newman y Thomson, 2005; Castro et al., 2008). Como consecuencia, puede producirse una reducción en la frecuencia de visitas o el tiempo que pasa el polinizador en cada flor y esto a su vez genera una disminución en la calidad del servicio de polinización (Irwin y Brody, 1998; Irwin, 2000; Dohzono et al., 2008).

Los efectos de los cambios en la cantidad y calidad de la recompensa se relacionan estrechamente con las capacidades sensoriales y los requerimientos energéticos de los visitantes florales (Goulson et al., 2001). Por ejemplo, algunos colibríes evitan visitar inflorescencias o plantas con una gran proporción de flores robadas, ya que a partir de un umbral éstas dejan de ser un recurso energéticamente rentable para estos animales con elevadas tasas metabólicas (Gass y Montgomerie, 1981; Irwin y Brody, 1998, 2000; González-Gómez y Valdivia, 2005). Estas aves poseen las capacidades sensoriales y cognitivas para detectar y evitar plantas robadas con escasas recompensas, pero los mecanismos que usan para ello son aún poco conocidos (Irwin, 2000). En otros sistemas, el robo de néctar reduce la cantidad de néctar disponible para los abejorros polinizadores afectando negativamente la distancia de dispersión de polen y la producción de semillas (Castro et al., 2008).

Sin embargo, hay también casos en los cuales el robo de néctar puede ser positivo para la reproducción de las plantas (Maloof e Inouye, 2000). Directamente, los robadores promueven la polinización cuando sacuden las flores o cuando contactan las estructuras sexuales de la flor con su cuerpo durante el forrajeo y ocasionalmente pueden incluso ser polinizadores más eficientes que los visitantes legítimos (Waser, 1979; Graves, 1982; Morris, 1996; Higashi et al., 1998; Navarro, 2000; Utelli y Roy, 2001; Zhu et al., 2010; Zhang et al., 2014). Indirectamente, la reducción en los niveles de néctar disponible causada por los robadores puede tener consecuencias positivas cuando los visitantes legítimos visitan un mayor número de flores o incrementen las distancias entre las plantas visitadas para suplir sus requerimientos energéticos promoviendo así un aumento en los niveles de entrecruzamiento genético de la población (Zimmerman y Cook, 1985; Richardson, 2004a; Singh et al., 2014).

Estos casos de estudio reflejan una gran complejidad en la cual diversos factores interactúan produciendo diferentes resultados para la reproducción de las plantas y a mayor escala para los sistemas de polinización y las interacciones planta-animal. Aunque se han propuesto algunos elementos claves que permitirían predecir la posible dirección de las consecuencias para las plantas (Stout et al., 2000; Burkle et al., 2007), una de las mayores dificultades para realizar generalizaciones radica en que en casi todos los estudios se tiene en cuenta solamente uno solo de los componentes del éxito reproductivo (bien el femenino o el masculino). En pocos casos se han estudiado simultáneamente ambos componentes y los resultados son contrastantes. Por ejemplo, es común que uno de los componentes se vea negativamente o positivamente afectado, mientras que en el otro componente no se observan cambios significativos (ver Irwin et al., 2010 para una revisión sobre casos con insectos robadores). También es notable que cuando se mide un mismo componente por medio de diferentes variables, los resultados no siempre apuntan a la misma dirección, dificultando generalizaciones. Por ejemplo, el robo de néctar puede afectar la cantidad de semillas producidas pero no su peso (Castro et al., 2008). Por estos motivos, para comprender en profundidad los diferentes factores relacionados con el robo de néctar y sus consecuencias para la reproducción de las plantas y los sistemas de polinización se requiere que los estudios involucren diferentes variables que permitan evaluar ambos componentes del éxito reproductivo de las plantas.

Adicionalmente hay que considerar que las plantas interactúan simultáneamente con una gran diversidad de organismos que pueden actuar como mutualistas o antagonistas a diferentes escalas ecológicas influenciando la reproducción, la dinámica de las poblaciones y por consiguiente su evolución (Karban y Strauss, 1993; Althoff et al., 2005). Considerando esto, es de esperar que los efectos de algunos organismos tengan consecuencias sobre otros miembros de las complejas redes de interacciones que ocurren en la naturaleza (Vázquez et al., 2009; Genini et al., 2010). Para lograr un mejor entendimiento y generar con ello la capacidad de proteger y conservar los vitales servicios de polinización actualmente amenazados, es urgente incorporar en los estudios la mayor cantidad de actores que pueden tener una influencia sobre la ecología y evolución de los sistemas polinización (Sahli y Conner, 2007; Kearns et al., 1998; Potts et al., 2010).

Protección contra el robo de néctar

En los casos en los cuales el robo de néctar es negativo para la reproducción de las plantas, es probable que éstas desarrollen mecanismos para evitar o reducir estos efectos y se ha propuesto que varios atributos florales podrían tener un papel importante para evitar visitas de los explotadores sin reducir la atracción a los mutualistas (Sapir y Dudley, 2013). La presencia de nectarios extraflorales o compuestos secundarios presentes en el néctar o los tejidos florales también pueden actuar como mecanismos defensivos reduciendo las visitas de los robadores de néctar (Adler, 2000; Adler e Irwin, 2005; Irwin and Adler, 2006; Kessler et al., 2008). También se ha propuesto que la agregación de las flores en inflorescencias, la presencia de brácteas o el engrosamiento de los tejidos florales pueden actuar como barreras físicas contra los robadores (Stiles, 1981; Inouye, 1983; Rojas-Nossa, 2007). No obstante, la efectividad de esos mecanismos para reducir el robo de néctar aún requiere una detallada evaluación (Irwin et al., 2010).

Objetivos y estructura de la tesis

A pesar de la importancia de los robadores de néctar para la reproducción de las plantas y la dinámica ecológica de los ecosistemas, pocos estudios empíricos han abordado este fenómeno a diferentes escalas ecológicas. Adicionalmente pocos sistemas han sido estudiados desde diversas perspectivas (pero ver Irwin y Brody, 1998, 2011), haciendo que la información sobre los robadores de néctar y sus efectos sea aún escasa, fragmentada y limitada a áreas geográficas muy puntuales. Por ello, la presente disertación explora algunos aspectos ecológicos y evolutivos del robo de néctar desde la escala microscópica de los granos de polen y las utraestructuras estigmáticas, pasando por interacciones entre pares de especies, involucrando y comparando mecanismos a nivel de comunidades y de interacciones multitróficas y finalmente llegando a una macro escala temporal y ecológica en la cual se analizan y comparan patrones a nivel de grandes regiones biogeográficas.

En los **Capítulos 2 y 3** estudiaremos las consecuencias del robo de néctar sobre los componentes femenino y masculino del éxito reproductivo de especies de plantas con diferentes historias de vida. En el **Capítulo 2** analizaremos los patrones de robo de néctar a lo largo de 12 años en tres poblaciones de *Petrocoptis grandiflora*, una planta autocompatible y endémica de la Península Ibérica. Además estudiaremos los efectos del robo de néctar sobre la producción de frutos y semillas, y sobre la distancia de donación de polen. En el **Capítulo 3** estudiaremos los efectos del robo de néctar sobre diferentes variables usadas para estimar los componentes femenino y masculino del éxito reproductivo de *Lonicera etrusca*, una especie polinizador-dependiente y común en ecosistemas mediterráneos.

En el **Capítulo 4** compararemos la dirección y la magnitud de los efectos de los herbívoros chupadores de sabia y de los robadores de néctar sobre la polinización de *L. etrusca*. Para ello se documentará la incidencia del robo de néctar y la herbivoría por áfidos en tres poblaciones de la Península Ibérica, y se analizarán las consecuencias directas e indirectas de estas interacciones sobre el servicio de polinización de la especie a diferentes escalas ecológicas (flor, individuo y población).

En el **Capítulo 5** se explorará en detalle el comportamiento de forrajeo de los visitantes florales de *L. etrusca*. Con base en aspectos de su comportamiento y su desempeño como vectores de polen estimaremos la calidad de la polinización de cada una de las especies de visitantes florales en términos de una visita a flores individuales. Adicionalmente, por medio de censos realizados a lo largo del periodo de floración durante tres años estimaremos el componente cuantitativo que permitirá calcular y comparar la importancia de los visitantes florales a nivel poblacional.

En el **Capítulo 6** cuantificaremos los niveles de robo de néctar en dos comunidades de plantas de regiones templadas en Europa y dos comunidades en ecosistemas Neotropicales. En todas las especies nectaríferas de estas comunidades se caracterizarán los diferentes atributos que se pueden relacionar con estos niveles robo con el fin de encontrar patrones en los factores que determinan las diferencias en la frecuencia de robo de néctar experimentada por plantas en ambientes contrastantes.

Finalmente, en el **Capítulo 7** se realizará un análisis integrado de los resultados obtenidos en los anteriores capítulos contextualizando el aporte de este trabajo al conocimiento de las interacciones planta-animal y delineando los principales retos para investigaciones futuras.

Chapter 2

Frequency of nectar robbery and consequences for the fitness of *Petrocoptis grandiflora*, a threatened plant species

Abstract

Nectar robbery is a common phenomenon in angiosperms with long tubular flowers or spurs. This behaviour has direct and/or indirect effects on the plant's reproduction and therefore may be considered as an important agent of evolution. However, its implications on female fitness and particularly on male fitness are restricted to a few plant species. Despite spatial and temporal variations in plant-robber interactions seem to be a constant, long term studies are needed to fully understand the effects of this phenomena for the evolution of angiosperms. In this study, we quantified the levels of nectar robbery at three populations along 12 years in *Petrocoptis grandiflora*, and assessed the effect of nectar robbery on seed set (female fitness) and on distance of pollen dispersion (male fitness). Nectar robbery levels between populations were consistent, but significant differences along years occurred. This could be affected by strong climate changes that caused low abundances and scarce foraging activity of robbers. Fruit setting and distance of pollen dispersion were positively affected by nectar robbery. Besides pollination as a possible direct consequence of robber's behavior and morphology, robbery could affect the reproductive success of this plant species indirectly, through a reduction of nectar standing crop of robbed flowers which promotes an increase of flying distances for legitimate visitors. Nectar robbery in *Petrocoptis grandiflora* is frequent, but highly variable over time. This variation could be caused by annual changes in climatic patterns that affect the population abundances of the main primary nectar robbers. In the studied system nectar robbery enhance both female and male fitness, probably through direct and indirect ways.

Introduction

Plant-pollinator interactions involve fine tuned complex arrays of historical and actual factors that allow the organisms to benefit from each other and persist. Contrary to stable and predictable entities, pollination systems are stochastic and highly variable over time and space (Petanidou and Potts, 2006). Such variation includes strong seasonal changes in diversity, composition and populational abundances of the pollinator assemblage (Herrera, 1988), as well as shifts in timing, duration and intensity of blooming periods; these factors are in turn, highly dependent on local and global climatic conditions (Memmott et al., 2007).

When attracting pollinators, flowers may also attract a wide array of non-pollinating visitors that are also vulnerable to such environmental changes. Nectar robbers are animals which use a perforation in the corolla to introduce the tongue and take nectar (Inouye, 1980). This behaviour is performed by short-tongued animals, such as some insects, birds, and mammals (Irwin et al., 2010), which otherwise do not have access to nectar kept at the base of long tubular corollas or spurs. This phenomenon is very common in angiosperms (Maloof and Inouye, 2000), and primary or secondary nectar robbery has been reported in more than 240 plant species of 59 different families (Irwin and Maloof, 2002). Despite the scarcity of long term studies assessing robbery levels in broad geographic scales and/or multispecies systems (Irwin and Maloof, 2002), spatial and temporal variations in plant-robber interactions also seem to be a constant. Most plant species experience different levels of robbery among sites, years and even within the same flowering season (Morris, 1996; Navarro, 2000; Irwin and Maloof, 2002). These changes in spatial and temporal robbery levels are apparently related to shifts in visitors' assemblage and changes in availability of alternative nectar resources (Irwin et al., 2010). However, the causes of such variation in robbery levels are practically unexplored.

Besides the common presence of robbers in diverse pollination networks, recent evidences show the relevance of nectar robbers for the reproductive fitness and evolution of several plant species (Castro et al., 2008, 2009; Navarro and Medel, 2009; Irwin et al., 2010). Complexity characterizes plant-pollinator-robber interactions and its direct and/or indirect consequences for plant reproduction (Maloof and Inouye, 2000; Irwin et al., 2010). Such consequences could be negative by reducing the reproductive fitness of the plant, positive by enhancing the reproductive fitness of the plant or even neutral. The latter has no significant differences for plant fitness and has been observed in several plant species (e.g., Maloof, 2001; Lasso and Naranjo, 2003; Richardson, 2004a). Negative consequences are frequent and were observed when robbers cause significant damages to floral tissues (McDade and Kinsman, 1980; Traveset et al., 1998), when the production of additional nectar implies an extraordinary effort to the plant

(Navarro, 1999), or when nectar robbery reduces the number or quality of legitimate visits by pollinators (Navarro, 2000; Irwin and Maloof, 2002; Kjonaas and Rengifo, 2006). Behaviour of pollinators could be directly modified by territoriality (Colwell et al., 1974; Roubik, 1982), or indirectly by reducing flower attractiveness due to scarce nectar recompense (Reddy et al., 1992; Newman and Thomson, 2005; Navarro and Medel, 2009). Otherwise, nectar robbery could have positive consequences for reproductive fitness, either in a direct or in an indirect way. Among direct positive effects, robbers also visit the flowers legitimately (Stiles et al., 1992; Arizmendi, 2001; Rojas-Nossa, 2007) or transfer pollen to the stigma while robbing nectar (Higashi et al., 1998; Navarro, 2000; Zhu et al., 2010). Indirect positive effects are associated with changes in the behaviour of legitimate visitors, increasing pollinator flight distances and promoting higher outcrossing rates (Zimmerman and Cook, 1985; Irwin, 2003), or incrementing pollinator visitation rates (Heinrich and Raven, 1972; Soberón and Martínez del Río, 1985; Cushman and Beattie, 1991). In hermaphroditic plants, the reproductive fitness is the result of both female and male functions. For this reason, it is relevant to understand the implications of nectar robbery on both components. Nevertheless, in comparison with studies addressing female fitness, the effects of nectar robbery on male fitness have been addressed only in a few cases (see Irwin et al., 2010). These evidences suggest that the behaviour of nectar robbers reduces the quantity of pollen removed and sired seeds (Irwin and Brody, 2000; Irwin, 2003; Castro et al., 2009), but increases pollen dispersal distances among plants (Maloof, 2001).

In the present work we used *Petrocoptis grandiflora* as a model to study spatiotemporal patterns of nectar robbery and its consequences for the plant reproductive fitness. *P. grandiflora* is a nectar producing plant visited by both pollinators and nectar robbers, and different robbery intensities were preliminarily reported (Navarro and Guitián, 2000). The objectives of the study were: 1) to assess the frequency and variability of nectar robbery among three populations of *P. grandiflora* along 12 years, and 2) to assess the consequences of nectar robbery in both female and male components of the reproductive fitness measured through fruit production and flow distance of pollen analogues, respectively.

Methods

Study system

Petrocoptis grandiflora (Caryophyllaceae) is a perennial herb of 15-30 cm height (Fig. 2.1). The flowers are arranged in simple or compound dichasia with an average of five flowers per inflorescence (Guitián et al., 1997). Despite highly variable, in average about 70 flowers are produced per plant. The purple calyx is about 10-14 mm long, and the corolla comprises five purple petals being up to 20 mm long (Castroviejo, 2012). The flower is protandric, with ten stamens and five styles and has a mean duration of 4 days. Flowering period begins in March and ends in June. According to Pollen:Ovules index (641.4 ± 89.6) and pollen transfer experiments, the species possesses a facultatively xenogam reproductive system, in which flowers pollinated with pollen from other plant have more chances to produce fruits and seeds (Navarro et al., 1993). The fruit is a unilocular capsule with little black seeds. The first mature fruits appear in June, and by the end of July all capsules are dehiscent (Guitián et al., 1997). *P. grandiflora* offers highly concentrated nectar (between 32.2 and 41.6 % depending on flower age) to a diverse array of floral visitors. The nectar is produced and accumulated at the base of the corolla in small quantities (0.5 ± 0.3 µl, with a sugar concentration of 41.5 ± 10.1 % w/w).

Figure 2.1. *Petrocoptis grandiflora* is an endemic perennial herb with long tubular flowers (a). The marks leaved by primary nectar robbers remain until fruit and seed development (b).

The main pollinators are *Anthophora acervorum* (Hymenoptera) and *Bombylius major* (Diptera). *Xylocopa violacea* (Hymenoptera) has been recorded as primary nectar robber (Navarro et al., 1993), and preliminary observations indicate that differences in its abundance may explain the differences in robbery levels observed among populations. Other robbers are *Bombus terrestris* and *B. jonellus* (Hymenoptera) with different degrees of importance between populations (Guitián et al., 1997). *P. grandiflora* is endemic to the northwest Iberian Peninsula, with a total distribution area barely exceeding 100 km^2. It grows almost exclusively in limestone crevices, overhangs and ledges (Fig. 2.1a), occurring in small scattered populations on dolomite outcrops in the El Bierzo region and the Montes Aquilianos (Navarro et al., 1993).

Study area

This study was made in the Ourense province (northwest Spain), at three locations: Vilardesilva (650 m a.s.l.), Cobas and Estrecho (both at 450 m a.s.l.). The area is characterized by a mosaic of habitats including cultivated land and small groups of houses, limestone outcrops, holm oak woodland (with *Quercus rotundifolia*, *Arbutus unedo* and *Quercus suber*), and Mediterranean scrub communities. The populations studied were all located on limestone caves and walls (Navarro et al., 1993). The climate is subhumid Mediterranean with a Central European tendency, having a mean annual temperature of 12.3 °C and an annual precipitation of 901 mm (Navarro et al., 1993).

Variation on nectar robbery among populations and years

The incidence of nectar robbery was analyzed in the three studied populations during 1992-1997, 2002, 2003 and 2006-2009. An average of 2732.1 flowers per year was examined in order to record presence or absence of holes made by robbers. Robbery levels were calculated as the percentage of flowers with holes on the total flowers analyzed.

Effects of nectar robbery on female fitness

We assessed 420-1090 old flowers randomly chosen on 60-100 plants, approximately 2 weeks after flowering and recorded whether or not each flower (robed or non-robbed) produced fruits. Nectar robbery in this species could be readily confirmed by examining the fruits 2-3 weeks after flowering, because robbers make a visible incision at the base of the corolla tube and calyx (Fig. 2.1b). Because the accrescent calyx persists in the fruit, the scar of the incision made by nectar robbers permits an easy determination of whether the former flower was robbed or not.

Male fitness: effects on pollen flow

In order to evaluate the effect of nectar robbery on male fitness (measured as pollen dispersal distances), fluorescent powdered dyes (Radiant Colour, Richmond, CA, USA) were used as pollen analogues (Waser and Price, 1982). In each population, three replicates, separated by distances of over 100 m, were set up at the same time. In each of the replicates two focal plants of similar dimensions with more than 20 flower buds each were protected with mosquito net to prevent nectar robbery. When flowers opened, the mosquito net was removed and, at each focal plant, two sets of flowers were prepared: (1) non-robbed flowers—fluorescent dye was applied on the stamens of 20 flowers protected at the base of the corolla using transparent tape to prevent nectar robbery; and (2) robbed flowers—fluorescent dye of a different colour was applied on the stamens of 20 flowers daily subjected to nectar robbery. Nectar robbery was performed by cutting a hole at the base of the corolla and removing the available nectar daily with micro-capillary tubes. Furthermore, in each replica different coloured dyes were used to avoid erroneous results. After 3 days, 50 flowers were examined by using a UV flashlight at several distances from each focal plant (1, 2, 3, 4, 5, 10, 25–50, 50–100 m) along two opposite linear transects. The presence/absence of each dye powder deposited on the stigma was recorded for each distance.

Statistical analysis

In order to analyze the variation of nectar robbery levels in three studied populations and along 12 years we performed a One-way ANOVA. The percentage of flowers with one or more holes made by robbers every year in every population (arcsine-square root transformed) was the response variable. The model included the year as the main factor and the population as a random effect factor. Due to the absence of homogeneity of variances a pairwise multiple comparisons of Games–Howell test between years was performed (Quinn and Keough, 2002).

To test differences in the fruit setting of robbed versus non-robbed flowers a Welch *t*-test was performed. In order to test the probability of fruit setting by robbed and non robbed flowers at the three populations and 12 monitoring years, we performed a logistic regression with setting fruit or not as a dichotomous response variable and robbery, population and year as predictors. Finally, we analyzed the effect of nectar robbery on the distance of pollen analogues dispersion using a One-way ANOVA. The frequency of dyes from robbed or not robbed flowers found in every distance category (arcsine-square root transformed) was used as the response variable. Treatment (robbed or not robbed) and distance were considered as fix effects. These analyses were carried out using SPSS 19.0 for Windows.

Additionally, with the aim to test spatiotemporal effects of nectar robbery on fruit setting we used a general linear mixed model (Baayen, 2008). The response was the percentage of flowers that produced fruits from robbed or non-robbed flowers (arcsine-square root transformed), over 12 years in three populations (n = 72) into which we included these two predictors as random effects and the robbery as fixed effect. The model was fitted in R (R Core Team, 2012) using the function lmer of the R-package lme4 (Bates et al., 2012).

Results

Variation on nectar robbery among populations and years

Nectar robbery in *P. grandiflora* was present at the three studied populations (Fig. 2.2), with an overall average of 38.4 % in 12 years (n = 32 785 flowers analyzed). No differences in robbery levels among populations were present ($F_{2, 22} = 0.046$; $P = 0.955$). Nevertheless, robbery levels were highly variable among years ($F_{11, 22} = 2.918$; $P = 0.016$), ranging between 1.8 % (s.d. = 1.45; $n = 3$) in 1997 and 51.6 % (s.d. = 10.53; $n = 3$) in 1994 (Fig. 2.2). In 1997 the robbery level was significantly lower in comparison with other years in which nectar robbery surpassed 70 %, such in 1994, 1996, 2002, 2003, 2006 and 2007 (Games-Howell test $P < 0.05$).

Table 2.1. Levels of nectar robbery and fruit set in three populations of *Petrocoptis grandiflora* over 12 years. Values are given as means ± standard deviation of the mean.

Population	Levels of nectar robbery (%)	Fruit set (%) Robbed	Fruit set (%) Non robbed	*n*
Cobas	37.8 ± 18.4	83.6 ± 11.4	57.7 ± 7.0	10967
Estrecho	38.9 ± 20.5	86.7 ± 7.4	56.5 ± 15.7	11072
Vilardesilva	36.7 ± 20.9	75.5 ± 23.9	55.7 ± 10.7	10746
General	37.8 ± 19.4	81.9 ± 16.1	56.7 ± 11.4	32785

Effects of nectar robbery on female fitness

The fruit setting of robbed and non robbed flowers presented statistical differences (Welch $t = 57.17$; $P < 0.001$). Nectar robbery had a positive effect on female fitness enhancing the probability of flowers to produce fruits. In average, 81.9 % of the robbed flowers produced fruits compared to 56.7 % of non robbed flowers setting fruits (Table 2.1). The OR (Odd Ratios) values of the logistic model indicated that robbed flowers had 4.06 more chances to produce fruits than non-robbed ones (Table 2.2). The model suggests that the fact of a flower being robbed or not was the variable which better predicts fruit set outputs, even over population or year. This result was consistent when incorporating populations and years as random effects in the GLMM model. We found that the fruit set of non-robbed flowers was 0.3 times lower than the fruit set of robbed flowers (estimate ± SE = -0.303 ± 0.040, PMCMC < 0.001).

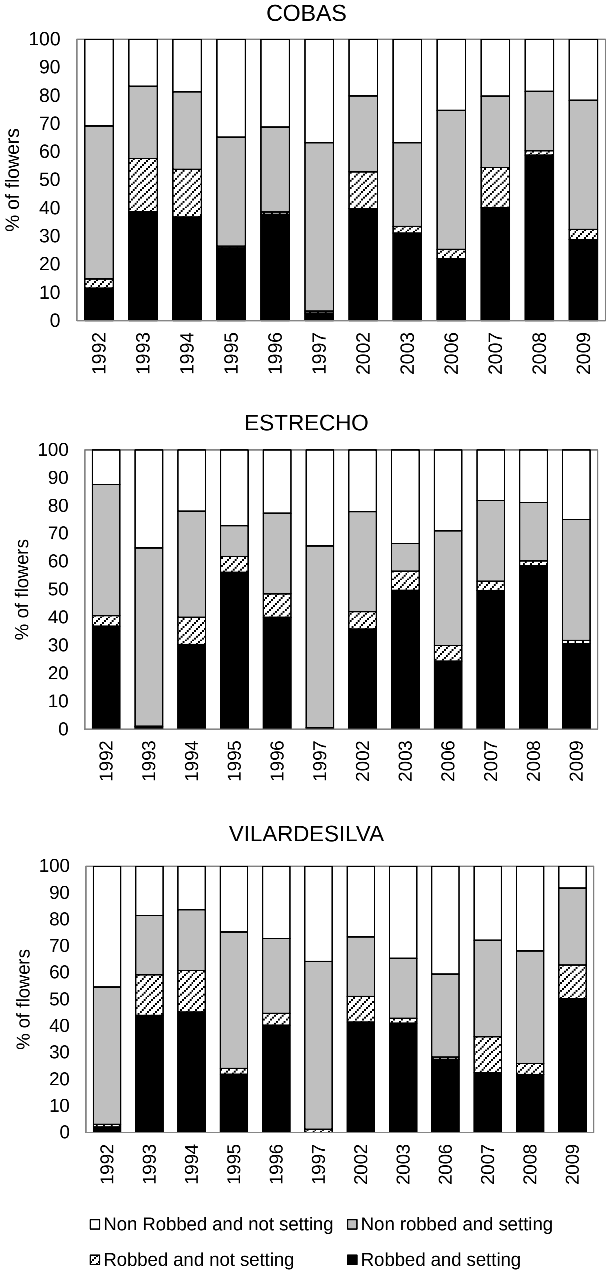


Figure 2.2. Percentage of fruit setting from flowers with and without nectar robbery at three populations of *Petrocoptis grandiflora* over 12 years.

Effects of nectar robbery on male fitness

Both robbed and non-robbed flowers of *P. grandiflora* dispersed pollen along the evaluated distances (Fig. 2.3). The distance from treated plants had significant effects on the frequency of pollen present in sampled plants ($F_{7,\,128} = 68.64$; $P < 0.001$). The interaction term (robbery * distance) was significant ($F_{7,\,128} = 4.28$; $P < 0.001$). In all cases, pollen flow was higher in the first meters and diminished as the distance to the focal plants increased. Robbed flowers dispersed pollen in lower proportions along the first 5 m (Fig. 2.3); after 5 m, the flow of pollen from non-robbed flowers was reduced, and the presence of fluorescent dye from robbed flowers was higher than dye from non-robbed flowers, indicating that robbed flowers dispersed pollen substitutes at longer distances than non-robbed flowers.

Table 2.2. Logistic regression analysis for the effects of nectar robbing, population and year on fruit set.

Variable	Coefficient	SE	χ^2	*P*	Odds Ratio
Robbery (1)	1.401	0.029	2398.517	<0.001	4.058
Population - Estrecho	-	-	37.759	<0.001	-
Population (1) – Cobas	0.186	0.030	37.638	<0.001	1.205
Population(2) - Vilardesilva	0.082	0.030	7.449	0.006	1.085
Year	0.002	0.002	9.251	0.002	1.006
Constant	-12.581	4.204	8.955	0.003	0.000

Discussion

Nectar robbery in flowers of *P. grandiflora* is frequent, but highly variable over time. In our study, a simultaneous decrease of robbery levels in all populations occurred in 1997. The same pattern was present in *Anthyllis vulneraria* present in the area (Navarro, 2000). Both plants share main legitimate visitors (e.g. *Anthopora acervorum*) as well as nectar robbers (e.g. *Bombus terrestris* and *Bombus major*). This suggests that the same phenomena affected the levels of robbery in this particular year. The most plausible explanation lies on a decrease of population abundances of these bees caused by adverse climatic conditions. Spring 1997 was characterized by very low temperatures, strong winds and rainy days (Navarro, 2000), which could affect some of the most important steps on hymenopterans workers recruitment and colonies maintenance. First, the hibernating queens could suffer freezing and die due to unusual very low temperatures. After awaking of the surviving bees and during the constitution of new colonies such climatic conditions could affect negatively the food resource stores through two ways: a) reducing the daily foraging time of queens and workers (Heinrich, 1975) and therefore the quantity of food they bring to the colony; and b) rain washes the pollen from anthers and produces dilution of nectar which could reduce the quality of the reward obtained in each visit. Such kind of shortage in quality and quantity of food resources for immature bees causes a reduction in number and size of workers and sexuals in bumblebee colonies (Schmid-Hempel and Schmid-Hempel, 1998). Thus, the result is a reduction in bee populations with its consequent diminishing in foraging activity, including nectar robbery.

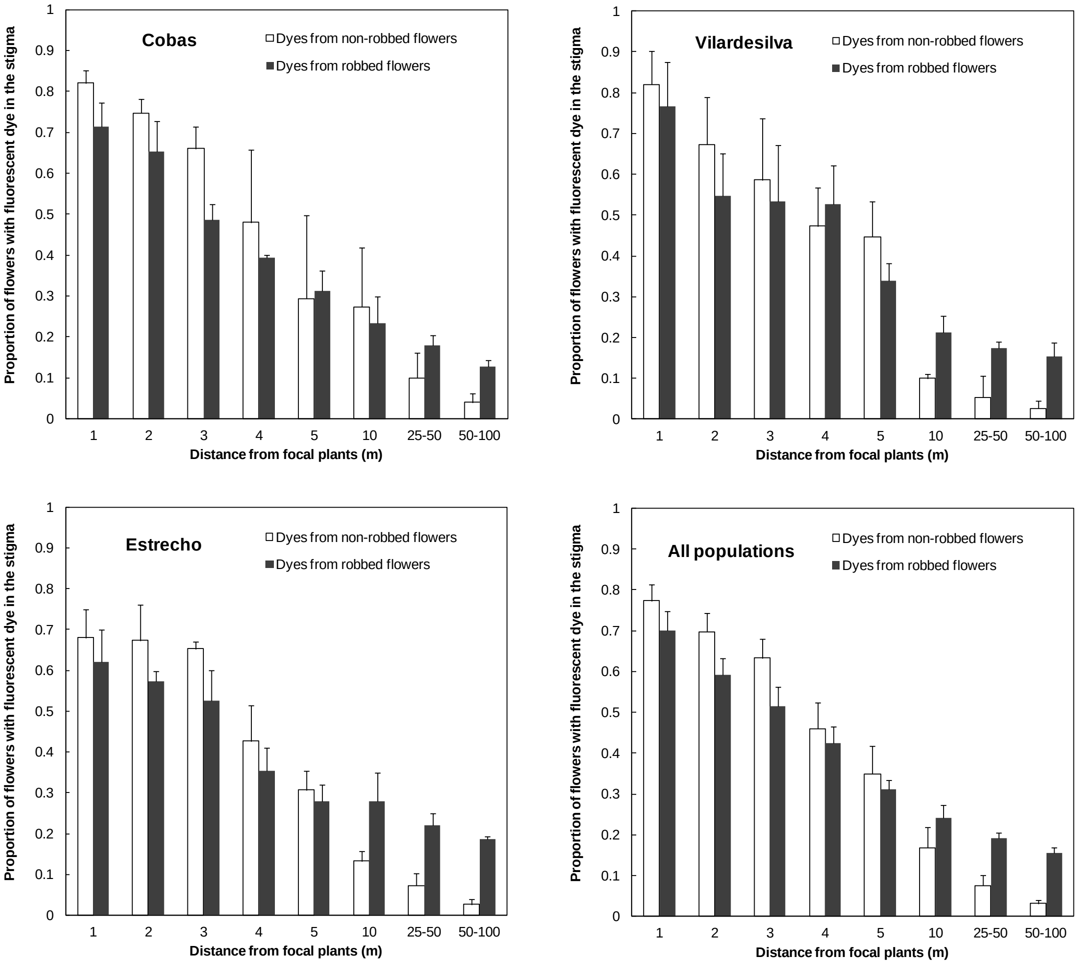


Figure 2.3. Fluorescent dye dispersion among robbed and non-robbed flowers of *Petrocoptis grandiflora*. The bars represent the average of flowers with fluorescent dye in the stigma as a function of the distance to the cluster of focal plants and the lines represent the standard deviation.

In some populations the annual patterns of robbery levels were not concordant. In 1992 very low robbery levels occurred in Cobas and Vilardesilva, while the level in Estrecho was near average. The next year this pattern was opposite with lower robbery levels in Estrecho and high levels in Cobas and Vilardesilva. This asymmetry in robbery levels between populations could be associated to changes in abundance and foraging activities of robbers (as in 1997). But it also reflects a more complex scenario related to the species assemblage, flowering phenology, local and annual abundance of main robbers, and their temporal patterns of activity and life cycle. It is remarkable that at Covas and Vilardesilva locations the main robbers are *B. terrestris* and *B. jonellus*, while at the Estrecho population *X. violacea* is almost the only robber (Navarro, 1992). Changes in abundances of these species at each locality could have been the cause for the particular patterns present in 1992 and 1993. There is probably also a strong influence of the floral phenology on robbery levels, particularly in those populations where *B. terrestris* is the main robber since this bumblebee causes flower larceny especially at the beginning of the flowering season of the whole plant community. This species is able to be active in adverse meteorological conditions, even in early spring when other insects are inactive (Lundberg, 1980). At that time of the year *P. grandiflora* is almost the only nectariferous plant susceptible to suffer robbery. After the blooming peak of *P. grandiflora* (approximately mid-spring) other plant species with high nectar rewards offer alternative resources to diverse floral visitors, including robbers (Navarro et al., 1993).

Most evidences suggest that nectar robbery is detrimental to plant fitness through diverse ways (Inouye, 1980; Irwin et al., 2001, 2010; Irwin and Maloof, 2002). Nevertheless, this study constitutes evidence of positive effects on both female and male reproductive success on a threatened Iberian species. We think that higher fruit-set in robbed flowers was caused by direct pollination of *X. violacea*, *B. terrestris* and *B. jonellus*. Since these bumblebees with big corporal size (Herrera, 1990) touch the reproductive structures of flowers during robbery activity. Robbers extracted nectar placing their bodies in inverted position to the direction of floral tube supporting the back of the abdomen above stamens and stigma. It is remarkable that differences of inflorescence morphology of different plant species seem to generate subtle changes in foraging behaviour. The flowers of *A. vulneraria* are arranged in capitula, across which the bumblebees are obligated to move in order to collect nectar from the base of the flowers (Navarro, 2000). Despite such differences in foraging behaviour, the robbers promoted pollen transfer between visited flowers of *P. grandiflora* as well as in *A. vulneraria*.

Other studies have documented pollination by robbers during foraging (Higashi et al., 1988). In self-compatible species the movements of the robber on the flower promote pollination caused by direct pass of pollen from stamens to stigmas of the same flower without a service of transport made by the animal (Kendall and Smith, 1976). Pollen transfer between plants as a consequence of nectar robbery has also been explained by different indirect ways. Some authors (e. g., Free and Butler, 1959; Hawkins, 1961; Koeman-Kwak, 1973) suggest that robbed flowers make accessible nectar that otherwise bees are not able to reach. As a consequence, bees also forage on pollen resulting in pollination.

Indirect impacts of robbery on pollen transfer are related with changes of pollinator behaviour with dissimilar results for plant reproduction (Irwin, 2003).

Heinrich and Raven (1972) proposed that in the presence of the lower nectar recompenses of robbed flowers, pollinators need to visit more flowers in order to satisfy their energetic requirements. For this reason, pollinators are forced to increase foraging distances, causing higher out-crossing and enhancing plant fitness (Zimmerman and Cook, 1985; Maloof, 2001). Until now, the evidences did not support this hypothesis and studies regarding pollen transfer of robbed flowers tended to conclude that robbery has no impact on male fitness (Morris, 1996), or that robbery is negative for male fitness (Irwin and Brody, 1999, 2000; Irwin, 2003; Castro et al., 2008). Conversely, nectar robbery enhanced male fitness of *P. grandiflora*, increasing the chance of pollen dispersion along larger distances. Such higher pollen flow could be attributed to the reduction in quantity of nectar reward of robbed flowers, which magnified the variability in nectar availability between flowers. Therefore, visitor foraging behaviour could have been modified, and such a change could have promoted an increase in the flight distances between plants, improving both female and male components of fitness of this threatened plant species.

In this study we found that nectar robbery is a frequent phenomena in different populations of *P. grandiflora*. However is highly variable over years. Such temporal variation could be related with changes in population abundances of the main primary nectar robbers (particularly *B. terrestris*), which are in turn related with weather conditions. Additionally, we present evidences that demonstrate a positive effect of nectar robbery for the female and male components of reproductive success of this threatened plant species of the Iberian Peninsula. This effect is probably mediated through both, direct (pollination performed by nectar robbers) and indirect ways (increment of the flying distances of pollinators).

Conclusions

Nectar robbery is common in different populations of the endemic plant *Petrocoptis grandiflora*, but highly variable across years. Despite that in most studied systems nectar robbers have negative consequences for the reproduction of the plants through direct and indirect mechanisms, in *P. grandiflora* nectar robbery has positive consequences. Robbed flowers have more chances to produce fruits (female component of the reproductive success) and disperse pollen at longer distances (male component) than non-robbed flowers. In this plant, nectar robbers perform pollination during robbery.

Chapter 3

Effect of nectar robbery on male and female reproductive success of a pollinator-dependent plant

Abstract

Nectar robbery is common among angiosperms with tubular flowers. Nectar robbers affect host fitness in different directions and magnitudes through direct and indirect ways and potentially constitute important pieces of plant-pollinator interactions. However, few studies measured the effects on both female and male components of reproductive success simultaneously leading to an incomplete idea of the consequences of this behaviour for host plants. Our aim is to assess the effect of nectar robbery on several variables used to characterize both components of reproductive success in *Lonicera etrusca*, a pollinator dependent plant with long tubular flowers that produce abundant nectar which is commonly exploited by nectar robbers. We experimentally assessed the effect of nectar robbery on pollen donation, fruit and seed production in four populations. We found that nectar robbery does not have negative consequences on the distance of pollen dispersal, quantity of exported pollen, fruit set, seed:ovule ratio and seeds' weight. Nectar robbers constitute an important part of the mutualistic interactions for *L. etrusca* and reveal the capacity of this plant to profit from an interaction that is antagonistic in other pollination systems. Diverse mechanisms that might allow the plant to compensate the energetic investment in terms of nectar taken by nectar robbers are: (1) the damages by robbers do not completely preclude the flower's functionality; (2) no significant changes in the behaviour of the pollinators as a consequence of robbery occur; (3) the robbers act as pollinators; and (4) the holes made by primary robbers promote higher visitation rates of secondary robbers that also pollinate while foraging for nectar and pollen in the same visit. The occurrence and relative importance of these mechanisms still require further research in order to get a better understanding of plant-floral visitor interactions.

Introduction

Since more than 87.5 % of angiosperms depend on floral visitors for sexual reproduction (Ollerton et al., 2011), mutualistic pollination interactions are key components for the subsistence of terrestrial ecosystems. Therefore, the current decline of diversity and abundance of pollinators is a threat to the stability of pollination services for crops and wild plants (Potts et al., 2010; Burkle et al., 2013). These organisms are part of complex interaction networks and the consequences for plant's reproduction and evolution are highly dynamic and context dependent (Gómez et al., 2007; Burkle and Alarcón, 2011). In plant-pollinator interactions both groups obtain benefits for their fitness (Waser and Price, 1983; Bronstein, 1994). But the rewards offered to pollinators are also exploited by other animals that are commonly thought as detrimental for plant reproduction (Bronstein, 2001), although some evidence suggests that occasionally those exploiters can be also efficient pollinators of host plants (Navarro, 2000; Fumero-Cabán and Meléndez-Ackerman, 2007; Zhang et al., 2014).

Nectar robbers are animals that use a hole, slit or tear in the perianth to reach the nectar accumulated within a flower (Inouye, 1980, 1983). The strength and direction of the consequences of this behaviour for plants' reproductive success depend on complex arrays of diverse factors that vary in time and space (Irwin and Maloof, 2002; Irwin et al., 2010). Some of those factors involve plant's mating system and level of pollen limitation, as well as the behaviour, morphology, and physiology of pollinators and robbers (Maloof and Inouye, 2000; Burkle et al., 2007; Castro et al., 2009; Navarro and Medel, 2009; Zhang et al., 2014). Net effects for plant reproduction range from negative to positive, and in some systems the consequences are considered to be neutral when no significant differences in plant fitness between robbed and non robbed flowers are observed (Morris, 1996; Maloof and Inouye, 2000; Richardson, 2004 a, b).

In most cases, nectar robbers have negative consequences for male or female success of plants through direct and indirect pathways, and are acknowledged as relevant participants altering pollination services (Irwin et al., 2001, 2010; González-Varo et al., 2013). Some negative direct effects include damages to reproductive organs that affect the flower's function (McDade and Kinsman, 1980; Traveset et al., 1998; Zhang et al., 2007; Milet-Pinheiro and Schlindwein, 2009). In other plants, the production of additional nectar implies an extraordinary effort that reduces resources for fruit and seed production (Navarro, 2001). Also, robbers are negative for male success when they cause significant losses of pollen during foraging (Navarro, 1999; Navarro et al., 2008; Irwin et al., 2010). The indirect effects encompass changes in the behaviour of pollinators that negatively affect pollen transfer and fruit or seed production. Negative indirect effects involve territorial defence (Roubik, 1982), changes in the visiting behaviour of pollinators that become secondary robbers in presence of holes made by primary robbers (Inouye, 1983; Roubik et al., 1985), or a decrease in visit frequency and time spent at the flower (Zimmerman and Cook, 1985; Irwin and Brody, 1998; Irwin, 2000). In all these cases, nectar robbery diminishes the quality of the pollination service causing a reduction in male success, female success, or both (Maloof and Inouye, 2000; Burkle et al., 2007; Irwin et al., 2010).

On the contrary, under certain circumstances nectar robbery can be positive for plant reproduction. Robbers can contribute directly to the pollination when the animal systematically contact anthers and stigmas during foraging (Higashi et al., 1988; Navarro, 2000; Utelli and Roy, 2001), and some studies have reported higher fruit or seed set caused by pollination performed by nectar robbers (Navarro, 2000; Zhu et al., 2010; Zhang et al., 2014). Indirectly, robbery may cause a reduction of nectar standing crop that indirectly compels the pollinators to increase the number of visited flowers or flying distances between plants, resulting in a potential increase of outcrossing levels in the population (Zimmerman and Cook, 1985). Unfortunately, very scant biological systems have been thoroughly studied making the information about nectar robbers still too scarce, fragmented and limited to punctual geographic areas to draw common patterns. Hence, a clear-cut distinction between legitimate visitors as beneficial and robbers as detrimental for the plants fitness needs to be carefully re-evaluated.

Lonicera etrusca is a host plant for a diversity of legitimate visitors, but a very high proportion of the total visits are performed by primary nectar robbers (Jordano, 1990; Guitián et al., 1993). As a result, at the end of the blooming season nearly all mature flowers present one or more holes made by robbers. Considering such high levels of nectar robbery, a reduction on some of the components of reproductive success would be expectable. However, through bagging experiments Guitián et al. (1993) found evidence suggesting that nectar robbery does not affect the fruit production of this species. Nevertheless, the consequences for plant fitness were measured only for one female component of success and no proper experimental manipulation was used to exclude nectar robbers. Because total plant fitness is the result of both female and male functions, it is relevant to measure the impacts of nectar robbery on both to fully understand its effect on plant reproduction. Nevertheless, few studies evaluated the impacts on female and male components simultaneously (Zimmerman and Cook, 1985; Maloof, 2001; Temeles and Pan, 2002; Richardson, 2004a). In these cases, divergent consequences for female and male components were found. This fact added to the lack of plant-floral visitor systems that have been thoroughly studied, make the knowledge about nectar robbers still too fragmented and limited to punctual geographic areas hindering to draw common patterns. In this study, we experimentally assess the effects of nectar robbery on several variables used to characterize both components of the plant's reproductive success to analyse how nectar robbers affect the reproduction of *L. etrusca*. Such understanding is fundamental to achieve a more complete perspective of the complex interplay between plants, pollinators and larcenists.

Methods

Study area

The study was conducted at El Bierzo region, North-west Spain. Two of the populations studied are located in the Natural Park Serra da Enciña da Lastra: Cobas A (567 m a.s.l.; 42°28'19''N, 6°50'17''W), and Cobas B (438 m a.s.l. 42°28'15''N, 6°49'26''W). A third population is located in La Barosa (590 m a.s.l. 42°29'50''N, 6°48'52''W), and the fourth at Carucedo (520 m a.s.l.; 42°29'6''N, 6°45'59''W). The region has a Mediterranean climate and the landscape is composed of a mosaic of habitats with cultivated lands and native vegetation, such as holm oak woodland (*Quercus ilex*, *Arbutus unedo* and *Quercus suber*), and different Mediterranean shrubland and pasture communities, many of them growing on former cultivated lands.

Study system

Lonicera etrusca Santi (Caprifoliaceae) is a climbing shrub native to the Mediterranean basin. In the northern Iberian Peninsula region, the blooming period starts in May and finishes in June (Guitián et al., 1993). Floral buds usually open at dusk and the fragrant flowers last three days until the sympetalous corolla falls off. The corolla has a white to pinkish colour at anthesis (Figs. 3.1a, b) and changes to yellowish-pinkish from the second day on. The flowers present long tubular corollas (32.9 ± 4.6 mm, n = 761. Chapter 4 this dissertation). Flowers are hermaphrodite with exerted stigma and five stamens with exerted anthers. Stigma is receptive at anthesis and anther maturation occurs the following day (Guitián et al., 1993). Flowers produce copious nectar that accumulates at the base of the corolla (4.1 ± 2.9 µl, 23 ± 4.1 % sugar concentration. Guitián et al., 1993). The fruit is a reddish berry with 5-7 seeds measuring about 5 × 3.5 mm (Fig. 3.1c). Based on controlled pollination experiments, Guitián et al. (1993) concluded that the species presents a self-compatible but insect dependent reproductive system. Levels of nectar robbery of *L. etrusca* were very high during the study. In spring 2010 up to 90.6 % of the flowers in Cobas A (*n* = 915), 100 % in Cobas B (*n* = 40) and 96.1 % in La Barosa (*n* = 7255) were robbed during the blooming season and significant damages to reproductive organs were infrequent (Chapter 4 this dissertation Rojas).

Macroglossum stellatarum is the most common legitimate visitor and probably the main pollinator of *L. etrusca* in the Iberian Peninsula (Jordano, 1990; Guitián et al., 1993). Other legitimate visitors include long-tongued bees and bumblebees, such as *Anthophora hispanica*, *Anthophora acervorum* and *Bombus vestalis* (Guitián et al., 1993), as well as nocturnal Lepidoptera (e. g. *Hyloicus pinastri*, *Sphinx ligustri*, *Ochropleura* *flammatra* and *Ochropleura forcipula*; Jordano, 1990). Besides nectarivorous visitors, some species of Diptera and Hymenoptera collect pollen, but in most cases they do not contact the stigma (Jordano, 1990). The holes made by primary robbers such as *Bombus terrestris* (Fig. 3.1b) and *Xylocopa violacea* are often used by other bumblebees and smaller bees that behave as secondary robbers (*sensu* Inouye, 1980).

Figure 3.1. *Lonicera etrusca* has long sympetalous flowers commonly robbed by hymenopterans. (a) shows the treatments with fluorescent dyes used to measure pollen dispersal by robbed flowers (pink dyes) and non-robbed flowers (green dyes). (b) *Bombus terrestris* bites the base of the corolla with the mandible and introduce the galea to rob nectar. The plant requires pollinators to produce fruits (c).

Effects of nectar robbery on male reproductive success: Distance of pollen dispersal

In order to evaluate the effect of nectar robbery on pollen dispersal range, we performed an experiment in spring 2011 using fluorescent powdered dyes (Radiant Colour, Richmond, CA, USA) as pollen analogues. It has been previously observed that dye transfer closely resembles pollen transfer by insects (see Adler and Irwin, 2006; and references therein). We conducted this experiment on two focal plants in the Carucedo population. The focal plants were chosen according to size, location in relation to other plants and number of flower buds. We bagged 100 buds in each plant with mosquito net to prevent visits. When flowers opened, the net was removed and, at each focal plant, two sets of flowers were treated on 3 consecutive days: (1) non-robbed flowers— the corollas of 50 flowers were protected using transparent tape to prevent nectar robbery and pink fluorescent dye was applied on their anthers with a brush (Fig. 3.1a); and (2) artificially robbed flowers— the corollas of 50 flowers were manually perforated, nectar was extracted with capillary micropipettes and yellow fluorescent dye was applied on their anthers. Every day at dusk we examined all open flowers present in the plants within a radius of 60 m from the focal plants using a UV flashlight. For each flower we recorded the presence of dye as well as the part of the flower where it had been placed in order to estimate the precision of the pollinators. Maximum precision was attained when the pollen substitute was found on the stigma only. Dyes were removed from flowers after each record to avoid recounting on the next day. The distance from each plant to the focal was measured, and the number of opened flowers was counted daily.

Effects of nectar robbery on male reproductive success: Quantity of pollen exported

We marked 90 plants in three populations (31 plants in Cobas A, 29 in Cobas B and 30 in La Barosa) at the beginning of the blooming period in 2010. Four treatments were applied to floral buds (two flowers per treatment per individual): (1) Non robbed flowers: the corollas were protected from nectar robbery with a transparent plastic-tape cover; (2) Robbed flowers: the corolla tube was artificially perforated with a micropipette, approximately in the same way (form and position) as the robbers do; (3) mixed treatment: the distal half of the corolla was protected as for treatment 1, and the proximal half perforated as for treatment 2; (4) control: unmanipulated flowers. Each treated flower was marked with indelible ink at the base of the calyx so that the mark does not affect the visitation. Threads with different colours were used to recognize treated branches within individuals.

To quantify pollen export from flowers under different treatments, anthers were carefully removed three days after anthesis by cutting the tip of the filaments to minimize the damage to the flower. All anthers of each treated flower were collected and preserved in vials with isotonic solution (ISOTON II Diluent, Beckman Coulter). At the laboratory, anthers were placed on a microscope slide with a drop of ISOTON II and all pollen grains were manually removed under a magnifying glass. Before analyzed, each sample was carefully placed in a plastic vial and immersed in a bath sonicator for 5 minutes in order to disaggregate the pollen clusters and to detach the grains from any fragments of anther tissue. The pollen sample was filtered with a 100 µm sieve and the volume was completed up to 20 ml with ISOTON II. Pollen grains in 1ml homogenized subsamples were counted with a particle counter (Multisizer 3 Coulter Counter, Beckman Coulter). The total number of grains per anther was calculated as the mean of three subsamples corrected by the dilution factor. The same procedure was performed in anthers of floral buds (90 plants, five buds each) to quantify the pollen in fresh unopened flowers. The number of pollen grains exported was calculated as the difference between the mean number of grains per anther in fresh opened flowers, minus the number of grains remaining in the anthers of the treated flowers.

Effects of nectar robbery on female reproductive success: Fruit set, seed to ovule ratio and seed weight

All the flowers treated in the previous experiment were monitored every 15 days until fruits matured. Mature fruits were collected and kept in 70 % ethanol until dissected in the laboratory. The number of viable seeds, aborted seeds and unmatured ovules was counted for each fruit. Seeds that looked viable were extracted from fruits, cleaned and dehydrated in an oven at 50 °C until constant weight. Seed weight was measured with an analytical balance (0.01 mg precision).

Statistical analyses

To test the effect of robbery on the distance of pollen dispersal we used a Generalized Linear Mixed Model (GLMM). We fitted the model using a Poisson error structure and log link function. Into this we included the treatment (robbed/non-robbed), the distance of the sampled plant to the treated plant, and the interaction term as fixed effects. The response variable was the number of flowers that were found with the respective dye on the stigma per sampled (peripheral) plant. The identity of the sampled plant and the date were included as random effects into the model. The number of flowers inspected per sampled plant (log-transformed) was included as an offset term into the model. Prior to fitting the model we z-transformed the distances to a mean of zero and a standard deviation of one. To determine the overall effect of treatment (i.e., the impact of treatment and/or its interaction with distance), we initially compared the fit of the full model with that of the null model lacking treatment and its interaction with distance but comprising all other effects and terms present in the full model. This comparison was based on a likelihood ratio test. The sample size for this analysis was 164 flowers from 40 plants, four days and two treatments.

To analyse the effect of nectar robbery on the quantity of pollen exported we followed several steps. First we used a one way ANOVA to compare differences in the quantity of pollen that was present in closed anthers among individuals. Then, we tested differences in pollen production per anther within individuals using a General Linear Model (GLM), including the individual identity as a random effect. Finally, to determine the potential influence of the four treatments (robbed, non-robbed, mixed, control) on the quantity of pollen donated, we used a GLMM. We included the treatment as a fixed effect. Plant identity, population and date were included as random effects. We used the estimated number of pollen grains exported per flower as response. We derived *P*-values using Markov chain Monte Carlo (MCMC) randomizations. We tested the significance of the random effects by removing them from the full model as described above and compared the fits of the two models using likelihood ratio tests. The sample size for this analysis was 220 flowers from 69 plants of three populations on 10 different days.

To analyze the effect of control, mixed, non robbed and robbed treatments on fruit set, number of viable seeds and total weight of viable seeds we used GLMM. We included the treatment, plant height, plant diameter (square-root transformed), the total number of flowers produced by the plant (log-transformed) and their interaction as fixed effects for fruit set and number of viable seeds models. For the total weight of viable seeds model we used treatment, total number of flowers produced by the plant (log-transformed), tube length and volume of nectar per flower as fixed effects. In all these models the plant identity was used as a random effect. Since the response for the first model (fruit set) was binary (mature vs. aborted), the model was fitted with binomial error structure and logit link function. The sample size for this analysis was 541 fruits from 64 plants. For the second model we used a Poisson error structure and log link function. To control for the number of ovules per ovary we included it as an offset term (log-transformed) into the model. To test specifically for the effect of treatment, we compared the full model with a null model lacking treatment but comprising all other terms in the full model using a likelihood ratio test. The sample size for this analysis was 105 fruits from 46 plants. The third model was fitted with a Gaussian error function and identity link. Prior to running the model we square-root transformed the seed weight and tube length (after subtracting the minimum tube length) and log transformed total number of flowers per plant and plant volume. Then, we z-transformed the predictors to a mean of zero and a standard deviation of one. The sample size was 87 seeds from 39 plants. We considered 0.05 as the level of significance. All models were fitted in R software using the packages lme4 (Bates et al., 2012) and languageR (Baayen, 2011).

Results

Distance of pollen dispersion

Both robbed and non robbed flowers dispersed more dye within the first 10 m than at any other distance (Fig. 3.2), the probability of pollen transfer decreased with the distance between treated and sampled plant (estimate ± SE = -1.01 ± 0.15, z = -6.76, $P < 0.001$). The full-null model comparison revealed that the frequency of dye deposition to stigmas of peripheral plants was not influenced by robbery ($\chi^2 = 2.01$, df = 2, $P = 0.366$).

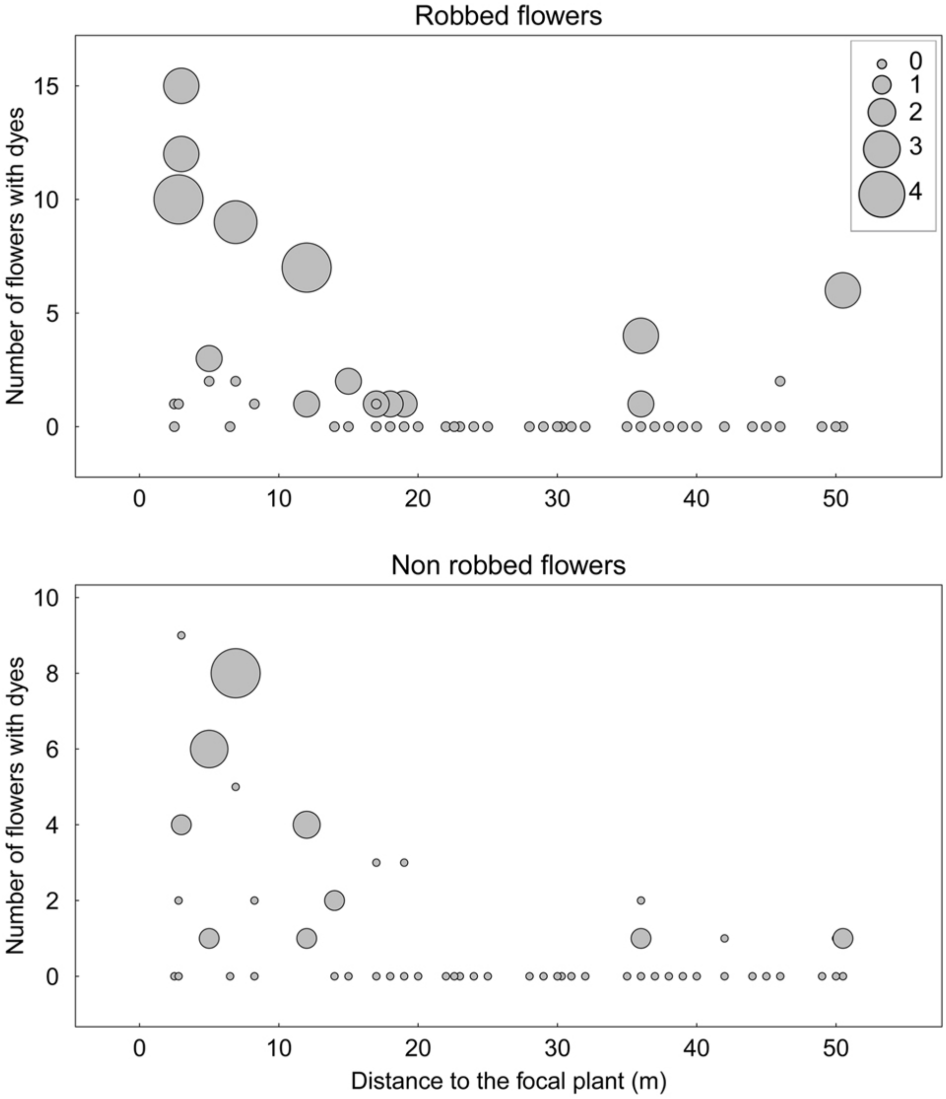


Figure 3.2. Number of flowers with fluorescent dye from non robbed flowers and robbed flowers, as a function of the distance from the focal (treated) plants. The size of the circles corresponds to the number of plants with dyes at each distance interval.

Quantity of pollen donated

We found differences in the quantity of pollen present in closed anthers among plants ($F_{73,\ 317} = 3.06$, $P < 0.001$) but not among flowers within plants ($F_{5,\ 53} = 0.88$, $P = 0.5$).The GLMM revealed that robbed flowers were able to export similar quantities of pollen than non robbed, mixed treatment or control flowers (Fig. 3.3a, PMCMC = 0.89). Other variables, such as the identity of the individual plant ($\chi^2 = 201.23$, df = 1, $P < 0.001$) and date when the anthers were collected ($\chi^2 = 1651.53$, df = 1, $P < 0.001$) had significant effects on the probability of pollen donation.

Fruit set

Despite than non robbed flowers presented higher fruit set than other treatments (Fig. 3.3b), the fruit production did not statistically differ between treatments (χ^2 = 2.83, df = 3, *P* = 0.419). More than 30% of robbed flowers were able to produce mature fruits (Fig. 3.3b) and nectar robbery had no significant effects on fruit setting. However, the probability to produce mature fruits was lower in Cobas A than in the other two populations (χ^2 = 6.01, df = 2, *P* = 0.049).

Seed:ovule ratio

Although *L. etrusca* presented an average of 10.6 ovules per flower (SD = 1.7, *n* = 92), most of the fruits produced only one or two viable seeds (mean ± SD = 1.6 ± 0.8, *n* = 88). Non robbed flowers produced occasionally fruits with five and six seeds, but the average Seed:ovule ratio was very similar between treatments (Fig. 3.3c). As for the fruit set, there was no significant effect of the treatment on the number of seeds per fruit (χ^2 = 2.30, df = 3, *P* = 0.51).

Seed weight

Despite the seeds of robbed flowers were slightly lighter than those from other treatments (Fig. 3.3d), nectar robbery was not significantly detrimental for the size of the seeds (*P*MCMC = 0.110).

Discussion

Considering the very high levels of nectar robbery that occurs in the studied populations and the high frequency of cases in which nectar robbers are detrimental for plant's reproduction (Irwin et al., 2010), we expected that robbers would generate changes in any of the components of reproductive success of *L. etrusca*. However, our results suggest that neither male nor female components are negatively affected by robbery. In only one previous study similar consequences of nectar robbery were observed (Morris, 1996). In his study, Morris (1996) reported that the foraging of two species of primary robber bumblebees did not modified the levels of pollen removal, fruit development or seed mass of *Mertensia paniculata*. This output was attributed to the particular behaviour of the bumblebees, which robbed flowers during the female phase but collected pollen during the male phase, performing cross pollination thanks to this mixed behaviour. Nevertheless, the marked dichogamy present in *M. paniculata* and the changes in behaviour of robbers observed by Morris (1996) do not occur in *L. etrusca*. In this species, primary robbers forage exclusively for nectar, and male and female phases overlap for an important part of the flowers' life (Jordano, 1990; Guitián et al., 1993). Therefore other mechanisms must be responsible for the outputs observed in our study.

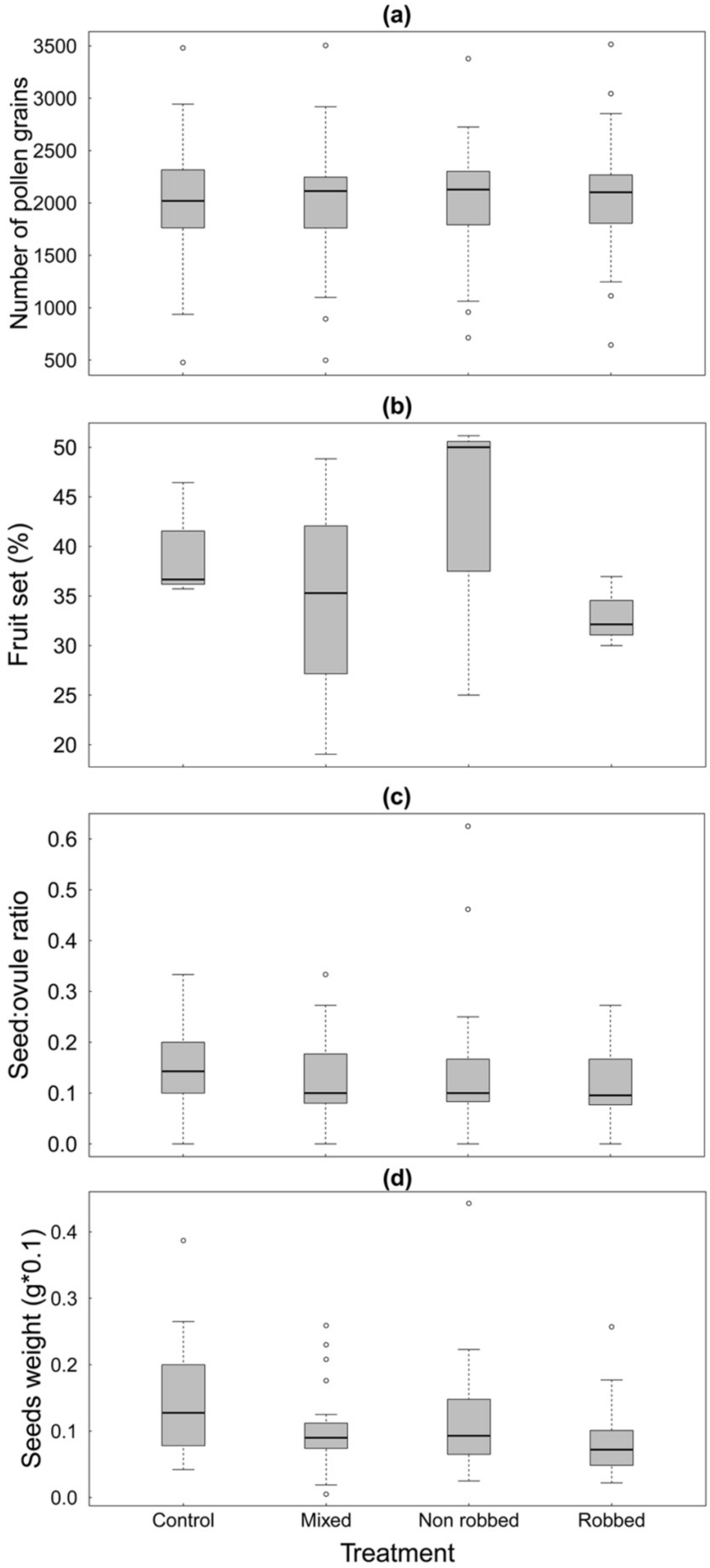


Figure 3.3. Effects of four treatments for the variables used to estimate the male and the female components of *Lonicera etrusca* reproductive success. (a) Quantity of pollen grains exported by treated flowers (b) Percentage of fruits produced by treated flowers (c) Seed: ovule ratio of treated flowers and (d) Total dry weight of the viable seeds per fruit from treated flowers. Shown are medians (horizontal lines), quartiles (boxes), 2.5 to 97.5 percentiles (vertical lines), and outliers (open dots).

In several pollination systems, nectar robbery diminishes the functionality of the flowers by shortening flower's life span, causing considerable damages to floral tissues and altering the attractiveness to pollinators (Traveset et al., 1998; Temeles and Pan, 2002; Rojas-Nossa, 2007; Zhang et al., 2007). Changes in attractiveness cause avoiding of robbed flowers or plants, or a decrease in visit frequencies by legitimate visitors that reduce pollination services (Zimmerman and Cook, 1985; Irwin and Brody, 1998; Irwin, 2000; Navarro, 2001). As a consequence, female and male components of reproduction result negatively affected, presenting a reduction in the quantity of sired seeds, pollen removal or distance of pollen dispersal (Irwin and Broody, 2000; Zhang et al., 2007; Castro et al. 2008; Irwin et al., 2010). However, since *L. etrusca* present a self-compatible reproductive system but pollen from other flower is required to produce fruits and seeds (Guitián et al., 1993), our results suggest that robbed flowers receive a similar pollination service in comparison with non robbed flowers. This indicates that robbery does not generate a reduction in flower's functionality and that the behaviour of legitimate visitors does not suffer significant alterations as a consequence of nectar robbery. Additionally, the small number of ovules per flower could contribute to achieve a maximum quantity of sired seeds with a reduced input of pollen brought by the floral visitors to the stigma.

We believe that the absence of negative effects of nectar robbery in this plant species, as well as in other species in which "neutral" effects have been recorded (Morris, 1996; Maloof and Inouye, 2000), are not due to a lack of effects of nectar robbery, but to the capacity of the plant to obtain some benefits, or at least not to result negatively affected by an interaction that is usually antagonistic for other plant species in different ecological and evolutionary contexts. This hypothesis is also supported by the fact that in systems in which nectar robbers do not cause significant changes for plant's reproduction is because they often perform pollination as well (Graves, 1982; Arizmendi et al., 1996; Morris, 1996; Utelli and Roy, 2001). In those cases robber's visiting behaviour and morphological adjustments between flowers and insects allow effective pollination that maintain or even enhance host reproductive success (Higashi et al., 1988; Zhu et al., 2010; Zhang et al., 2014).

In the Mediterranean ecosystems, the primary robbers *X. violacea* and *B. terrestris* pollinate the flowers of *Anthyllis vulneraria* and increase fruit production (Navarro, 2000). These robust bees systematically contact the reproductive flower parts while moving from one flower to the next in the inflorescences or when placing the body to open the hole and extract the nectar. The same could be occurring in *L. etrusca*, facilitated by the arrangement of the flowers in compact inflorescences that facilitate the pollination process when the insects crawl between flowers to pierce the base of the corolla and take the nectar. Similarly in other systems, it has been suggested a possible relationship between pollination by robber birds and the arrangement of flowers in dense inflorescences that allow pollen transfer while the bird pierce the flower and extract nectar (Graves, 1982; Arizmendi et al., 1996). Additionally, the robbed flowers of *L. etrusca* also receive visits from secondary nectar robbers that extract nectar through the perforations made by primary robbers (Jordano, 1990). These visitors (such as *Megachile* and *Lassioglossum* bees) commonly forage for nectar and collect pollen from anthers during the same visit. Therefore, the plant could be indirectly beneficiated by primary nectar robbery, since it allows the addition of potential pollen vectors to the system (Zimmerman and Cook, 1985; Morris, 1996; Irwin et al., 2001; Richardson, 2004; Newman and Thompson, 2005). The floral morphology, particularly the exertion of stigma and anthers, might facilitate pollination by a higher diversity of animals than expected by the 'sphingophilous syndrome' characteristic to several species of the *Lonicera* genus (Miyake and Yahara, 1998; Miyake et al., 1998).

Based on the evidence obtained in this study we consider that in several systems nectar robbers are an important part of the mutualistic plant-animal interactions, and a combination of mechanisms allows plants to compensate the energetic investment in nectar exploited by robbers. We hypothesize that some of those mechanisms are: a) Nectar robbers do not damage significantly the reproductive structures or reduce flower life span or other components of floral attractiveness as they do in other systems; b) Considering that robbed flowers receive enough cross-pollen to fully form viable seeds and develop mature fruits, this is an indication that flower's functionality and behaviour of legitimate visitors is not significantly affected by robbers. This could be favoured by the foraging activity patterns of robbers and legitimate visitors, since robbery is usually performed at midday, after the visits of crepuscular sphingids that occur right after anthesis at late afternoon (Jordano, 1990; Guitián et al., 1993); moreover, c) the main primary robbers (*X. violacea* and *B. terrestris*) are effective pollinators of other plant species which coexist with *L. etrusca* in the studied populations (Navarro, 2000), and they could also act as pollinators of this plant species. Pollination performed by robbers is facilitated by the arrangement of flowers in inflorescences and exerted reproductive structures that allow contact with the insect's body during nectar foraging. Finally, d) primary robbers make accessible a new resource to small bees that behave as secondary nectar robbers and in turn they can contribute to pollination when gather pollen during the same visit (Newman and Thomson, 2005). All those scenarios are plausible and not mutually exclusive but the feasibility and relative importance of each still need to be carefully evaluated.

Detailed studies such as presented here, analyzing the effect of nectar robbery on male and female reproductive success, are necessary to broad our knowledge on the ecological and evolutionary consequences that these illegitimate visitors can have on flowering plants for whose floral morphology are not adapted.

Conclusions

Despite that *L. etrusca* presents high levels of nectar robbery and that for most plant species robbers are negative for reproduction through direct or indirect mechanisms (Irwin et al., 2010), we found no evidence of detrimental consequences in any of the five variables used to estimate male and female reproductive success. A similar output was observed previously in only one plant species (Morris, 1996). However, in that case, bumblebees present a mixed behaviour. They rob nectar during the flower's female phase but perform legitimate visits when collecting pollen during the male phase pollinating thus the flowers of *M. paniculata* (Morris, 1996). In *L. etrusca* primary nectar robbers forage exclusively for nectar and no mixed behaviours among robbery and legitimate visits occur. Therefore, we propose that a combination of morphological and ecological mechanisms allow plants to compensate the energetic investment in nectar exploited by robbers. The evidence suggests that under diverse ecological and evolutionary scenarios the interactions among plants, legitimate visitors and robbers are very complex, and the simplistic idea of robbers as cheaters of mutualistic pollination interactions must be carefully evaluated before making assumptions about the consequences for plants reproduction.

Chapter 4

Nectar robbers and aphids: Opposite forces in a pollinator-dependent plant

Abstract

The direction and magnitude of the effects of animal-plant interactions depend on diverse ecological and physiological factors highly dynamic in time and space and that rarely occur in isolation. *Lonicera etrusca* is a pollinator dependent plant strongly attacked by sap-sucking herbivores and nectar robbers along its native range. Although aphids are important pests in several cultures and nectar robbers have commonly detrimental effects on host plants, the consequences their consequences for plants that require attracting pollinators to reproduce are unknown. The aims of our study are to document the incidence of nectar robbery and herbivory by aphids and to analyze direct and indirect (i.e. via pollinators) consequences of these interactions for the reproduction on a pollinator-dependent species. Although both nectar robbers and herbivores are present in three studied populations and most flowers were either damaged by aphids or robbed by hymenopterans or coleopterans, very few were damaged and robbed simultaneously. Nectar robbery cause changes in volume and concentration of nectar. However, the net effect for the plants is positive. Hymenopterans nectar robbers perform cross-pollination while robbing without harming reproductive structures. Oppositely, herbivory by aphids causes a reduction in the dimensions of the floral structures, the quantity and quality of pollen and ovules, and generates changes in the stigmatic papillae. Additionally, those damaged flowers have null nectar production, reducing floral visits from pollinators and thus affecting indirectly the quality of the pollination service received by plants. As a result, herbivory generates a diminishing in fruit set and weight of the seeds produced in damaged flowers but also those produced in healthy flowers in plants highly attacked by aphids. Since aphids feed on phloem sap, the loss of important quantities of nutritional materials is the most likely cause for the damages observed and the resulting reduction in the reproductive success of *L. etrusca*.

Introduction

Plants interact simultaneously with a diversity of organisms that act as selective agents by influencing directly and indirectly their reproductive success (Karban and Strauss, 1993; Althoff et al., 2005). These effects rarely occur in isolation, and some organisms may alter plant's population dynamics and evolutionary traits but also can have impacts on other members of the interaction networks (Vázquez et al., 2009; Genini et al., 2010).

Lonicera etrusca is a common shrub of the Mediterranean basin important as a food resource for many nectarivorous and frugivorous animals (Jordano, 1990). The flowers require visits from insects for pollination and fruit setting (Guitián et al., 1993). They are visited by a diversity of lepidopterans, hymenopterans, dipterans and coleopterans that collect nectar and pollen through the entrance of the corolla, but are also visited by insects that behave as nectar robbers (Jordano, 1990; Guitián et al., 1993; Chapter 3 this dissertation). This means that some are animals consume floral nectar by removing it through holes bitten in the flower by themselves or by other robbers (Inouye, 1980). In other plants, nectar robbers commonly do not perform pollination and are negative for plant's reproduction (Maloof and Inouye, 2000; Irwin et al., 2010). However, in *L. etrusca* nectar robbery is not detrimental for the reproduction despite that in some populations of more than 70 % of the flowers present holes made by robbers (Guitián et al., 1993; Chapter 3 this dissertation).

The consequences of nectar robbery can range from negative to positive for plant's reproductive success depending on complex interplaying factors related mainly to the identity and life histories of plants, pollinators and robbers (Irwin et al., 2001; Burkle et al., 2007). For instance, nectar robbers reduce fitness damaging the reproductive organs of the flower, reduce flower lifespan, induce seed abortion, or drain resources available for fruit and seed production (Traveset et al., 1998; Navarro, 2001; Zhang et al., 2007, 2011). Also, they cause changes in the foraging behaviour of pollinators that indirectly promotes a reduction in male and/or female components of reproductive success (Irwin and Brody, 1999, 2000; Irwin et al., 2001; Castro et al., 2008).

However, nectar robbery is not allays negative. In some cases, robbery can be positive for the plants. This occurs mainly when robbers perform pollination, or promote changes in pollinators' behaviour that increase outcrossing levels (Waser, 1979; Higashi et al., 1988; Maloof, 2001; Zhu et al., 2010; Zhang et al., 2014). However, not always the effect of robbers on plant reproduction is completely negative or positive, but sometimes lies somewhere in a continuum with an intermediate point (i.e. neutral effect) where no changes on female or male components of reproductive success are detected (Zimmerman and Cook, 1985; Temeless and Pan, 2002; Richardson, 2004). There are some documented cases of simultaneous neutral effects of robbers on both components of plant reproduction, but are scarce and require further exploration (Morris, 1996; Chapter 3 this dissertation). Nevertheless it is likely that this 'neutral' consequence is not due merely to a lack of effects of robbers, but involves complex mechanisms that allow the plant to balance the energetic loss caused by the high quantities of nectar consumed by these illegitimate floral visitors (see Chapter 3 this dissertation).

Besides floral visitors, the reproductive success of *L. etrusca* is also under the influence of herbivores such as aphids that presumably affect floral development and cause considerable losses of flowers and fruits (Jordano, 1990). However, until now there is a lack of data on the subject and is still unclear the ecological pathways in which aphids modify the reproductive outputs of this plant.

Aphids (Hemiptera, Aphioidea) constitute the largest group of sap-sucking herbivores. These animals extract phloem from sieve tube elements with their stylet-like mouth parts (Pollard, 1972). The consequences of these herbivores are usually detrimental for the host plants because aphids drain energy and nutrients, are vectors of infections, induce the production of leave galls and in few cases cause defoliation (Dixon, 1971; Larson, 1998; Power and Flecker, 2003). Despite they attack many plant species including several crops (Blackman and Eastop, 1984; Van Emden and Harrington, 2007), the knowledge about plant responses, resistance mechanisms and particularly the effects of aphids on the reproduction of wild plant species is still incipient (but see Snow and Stanton, 1988; Moran and Thompson, 2001). Since the effects of aphids on host plants are strongly influenced by interactions with diverse organisms, is necessary to explore the mechanisms through which these herbivores affect pollination services (Goggin, 2007).

Therefore, *L. etrusca* is an excellent case of study of the effects of both nectar robbery and herbivory, since the plant is commonly under the influence of both kinds of interactions simultaneously (Jordano, 1990). Besides it has long sympetalous flowers that require pollination by insects for reproduction (Guitián et al., 1993). Thus, the aims of our study were: a) to assess the incidence of nectar robbery and herbivory by aphids in *L. etrusca* natural populations, and b) to analyze direct and indirect (i.e. via pollinators) consequences of these interactions for the reproduction of this pollinator-dependent species. To achieve this, we studied and compared the effects of both kinds of interactions on floral traits and plant fitness separately, then analyzed the effect of herbivory on floral visitors, and finally we analyzed the direct and indirect effects of both interactions simultaneously on the quality of pollination service received by the plant.

Methods

Study system

Lonicera etrusca G. Santi (Caprifoliaceae) is a Mediterranean shrub that grows at forest margins as well as in abandoned cultures. In the study site the blooming period begins in the first week of May and finishes on the first week of June (Guitián et al., 1993). Flowers open at dusk and last three days (Jordano, 1990). The plant is able to form fruits when xenogamous and geitonogamous pollination occurs. Nevertheless, no fruits are produced when stigmas receive pollen from the same flower, making the plant dependent on pollinators to reproduce (Guitián et al., 1993). Besides lepidopterans, dipterans, coleopterans and long-tongued bees, several species of primary nectar robbers (*Xylocopa violacea* and *Bombus terrestris*) are common visitors (Jordano, 1990; Guitián et al., 1993). As a consequence of nectar robbery, particular marks are left at the corolla.

The aphid *Hyadaphis passerinii* (del Guercio) (Aphidinae: Macrosiphini) is widespread in Europe, predominantly in the Mediterranean Region, Middle East, Pakistan and India and was introduced to Southern Africa, Australia, New Zealand, North and South America (Blackman and Eastop, 2006). The species was reported as a parasite of some species of the *Lonicera* genus particularly *L. caprifolium* and *L. periclimenum*, forming colonies in early spring. When winged individuals are produced during the summer, they migrate to other plants of the genera *Daucus*, *Coniium* and *Pastinaca*. Colonies on *Lonicera* then die out, and re-colonization occurs next autumn. The attack of *H. passerinii* causes damages on shoot growth and flower development (Alford, 2012). Hereafter the flowers damaged by aphids will be referred to as "damaged flowers", to differentiate them from "healthy flowers" which were undamaged.

Study area

The study was conducted at El Bierzo region, North-west Spain. Two populations are located in the Natural Park Serra da Enciña da Lastra, municipality of Rubiá (Cobas A: 567 m asl; 42°28'19''N, 6°50'17''W and Cobas B: 438 m asl 42°28'15''N, 6°49'26''W), at the Orense province. A third population is located in La Barosa (590 m asl 42°29'50''N, 6°48'52''W) and another one in Carucedo (520 m asl; 42°29'6''N, 6°45'59''W) at the León province. The region present a Mediterranean climate and the vegetation is composed of cultivated lands and native vegetation, such as holm oak woodland (predominantly *Quercus ilex*, *Arbutus unedo* and *Quercus suber*), and shrub communities (Guitián et al., 1993; Navarro et al., 1993).

Incidence of nectar robbery and herbivory

We made observations of the species that behave as nectar robbers along transects. To assess the quantity of flowers affected by herbivory we delimited three transects of 300 m length and 5 m width, one transect per population in Cobas A, Cobas B and La Barosa. In May and June 2012 we counted the total number of flowers within the transects. There, we also inspected a random sample of flowers (914 flowers in Cobas A, 1192 in Cobas B and 597 in La Barosa) to estimate the frequency of flowers with one or more holes made by robbers and flowers damaged by aphids. To analyze the effect of the distance between individuals on the percentage of damaged flowers 30 plants per population were marked and the distance to their nearest neighbour was measured. For these plants the level of aphid infestation was estimated as the percentage of damaged flowers per plant.

Effects of nectar robbery on floral traits and plant fitness

Floral traits-. To characterize the damages produced by nectar robbers we made detailed observations of their floral visiting behaviour. Additionally, we analyzed whether stigmas, styles or anthers presented structural damages caused by robbers in 300 flowers in the transects (100 per population). To compare the volume and sugar concentration of nectar in flowers with and without nectar robbery we extracted the nectar and then bagged 47 healthy first day flowers (26 non-robbed flowers, 21 robbed flowers) from 30 individuals. All nectar accumulated after 24 hours was extracted with 1 μl capillary micropipettes. Sugar concentration was estimated with a portable refractometer (Fisher Scientific TM, 0–32 %).

Plant fitness-. To test whether primary nectar robbers perform cross pollination we compared the performance of robbers as pollinators against the results of three different types of hand pollination as follows: 100 floral buds in 10 plants (10 buds per plant) were protected with mosquito net bags (1 mm eye mesh) in order to prevent visits by insects. After anthesis, three types of pollen were randomly assigned and added to the stigmas of 60 flowers: pollen of the same flower (autogamy, $n = 20$), pollen from different flowers of the same plant (geitonogamy, $n = 20$), and a mixture of pollen from 5 other plants at least 10-15 m apart (xenogamy, $n = 20$). The remaining 40 virgin flowers were unbagged just to allow one single visit from a primary nectar robber. After treatment or visit each flower was collected in a sterile vial to allow pollen germination and growing of pollen tubes. After 24 hours at room temperature the stigmas were removed and preserved in 70% ethanol. At the laboratory the stigmas were immersed into a NaOH 8M solution during 48 hours at 24 °C, rinsed with distilled water and stained during 24 hours with aniline blue (see Kearns and Inouye, 1993). Finally, the stigmas were placed on microscope slides, submerged into a drop of glycerine and protected from light. Using a microscope with UV light, pollen grains on stigma and growing pollen tubes were quantified. We counted the number of pollen tubes at three positions along the style: top (right under stigma), middle (at half of the style) and base (at the bottom of the style, just before they reach the ovary).

Effects of herbivory on floral traits and plant fitness

Floral traits-. In order to characterize and compare floral morphology in healthy versus damaged flowers, 146 fresh non-robbed flowers (63 damaged and 83 healthy flowers) from 40 individual plants present in the four populations (1 to 8 flowers per plant) were measured with a digital calliper (0.01 mm precision). The floral characters measured were: total corolla length, tube length, tube diameter, stamen length (as the longest filament length) and pistil length (Fig. 4.1).

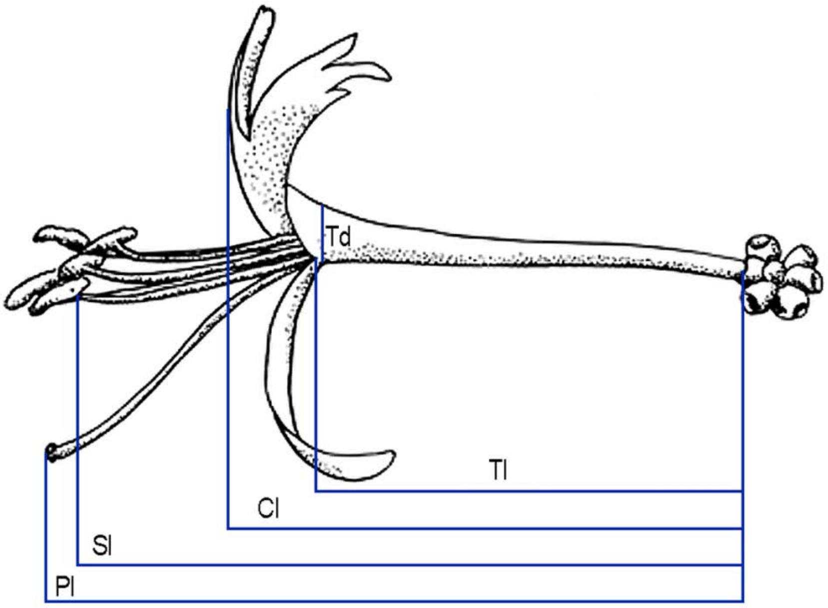


Figure 4.1. Morphological variables characterized for the flowers of *Lonicera etrusca*: Pistil length (Pl), stamen length (Sl), corolla length (Cl), tube length (Tl) and tube diameter (Td).

To characterize the number of ovules per flower, 40 non-robbed flowers (20 damaged and 20 healthy flowers) randomly taken from a different plant were collected and preserved in vials with ethanol 70 %. In the laboratory the ovaries were dissected and the number of ovules was counted under a stereoscopic microscope. To measure the ovule size, a random sample of five ovules per flower (n = 200 ovules from 40 plants) was placed on a microscope slide with a millimetric scale and photographed. Finally we measured length and width of each ovule with the program ImageJ 1.46r for Windows.

To quantify the number of pollen grains per anther, 355 floral buds were collected and preserved in vials with ethanol 70 %. At the laboratory, one random anther per flower was placed on a microscope slide with a drop of isotonic solution (ISOTON II) and all pollen grains were manually removed under a stereoscopic microscope. Before analyzed, each sample was carefully placed into a plastic vial and immersed in a sonicator bath for 5 minutes in order to disaggregate pollen clusters and to detach any remnant of anther tissue. Each pollen sample was filtered with a 100 μm sieve and the volume was completed up to 20 ml with ISOTON II. Pollen grains in 1 ml homogenized subsamples were counted with a particle counter (Beckman Coulter Multisizer 3). The total number of grains per anther was calculated as the average of three subsamples corrected by the dilution factor. In order to characterize the pollen size, one anther per flower, each flower from every 20 different plants was dissected under a stereoscopic microscope (10 anthers of damaged flowers and 10 anthers of healthy flowers) and placed on microscope slides with a glycerine drop. Measurements of equatorial and polar axis of 10 pollen grains per anther (n = 100) were made with a reticle in the light microscope.

To compare the microscopic structure of pollen and stigma between healthy and damaged flowers, a sample of 30 healthy and 30 damaged flowers (each from a different plant) was randomly taken and preserved in 70 % ethanol. At the laboratory pollen and stigmas were separated and dehydrated with increasing concentrations of aqueous ethanol solutions (70 - 100 %). Afterwards ethanol was replaced with amiloacetate (successive amiloacetate-ethanol solutions 1:3, 2:2, 3:1). Finally, samples were subjected to critical point drier and mounted on metallic stubs. Pollen samples were coated with a gold/palladium film at high vacuum in a sputtering chamber. Samples were then observed and photographed with an environmental scanning electron microscope in low vacuum mode, operating at 15 kV.

To compare the volume and sugar concentration of nectar in damaged versus undamaged flowers by herbivory we bagged 110 floral buds (83 healthy and 27 damaged flowers) from 90 individuals and 24 hours after anthesis we performed same procedure before described to characterize the volume of nectar produced and its sugar concentration.

Plant fitness-. To assess the effect of herbivory by aphids on the number of pollen grains on stigma and pollen tubes reaching the ovary, a random sample of 60 flowers on the third day after anthesis (30 healthy and 30 damaged flowers, one per individual) was collected and placed in sterile vials. Third day flowers were visually identified by their yellow perianth and dehiscent anthers. After 24 hours at room temperature, samples were preserved in ethanol 70 %. At the laboratory pistils were immersed in a NaOH 8M solution during 48 hours at 24 °C, rinsed with distilled water and stained with aniline blue solution during 24 hours (Kearns and Inouye, 1993). Finally, pistils were placed on microscope slides, submerged in a drop of glycerine and protected from light. Using a microscope with UV light, pollen grains on stigma and growing pollen tubes at the base of the style were counted.

To estimate the effect of herbivory by aphids on plant fitness (female function), 120 inflorescences (60 with healthy and 60 with damaged floral buds) were marked in 30 individuals (minimum 1 and maximum 6 inflorescences per individual) at the Carucedo population in June 2011. To control for possible architectural effects of fruit production related to differential resource-availability (Diggle, 1995), the position of the inflorescence in the branch was recorded as apical (n = 45), or lateral of first (n = 22), second (n = 23) or third order (n = 30). After fruit maturation, the inflorescences were collected and preserved in ethanol 70 %. At the laboratory the number of ripe fruits and ovaries per inflorescence was counted. One ripened fruit per inflorescence was dissected and the number of viable seeds and undeveloped ovules was counted. Finally, viable seeds were extracted from fruits, cleaned and dehydrated in an oven at 50 °C until constant weight. Seed weight was measured with an analytical balance (0.01 mg precision). These data were analyzed in two different ways. First, we compared the number and weight of the seeds produced in healthy and damaged flowers in order to analyze the effects of herbivory on seeds. For the second analysis we used the weight of the seeds produced in healthy flowers only to detect changes at the plant level. For this analysis the predictor variable consisted in two levels of infestation by aphids: plants without aphids vs. highly infested plants (more than 90 % of the flowers damaged).

Effects of herbivory on floral visits

To study the effects of damages by aphids on the behaviour of floral visitors we performed an experiment in spring 2011 using fluorescent dye powder fluorescent (Radiant Colour, Richmond, CA, USA) as pollen analogues. We conducted three replicates, each with two focal plants in the Carucedo population. The focal plants were chosen according to size, position in relation to other plants and number of flowers. We bagged 100 flower buds with mosquito net bags to prevent visits. Next day after anthesis the bags were removed and fluorescent dyes were applied on anthers with a fine paintbrush. This procedure was performed once every morning during 4 consecutive days at each focal plant. Every day at dusk we examined all open flowers in plants within a radius of 60 m from the focal plants using a UV flashlight, and recorded the presence of fluorescent dyes on their stigmas. Dyes were removed after recording to avoid recounting on the next day. The distance and percentage of damaged flowers at each peripheral plant was assessed.

To analyze the effect of herbivory on nectar robbery we inspected a random sample of flowers in the transects (358 damaged flowers and 505 healthy flowers). To estimate the effect of aphids on nectar robbery at the plant level we randomly chose

115 plants in the same populations and assessed the proportion of robbed and damaged flowers per plant.

Direct and indirect effects of nectar robbery and herbivory on pollination quality

We made observations of floral visitor's behaviour from May to June of 2010 and 2011, in 100 marked plants at Cobas A, Cobas B and La Barosa populations (approximately 35 plants per population). In each plant we made 5 minutes censuses of floral visitors accumulating a total of 70 hours and 9 minutes of observation (24 hours 16 minutes in Cobas A, 23 hours 45 minutes in Cobas B and 22 hours 8 minutes in La Barosa). These observations were useful to calculate the visit rate of each visitor species to each plant as the number of individuals per minute multiplied by the number of visited flowers per plant. Then, this value was multiplied by the qualitative component of pollination effectiveness of each visitor species to calculate the pollination service delivered by each visitor species at each plant. The value of the qualitative component represents the contribution of the visitor for both male and female components of the pollination process in terms of one single visit by one individual (for a complete explanation of the way to calculate qualitative component for each visitor species see Chapter 5 this dissertation). The pollination quality received per plant was calculated as the sum of pollination service delivered by all species that visited the flowers of each plant. To analyze possible causal relationships among floral display, level of herbivory and quality of the pollination service we quantified the open flowers, the flowers damaged by aphids and the robbed flowers present at each plant after the observations of floral visitors.

Statistical analysis

Incidence of nectar robbery and herbivory

A non-parametric Kendall's Tau correlation was used to analyze the relationship between the distance from the plants to their nearest neighbour and the proportion of damaged flowers per plant (arcsine-square root transformed).

Effects of nectar robbery on floral traits and plant fitness

Floral traits-. The volume of nectar of robbed and non robbed flowers was log transformed to achieve normality. Then, we performed *t*-Student tests to analyze the influence robbery on nectar traits (volume and sugar concentration).

Plant fitness-. To analyse the section of the style in which tube attrition takes place, we calculated the Pearson product-moment correlation coefficient (r) between the number of pollen grains deposited on the stigma and the number of pollen tubes growing at three different longitudes of the style. To assess the role of nectar robbers as pollinators, we used a Generalized Linear Model (GLM) with negative binomial error structure and log link function to analyze the differences in the number of pollen tubes reaching the base of the style after xenogamous, geitonogamous and autogamous hand pollinations, and pollination by nectar robbers. The log transformed number of pollen grains deposited on the stigma was included as an offset term into the model. The model was fitted using the function glm.nb of the package MASS (Venables and Ripley, 2002). To test for the overall effect of the type of pollination we compared the full model with a model comprising only the intercept using a likelihood ratio test.

Effects of herbivory on floral traits and plant fitness

Floral traits-. To analyze differences in the number of pollen grains per anther between healthy and damaged flowers we performed a *t*-Student test for non homocedastic variances. Mean pollen size (equatorial and polar axis) was averaged for each individual and compared between damaged and undamaged flowers with a *t*-Student test for non homocedastic variances based on 10 000 bootstrap samples to correct non-normality. The same analysis was performed to compare the quantity of ovules per flower.

Plant fitness-. We performed an ANOVA with 10 000 bootstraps to compare the quantity of pollen grains received by the stigmas of healthy and damaged flowers. The number of pollen tubes reaching the ovary was analyzed with an Analysis of Covariance (ANCOVA) with 10 000 bootstraps. The quantity of pollen on stigma was used as a covariable.

We used Generalized Mixed Linear Models (GLMM) to analyse whether the damage by herbivores had an effect on the number of ripened fruits produced per inflorescence. The type of flower (healthy or damaged) was included as a fixed effect. The plant identity and the position of the inflorescence at the branch were used as random effects. The model was fitted with Poisson error structure and logit link function. The number of ovaries per inflorescence was included as an offset term (square root transformed) into the model. To test specifically for the effect of herbivory, we compared the full model with a null model lacking the type of flower but comprising all other terms in the full model. This comparison was made using a likelihood ratio test. Because the number of produced fruits from damaged flowers was very low, statistical analysis of quantity or weight of seeds was not possible. To test for differences in the weight of seeds produced in healthy flowers from plants without damages by aphids versus plants with flowers damaged by aphids, we performed a *t*-Student test.

Effects of herbivory on floral visits

To analyse whether herbivory by aphids affected the visitation by pollinators we fitted a GLM with Poisson error structure and logit link function. The number of dyed flowers was the response, the frequency of damaged flowers (square root transformed) the predictor variable, and the distance from the focal plant (log transformed) an offset term. Differences in the probability of a flower to be robbed considering if it was healthy or damaged were analyzed with a χ^2 with Yates correction. A non-parametric Kendall's Tau correlation was used to study the relationship between the proportions of robbed and damaged flowers per plant (both arcsine-square root transformed).

Direct and indirect effects of nectar robbery and herbivory on pollination quality

We used Confirmatory Path Analysis (CPA) to test the relative importance of herbivory by aphids and nectar robbery on the quality of the pollination service received in 99 plants. Since this analytical tool implies several steps we describe in detail the implementation in our analysis. Besides, considering that the floral display is a relevant trait for the attraction of floral visitors, we included the total number of open flowers per plant as a predictor into the models. We tested a priory hypotheses using the d-separation method for path analysis developed by Shipley (2000). To perform this analysis we first obtained the "basis set" by testing the significance of relationships among predictor variables (number of total open flowers, damaged flowers and robbed flowers) with a GLM with Poisson error structure and log link function. Then we drew directed acyclic graphs (DAG) for all possible models derived from the basis set by including the response variable (pollination quality) and linking it with one or two of the predictor variables (Shipley, 2004; Grace, 2006). For the resulting six models we extracted the non-adjacent pairs and causal parents to obtain the minimum set of conditional independence statements and identified the statistical relationships to test in each model. We used GLM with Poisson error structure and log link function to obtain the p values of each non-adjacent pair when conditioning on other variables. With those *P* values we calculated Fisher's C statistic and its p value which is useful for testing the goodness of fit for the path models. Models with p values ≥ 0.05 for the Fisher's C statistic can be accepted as likely causal explanations of the relationships found among the variables in the dataset (Gonzalez-Voyer and von Hardenberg, 2014). Afterwards, we calculated the C statistic Information criterion (CICc) of each model and subtracted the minimum CICc to obtain the Δ CICc of the six models. Models with Δ CICc < 2 were considered to have substantial support of empirical data among all tested models. To calculate the model path coefficients we standardized the data and performed GLM for all the relationships present in the most likely models.

All analyses were performed with SPSS 19.0.0 for Windows except GLM and GLMM that were fitted with R.

Results

Incidence of nectar robbery and herbivory

The hymenopterans *Bombus terrestris*, *Xylocopa violacea* and *Xylocopa cantabrita*, and the coleopterans *Oxythyrea funesta* and *Tropinota hirta* consumed nectar by biting holes or tearing the corolla (Figs. 4.2a, b), behaving as primary nectar robbers (*sensu* Inouye 1980).

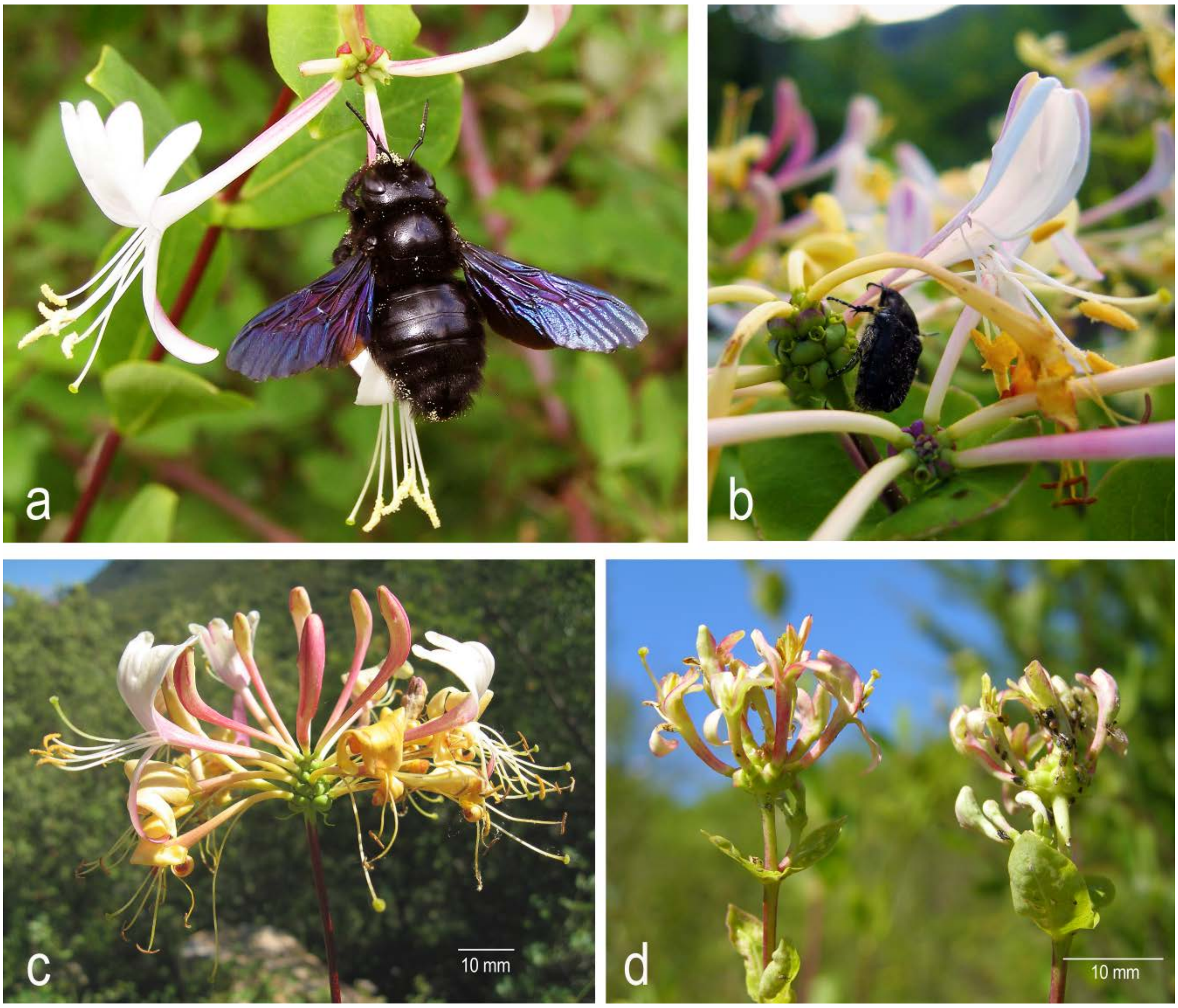


Figure 4.2. *Lonicera etrusca* lives under the exploitation pressures of nectar robbers and sap-sucking herbivores. *Xylocopa violacea* makes a slit on the base of the corolla with its sharp mouth parts and use it to rob nectar (a). The coleopteran *Oxythyrea funesta* tears the corolla from the entrance to the base to rob nectar (b) producing sever damages to the perianth and reproductive structures. The nectar robbers foraged almost exclusively in healthy flowers of *L. etrusca* (c). As a consequence of herbivory by the aphid *Hyadaphis passerinii* the flowers result damaged. In some branches these herbivores remain in damaged flowers during the blooming season (d right).

The aphids *Hyadaphis passerinii* consumed sap in branches and pedicels of fresh leaves at early spring. After the first infestation most aphids leaved plants at the beginning of the blooming. Occasionally they were also present along the blooming season feeding in damaged buds and flowers, but not in healthy flowers (Fig. 4.2c, d).

Both nectar robbers and herbivores were present in three studied populations of *Lonicera etrusca* (Fig. 4.3). 43.5 % of the total flowers found in three populations (n = 7305) presented one or more holes made by primary nectar robbers and 38.7 % presented damages induced by aphids. Nectar robbery affected a higher proportion of flowers in La Barosa population, while the levels of herbivory by aphids were higher for Cobas B population. We did not detect a significant correlation between the distance among plants and the level of aphid infestation (τ = -0.04, P = 0.594, n = 98). Remarkably, nectar robbery occurred almost exclusively in healthy flowers that were not affected by aphids' attack.

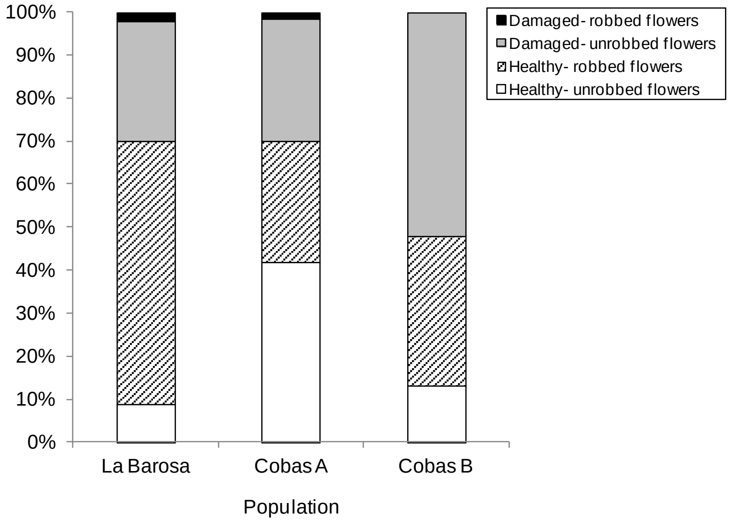


Figure 4.3. Percentage of flowers with one or more holes made by primary nectar robbers (robbed flowers) and with damages produced by aphids' herbivory (damaged flowers) in three studied populations of *Lonicera etrusca.*

Effects of nectar robbery on floral traits and plant fitness

Floral traits-. Hymenopterans nectar robbers opened holes at the base of the corolla (Fig. 4.2a). Although some flowers presented several holes, the reproductive structures did not result injured in 300 flowers inspected. On a different manner, the coleopterans produced a longitudinal tear from the entrance to the base of the flower causing a considerable damage to the corolla and usually cut or injured the style (Fig. 4.2b). Nevertheless, the impact of the latter on a population basis was minimal, since their marks were found only in a 2.2 % of the flowers analyzed in three populations.

The analysis of nectar production revealed that nectar robbery significantly reduced the synthesis of nectar, since after one day non-robbed flowers present twofold nectar volume in comparison with robbed flowers (Table 4.1). Although the mean sugar concentration was higher in robbed flowers in comparison with non-robbed flowers, the statistical difference among them was not significant.

Plant fitness-. The results of pollen tubes growing experiment revealed that for all treatments, the quantity of pollen tubes growing in the style beneath the stigma (Fig. 4.4a, d) was positively correlated with the quantity of pollen grains deposited on stigma (Pearson's $r = 0.895$, $P < 0.001$, $n = 48$). At the middle of style (Fig. 4.4b, e) the number of tubes decreased and the correlation became non significant ($r = 0.076$, $P = 0.608$, $n = 48$). The number of pollen tubes reaching the ovary (Figs. 4.4c, f) was correlated with the quantity of pollen tubes at the middle of the style ($r = 0.848$, $P < 0.001$, $n = 48$), but not with the pollen on the stigma ($r = 0.144$, $P = 0.328$, $n = 48$).

The number of pollen tubes reaching the ovary after one visit of *B. terrestris* or *X. violacea* (Fig. 4.4f) was significantly higher in comparison to autogamous self-pollination (GLM estimate ± SE = 1.053 ± 0.36, $z = 2.92$, $P = 0.003$ and estimate ± SE = 1.082 ± 0.374, $z = 2.892$, $P = 0.003$ respectively), demonstrating that both species of primary nectar robbers perform cross pollination in *L. etrusca*. We also found significant differences in the quantity of pollen tubes that reached the ovary after xenogamous, geitonogamous, autogamous crossings and nectar robbery (likelihood ratio test: $\chi^2 = 29.815$, df = 3, $P < 0.001$).

Effects of herbivory on floral traits and plant fitness

Floral traits-. Herbivory by aphids in branches and young floral buds induced severe changes in floral development. As a consequence both floral morphology and rewards resulted strikingly affected by these herbivores (Table 4.1). Damaged flowers were significantly smaller than healthy flowers in all morphological variables characterized (Fig. 4.2c, d). The size and quantity of pollen and ovules were also significantly lower in damaged flowers (Table 4.1). Pollen grains of damaged flowers were smaller and malformed in comparison with grains of healthy flowers (Fig. 4.5a, b, c). Also, the stigmatic papillae of damaged flowers presented deformations that reduced the area for pollen reception on the stigmatic surface (see Fig. 4.5d, e, f, g, h). Remarkably, the nectar production was severely affected by aphids, with null production of nectar in 27 damaged flowers examined (Table 4.1).

Plant fitness-. Contrary to the reproductive outputs of nectar robbery, herbivory by aphids was detrimental for the pollination process of *L. etrusca*. Damaged flowers received significantly less pollen grains on stigmas than healthy flowers ($F_{1,\ 59} = 157.99$, $P < 0.001$. See Fig. 4.6a). Furthermore, the few pollen grains that arrived to the stigma produced a very small quantity of pollen tubes able to grow down to the ovary ($F_{2,59} = 13.843$, $P < 0.001$. See Fig. 4.6b).

Table 4.1. Changes in floral traits caused by nectar robbers and aphids. Morphometric measurements are reported in mean ± standard deviation mm (*n*). Statistical significance of t-test: ** $P < 0.001$; * $P \leq 0.05$; ns (not significant) $P > 0.05$.

Herbivore group Floral trait	**Flower type**		***t*-test**
Nectar robbers	**Nonrobbed**	**Robbed**	
Nectar volume (µl)	4.2 ± 3.7 (26)	1.9 ± 2.5 (21)	*
Sugar concentration (°Brix)	24.0 ± 7.2 (26)	31.1 ± 21.5 (21)	ns
Aphids	**Healthy**	**Damaged**	
Nectar volume (µl)	4.7 ± 4.1 (83)	0 (27)	
Sugar concentration (°Brix)	21.1 ± 6.3 (83)	-	
Total corolla length	30.9 ± 2.6 (83)	12.5 ± 2.3 (63)	**
Tube length	25.6 ± 1.8 (83)	9.2 ± 3.3 (63)	**
Tube diameter	2.4 ± 0.3 (83)	1.5 ± 0.3 (55)	**
Pistil length	42.7 ± 2.8 (83)	14.8 ± 3.7 (63)	**
Stamens length	39.4 ± 3.0 (83)	12.6 ± 3.0 (63)	**
Ovules/flower	11 ± 1.2 (20)	8.8 ± 2.3 (20)	**
Ovule length	1.3 ± 0.1 (100)	1.1 ± 0.1 (100)	**
Ovule width	0.8 ± 0.1 (100)	0.6 ± 0.1 (100)	**
Pollen grains per anther	2337 ± 379.1 (325)	1447 ± 327 (30)	**
Pollen equatorial axis (µm)	71.1 ± 3.5 (50)	59.4 ± 5.3 (50)	**
Pollen polar axis (µm)	63.2 ± 3.4 (39)	55.2 ± 5.2 (50)	**

Aphid's attack affected the development not only of single flowers but also the development of all flowers into the inflorescence. As a consequence, the number of ripen fruits was significantly lower for inflorescences with damaged flowers than those with healthy flowers ($\chi^2 = 41.213$, df = 1; $P < 0.001$. Fig. 4.6c). Although damaged flowers produce a similar quantity of seeds compared to healthy flowers (similar average of Seed:ovule ratio observed in Fig. 4.6d), the seeds produced by damaged flowers were noticeable lighter than seeds from healthy flowers (Fig. 4.6e). At individual plant level, healthy flowers in plants attacked by aphids produced lighter seeds than those in plants that were not affected by aphids ($t = 2.89$, $P = 0.005$, df = 85 and Fig. 4.6f).

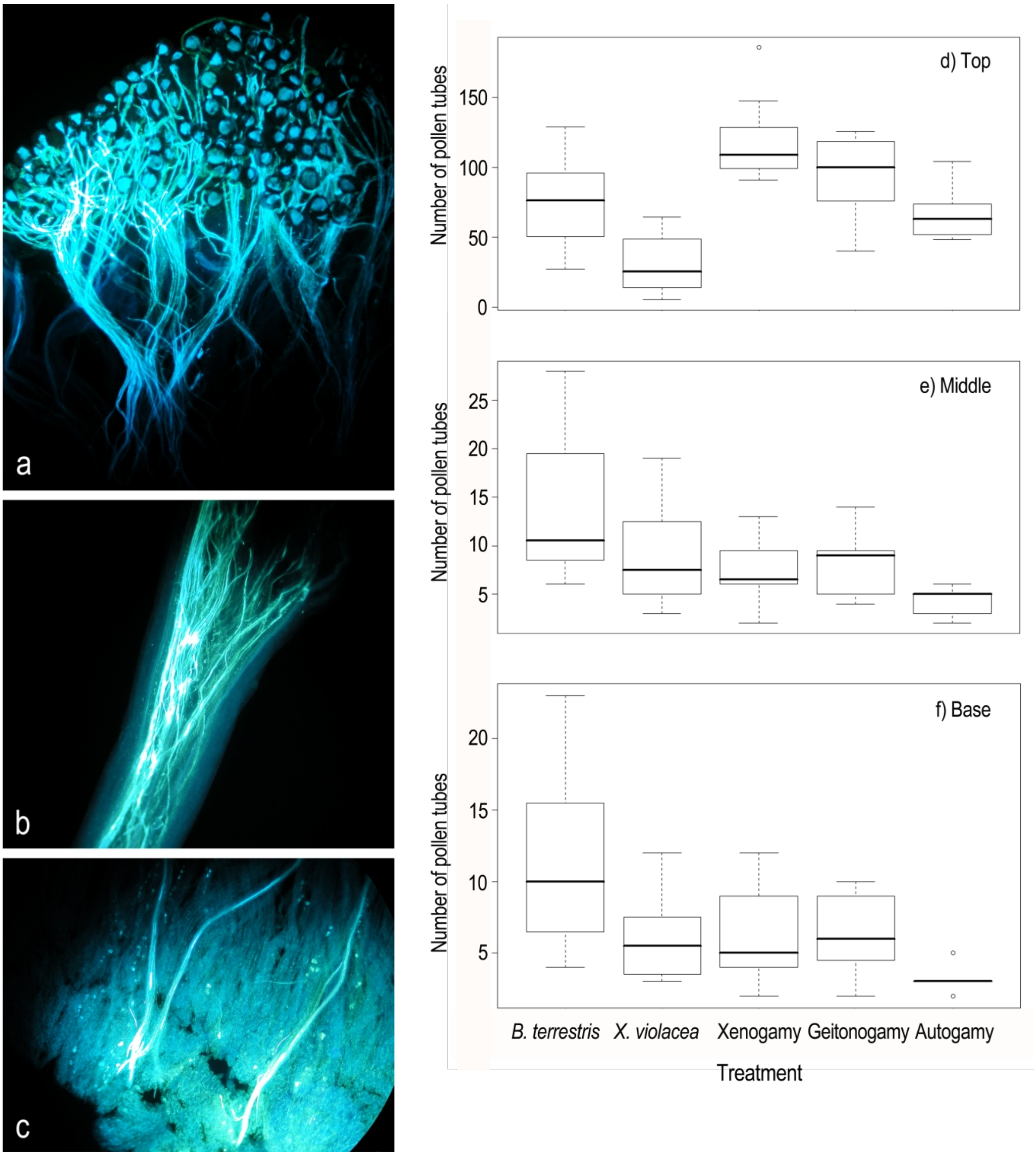


Figure 4.4. Pollen tubes growing along the style of *Lonicera etrusca* after one visit of a primary nectar robber or three treatments of pollen supplementation. (a) Pollen grains germinating with pollen tubes growing through the stigma. b) Pollen tubes at the middle of style. (c) Pollen tubes at the base of the style reaching the ovary. (d) Number of pollen tubes at the top of the style. (e) Number of pollen tubes at the middle of the style. (f) Number of pollen tubes at the base of the style. Box plots represent are medians (horizontal lines), quartiles (boxes), 0.25 - 1.5 IQR and 0.75 + IQR (whiskers), and outliers (dots).

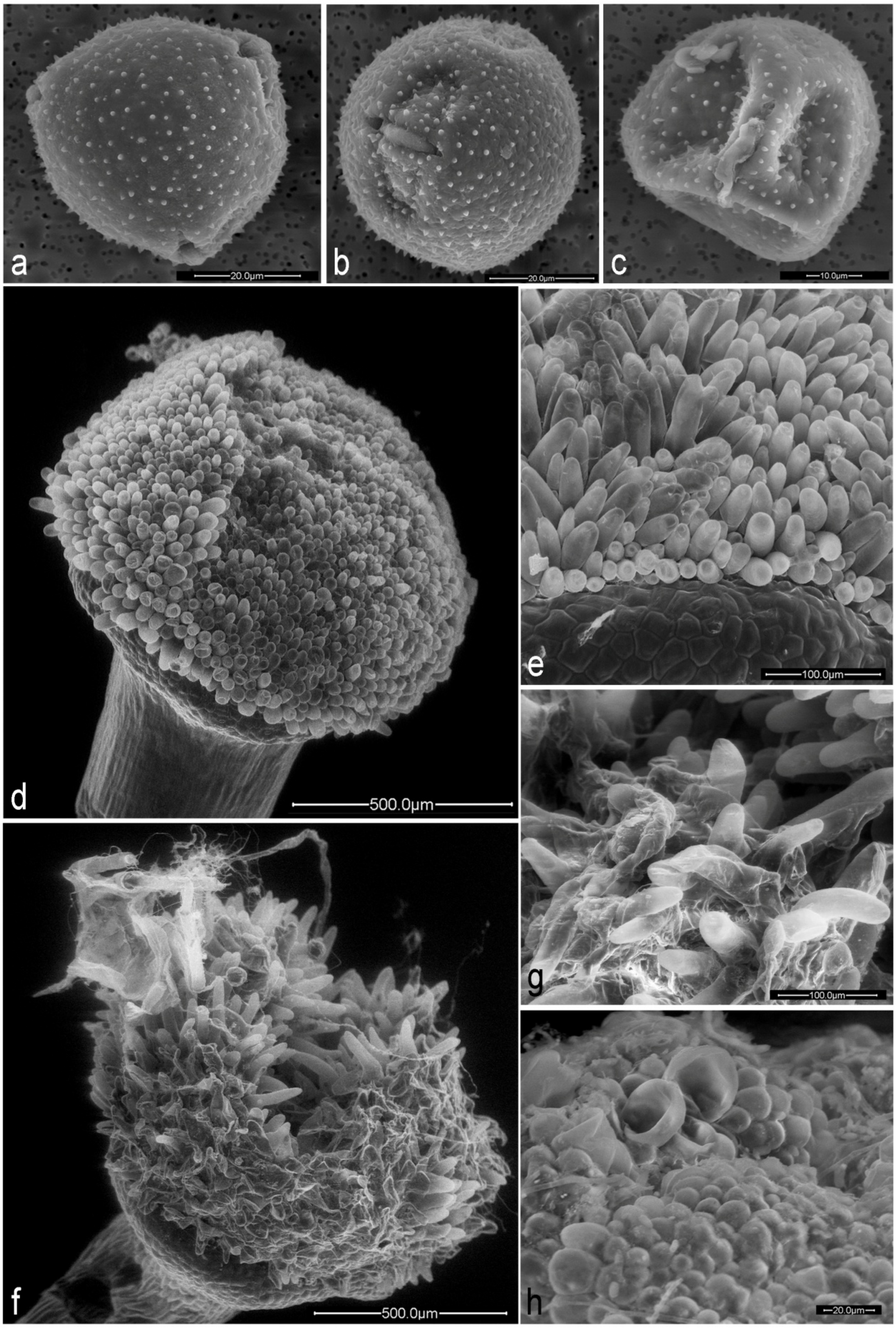


Figure 4.5. Morphology of pollen grains and stigmas of *Lonicera etrusca* at SEM. (a) healthy pollen grain in polar view; and (b) healthy pollen in semi-equatorial view; (c) pollen grain from a damaged flower; (d) stigmatic surface of a healthy flower; (e) detail of stigmatic papillae of a healthy flower; (f) stigmatic surface of a damaged flower; (g) and (h) detailed view of damaged stigmatic papillae.

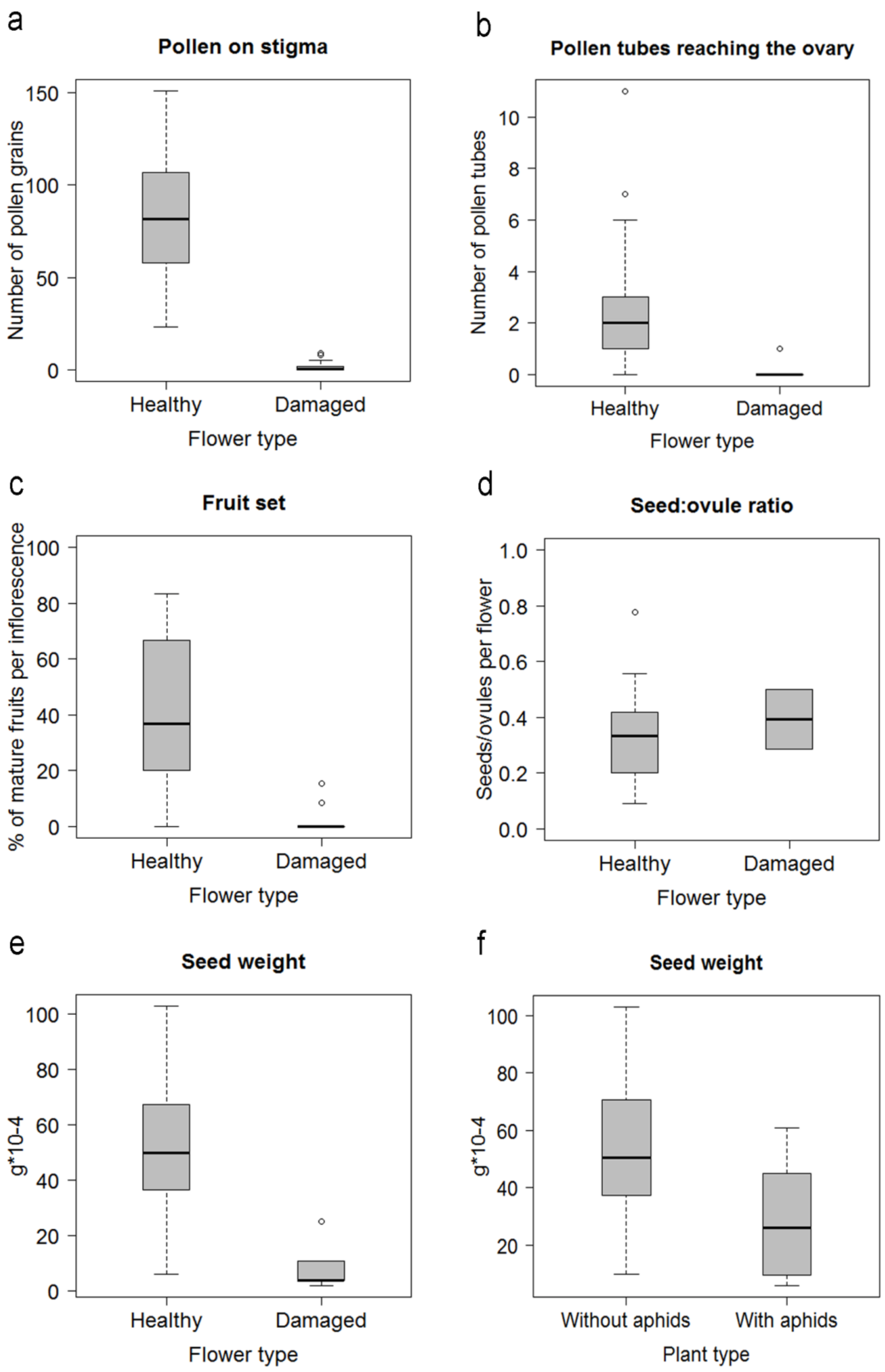


Figure 4.6. Consequences of herbivory by aphids for different parts of the pollination and post-pollination processes. (a) deposition of pollen on stigmas; (b) pollen tubes growing down to the ovary; (c) fruit setting; (d) number of seeds/number of ovules per ovary (Seed:ovule ratio); (e) weight of the seeds of healthy and damaged flowers; (f) weight of the seeds of healthy flowers from plants without damages by aphids and plants with damages.

Effects of herbivory on floral visits

The experiment with fluorescent dyes revealed that the percentage of damaged flowers by aphids per plant reduced significantly the chance of the flowers to be visited by pollinators (GLM estimate + SE = -0.45 + 0.16, z = -2.87, $P < 0.001$, $n = 1144$ flowers). In plants with more than 88 % of damaged flowers, no fluorescent dyes were found even on healthy flowers.

Our results revealed that herbivory prevented nectar robbery. The analysis of flowers along transects demonstrated that damaged flowers had lower chances to be visited by nectar robbers than healthy flowers ($\chi^2 = 304.5$, df = 1, $P < 0.001$). These differences were noticeable and only 1.1 % ($n = 2825$) of the damaged flowers presented holes made by primary robbers, while 58.8 % ($n = 4480$) of the healthy flowers presented one or more perforations made by these floral visitors. The few cases recorded of robbery in damaged flowers were performed by coleopterans. Additionally, the proportion of robbed flowers at the plant level was negatively correlated with the proportion of damaged flowers ($\tau = -0.506$, $P < 0.001$, $n = 115$).

Direct and indirect effects of nectar robbery and herbivory on pollination quality

We tested six theoretical models that describe the causal relationships among herbivory by aphids, nectar robbery and floral display, on the pollination quality received per plant (Appendix 1). Models four, six and five have a $\Delta \mathrm{CIC}_c < 2$, meaning that these theoretical models have significant support by the empirical data (Appendix 2). Since for all of them the three common predictor variables were redundant and the models only differed in the single path among one of the predictors and the response variable (pollination quality), for practical reasons and interpretability, we summarized the three models as shown in Figure 4.7. This analysis revealed that the floral display (i.e. number of open flowers per plant) had significant and positive effects on the pollination quality received by the plants and particularly on the number of robbed flowers per plant. Nectar robbery in turn has a direct and positive effect on pollination quality.

On the contrary the number of flowers damaged by aphids per plant was not related with the number of open flowers, and did not have a significant direct effect on pollination quality (Fig. 4.7). Instead, herbivory by aphids had a negative effect on nectar robbery thus affecting indirectly pollination.

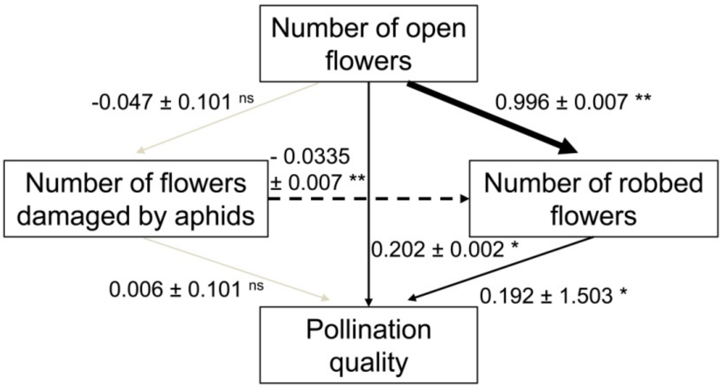


Figure 4.7. Summary for the three best-fit path models (with $\Delta \mathrm{CIC} < 2$) representing the magnitude and direction of direct and indirect effects of floral display (number of open flowers), herbivory by aphids and herbivory by nectar robbers on the quality of pollination received per plant. Numbers represent the value of each standardized path coefficient (SPC) ± standard error and its statistical significance: $P > 0.05$ are non significant (ns) and statistical significances at $P \leq 0.05$ (*) and $P < 0.001$ (**). The colour of the arrow represents the statistical significance: gray arrows are non significant SPC and black arrows represent significant SPC. Solid arrows are positive SPC while dashed lines symbolize negative SPC. The width of the arrow indicates the magnitude of the SPC: thin lines represent $\text{SPC} \leq 0.5$ and thick lines represent $\text{SPC} > 0.5$.

Discussion

Incidence of nectar robbery and herbivory

Nectar robbery and herbivory by aphids are common to several angiosperms (Blackman and Eastop, 1984; Van Emden and Harrington, 2007; Irwin et al., 2010). However, their incidence and consequences for the pollination process and the reproductive success of the same plant species simultaneously are studied here for the first time.

We found differences in nectar robbery levels among plants and populations of *L. etrusca*. Similar variations in the occurrence and intensity of nectar robbery are common to different plant-robber systems (Navarro, 2000; Irwin and Maloof, 2002; Price et al., 2005). These differences can be attributed to changes in the population abundances of robbers, presence and abundance of alternative floral resources, or intraspecific differences in floral morphology and nectar production that make some individuals or flowers more suitable than others (Urcelay et al., 2006; Castro et al., 2009; Navarro and Medel, 2009; Chapter 2 this dissertation).

Additionally, the CPA revealed that the robbery frequency strongly related with the number of flowers per plant. Although few studies have studied the effects of floral display on the behaviour of robbers at species or community levels (Goulson et al., 1998; Rojas-Nossa, 2013), a common pattern arises. As many other floral visitors, nectar robbers are more attracted to plants with higher number of flowers (i.e. larger floral display). This is a result of two main processes: on one side a higher concentration of flowers allows easier detection by insects, which are guided by visual and chemical cues; and second, a higher density of flowers reduces the energetic investment by foragers since the searching effort is reduced when resources are concentrated (Eckhart, 1991; Mitchell et al., 2004; Rojas-Nossa, 2013).

Despite the importance of aphids as pests in crops and ornamental plants (Blackman and Eastop, 1984; Van Emden and Harrington, 2007), the effects of sap-sucking herbivores on wild plants are poorly known. This is in part because the consequences for host reproduction are less obvious than leaf-chewing herbivores (Snow and Stanton, 1988). The strong impacts of aphids as herbivores are related to the fact that they can affect high percentages of branches of the plants thanks to very high rates of population growing (Kennedy and Stroyan, 1959). Our results indicate that the intensity of infestation is not related to the distance among individuals. Aphids use chemical cues to determine environmental conditions, particularly related to host and mate location, and are able to detect the physiological condition of the plants (Pickett et al., 1992).Then, factors involved in plant's resistance beyond the focus of our study, such as environmental and/or genetic conditions, might be related to differences in the intensity of infestation (Maddox and Cappucino, 1986; Smith and Boyko, 2007).

Effects of nectar robbery on floral traits and plant fitness

Robbed flowers of *L. etrusca* present low volumes of nectar slightly more concentrated than non-robbed flowers. The evaporation of water from nectar through the perforations made by robbers is one of the most likely explanations for such changes (Pleasants, 1983; Irwin and Brody, 1998). Reduction in volume and increasing in concentration of nectar caused by nectar robbery occur in other plant species, but the indirect consequences for plant reproduction differ. Depending on floral visitor's behaviour, such changes alter visitation preferences of pollinators affecting plant's reproductive success. For instance, in *Polygala vayredae* and *Puya coerulea* changes on nectar caused by robbers are related to a diminishing in attractiveness for pollinators that generates a reduction in the pollen flow, and the quantity of fruits and seeds produced (Castro et al., 2008; González-Gómez and Valdivia, 2005). However, for *L. etrusca* we found no evidence of obvious negative effects of the changes in nectar production caused by robbery.

Commonly nectar robbers are detrimental for the fitness of host plants through direct and indirect mechanisms (Irwin et al., 2010). Nevertheless, a growing body of experimental evidence suggests that in some instances robbers have neutral or even positive effects on plants (Maloof and Inouye, 2000; Chapters 2 and 3 this dissertation). This occurs when robbers contact the reproductive structures during the robbery process, and occasionally they are more efficient pollinators than legitimate visitors (Higashi et al., 1998; Navarro, 2000; Zhu et al., 2010; Zhang et al., 2014). *Bombus terrestris* and *Xylocopa violacea* are common nectar robbers in many native and cultivated species (Maloof and Inouye, 2000; Goulson, 2003; Castro et al., 2009). In some plants and under particular circumstances several species of both genera perform pollination while robbing (Navarro, 2000; Sampson et al., 2004). Several hypothesis were proposed to explain the neutral effect of nectar robbery observed on both male (measured as quantity and distance of pollen dispersal), and female (fruit set, seed-ovule ratio and seed weight) functions of reproductive success of *L. etrusca* (Chapter 3 this dissertation). Nevertheless, the mechanism was not clarified until now. In this study we present conclusive evidences of cross-pollination mediated by *Bombus terrestris* and *Xylocopa violacea* while acting as primary nectar robbers. This output is favoured by big body size and foraging behaviour of these insects, since they might contact the exerted anthers and stigma while move between flowers and inflorescences.

Effects of herbivory on floral traits and plant fitness

In other study systems the effects of herbivory are highly dynamic and the direction and magnitude for host plant's fitness depend on the identity of the participants in the interactions and their capacities to respond to diverse environmental pressures (Brody, 1997; García and Ehrlén, 2002; Gómez, 2005). For instance, foliar and floral herbivores reduce the production and quality of pollen and destroy reproductive structures affecting directly plant reproduction and thus modifying the population structure (Quesada et al., 1995; Strauss et al., 1996; Wise and Cummins, 2006). Herbivores also impact indirectly host fitness by inducing changes in plant-pollinator interactions (Herrera, 2000; Poveda et al., 2003; Gómez, 2005). For example, they reduce the number of flowers or affect the development of floral structures and rewards involved in attraction to pollinators diminishing host reproductive success (Lehtilä and Strauss, 1997; Strauss, 1997; Krupnick et al., 1999). However, herbivory is not always detrimental for the host. Several plants are able to cope with herbivory and present mechanisms to avoid, tolerate or compensate the effects of this interaction (Strauss and Agrawal, 1999; Brody and Irwin, 2012); and in some occasions plants achieve a higher reproductive success in the presence of herbivores (Strauss, 1991; Poveda et al., 2003).

In *L. etrusca*, the flowers that develop in branches attacked by aphids before the blooming season suffer severe detrimental changes in morphology and rewards for pollinators. Those changes include a reduction in quantity and quality of pollen and ovules, deformations in the stigmatic papillae, reduction in length and diameter of the corolla, length of stamens and pistils, and a total disruption of nectar production. Previous studies detected a decrease in numbers of staminated flowers, number and size of pollen and ovules, and a reduced size of corolla and petals as consequence of chewing herbivores (Strauss, 1997; Krupnick and Weis, 1999; Lehtilä and Strauss, 1999; Mothershead and Marquis, 2000), and a reduction of the number of flowers caused by aphids (Snow and Stanton, 1988). Since phloem sap transports the substances necessary for plant development (Douglas, 2006), it is likely that the foraging by aphids causes the loss of important quantities of nutritional materials for flower development causing the malformations observed in floral structures. Additionally, the cessation of nectar production is related to possible damages to nectaries thus physically preventing nectar synthesis, and also with the loss of raw materials required for nectar production.

Besides the behaviour of aphids as folivorous (i.e. feeding on branches and pedicels of leaves), we also observed that in some occasions these insects behave as florivorous (i.e. feeding on flowers) during the blooming season. This only occurred in damaged flowers and we never observed aphids in healthy flowers. We infer that in these cases the infestation was present since the early spring when buds were still not developed and lasted along bud development and blooming season and not only as a re-infestation of these branches as reported in the literature (Alford, 2012). Additionally, the permanent presence of aphids in certain inflorescences might potentiate the negative effects for the plant since a continuous depletion of nutritional resources long after the first infestation occurs. Besides, it may deter visits of pollinators with the visual and learning capacities to associate the presence of aphids with scarce floral recompenses; and also because both living individuals and exuviae inside the floral tube might act as a mechanical barrier for pollinators. Additionally, the presence of aphids in the plants also attracts ants that feed on honey-dew and spiders that predate aphids (Rojas-Nossa personal observations). Ants actively defend aphids against predators, and can modify pollinators' activity in the flowers leading to changes in reproductive success of the host plants (Ohm and Miller, 2014).

Although a reduction in nectar synthesis caused by herbivores was observed previously in *Isomeris arborea* (Krupnick et al., 1999), it is remarkable the total disruption of nectar production found in *L. etrusca*. Since nectar is the main recompense offered to most pollinators of this species (Jordano, 1990; Guitián et al., 1993), a modification in the pollination process would be expected.

Effects of herbivory on floral visits

To our knowledge no study tested before the consequences of sap-sucking herbivores on floral visitors such as nectar robbers. However, damages produced by florivorous larvae of Lepidoptera are associated with lower flower visitation by the nectarivorous bumblebee *Bombus hortorum* to the flowers of *Tropaeolum majus* (Goulson et al., 2007). Our study demonstrates that nectarivorous floral visitors avoid visiting damaged flowers and also healthy flowers in heavily attacked plants. Since the only robbers that visited damaged flowers were coleopterans we presume that these insects have a lower capacity than hymenopterans to distinguish and associate the changes in floral features with the poorer rewards found in damaged flowers.

Direct and indirect effects of nectar robbery and herbivory on pollination quality

Opposite to the direct benefit of nectar robbers as pollinators of *L. etrusca*, aphids negatively affected diverse aspects of the floral functionality with detrimental consequences for the reproduction of the plant by direct and indirect ways. Directly, herbivory by aphids diminishes the quality and quantity of ovules and pollen and affects the micro-structural morphology of stigmatic surface, altering pollen donation and deposition on stigmas. These herbivores consume resources necessary for fruit and seed development and caused a reduction in the weight of seeds not only in branches that were directly under herbivory, but also in healthy branches of attacked plants. This reveals that these herbivores modify the resource budget of entire plants and not only in the branches where aphids are foraging.

Indirectly, herbivory causes the disruption of nectar production that produces a reduction in the pollination quality of *L. etrusca*. A decrease of pollinator visit rates caused by foliar and floral herbivory was observed in other systems as a consequence of the reduction on the floral display, shortage of pollen and nectar rewards, and production of secondary compounds (Foster, 1984; Strauss, 1997; Lehtilä and Strauss, 1997; Krupnick et al., 1999). The production of anti-herbivore defences and the presence of trade-offs between plant defence, growing and reproduction (Koricheva, 2002; Strauss and Irwin, 2004) are some of the mechanisms worth of consideration in future studies in this field.

The effects of herbivory by aphids on the fitness of wild plant species was previously studied in few occasions, but studies that analyze its effect on pollination service are particularly scarce (Snow and Stanton, 1988; Strauss and Irwin, 2004; Strauss and Whittall, 2006). To our knowledge, this is the first study that reveals that herbivory by aphids has profound negative impacts on host plants via pollinators, as well as the detrimental consequences for male and female components of reproductive success through several physiological and ecological mechanisms. This result is particularly remarkable considering the high incidence of damaged flowers found in the studied populations as well as in other populations within the geographical range of the species. Further research regarding the consequences for the population structure or the evolution of floral traits and physiological trade-offs are required to extend our knowledge on the effects of mutualistic and antagonistic interactions in multi-species dynamical environments.

Conclusions

Nectar robbery and herbivory by aphids influence the reproduction of *L. etrusca* in opposite directions. Nectar robbers have direct positive effects since they perform cross-pollination without damaging the reproductive structures and do not cause an indirect reduction in pollination services. Aphids' foraging generates substantial changes on flower morphology, quantity and quality of pollen and ovules and cause the total disruption of nectar production. Such changes cause decreases in visits by pollinators (including nectar robbers) thus reducing indirectly the quality of pollination service. As a result, the quantity of pollen deposited on stigmas, the quantity of pollen tubes reaching the ovaries, the fruit set and the weight of the seeds are considerable lower in flowers damaged by these herbivores.

Chapter 5

Complex pollination landscape: Nectar robbers as effective pollinators

Abstract

The results of nectar robbing for the fitness of the host plants can range from negative to positive and depend on complex arrays of ecological and morphological traits of plants and visitors. The Etruscan honeysuckle (*Lonicera etrusca*) is a pollinator-dependent plant with flowers apparently specialized in pollination by hawkmoths. However, besides hawkmoths, its flowers are visited by a broad diversity of insects that forage for nectar and pollen. Nectar robbers are common exploiters of the abundant nectar rewards offered by the plant to pollinators and recent evidences suggest that these animals are not detrimental for plant's reproduction, but on the contrary perform cross-pollination while robbing. This represents a paradox in the classical understanding of floral specialization and constitutes an ideal case to evaluate the validity of the pollination syndromes as one of the fundamental concepts on pollination biology. Thus, the aim of this study is to compare the effectiveness of different floral visitors of *L. etrusca* focusing on the mechanisms that allow pollination by nectar robbers. To achieve it we made detailed observations of the behaviour of floral visitors, analyzed pollen loads transported on their bodies and compared the qualitative and quantitative components of pollinators' effectiveness. Based on previous observations and floral traits we expected that hawkmoths were the most effective pollinators. Indeed, the broad-bordered bee hawkmoth (*Hemaris fuciformis*) has the highest value for the qualitative component. However, the rarity of its visits reduced its effectiveness as pollinator. Two groups of hymenopterans: legitimate visitors and primary nectar robbers, were the most effective pollinators of *L. etrusca*. Pollination by nectar robbers is allowed by the exertion of floral reproductive structures and the grouping of flowers into inflorescences, as well as by the big body size, the foraging behaviour and the high visitation rate just after anthesis of hymenopterans primary nectar robbers. On the contrary, coleopterans that behave as primary robbers caused damages in the flower's reproductive structures and promoted high levels of self-pollination, performing the lowest pollination service for the plant among all visitors.

Introduction

Sexual reproduction of 87.5% angiosperms on earth relies on animals as pollen vectors (Ollerton et al., 2011). In return for this delivery service animals usually obtain resources in form of nectar, pollen, oils and fragrances. Besides these rewards, flowers possess long distance attraction methods (including visual and olfactory traits) and mechanisms that assure a higher specificity in the removal and deposition of pollen enhancing plant fertility (Harder and Barrett, 1996; Armbruster et al., 2009; Barrett, 2010). Such specialization process may be driven by differences in pollinators' performance resulting in plants specialized to their most effective pollinators (Stebbins, 1970; but see also Herrera 1996; Aigner, 2001, 2004, 2006). However, despite their specialization degree, flowers of most plant species receive visits from a diversity of animals that differ in their performance as pollinators and its consequent contribution to plant fitness (Jordano, 1987; Waser et al., 1996; Johnson and Steiner, 2000; Fenster et al., 2004). Considering the relevance of pollination services for the reproduction of plants and ecosystem functioning, it is of concern to measure and compare pollination effectiveness of floral visitors to understand the ecological and evolutionary paths of these mutualistic interactions. Such knowledge is fundamental in order to design management and conservation plans that take into account vital pollination ecosystem services (Kearns et al., 1998; Sahli and Conner, 2007; Potts et al., 2010).

Pollination effectiveness is usually calculated as the product of two components that reflect different ecological scales: the qualitative and quantitative components. The qualitative component is the basic unit and represents the effect of the interaction on plant fitness as the result of one single visit of one individual visitor to a single flower (see Herrera, 1987; Ne'eman et al., 2010). It is usually estimated either as the result of the pollination process in terms of the quantity of pollen deposited on stigma, or as the result of pollination and post-pollination processes by quantifying the resulting plant's reproductive success (Rodríguez-Rodríguez et al., 2013). According to the reproductive strategy of the plant these two approaches might be more or less directly related. For instance, stigmatic pollen deposition is a direct measure of pollinator contribution to female fitness in self-compatible species in which all pollen grains have similar chances to fertilize the ovules and sire seeds (Ne'eman et al., 2010).

However, in self-incompatible species the pollen grains placed on a stigma might differ in their quality to fertilize ovules according to their origin (Aizen et al., 1990). Visitors may cause self-pollination by displacing pollen from anthers to stigma of the same flower or by visiting several flowers within the same plant during a foraging bout (Galen et al., 1989). The variability in the frequency of self-pollination induced by the visitors is related to floral and plant traits such as presence and degree of dichogamy and herkogamy, quantity and disposition of open flowers per inflorescence or individual, etc., but also with morphological and behavioural attributes of the visitors such as territoriality, movement patterns, or time spent at each flower among others (Stiles, 1975; Webb and Lloyd, 1986; Lloyd and Schoen, 1992). Therefore, in such cases it is meaningful to involve behavioural traits of floral visitors to achieve a more accurate estimation of the qualitative component of pollinators' effectiveness. Otherwise, the quantitative component estimates the frequency of the interaction assessing the effect of a population of visitors on the pollination of the plant (Herrera, 1989; Vázquez et al., 2005).

Long tubular corollas or spurs have evolved as mechanisms to restrict visits only to animals with long proboscises, tongues or beaks, which are able to reach the large amounts of nectar accumulated at the base of the tube (Stiles, 1981; Nilsson, 1988; Fenster et al., 2004). Nevertheless, some animals with short mouth parts have found ways to surpass these morphological constraints imposed by the plant and gain access to the reward through 'illegitimate' ways. These animals are named floral larcenists (Irwin et al., 2010) and because in many occasions extract nectar profiting the reward without providing the pollination service in return, they have been considered cheaters of the plant-animal mutualism (Maloof and Inouye, 2000). There are different kinds of floral larcenists according to their particular behaviour of nectar foraging: primary nectar robbers make a perforation at the corolla through which they extract nectar; secondary nectar robbers use the perforations made by primary nectar robbers to reach nectar; and nectar thieves that have access to the nectar through the mouth of the flower as legitimate visitors do, but pollination does not occur due to a morphological mismatch between visitor and floral structures (Inouye, 1980, 1983).

Nectar robbers are common visitors of many species and their foraging behaviour may modify significantly the plant's reproductive success through direct and indirect ways (Maloof and Inouye, 2000; Irwin et al., 2010). In most cases, nectar robbery reduces male and/or female fitness by damaging the reproductive organs of the flower (Traveset et al., 1998), reducing resources for fruiting and seed siring (Navarro, 2001) or by changing the behaviour and preferences of legitimate pollinators (see Maloof and Inouye, 2000; Irwin et al., 2001; Burkle et al., 2007; Irwin et al., 2010 for comprehensive revisions). However, nectar robbers can directly enhance plant's fitness when they pollinate the flowers (Graves, 1982; Higashi et al., 1988; Navarro, 2000; Fumero-Cabán and Meléndez-Ackerman, 2007; Zhu et al., 2010; Zhang et al., 2014). Nectar robbing has also indirect positive effects when the reduction of nectar levels forces the legitimate pollinators to increase the number of visited flowers and/or flight distances, resulting in a potential increase of outcrossing levels in the population (Zimmerman and Cook, 1985; Maloof, 2001). In some cases the effects for the host plant are neutral; this means that no significant changes, neither positive nor negative, are observed in female and/or male components of reproductive success as a consequence of nectar robbing (Navarro et al., 1993; Arizmendi et al., 1996; Morris, 1996).

Lonicera etrusca has fragrant flowers with long tubular corollas that open at dusk (Jordano, 1990). They are mainly pollinated by Hawkmoths but are also visited by a variety of insects with diverse foraging behaviours (Guitián et al., 1993). Legitimate visitors include several nectarivorous species of lepidopterans and hymenopterans. While other visitors, such as syrphids, wasps and coleopterans forage for pollen. However, the most remarkable characteristic of this system is the high frequency of visits by nectar robbers (Guitián et al., 1993). Despite of that, experimental evidences indicate that nectar robbing does not cause a reduction on male or female reproductive success (Chapter 3 this dissertation). One hypothesis to explain it was proposed by Jordano (1990), who observed that nectar robbers forage on *L. etrusca* next day after anthesis, leaving enough time for the legitimate visitors to pollinate the flowers. Nevertheless, recent evidences support the idea that the observed neutral effects of robbing on plant fitness are related to the fact that the main hymenopteran primary robbers perform crossed pollination (Chapter 4 this dissertation), same as observed in other coexisting plant species (Guitián et al. 1994, Navarro, 2000).

It is puzzling that despite of the apparent specialization for Hawkmoth pollination reflected in floral features that characterizes the species of *Lonicera* genus (Miyake and Yahara, 1998; Miyake et al., 1998), *L. etrusca* is pollinated by short tongued bees through illegitimate visits. In this case, the concept of pollination syndromes (Faegri and van der Pijl, 1979) is not totally appropriate to predict the possible pollinators of this species by observing its floral features. Nevertheless, a thorough comparison of the pollinators' effectiveness is required to fully understand in which extant this system disagree with this fundamental concepts in pollination biology. Therefore, the objectives of this study are to characterize and compare the pollination effectiveness of different floral visitors, and elucidate the mechanisms that allow pollination by nectar robbers. To achieve this, we characterized the behaviour of floral visitors through careful field observations and estimated the qualitative and quantitative components of pollination effectiveness for different groups of floral visitors in *L. etrusca*.

Methods

Study system

Lonicera etrusca Santi (Caprifoliaceae) is a climbing shrub native to the Mediterranean basin (Guitián et al., 1993). Its blooming period starts in May and finishes in June. Anthesis occurs at late afternoon, usually one hour before sunset, and the fragrant flowers remain open for three days. Stigma is receptive since anthesis, and anthers' dehiscence occurs next day (Jordano, 1990). The flowers are hermaphrodite with a long tubular corolla (mean tube length = 25.6 ± 1.8 mm), and exerted reproductive structures (Chapter 4 this dissertation). The species presents a self-compatible insect-dependent reproductive system in which autogamous crossings do not produce fruits, while xenogamous and geitonogamous crossings produce 65.5 and 43.1 % fruit set respectively (Guitián et al., 1993). The flowers produce abundant nectar that accumulates at the base of the corolla (4.2 ± 3.7 µl, 24 ± 7.2 % sugar. Chapter 4 this dissertation) and it is consumed by a variety of floral visitors (Jordano, 1990; Guitián et al., 1993). Legitimate visitors include hawkmoths (e. g. *Macroglossum stellatarum*), long-tongued bees and bumblebees (e. g. *Anthophora hispanica*, *Anthophora acervorum* and *Bombus vestalis*), as well as nocturnal lepidopterans (e. g. *Hyloicus pinastri*, *Sphinx ligustri*, *Ochropleura flammatra* and *Ochropleura forcipula*). Another common group of visitors are big hymenopterans such as *Bombus terrestris* and *Xylocopa violacea*, which behave as primary nectar robbers, making holes in the corolla to extract nectar. These holes are often re-used by smaller bumblebees and bees which behave as secondary nectar robbers. Besides nectarivorous visitors, several dipterans and hymenopterans also visit the flowers to collect pollen and in most cases they do not contact the stigma (Jordano, 1990).

Study area

The study was conducted in three populations at El Bierzo region, North-West Spain. Two populations were located in the Natural Park Serra da Enciña da Lastra, municipality of Rubiá (567 m asl; 42°28'19''N, 6°50'17''W and 438 m asl 42°28'15''N, 6°49'26''W). A third population was located in La Barosa (590 m asl 42°29'50''N, 6°48'52''W). The region present a Mediterranean climate and the landscape is composed of crops and native vegetation, such as holm oak woodland (predominantly *Quercus ilex*, *Arbutus unedo* and *Quercus suber*), and scrub communities (Guitián et al., 1993, Navarro et al., 1993).

Behaviour of floral visitors

We made observations of floral visitors' behaviour from May to June of 2010, 2011 and 2012, in 90 marked plants at the three studied populations (30 plants per population). In each plant we made 10 min censuses of floral visitors accumulating a total of 70 hours and 9 minutes of observation (24 hours 16 minutes in Cobas A, 23 hours 45 minutes in Cobas B and 22 hours 8 minutes in La Barosa). We also quantified the number of available open flowers per plant at the moment of the census. The variables used to characterize visitor behaviour were: hour of visit, number of visited flowers per plant, type of visit (legitimate, primary nectar robbing, secondary nectar robbing, thieving, pollen consume or gathering), duration of visit (in seconds), and damages to reproductive structures of the flowers caused by the visitor. These observations were useful to characterize the behaviour of each visitor species, but also to define groups of species (which we called 'functional groups') based on their behavioural and taxomical affinities. Additionally, in those records in which detailed observations were possible we also quantified the number of visits in which the insect contacted anthers and/or stigma, and if the visitor promoted contact between anthers and stigma of the same flower. We accumulated a total of 621 detailed observations (called DObs in following sections) and they were useful to calculate the qualitative component of the pollination effectiveness of each visitor species.

Qualitative component of pollinator effectiveness

In order to calculate the qualitative component of the effectiveness of each visitor species as pollinator we estimated the number of pollen grains deposited on the stigma and removed from the anthers after a single visit (see below). Additionally we included some correction factors in the calculation to correct the losses of pollen due to self-pollination or by consumption by the visitor. Since no fruits are formed with autogamous crossings (Guitián et al., 1993), we considered self-pollination as a double waste for the plant: for male function because these pollen grains will not arrive to any stigma of another flower (Rathcke, 1983); and for the female function because autogamous pollen compete with the allogamous pollen for stigmatic surface and for space for pollen tubes into the style (Inouye et al., 1994).

The qualitative component term includes the contribution of the pollinators to the male and female functions of the fitness, as was calculated as:

$$Qualitative\ component = male\ function * female\ function$$

Male function-. Since a variable amount of pollen might be lost due to selfing or consumption (Inouye et al., 1994), we included into our calculation of male function two correction factors for each pollinators' species: The Coefficient of Autopollination (CAT) and the relative Coefficient of each species as vector of pollen to stigmas (CVSr).

The contribution for the male function by each pollinator species was calculated as:

$$Male\ function = \frac{PRv}{Pa} * (1 - CAT) * CVSr$$

Where:

PRv is the number of pollen grains removed per visit.

Pa is the number of pollen grains produced per anther.

CAT is the Coefficient of Autopollination.

and CVSr is the relative Coefficient of each species as Vector of pollen to Stigmas.

The number of pollen grains removed per visit (PRv) was assessed for each individual insect sampled. It was calculated as:

$$PRv = Pa - (Pc + Pv)$$

Where:

Pa is the number of pollen grains produced per anther.

Pc is the number of pollen grains pollen in control flowers (without visits). It was used as an estimative of pollen loss caused by manipulation and abiotical agents during the experiment.

Pv is number of pollen grains pollen remaining in anthers after one visit.

To quantify Pc and Pv we marked and bagged 170 floral buds in 10 plants (20 flowers per plant in average); next morning after anthesis each flower was exposed to one unique visit from one visitor except in control flowers that were kept bagged. Anthers were collected and preserved in isotonic solution (ISOTON II Diluent, from Beckman Coulter) in Eppendorf microcentrifuge tubes. At the laboratory, anthers were dissected on a microscope slide and pollen grains were removed under a magnifying glass. Each sample was placed in a plastic vial and immersed in a sonicator bath for 5 minutes in order to disaggregate clustered pollen grains and to detach the grains from any remnant of anther tissue. The samples were filtered using a 100 μm filter and the volume was completed until 20 ml with isotonic solution. Pollen grains in 1 ml homogenized subsamples were counted with a particle counter (Beckman Coulter Multisizer 3). We calculated the number of grains in each sample as the average of three counter runs corrected by the dilution factor. The same procedure was performed in one closed anther of six floral buds from the same plants to quantify the number of pollen grains produced per anther (Pa).

The Coefficient of Autopollination (CAT) was useful to correct the loss due to the pollen transfer between anthers and stigma of the same flower and was calculated as:

$$CAT = \frac{CSA}{DObs}$$

Where:

CSA is the number of records in which the species promote contact between anthers and stigma (in this order).

Dobs is the number of detailed observations.

Additionally we calculated the relative Coefficient of each species as vector of pollen to stigmas (CVSr) which reflects the capacity of the visitor species to transport pollen on its body and the chance to deliver it to a stigma. CVSr has values between 0 and 1 and was calculated as:

$$CVSr = \frac{CVS}{\max CVS}$$

Where:

max CVS is the highest value of CVS of all species.

CVS is the Coefficient of each species as vector of pollen to stigmas and was assessed as:

$$CVS = PB * \frac{CBS}{DObs}$$

Where:

PB is the average number of pollen grains on insects' bodies per species.

CBS is the number of records in which the body's visitor contacts stigmas.

DObs is the number of detailed observations.

In order to assess the number of grains on insects's body (PB) we captured floral visitors with entomological nets. Samples of pollen on their bodies were taken in basic fuchsin jelly (Kearns and Inouye, 1993) and preserved in plastic vials. For specimens bigger than 0.5 mm pollen samples were taken from different parts of the body (head, thorax, abdomen, legs and in lepidopterans also from tongue). At the laboratory the samples were gently heated and fixed on microscope slides. Finally we counted the number of conspecific (i.e. of *L. etrusca*) and heterospecific (i.e. other plant species) pollen grains with a light microscope. To estimate CVS the pollen of different parts of the body was pooled.

Female function-. The female function for self-compatible species is commonly estimated as the quantity of pollen deposited per single visit (Ne'eman et al., 2010). However, *L. etrusca* presents differences in the chances to produce fruits and seeds according to the origin of pollen (see Methods section for a description of species' reproductive system). Since stigmatic pollen load (*sensu* Inouye et al., 1994) deposited in one single visit might contain different relative amounts of pollen from the same flower (autogamous selfing), same individual (geitonogamous crossings) or other individuals (xenogamous crossings). The coefficient CAT (explained in previous section) was used to correct quality's visitor in terms of the autogamous selfing it promotes, while the Coefficient for Geitonogamy and Allogamy (CGA) was developed to involve geitonogamy and its associated reduction in plant fitness.

The female function by each visitor was calculated as:

$$Female\ function = \frac{PDv}{\max PDv} * (1 - CAT) * CGA$$

Where:

max PDv is the highest PDV of any visitor.

PDv is the number of pollen grains deposited on stigma per visit.

CAT is the Coefficient of Autopollination.

CGA is the Coefficient for Geitonogamy and Allogamy.

In order to quantify the number of pollen grains deposited on stigma after one visit (PDv), we collected stigma and style from the same flowers used to calculate the number of pollen grains removed per visit explained previously. The female structures were squashed on microscope slides and fixed with transparent nail polish. Pollen grains deposited on stigma and style were counted in the light microscope at the laboratory, distinguishing between conspecific and heterospecific pollen.

Since Guitián et al. (1993) demonstrated that geitonogamous crossings have lower fruit set than allogamous crossings, and that we observed many insects visiting more than one flower per plant, we developed the Coefficient for Geitonogamy and Allogamy (CGA) for each visitor species. This coefficient represents the average proportion of geitonogamous vs. allogamous pollen deposited on the stigma by the visitor based on the foraging behaviour at individual plants and a weighing of the value of each pollen type. We assumed that when an animal visits the first flower at one plant all the pollen deposited comes from another plant. Therefore, for those visitor species that visited only one flower per plant the CGA is equal to 1. Since pollinators do not deposit all pollen that they carry in one single visit, but a fraction of it (see Robertson, 1992 and references therein), we assumed that when the animal visits more than one flower successively in the same plant the quantity of allogamous pollen decreases and geitonogamous pollen increases at an exponential rate. This means that CGA depends on two factors: first, on the ratio of pollen deposited in one single visit vs. the pollen transported on insect's body; and second, on the average number of visited flowers per foraging bout of each visitor species.

Thereby, to calculate CGA we first estimated the average Rate of Pollen Loss (RPL) for each species. We assumed that when an animal visits the first flower all the pollen deposited is allogamous (i.e. $f_{(0)} = 1$). Then, we calculated and plotted the gradual reduction in the proportion of allogamous pollen as the insects visit a sequence of 50 flowers using the formula:

$$f_{(x)} = e^{\ln\,(1-RPL)\,*\,x}$$

Where:

x is the number of the flower visited in the sequence.

RPL was calculated as:

$$RPL = \frac{PDv}{PB}$$

Where:

PDv is the number of pollen grains deposited on stigma per visit.

PB is the average number of pollen grains on insects' bodies per species (the way to obtain both parameters was previously explained).

Then, the mean number of flowers visited per plant per species (obtained from observations of floral visitor's behaviour as explained above) was used to calculate the average proportion of allogamy in the pollen pool (*sensu* Robertson, 1992) as:

$$Allogamy = \int_{1}^{n} f(x)\,dx$$

Where:

n = mean number of flowers visited in a bout per plant per species

Then, we calculated the average proportion of geitonogamy in the pollen pool (*sensu* Robertson 1992) by subtracting the proportion of allogamous pollen from the total area delimited by the average number of flowers visited per bout as:

$$Geitonogamy = 100 * (n - 1) - \ Allogamy$$

Afterwards, we expressed Allogamy and Geitonogamy as a frequency (a number between 0 – 1) of the total area. Since the fruit set of geitonogamous crossings is 43.1 % and allogamous crossings is 65.5 % (Guitián et al., 1993), we assumed that Allogamy has the maximum output (i. e. equal to 1), and Geitonogamy has a value of 0.66. Therefore, the frequency of Geitonogamy was multiplied by 0.66 as it represents the reduction in plant's success in comparison with Allogamy. Finally, we calculated the Coefficient for Geitonogamy and Allogamy (CGA) as:

$$CGA = frequency\ of\ Allogamy + 0.66 * frequency\ of\ Geitonogamy$$

Quantitative component of pollinator effectiveness

The quantitative component of the effectiveness of a species measures the frequency of discrete mutualistic events (Rodríguez-Rodríguez et al., 2013). We calculated this component as:

$$Quantitative\ component = \frac{V}{MO} * VF$$

Where:

V is the number of visits of each species.

MO is the minutes of observation in each sampling period per plant.

VF is the number of visited flowers per plant.

The terms of this equation were calculated from the observations of visitor's behaviour explained in the previous section.

Pollination effectiveness of floral visitors

Finally, pollinator effectiveness for each visitor species was calculated as:

$$Pollinator\ effectiveness = Qualitative\ component * Quantitative\ component$$

Statistical analyses

To compare the time of visit spent per flower (log transformed) between species and groups we used Generalized Linear Model (GLM), and Games-Howell post-hoc analyses for non homoscedastic data were performed. Statistical differences in the number of visited flowers per plant (log transformed) between functional groups were evaluated using an Analysis of Variance (ANOVA) with bootstraps (10 000 iterations) to correct for non-normality; Mann-Whitney pairwise comparisons were performed in order to detect significant differences between pairs of functional groups.

To compare the quantity of pollen grains (log transformed) of *L. etrusca* and other plant species on the body of different visitors and by groups, we performed GLM analysis. The same analysis was performed to compare the pollen carried in different parts of the body of visitors.

In order to test for differences in the contribution of pollinators' species and groups to male function and the final qualitative component of pollination effectiveness, we transformed (arcsine-square root) the response variables and used a univariant ANOVA with bootstraps randomization (10 000 iterations) to correct for non-normality. To test statistical differences in the female function for species and functional groups we transformed the variables (arcsine-square root) and performed an ANOVA. The analyses were performed with SPSS 19.0.0 for Windows (IBM Corp., 2010).

RESULTS

Behaviour of floral visitors

Foraging behaviour: functional groups –. A total of 23 insect species visited the flowers of *Lonicera etrusca* (Table 5.1). Most of them foraged for nectar whether by performing legitimate visits, nectar robbing or nectar thieving. Some species also foraged for pollen consuming it at the anthers or gathering and transporting it on their bodies. Primary nectar robbing was the most frequent behaviour in visiting rate. Visits of three species of hymenopterans nectar robbers (*Bombus terrestris*, *Xylocopa violacea* and *X. cantabrita*) accounted for 54.5 % of all records. *B. terrestris* was the commonest visitor. It made heart-shape perforations on the base of the flowers by biting with the mandibulae and then introduced the tongue to extract nectar (Fig. 5.1a). On some occasions *B. terrestris* behaved as secondary nectar robber, but because of the low frequency of this behaviour and the difficulty in observing whether the individual performed primary or secondary nectar robbery we considered all visits by this species as primary robbery. *X. violacea* and *X. cantabrita* used their mouth parts to make longitudinal slits on the corolla near the base, clearly differentiable of the perforations made by *B. terrestris*. Sometimes *X. violacea* and *X. cantabrita* robbed almost ripened floral buds. During the robbing process these species commonly placed the body parallel to the corolla, with the head directed to the base of the flower and the abdomen to the distal aperture (Fig. 5.1b). During the process of landing on an inflorescence, crawling between flowers and flower visiting, they contacted both female and male reproductive structures (Table 5.1), particularly with the abdomen (Fig. 5.2). They pierced one or more times each flower to extract all nectar accumulated and then crawled to another flower of the same inflorescence, visiting in average more than 6 flowers per plant (Table 5.1). Primary robbers commonly made new perforations even if the flower was already pierced. As a result, some flowers may present several holes performed on different visit events, often made by different robber species. However, we never observed damages to the reproductive structures (stamens or pistils) as a consequence of the foraging behaviour of these species.

On a different way, the coleopterans *Oxythirea funesta* and *Tropinota hirta* torn the corolla longitudinally from the entrance down to the base to reach for the nectar (Fig. 5.1c). This robbing behaviour produced severe damages to the corolla and frequently also to the filaments of stamens and/or the style. Coleopterans primary robbers have low visits frequency (Table 5.1) and made longer visits per flower in comparison with legitimate visitors and hymenopterans primary robbers ($F_{5,\ 278} = 55.98$; $P < 0.001$; Games-Howell $P < 0.05$); and visited fewer flowers per plant than other groups of visitor species ($F_{5,\ 173} = 22.106$, $P < 0.001$; $U = 0.00$, $P < 0.05$, $n = 10$ with legitimate hymenopterans; $U = 0.3$, $P < 0.01$, $n = 33$ with legitimate lepidopterans; and $U = 9$, $P < 0.05$, $n = 71$ with primary hymenopteran robbers). Additionally, during the corolla tearing and nectar feeding process these visitors very often forced the contact between anthers and stigma causing a high percentage of self-pollination (Table 5.1). Moreover, unlike hymenopterans primary robbers which consumed exclusively nectar, coleopterans primary robbers also ate pollen from anthers after foraging for nectar. Based on these differences hymenopterans and coleopterans primary nectar robbers were considered as different functional groups.

The second most frequent group of visitors performed legitimate visits (i.e. accessing to the nectar through the floral mouth). This group comprise long tongued hymenopterans and lepidopterans that foraged exclusively for nectar and contacted the stigma in more than 60 % of the visits (Fig. 5.1d, e and Table 5.1). The visits performed by this group were two times faster than visits performed by primary robbers. Although we found significant differences in the number of flowers visited per plant between visitor groups ($F_{5,\ 173} = 22.106$, $P < 0.001$), pairwise comparisons revealed that legitimate visitors visited similar quantities of flowers per plant than hymenopterans primary robbers ($U = 225$, $P = 0.81$, $n = 75$). However, unlike robbers, legitimate visitors commonly flew between flowers even in the same inflorescence. *Anthophora acervorum* made the visits in two phases: first introduced its head into the flower while still in flight, and then perched to extract nectar. Whether the flower was recently visited by another insect, this bee leaved without perching. This suggests that they made a first inspection for the presence of nectar and only perched when there was enough accumulated nectar.

Otherwise, *Macroglossum stellatarum* and *Hemaris fuciformis* always extracted nectar while sustaining hovering flight (Fig. 5.1e). Their visits were significantly faster than other groups of visitors such as pollen consumers, primary robbers and hymenopterans secondary robbers (post-hoc Games-Howell test $P < 0.05$). At occasions, *M. stellatarum* visited flower buds that begun anthesis but still without protruding reproductive structures.

Five species of coleopterans and dipterans consumed pollen directly from anthers (Fig. 5.1f, g). They perched on stamens and occasionally contacted stigmas during the pollen foraging process. This group visited fewer flowers per plant than other visitors ($F_{5,173} = 22.106$, $P < 0.001$; pair wise comparisons $U = 8$, $P < 0.001$, $n = 40$ with legitimate hymenopterans; $U = 78$, $P < 0.001$, $n = 63$ with legitimate lepidopterans; and $U = 194$, $P < 0.001$, $n = 101$ with nectar robber hymenopterans). This group also made longer visits per flower in comparison with legitimate visitors and primary nectar robber hymenopterans ($F_{5,\,278} = 55.98$; $P < 0.001$; Games-Howell $P < 0.001$). These coleopterans often spent long periods of apparent inactivity on the flowers and seldom used flowers as mating areas. In two opportunities dipterans were observed eating pollen placed from the stigma.

Three species of hymenopterans and two nocturnal lepidopterans behaved as secondary nectar robbers (Table 5.1). Besides nectar, small bees (such as *Lasioglossum* spp., *Ceratina* spp. and *Halictus* spp.) also foraged for pollen during the same visit. They usually perched on anthers, filaments and style while collecting pollen and stored it on the ventral region (Fig. 5.1h). While doing this, they occasionally touched the stigmas. Then they crawled along the external part of the flowers searching for perforations made by primary nectar robbers to extract nectar. Nocturnal lepidopterans also used existing perforations to extract nectar, but we never observed any contact with reproductive structures.

Anaspis frontalis is a small coleopteran that entered through the distal aperture of the flower and crawled along the inner corolla to reach the nectar. Eventually this species also foraged for pollen contacting anthers, but as occurred with nocturnal lepidopterans they never contacted stigmas while foraging. For this reason, added to their low visit frequencies, *A. frontalis* and nocturnal lepidopterans were not included in subsequent analysis of pollination effectiveness.

Table 5.1. Foraging behaviour and visit frequency of 23 insect species in the flowers of *Lonicera etrusca* at three populations of the Iberian Peninsula. * n = nectar, n-p = nectar and pollen, p = pollen; ‡ time in seconds ± standard deviation (*n*); ***n***§ = number of observations to calculate percentage of contact of anthers, stigmas and self-pollination.

Behaviour	Taxonomic group	Visitor species	Consumed resource*	% visits (n = 1463)	Flowers per plant	Time of visit‡	% Contact of anthers	% Contact of stigmas	% self-pollination	*n*§
Legitimate visits	Hymenoptera	*Anthophora acervorum*	n	15	13.8	3.2 ± 3.2 (73)	22.2	72.2	5.6	18
		Bombus hortorum	n	4.8	23.3	23.9 ± 28 (6)				
		Eucera longicornis	n	1.1	16					
		Anthophora plumipes	n	0.9	4.3	3.6 ± 0.3 (2)				
	Lepidoptera	*Macroglossum stellatarum*	n	11.6	14.2	3.6 ± 2.5 (27)	14.3	52.4	0	21
		Hemaris fuciformis	n	0.1	2	4.2 ± 3 (3)	60	60	0	5
		Papilio machaon	n	0.1	2		100	100	0	1
Primary robbery	Hymenoptera	*Bombus terrestris*	n	36.2	11.5	8.3 ± 4.9 (51)	14.7	24.8	6.2	129
		Xylocopa violacea	n	17	13.1	6.5 ± 4 (63)	31.9	26.8	5.8	138
		Xylocopa cantabrita	n	1.3	6.3	11.6 ± 11 (16)	9.1	6.1	3.0	33
Primary robbery and pollen consumption	Coleoptera	*Oxythyrea funesta*	n-p	0.9	1	219.5 ± 137.7 (4)	25.6	2.6	59.0	39
		Tropinota hirta	n-p	0.1	1					
Secondary robbery	Lepidoptera	*Scopula subpunctaria*	n	0.4	2	56 (1)	0	0	0	6
		Pleurota	n	0.1	1	14 (1)	0	0	0	4
	Hymenoptera	*Lasioglossum*	n-p	5.3	3.2	55 ± 61.8 (8)	59.3	13.0	3.7	54
		Ceratina	n-p	0.8	1.2	93.6 ± 99.1 (7)	54.5	3.0	6.1	33
		Halictus	n-p	0.3	1		11.1	11.1	0	9

Pollen consumption	Coleoptera	*Cardiophorus gramineus*	p	1.6	1.3	36 ± 10.6 (3)	51.6	16.1	16.1	31
		Oedemera lurida	p	0.3	1.7	60 ± 35.3 (4)	64.3	3.6	0	28
	Diptera	*Episyrphus balteatus*	p	0.7	1.7	47 ± 45.2 (5)	79.2	20.8	25	24
		Scaeva pyrastri	p	0.5	1.8	74 ±56.6 (2)	62.5	25.0	6.3	16
		Diptera	p	0.4	6	8.7 ± 4.2 (3)	33.3	33.3	33.3	3
Nectar thievery and pollen consumption	Coleoptera	*Anaspis frontalis*	n-p	0.3	1	98 (1)	84.6	0	7.7	13

Figure 5.1. The flowers of *Lonicera etrusca* were visited by a high diversity of insects with different behaviours. a) *Bombus terrestris* performing primary nectar robbery. b) *Xylocopa violacea* performing primary nectar robbery. The big size and the position of the body facilitated the contact between abdomen and floral organs. c) *Oxytyrea funesta* tore the corollas from entrance to the base to rob nectar and commonly damaged the reproductive structures of the flower. d) Long tongued bees, such as *Bombus hortorum*, visited the flowers legitimately contacting very oft anthers and stigmas. e) Hawkmoths, such as *Macroglossum stellatarum* visited the flowers legitimately. f) *Cardiophorus gramineus* and other small beetles, as well as syrphid flies such this *Scaeva pyrastri* (g), consumed pollen directly from the anthers and commonly produced contact of stigma and anther of the same flower promoting self-pollination. h) Small bees, such as this *Ceratina*, collected pollen and gathered at the abdomen to take it to the colony. During the same visit they usually searched along the corolla for holes made by primary robbers in order to extract nectar, behaving as secondary nectar robbers.

Figure 5.2. Hymenopteran primary robbers contact stamens and stigmas with abdomen and legs during landing, movement between flowers and nectar robbery process. a, b y c) *Xylocopa violacea.* d and e) *Bombus terrestris.*

Daily temporal dynamics of floral visitors-. Legitimate visitors presented a diurnal bimodal pattern of foraging activity (Fig. 5.3). Maximum activity of hymenopterans occurred at the late afternoon (20:00 - 22:00 h). This pattern was highly influenced by the foraging behaviour of *A. acervorum*, which foraged after midday and was the only active legitimate hymenopteran visitor at the evening. Lepidopterans presented a main activity peak after midday (14:00 - 16:00 h). *M. stellatarum* showed a bimodal activity pattern, with highest visitation rate after midday and another peak at the evening (18:00 - 20:00 h). Primary nectar robbers were diurnal and presented highest visitation rates at evening, similar to the foraging pattern of legitimate visitors. It was remarkable that the main two species of primary robbers presented asynchronous foraging patterns: activity of *B. terrestris* started in the morning (08:00 - 12:00 h), decreasing at midday (12:00 - 14:00 h) and increased again until reaching a maximum at the late evening being the only primary robber active during this period; on the other hand, *X. violacea* had the highest visitation rate after midday and was inactive during early morning and evening. The two activity peaks of secondary robbers correspond to different insect groups: bees were active after midday, while moths were active during the night (after 22:00 h). Pollen foragers had low visitation rates in comparison with other visitors and were active late in the evening.

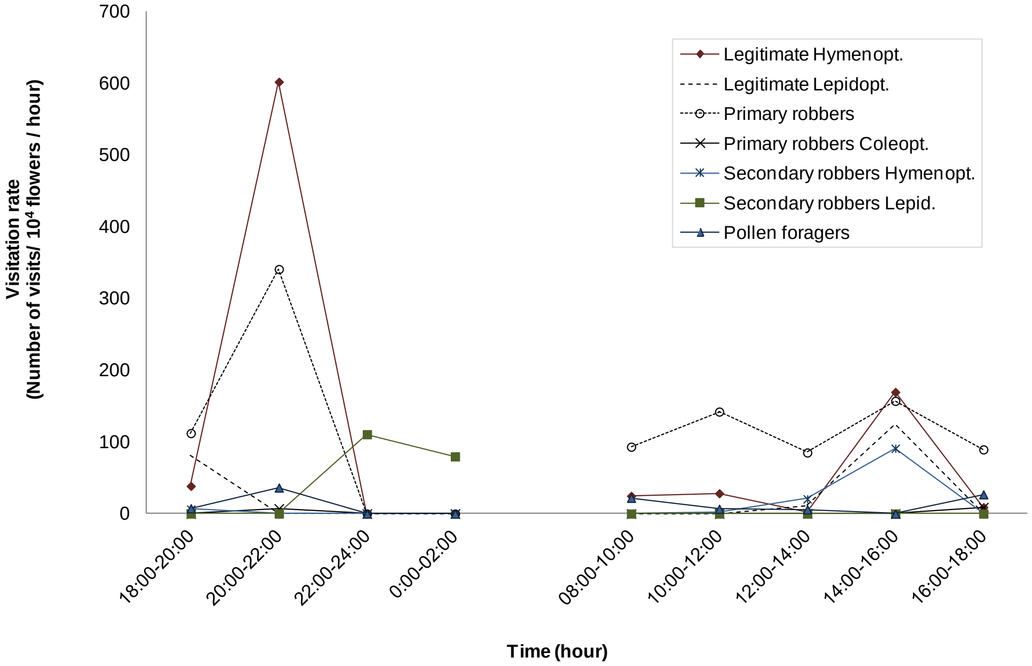


Figure 5.3. Diurnal and nocturnal activity patterns of visitor groups since the anthesis of *L. etrusca*'s flowers.

Qualitative component of pollinator effectiveness

Male function–. As expected from foraging behaviour, small bees (that behaved as pollen gatherers and secondary robbers) removed from anthers large quantities of pollen grains (Fig. 5.4a), whereas primary nectar robbers removed the least amount of pollen in comparison with other visitors. They removed significantly less pollen from anthers than legitimate lepidopterans and secondary robbers (*P*MCMC = 0.003 and *P*MCMC = 0.018 respectively).

The pollen carryover analysis of 208 pollen samples revealed that 18 visitor species transported pollen of *L. etrusca* on the body. However, we found significant differences in the quantity of pollen transported by species ($F_{17, 86} = 5.21$, $P < 0.001$) and functional groups ($F_{5, 86} = 4.77$, $P = 0.001$). Pollen gatherers, legitimate visitors (both bees and butterflies) and primary nectar robbers transported higher quantities of pollen, while pollen eaters and coleopterans primary robbers transported very low quantities of pollen on their bodies (Fig. 5.4b). Legitimate butterflies tended to carry more pollen of *L. etrusca* on abdomen and thorax with minimal mixture of pollen from other species. Legitimate bees transported more pollen of *L. etrusca* on thorax, head and legs, although the quantity of pollen from other species was 4.2 times greater. Nectar robbers transported pollen of *L. etrusca* particularly on abdomen and legs. When analyzed the log transformed quantity of pollen grains transported by the species in different body parts, we found a remarkable variation of between individuals of all species and functional groups, but no significant differences in the quantity of pollen transported in different parts of the body were found, except in *Hemaris fuciformis* which transported lower pollen quantities at the tongue (Table 5.2).

We found statistical differences in pollen transport of heterospecific pollen on the bodies of visitors ($F_{1, 17} = 2.062$, $P = 0.019$ for species and $F_{1, 5} = 4.26$, $P = 0.002$ for functional groups). Primary robbers and legitimate hymenopterans transported significantly higher mixed pollen loads (Fig. 5.4b) in comparison with other visitor groups such as pollen foragers ($P < 0.001$). However, the quantity of heterospecific pollen deposited on stigmas by these visitors was low (mean = 0.61, sd = 0.16, n = 74 for primary robbers and 0.09, sd = 0.05, n = 33 for legitimate hymenopterans).

Male function of the qualitative component of pollination effectiveness (Fig. 5.5) comprised the main aspects of pollen removal and the chance to be transferred to a stigma. Therefore, groups such as pollen gatherers or pollen foragers which removed high quantities of pollen mostly for consume (Fig. 5.4a), and that additionally had low chances to contact stigma during foraging (Table 5.1), contributed at lower levels to male function in comparison to the original patterns of pollen removal. We found significant differences in the male function between species ($F_{10, 211} = 68.61$, $P < 0.001$) and functional groups ($F_{5, 211} = 32.094$, $P < 0.001$). Legitimate hymenopterans presented the highest values for male function while coleopterans robbers presented the lowest values (Fig. 5.5).

Female function–. We found significant differences in the contribution to the female function between species and functional groups ($F_{10, 200} = 2.46$, $P = 0.009$ and $F_{5, 199} = 2.42$, $P = 0.037$ respectively). Legitimate lepidopterans and coleopterans primary nectar robbers presented higher values than other functional groups (Fig. 5.5). Otherwise, primary robbers were less specific in pollen deposition, since they deposited higher quantities of pollen on style (Fig. 5.4c). However, no statistical differences in the pollen deposited on styles between groups were found (*P*MCMC > 0.05 between all pairs of groups).

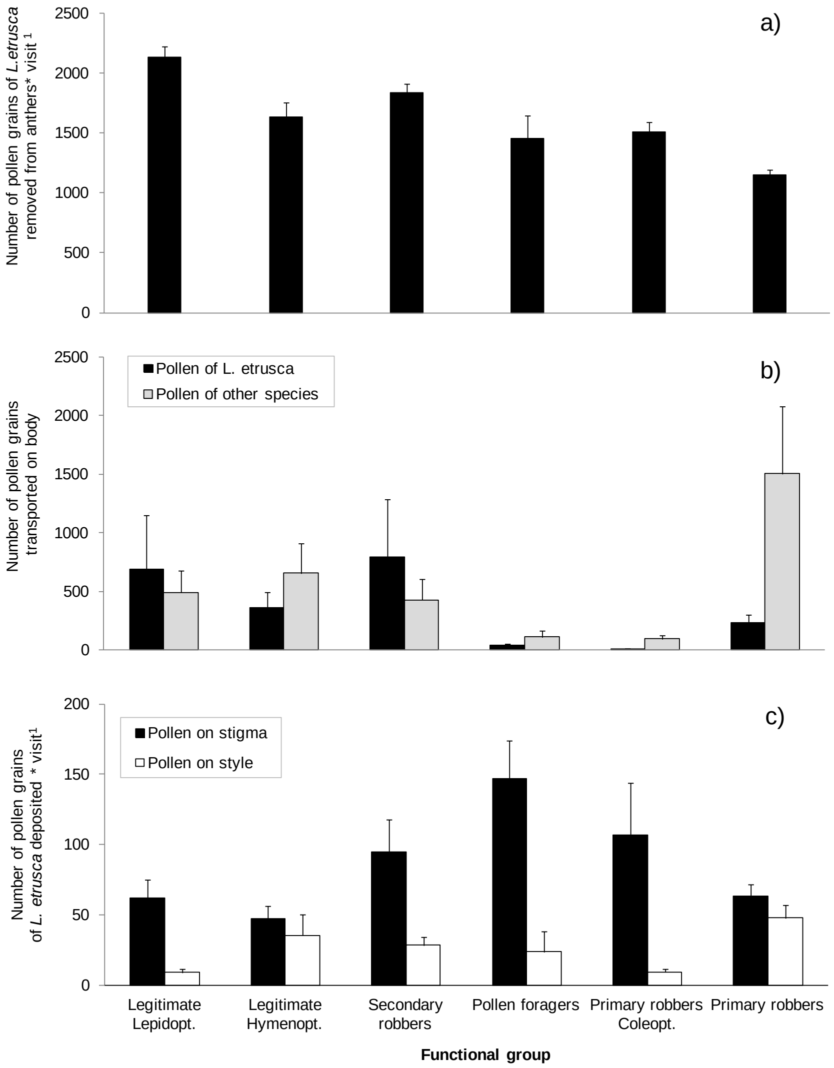


Figure 5.4. Pollen transference process promoted by floral visitors in *Lonicera etrusca*. a) Pollen removal from anthers per visit. b) Quantity of pollen of *L. etrusca* and other plant species on the body of floral visitors. c) Quantity of conspecific pollen deposited on stigma and style per visit by groups of visitors.

Qualitative component of pollinator effectiveness

Visitor species and functional groups presented significant differences in the qualitative component of pollination effectiveness ($F_{9,\ 181} = 23.328$, $P < 0.001$ for species and $F_{5,\ 181} = 4.509$, $P = 0.001$ for groups). The Broad-bordered Bee Hawkmoth (*H. fuciformis*) has the highest value on the qualitative component (Fig. 5.6). The opposed extreme in the qualitative component corresponds to the coleopteran primary robber (*O. funesta*), which had the lowest value.

Table 5.2. GLM for the pollen quantity transported on different body structures by four common visitors. Asterisk represents significant relationships with $P \leq 0.05$.

Species	Pollen transport	F	*P*
A. acervorum	Part of the body	2.74	0.69
	Individual	8.47	<0.001*
H. fuciformis	Part of the body	3.63	0.017*
	Individual	2.63	0.025*
B. terrestris	Part of the body	1.47	0.23
	Individual	5.52	<0.001*
X. violacea	Part of the body	2.13	0.12
	Individual	4.64	<0.001*

Quantitative component of pollinator effectiveness

We found highly significant differences in the quantitative component at both ecological levels, species ($F_{18,\ 106} = 3.06$, $P < 0.001$) and functional groups ($F_{5,\ 119} = 6.51$, $P < 0.001$). Primary nectar robbers and legitimate visitors (lepidopterans and hymenopterans) presented the highest values of the quantitative component (Fig. 5.6).

Pollination effectiveness of floral visitors

A. acervorum (a hymenopteran legitimate visitor) was the most efficient pollinator of *L. etrusca* (Fig. 5.6). Two species of hymenopterans primary robbers were the second most important pollinators and presented similar values on both components between them. This result presented a sharp contrast with the other group of primary nectar robbers, the coleopterans which had very low values on both, qualitative and quantitative components, that reflected the high levels of self pollination promoted by them, as well as a low percentage of stigmas contact and low visitation rates (Table 5.1).

Legitimate lepidopterans presented divergent patterns of effectiveness. *H. fuciformis* had the highest value for the qualitative component of any species, but the low visitation rate reduced its effectiveness as pollinator, which was similar to the effectiveness of *X. violacea*. On the other hand, *M. stellatarum* presented intermediate to low values for the qualitative component but given that was a common visitor the quantitative values were moderately high, resulting in an intermediate value of pollination effectiveness. Different species of small bees behaving as secondary robbers also presented differences in their importance as pollinators changing in both dimensions of pollination effectiveness.

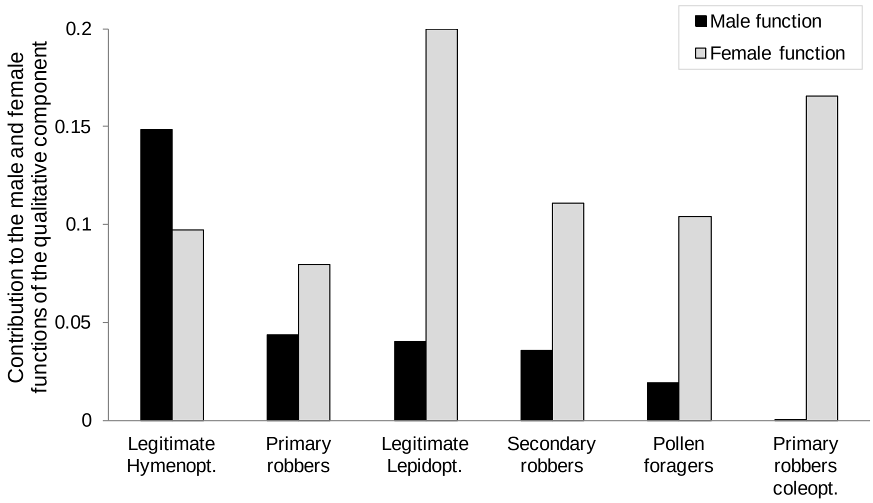


Figure 5.5. Contribution of visitor groups to female and male functions of the quantitative component.

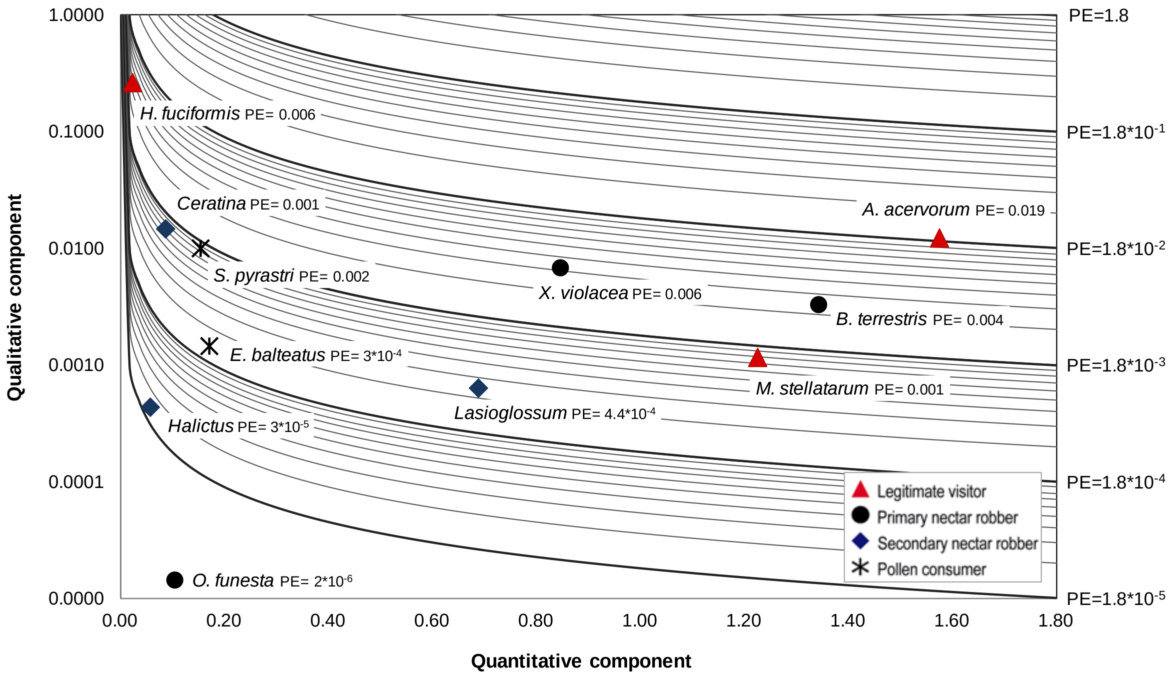


Figure 5.6. Pollination effectiveness landscape of *Lonicera etrusca's* pollinators. Isoclines represent all combinations of the quantitative and qualitative components with same values for pollination effectiveness (PE).

Discussion

Effectiveness of floral visitors as pollinators

According with previous observations of floral visitors and floral features of *L. etrusca* (Jordano, 1990; Guitián et al., 1993; Chapter 4 this dissertation), we expected that crepuscular legitimate lepidopterans (particularly the Hawkmoth *Macroglossum setellatarum*) were the most efficient pollinators. Instead, *M. stellatarum* has low or medium pollination effectiveness in comparison with hymenopterans that behave as legitimate visitors (particularly *Anthophora acervorum*) or primary nectar robbers (such as *Xylocopa violacea* and *Bombus terrestris*). A similar tendency was observed in other plants (Mayfield et al., 2001; Fumero-Cabán and Meléndez-Ackerman, 2007), whose flowers are apparently specialized for a certain group of pollinators but thorough examinations of plant-visitor interactions revealed that unexpected pollinators were more efficient than expected by its pollination syndrome (*sensu* Proctor et al., 1996). This result is consistent with the evidences accumulated in last decades about the rarity of extreme generalization or specialization in pollination mutualistic interactions (e.g. Mayfield et al., 2001; Olesen and Jordano, 2002; Fenster et al., 2004; Waser and Ollerton, 2006).

The differences observed in the importance of different groups of floral visitors between this study and previous studies regarding the pollination ecology of *L. etusca* (Guitián et al., 1993), might be related to the scope of the studies to some extent, but mainly rely in temporal and spatial dynamics of interacting communities. By one side, we assessed two components of pollination effectiveness (i.e. qualitative and quantitative components), while in previous studies researchers based their conclusions exclusively in the number of floral visits (Jordano, 1990; Guitián et al., 1993). Also, differences in visitation rates between geographical regions result from changes in species composition and population abundances of visitor assemblages (Moeller, 2006; Gómez et al., 2009). When comparing the number of floral visits in the same region in different studies (i.e. this study and Guitián et al., 1993), the proportion of visits by legitimate hymenopterans and lepidopterans changed considerably across years. In fact, changes in population abundances of visitors are common for many pollination systems (Price et al., 2005; Vázquez et al., 2009 and references therein). Therefore, besides geographical differences in visitor assemblages, the year-to year variation of population abundances associated to visitation rates constitute a very important factor to consider when searching of general patterns in plant-pollinator interactions.

A remarkable result was the strong asymmetry in the contribution of both components of pollination effectiveness found in the Hawkmoth *Hemaris fuciformis*. This species has the highest value on the qualitative component, resulting from its notable contribution for the male function of qualitative component, its importance as pollen vector observed in body pollen loads and its specificity in depositing conspecific pollen on stigma. This means that in a single floral visit basis the species has the highest effect on pollen removal and deposition, probably caused by a strong correspondence in morphology between flower and visitor. In general, the species of *Lonicera* genus are broadly recognized by sharing a coevolutionary history with Hawkmoths that shaped floral characters resulting in the typical "sphingophilous flowers" (Miyake and Yahara, 1998; Miyake et al., 1998; Larson et al., 2002). However, the frequency of the interaction (i.e. quantitative component) is determining for the output of mutualistic relationships rather than differences in the effect per visit (Vázquez et al., 2005; Fumero-Cabán and Meléndez-Ackerman, 2007; Sahli and Conner, 2007). In this case, a very low visitation rate caused a moderated pollination effectiveness of this floral visitor. The effectiveness of this pollinator was similar to the hymenopterans primary robbers which had lower values for the qualitative component, but whose values for the quantitative component were medium to high.

The differences in pollination effectiveness observed between the hymenopterans and coleopterans primary robbers suggest that changes in the particular behaviour of robbers might have opposite consequences for the pollination of the plant. For instance, despite that hymenopterans pierced an important quantity of flowers, even leaving several holes at the same flower, the flowers were able to receive subsequent visits (Rojas-Nossa personal observation) and no damages to the reproductive organs were observed. On the contrary, the coleopterans (*Oxythyrea funesta* and *Tropinota hirta*) produce severe damages to the corolla, stamens and style during nectar robbery and additionally consumed pollen. This behaviour probably affects the attractiveness for other visitors and diminishes fecundation rates, as has been recorded in other systems with robbers behaving in a similar way (McDade and Kinsman, 1980; Traveset et al., 1998; Rojas-Nossa, 2007). Also, the exaggerated visit time and the high frequency of contact between anthers and stigmas produced by coleopterans in the flowers produce high levels of self-deposition of pollen. As a consequence these visitors have a very low quality as pollinators. This fact combined to the extreme differences in the visitation rates (and therefore in the quantitative component) produced marked differences of pollination effectiveness among coleopterans and hymenopterans primary nectar robbers.

Nectar robbers as efficient pollinators

Commonly nectar robbers are detrimental for the fitness of host plants through direct and indirect mechanisms (Irwin et al., 2010). Nevertheless, a growing body of experimental evidence suggests that robbers have neutral or even positive effects on plants by pollinating flowers when contact the reproductive structures during the robbery process ("robber-like pollinators" *sensu* Higashi et al., 1998), being in some cases more efficient pollinators than legitimate visitors (Zhang et al., 2014). *Bombus terrestris* and *Xylocopa violacea*, are common nectar robbers in native and cultivated plant species (Maloof and Inouye, 2000; Goulson, 2003; Castro et al., 2009; Irwin et al., 2010 and references therein), and in several plants perform pollination while behave as primary nectar robbers (Higashi et al., 1998; Navarro, 2000; Zhang et al., 2014). In *L. etrusca* there is evidence that nectar robbery does not have a negative effect on male (measured as quantity and distance of pollen dispersal), or female functions (fruit set, seed-ovule ratio and seed weight) of reproductive success (Chapter 3 this dissertation), and that this species promote cross-pollination during nectar robbing (Chapter 4 this dissertation). Some hypothesis to explain these results were proposed, but the factors and processes involved were not clarified until now.

Hymenopterans primary robbers were the second most efficient group of pollinators of *L. etrusca*. This output is due in part to the high visitation rates by both species at the study site. A similar pattern was observed in other studies made in the Iberian Peninsula where the nectar robbers performed a high percentage of the visits observed (Jordano, 1990; Guitián et al., 1993). This reveals, that nectar robbing in *L. etrusca* is not a casual phenomenon, but reiterative and apparently variable in its distribution area and across years.

Besides the importance of the high visitation rates to determine the pollination effectiveness of hymenopterans primary robbers (i.e. high values for the quantitative component), it is remarkable the relatively high quality of the pollination service performed by them. These insects provide a better pollination service in terms of the quality of pollen deposited in comparison with the supplementation of xenogamous pollen treatment and that one single visit is enough to fertilize all ovules present in one flower (Chapter 4 this dissertation). Our observations of foraging behaviour and the high quantity of pollen transported on abdomen and legs indicate that the pollen transfer between flowers and robbers mainly occurs during the process of piercing and nectar extraction, but also during landing to the flowers and movements between flowers in the inflorescences. Similar results were found in other plant species in which nectar robbers enhanced female fitness through pollination (Macior, 1966; Waser, 1979; Higashi et al., 1988; Arizmendi et al., 1996; Morris, 1996; Navarro, 2000). In all these cases, the foraging behaviour, and the morphological aspects of flowers and robbers (such as exerted reproductive structures, arrangement of flower in inflorescences and big body size), are key factors that facilitate pollen transfer. Also, since most of the visits by *B. terrestris* are made just after anthesis our results regarding the activity patterns of nectar robbers do not totally fit Jordano's hypothesis about the visits performed next day after anthesis as an explanation to the absence of adverse effects of nectar robbing on the fitness of this plant, at least with regard to this robber species. The observed visitation pattern favours pollination by nectar robbers because many visits are performed just after anthesis when the stigmas are already receptive but anthers are still closed, producing minimal self-pollen deposition and higher outcrossing levels.

Besides the direct effect of hymenopterans primary nectar robbers as pollinators, their effects might also be indirect since they create new ways of access to floral nectar that otherwise would not be accessible for secondary robbers (Newman and Thomson, 2005). Small bees usually collect pollen and forage for nectar during the same visit, behaving as secondary nectar robbers (*sensu* Inouye, 1980). These species are to some extent positive for the reproduction of *L. etrusca*, acting as low-effective pollinators, but could also be detrimental by direct and indirect mechanisms (Irwin et al., 2010). Negative impacts on host fitness involve reducing the quantity of pollen available for seed siring, since they gather and carry pollen to the nest for feeding (Inouye et al., 1994). And also, their foraging activity may cause a reduction in nectar standing crop that diminishes both the quantity and the quality of visits performed by more efficient pollinators (Maloof and Inouye, 2000).

Considering that hymenopterans primary robbers are common visitors of *L. etrusca* along an important part of its distribution range, that are responsible for a high proportion of all floral visits received, and that they affect the reproduction by direct and indirect paths. Further research must address the effects of robbers as potential drivers of the evolution of floral and plant traits (Navarro and Medel, 2009; Irwin et al., 2010; Zhang et al., 2014).

Conclusions

Despite *L. etrusca* shows a floral morphology apparently specialized to the pollination of Hawkmoths, its flowers are visited and pollinated by a high diversity of insects that differ in their pollination effectiveness. The most important pollinators of this plant are big size hymenopterans that behave as legitimate visitors (*Anthophora acervorum*) or primary nectar robbers (*Bombus terrestris* and *Xylocopa violacea*). These species carried high quantities of pollen on the body, particularly on abdomen and legs. The pollen removal from anthers and deposition on stigmas occurred during the displacement of these visitors between flowers, as well as during the robbery process (i.e. corolla piercing and nectar intake). This output is favoured by the exerted reproductive structures of the flower and the grouping of flowers into inflorescences. Also, big body size and foraging behaviour facilitated contact between the body of robbers and the floral reproductive organs. *B. terrestris* presents the highest visitation rate of at the end of the afternoon just after anthesis. This allows cross pollination, since at that moment stigmas are already receptive but anthers are still closed, producing minimal self-pollination. Two species of coleopterans (*Tropinota hirta* and *Oxythyrea funesta*) also performed primary nectar robbery. But at difference with hymenopterans, they produced severe damages to the reproductive floral organs, transported few pollen grains, promoted high levels of auto-pollination and visited few flowers. As consequence, the pollination effectiveness performed by coleopterans was significantly lower than by hymenopterans. This reflects the importance of the particular behaviour and visit frequency for determine the effects of robbery for host plant fitness.

Chapter 6

Nectar robbery: a common phenomenon mainly determined by accessibility constrains and density of energetic rewards

Abstract

Nectar robbers are animals that use a hole made in the perianth to extract nectar. Since robbers may modify plant fitness they play important ecological roles by driving floral evolution, shaping populations' structure and influencing community dynamics. Although nectar robbery is widespread in angiosperms, the causes and ecological implications of this behaviour at large ecological scales remain practically unexplored. Our aims are to study the frequency of nectar robbery in plants of temperate and tropical regions and its association with plant traits. We characterised the frequency of nectar robbery in 88 plant species of different biogeographical regions (Mediterranean, Alps, Antilles and Andes) and identified the species of floral visitors that behave as nectar robbers. We analysed associations between the levels of robbery with floral morphology, production and density of energetic rewards, mechanisms of protection against potential nectar robbers and plant's life form. Nectar robbery was present in all sampled sites, revealing that this phenomenon is ubiquitous in animal-dependent pollination systems and more frequent than the literature about plant-animal interactions suggests. Within communities one or two plant species present very high levels of robbery while other species have intermediate to low robbery. Plants with long flowers, abundant nectar and high density of energetic offer are more prone to be robbed by both insects and birds. A high aggregation of the flowers in the plants, and the presence of long calyxes and bracts are effective mechanical barriers that reduce nectar robbery by insects and in lesser extent by birds. Birds robbed predominantly less aggregated fleshy flowers from trees, while insects robbed mostly narrow flowers with intermediate aggregation. Besides the morphological constraints that operate at a single flower basis, this study reveals that nectar robbery is a phenomenon highly density-dependent that reflects mechanisms that operate at higher ecological scales.

Introduction

Nectar robbers are animals that obtain nectar through perforations in the flower's perianth made by themselves or by other robber (Inouye, 1980). This particular feeding behaviour has diverse consequences for plant's reproductive success that differ in strength and direction, ranging from none to high impact that can be negative to positive for host fitness (for detailed reviews see Maloof and Inouye, 2000; Irwin et al., 2010). For this reason nectar robbers are considered as one of the selective forces that drive plant's evolution shaping floral characteristics, populations structure and community dynamics (Irwin et al., 2001; Urcelay et al., 2006; Navarro and Medel, 2009).

Nectar robbery is common in angiosperms and has been observed in many systems around the globe except in Antarctica (Irwin and Maloof, 2002). It occurs mostly in long tubular flowers or flowers with spurs in which nectar is kept out of reach from animals with short proboscis (Lara and Ornelas, 2001; Irwin et al., 2010). However, not all plants with concealed nectar are equally robbed, and a great variation among individuals, species, sites, seasons and years occurs (Arizmendi, 2001; Irwin et al., 2001). Although some plant species are only occasionally robbed, other plants are more prone to experience robbery and in some systems the impact is so high that nearly all open flowers within a population present holes made by these floral visitors (Maloof and Inouye, 2000). Such differences in the proportion of robbed flowers among species are attributable to temporal or spatial changes in abundances of robbers or availability of floral resources, but also to particular characteristics of the plants that make them more prone to be robbed (Navarro, 2000; Irwin and Maloof, 2002). However, the factors that determine the differences in the frequency of robbery within communities have been practically unexplored. To our knowledge one previous work provided preliminary insights on the importance of corolla length and flowers' abundance to determine robbery in tropical plant communities (Rojas-Nossa, 2013). Considering the relevance of these exploiters for the reproduction and evolution of the plants and thus their impact on the dynamics of entire ecosystems, the plants' traits that explain the variation in robbery frequency require particular attention (Irwin et al., 2001; Irwin and Maloof, 2002).

Although nectar robbery has been observed in diverse taxonomical groups, the most common robbers are insects and birds (Irwin et al., 2010). Several species of hymenopterans (particularly of the genera *Xylocopa* and *Bombus*), and coleopterans in diverse ecosystems have been observed behaving as nectar robbers of bird or insect-pollinated plants (Utelli and Roy, 2001; Zhu et al., 2010; Teppner, 2011). Among birds, the flowerpiercers (*Diglossa* genus) are very specialized nectar robbers (Schondube and Martínez Del Rio, 2004). They have a particular bill and tongue morphology that allows them to hold the flower with their hooked maxilla while pierce with the sharp mandible and introduce repeatedly the tongue to extract nectar

(Villeumier, 1969; Schondube and Martínez Del Rio, 2003). These birds live in sympatry with hummingbirds in the mountain forests of South and Central America sharing nectar resources (Arizmendi, 2001; Navarro, 2001; Rojas-Nossa, 2007; Navarro et al., 2008).

Even though both groups of robbers (insects and birds) feed on floral nectar, their cognitive, behavioural, sensory, morphological and physiological traits related to nectarivory differ. As a consequence, they can use different plant traits as cues to find nectar and discriminate among less or more rewarding flowers and species, driving selection on different characteristics of the plants they visit. For example, floral morphology is a character strongly associated to the quality of the reward and the frequency of nectar robbery by both animal groups, but other traits such as floral orientation and flowers' density have been related to higher frequency of robbery either by birds or insects (Lara and Ornelas, 2001; Rojas-Nossa, 2007, 2013; Castro et al., 2009). Otherwise, some plants present traits that act as mechanisms to reduce nectar robbery. The presence of extrafloral nectaries or chemical compounds in nectar and flower tissues reduces visits by nectar robber insects (Adler, 2000; Adler and Irwin, 2005; Kessler et al., 2008). Also it has been suggested that flowers densely arranged, modifications of floral parts and presence of structures (such as thick floral parts, or bracts), might act as efficient physical constraints to protect nectar from larcenists (Stiles, 1981; Inouye, 1983; Rojas-Nossa, 2007; Whitney et al., 2009). However the effectiveness of such mechanical barriers to diminish nectar robbery has been not systematically tested (Irwin et al., 2010).

Despite nectar robbers' relevance to understand evolution and stability of pollination interactions, scant biological systems have been studied from different perspectives (but see Irwin and Brody, 1998, 2011; and references therein), making the information about nectar robbers still too scarce, fragmented and limited to punctual geographic areas hindering to infer common patterns. Only a few empirical and theoretical approaches have attempted to disentangle the ecological complexity of plants-pollinators-larcenists interactions at community or system levels (Arizmendi, 2001; Rojas-Nossa, 2013; Wang, 2013; Maruyama et al., 2015; Wang et al., 2015), but is still more remarkable the absence of studies that compare the characteristics among robbed and non robbed plants within communities that could lead to generalizations regarding the causes of this phenomenon in broader ecological and evolutionary contexts. Therefore, our aims are: a) to study and compare the frequency of nectar robbery in plant species within four temperate and tropical communities in which nectar robbers are mainly insects or birds, and b) to detect which floral and plant traits (including morphology, nectar reward, density of energetic offer, presence of protection mechanisms against nectar robbers, and plant life form), are associated with the frequency of robbery by each animal group within these communities.

Methods

Study sites

This study was performed in four communities of plants analysing only those species whose flowers had concealed nectar into a spur, cup or tube ≥ 3.6 mm length formed by the corolla, the calyx or both. This limit value corresponds to the minimum length of the proboscis or beaks for the legitimate pollinators reported for the whole of studied communities (see Herrera, 1989). The work was made in two communities at two different temperate biogeographical regions: Mediterranean and Alps; and two of tropical regions: Antilles and Andes.

The Mediterranean community was studied in three sites of the Iberian Peninsula (Fig. 6.1a). Two sites were located in the Natural Park Serra da Enciña da Lastra, municipality of Rubiá, Ourense, Spain (567 m a.s.l.; 42°28'19''N, 6°50'17''W and 438 m a.s.l.; 42°28'15''N, 6°49'26''W). The third site was located in La Barosa, León, Spain (590 m a.s.l.; 42°29'50''N, 6°48'52''W). The area presents a Mediterranean climate with mean annual temperature of 12.3ºC and mean rainfall 901 mm (Guitián et al., 1994). The landscape is composed of crops and native vegetation, such as holm oak woodland (predominantly *Quercus ilex*, *Arbutus unedo* and *Quercus suber*), and scrub communities (Guitián et al., 1993). The field work spanned from May to June in 2010, 2011 and 2012.

The alpine community was studied in two sites of the north-eastern Calcareous Alps (Fig. 6.1b). Both sites in the Rax mountain, one in Reichenau an der Rax, and the other in Schwarzau im Gebirge, Lower Austria, Austria (1625 m a.s.l.; 47°43'02''N, 15°45'42''E and 1820 m a.s.l.; 47°42'54''N, 15°42'11''E respectively). Mean annual temperature is 1°C and annual precipitation averages 2000 mm (Dullinger et al., 2011). The area is covered by natural vegetation and consists in a matrix of alpine meadows with scattered patches of different sizes dominated by small shrubs and trees (such as *Pinus mugo*, *Picea abies* and *Larix decidua*). In this community the field work was made in August 2012.

The Antillean community was studied at two localities of Cuba (Fig. 6.1c). One in Tapaste, La Habana (250 m a.s.l.; 23º00'44''N, 82º06'10'W), and the second site in La Laguna, Mayabeque (71 m a.s.l.; 71 23º08'34''N, 81º49'05''W). The region presents mean annual temperature of 25.5ºC and mean annual rainfall of 1300 mm. The landscape is karstic and covered by a heterogeneous vegetation including littoral microphylous forests, mesophylous semideciduous forests, evergreen forests, riparian forests, secondary forests and scrublands mixed with fields and pasturelands (Vale et al., 2011). In this community the field work was made in August 2010.

The Andean community was sampled in the Northern Andes at two sites (Fig. 6.1d). Both sites are located in the Cerros Orientales of Bogotá, Cundinamarca, Colombia (2700 - 3150 m a.s.l.; 4°49’ 22’’N, 74° 01’ 10’’W and 2800 - 3190 m a.s.l.; 4º48’41’’N, 74º00’36’’W). The area presents a mean annual temperature of 14 °C and annual precipitation of 1038 mm (Rojas-Nossa, 2007). The high Andean forest is present in the lower and humid areas of the hills with dominance of trees such as *Weinmania tomentosa* and *Clusia multiflora*. The elfin forest is present in higher areas and is characterised by shrubbery vegetation with dominance of composites and ericads. In this community the field work was made from March 2003 to April 2004 and from November 2011 to June 2012.

Figure 6.1. Panoramic view of the studied sites. a) Mediterranean community. Natural Park Serra da Enciña da Lastra (Galicia, Spain). b) Alpine community. North-eastern Calcareous Alps (Lower Austria, Austria). c) Antillean community. Littoral evergreen microphylous forest at La Laguna site (Mayabeque, Cuba). c) Andean community. Elfin and high Andean forests of the Cerros Orientales of Bogotá (Cundinamarca, Colombia).

Identity of nectar robbers

To identify the species that behave as primary nectar robbers we made observations of the floral visitors and their behaviour along transects of 300–1000 m length in each site. Nectar robbers were identified by capturing insects or by visual observation of birds with binoculars (10 x 25) from 6 a.m. to 6 p.m. in the tropical communities, and from 8 a.m. to 8 p.m. at the temperate communities. Since in the Mediterranean and Antillean communities some plant species might have nocturnal floral visitors we extended there our observations from 10 p.m. to 1 a.m. Besides, we characterised the type of hole made by robbers, which was useful to differentiate robbery by certain species and to assign confidently cases of nectar robbery from florivory. We accumulated 70 hours and 9 minutes of observations in the Mediterranean community, 21 hours 19 minutes in the Alpine community, 14 hours and 25 minutes in the Antillean community, and 148 hours and 28 minutes in the Andean community. The differences in the sampling effort were useful to analyse whether nectar robbery was detected only in thoroughly sampled sites or if it was also detected through rapid assessments, as for study patterns in the nectar robbery within communities.

Levels of nectar robbery

To assess the level of nectar robbery in each plant species we carefully observed the presence of perforations made by robbers in a sample of flowers randomly selected along the transects. In average 123.2 flowers per species were analysed for perforations. We inspected a total of 10 838 flowers of 88 plant species. The level of nectar robbery was calculated as the number of flowers with one or more holes divided by the number of analysed flowers per species.

Plant traits

To characterise floral morphology, a sample of approximately 30 fresh flowers of each species was randomly selected. We measured a total of 3915 flowers. The floral characters assessed were: total corolla length, tube length and tube diameter tube diameter at the opening (see Fig. 4.1 of Chapter 4 this dissertation). As a measure of the narrowness of the corolla tube we calculated the diameter / length of the tube. Thus, smaller values of this variable correspond to narrow floral tubes, while numbers around 1 or more correspond to broad corollas. The flower orientation was measured in 1–10 flowers per species measuring the angle with respect to a line perpendicular to the ground. With this aim we placed a small pendulum attached to a thread near the flower, measured the angle with a protractor and then transformed it to radians; flowers oriented totally upwards have 0 rad; flowers horizontally oriented have 1.57 rad and flowers perfectly pendent have 3.14 rad. We also characterised flower's symmetry (zygomorphous or actinomorphous) and corolla type (dialipetalous or gamopetalous).

To estimate the effectiveness of chemical or physical barriers against nectar robbery we characterised presence/absence of substances (such as latex) secreted by floral tissues when the corolla is mechanically damaged. Also, we estimated presence/absence of protective structures (such as calyx, hairy calyxes, or bracts) that covered at the least the basal part of the corolla where nectar is concealed, and classified the flowers based on the thickness of the perianth as thin (≤ 1 mm) or fleshy (> 1 mm thickness). We characterised the aggregation of flowers within inflorescences and plants using a semi-qualitative scale with three values: low aggregation for flowers with distances ≥ 30 mm; medium aggregation for flowers with distances between 5–29 mm; and high aggregation for flowers with ≤ 5 mm distance one each other.

Besides, we characterised plant's life form as tree, shrub, herb, parasite or epiphyte and in sites where exotic plants occur we included this variable (i.e. exotic or native) into the analysis.

Flower density and nectar rewards

We quantified the density of flowers (in terms of flowers / m^2) of each plant species at each site. With this aim we delimited one transect per site of 10 m wide and 300 - 1700 m length (according to the environmental complexity of the vegetation). In each transect we counted all open flowers of each species. Finally we averaged the flowers / m^2 of all sites for each community.

To assess the volume and sugar concentration of nectar we bagged in average 20 fresh flowers randomly selected of each species (one per plant) with mosquito net bags. After 24 hours we extracted and measured all nectar accumulated within the flower with 0.5, 1, 2, 5, 10 µl capillary micropipettes (according to the size of the flower and the produced volume). Sugar concentration was characterised with a portable refractometer (Fisher Scientific TM, 0–32 ºBrix). For flowers with nectar concentration higher than 32 ºBrix, we diluted nectar with a known volume of distilled water, take the measurement and then calculated the original concentration. To calculate the kilojoules per flower produced in 24 hours we used the method described in Prys-Jones and Corbet (1991). The mean energetic value per flower (kJ / flower) was multiplied by the density of flowers (flowers / m^2) to express the density of energetic reward offered by each plant species in terms of kilojoules per m^2 (kJ / m^2).

Data analysis

We used an arcsine-root transformation for flower orientation. Other variables were non-transformed. Then, we performed Principal Components Analysis (PCA) for all quantitative and qualitative variables at each community using the package FactoMineR version 1.28 for R (Lê et al., 2008). Finally, we analysed the relationship between the levels of nectar robbery as response, and the first factors of the PCA that explained more than 72 % of the variance in the data as predictors. With this aim we fitted Generalized Linear Models (GLM) for each community using a binomial errors structure and log link function in R software.

To assign a positive or negative association for certain plant traits and the levels of nectar robbery in each community, we first analysed the contribution of each variable for one of the first PCA factors. To choose in which factor each trait contributes more, we used the following criteria: for continuous variables we considered traits with contributions higher than 10; for categorical variables we considered traits with a $p \leq 0.05$ for the v-test (see Supplementary material Appendices1 - 4). We used the sign of the Coordinate to determine the direction of the association among trait and that particular factor. Then, we used the sign of the estimates of those factors that contributed significantly (i.e. with a $p \leq 0.05$) to the GLM models. Finally, to assess the direction (positive or negative) of the influence of each factor on the robbing level signs were multiplied.

Results

Levels of nectar robbery in plants and identity of nectar robbers

The Mediterranean community was composed by 27 species of 14 families (Fig. 6.2a). 51.9 % of 27 species presented levels of nectar robbery ≤ 0.2. The frequency of robbery in *Lonicera etrusca* was strikingly higher than in all other species of the community with more than the half of the flowers presenting one or more holes made by several insect species (Figs. 6.2a and 6.3a), such as the hymenopterans *Xylocopa cantabrita*, *X. violacea*, and *B. terrestris*, or the coleopterans *Oxythyrea funesta* and *Tropinota hirta*. We did not detect any sign of nectar robbery in 44.4 % of the nectariferous species of this community (Figs. 6.2a and 6.3i-l).

The Alpine community was represented by 12 species of 7 families (Fig. 6.2b). At difference with the Mediterranean community, most species did not present signs of floral larceny by primary nectar robbers. Only *Anthyllis vulneraria* presented intermediate levels of robbery by the bumblebee *Bombus terrestris*, while *Gentiana nivalis* presented low levels of robbery (Figs. 6.2b and 6.4a, b). Despite that species such as *Gentianella campestris*, *Aconitum napellus*, *Dianthus alpinus*, *Campanula alpina*, *Euphrasia officinalis*, *Rhinanthus serotinus* or *Pedicularis rosea* were common in the area we did not detect signs of nectar robbery in their flowers (Figs. 6.4c-i).

In the Antilles only 9 species of 6 families fulfilled the characteristics defined to be studied in the community at the time the study was conducted (Figs. 6.2c). The pattern of robbery was similar to the Alpine community with one species (*Tecoma stans*) having intermediate levels of robbery and another (*Ipomoea nil*) with low levels

(Figs. 6.2c and 6.5a). In this community the primary robbers were one species of carpenter bee (*Xylocopa cubaecola*) and one hummingbird (*Chlorostilbon ricordii*). Other common species in this community such as *Ipomoea angulata*, *Rhytidophyllum wrightianum*, *Hamelia patens* or *Rondeletia odorata* did not present holes in their flowers (Figs. 6.2c and 6.5b-e).

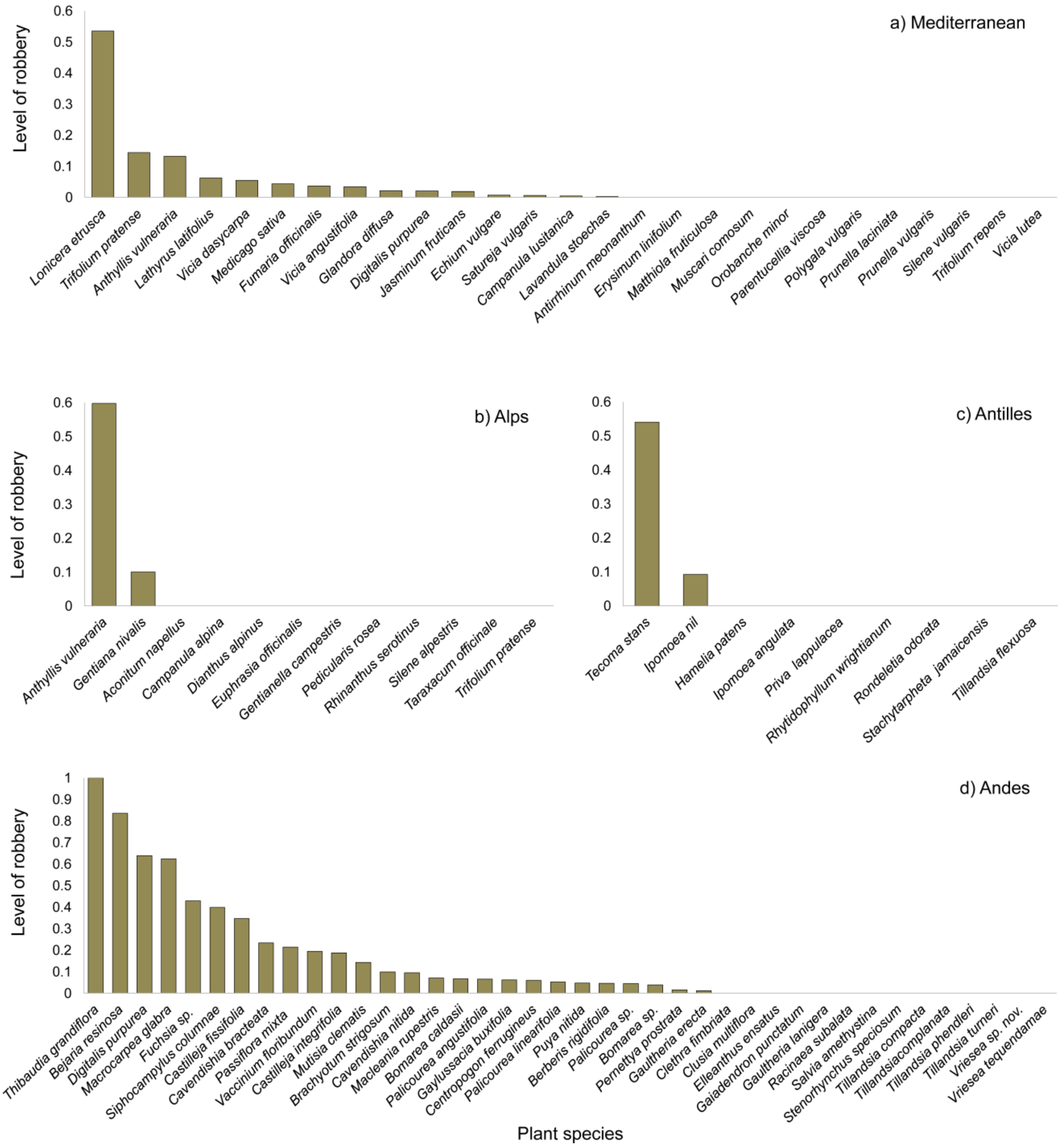


Figure 6.2. Levels of nectar robbery (number of flowers with ≥ 1 holes made by nectar robbers / total number of inspected flowers) in a) 27 species of the Mediterranean community, b) 12 species of the Alpine community, c) 9 species of the Antillean community and d) 40 species of the Andean community.

Figure 6.3. Plants present in the Mediterranean community. In *Lonicera etrusca* (a) more than 50 % of the flowers presented several holes performed by hymenopterans and coleopterans. Other nectariferous species such as *Trifolium repens* (b), *Vicia dasycarpa* (c), *Digitalis purpurea* (d), *Anthyllis vulneraria* (e), *Vicia angustifolia* (f), *Jasminum fruticans* (g), *Lathyrus latifolius* (h) or *Satureja vulgaris* (i) were less frequently robbed; while in *Prunella laciniata* (j), *Antirrhinum meonanthum* (k) or *Parentucellia viscosa* (l) we did not observe holes made by nectar robbers.

Figure 6.4. Plants present in the Alpine community. *Anthyllis vulneraria* with holes made by *Bombus terrestris* (a). *Gentiana nivalis* (b) presented low levels of robbery. Other species with concealed nectar such as *Gentianella campestris* (c), *Aconitum napellus* (d), *Dianthus alpinus* (e), *Campanula alpina* (f), *Euphrasia officinalis* (g), *Rhinanthus serotinus* (h) or *Pedicularis rosea* (i) were not robbed.

Figure 6.5. Plants present in the Antillean community. *Tecoma stans* with slits made by *Xylocopa cubaecola* and holes made by *Chlorostilbon ricordii* (a). Other species with concealed nectar such as *Ipomoea angulata* (b*), *Rhytidophyllum wrightianum* (c*), *Hamelia patens* (d*) or *Rondeletia odorata* (e*) were not robbed. (* Photography by V. Ferrero and D. Rojas).

The Andean community was highly diverse and comprised 40 nectariferous species of 18 families. 66 % of species were robbed in different degrees that ranged from 0.12 in to 1 (Fig. 6.2d). Two species (*Thibaudia grandiflora* and *Bejaria resinosa*) presented very high levels of robbery (Figs. 6.2d and 6.6a, b). Plants such as *Macleania rupestris*, *Passiflora mixta*, *Castilleja fisifolia*, *Macrocarpaea glabra*, *Cavendishia bracteata*, *C. nitida*, *Brachyotum strigosum* or *Centropogon ferrugineus* showed intermediate or low levels of nectar robbery (Figs. 6.2d and 6.6c-j). Despite that flowers of some species were abundant (e.g. *Gaiadendron punctatum* or *Clusia multiflora*), and/or produced large amounts of nectar (e.g. some Bromeliaceae), we did not detect any sign of nectar robbery in their flowers (Figs. 6.2d and 6.6k, l). Primary robbers in this community were the passerines *Diglossa albilatera*, *D. humeralis*, *D. lafresnayii*, *D. caerulescens*, and *D. cyanea*.

Figure 6.6. Plants present in the Andean community. The flowers of the tree *Thibaudia grandiflora* (a) and the shrub *Bejaria resinosa* (b) presented very high levels of robbery performed by *Diglossa*. Other nectariferous species such as *Macleania rupestris* (c), *Passiflora mixta* (d), *Castilleja fisifolia* (e), *Macrocarpea glabra* (f), *Cavendishia bracteata* (g), *C. nitida* (h), *Brachyotum strigosum* (i) or *Centropogon ferrugineus* (j) were less frequently robbed; while in several bromeliad species such as *Tillandsia turneri* (k) or *Vriesea tequendamae* (l) we did not observe holes made by nectar robbers.

Floral characteristics associated to nectar robbery

Mediterranean community-. The levels of robbery in the Mediterranean plants were significantly associated to the three first principal components (factors) that explained 72.8 % of the variance (Table 6.1). The first factor was mostly defined by positive associations with morphological variables, such as tube diameter, corolla length, tube length and orientation (Table 6.2 and Appendix 3). Remarkably, the presence of long calyxes and bracts and high aggregation of the flowers were negatively associated to the first factor and thus with robbery, suggesting that these features act as mechanical barriers reducing nectar robbery in the plants. Thus, species with long flowers oriented horizontally to downwards and without mechanical barriers were more frequently robbed. The second factor was mainly determined by variables related to the energetic offer in the environment (kJ / m^2), the number of flowers per unit area, the narrowness of the tube and the nectar produced per flower. This factor is also significantly associated with robbery levels (Table 6.1), meaning that those plants that offer dense and high nectar rewards, present in narrow flowers are more prone to be robbed (Table 6.2, Fig. 6.7).

The tube diameter and the particular morphology and behaviour of robbers were relevant to determine the levels of nectar robbery in certain plants regardless of the flower length or the nectar production. For instance, *Digitalis purpurea* and *Lonicera etrusca* had very long flowers (mean ± SD = 46.9 ± 4.1 and 32.9 ± 4.6 mm respectively) and copious nectar production (mean ± SD = 5.9 ± 9.4 and 5.2 ± 3.7 μl respectively). However, the big diameter of *D. purpurea* (mean ± SD = 14.9 ± 5.6 mm) allowed that common visitors such as *Bombus terrestris* enter into the flower to extract nectar without performing holes in the corolla. In fact, we only recorded nectar robbery by *Xylocopa violacea* in this species. Differently, the narrow aperture of *L. etrusca* (mean ± SD = 2.5 ± 0.4 mm) imposes floral visitors with medium to big body size the necessity to have a long proboscis to extract nectar through the entrance of the flower or perform larceny to have access to the reward.

Alpine community-. Three PCA factors explained 73.2 % of the variance (Table 6.1). The levels of robbery were significantly associated with the first and third factors of the PCA, but not with the second one (Table 6.1). The first factor was determined mostly by positive associations with morphological variables, such as tube diameter, total corolla length, tube length and diameter / length of the tube (Appendix 4). Thus, plants with long tubular flowers and broad tube diameter presented higher levels of nectar robbery (Table 6.2 and Fig. 6.8). At difference with the Mediterranean community, the flower orientation, the density of the energetic offer and the quantity of flowers / m^2 (i.e. variables highly related with second factor of the PCA), were not determinant to explain the observed levels of robbery. Despite that *Aconitum napellus* represented an abundant nectar resource with high caloric offer we did not observe signs of nectar robbery in its flowers during the sampling.

Antillean community-. In the Antillean plants, the first two PCA factors explained 74.8 % of the variance (Table 6.1). The first factor was mostly determined by energetic offer, nectar traits, orientation, narrowness, floral symmetry and consistency, symmetry, flower consistency, and plant's life form (Appendix 5). The GLM analysis revealed that this factor was not significantly associated to the levels of nectar robbery (Table 6.1). Otherwise, morphological traits (such as tube diameter, total flower length and tube length) and low aggregation of the flowers were the most relevant variables to determine the second PCA factor in this community, which was significantly and positively associated to the levels of robbery in the plants (Appendix 5 and Table 6.1). Hence, bigger and less aggregated flowers showed higher robbery frequency (Table 6.2, Fig. 6.9). One case was remarkable for the absence of robbery despite that the characteristics of the plants in the field made us to expect to find nectar robbery. The endemic gesneriad *Rhytidophyllum wrightianum* presents relatively long and narrow gamopetalous corollas (mean corolla length ± SD = 16.3 ± 1.3 mm, mean tube length ± SD = 14.0 ± 1.6 mm and mean tube diameter ± SD = 7.0 ± 0.5 mm), produces copious quantities of nectar (mean µl ± SD = 28.8 ± 27.7), and was the most common nectariferous plant in the Tapaste site (flowers / m^2 = 0.08). Their flowers were legitimately visited by the hummingbird *Chlorostilbon ricordii* during the day, sphingids at dusk and the bat *Monophyllus redmanii* during the night, representing a rich source of nectar for a broad diversity of nectarivorous animals. Although some individuals presented florivory by Lepidoptera larvae, we did not find signs of nectar robbery by *Xylocopa cubaecola* despite that this carpenter bee robbed *Ipomoea nil* flowers nearby. The species has not bracts or long calyx that could act as a mechanical barrier for robbers, but has a thick corolla covered with sticky trichomes (Fig. 6.5c).

Andes-. In the Andean community three factors accumulated the 72.5 % of the variance and were significantly and positively associated to the levels of nectar robbery (Table 6.1). Tube length, total corolla length, nectar volume, flower aggregation and plant's life form contributed to the first factor (Appendix 6). The density of energetic rewards (kJ / m^2), the quantity of flowers / m^2, flower consistency and orientation contributed mostly to the second factor. Since this factor had the highest estimate value on the model (Table 6.1), these variables were more important to explain the levels of robbery observed in this community. Hence, plants with high quantity of flowers that represented an abundant nectar source in the area (kJ / m^2) presented higher robbery levels (Fig. 6.10). Tube diameter did not contribute to the same factor with other morphological variables as occurred in the other communities. For the construction of the third factor were important the tube diameter, the narrowness, the flower type (i.e. dialipetalous/sepalous or gamopetalous/sepalous), plant's life form and presence of latex. The contribution of the variables to these factors and the sign of the estimate on the GLM model revealed that plants with dense and abundant nectar rewards, with long pendant dialipetalous flowers were more prone to be robbed (Table 6.2, Fig. 6.10).

Despite a non significant relation between the presence of protective structures and the first three factors (Appendix 6), species with strong bracts and generous nectar production kept in the base of long and narrow corollas presented low (in the case of *Puya nitida*) or null robbery (such as all species of *Tillandsia* and *Vriesea*. See Figs. 6.2d and 6.10). Remarkably, the thickness of the corolla does not seem to represent a physical barrier to flowerpiercers. On the contrary, plant species with thick flowers, such as those present in the ericads *Thibaudia grandiflora*, *Bejaria resinosa*, *Cavendishia nitida* or *Macleania rupestris*, presented a significant association experiencing intermediate to highly levels of nectar robbery (Fig. 6.2d). The aggregation of the flowers was significantly associated to the first factor of the PCA and thus to the levels of robbery (Appendix 6, Table 6.1). Plants with less aggregated flowers presented higher frequency of robbery (Table 6.2 and Fig. 6.10). On the contrary, highly aggregated flowers present low levels of robbery, reflecting that this trait constitutes an efficient mechanical barrier to reduce nectar robbery by birds.

Table 6.1. Results for the association between the levels of robbery and the morphological and nectar traits of four temperate and tropical plant communities. Given values are the percentage of variance explained by the first factors of the Principal Component Analysis (PCA) and results of the Generalized Linear Model (GLM) that analyze the association of the levels of nectar robbery with each factor. The values in the GLM columns correspond to the estimates for each factor and their significance (** for *P* ≤ 0.001; * for *P* ≤ 0.05; ^{NS} for *P* > 0.05).

	Mediterranean		**Alps**		**Antilles**		**Andes**	
Factor	**PCA**	**GLM**	**PCA**	**GLM**	**PCA**	**GLM**	**PCA**	**GLM**
1	36.40	0.11*	32.71	0.61**	42.00	0.16^{NS}	33.96	0.32**
2	20.63	0.49**	26.26	0.13^{NS}	32.78	1.22*	22.14	0.54**
3	15.77	-0.85**	14.21	0.72**	-	-	16.40	0.27**

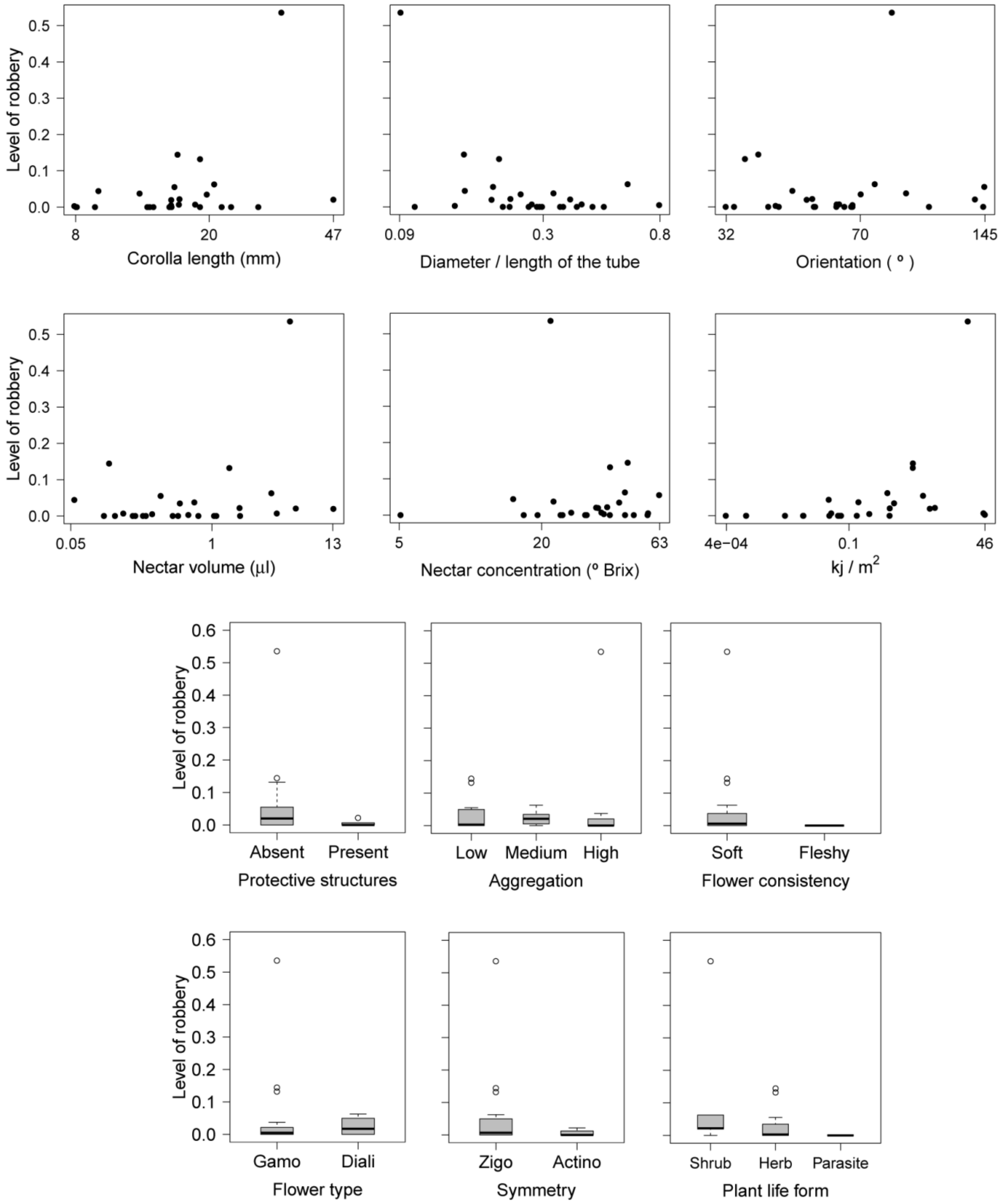


Figure 6.7. Association between plant's traits and the levels of nectar robbery in a Mediterranean plant community.

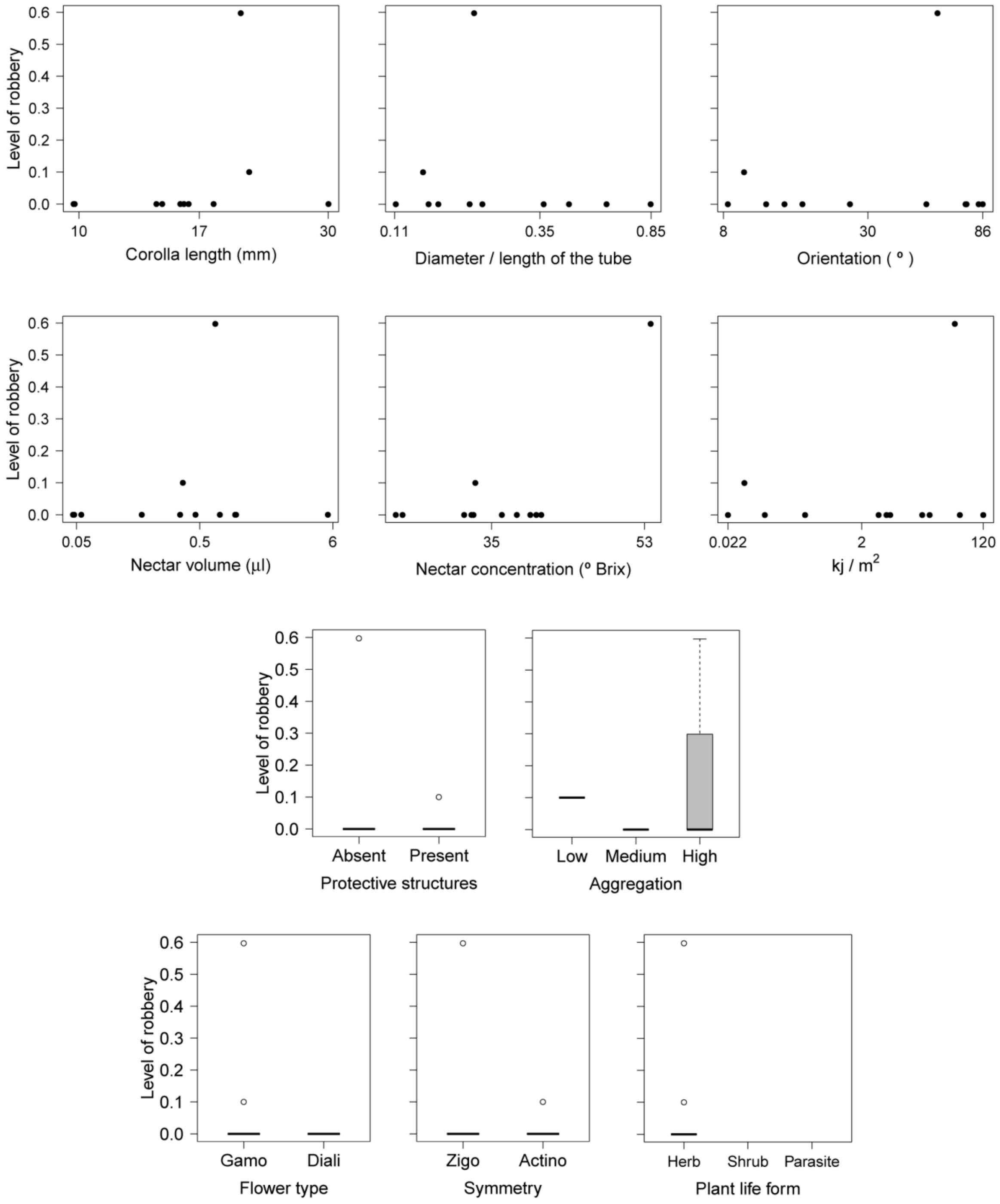


Figure 6.8. Association between plant's traits and the levels of nectar robbery in an Alpine plant community.

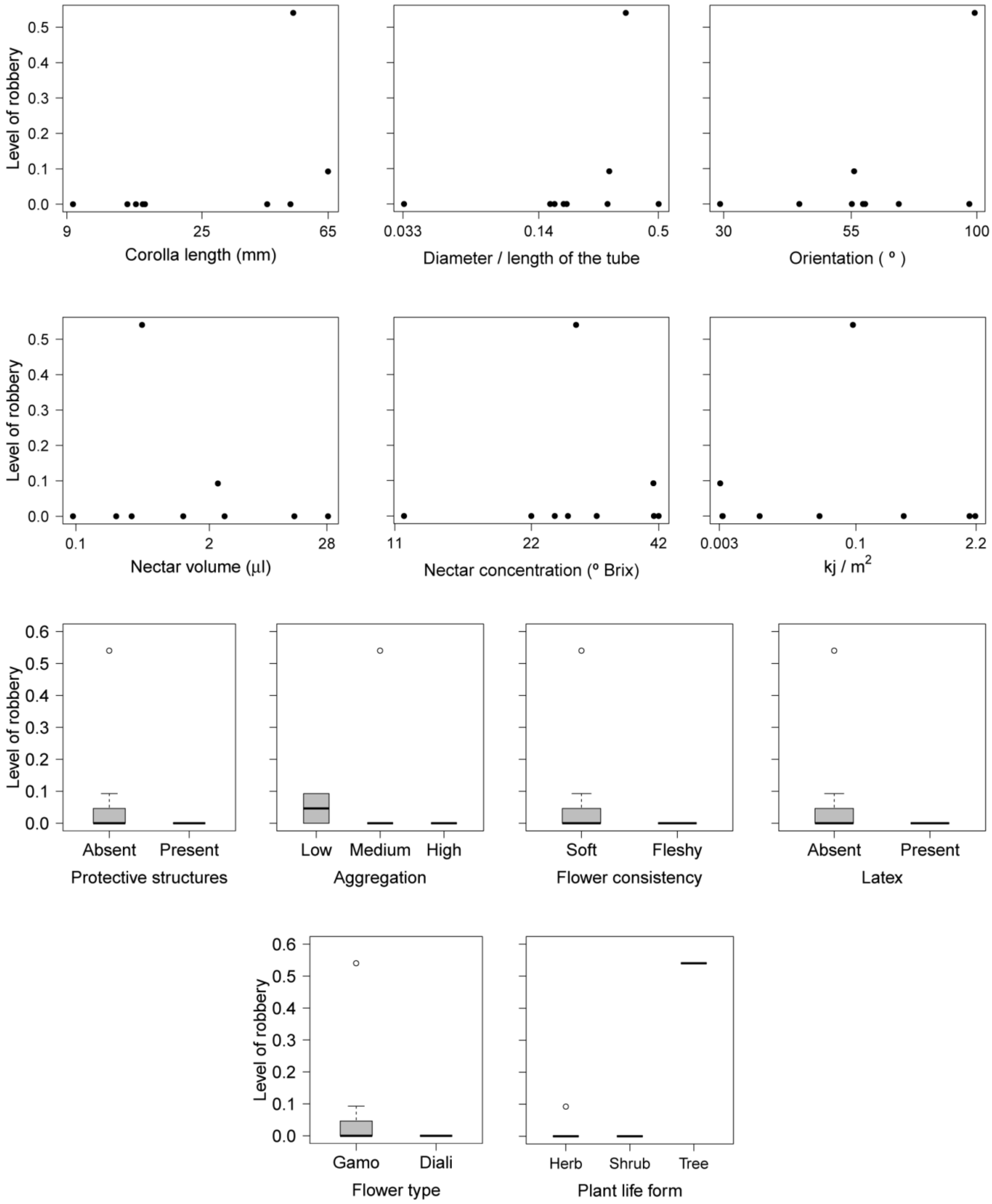


Figure 6.9. Association between plant's traits and the levels of nectar robbery in an Antillean plant community.

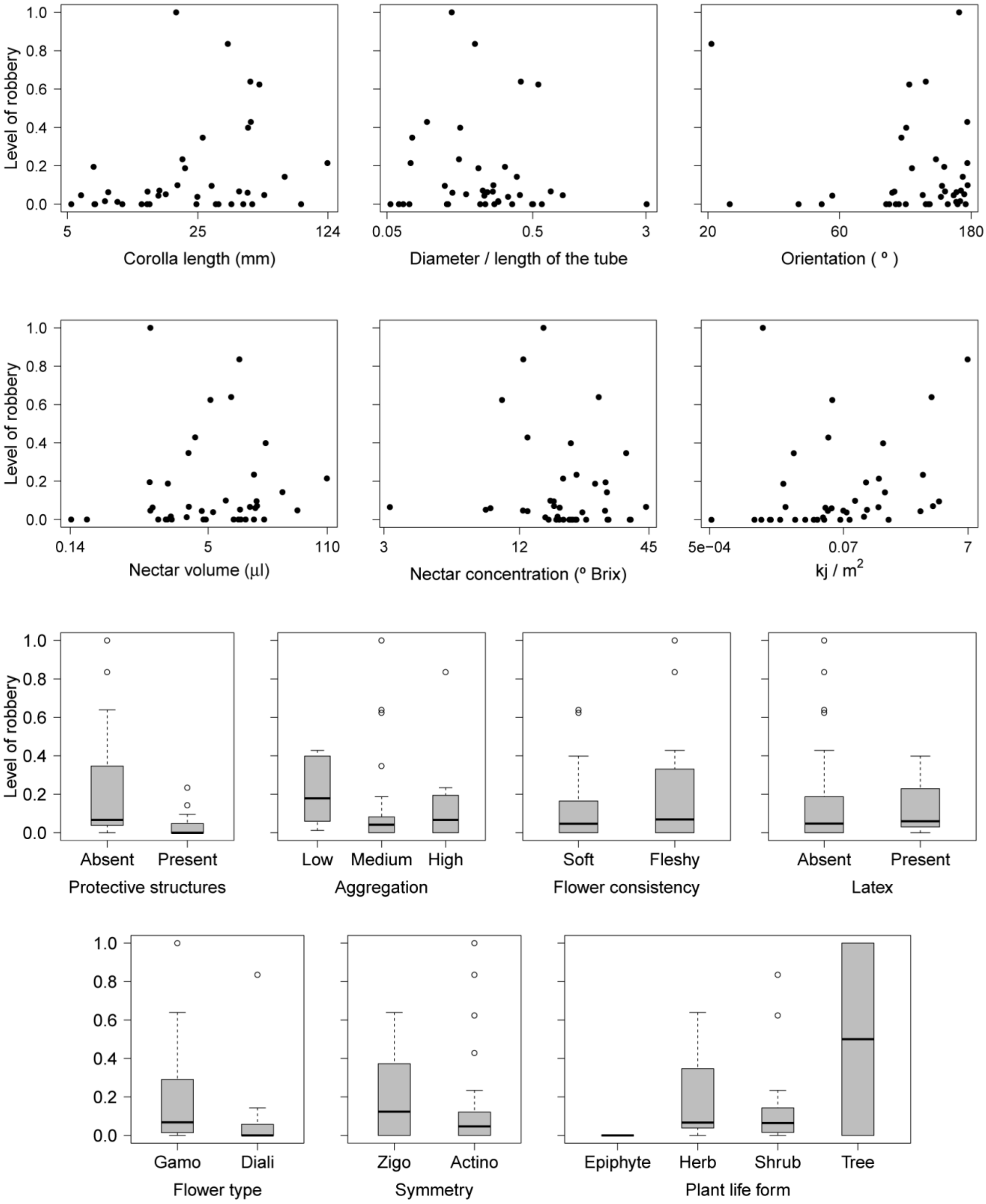


Figure 6.10. Association between plant's traits and the levels of nectar robbery in an Andean plant community.

Table 6.2. Direction and significance of the association between levels of nectar robbery and plant traits in four plant communities of temperate and tropical regions. The symbols resume the information extracted from the contribution of each variable to the PCA factors (Table 6.1) and their significance on GLM models (Appendices 3 - 6). Blue cells with + symbols represent positive associations, green cells with - symbols represent negative associations, and grey cells with x symbols represent traits that do not explain the levels of nectar robbery for that particular community, and empty cells represent cases in which only one or none of the states were present for a certain variable and therefore it was not included in the analysis. See Methods section for a detailed description of the way we assigned symbols.

Floral morphology	**Mediterranean**	**Alps**	**Antilles**	**Andes**
Corolla length	+	+	+	+
Tube length	+	+	+	+
Tube diameter	+	+	+	+
Diameter / length of the tube	-	+	x	+
Orientation	+	x	x	-
Actinomorphous symmetry	x	x	x	x
Zygomorphous symmetry	x	x	x	x
Dialipetalous/sepalous flower	x	x	x	+
Gamopetalous/sepalous flower	x	x	x	-
Energetic reward				
Nectar volume	+	x	x	+
Nectar concentration	x	x	x	x
Number of flowers / m^2	+	x	x	+
kJ / m^2	+	x	x	+
Barriers against nectar robbers				
Protective structures present	-	x	x	x
Protective structures absent	+	x	x	x
High flower aggregation	-	x	x	-
Medium flower aggregation	+	x	x	x
Low flower aggregation	x	x	+	+
Fleshy consistency	x		x	+
Thin consistency	x		x	-
Latex present				+
Latex absent				-
Plant life form				
Epiphyte	x		x	x
Herb	x		x	+
Parasite	x			
Shrub	x		x	-
Tree			x	+
Exotic	x			x
Native	x			x

Discussion

Identity and behaviour of primary nectar robbers in temperate and tropical plants

Several species of *Xylocopa* and *Bombus* are common primary nectar robbers in diverse plant communities worldwide (see Maloof and Inouye, 2000; Goulson, 2003; Zhu et al., 2010; Zhang et al., 2011; among others). Our results reveal that most of the plant species robbed by *Xylocopa violacea*, *X. cubaecola* and *Bombus terrestris*, have tubular corollas longer than their tongues (mean tongue length 7.7 mm for *B. terrestris* workers and 9.8 mm for *X. violacea,* in Herrera, 1989). However, in this study we found that these insects also act as nectar robbers in flowers shorter than their tongues, such as *B. terrestris* in *Fumaria officinalis*, or *X. violacea* in *Echium vulgare*, *Campanula lusitanica* and *Lathyrus latifolius*. This means that morphological and physiological constraints other than merely the match between flower tube length and proboscis length can also determine the foraging strategy of these insects. For example, the diameter of the tube and particularly its relation with the tube length is important to determine the way in which potential robbers access the nectar. Broad entrances allow smaller hymenopterans to enter into de corolla accessing nectar without the need to perforate corollas, as we observed *B. terrestris* in *Digitalis purpurea*, several species of *Campanula* or *Gentianella campestris* in temperate communities. In contrast, bigger hymenopterans (such as *X. violacea*) rob nectar in *D. purpurea*. Therefore, besides the match flower-tongue, differences in body size in hymenopterans are relevant to determine the nectar foraging strategy.

Although diverse birds in tropical and temperate regions have been recorded behaving as primary and secondary nectar robbers (Irwin et al., 2010; Anderson et al., 2011), flowerpiercers (*Diglossa* genus) are passerines considered highly specialized nectar robbers (Schondube and Martínez del Rio, 2004). Nevertheless, they do not always behave as larcenists and several species present changes in the strategy of nectar extraction according to bill morphology, flower length, orientation and narrowness of the tube. Flowerpiercers with shorter and hooked bills make robbery more frequently than species with longer and less hooked bills (Rojas-Nossa, 2007). These birds commonly rob flowers with narrow tubes that are larger than their extended tongues, but perform ‘legitimate’ visits (i.e. introducing the mandible and the tongue through the entrance of the flower) in Andean plants with short flowers. This explains the low levels of nectar robbery found in this study and the high frequency of pollen carryover by flowerpiercers of Andean plants such as *Clusia multiflora*, *Macleania rupestris* or *Brachyotum strigosum* previously observed (Rojas-Nossa, 2007). As a consequence, *Diglossa* are likely pollinators of flowers with short corollas that are visited legitimately (Stiles et al., 1992; Arizmendi, 2001; Rojas-Nossa, 2007), but their effectiveness as pollinators has not been compared with other floral visitors (but see Navarro et al., 2008).

Hummingbirds are broadly recognized by its harmonious interactions with their nutritious plants. Still, a growing body of evidence reveals that some species regularly perform primary and/or secondary nectar robbery (Navarro, 1999; Lara and Ornelas, 2001; Kjonaas and Rengifo, 2006). We found that the Antillean hummingbird *Chlorostilbon ricordii* perform primary robbery in the long flowers of *Tecoma stans*. These evidences indicate that even very specialized nectar robbers (such as flowerpiercers) or specialized pollinators (such as hummingbirds) present a remarkable variability in foraging behaviour changing from legitimate visitors to nectar robbers depending on their own attributes and several morphological aspects of the flowers. In these cases, the match between the length of the tube and the length of birds' bill and tongue is important to predict the foraging strategy of birds, the frequency of nectar robbery and the consequences of this visiting behaviour for the reproductive success of tropical plants. Further studies are required to understand the ecological and evolutionary implications of this variability on the structure of communities and the stability of trophic networks.

Levels of nectar robbery in plant communities

In our study, all sampled sites presented plants with nectar robbery independently of the biogeographical region and the sampling effort, revealing that this phenomenon is ubiquitous in animal-dependent pollination systems and more frequent than the literature about plant-animal interactions suggests (see Irwin et al., 2010; for the latest review). The patterns of the levels of nectar robbery within communities (Fig. 1) reflect mechanisms related to the diversity of nectariferous plants and robbers, but also with differences in sampling effort. Two communities (Mediterranean and Andes) were thoroughly sampled along the blooming period and across multiple years, while other two communities (Alps and Antilles) were "snapshots" of one moment during the blooming season. Despite such differences, all these communities share a common pattern: one plant species (or two in the case of the Andean community) presents very high levels of robbery in comparison with other species. This pattern is particularly pronounced in the Mediterranean, Alpine and Antillean communities, where *Lonicera etrusca*, *Anthyllis vulneraria* and *Tecoma stans* were the most robbed plants respectively. The first two species also present nectar robbery in other localities (Jordano, 1990; Guitián et al., 1993; Navarro, 2000), suggesting that this interaction is common along the plants' distribution area.

When comparing our results with previous works we found that changes in the abundance of flowers of different species promote sequential facilitation of nectar robbery. *Petrocoptis grandiflora* is a native plant present in the same Mediterranean area sampled in this study. It presents higher levels of robbery at early spring during the first time of the blooming season (Navarro et al., 1993). Afterwards, the robbery levels in this plant diminish simultaneously with an increase in the robbery of *Lonicera etrusca*, which has a blooming peak at the end of spring (Guitián et al., 1993). This kind of processes are common in diverse systems in which competition for pollination services shape the blooming season of co-existing plants (Waser and Real, 1979). Irwin et al. (2001) proposed that cases of sequential facilitation could occur also in plant-robber interactions. We here give the first evidences of this phenomenon, but the mechanisms and their consequences still require further research.

In the Andean community, the richness of plant species was higher than in other communities. There, a higher proportion of plants in the community presented nectar robbery. Also, several species had intermediate robbery levels (between 0.1 and 0.6). In a similar way, in the mountain forests of Mexico almost all hummingbird pollinated plants were robbed by passerines (Arizmendi, 2001). This is a consequence of ecological and evolutionary processes. At the Andes, ornithophily is the most important pollination mode for several groups of plants and most of them have flowers specialized for pollination by hummingbirds (Luteyn, 1989; Kessler and Krömer, 2000). High Andean hummingbirds, in turn, present a very wide spectrum of bill morphologies, ranging from very short (7.6 mm in the case of *Ramphomicron microrhynchum*) to the longest bill in the avian world in relation to its body mass (100.4 mm length in *Ensifera ensifera*) (Gutiérrez et al., 2004). Flowerpiercers share nectar resources with hummingbirds, and besides the potential effects for the reproduction of plants, they are common members of the mountain Neotropical avifaunas (Arizmendi, 2001; Rojas-Nossa, 2013). This raises a complex scenario of evolutionary processes in which passerine nectar robbers might have important roles for shaping the structure of Andean communities.

Plant traits and their association with nectar robbery levels

Morphology and nectar characteristics-. In all studied temperate and tropical plant communities the levels of nectar robbery are associated with morphological features of the flowers and the quantity of nectar produced. Usually, plants with long tubular flowers that produce abundant nectar were more prone to undergo high levels of robbery by both insects and birds. This result was expected considering the patterns observed in single species. For instance, under natural conditions Castro et al. (2009) found positive relations between flower size and robbery levels in *Polygala vayredae* by several insects; while Navarro and Medel (2009) found that the probability of nectar robbery by *Xylocopa cubaecola* increased with the length and the diameter of the flowers of *Duranta erecta*. Similarly, hummingbirds are more likely to rob longer artificial and natural flowers (Lara and Ornelas, 2001; Maruyama et al., 2015). The length of the tube is, in fact, one of the most likely traits in plants that explain the causes of nectar robbery for several reasons. For animals with short proboscis the long tubular corollas with narrow apertures act as a mechanical hindrance to access nectar. Also, longer flowers tend to have bigger nectaries and consequently produce more nectar (Ornelas et al., 2007; Castro et al., 2009). Besides, length and narrowness of the tube can contribute to keep higher quantities of nectar since bigger flowers can keep bigger volumes of nectar that evaporates at lesser rates (Corbet, 2003). Thus, larger flowers are more profitable source of nectar in terms of one visit.

Other morphological traits were also significant to explain the levels of nectar, but the direction of the relation was different for communities with insects or birds as nectar robbers (Table 6.2). Birds robbed predominantly fleshy and broad flowers from trees, while insects robbed mostly narrow flowers (small diameter / length of the tube) with intermediate aggregation. Trees are particularly robbed by flowerpiercers because they need branches, pedicels or inflorescences strong enough to support their weight while forage for nectar, unlike hummingbirds which can rob flowers in hovering flight (see Maruyama et al., 2015). Also, some Andean plants with very broad flowers, such as *Macrocarpaea glabra* were highly robbed by flowerpiercers (Fig. 6.2). For these birds the length of the corolla is the most important trait to determine the foraging strategy (Rojas-Nossa, 2007). In several species there is a positive allometric relationship between the length of the flower and the diameter of the tube, meaning that longer flowers commonly have also broader tubes (Navarro and Medel, 2009). However, our results suggest that length and diameter might vary separately in different communities. In the Mediterranean and the Alpine communities both traits were grouped into the same PCA factor, while in the Andean and the Antillean communities contributed mostly to different factors (Appendices 1 - 4). This reveals that ornithophilous pollination and robbery by birds could impose a selection on diameter independent of the selection on tube length. Such differences in the patterns among insect- robbed *vs.* bird-robbed plants are related with idiosyncratic characteristics in foraging behaviour and morphological constraints according to the floral visitor fauna (including both legitimate visitors and larcenists) of each biogeographical region, and the evolutionary trends on different plant traits imposed by them (see Anderson and Johnson, 2008; Thomson and Wilson, 2008; for examples on ecological and evolutionary trends on plant-pollinator morphologies).

Density of the energetic offer-. Besides the morphological constraints that operate at a single flower visit basis, our study reveal that nectar robbery is a phenomenon highly density-dependent reflecting mechanisms that operate at higher ecological scales. We found positive and significant associations between the density of flowers (number of flowers / m^2) or density of the energetic offer (kJ / m^2) with the levels of robbery in those communities in which we encompassed whole blooming cycles (*i. e.* Mediterranean and Andes). A similar pattern was also observed in a previous work made in tropical plants, where the number of flowers / hectare was significantly related to the proportion of robbed flowers by passerines (Rojas-Nossa, 2013). Commonly, the size of floral display (i.e. number of open flowers) and the density of other nectar sources influence the attractiveness to floral visitors affecting plant's reproductive success (Brys and Jacquemyn, 2010). Goulson et al. (1998) observed that *Bombus terrestris* visited more inflorescences in plants with larger floral displays. The higher attractiveness of plants that offer concentrated resources is mostly explained by the energetic economy of floral visitors. Denser resources are easier to detect and the time to move between the flowers decreases, thus reducing both the energetic investment during foraging and the risks of predation (Eckhart, 1991).

We have not found robbery traces in some species which either have the typical robbery syndrome (i.e. long tubes, with high density of flowers and energetic reward, high nectar production and low to median flower aggregation), or have been reported as robbed somewhere else. For instance, studies in other localities reported nectar robbery in *Tecoma stans* (Lasso and Naranjo, 2003), *Aconitum napellus* (Mayer et al., 2014) or *Rhinanthus serotinus* (Kwak, 1978). Similar geographical and temporal differences in the levels of robbery were observed in diverse plant species in which insects are the main robbers (Utelli and Roy, 2001; Irwin and Maloof, 2002; Price et al., 2005). Such variation could be explained by the local availability of alternative nectar resources or changes in abundances of floral visitors within and among seasons (Navarro, 2000; Irwin et al., 2001).

Barriers against nectar robbery-. In cases when robbers have negative consequences for host's fitness, protective mechanisms against robbery could imply selective advantages for plants (Adler, 2000). Different chemical substances present in nectar and flower tissues might deter less efficient pollinators or exploiters such as nectar robbers (Kessler et al., 2008). However, the presence of chemical deterrents is not always positive for plants' reproduction. Some evidences show that in high concentrations, secondary compounds can reduce the frequency of visits and the time spent at the flower, diminishing pollen donation and representing more costs than benefits for the plant (Adler and Irwin, 2005). Despite we included in our models the production of latex in floral tissues, we have not found a common pattern. Detailed approaches that analyse the chemical nature of secondary compounds in tissues and/or nectar are required to test their efficiency as deterrents against nectar robbery.

The results reveal that highly aggregated flowers are less robbed. This suggests that this trait constitutes a physical barrier for both insects and birds, since they find difficulties to find the base of the corollas and rob nectar. The arrangements of flowers and the presence of bracts in the bromeliads were proposed as defence mechanisms against robbery (Stiles, 1981; Rojas-Nossa, 2007). Our findings support this hypothesis, since the aggregation of flowers and low density of flowers in the environment are the most likely explanation to the low or null robbery observed in several Andean bromeliads. Besides, long calyxes and bracts significantly diminish nectar robbery by *Bombus terrestris* and *Xylocopa violacea* in the Mediterranean community. Despite that several authors suggested that some plants might have mechanical barriers against nectar robbery (Guerrant and Fiedler, 1981; Roubik, 1982; Lara and Ornelas, 2001), to our knowledge this study is the first evidence of the efficacy of physical barriers to reduce primary nectar robbery by insects and birds.

Inouye (1983) proposed that thick corollas could diminish robbery by bees. We did not find a significant association of the levels of robbery by insects with this floral feature probably because very few plants with thick corollas and profitable nectar rewards were present in the studied communities. Nevertheless, in the Andean community we found the opposite pattern to the expected by Inouye's hypothesis. There, fleshy flowers (mainly ericads) presented medium to high levels of robbery. This trait might therefore not be acting as a barrier, but on the contrary might facilitate the manipulation of the flower by perching birds (particularly flowerpiercers). Additionally, considering the fact that *Diglossa lafresnayii* has been observed making scars on the bark of *Baccharis arbutifolia* to drain and drink the sap (Martin et al., 2009), it is expectable that these birds have not only the bill morphology but also a powerful mouth musculature to perforate thick corollas or flowers with long calyxes.

The floral morphology, the high quantity of produced nectar and the abundance of flowers of the Antillean *Rhytidophyllum wrightianum* made us to expect robbery by *Xylocopa cubaecola*, but this was not the case. Several hypotheses might explain the absence of the typical slits made by this robber. One possibility is the presence of constitutive defences, such as the sticky trichomes observed on the corolla, or induced chemical defences in nectar and floral tissues that act as barriers against nectar robbers. The production of these compounds could be induced by the presence of florivores commonly observed in these plants, as occur in other plant species under herbivory pressure (Adler, 2000; Adler and Irwin, 2005). Another hypothesis proposed by Irwin et al. (2004) is that the low concentration of sugars in nectar makes the flowers unattractive for hymenopterans without deterring the main pollinators (i.e. hummingbirds and bats in this case) adapted to feed on nectar with lower sugar concentrations. Also, the flowers of *R. wrightianum* have thick corollas that might represent a mechanical hindrance to robbers as Inouye (1983) suggested. The relative importance of these mechanisms to reduce nectar robbery in this and other plant species and the consequences on plant traits' evolution is a promising field to explore.

Conclusions

This study is the first field approach devoted to disentangle the inherent complexity of plant-nectar robbers systems in broad ecological and geographical scales. Our results reveal that nectar robbery is a frequent phenomenon in angiosperms with long tubular corollas and abundant flowering, but highly variable in time and space. Within plant communities one or two species are highly robbed and others have intermediate to low levels of nectar robbery according to the density of flowers, and floral and nectar traits. The length of the tube, the quantity of nectar produced, and the low or medium aggregation of flowers are positively related to the frequency of robbery by insects and birds. Also, the distribution and abundance of nectar resources in the environment determine the levels of nectar robbery in plants by both animal groups. Other plant traits have opposite effects when robbers are insects or birds. For instance, insects rob more frequently pendant and narrow flowers while birds rob more erect and broad flowers. This is related to differences in foraging behaviour and morphological constraints. Here we present new evidences of the effectiveness of mechanical barriers against nectar robbers. A high aggregation of the flowers and the presence of long calyxes and bracts reduced nectar robbery by insects and in lesser extent to birds. Although morphological constraints are important to determine the strategy of nectar extraction by nectar robbers in a single flower basis, nectar traits and particularly the density of energetic offer in the environment are the most relevant characters to explain the levels of nectar robbery observed in temperate and tropical plant communities.

Capítulo 7

Discusión general

Consecuencias del robo de néctar para la reproducción de las plantas

Aunque la última revisión sobre el tema y evidencias posteriores indican que los casos en los cuales los insectos robadores de néctar ejercen una influencia negativa sobre el éxito reproductivo de las plantas robadas son muy comunes (Irwin et al., 2010; Wang et al., 2013), el estudio de un mayor número de casos incluyendo también a las aves como robadoras de néctar demuestra que la influencia de estos visitantes florales puede ser positiva en más sistemas de los esperados (ver por ejemplo Fumero-Cabán y Meléndez-Ackerman, 2007; Zhu et al., 2010; Singh et al., 2014; Zhang et al., 2014). En el caso de *P. grandiflora* los robadores tienen un efecto positivo aumentando la producción de frutos y semillas y la distancia de flujo de polen (Figs. 2.2 y 2.3, Capítulo 2 de esta tesis). En *Lonicera etrusca* el robo no tiene consecuencias negativas pero tampoco aumenta el éxito reproductivo de la planta medido a través de diferentes variables (Figs. 3.2 y 3.3, Capítulo 3 de esta tesis). En ambos casos, los robadores de néctar actúan como polinizadores y además influyen en el comportamiento de otros visitantes florales. Por ejemplo, al abrir perforaciones en la flor hacen accesible un recurso que anteriormente no lo era. Esto es aprovechado por pequeñas abejas de los géneros *Ceratina*, *Lassioglossum* y *Halictus*, quienes a pesar de de baja su efectividad (Fig.5.6 Capítulo 5 de esta tesis), contribuyen al proceso de polinización de la planta cuando se comportan como robadores secundarios de néctar y colectan polen durante la misma visita. Dado que los robadores primarios de néctar promueven la inclusión de nuevos participantes en la red de interacciones se les ha considerado "ingenieros de recursos" y ello puede mitigar los potenciales efectos negativos de los robadores primarios (Newman y Thomson, 2005).

Esta tesis revela que en los casos en los cuales el robo de néctar resulta ser "neutro" para la reproducción de las plantas, los robadores en realidad tienen un efecto positivo ya que contribuyen directamente o indirectamente al proceso de polinización. No obstante, la respuesta de la planta (es decir, si se aumenta o no su éxito reproductivo) se relaciona con factores idiosincráticos, como el sistema de auto-compatibilidad (ya notado anteriormente por Burkle et al., 2007), y con la efectividad de los robadores como polinizadores relacionada con aspectos como su morfología, comportamiento y abundancia.

El ajuste morfológico entre polinizadores y flores usualmente se estudia a partir de estructuras especializadas en el proceso de toma de néctar, tales como el pico en aves o la lengua en insectos y murciélagos (ver por ejemplo Stiles, 1981; Nilsson, 1988; Temeles et al., 2002; Muchhala, 2006; Wester y Claβen-Cockhoff, 2006). No obstante, los sistemas de polinización estudiados en los Capítulos 2 a 4 de esta tesis reflejan un ajuste morfológico menos obvio, refinado y conocido, entre la longitud del cuerpo del insecto y la longitud de las estructuras reproductivas de la flor. De este modo, en especies parcial o totalmente auto-compatibles en los cuales el ajuste morfológico entre los robadores y la flor es mayor (como en *P. grandiflora*), el forrajeo de los robadores de néctar contribuye al aumento del éxito reproductivo de la planta. Por otra parte, en plantas con sistemas polinizador-dependientes que requieren el transporte de polen de otra flor para reproducirse, y en las cuales el ajuste morfológico entre el cuerpo de los robadores y las estructuras reproductivas es menor (como en *L. etrusca*. Figs. 3.1b, 4.2a, 5.1a, b), la polinización mediada por los robadores no se traduce en una mayor producción de frutos y semillas. No obstante, aspectos como la viabilidad de esas semillas y el éxito biológico de la descendencia requieren ser analizados en posteriores estudios.

La polinización cruzada de *L. etrusca* por himenópteros robadores primarios de néctar sucede durante el proceso de movimiento entre las flores y ubicación del cuerpo para abrir la perforación y toma de néctar a través de éstas (como se ilustra en la Figura 5.2 del Capítulo 5). El comportamiento de los himenópteros, su gran tamaño y las estructuras reproductivas de la flor ubicadas fuera del tubo floral facilitan el transporte de polen en el abdomen y las patas de los insectos a otras flores. Entonces, considerando que estas especies son visitantes florales comunes en diferentes poblaciones (ver Jordano, 1990; Guitián et al., 1993; y Capítulo 5 de esta disertación) y que afectan la reproducción de la planta de forma directa e indirecta (ver Capítulos 3 a 5), es relevante considerar a estos robadores de néctar como potenciales agentes selectivos en este y otros sistemas con características similares. Futuros trabajos deben incluir y evaluar el efecto de estos visitantes florales para la evolución de caracteres florales tales como la longitud y el diámetro del tubo floral, la longitud de las flores en inflorescencias.

Al analizar las características de las flores de *L. etrusca* y la literatura sobre la polinización en otras especies del género *Lonicera* (Miyake y Yahara, 1998; Miyake et al., 1998), se esperaría un alto grado de especialización para la polinización por esfíngidos. En efecto, una especie de esfíngido (*Hemaris fuciformis*) fue el polinizador de mayor calidad en términos de una visita a una flor (componente cualitativo de la efectividad de la polinización. Ver Fig. 5.5, Capítulo 5 de esta tesis). No obstante, al incluir el aporte en términos poblacionales (i.e. componente cuantitativo) en la estimación de la efectividad de los diferentes visitantes florales, los himenópteros que se comportan como visitantes legítimos (*Anthophora acervorum*), dos especies de robadores primarios (*Xylocopa violacea* y *Bombus terrestris*) y un esfíngido (*Macroglossum stellatarum*) fueron los polinizadores más efectivos de la planta (ver Fig. 5.5, Capítulo 5 de esta tesis). Este resultado se debe en parte a la mayor eficiencia de los himenópteros en el transporte de polen (Fig. 5.4, Capítulo 5 de esta tesis), pero especialmente se relaciona con la mayor tasa de visitas florales por estas especies. Por el contrario, los coleópteros *Oxythyrea funesta* y *Tropinota hirta* que también se comportan como robadores primarios de néctar, fueron los polinizadores menos eficaces y adicionalmente tienen efectos negativos para el funcionamiento de la flor, dado que rasgan la corola en toda su longitud, consumen polen y frecuentemente dañan las estructuras reproductivas (Fig. 4.2b, Capítulo 4 de esta tesis). Esto demuestra que aunque tanto himenópteros como coleópteros presentan un comportamiento aparentemente similar (son robadores primarios de néctar), diferencias en la forma de extracción de néctar, asociadas a su morfología y comportamiento, tienen efectos contrastantes para el proceso de polinización de las plantas.

Explotadores: fuerzas opuestas para la reproducción de las plantas

En un sentido amplio, los herbívoros que consumen partes de las plantas, tales como tejidos, néctar, polen, fluidos, madera o frutos y que aparentemente no proveen ningún servicio a cambio se consideran “explotadores” de las plantas y sus interacciones con organismos mutualistas (Bronstein, 2001). Esto puede implicar costos significativos para los mutualismos que se traducen en la reducción de la reproducción para alguna de las partes de la interacción.

Aunque tanto los robadores de néctar como los herbívoros chupadores de sabia son dos tipos de explotadores comunes en angiospermas (Irwin et al., 2010; Blackman e Eastop, 1984; Van Emden y Harrington, 2007), su incidencia y las consecuencias para el proceso de polinización se analizan y comparan por primera vez en este trabajo (Capítulo 4). Ambos tipos de explotadores ocurren con frecuencia en diferentes poblaciones de *L. etrusca* y los efectos para la reproducción de la planta son opuestos. Mientras que los himenópteros robadores primarios de néctar son positivos, ya que no dañan las estructuras reproductivas ni generan cambios considerables en el funcionamiento de la flor y además promueven la polinización cruzada durante el robo (Capítulos 4 y 5 de esta tesis), los áfidos consumen sabia y esto desencadena una serie de cambios en las flores y las recompensas para los diferentes visitantes florales, disminuyendo el servicio de polinización y generando finalmente una reducción del éxito reproductivo de la planta (Tabla 4.1 y Figs. 4.2d, 4.5, 4.6 y 4.7, Capítulo 4 de esta tesis).

Los áfidos (Hemiptera: Aphioidea) extraen importantes cantidades de energía y nutrientes, son vectores de infecciones, inducen la producción de agallas e incluso causan defoliación de sus plantas hospederas (Dixon, 1971; Larson, 1998; Power y Flecker, 2003). Además disminuyen la capacidad reproductiva de las plantas afectando su interacción con los polinizadores a través de diferentes mecanismos. Por ejemplo, estos herbívoros causan una reducción en el número de flores producidas en *Raphanus sativus* (Snow y Stanton, 1988), o atraen hormigas que modifican la conducta de los polinizadores de varias especies de cactus (Ohm y Miller, 2014). En el caso de *L. etrusca*, la pérdida de recursos que genera la herbivoría por áfidos en ramas y pedicelos antes del periodo de floración es la explicación más viable a los cambios observados en el tamaño floral, el tamaño y la cantidad de óvulos y polen, la deformación de las papilas estigmáticas, y especialmente en el cese de producción de néctar en las flores que se desarrollan en ramas con herbivoría. Los efectos observados tanto en flores como inflorescencias dañadas, como en flores sanas presentes en plantas altamente afectadas, demuestran que estos herbívoros disminuyen la calidad del servicio de polinización (mediado tanto por visitantes legítimos como por los robadores primarios de néctar).

Variación espacial y temporal del robo de néctar

La variación espacial y temporal de la frecuencia de robo de néctar es común en diferentes plantas de diversas regiones geográficas (Navarro, 2000; Irwin y Maloof, 2002; Price et al., 2005). En este estudio, encontramos que en *Petrocoptis grandiflora* y *Lonicera etrusca* también ocurre una importante variación en la frecuencia de robo de néctar (ver Fig. 2.2 del Capítulo 2 y Fig. 4.3 del Capítulo 4) tanto entre las poblaciones estudiadas (para ambas especies), como a lo largo de un monitoreo de 12 años (para *P. grandiflora*). Ésta variación es atribuida a diferentes factores relacionados con cambios en las abundancias poblacionales de los robadores, con la presencia y abundancia de recursos alimenticios alternativos y con diferencias intra e interespecíficas en las características florales que hacen más apetecibles algunas plantas que otras (Urcelay et al., 2006; Castro et al., 2009; Navarro y Medel, 2009).

Tal variación es igualmente evidente entre especies plantas que co-existen y comparten los visitantes florales. En la misma localidad algunas especies experimentan elevados niveles de robo, mientras que en otras especies nectaríferas el robo de néctar es poco frecuente o no ocurre (ver Maloof e Inouye, 2000; Irwin et al., 2010 para revisiones sobre el tema). No obstante, las causas y en especial, las características de las plantas asociadas a los diferentes niveles de robo han sido prácticamente inexploradas hasta ahora (Irwin et al., 2001; Rojas-Nossa, 2013).

Características de las plantas asociadas al robo de néctar

Algunos rasgos morfológicos, como las corolas tubulares largas o los espolones, han evolucionado como mecanismos para recibir visitas florales solamente de aquellos animales con la capacidad morfológica para acceder al néctar acumulado en la base de la flor, promoviendo una mayor eficiencia en el proceso de polinización (Stebbins, 1970; Aigner, 2001, 2004, 2006). Así, la presencia de estas estructuras "especializadas" implica que los animales con probóscides o lenguas cortas no pueden acceder al néctar por la boca de la flor. Sin embargo en algunos casos, éstos poseen la capacidad morfológica y la flexibilidad en el comportamiento para acceder a la recompensa a través de perforaciones en la base de la corola. Es decir, que las limitaciones morfológicas para acceder al néctar son la más básica y obvia causa del robo de néctar.

Este estudio revela que los niveles de robo no se asocian solamente con la presencia de néctar acumulado en corolas tubulares o espolones como se asumía hasta ahora (Irwin et al., 2010), sino que otras características son importantes para determinar los niveles de robo de néctar experimentado por especies de comunidades templadas y tropicales. A una escala ecológica fina, los niveles de robo de néctar se asocian con las características morfológicas de las flores y con la cantidad de néctar producido (Capítulo 5). Esto apoya las evidencias encontradas en especies estudiadas aisladamente, en las cuales se encontró que las flores más grandes y con mayor producción de néctar sufrían robo de néctar con mayor frecuencia (Urcelay et al., 2006; Castro et al., 2009; Navarro y Medel, 2009). Además, flores densamente agrupadas son robadas con menor frecuencia por insectos y aves (Tabla 6.2 y Apéndices 3 y 6), ya que las bases de las corolas se protegen unas a otras actuando como barreras físicas que dificultan el acceso de los robadores de néctar. Adicionalmente, las brácteas y los cálices largos y engrosados también son barreras físicas eficientes para reducir la frecuencia de robo por insectos y en menor grado por aves (Figs. 6.7 y 6.10, Capítulo 6 de esta tesis). Aunque diferentes autores propusieron que la modificación de los atributos florales podría actuar como un mecanismo de tolerancia o defensa contra el hurto floral (Guerrant y Fiedler, 1981; Stiles, 1981; Roubik, 1982; Inouye, 1983; Newman y Thomson, 2005; Rojas-Nossa, 2007), en esta tesis se demuestra y compara por primera vez su efectividad en comunidades de plantas sometidas a presiones de robo de néctar por insectos y aves.

A una escala ecológica más amplia, el robo de néctar se asocia positivamente con la densidad energética ofrecida por las plantas (Capítulo 6 de esta tesis). Un patrón similar fue observado en ambientes tropicales en los cuales el número de flores por hectárea se relacionó positivamente con la frecuencia de robo por aves paseriformes (Rojas-Nossa, 2013). Comúnmente el despliegue floral es reconocido como un mecanismo para la atracción de polinizadores (Goulson et al., 1998; Makino et al., 2007; Brys y Jacquemyn, 2010). En esta tesis se demuestra que este factor también actúa para atraer a los robadores de néctar. Además de una mayor facilidad para encontrar los recursos más concentrados, la preferencia por altas densidades florales de una misma especie se relaciona con aspectos energéticos, ya que los animales requieren invertir una menor cantidad de energía para desplazarse entre flores cercanas y disminuir así el riesgo de depredación (Eckhart, 1991; Mitchell et al., 2004). Este trabajo constituye la base para el entendimiento de las características de las plantas asociadas al robo de néctar. Sin embargo, se requieren también estudios basados en la eficiencia energética de diferentes estrategias de extracción de néctar (visitas legítimas, robo primario o secundario) para entender los condicionantes del robo de néctar desde la perspectiva del animal, ya que muchos de los mecanismos que subyacen a los patrones observados parecen tener más relación con la eficiencia del forrajeo de los animales y los procesos competencia por explotación, más que procesos de competencia por interferencia (aunque estos últimos también ocurren).

Es notable que el robo de néctar ocurre en prácticamente todos los ambientes en los cuales las plantas presentan abundante néctar y flores tubulares, reflejando que es un fenómeno ubicuo y más común en angiospermas de lo que la literatura sobre interacciones planta-polinizador ha reflejado hasta ahora. A pesar de las limitaciones metodológicas inherentes al muestreo de áreas geográficas tan amplias como las estudiadas en el Capítulo 6 de esta tesis, los resultados muestran que prácticamente todas las especies susceptibles de sufrir robo de néctar son efectivamente robadas. No obstante, encontramos algunas excepciones, como las bromeliáceas de los ambientes montañosos del Neotrópico que poseen brácteas que actúan como una barrera eficiente contra el robo de néctar. En otras especies, además del efecto de las barreras físicas se pueden presentar también mecanismos químicos para reducir el robo de néctar (Baker, 1977; Adler, 2000; Kessler et al., 2008). Así, la ausencia de robo en plantas como la gesneriácea antillana *R. wrigthianum* puede representar un caso en el cual el engrosamiento de los tejidos florales, la presencia de tricomas glandulares o la producción de compuestos secundarios son potenciales mecanismos para reducir o evitar el robo de néctar. La presencia y funcionamiento de estos mecanismos representa un interesante campo de estudio hasta ahora poco explorado.

Estudios detallados que evalúen los efectos de los explotadores sobre ambos componentes del éxito reproductivo son necesarios para ampliar nuestro conocimiento sobre el funcionamiento de las redes de interacciones mutualistas y antagonistas. Así mismo, se requieren estudios multi-tróficos que involucren diferentes escalas ecológicas y temporales como los presentados en esta tesis con el fin de obtener una idea más completa de estos complejos fenómenos biológicos. Finalmente, quisiéramos llamar la atención de los investigadores para que observen y analicen con mente abierta el verdadero papel de los robadores de néctar en la ecología y evolución de los sistemas de polinización, ya que las evidencias presentadas en esta tesis demuestran que estos visitantes florales son piezas importantes para el funcionamiento de las interacciones planta-animal a menudo en direcciones diferentes a las esperadas.

Conclusiones

1. Los robadores de néctar son explotadores de las interacciones planta-polinizador. Sin embargo, las consecuencias de este comportamiento sobre la reproducción de las plantas no siempre son negativas. En algunas especies de plantas sus efectos son positivos y opuestos a los efectos de otros herbívoros explotadores. Esto tiene potenciales implicaciones para la evolución de mecanismos de tolerancia y resistencia en las plantas.

2. Los áfidos son herbívoros comunes en diferentes poblaciones de *Lonicera etrusca*. Estos explotadores consumen sabia de las ramas y los pedicelos foliares y causan un déficit de recursos que ocasiona posteriores cambios en el desarrollo floral: generan una disminución en el tamaño y la cantidad de polen y óvulos, afectan la estructura de las papilas estigmáticas y producen malformaciones de los granos de polen. Adicionalmente causan una disminución en el tamaño de las estructuras florales y una disrupción total de la producción de néctar. Por consiguiente, la herbivoría por áfidos afecta el proceso de polinización y esto tiene consecuencias negativas para la producción de frutos y semillas.

3. El robo de néctar tiene efectos positivos o neutros (es decir, no hay un aumento ni una disminución) sobre uno o ambos componentes del éxito reproductivo de las plantas mediante dos mecanismos: los robadores de néctar actúan como polinizadores o modifican el comportamiento de los visitantes legítimos. Las consecuencias de estos mecanismos se relacionan con el sistema reproductivo de la planta y a la cantidad de óvulos presentes en la flor. Así, en aquellos sistemas de polinización en los cuales se producen ambos mecanismos simultáneamente, los efectos de los robadores son aditivos a los efectos de los visitantes legítimos cuando las plantas tienen sistemas reproductivos auto-compatibles y mayor número de óvulos por flor (como en el caso de *Petrocoptis grandiflora*), produciéndose aumentos en el éxito reproductivo en presencia del robo de néctar. A diferencia, en plantas polinizador-dependientes con menores

cantidades de óvulos por flor (como en el caso de *L. etrusca*), la actividad de los robadores no genera un aumento en la donación de polen o la producción de frutos y semillas.

4. Aunque *L. etrusca* presenta una morfología floral aparentemente especializada para la polinización por esfíngidos, sus flores son visitadas y polinizadas por una gran diversidad de insectos que difieren en su eficiencia de polinización. Los polinizadores más importantes de esta planta son grandes himenópteros que se comportan como visitantes legítimos (*Anthophora acervorum*), himenópteros robadores primarios de néctar (*Bombus terrestris* y *Xylocopa violacea*) y el esfíngido visitante legítimo *Macroglossum stellatarum*.

5. A pesar de una aparente discordancia entre la morfología floral y la morfología de la probóscide de los robadores de néctar, los ajustes morfológicos que permiten la polinización por parte de estos visitantes florales involucran aspectos que hasta ahora fueron poco explorados. En el caso de *P. grandiflora* y *L. etrusca* la ubicación de las estructuras reproductivas fuera del tubo de la corola y el gran tamaño corporal de los principales robadores (*X. violacea* y *B. terrestris*) permite el contacto entre el abdomen del animal y las anteras y el estigma de las flores durante el forrajeo. Entonces, la posición de las estructuras reproductivas es una de las características florales sobre las cuales los robadores son potenciales agentes selectivos.

6. Las diferencias en la morfología y el comportamiento de forrajeo de los robadores primarios tienen efectos contrastantes para el proceso de polinización de *L. etrusca*. Los himenópteros que se comportan como robadores primarios de néctar no causan daños considerables a las estructuras reproductivas de la flor y además actúan como polinizadores efectivos. Por el contrario, los coleópteros que se comportan como robadores primarios son muy poco eficaces como polinizadores y ejercen presiones negativas sobre la planta consumiendo polen, promoviendo la auto-polinización y causando daños severos a la corola y a las estructuras reproductivas de la flor que quedan inservibles para posteriores visitas.

7. El robo de néctar es un fenómeno frecuente en angiospermas con flores tubulares largas y abundante floración, pero altamente variable en el tiempo y el espacio.

8. En las comunidades de plantas de áreas templadas y tropicales comúnmente una o dos especies son altamente robadas, mientras que otras presentan niveles intermedios y bajos de robo de néctar de acuerdo a la densidad de flores en el ambiente, y las características florales y del néctar.

9. El robo de néctar está mediado por la dificultad del visitante para acceder legítimamente al néctar (morfología y comportamiento). A una escala ecológica fina, la morfología floral y la agregación de las flores constituyen los caracteres más relevantes que determinan la frecuencia de robo en las plantas. A una escala ecológica más amplia el robo de néctar es un fenómeno denso-dependiente que se produce con más frecuencia en aquellas especies de plantas que representan un recurso abundante.

10. La presencia de barreras físicas, como brácteas o cálices fuertes y alargados, actúa como un mecanismo eficiente para reducir el robo de néctar por parte de insectos y en menor grado por aves.

11. El robo de néctar es un fenómeno ubicuo que se produce tanto en comunidades de regiones templadas como tropicales, con mayor frecuencia de lo que la literatura sobre las interacciones planta-animal había reflejado hasta ahora. Además, considerando que los robadores tienen efectos directos e indirectos sobre la reproducción de las plantas y esto tiene repercusiones sobre las dinámicas poblacionales, la evolución de los caracteres florales y las redes de interacciones planta-animal, es necesario considerar e incorporar a los robadores como piezas importantes en los estudios de las interacciones de polinización.

Conclusions

1. Nectar robbers are exploiters of the plant-pollinator interactions. Nevertheless, the consequences of this behaviour for the reproduction of the plants are not always negative. In some plant species the effects of robbers are positive and opposite to the effects of other herbivores exploiters. This has potential implications for the evolution of tolerance and resistance mechanisms in plants.

2. Aphids are common herbivores in different populations of *Lonicera etrusca*. These exploiters feed on sap from branches and leaf pedicels causing a shortening in the resources for flower development: they cause a reduction in the size and quantity of pollen and ovules, affect the structure of stigmatic papillae and produce malformations of the pollen grains. Besides, they cause a reduction in the size of flower's structures and a total cessation of nectar production. Therefore, herbivory by aphids affects the pollination process and this has negative consequences for the production of fruits and seeds.

3. Nectar robbery has positive or neutral (i.e. there is not an increase nor a diminishing) effects in one or both components of plant's reproductive success through two mechanisms: nectar robbers perform pollination or modify the behaviour of legitimate visitors. The consequences of these mechanisms are related to the reproductive system of the plant and the quantity of ovules present in the flower. Thus, in those pollination systems in which both mechanisms occur simultaneously, the effects of robbers are additive with the effects of legitimate visitors when plants have self-compatible reproductive systems and higher number of ovules per flower (such as in the case of *Petrocoptis grandiflora*). In that case, an increasing in the reproductive success in presence of robbers occurs. At difference, in pollinator-dependent plants with less ovules per flower (such as in the case of *L. etrusca*), the activity of nectar robbers does not cause an increase in pollen donation or fruit and seed set.

4. Although *L. etrusca* shows a floral morphology apparently specialized to the pollination of Hawkmoths, its flowers are visited and pollinated by a high diversity of insects that differ in their pollination effectiveness. The most important pollinators of this plant are big size hymenopterans that behave as legitimate visitors (*Anthophora acervorum*), hymenopterans primary nectar robbers (*Bombus terrestris* and *Xylocopa violacea*) and the Hawkmoth legitimate visitor *Macroglossum stellatarum*.

5. Despite an apparent mismatch between floral morphology and proboscid morphology of the nectar robbers, the morphological arrangements that allow pollination by these floral visitors involve aspects that have been barely explored. In the case of *P. grandiflora* and *L. etrusca* the exert reproductive structures of the flower and big size of the main robbers (*X. violacea* y *B. terrestris*) allows contact between the abdomen of the animal and the anthers and stigma of flowers during foraging. Thus, the position of reproductive structures is one of the floral traits on which the robbers are potential selective agents.

6. Differences in morphology and foraging behaviour of primary robbers have contrasting effects for the pollination process of *L. etrusca*. Hymenopterans that behave as primary nectar robbers do not cause considerable damages to the flower's reproductive structures and also are effective pollinators. At difference, coleopterans that behave as primary robbers are very ineffective as pollinators and exert negative pressures by consuming pollen, promoting self-pollination and causing severe damages to the reproductive structures and the corolla leaving the flower remaining unable for subsequent visits.

7. Nectar robbery is a frequent phenomenon in angiosperms with long tubular corollas and abundant flowering, but highly variable in time and space.

8. Within plant communities one or two species are highly robbed and others have intermediate to low levels of nectar robbery according to the density of flowers, and floral and nectar traits.

9. Nectar robbery is mediated by the difficulty that the visitor has to access legitimate to nectar (morphology and behaviour). At an ecological fine scale, floral morphology and aggregation of flowers are the most important characters that determine the frequency of robbery in plants. On a larger ecological scale,

nectar robbery is a density-dependent phenomenon that occurs most frequently in those plant species that represent abundant resources.

10. The presence of physical barriers such as bracts or strong elongated calyces, acts as an efficient mechanism that reduce nectar robbery by insects and, in lesser extent, by birds.

11. The nectar robbery is a ubiquitous phenomenon that occurs in temperate and tropical plant communities, more frequently than the literature about plant-animal interactions has reflected so far. Further, considering that the robbers have direct and indirect effects on plant reproduction and this has impacts on population dynamics, evolution of floral traits and plant-animal networks, it is necessary to consider and incorporate robbers as important pieces in the studies of pollination interactions.

Resumen

Las plantas interactúan simultáneamente con una gran diversidad de organismos que pueden influir en su éxito biológico y actuar como agentes selectivos. En las interacciones mutualistas de polinización las plantas se benefician del transporte de polen entre las flores, mientras que los animales obtienen recursos como recompensa (Faegri y van der Pijl, 1979). Este proceso reviste tal importancia que la reproducción sexual de aproximadamente 88 % de las angiospermas depende de los animales como polinizadores (Ollerton et al., 2011). Sin embargo, recompensas como el néctar son también consumidas por organismos explotadores quienes aparentemente no proveen ningún beneficio en retorno (Bronstein, 2001). Algunos de estos organismos consumen directamente partes de la flor (florívoros), o las recompensas nutritivas ofrecidas por la planta (en el caso de los robadores, los ladrones de néctar o los ladrones de polen) reduciendo su éxito reproductivo (Maloof e Inouye, 2000; McCall e Irwin, 2006). Pero hay también explotadores que consumen otras partes de la planta y que perjudican su reproducción a través de rutas fisiológicas que no son tan obvias. Por ejemplo, el consumo de tejidos fotosintéticos por herbívoros masticadores puede generar una reducción en el número de flores estaminadas, el número y el tamaño de polen y óvulos o la reducción en el tamaño de las flores afectando, en ocasiones, al proceso de polinización (Strauss, 1997; Krupnick y Weis, 1999; Lehtilä y Strauss, 1999; Mothershead y Marquis, 2000). En otros casos el forrajeo de savia por herbívoros chupadores causa una disminución en la producción de flores y frutos (Snow y Stanton, 1988). No obstante, las consecuencias de los explotadores para los organismos implicados en las interacciones mutualistas no siempre son negativas. En determinadas condiciones, las interacciones de explotación pueden generar incrementos en el éxito reproductivo de las plantas de acuerdo al tipo de recurso consumido, el comportamiento de forrajeo de los explotadores y una serie de atributos propios de los participantes en la interacción (ver por ejemplo Maloof e Inouye, 2000; Burkle et al., 2007; Irwin et al., 2010). Así, a medida que se profundiza en el conocimiento de estos organismos y sus consecuencias, se hace más evidente la importancia que tienen las interacciones de explotación sobre el funcionamiento y la estabilidad de las redes mutualistas (Ferriere et al., 2002; Genini et al., 2010).

Los robadores de néctar son visitantes florales que usan perforaciones en la corola para extraer el néctar acumulado en la base de los tubos florales o en los espolones. Por lo tanto no acceden “legítimamente” por la abertura natural de la flor (Inouye, 1980, 1983). Estas perforaciones son realizadas por los robadores primarios de néctar

con las estructuras bucales. A menudo, las perforaciones son también usadas por los robadores secundarios que pueden ser individuos de la misma especie o de una especie diferente a la que abrió la perforación (Irwin et al., 2010). Estas perforaciones son evidencias informativas que permiten hacer inferencias sobre el comportamiento de los robadores primarios incluso mucho tiempo después de que se produjo la visita a la flor (Rojas-Nossa, 2007; Teppner, 2011). En parte gracias a esto, las interacciones entre plantas, polinizadores y robadores de néctar son sistemas ideales para entender las consecuencias de diferentes tipos de visitantes florales en la ecología y evolución de los sistemas de polinización.

Una gran diversidad de insectos, aves y mamíferos nectarívoros se comportan como robadores primarios y/o secundarios de néctar (Maloof e Inouye, 2000; Irwin et al., 2010). A través de mecanismos directos e indirectos el robo de néctar puede tener consecuencias de diferente sentido y magnitud sobre la reproducción de las plantas de acuerdo a las características de los participantes en las interacciones (Maloof e Inouye, 2000). En muchos casos los robadores tienen efectos negativos para el éxito reproductivo de las plantas (Irwin et al., 2001, 2010). Directamente, pueden causar pérdidas de polen, dañar los órganos reproductivos o reducir la vida útil de la flor (McDade y Kinsman, 1980; Milet-Pinheiro y Schlindwein, 2009; Zhang et al., 2009b). También pueden promover la producción extra de néctar que se traduce en una reducción de los recursos para la producción de frutos y semillas (Navarro, 2001). Indirectamente, los robadores de néctar pueden impedir el acceso de los visitantes legítimos a las flores por territorialidad (Roubik, 1982). Además, el forrajeo de los robadores puede causar un descenso en los niveles de néctar disponibles para los visitantes florales que ocasiona cambios en su comportamiento. Por ejemplo, disminuyen o evitan por completo visitar plantas con altos niveles de robo, o reducen el tiempo de la visita en cada flor (Irwin y Brody, 1998; Irwin, 2000; Dohzono et al., 2008; Maruyama et al., 2015). En estos casos la calidad del servicio de polinización puede verse reducida. Sin embargo, el robo de néctar no siempre es negativo y en ocasiones el éxito reproductivo de las plantas es igual o incluso mayor bajo la acción de los robadores que sin ellos (Higashi et al., 1998; Utelli y Roy, 2001; Zhu et al., 2010; Singh et al., 2014). Esto ocurre cuando los robadores actúan como polinizadores de las plantas, o indirectamente cuando la reducción en los niveles de néctar promueve que los visitantes legítimos visiten un mayor número de flores a mayores distancias, favoreciendo el entrecruzamiento genético de la población (Zimmerman y Cook, 1985; Morris, 1996; Navarro, 2000; Zhang et al., 2014). Como resultado de los cambios en el éxito reproductivo de las plantas, los robadores de néctar pueden actuar como agentes selectivos, modificar las dinámicas poblacionales, estabilizar las redes de interacciones mutualistas y constituir piezas importantes para el mantenimiento de los sistemas de polinización (Navarro y Medel, 2009; Wang et al., 2015). No obstante, pocos sistemas biológicos han sido detalladamente estudiados haciendo la información sobre los robadores aún escasa, fragmentada y limitada a áreas geográficas puntuales, impidiendo generalizaciones.

El objetivo general de esta disertación es estudiar las implicaciones ecológicas y evolutivas del robo de néctar desde una perspectiva multiescala. El análisis del estado actual del conocimiento sobre este tema presentado en el Capítulo 1 permitió detectar los principales vacíos en el conocimiento de los sistemas planta-polinizador-robador que nos llevaron a delinear los objetivos específicos y los métodos de esta tesis y que corresponden a las siguientes secciones. En los Capítulos 2 y 3 analizamos detalladamente las consecuencias del robo de néctar sobre los componentes femenino y masculino del éxito reproductivo de dos especies de plantas presentes en la Península Ibérica con historias de vida contrastantes. En el Capítulo 2 estudiamos la variabilidad espacial y temporal en la frecuencia de robo en *Petrocoptis grandiflora*, una hierba rupícola, auto-compatible y de distribución restringida. Cuantificamos los niveles de robo de néctar en tres poblaciones a lo largo de 12 años y analizamos su efecto sobre la producción de frutos y semillas, y sobre la distancia de dispersión de polen. En el Capítulo 3 estudiamos y comparamos el efecto del robo de néctar sobre el éxito reproductivo de *Lonicera etrusca*, un arbusto escandente, polinizador-dependiente y común en ecosistemas Mediterráneos. Cinco variables fueron usadas para caracterizar los componentes femenino y masculino del éxito reproductivo de la planta y evaluar los cambios relacionados con el robo de néctar. En el Capítulo 4 estudiamos la dirección y la magnitud de los efectos de dos tipos de explotadores: los robadores de néctar y los herbívoros chupadores de sabia sobre el servicio de polinización de *L. etrusca*. Evaluamos los efectos directos e indirectos de estos explotadores sobre la calidad de la polinización con un análisis de vías. El estudio detallado del comportamiento de forrajeo de los diferentes visitantes florales de *L. etrusca* nos permitió explorar e incorporar los diferentes aspectos que determinan su efectividad como polinizadores. Dado que *L. etrusca* requiere polinización por insectos para producir frutos y semillas (Guitián et al. 1993), en el Capítulo 5 desarrollamos y proponemos un modelo que permite calcular y comparar la efectividad de la polinización por diferentes visitantes florales en plantas con sistemas reproductivos polinizador-dependientes. Para desarrollar este modelo usamos como base conceptual los modelos propuestos para plantas auto-compatibles (ver Herrera, 1987; Ne'eman et al., 2010; entre otros). El modelo involucra variables relacionadas con el comportamiento de los polinizadores y su aporte a los componentes masculino (donación de polen) y femenino (deposición de polen proveniente de otra flor sobre el estigma) de la polinización. Este modelo permite hacer inferencias de los procesos a diferentes escalas ecológicas. A lo largo de la tesis fue usado para analizar el efecto de visitas únicas a una flor (componente cualitativo de la polinización presentado en el Capítulo 5), también para medir y analizar la calidad de la polinización recibida por los individuos (usado en el Capítulo 4) y para estimar la efectividad de los polinizadores en términos poblacionales (representado en el paisaje de polinización del Capítulo 5). Debido a que uno de los mayores vacíos detectados es la falta de estudios que faciliten el análisis de los patrones relacionados con el robo de néctar a nivel de comunidades y más aún a nivel de grandes regiones geográficas, en el Capítulo 6 estudiamos y comparamos la frecuencia de robo de néctar por insectos y aves en cuatro comunidades de plantas de regiones templadas y tropicales. Evaluamos la asociación entre la frecuencia de robo en las especies dentro de las comunidades con las características relacionadas con los visitantes florales, tales como la morfología de la flor, la producción de néctar, la densidad de oferta energética, la presencia de físicas o químicas contra robadores de néctar y la forma de vida de la especie. Finalmente, en el Capítulo 7 hacemos una discusión general en la cual se presentan los principales aportes de esta tesis con relación al estado del conocimiento del tema y se proponen líneas de estudio para futuros trabajos.

Los principales resultados, discusión y conclusiones de los capítulos se describen a continuación.

En el Capítulo 2 encontramos que los niveles de robo de néctar en *P. grandiflora* fueron similares entre las poblaciones estudiadas, pero cambiaron a lo largo de 12 años de monitoreo (Fig. 2.2 y Tabla 2.1). En otros sistemas se ha documentado también una gran variabilidad en los niveles de robo experimentado por diferentes individuos, parches, o poblaciones de la misma especie, o entre especies de una misma comunidad (Irwin et al., 2001; Irwin y Maloof, 2002; Rojas-Nossa, 2013). Se han planteado diferentes hipótesis para explicar esta variación, tales como cambios temporales o espaciales en la disponibilidad de recursos alternativos, cambios temporales en las abundancias poblacionales de los robadores, o diferencias en las características de las plantas que las hacen más apetecibles para los robadores (Navarro, 2000; Irwin y Maloof, 2002; Rojas-Nossa, 2013). En las tres poblaciones estudiadas de *P. grandiflora*, ocurrieron bajas frecuencias de robo de néctar en algunos años (Fig. 2.2). Simultáneamente también ocurrieron descensos en los niveles de robo de néctar en las poblaciones de *Anthyllis vulneraria* estudiadas en la misma región por Navarro (2000). Es factible que cambios abruptos en las condiciones climáticas al inicio de la primavera afectaron las abundancias poblacionales de los principales robadores primarios de néctar de ambas plantas (los himenópteros *Xylocopa violacea* y *Bombus terrestris*) y esto causó las bajas frecuencias de robo de néctar registradas observadas en esos años.

Los resultados obtenidos en el Capítulo 2 de esta disertación muestran que el robo de néctar es positivo para los componentes femenino y masculino del éxito reproductivo de *P. grandiflora*. El incremento en la fructificación y la distancia de dispersión de polen están relacionadas con el efecto directo de los principales robadores primarios (*Xylocopa violacea*, *Bombus terrestris* y *B. jonellus*), quienes efectúan la polinización durante el robo de néctar (Navarro y Guitián, 2000). Mecanismos indirectos, como el aumento de las distancias de forrajeo o las frecuencias de visita de los visitantes legítimos debidas a la reducción de la cantidad de néctar en las flores robadas, también pueden contribuir al aumento en el éxito reproductivo observado en esta especie. A diferencia, en *Lonicera etrusca* el robo de néctar por *X. violacea* y *B. terrestris* no causó cambios (ni positivos ni negativos) en ninguna de las variables usadas para estimar los componentes femenino y masculino del éxito reproductivo (Capítulo 3). Ambas plantas co-existen en la localidad estudiada en la Península Ibérica y comparten los principales robadores primarios de néctar. El análisis de los tubos polínicos producidos después de una visita de estos robadores primarios a las flores de *L. etrusca* demuestra que éstos promueven la polinización cruzada mientras se desplazan entre las flores, perforan la base del perianto y extraen néctar (Capítulo 4). Es decir, que los robadores primarios de néctar son polinizadores de ambas plantas, pero las diferencias en el sistema de auto-compatibilidad y en el ajuste morfológico insecto-flor determinan los cambios en el éxito reproductivo de la planta. Tal ajuste morfológico es menos obvio, refinado y conocido que los casos de polinización por visitantes legítimos. En él intervienen aspectos como la longitud del cuerpo del insecto y la longitud de las estructuras reproductivas que están ubicadas fuera del tubo floral y permiten que los robadores transporten polen entre las flores promoviendo la polinización favoreciendo la reproducción de las plantas robadas. Además de las consecuencias positivas directas que tienen los robadores primarios como polinizadores, la ausencia de efectos negativos en las especies estudiadas se relaciona con varios factores. Por una parte estos robadores no producen daños significativos a las estructuras reproductivas y con ello no reducen la funcionalidad de la flor. Por otra parte, aunque encontramos que las flores robadas de *L. etrusca* producen menos néctar de mayor concentración (Tabla 4.1), estos cambios no generan una disminución en el servicio de polinización. Además, las perforaciones hechas por los robadores primarios de néctar son aprovechadas por pequeñas abejas de los géneros *Ceratina*, *Lassioglossum* y *Halictus* que comúnmente se comportan como robadoras secundarias de néctar y recolectoras de polen durante la misma visita (Capítulo 5). Aunque la efectividad de polinización de estas pequeñas abejas es baja, éstas contribuyen al proceso de polinización (Fig. 5.6). De este modo, los robadores primarios de néctar tienen efectos directos e indirectos sobre la reproducción de esta especie polinizador-dependiente. Aunque frecuentemente el robo de néctar tiene efectos negativos para alguno de los dos componentes del éxito reproductivo de las plantas robadas (Irwin et al., 2010), el estudio de un mayor número de casos incluyendo también a las aves como robadoras de néctar demuestra que la influencia de estos visitantes florales puede ser positiva en más sistemas de los esperados (Fumero-Cabán y Meléndez-Ackerman, 2007; Zhu et al., 2010; Singh et al., 2014; Zhang et al., 2014).

Por el contrario, los áfidos chupadores de sabia *Hyadaphis passerinii* atacan las ramas y los peciolos de *L. etrusca* antes de la floración y generan cambios morfológicos y fisiológicos que producen una posterior reducción en el servicio de polinización (Capítulo 4). A pesar de que los áfidos son plagas comunes en plantas cultivadas, los efectos sobre las plantas nativas y en particular sobre las interacciones de polinización prácticamente inexploradas (pero ver Snow y Stanton, 1988). La herbivoría por áfidos causa una reducción en las dimensiones de las estructuras florales, la cantidad y la calidad de polen y óvulos, y genera cambios en las papilas estigmáticas (Tabla 4.1 y Figs. 4.2 y 4.5). Además, las flores dañadas no producen néctar. Estos cambios generan una reducción en las visitas florales de los polinizadores (incluyendo los robadores primarios de néctar) afectando indirectamente la calidad del servicio de polinización recibida por las plantas (Fig. 4.7). Como resultado, la herbivoría genera una disminución en la producción de frutos y peso de las semillas producidas en flores dañadas y también en el peso de las semillas producidas en las flores sanas de aquellas plantas que altamente atacados por áfidos (Fig. 4.6). En otras plantas estos herbívoros son vectores de infecciones, inducen la producción de agallas, causan defoliación y causan una reducción en la cantidad de flores (Dixon, 1971; Snow y Stanton, 1988; Larson, 1998; Power y Flecker, 2003). Dado que estos herbívoros se alimentan de savia y ésta transporta sustancias necesarias para el desarrollo de las plantas (Douglas, 2006), la pérdida de cantidades importantes de recursos para la formación de flores, frutos y semillas es la causa más probable de los daños observados en las flores y la consiguiente reducción en el éxito reproductivo de *L. etrusca*. Este estudio constituye la primera evidencia de las consecuencias negativas de estos explotadores para el desarrollo floral, la producción de recompensas y el servicio de polinización en una especie nativa.

A pesar de que las flores de *L. etrusca* presentan características asociadas con un sistema especializado para la polinización por esfíngidos (Jordano, 1990; Guitián et al., 1993), el servicio de polinización es mediado por una gran diversidad de insectos (Capítulo 5). Efectivamente, una especie de esfíngido (*Hemaris fuciformis*) es el polinizador de mayor calidad en términos de una visita a una flor (Fig. 5.6). No obstante, en términos poblacionales, los himenópteros visitantes legítimos y robadores primarios son polinizadores altamente efectivos de *L. etrusca* debido a su comportamiento, su morfología y a la alta frecuencia de visitas florales. En contraste, los coleópteros que se comportan como robadores primarios tienen una baja efectividad como polinizadores y causan daños considerables a la flor y las estructuras reproductivas. Este resultado demuestra que dos grupos que aparentemente se comportan de forma similar (i.e. robadores primarios de néctar) tienen consecuencias opuestas para la reproducción de una misma especie de planta. Estas diferencias se relacionan con su morfología, su comportamiento y sus abundancias poblacionales, reflejando la importancia de estudiar en detalle los sistemas de polinización analizando y comparando objetivamente el papel de los robadores de néctar en las interacciones planta-animal.

Notablemente, el robo de néctar ha sido observado en una gran diversidad de angiospermas alrededor del globo (Fig. 1.2 del Capítulo 1). Se había sugerido que la causa más probable del robo de néctar eran las limitaciones morfológicas que tienen los animales con lenguas cortas para extraer néctar en flores con el néctar “escondido” (Irwin et al., 2010). También se ha observado que alta una alta variación temporal, espacial y taxonómica en la frecuencia de robo de néctar. Es decir, que mientras que en algunas plantas se observa robo de néctar ocasionalmente, en otros casos prácticamente todas las flores de una población están perforadas (Roubik et al., 1985;

Guitián et al., 1994; Maloof e Inouye, 2000; Utelly y Roy, 2001). En este trabajo evaluamos por primera vez las características de las plantas relacionadas con las diferencias en los niveles de robo observados en las plantas de comunidades de diferentes regiones biogeográficas. El estudio de cuatro comunidades de plantas en regiones templadas y tropicales presentado en el Capítulo 6 demuestra que a una escala fina la morfología floral, las características del néctar y la presencia de mecanismos físicos que actúan como barreras contra el robo de néctar son caracteres relevantes para determinar la frecuencia de este fenómeno en las plantas (Tabla 6.2). A una mayor escala ecológica, el robo de néctar es un fenómeno denso-dependiente que se produce con más frecuencia en aquellas especies de plantas que representan un recurso abundante. Sugerimos que en futuros estudios se considere a los robadores de néctar como piezas clave en las interacciones de polinización y que se evalúe el efecto su sobre la evolución de los caracteres florales relacionados con la variabilidad intra e interespecífica en los niveles de robo de néctar, involucrando niveles multitróficos y multiescala en los estudios que permitan obtener una información más completa de este complejo fenómeno y sus consecuencias para el funcionamiento de los mutualismos.

References

Adler, L. S. 2000. The ecological significance of toxic nectar. Oikos 91: 409–420.

Adler, L. S., Irwin, R. E. 2005. Ecological costs and benefits of defenses in nectar. Ecology 86: 2968–2978.

Adler, L. S, Irwin, R. E. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. Annals of Botany 97: 141–150.

Aigner, P. A. 2001. Optimality modeling and fitness trade-offs: When should plants become pollinator specialists? Oikos 95: 177–184.

Aigner, P. A. 2004. Floral specialization without trade-offs: optimal corolla flare in contrasting pollination environments. Ecology 85: 2560–2569.

Aigner, P. A. 2006 The evolution of specialized floral phenotypes in a fine-grained environment. In: Plant–pollinator interactions, from specialization to generalization (N. M. Waser and J. Ollerton eds.), pp. 23–46. University of Chicago Press, Chicago.

Aizen, M. A., Searcy, K. B., Mulcahy, D. L. 1990. Among- and within-flower comparisons of pollen tube growth following self-and cross-pollinations in *Dianthus chinensis* (Caryophyllaceae). American Journal of Botany 77: 671–676.

Alford, D. V. 2012. Pests of ornamental trees, shrubs and flowers. Manson Publishing Ltd, London.

Althoff, D. M., Segraves, K. A., Pellmyr, O. 2005. Community context of an obligate mutualism: pollinator and florivore effects on *Yucca filamentosa*. Ecology 86: 905–913.

Anderson, B., Johnson, S. D. 2008. The geographical mosaic of coevolution in a plant–pollinator mutualism. Evolution 62: 220–225.

Anderson, S. H., Kelly, D., Ladley, J. J., Molloy, S., Terry, J. 2011. Cascading effects of bird functional extinction reduce pollination and plant density. Science 331: 1068–1071.

Arizmendi, M. C. 2001. Multiple ecological interactions: nectar robbers and hummingbirds in a highland forest in Mexico. Canadian Journal of Zoology 79: 997–1006.

Arizmendi, M. C., Dominguez, C., Dirzo, R. 1996. The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. Functional Ecology 10: 119–127.

Armbruster, W. S., Hansen, T. F., Pélabon, C., Pérez-Barrales, R., Maad, J. 2009. The adaptive accuracy of flowers: measurement and microevolutionary patterns. Annals of Botany 103: 1529–1545.

Askins, R. A., Ercolino, K. M., Waller, J. D. 1987. Flower destruction and nectar depletion by avian nectar robbers on a tropical tree, *Cordia sebestena*. Journal of Field Ornithology 58: 345–349.

Aximoff, I. A., Freitas, L. Composição e comportamento de aves nectarívoras em *Erythrina falcata* (Leguminosae) durante duas florações consecutivas com intensidades diferentes. Revista Brasileira de Ornitologia 17: 194–203.

Baayen, R. H. 2008. Analyzing Linguistic Data. Cambridge University Press, Cambridge.

Baayen, R. H. 2011. languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics". Version 1. http:// CRAN.R-project.org/package = language

Baker, H. G. 1977. Non-sugar chemical constituents of nectar. Apidologie 8: 349–356.

Barrett, S. C. H. 2010. Darwin's legacy: the forms, function and sexual diversity of flowers. Philosophical Transaction of the Royal Society B 365: 351–368.

Barrows, E. M. 1980. Robbing of exotic plants by introduced carpenter and honey bees in Hawaii, with comparative notes. Biotropica 12: 23–29.

Bates, D., Maechler, M., Bolker, B. 2012. lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0.http://CRAN.R-project.org/ package=lme4.

Blackman, R. L., Eastop, V. F. 1984. Aphids on the world's crops. An identification and information guide. John Wiley & Sons Ltd, West Sussex.

Blackman, R. L., Eastop, V. F. 2006. Aphids on the World’s Herbaceous Plants and Shrubs. John Wiley & Sons Ltd, West Sussex.

Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology 78: 1624–1631.

Brody, A. K., Irwin, R. E. 2012. When resources don't rescue: flowering phenology and species interactions affect compensation to herbivory in *Ipomopsis aggregata*. Oikos 121: 1424–1434.

Brody, A. K., Irwin, R. E., McCutcheon, M., Parsons, E. 2008. Interactions between nectar robbers and seed predators mediated by a shared host plant, *Ipomopsis aggregata*. Oecologia 155: 75–84.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology & Evolution 9: 214–217.

Bronstein, J. L. 2001. The exploitation of mutualisms. Ecology Letters 4: 277–287.

Brys, R., Jacquemyn, H. 2010. Floral display size and spatial distribution of potential mates affect pollen deposition and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). Plant Biology 12: 597–603.

Burkle, L. A, Alarcón, R. 2011. The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. American Journal of Botany 98: 528–538.

Burkle, L. A, Marlin, J. C, Knight, T. M. 2013. Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. Science 339: 1611–1615.

Burkle, L. A., Irwin, R. E., Newman, D. A. 2007. Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. American Journal of Botany 94: 1935–1943.

Castro, S., Silveira, P., Navarro, L. 2008. Consequences of nectar robbing for the fitness of a threatened plant species. Plant Ecology 199: 201–208.

Castro, S., Silveira, P., Navarro, L. 2009. Floral traits variation, legitimate pollination, and nectar robbing in *Polygala vayredae* (Polygalaceae). Ecological Research 24: 47–55.

Castroviejo, S. 2012. Flora Ibérica: plantas vasculares de la Península Ibérica e Islas Baleares. Real Jardín Botánico, Madrid.

Chittka, L., Leadbeater, E. 2005. Social learning: public information in insects. Current Biology 15: R869–R871.

Colwell, R. K., Betts, B. J., Bunnell, P., Carpenter, F. L., Feinsinger, P. 1974. Competition for the nectar of *Centropogon valerii* by the hummingbird *Colibri thalassinus* and the flower-piercer *Diglossa plumbea*, and its evolutionary implications. Condor 76: 447–454.

Corbet, S. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Apidologie 34: 1–10.

Cotton, P. A. 1998. Coevolution in an Amazonian hummingbird-plant community. Ibis 140: 639–646.

Cushman, J. H., Beattie, A. J. 1991. Mutualisms: assessing the benefits to host and visitors. Trends in Ecology and Evolution 6: 191–195.

Crepet, W. L., Niklas, K. J. 2009. Darwin’s second “abominable mystery”: Why are there so many angiosperm species? American Journal of Botany 96: 366–381.

Darwin, C. 1859. The Origin of Species. Penguin Books, London.

Darwin, C. 1876. The effects of cross and self-fertilisation on the vegetable kingdom. Appelton, New York.

Dellinger, A. S., Penneys, D. S., Staedler, Y. M., Fragner, L., Weckwerth, W., Schönenberger, J. 2014. A specialized bird pollination system with a bellows mechanism for pollen transfer and staminal food body rewards. Current Biology 24: 1615–1619.

Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. Annual Review of Ecology and Systematics 26: 531–552.

Dixon, A. F. G. 1971. The role of aphids in wood formation. I. The effect of the sycamore aphid, *Dreopanosiphum platanoides* (Schr.) (Aphididae), on the growth of sycamore, *Acer pseudoplatanus* (L.). Journal of Applied Ecology 8: 165–179.

Dohzono, I., Kunitake, Y. K., Yokoyama, J., Goka, K. 2008. Alien bumble bee affects native plant reproduction through interactions with native bumble bees. Ecology 89: 3082–3092.

Douglas, A. E. 2006. Phloem-sap feeding by animals: problems and solutions. Journal of Experimental Botany 57: 747–754.

Dullinger, S., Mang, T., Dirnböck, T., Ertl, S., Gattringer, A., Grabherr, G., Leitner, M., Hülber, K. 2011. Patch configuration affects alpine plant distribution. Ecography 34: 576–587.

Eckhart, V. M. 1991. The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). Evolutionary Ecology 5: 370–384.

Ehrlich, P., Raven, P. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586–608.

Faegri, K., van der Pijl, L. 1979. The principles of pollination ecology. Pergamon Press Ltd, Oxford.

Feinsinger, P., Colwell, R. K. 1978. Community organization among neotropical nectar-feeding birds. American Zoologist 18: 779–795.

Feinsinger, P., Beach, J. H., Linhart, Y. B., Busby, W. H., Murray, G. 1987. Disturbance, pollinator predictability, and pollination success among Costa Rican cloud forest plants. Ecology 68:1294–1305.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., Thomson, J. D. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution, and Systematics 35: 375–403.

Ferriere, R., Bronstein, J. L., Rinaldi, S., Law, R., Gauduchon, M. 2002. Cheating and the evolutionary stability of mutualisms. Proceedings of the Royal Society B: Biological Sciences 269: 773–780.

Ferrière, R., Gauduchon, M., Bronstein, J. L. 2007. Evolution and persistence of obligate mutualists and exploiters: competition for partners and evolutionary immunization. Ecology Letters 10: 115–126.

Foster, W. A. 1984. The distribution of the sea-lavender aphid *Staticobium staticis* on a marine saltmarsh and its effect on host plant fitness. Oikos 42: 97–104.

Frame, D. 2003. Generalist flowers, biodiversity and florivory: implications for angiosperm origins. Taxon 54: 681–685.

Free, J. B., Butler, C. G. 1959. Bumblebees. Collins, London.

Friis, E. M., Crane, P. R., Pedersen, K. R. 2011. Early flowers and angiosperm evolution. Cambridge University Press, Cambridge.

Fumero-Cabán, J. J., Meléndez-Ackerman, E. J. 2007. Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). American Journal of Botany 94: 419–424.

Galen, C., Gregory, T., Galloway, L. F. 1989. Costs of self-pollination in a self-incompatible plant, *Polemonium viscosum*. American Journal of Botany 76: 1675–1680.

García, M. B., Ehrlén, J. 2002. Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. American Journal of Botany 89: 1295–1302.

Gass, C. L., Montgomerie, R. D. 1981. Hummingbird foraging behavior:

decision-making and energy regulation. In: Foraging behavior: ecological, ethological, and psychological approaches (A. C. Kamil and T. D. Sargent, eds.), pp. 150–194. Garland STPM Press, New York.

Geerts, S., Pauw, A. 2009. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. South African Journal of Botany 75: 699–706.

Genini, J., Morellato, L. P. C., Guimarães Jr., P. R., Olesen, J. M. 2010. Cheaters in mutualism networks. Biology letters 6: 494–497.

Gerling, D., Velthuis, H. H. W., Hefetz, A. 1989. Bionomics of the large carpenter bees of the genus *Xylocopa*. Annual Review of Entomology 34: 163–190.

Gill, G. E., Fowler, R. T., Mori, S. A. 1998. Pollination Biology of *Symphonia globulifera* (Clusiaceae) in Central French Guiana. Biotropica 30: 139–144.

Glover, B. J. 2007. Understanding flowers and flowering: an integrated approach. Oxford University Press, Oxford.

Goggin, F. L. 2007. Plant–aphid interactions: molecular and ecological perspectives. Current Opinion in Plant Biology 10: 399–408.

Gómez, J. M. 2005. Non-additive effects of herbivores and pollinators on *Erysimum mediohispanicum* (Cruciferae) fitness. Oecologia 143: 412–418.

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M. 2007. Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. Oecologia 153: 597–605.

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M., Camacho, J. P. M. 2008. Association between floral traits and rewards in *Erysimum mediohispanicum* (Brassicaceae). Annals of Botany 101: 1413–1420.

Gómez, J. M., Perfectti, F., Bosch, J., Camacho, J. P. M. 2009. A geographic selection mosaic in a generalized plant–pollinator–herbivore system. Ecological Monographs 79: 245–264.

González-Gómez, P. L., Valdivia, C. E. 2005. Direct and indirect effects of nectar robbing on the pollinating behavior of *Patagona gigas* (Trochilidae). Biotropica 37: 693–696.

González-Varo, J. P., Biesmeijer, J. C., Bommarco, R., Potts, S. G., Schweiger, O., Smith, H. G., Steffan-Dewenter, I., Szentgyörgyi, H., Woyciechowski, M., Vilà, M. 2013. Combined effects of global change pressures on animal-mediated pollination. Trends in Ecology & Evolution 28: 524–530.

Gonzalez-Voyer, A., von Hardenberg, A. 2014. An Introduction to Phylogenetic Path Analysis. In: Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology (L. Z. Garamszegi ed.), pp. 201–229. Springer-Verlag, Berlin.

Goulson, D. 2003. Effects of introduced bees on native ecosystems. Annual Review of Ecology, Evolution, and Systematics 34: 1–26.

Goulson, D., Chapman, J. W., Hughes, W. O. 2001. Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. Journal of Insect Behavior 14: 669–678.

Goulson, D., Cruise, J. L. Sparrow, K. R., Harris, A. J. Park, K. J. Tinsley, M. C., Gilburn, A. S. 2007. Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees. Behavioural Ecology and Sociobiology 61: 1523–1529.

Goulson, D., Park, K. J., Tinsley, M. C., Bussière, L. F., Vallejo-Marin, M. 2013. Social learning drives handedness in nectar-robbing bumblebees. Behavioral Ecology and Sociobiology 67: 1141–1150.

Goulson, D., Stout, J. C., Hawson, S. A., Allen, J. A. 1998. Floral display size in comfrey, *Symphytum officinale* L. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set. Oecologia 113: 502–508.

Grace, J. B. 2006. Structural equation modeling and natural systems. Cambridge University Press, Cambridge.

Graves, G. R. 1982. Pollination of *Tristerix mistletoe* (Loranthaceae) by *Diglossa* (Aves, Thraupidae). Biotropica 14: 316.

Guerrant Jr, E. O., Fiedler, P. L. 1981. Flower defenses against nectar-pilferage by ants. Biotropica 13: 25–33.

Guitián, J., Navarro, L., Guitián, P., Sánchez, J. M. 1997. Variation in floral morphology and reproductive success in *Petrocoptis grandiflora* (Caryophyllaceae). Annales Botanici Fennici 34: 35–40.

Guitián, J., Sánchez, J. M., Guitián, P. 1994. Pollination ecology of *Petrocoptis grandiflora* Rothm. (Caryophyllaceae); a species endemic to the north-west part of the Iberian Peninsula. Botanical Journal of the Linnean Society 115: 19–27.

Guitián, P., Guitián, J., Navarro, L. 1993. Pollen transfer and diurnal versus nocturnal pollination in *Lonicera etrusca*. Acta Oecologica 14: 219–227.

Gutiérrez, A., Rojas-Nossa, S. V., Stiles, F. G. 2004. Dinámica anual de la interacción colibrí-flor en ecosistemas altoandinos. Ornitología Neotropical 15: 1–9.

Gutiérrez-Zamora, A. 2008. Las interacciones ecológicas y estructura de una comunidad altoandina de colibríes y flores en la Cordillera Oriental de Colombia. Ornitología Colombiana 7: 17–42.

Harder, L. D., Barrett, S. C. H. 1996. Pollen dispersal and mating patterns in animal-pollinated plants. In: Floral Biology (D. G. Lloyd and S. C. H. Barrett eds), pp. 140–190. Chapman and Hall, New York.

Hawkins, R. P. 1961. Observations on the pollination of red clover by bees. I. The yield of seed in relation to the numbers and kinds of pollinators. Annals of Applied Biology 49: 55–65.

Heinrich, B., Raven, P. H. 1972. Energetics and pollination ecology. Science 176: 597–602.

Heinrich, B. 1975. Energetics of pollination. Annual Review of Ecology and Systematics 6: 139–170.

Herrera, C. M. 1987. Components of pollinator 'quality': comparative analysis of a diverse insect assemblage. Oikos 50: 79–90.

Herrera, C. M. 1988. Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. Biological Journal of the Linnean Society 35: 95–125.

Herrera, C. M. 1989. Pollinator abundance, morphology, and flower visitation rate: analysis of the ''quantity'' component in a plant-pollinator system. Oecologia 80: 241–248.

Herrera, C. M. 1990. Daily pattern of pollinator activity, differential pollinating effectiveness, and floral resource availability, in a summer-flowering Mediterranean shrub. Oikos 58: 277–288.

Herrera, C. M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Floral Biology. (D. G. Lloyd and S. C. H. Barrett eds.), pp. 65–87. Chapman and Hall, New York.

Herrera, C. M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. Ecology 81: 2170–2176.

Higashi, S., Ohara, M., Arai, H., Matsuo, K. 1988. Robber-like pollinators: overwintered queen bumblebees foraging on *Corydalis ambigua*. Ecological Entomology 13: 411–418.

Hughes, N. F. 1982. Palaeobiology of angiosperm origins. Cambridge University Press, Cambridge.

IBM Corp. 2010. IBM SPSS Statistics for Windows, Version 19.0. IBM Corp.

Inouye, D. W. 1980. The terminology of floral larceny. Ecology 61: 1251–1253.

Inouye, D. W. 1983. The ecology of nectar robbing. In: The Biology of Nectaries (B.

Beattey, T. Elias eds.), pp. 153–173. Columbia University Press, New York.

Inouye, D. W., Gill, D. E., Dudash, M. R., Fenster, C. B. 1994. A model and lexicon for pollen fate. American Journal of Botany 81: 1517–1530.

Irwin, R. E. 2000. Hummingbird avoidance of nectar-robbed plants: spatial location or visual cues. Oikos 91: 499–506.

Irwin, R. E. 2003. Impact of nectar robbing on estimates of pollen flow: conceptual predictions and empirical outcomes. Ecology 84: 485–495.

Irwin, R. E. 2006. The consequences of direct versus indirect species interactions to selection on traits: Pollination and nectar robbing in *Ipomopsis aggregata*. American Naturalist, 167, 315–328.

Irwin, R. E., Adler, L. S. 2006. Correlations among traits associated with herbivore resistance and pollination: implications for pollination and nectar robbing in a distylous plant. American Journal of Botany 93: 64–72.

Irwin, R. E., Adler, L. S., Brody, A. K. 2004. The dual role of floral traits: pollinator attraction and plant defense. Ecology 85: 1503–1511.

Irwin, R. E., Brody, A. K. 1998. Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. Oecologia 116: 519–27.

Irwin, R. E., Brody, A. K. 1999. Nectar-robbing bumblebees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). Ecology 80: 1703–1712.

Irwin, R. E., Brody, A. K. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. Ecology 81: 2637–2643.

Irwin, R. E., Brody, A. K. 2011. Additive effects of herbivory, nectar robbing and seed predation on male and female fitness estimates of the host plant *Ipomopsis aggregata*. Oecologia 166: 681–692.

Irwin, R. E., Brody, A. K., Waser, N. M. 2001. The impact of floral larceny on individuals, populations, and communities. Oecologia 129: 161–168.

Irwin, R. E., Bronstein, J. L., Manson, J. S., Richardson, L. 2010. Nectar Robbing: Ecological and Evolutionary Perspectives. Annual Review of Ecology and Systematics 41: 271–92.

Irwin, R. E., Galen, C., Rabenold, J. J., Kaczorowski, R., McCutcheon, M. L. 2008. Mechanisms of tolerance to floral larceny in two wildflower species. Ecology 89: 3093–3104.

Irwin, R. E., Maloof, J. E. 2002. Variation in nectar robbing over time, space, and species. Oecologia 133: 525–533.

Irwin, R. E., Strauss, S. Y., Storz, S., Emerson, A., Guibert, G. 2003. The role of herbivores in the maintenance of a flower color polymorphism in wild radish. Ecology 84: 1733–1743.

Johnson, S. D., Steiner, K. E. 2000 Generalization versus specialization in plant pollination systems. Trends in Ecology & Evolution 15: 140–143.

Jordano, P. 1987. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. American naturalist 129: 657–677.

Jordano, P. 1990. Biología de la reproducción de tres especies del género *Lonicera* (Caprifoliaceae) en la Sierra de Cazorla. Anales del Jardín Botánico de Madrid 48: 31–52.

Karban, R., Strauss, S. Y. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. Ecology 74:39–46.

Kearns, C. A., Inouye, D. W. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot.

Kearns, C. A., Inouye, D. W., Waser, N. 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. Annual Review of Ecology and Systematics 29: 83–112.

Kendall, D. A, Smith, B. D. 1976. The pollinating efficiency of honeybee and bumblebee visits to flowers of the runner bean *Phaseolus coccineus* L. Journal of Applied Ecology 13: 749–752.

Kennedy, J. S., Stroyan, H. L. G. 1959. Biology of aphids. Annual Review of Entomology 4: 139–160.

Kessler, D., Gase, K., Baldwin, I. T. 2008. Field experiments with transformed plants reveal the sense of floral scents. Science 321: 1200–1202.

Kessler, M., Krömer, T. 2000. Patterns and ecological correlates of pollination modes among bromeliad communities of Andean forests in Bolivia. Plant Biology 2: 659–669.

Kjonaas, C., Rengifo, C. 2006. Differential effects of avian nectar-robbing on fruit set of two Venezuelan Andean cloud forest plants. Biotropica 38: 276–279.

Kodric-Brown, A., Brown, J. H., Byers, G. S., Gori, D. F. 1984. Organization of a tropical island community of hummingbirds and flowers. Ecology 65: 1358–1368.

Koeman-Kwak, M. 1973. The pollination of *Pedicularis palustris* by nectar thieves (short-tongued bumblebees). Acta Botanica Neerlandica 22: 608–615.

Koricheva, J. 2002. Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. Ecology 83: 176–190.

Kort, E. 2014. Package 'rtiff'. http://www.libtiff.org.

Krupnick, G. A., Weis, A. E. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. Ecology 80: 135–149.

Krupnick, G. A., Weis, A. E., Campbell, D. R. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. Ecology 80: 125–134.

Kwak, M. M. 1978. Pollination, hybridization and ethological isolation of *Rhinanthus minor* and *R. serotinus* (Rhinanthoideae: Scrophulariaceae) by bumblebees (*Bombus* Latr.). Taxon 27: 145–158.

Lara, C., Ornelas, J. F. 2001. Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. Oecologia 128: 263–273.

Larson, K. C., Fowler, S. P., Walker, J. C. 2002. Lack of pollinators limits fruit set in the exotic *Lonicera japonica*. The American Midland Naturalist 148: 56–62.

Larson, K. C. 1998. The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. Oecologia 115: 161–166.

Lasso, E. M., Naranjo, L. 2003. Effect of pollinators and nectar robbers on nectar production and pollen deposition in *Hamelia patens* (Rubiaceae). Biotropica 35: 57–66.

Lê, S., Josse, J., Husson, F. 2008. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 25: 1–18.

Leadbeater, E., Chittka, L. 2008. Social transmission of nectar-robbing behaviour in bumble-bees. Proceedings of the Royal Society B: Biological Sciences 275: 1669–1674.

Lehtilä, K., Strauss, S. Y. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. Oecologia 111: 396–403.

Lehtilä, K., Strauss, S. Y. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. Ecology 80: 116–124.

Lloyd, D. G., Schoen, D. J. 1992. Self- and Cross-fertilization in plants. I. Functional dimensions. International Journal of Plant Sciences 153: 358–369.

Lundberg, H. 1980. Effects of weather on foraging-flights of bumblebees (Hymenoptera, Apidae) in a sublapine/alpine area. Holarctic Ecology 3: 104–110.

Luteyn, J. L. 1989. Speciation and diversity of Ericaceae in neotropical montane vegetation. In: Tropical forests–botanical dynamics, speciation and diversity (L. B. Holm-Nielsen, I. C. Nielsen and H. Balslev eds.), pp. 103–126. Academic Press Limited, London.

Macior, L. W. 1966. Foraging behavior of *Bombus* (Hymenoptera: Apidae) in relation to *Aquilegia* pollination. American Journal of Botany 53: 302–309.

Maddox, G. D., Cappuccino, N. 1986. Genetic determination of plant susceptibility to an herbivorous insect depends on environmental context. Evolution 40: 863–866.

Makino, T. T., Ohashi, K., Sakai, S. 2007. How do floral display size and the density of surrounding flowers influence the likelihood of bumble bee revisitation to a plant? Functional Ecology 21: 87–95.

Maloof, J. E. 2001. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. American Journal of Botany 88: 1960–1965.

Maloof, J. E., Inouye, D. W. 2000. Are nectar robbers cheaters or mutualists? Ecology 81: 2651–2661.

Martin, P. R., Bonier, F., Moore, I. T. 2009. First observation of sap well use and maintenance by the glossy flowerpiercer (*Diglossa lafresnayii*) (Thraupidae). Wilson Journal of Ornithology 121: 213–215.

Maruyama, P. K. Vizentin-Bugoni, J., Dalsgaard, B., Sazima, I., Sazima, M. 2015. Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. Oecologia 177: 1–11. DOI: 10.1007/s00442-015-3275-9.

Mayer, C., Dehon, C., Gauthier, A. L., Naveau, O., Rigo, C., Jacquemart, A. L. 2014. Nectar robbing improves male reproductive success of the endangered *Aconitum napellus* ssp. *lusitanicum*. Evolutionary Ecology 28: 669–685.

Mayfield, M. M., Waser, N. M., Price, M. V. 2001. Exploring the 'most effective pollinator principle' with complex flowers: bumblebees and *Ipomopsis aggregata*. Annals of Botany 88: 591–596.

McCall, A. C., Irwin, R. E. 2006. Florivory: the intersection of pollination and herbivory. Ecology Letters 9: 1351–1365.

McDade, L. A., Kinsman, S. 1980. The impact of floral parasitism in two neotropical hummingbird-pollinated plant species. Evolution 34: 944–958.

McDade, L. A., Weeks, J. A. 2004. Nectar in hummingbird–pollinated Neotropical plants II: interactions with flower visitors. Biotropica 36: 216–230.

Memmott, J., Craze, P. G., Waser, N. M., Price, M. V. 2007. Global warming and the disruption of plant-pollinator interactions. Ecology Letters 10: 710–717.

Milet-Pinheiro, P., Schlindwein, C. 2009. Pollination in *Jacaranda rugosa* (Bignoniaceae): euglossine pollinators, nectar robbers and low fruit set. Plant Biology 11: 131–141.

Mitchell, R. J., Karron, J. D., Holmquist, K. G., Bell, J. M. 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. Functional Ecology 18:116–124.

Miyake, T., Yahara, T. 1998. Why does the flower of *Lonicera japonica* open at dusk? Canadian Journal of Botany 76: 1806–1811.

Miyake, T., Yamaoka, R., Yahara, T. 1998. Floral scents of hawkmoth-pollinated flowers in Japan. Journal of Plant Research 111: 199–205.

Moeller, D. A. 2006. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self pollination. Ecology 87: 1510–1522.

Moller, W. 1932. Die Zungen der kostarizensischen Zuckervogel. Zeitschrift fur Mikroskopisch-Anatomische Forschung 4: 363–417.

Moran, P. J., Thompson, G. A. 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defence pathways. Plant Physiology 125: 1074–1085.

Morris, W. F. 1996. Mutualism denied? Nectar-robbing bumble bees do not reduce female or male success of bluebells. Ecology 77: 1451–1462.

Mothershead, K., Marquis, R. J. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. Ecology 81: 30–40.

Muchhala, N. 2006. Nectar bat stows huge tongue in its rib cage. Nature 444: 701–702.

Navarro, L. 1992. Biología de la reproducción de un endemismo del N. W. Ibérico: *Petrocoptis grandiflora* Rothm. (Caryophyllaceae). PhD Thesis, Universidad de Santiago de Compostela, Spain.

Navarro, L. 1999. Pollination ecology and effect of nectar removal in *Macleania bullata* (Ericaeae). Biotropica 31: 618–625.

Navarro, L. 2000. Pollination ecology of *Anthyllis vulneraria* subsp. *vulgaris* (Fabaceae): nectar robbers as pollinators. American Journal of Botany 87: 980–985.

Navarro, L. 2001. Reproductive biology and effect of nectar robbing on fruit production in *Macleania bullata* (Ericaceae). Plant Ecology 152: 59–65.

Navarro, L., Guitián, J. 2000. Variación en el robo de néctar y efecto en la fructificación en *Petrocoptis grandiflora* Rothm. (Caryophyllaceae). In: Ecología Latinoamericana (J. E. Péfaur, ed.). Mérida.

Navarro, L., Guitián, J., Guitián, P. 1993. Reproductive biology of *Petrocoptis grandiflora* Rothm. (Caryophyllaceae): a species endemic to northwest Iberian Peninsula. Flora 188: 253–261.

Navarro, L., Guitián, P., Ayensa, G. 2008. Pollination ecology of *Disterigma stereophyllum* (Ericaceae) in south-western Colombia. Plant Biology 10: 512–518.

Navarro, L., Medel, R. 2009. Relationship between floral tube length and nectar robbing in *Duranta erecta* L. (Verbenaceae). Biological Journal of the Linnean Society 96: 392–398.

Ne'eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S. G., Dafni, A. 2010. A framework for comparing pollinator performance: effectiveness and efficiency. Biological Reviews 85: 435–451.

Newman, D. A., Thomson, J. D. 2005. Effects of nectar robbing on nectar dynamics and bumble bee foraging strategies in *Linaria vulgaris* (Scrophulariaceae). Oikos 110: 309–320.

Nilsson, L. A. 1988. The evolution of flowers with deep corolla tubes. Nature 334:147–149.

Ohm, J. R., Miller, T. E. 2014. Balancing anti-herbivore benefits and anti-pollinator costs of defensive mutualists. Ecology 95: 2924–2935.

Olesen, J., Jordano, P. 2002. Geographic patterns in plant-pollinator mutualistic networks. Ecology 83: 2416–2424.

Ollerton, J., Winfree, R., Tarrant, S. 2011. How many flowering plants are pollinated by animals? Oikos 120: 321–326.

Ornelas J. F. 1994. Serrate tomia: an adaptation for nectar robbing in hummingbirds? The Auk 111: 703–110.

Ornelas, J. F., Ordano, M., de Nova, A. J., Quintero, M. E., Garland, T. Jr. 2007. Phylogenetic analysis of interspecific variation in nectar of hummingbird-visited plants. Journal of Evolutionary Biology 20: 1904–1917.

Pelayo, R. C., Rengifo, C., Soriano, P. J. 2011. Avian nectar robbers of *Passiflora mixta* (Passifloraceae): do they have a positive effect on the plant? Interciencia 36: 587–592.

Petanidou, T., Potts, S. G. 2006. Mutual use of resources in Mediterranean plant-pollinator communities: How specialized are pollination webs? In: Plant-pollinator interactions from specialization to generalization (N. Waser and J. Ollerton eds.), pp. 220–224. Chicago Press, Chicago.

Pickett, J. A., Wadhams, L. J., Woodcock, C. M., Hardie, J. 1992. The chemical ecology of aphids. Annual Review of Entomology 37: 67–90.

Pleasants, J. M. 1983. Nectar production patterns in *Ipomopsis aggregata*

(Polemoniaceae). American Journal of Botany 70: 1468–1475.

Pollard, D. G. 1972. Plant penetration by feeding aphids (Hemiptera, Aphidoidea): a review. Bulletin of Entomological Research 62: 631–714.

Potts, S. G, Biesmeijer, J. C, Kremen, C., Neumann, P., Schweiger, O., Kunin, W. E. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25: 345–353.

Poveda, K., Steffan-Dewenter, I., Scheu, S., Tscharntke, T. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. Oecologia 135: 601–605.

Power, A. G., Flecker, A. S. 2003. Virus specificity in disease systems: are species redundant. The importance of species: perspectives on expendability and triage (P. Kareiva and S. A. Levin eds.), pp. 330–346. Princeton University Press, Princeton.

Price, M. V., Waser, N. M., Irwin, R. E., Campbell, D. R., Brody, A. K. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. Ecology 86: 2106–2116.

Proctor, M., Yeo, P., Lack, A. 1996. The natural history of pollination. Harper Collins, London.

Prys-Jones, O. E., Corbet, S. A. 1991. Bumblebees, The Richmond Publishing Co. Ltd, Slough.

Quesada, M., Bollman, K., Stephenson, A. G. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. Ecology 76:437–443.

Quinn, G. P, Keough, M. J. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. In: Pollination Biology (L. Real ed.), pp. 305– 329. Academic Press, Orlando.

Raven, P. H. 1977. A suggestion concerning the Cretaceous rise to dominance of the angiosperms. Evolution 31: 451–452.

Reddy, T. B, Rangaiah, K., Reddi, E. U. B., Reddi, C. S. 1992. Consequences of nectar robbing in the pollination ecology of *Vitex negundo* (Verbenaceae). Current Science 62: 690–691.

Renner, S. 1983. The widespread occurrence of anther destruction by *Trigona* bees in Melastomataceae. Biotropica: 251–256.

Richardson, S. C. 2004a. Are nectar-robbers mutualists or antagonists? Oecologia 139: 246–254.

Richardson, S. C. 2004b. Benefits and costs of floral visitors to *Chilopsis linearis*: pollen deposition and stigma closure. Oikos 107: 363–375.

Rico-Guevara, A., Rubega, M. A. 2011. The hummingbird tongue is a fluid trap, not a capillary tube. Proceedings of the National Academy of Sciences 108: 9356–9360.

Robertson, A. W. 1992. The relationship between floral display size, pollen carryover and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). Biological Journal of the Linnean Society 46: 333–349.

Rodríguez-Rodríguez, M. C., Jordano, P., Valido, A. 2013. Quantity and quality components of effectiveness in insular pollinator assemblages. Oecologia 173: 179–190.

Rojas-Nossa, S. V. 2005. Ecología de la comunidad de pinchaflores (Aves: *Diglossa* y *Diglossopis*) en un bosque altoandino. Master thesis. Universidad Nacional de Colombia, Bogotá, Colombia.

Rojas-Nossa, S. V. 2007. Estrategias de extracción de néctar por pinchaflores (Aves: *Diglossa* y *Diglossopis*) y sus efectos sobre la polinización de plantas de los altos Andes. Ornitología Colombiana 5: 21–39.

Rojas-Nossa, S. V. 2013. Asociación entre el robo de néctar y las características florales en una comunidad montana de los Andes colombianos. Revista Ecosistemas 22: 107–112.

Roubik, D. W. 1982. The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. Ecology 63: 354–360.

Roubik, D. W., Holbrook, N. M., Parra, G. V. 1985. Roles of nectar robbers in reproduction of the tropical treelet *Quassia amara* (Simaroubaceae). Oecologia 66: 161–167.

Sahli, H. F., Conner, J. K. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanismum* (Brassicaceae). American Journal of Botany 94: 203–209.

Sampson, B. J., Danka, R. G., Stringer, S. J. 2004. Nectar robbery by bees *Xylocopa virginica* and *Apis mellifera* contributes to the pollination of rabbiteye blueberry. Journal of Economic Entomology 97: 735–740.

Sapir, N., Dudley, R. 2013. Implications of floral orientation for flight kinematics and metabolic expenditure of hover-feeding hummingbirds. Functional Ecology 27: 227–235.

Schmid-Hempel, R., Schmid-Hempel, P. 1998. Colony performance and immunocompetence of a social insect, *Bombus terrestris*, in poor and variable environments. Functional Ecology 12: 22–30.

Schondube, J. E., Martínez Del Rio, C. 2003. The Flowerpiercers' hook: an experimental test of an evolutionary trade-off. Proceedings of the Royal Society B: Biological Sciences 270: 195–198.

Schondube, J. E., Martínez Del Rio, C. 2004. Sugar and protein digestion in flowerpiercers and hummingbirds: a comparative test of adaptive convergence. Journal of Comparative Physiology B 174: 263–273.

Simpson, B. B., Neff, J. L. 1981. Floral rewards: alternatives to pollen and nectar. Annals of the Missouri Botanical Garden 68: 301–322.

Singh, V. K., Barman, C., Tandon, R. 2014. Nectar robbing positively influences the reproductive success of *Tecomella undulata* (Bignoniaceae). PloS one 9: e102607.

Sherry, D. F. 2008. Social learning: nectar robbing spreads socially in bumble bees. Current Biology 18: R608–R610.

Shipley, B. 2000. A new inferential test for path models based on directed acyclic graphs. Structural Equation Modeling: A Multidisciplinary Journal 7: 206–218.

Shipley, B. 2004. Cause and Correlation in Biology A User's Guide to Path Analysis, Structural Equations and Causal Inference. Cambridge University Press, Cambridge.

Skutch, A. F. 1954. Life histories of Central American birds: *Diglossa plumbea*. Pacific Coast Avifauna 31: 421–436.

Smith, C. M., Boyko, E. V. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata 122: 1–16.

Snow, A. A., Stanton, M. L. 1988. Aphids limit fecundity of a weedy annual (*Raphanus sativus*). American Journal of Botany 75: 589–593.

Soberón, J., Martínez del Rio, C. 1985. Cheating and taking advantage. In: The biology of mutualism: Ecology and evolution (D. Boucher ed.), pp. 192–213. Oxford University Press, New York.

Sprengel, C. K. 1793. The secret of nature in the form and fertilization of flowers discovered. Friedrich Vieweg Altere, Berlin.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. Annual Review of Ecology and Systematics 1: 307–326.

Stiles, F. G. 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. Ecology 56: 285–301.

Stiles, F. G. 1977. Coadapted competitors: flowering seasons of hummingbird food plants in a tropical forest. Science 198: 1117–1178.

Stiles, F. G. 1981. Geographical aspects of bird-flower coevolution, with particular reference to Central America. Annals of the Missouri Botanical Garden 68: 323–351.

Stiles, F. G. 1985. Seasonal patterns and coevolution in the hummingbird-flower community of a Costa Rican subtropical forest. Ornithological Monographs 757–787.

Stiles, F. G., Ayala A. V., Girón, M. 1992. Polinización de las flores de *Brachyotum* (Melastomataceae) por dos especies de *Diglossa* (Emberizidae). Caldasia 17: 47–54.

Stout, J. C., Allen, J. A., Goulson, D. 2000. Nectar robbing, forager efficiency and seed set: bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). Acta Oecologica 21: 277–283.

Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. Ecology 72: 543–558.

Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. Ecology 78: 1640–1645.

Strauss, S. Y., Agrawal, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14: 179–185.

Strauss, S. Y., Conner, J. K., Rush, S. L. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. American Naturalist 147: 1098–1107.

Strauss, S. Y., Irwin, R. E. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. Annual Review of Ecology, Evolution, and Systematics 35: 435–466.

Strauss, S. Y., Whittall, J. B. 2006. Non-pollinator agents of selection on floral traits. In: Ecology and evolution of flowers (L. D. Harder, S. C. H. Barrett eds.), pp. 120–138. Oxford University Press, Oxford.

Temeles, E., Pan, I. 2002. Effect of nectar robbery on phase duration, nectar volume, and pollination in a protandrous plant. International Journal of Plant Sciences 163: 803–808.

Temeles, E. J., Linhart, Y. B., Masonjones, M., Masonjones, H. D. 2002. The role of flower width in hummingbird bill length-flower length relationships. Biotropica 34: 68–80.

Teppner, H. 2011. Flowers of Boraginaceae (*Symphytum, Onosma, Cerinthe*) and *Andrena symphyti* (Hymenoptera-Andrenidae): morphology, pollen portioning, vibratory pollen collection, nectar robbing. Phyton 50: 145–180.

Thomson, J. D., Wilson, P. 2008. Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. International Journal of Plant Sciences 169: 23–38.

Traveset, A., Willson, M. F. Sabag, C. 1998. Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. Functional Ecology 12: 459–464.

Urcelay, C., Morales, C. L., Chalcoff, V. R. 2006. Relationship between corolla length and floral larceny in the South American hummingbird-pollinated *Campsidium valdivianum* (Bignoniaceae). Annales Botanici Fennici 43: 205–211.

Utelli, A. B., Roy, B. A. 2001. Causes and consequences of floral damage in *Aconitum lycoctonum* at high and low elevations in Switzerland. Oecologia 127: 266–273.

Vale, Á., Rojas, D., Álvarez, J. C., Navarro, L. 2011. Breeding system and factors limiting fruit production in the nectarless orchid *Broughtonia lindenii*. Plant Biology 13: 51–61.

Van Emden, H. F., Harrington, R. 2007. Aphids as crop pests. CABI, Wallingford.

Vázquez, D. P., Blüthgen, N., Cagnolo, L., Chacoff, N. P. 2009. Uniting pattern and process in plant–animal mutualistic networks: a review. Annals of Botany 103: 1445–1457.

Vázquez, D. P., Morris, W. F., Jordano, P. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. Ecology Letters 8: 1088–1094.

Venables, W. N., Ripley, B. D. 2002. Modern Applied Statistics with S. Springer, New York.

Villeumier, F. 1969. Systematics and evolution in *Diglossa* (Aves: Coerebidae). A.M.N.H. Novitates No. 2831.

Vogt, C. A. 2006. Secondary nectar robbing, a previously unsubstantiated foraging behavior of the Cinereous Conebill (*Conirostrum cinereum*). Ornitologia Neotropical 17: 613–617.

Wang, Q., Li, Y., Pu, X., Zhu, L., Tang, Z., Liu, Q. 2013. Pollinators and nectar robbers cause directional selection for large spur circle in *Impatiens oxyanthera* (Balsaminaceae). Plant systematics and evolution 299: 1263–1274.

Wang, Y. 2013. Dynamics of plant-pollinator-robber systems. Journal of Mathematical Biology 66: 1155–1177.

Wang, Y., Wu, H., Wang, S. 2015. Invasibility of nectar robbers in pollination-mutualisms. Applied Mathematics and Computation 250: 908–919.

Waser, N. M. 1979. Pollinator availability as a determinant of flowering time in ocotillo (*Fouquieria splendens*). Oecologia 39:107–121.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., Ollerton, J. 1996. Generalization in pollination systems, and why it matters. Ecology 77: 1043–1060.

Waser, N. M., Ollerton, J. 2006. Plant-pollinator interactions: from specialization to generalization. The University of Chicago Press, Chicago.

Waser, N. M., Price MV. 1982. A comparison of pollen and fluorescent dye carryover by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae). Ecology 63: 1168–1172.

Waser, N. M., Price MV. 1983. Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. In: Handbook of experimental pollination biology (C. Jones and R. Little eds.), pp. 341–359. Van Nostrand Reinhold Company Inc., New York.

Waser, N. M., Real, L. A. 1979. Effective mutualism between sequentially flowering plant species. Nature 281: 670–672.

Webb, C. J., Lloyd, D. G. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. New Zealand Journal of Botany 24: 163–178.

Wester, P., Claßen-Bockhoff, R. 2006. Hummingbird pollination in *Salvia haenkei* (Lamiaceae) lacking the typical lever mechanism. Plant Systematics and Evolution 257: 133–146.

Whitney, H., Federle, W., Glover, B. 2009. Grip and slip: mechanical interactions between insects and the epidermis of flowers and flower stalks. Communicative & Integrative Biology 2: 505–508.

Wikström, N., Savolainen, V., Chase, M. W. 2001. Evolution of the angiosperms: calibrating the family tree. Proceedings of the Royal Society B: Biological Sciences 268: 2211–2220.

Wilson, W. G., Morris, W. F., Bronstein, J. L. 2003. Coexistence of mutualists and exploiters on spatial landscapes. Ecological Monographs, 73: 397–413.

Wise, M. J., Cummins, J. J. 2006. Strategies of *Solanum carolinense* for regulating maternal investment in response to foliar and floral herbivory. Journal of Ecology 94: 629–636.

Zhang, C., Irwin, R. E., Wang, Y., He, Y. P., Yang, Y. P., Duan, Y. W. 2011. Selective seed abortion induced by nectar robbing in the selfing plant *Comastoma pulmonarium*. New Phytologist 192: 249–255.

Zhang, Y. W., Robert, G., Wang, Y., Guo, Y, H. 2007. Nectar robbing of a carpenter bee and its effects on the reproductive fitness of *Glechoma longituba* (Lamiaceae). Plant Ecology 193: 1–13.

Zhang, Y. W., Wang, Y., Guo, Y. 2007. Effects of nectar-robbing on plant reproduction and evolution. Frontiers of Biology in China 2: 443–449.

Zhang, Y. W., Yang, C. F., Zhao, J. M., Guo, Y. H. 2009a. Selective nectar robbing in a gynodioecious plant (*Glechoma longituba*) enhances female advantage. Journal of evolutionary biology 22: 527–535.

Zhang, Y. W., Yu, Q., Zhao, J. M., Guo, Y. H. 2009b. Differential effects of nectar robbing by the same bumble-bee species on three sympatric *Corydalis species* with varied mating systems. Annals of Botany 104: 33–39.

Zhang, Y. W., Zhao, J. M., Inouye, D.W. 2014. Nectar thieves influence reproductive fitness by altering behaviour of nectar robbers and legitimate pollinators in *Corydalis ambigua* (Fumariaceae). Journal of Ecology 102: 229–237.

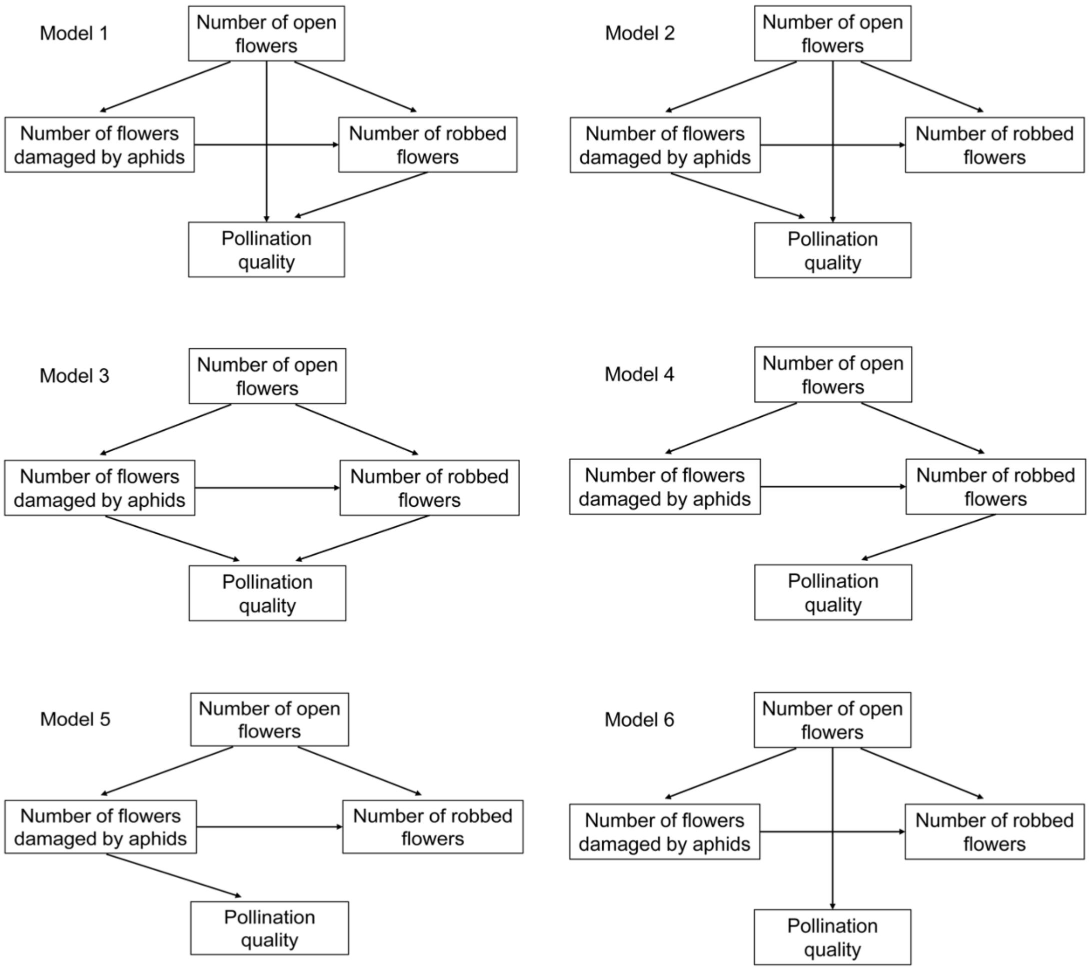
Zhang, Y. W., Zhao, J. M., Yang, C. F., Gituru, W. R. 2011. Behavioural differences between male and female carpenter bees in nectar robbing and its effect on reproductive success in *Glechoma longituba* (Lamiaceae). Plant Biology 13: 25–32.

Zhu, X. F., Wan, J. P., Li, Q. J. 2010. Nectar robbers pollinate flowers with sexual organs hidden within corollas in distylous *Primula secundiflora* (Primulaceae). Biology Letters 6: 785–787.

Zimmerman, M., Cook, S. 1985. Pollinator foraging, experimental nectar-robbing and plant fitness in *Impatiens capensis*. The American Midland Naturalist 113: 84–91.

Appendices

Appendix 1. Directed Acyclic Graphs for the six tested theoretical models that describe causal relationships among floral display, herbivory by aphids and herbivory by nectar robbers on the pollination quality received per plant.



Appendix 2. Results of the Confirmatory Path Analysis with the d-separation method for the 6 hypothetical cause-effect models depicted in Appendix 1. * Models with Δ CICc < 2 have significant support by the empirical data.

Model	Non-adjacent pairs	Causal parents	Conditional Independence statements	Statistical models	C	C-*p* val	CICc	Δ CICc
1	[(A,P)]	[{O,R}]	[(A,P) {O,R}]	P~O+R+A	0.047	0.977	20.069	2.224
2	[(R,P)]	[{O,A}]	[(R,P) {O,A}]	P~O+A+R	0.175	0.916	20.197	2.352
3	[(O,P)]	[{A,R}]	[(O,P) {A,R}]	P~A+R+O	0.198	0.906	20.221	2.376
4	[(O,P),(A,P)]	[{R}, R,O}]	[(O,P) {R}, (A,P) {R,O }]	P~R+O P~R+O+A	0.245	0.993	17.845	0.000*
5	[(O,P),(R,P)]	[{A}, A,O}]	[(O,P) {A}, (R,P) {A,O}]	P~A+O P~A+O+R	0.531	0.970	18.131	0.286*
6	[(A,P),(R,P)]	[{O},{O,A}]	[(A,P) {O}, (R,P) {O,A}]	P~O+A P~O+A+R	0.296	0.990	17.896	0.051*

Appendix 3. Contribution of morphological and nectar variables to the first three PCA factors that resume the variability in the data of the Mediterranean plant community. The values for continuous variables correspond to the coordinates (Coord.) and the contribution of each variable to the factors. The reported values for categorical variables correspond to the coordinates (Coord.) and the v-test which is a criterion with a Normal distribution. * Significant values for v-test ≥ |1.7| with $P \leq 0.05$ and $n = 27$.

Variables	Factor 1		Factor 2		Factor 3	
Continuous variables	**Coord.**	**Contr.**	**Coord.**	**Contr.**	**Coord.**	**Contr.**
Number of flowers / m^2	-0.39	4.74	0.74	29.40	0.40	1.20
Corolla length	0.89	24.06	0.27	0.81	-0.08	0.41
Diameter / length of the tube	0.39	4.66	-0.45	10.86	0.64	8.58
Tube length	0.86	22.69	0.35	6.54	-0.24	4.18
Tube diameter	0.91	25.41	-0.03	0.05	0.21	3.11
Nectar volume	0.40	4.94	0.44	10.64	-0.18	2.21
Nectar concentration	0.15	0.70	-0.15	1.18	0.77	1.30
kJ / m^2	-0.25	1.98	0.83	37.51	0.35	8.70
Orientation	0.60	10.83	0.02	0.03	0.07	0.31
Categorical variables	**Coord.**	**v.test**	**Coord.**	**v.test**	**Coord.**	**v.test**
Actinomorphous symmetry	0.01	0.02	-0.36	-0.79	-0.12	-0.3
Zygomorphous symmetry	-0.004	-0.02	0.13	0.79	0.04	0.3
Fleshy consistency	-0.76	-0.42	-0.52	-0.38	-1.03	-0.87
Soft consistency	0.03	0.42	0.02	0.38	0.04	0.87
Dialipetalous/sepalous flower	-0.37	-0.67	-0.48	-1.16	-0.22	-0.60
Gamopetalous/sepalous flower	0.16	0.67	0.20	1.16	0.09	0.60
Protective structures present	-0.88	-1.75*	0.35	0.93	0.20	0.61
Protective structures absent	0.44	1.75*	-0.18	-0.93	-0.10	-0.61
High aggregation	-0.99	-2.32*	-0.04	-0.13	0.04	0.13
Low aggregation	0.46	0.69	-0.33	-0.66	0.40	0.90
Medium aggregation	0.82	1.77*	0.24	0.70	-0.28	-0.91
Exotic	2.42	-0.95	-0.42	-0.31	-1.54	-1.30
Native	0.07	0.95	0.02	0.31	0.06	1.30
Herb	-0.13	-0.67	-0.06	-0.41	-0.003	-0.02
Parasite	0.11	0.06	-1.14	-0.83	0.53	0.45
Shrub	0.51	0.69	0.47	0.85	-0.09	-0.1

Appendix 4. Contribution of morphological and nectar variables to the first three PCA factors that resume the variability in the data of the Alpine plant community. The values for continuous variables correspond to the coordinates (Coord.) and the contribution of each variable to the factors (Contr.). The reported values for categorical variables correspond to the coordinates (Coord.) and the v-test which is a criterion with a Normal distribution. * Significant values for v-test ≥ |1.78| with $P \leq 0.05$ and $n = 12$.

Variables	**Factor 1**		**Factor 2**		**Factor 3**	
Continuous variables	**Coord.**	**Contr.**	**Coord.**	**Contr.**	**Coord.**	**Contr.**
Number of flowers / m^2	-0.36	4.48	0.73	22.55	-0.43	4.23
Corolla length	0.78	20.80	-0.30	3.30	-0.04	0.10
Diameter / length of the tube	0.69	16.03	0.28	3.23	-0.39	2.10
Tube length	0.77	19.86	-0.34	4.79	0.08	0.47
Tube diameter	0.88	26.53	0.12	0.61	-0.34	9.16
Nectar volume	0.19	1.17	0.25	2.60	0.79	9.26
Nectar concentration	0.53	9.41	0.30	3.70	0.37	0.53
kJ / m^2	0.08	0.19	0.81	27.83	-0.03	0.07
Orientation	0.21	1.53	0.86	31.40	0.23	4.08
Categorical variables	**Coord.**	**v.test**	**Coord.**	**v.test**	**Coord.**	**v.test**
Actinomorphous symmetry	1.05	1.72	-0.85	-1.54	-0.42	1.05
Zygomorphous symmetry	-0.75	-1.72	0.61	1.54	0.30	1.05
Dialipetalous/sepalous flower	-0.18	-0.20	-0.55	-0.68	1.04	1.77
Gamopetalous/sepalous flower	0.06	0.20	0.18	0.68	-0.35	1.77
Protective structures present	0.24	0.40	-0.65	-1.19	-0.18	0.45
Protective structures absent	-0.17	-0.40	0.47	1.19	0.13	0.45
High aggregation	-0.89	-0.99	-0.22	-0.28	-0.02	0.03
Low aggregation	0.38	0.22	-2.22	-1.44	0.23	0.20
Medium aggregation	0.28	0.78	0.36	1.10	-0.02	0.09

Appendix 5. Contribution of morphological and nectar variables to the first two PCA factors that resume the variability in the data of the Antillean plant community. The values for continuous variables correspond to the coordinates (Coord.) and the contribution of each variable to the factors. The reported values for categorical variables correspond to the coordinates (Coord.) and the v-test which is a criterion with a Normal distribution. * Significant values for v-test $\geq |1.8|$ with $P \leq 0.05$ and $n = 9$.

Variables	**Factor 1**		**Factor 2**	
Continuous variables	**Coord.**	**Contr.**	**Coord.**	**Contr.**
Number of flowers / m^2	0.60	9.59	0.01	0.004
Corolla length	-0.37	3.67	0.87	25.56
Diameter / length of the tube	0.75	14.71	0.59	11.67
Tube length	-0.43	4.96	0.82	23.03
Tube diameter	0.03	0.02	0.96	31.25
Nectar volume	0.69	12.69	0.38	4.89
Nectar concentration	-0.79	16.56	0.27	2.48
kJ / m^2	0.90	21.62	0.18	1.11
Orientation	0.78	16.19	-0.004	0.00
Categorical variables	**Coord.**	**v.test**	**Coord.**	**v.test**
Actinomorphous symmetry	-0.80	-2.18*	-0.33	-1.02
Zygomorphous symmetry	2.80	2.18*	1.15	1.02
Fleshy consistency	2.40	1.87*	-0.46	-0.41
Soft consistency	-0.69	-1.87*	0.13	0.41
Dialipetalous/sepalous flower	-1.85	-0.95	-0.49	-0.28
Gamopetalous/sepalous flower	0.23	0.95	0.06	0.28
Protective structures present	-1.16	-0.90	-1.13	-1.00
Protective structures absent	0.33	0.90	0.32	1.00
High aggregation	0.71	0.37	-1.49	-0.87
Low aggregation	-1.42	-1.11	2.43	2.14*
Medium aggregation	0.36	0.73	-0.56	-1.31
Latex absent	0.27	0.71	-0.14	-0.43
Latex present	-0.92	-0.71	0.49	0.43
Epiphyte	-1.85	-0.95	-0.48	-0.28
Herb	-1.09	-1.41	0.30	0.44
Shrub	1.84	1.89*	-0.80	-0.94
Tree	0.69	0.35	1.70	1.00

Appendix 6. Contribution of morphological and nectar variables to the first three PCA factors that resume the variability in the data of the Andean plant community. The values for continuous variables correspond to the coordinates (Coord.) and the contribution (Contr.) of each variable to the factor. The reported values for categorical variables correspond to the coordinates (Coord.) and the v-test which is a criterion with a Normal distribution. * Significant values for v-test ≥ |1.65| with $P \leq 0.05$ and $n = 40$.

Variables	Factor 1		Factor 2		Factor 3	
Continuous variables	**Coord.**	**Contr.**	**Coord.**	**Contr.**	**Coord.**	**Contr.**
Number of flowers / m^2	-0.32	3.42	0.86	36.76	-0.08	0.41
Corolla length	0.95	29.35	0.17	1.47	-0.003	0.001
Diameter / length of the tube	-0.18	1.06	-0.02	0.02	0.86	19.96
Tube length	0.96	29.65	0.19	1.81	-0.07	0.35
Tube diameter	0.47	7.25	0.13	0.85	0.71	34.10
Nectar volume	0.82	21.98	0.10	2.10	0.05	0.19
Nectar concentration	-0.17	0.98	-0.21	2.16	0.31	6.26
kJ / m^2	-0.10	0.35	0.88	38.80	-0.15	1.47
Orientation	0.43	5.97	-0.57	16.09	-0.33	7.25
Categorical variables	**Coord.**	**v.test**	**Coord.**	**v.test**	**Coord.**	**v.test**
Actinomorphous symmetry	0.07	0.49	0.05	0.40	-0.04	-0.44
Zygomorphous symmetry	-0.27	-0.49	-0.18	-0.40	0.17	0.44
Fleshy consistency	-0.60	-1.41	0.60	1.72*	0.36	1.21
Soft consistency	0.26	1.41	-0.26	-1.72*	-0.16	-1.21
Dialipetalous/sepalous flower	0.25	0.74	0.03	0.12	0.40	1.66*
Gamopetalous/sepalous flower	-0.17	-0.74	-0.02	-0.12	-0.26	-1.66*
Protective structures present	0.32	0.85	-0.20	-0.63	-0.14	-0.51
Protective structures absent	-0.17	-0.85	0.11	0.63	0.07	0.51
High aggregation	-1.14	-2.36*	0.79	2.00	-0.26	-0.76
Low aggregation	1.96	2.96*	-0.13	-0.23	-0.36	-0.77
Medium aggregation	-0.02	-0.07	-0.30	-1.60	0.20	1.24
Exotic	1.02	0.59	1.17	0.82	1.19	0.98
Native	-0.03	-0.59	-0.03	-0.82	-0.03	-0.98
Epiphyte	0.46	0.75	-0.41	-0.84	-0.54	-1.27
Herb	0.76	1.88*	-0.17	-0.51	0.29	1.02
Shrub	-0.64	-2.07*	0.33	1.32	-0.25	-1.16
Tree	-0.75	-0.62	-0.44	-0.44	2.26	2.65*
Latex present	0.23	0.23	0.17	0.21	1.62	2.35*
Latex absent	-0.02	-0.23	-0.01	-0.21	-0.13	-2.35*