Efecto de la hormiga tejedora, *Oecophylla smaragdina*, sobre la trayectoria evolutiva de los mutualismos planta-polinizador en el Sudeste Asiático

Tesis doctoral 2014

Francisco García Gonzálvez

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Memoria presentada por Francisco García Gonzálvez para optar a Grado de Doctor por la Universidad de Granada.

El Doctorando

Francisco García Gonzálvez

Granada, Febrero 2014

El doctorando Francisco García Gonzálvez y el director de la tesis Miguel Ángel Rodríguez-Gironés garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección del director de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

de Febrero de 2014, Almería

Director/es de la Tesis

Doctorando

Fdo.:

Fdo.:

A mis padres,

los grandes maestros de mi vida

A Miguel,

por su dedicación y comprensión

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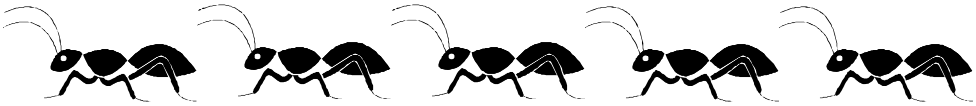


RESUMEN

Dos de los principales factores que afectan las estrategias de forrajeo de los animales son la distribución de los recursos y el riesgo de depredación. Aunque en un principio se asumió que el riesgo de depredación al que estaban sometidos los polinizadores era demasiado bajo para afectar su comportamiento, numerosos estudios realizados durante la última década muestran que los polinizadores presentan estrategias antipredatorias durante el forrajeo. La mayoría de las investigaciones sobre el efecto de los depredadores emboscados en flores sobre la evolución del comportamiento antipredatorio en polinizadores se han centrado en las arañas cangrejo, el depredador más común en prados de climas templados. Sin embargo, parece poco probable que las arañas cangrejo tengan un efecto sobre la evolución del mutualismo planta-polinizador. Para que los depredadores ejerzan presiones selectivas suficientemente potentes para inducir cambios en la interacción planta-polinizador, se requiere un sistema en el que los depredadores se presenten en abundancia y es por ello que esta tesis utiliza como modelo de estudio la hormiga tejedora *Oecophylla smaragdina*.

El objetivo de la presente tesis es estudiar el papel de la hormiga tejedora como depredador emboscado en flores en los bosques del Sudeste Asiático, profundizando en el efecto que tiene su presencia sobre las redes de interacción planta-polinizador, en la evolución de sistemas de polinización particulares así como en el desarrollo de estrategias antipredatorias en polinizadores.

En el capítulo 1 evaluamos el posible papel que desempeña la hormiga tejedora, *Oecophylla smaragdina*, sobre las interacciones planta-polinizador en el Sudeste Asiático. A través de su rango de distribución, la hormiga tejedora fue observada en

múltiples ocasiones emboscada en flores de numerosas especies de plantas e interaccionando directamente con el mutualismo planta-polinizador. Como consecuencia, y debido a su movilidad y abundancia en los hábitats donde se presenta, la hormiga tejedora tiene el potencial de moldear la trayectoria ecológica y evolutiva de las interacciones planta-polinizador.

En el capítulo 2 estudiamos empíricamente el efecto de la hormiga tejedora sobre la estructura de las redes de polinización. Encontramos que comunidades con hormiga presentaron un menor tamaño de red que comunidades sin hormiga. Además, redes construidas a partir de comunidades con hormiga mostraron una disminución en la asimetría de la red, en el coeficiente de agrupamiento y en el anidamiento de la red. Estos resultados evidencian la importancia de incluir el efecto de los depredadores en la estima de los parámetros de las redes de polinización.

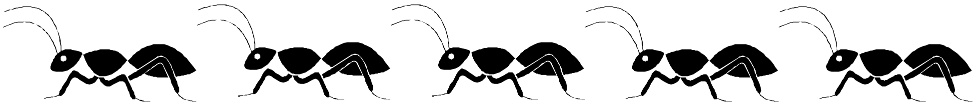
En el capítulo 3 estudiamos las consecuencias ecológicas y evolutivas que tiene la presencia de la hormiga tejedora sobre su planta hospedadora *Melastoma malbathricum*. Encontramos que la hormiga tejedora es atraída por las flores de *M. malabathricum* generando una partición del recurso en los polinizadores. Mientras que las abejas *Xylocopa*, polinizadores efectivos de *M. malabathicum* e invulnerables al ataque de las hormigas, visitaron fundamentalmente plantas con hormiga, la abeja solitaria *Nomia strigata*, polinizador poco efectivo de *M. malabathricum*, forrajeó mayoritariamente plantas sin hormiga. Como resultado, aquellas plantas con hormiga mostraron un mayor éxito reproductivo que plantas sin hormiga. Estos resultados sugieren que, dependiendo del contexto ecológico, los depredadores emboscados en flores pueden tener un efecto positivo o negativo en el éxito reproductivo de las plantas que los albergan.

En el capítulo 4 estudiamos cómo la variabilidad en la abundancia de los polinizadores puede afectar la trayectoria evolutiva de la interacción hormiga-planta-polinizador. Mientras que en 2010 encontramos que *Nomia strigata* concentraba sus esfuerzos de forrajeo en plantas sin nido de hormiga y *Xylocopa* visitaba plantas con hormiga, en 2012, cuando la abundancia de abejas fue menor, *Xylocopa* mostró una tasa de visitas similar en plantas con y sin hormiga. Consecuentemente en 2012 el éxito reproductivo fue similar en plantas con y sin hormiga. Estos resultados manifiestan una variabilidad en la magnitud y dirección de las presiones selectivas a través del tiempo en la interacción hormiga tejedora-planta-polinizador.

En el capítulo 5 estudiamos cómo las abejas solitarias usan la información procedente de la hormiga tejedora para detectar, evaluar y responder al riesgo de depredación. Encontramos que la abeja solitaria, *Nomia strigata*, examinó durante más tiempo flores con señales procedentes de la hormiga tejedora que flores con señales de la hormiga no depredadora, *Polyrhachis dives*, o flores sin señales. Además, mientras que en presencia de señales químicas de la hormiga tejedora *N. strigata* realizó vuelos alrededor de la flor, la abeja se cernió cuando la flor contuvo exclusivamente señales visuales. *N. strigata* rechazó visitar significativamente más flores con señales visuales que químicas procedentes de la hormiga tejedora. Estos resultados sugieren que las señales químicas proporcionan a las abejas solitarias una buena información sobre la naturaleza de la hormiga. Las señales visuales, además de proporcionar esta información, les informan con precisión de la procedencia de la señal.

Los resultados de la presente tesis demuestran que la hormiga tejedora, *Oecophylla smaragdina*, emplea comúnmente flores de diversas especies de plantas como plataforma de caza de polinizadores. Esta estrategia de caza de la hormiga tejedora tiene un efecto importante sobre el comportamiento antipredatorio de los polinizadores y éste, a su vez, puede tener implicaciones en la estructura de las redes de polinización. Además, la hormiga tejedora puede llegar a generar presiones selectivas suficientemente potentes para afectar al éxito reproductivo de las plantas que la albergan dirigiendo, de esta manera, la evolución de sistemas de polinización particulares. Análogamente al papel de la hormiga tejedora como depredador emboscado en flores de bosques del Sudeste Asiático, futuros estudios deberán evaluar si otras especies de hormigas depredadoras ejercen un efecto sobre la trayectoria evolutiva de los mutualismos planta-polinizador en bosques tropicales africanos y americanos.

INTRODUCCIÓN GENERAL



INTRODUCCION

La polinización es el proceso de transferencia del polen desde los estambres hasta el estigma o parte receptiva de las flores, donde germina, fecunda los óvulos y, en última instancia, se producen las semillas. Este proceso de transferencia de polen es realizado por el aire, el agua y los animales. Más concretamente, de las 250.000 especies estimadas de angiospermas, entre el 70 y el 90% son polinizadas por animales, y de éstas, el 67% por insectos (Buchmann and Nabhan 1996, Kearns, Inouye et al. 1998). De la presencia de polinizadores depende, por tanto, la producción de descendencia en plantas con flores visitadas por animales.

Por otro lado, el comportamiento de los polinizadores determina el flujo de genes entre plantas, lo que determina, a su vez, la depresión por endogamia a la que las plantas están sujetas (Schoen 1982, Linhart, Busby et al. 1987). La actividad de la mayoría de los polinizadores disminuye esta depresión ya que, por lo general, al aumentar la distancia de trasferencia de polen de una a otra planta se incrementa la viabilidad de la progenie (Richards 1986, Johannsson, Gates et al. 1998). Además, a mayor flujo de genes, mayor variabilidad genética en la población permitiendo una mayor facilidad para la adaptación al medio por selección natural (Morran, Parmenter et al. 2009). Por tanto, los polinizadores no sólo juegan un papel fundamental en el funcionamiento de la mayoría de ecosistemas terrestres, sino que además determinan también la trayectoria evolutiva de las plantas con polinización animal.

Desde hace más de un siglo los ecólogos han mostrado gran interés en el estudio de las interacciones planta-polinizador. Los primeros estudiosos acuñaron el término “síndrome de polinización” refiriéndose al conjunto de caracteres de las flores destinados a atraer a un tipo particular de polinizador excluyendo a otros visitantes florales que podrían consumir los recursos sin realizar una polinización efectiva (Faegri and Van der Pijl 1978). El concepto de síndrome de polinización implica, además, la aceptación de que existe especialización entre plantas y sus polinizadores (Waser, Chittka et al. 1996). No obstante, estudios más recientes demostraron que en la mayoría de casos, el ajuste entre las características florales y el polinizador no es tan específica, y que distintos grupos de polinizadores tienen la capacidad de polinizar una determinada flor (Johnson and Steiner 2000). Por ello, actualmente se considera que la polinización de la mayoría de plantas con flores se caracteriza por una moderada generalización más que por la especialización. Esta visión promueve una nueva aproximación en la ecología de la polinización donde las interacciones entre plantas y polinizadores se estudian en forma de complejas redes mutualistas.

La mayoría de los polinizadores son animales que forrajean en busca de polen y/o néctar(las excepciones incluyen, entre otras, animales buscando pareja – Schiestl, Ayasse et al. 1999 – o lugares de ovoposición – Thompson and Pellmyr 1992). Dado que el riesgo de depredación ejerce un gran efecto sobre las estrategias de forrajeo (Lima and Dill 1990), cabe esperar que afecte igualmente a los polinizadores. No obstante, aunque las estrategias de forrajeo de los polinizadores han sido objeto de numerosos estudios (Pyke 1978, Heinrich 1979, Pyke 1979, Chittka, Gumbert et al. 1997), el efecto del riesgo de depredación sobre las mismas ha recibido poca atención al asumirse tradicionalmente que la depredación era demasiado infrecuente para afectar el comportamiento de los polinizadores (Pyke 1979; Miller & Gass 1985). Sólo en las últimas décadas algunos estudios han demostrado que, pese a la baja tasa de encuentro entre depredadores y polinizadores, éstos últimos exhiben comportamientos antipredatorios durante las tareas de forrajeo (Dukas 2001, Dukas and Morse 2003, Heiling and Herberstein 2004, Dukas 2005). Por tanto, si partimos de la premisa de que el riesgo de depredación puede modificar el comportamiento de los polinizadores, los depredadores podrían imponer indirectamente presiones selectivas sobre los rasgos florales de las plantas.

En este sentido Wasserthal (1997) trabajó en la idea de que los depredadores están indirectamente relacionados con la evolución de corolas profundas. Él sugirió que cuando los depredadores son abundantes, los polinizadores con probóscide larga gozarán de un mayor éxito reproductivo que los polinizadores de probóscide corta, al ser capaces de extraer néctar sin aproximarse demasiado a la flor. Por tanto, la ventaja selectiva que ofrecen los polinizadores de probóscide larga frente a los depredadores favorecerá, a su vez, la evolución de flores con corolas largas.

La presencia de depredadores emboscados en flores podría, además, favorecer la partición de recursos cuando los polinizadores se enfrentan a un compromiso entre maximizar su tasa de ingesta y minimizar el riesgo de depredación. Si, por ejemplo, en una misma comunidad de polinizadores existen unos más susceptibles que otros al riesgo de depredación, un determinado rasgo floral asociado al depredador podría evolucionar si los polinizadores más susceptibles a los depredadores son menos eficaces para la planta que los que sufren menos el riesgo de depredación (Rodríguez-Gironés & Santamaría 2004,2005).

Estudios llevados a cabo mayormente en zonas templadas demuestran que predadores emboscados en flores, tales como arañas cangrejo, mantis e insectos hemípteros, pueden afectar el éxito reproductivo de la planta que los alberga (Dukas and Morse 2005) a pesar de que en la mayoría de los sistemas descritos la proporción de plantas que albergan depredadores es muy baja. Sin embargo, esta baja densidad no impide la evolución de comportamientos antipredatorios ya que (1) durante sus tareas de forrajeo muchos polinizadores visitan gran cantidad de flores para satisfacer sus necesidades, por lo que en algún momento encontraran algún depredador emboscado en una flor y (2) el costo de no detectar/evitar un depredador es muy alto. Estas dos condiciones, no obstante, no se cumplen para las plantas. Para que los depredadores ejerzan presiones selectivas sobre las plantas que los albergan tenemos que buscar un sistema en el que los depredadores emboscados en flores se presenten en abundancia. Por esta razón, la presente tesis propone como modelo de depredador la hormiga tejedora, *Oecophylla smaragdina*.

La hormiga tejedora tiene una amplia distribución, extendiéndose desde el NW de la India al NE de Australia, siendo muy abundante en los bosques tropicales de todo su rango (Azuma, Ogata et al. 2006). Se considera una especie muy agresiva y depredador clave de pequeños animales (Holldobler and Wilson 1978). Además, por su agresividad, es muy utilizada para el control biológico de plagas (Van Mele 2008). Aunque no se ha estudiado su papel como depredador emboscado en flores, se ha documentado que árboles de la especie *Nephelium lappaceum* con nidos de hormiga tejedora reciben menos visitas de sus polinizadores que los árboles sin nido (Tsuji, Hasyim et al. 2004). Aunque Tsuji et al. explican esta diferencia como una consecuencia del carácter territorial de la hormiga, Santamaría & Rodríguez-Gironés (comunicación personal) observaron hormigas tejedoras emboscadas en flores y capturando abejas (*Apis mellifera* y *Trigona spp.*) en plantas sin nido de hormiga. En este caso podemos descartar que se trate de un comportamiento territorial.

Además de su abundancia, la hormiga tejedora constituye un excelente modelo de estudio por las siguientes razones:

1. Se trata de un depredador que puede actuar emboscado en flores.
2. Es fácil de manipular. Se pueden excluir hormigas en determinadas plantas o puede trabajarse con ellas en el laboratorio.

3. Es móvil. Puede desplazarse en busca de las flores donde obtiene un mayor número de presas.
4. Adapta su estrategia al tipo de presa más abundante (Santamaría y Rodríguez-Gironés, datos no publicados). La hormiga tejedora caza en solitario abejas de pequeño tamaño (*Trigona spp.*) y en grupo abejas de gran tamaño (*Apis spp.*)

JUSTIFICACION Y OBJETIVOS

Mientras que hoy en día es incuestionable que los depredadores emboscados en flores influyen en el comportamiento de los polinizadores (Dukas 2001, Schmalhofer 2001, Dukas and Morse 2003, Dukas 2005) la idea de que arañas cangrejo u otros organismos que utilizan flores como plataforma de caza puedan afectar a la evolución de los rasgos es menos convincente. A diferencia de ecosistemas de climas más templados, en los trópicos las hormigas son extraordinariamente abundantes (Davidson, Cook et al. 2003) y establecen numerosas interacciones mutualistas y/o parasíticas con otros organismos, influyendo en la dinámica evolutiva del ecosistema (Yu 2001). En el trópico de Asia concretamente, una de las especies más frecuentes, la hormiga tejedora, establece una interacción mutualista con su planta hospedadora al consumir organismos herbívoros. Sin embargo, esta especie de hormiga es un depredador generalista que no solamente ataca a organismos herbívoros sino que además captura polinizadores emboscada en flores. Debido a la abundancia, ubicuidad y agresividad de esta hormiga en el SE Asiático es muy probable que su presencia en determinadas especies vegetales haya ejercido una presión selectiva lo suficientemente potente como para moldear la evolución de los rasgos de polinizadores y flores, mitigando de alguna manera los efectos negativos que la hormiga tiene sobre dicha interacción. Por tanto esta tesis pretende estudiar el efecto del riesgo de depredación sobre el comportamiento de los polinizadores, así como las consecuencias del comportamiento antipredatorio de los polinizadores en la estructura de las redes de interacción planta-polinizador y la evolución de los rasgos florales.

Posible papel de la hormiga tejedora, *Oecophylla smaragdina*, en la estructura de las interacciones planta-polinizador en el Sudeste Asiático (capítulo 1)

Aunque las arañas cangrejo son el principal sistema modelo para estudiar el efecto de los depredadores emboscados en flores sobre las interacciones planta-polinizador (Morse 2007), sus implicaciones sobre dichas interacciones son ampliamente discutidas debido a su baja densidad en prados de zonas templadas. Por el contrario, la hormiga tejedora, debido a su gran movilidad y ubicuidad en bosques tropicales del Sudeste Asiático, podría jugar un papel fundamental conformando las trayectorias ecológicas y evolutivas de las interacciones planta-polinizador. En este primer capítulo el principal objetivo es documentar el uso que la hormiga tejedora hace de los distintos tipos de flores como plataforma de caza. Para ello se realizaron observaciones en campo de la interacción hormiga tejedora con el mutualismo planta-polinizador, documentando aquellas especies vegetales en las que se cumplieron las siguientes condiciones: que estuviesen en floración, que fuesen patrulladas por *Oecophylla smaragdina* y que recibiesen la visita de polinizadores. Dichas observaciones se realizaron en diferentes localidades del SE Asiático, desde el sur de China hasta el norte de Australia pasando por Malasia, Singapur e Indonesia, cubriendo de esta manera gran parte del rango de distribución de la hormiga tejedora.

Predadores inducen cambios estructurales en redes mutualistas (capítulo 2)

Tradicionalmente las redes mutualistas han sido consideradas como entidades independientes dentro del ecosistema. El impacto que otras interacciones, tales como parasitismo o depredación, pueda tener sobre las propiedades topológicas de las redes ha sido desatendido por razones logísticas. Debido a que se ha sugerido que la estructura de las redes de polinización podría depender del riesgo de depredación al que los polinizadores están sometidos, en este capítulo examinamos empíricamente el papel de los depredadores, y más concretamente el papel que desempeña la hormiga tejedora, *Oecophylla smaragdina*, estructurando las redes de polinización. Para ello creamos artificialmente 8 comunidades vegetales compuestas por 6 especies de plantas procedentes de un vivero. La hormiga tejedora tuvo acceso a la mitad de las comunidades mientras que el acceso a la otra mitad estuvo limitado mediante el uso de tanglefoot. Las comunidades se dispusieron en el espacio de manera que tanto las comunidades con hormiga como las comunidades sin hormiga estuviesen expuestas a condiciones ambientales similares. Registramos las interacciones flor-polinizador observadas durante censos en estas comunidades artificiales. Además, se anotó el número de flores por comunidad y el número de hormigas que, en un momento dado, encontramos sobre las flores de la comunidad.

Las flores de *Melastoma malabathricum* atraen hormigas tejedoras que disuaden a los polinizadores menos efectivos (capítulo 3)

Muchas flores producen sustancias repelentes que evitan que las hormigas patrullen sobre las flores y ahuyenten a los polinizadores (Willmer and Stone 1997, Junker, Chung et al. 2007). Sin embargo, cuando el polinizador más efectivo para una determinada especie de planta no es vulnerable al ataque de las hormigas, la planta debería beneficiarse de la presencia de las hormigas que disuadan a los polinizadores menos eficaces. En este capítulo estudiamos el efecto de las hormigas sobre las interacciones planta-polinizador cuando el principal polinizador es menos vulnerable a la depredación que los polinizadores menos eficaces. Estudiamos también las consecuencias evolutivas de la interacción hormiga-polinizadores para la planta. Para ello observamos durante un tiempo determinado el número de visitas de los visitantes florales en plantas con y sin hormigas. Además, determinamos la efectividad de los polinizadores a través de la producción de frutos y semillas. La eficiencia de los polinizadores extrayendo polen de las anteras fue analizada mediante conteos de granos de polen remanente en las anteras tras la visita de un polinizador. El éxito reproductivo de plantas con y sin hormigas fue estudiado marcando un determinado número de flores por planta y cuantificando la producción de frutos y semillas. Además, comprobamos la presencia de sustancias atrayentes/repelentes en las flores de la planta hospedadora mediante la metodología descrita por Ghazoul (2001).

La variabilidad en la abundancia de las abejas puede afectar la trayectoria evolutiva de las interacciones tritróficas (capítulo 4)

Las interacciones planta-polinizador están sujetas a una heterogeneidad en las fuerzas selectivas cuando la composición de la comunidad de polinizadores varía a través del espacio y del tiempo. Los depredadores, así mismo, constituyen una fuerza significativa en la evolución de las interacciones entre plantas y polinizadores. En este capítulo evaluamos si las interacciones entre plantas, polinizadores y depredadores también exhiben un mosaico espaciotemporal de fuerzas selectivas. Para ello replicamos dos años más tarde, cuando la densidad de polinizadores fue considerablemente menor, parte del experimento descrito en el capítulo 3. Inicialmente determinamos la nueva tasa de visitas de los polinizadores a plantas con y sin hormigas así como la producción de frutos y semillas en ambas plantas. Posteriormente comparamos las diferencias temporales en la tasa de visitas de polinizadores a flores con y sin hormiga así como el éxito reproductivo de ambos tipos de planta.

Ver para creer: contenido informativo y respuesta comportamental a señales visuales y químicas (capítulo 5)

La evitación de los depredadores y el forrajeo a menudo suponen demandas conflictivas. Los animales pueden disminuir el riesgo de mortalidad localizando a los depredadores, pero este proceso implica una disminución en el tiempo de forrajeo y por tanto de la tasa de ingesta. El objetivo de este capítulo es estudiar cómo las presas deben usar la información para detectar, evaluar y responder al riesgo de depredación desde la perspectiva del forrajeo óptimo. Para ello desarrollamos un modelo matemático que sugiere que las abejas solitarias deberían incrementar el tiempo que invierten examinando flores en respuestas a señales procedentes de depredadores. Además, estudiamos el comportamiento de una abeja solitaria, *Nomia strigata*, en su hábitat natural en respuesta a señales químicas y visuales procedentes de la hormiga tejedora. Para ello ofrecimos a las abejas flores vírgenes control y con información visual u olfativa asociada a la presencia de hormigas tejedoras o de una hormiga que no ataca a las abejas, *Polyrhachis dives*. Grabamos la respuesta de la primera abeja que se acercó a cada flor y analizamos los vídeos mediante un software (Picture Motion Browser) que nos permitió analizar secuencias de vídeo fotograma a fotograma.

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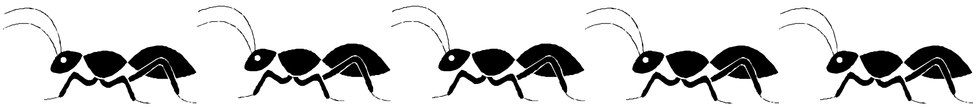
CAPÍTULO 1



Possible role of weaver ants, *Oecophylla smaragdina*, in shaping plant-pollinator interactions in SE Asia

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Abstract

1. Although theoretical models suggest that ambush predators could have a wide range of ecological and evolutionary effects on plant-pollinator interactions, these models require predators that are both abundant and mobile. Crab spiders, the main model system for studying the effects of ambush predators on plant-pollinator interactions, are neither of these.

2. The weaver ant *Oecophylla smaragdina* is a keystone predator in SE Asian forests. It uses the flowers of a wide range of native and introduced species as hunting platforms for incoming pollinators. Weaver ants affect the behaviour of flower visitors and the reproductive success of their host plants.

3. Due to their ubiquity and mobility, *O. smaragdina* ants satisfy the assumptions of the theoretical models. They have the potential to affect the structure of pollination networks.

4. *Synthesis*. *Oecophylla smaragdina* in SE Asia, and possibly other predatory ant species in African and American tropical forests, may play a key role in shaping the ecological and evolutionary trajectories of plant-pollinator interactions.

Introduction

One decade ago, the opening statement of a ground-breaking paper could claim that the effects of predation on pollinators had been largely ignored (Dukas 2001). Since then it has been suggested that ambush predators – mainly crab spiders, praying mantises and hemipteran bugs that wait on or under flowers for the arrival of unsuspecting pollinators – could affect the distribution patterns of plant species (Suttle 2003), the foraging decisions of pollinators (Jones 2010), the evolution of flower colours (Abbott 2010), the evolution of plant traits that attract ambush predators (Higginson *et al.* 2010), and even play a role in regulating the populations of solitary bees (Rodríguez-Gironés 2012).

Ambush predators, particularly crab spiders, have been shown to affect the behaviour of insect pollinators at the inflorescence, plant and patch levels (Dukas 2001; Schmalhofer 2001; Dukas & Morse 2003; Dukas 2005), and today it is undisputed that they can alter the behaviour of insect pollinators – although this effect is not universal (Brechbühl *et al.* 2010; Llandres & Rodriguez-Girones 2011). Crab spiders may even have top-down indirect effects on the fitness of the plants that harbour them (Suttle 2003; Gonçalves-Souza *et al.* 2008). It is less clear, however, whether they can affect the population dynamics or evolutionary trajectories of their host plants. There is a simple reason why a given abundance of ambush predators should have stronger evolutionary effects on pollinators than plants. Individuals of many species of insect pollinators, particularly bees, can visit thousands of flowers during their lifetime (Müller *et al.* 2006). As a result, the probability that a bee will have a fatal encounter with a predator is much greater than the proportion of flowers harbouring them; it will equal the proportion of flowers with predators multiplied by the number flowers visited in the pollinator’s lifetime. Hence, bees are likely to experience high selective pressures to avoid predator-harbouring flowers even when the predators occur at low densities (Rodríguez-Gironés 2012; Rodríguez-Gironés & Bosch 2012).

On the other hand, ambush predators only affect the fitness of the plants hosting them or those in their immediate neighbourhood (i.e. in the patch that pollinators are likely to avoid upon detection of the predator's presence). If ambush predators are scarce, few plant individuals will be affected by their presence and, even if they have strong effects on the fitness of these plants, they will represent a weak selection force. As a consequence, we should only expect predators to affect the population dynamics or evolutionary trajectory of their host plant if they are sufficiently abundant. Indeed, the suggestion that the anti-predator response of pollinators could have important effects for plant community composition or for the evolution of floral traits relies on the assumption that ambush predators are both abundant and mobile. As an example of the importance of abundance, population growth of the invasive plant *Leucanthemum vulgare* was only affected significantly by the presence of *Misumenops schlingeri* at occupancy levels above 12% (Suttle 2003), considered to be a high density for crab spiders (Schmalhofer 2001; Dukas & Morse 2003; Llandres *et al.* 2012). As for the importance of mobility, ambush predators could exclude pollinators from rich flowers if they were able to track resources in the environment (Jones 2010). But only mobile predators can track resources and the crab spiders that feed on pollinators are rather sedentary (Morse 2007). For instance, female *Misumenops schlingeri* remained on individual inflorescences for up to two weeks (Suttle 2003).

To summarise, although theory suggests that ambush predators could play an important role in shaping the patterns of plant-pollinator interactions, studies with crab spiders provide little support for this claim. Mantises and hemipteran bugs have been much less studied in this context, but they tend to occur at even lower densities on flowers, which makes them even less likely to affect plant community composition through the behavioural changes they induce in foraging pollinators. Our hypothesis, therefore, is that where crab spiders are the main predators ambushing at flowers, as is the case in temperate meadows, ambush predators can have large effects on pollinator behaviour, but they will have only modest effects on plant populations and plant-pollinator interactions at the community level. Things, however, can be otherwise in the tropics, due to the presence of highly mobile, ubiquitous ambush predators: aggressive predatory ants. Although ants can affect plant fitness in a diversity of ways, such as by damaging flowers (Galen & Geib 2007), predating on seeds or dispersing them (Brown & Human 1997), and removing herbivores (Llandres *et al.* 2010), in what follows we concentrate on their effects on plant-pollinator interactions.

Ants are abundant and diverse in most habitats. Many ant species are opportunistic foragers, consuming flower nectar when available (Herrera *et al.* 1984). In particular, certain species are able to displace, through interference or exploitation competition, other flower visitors (Lach 2007; Lach 2008). Ants can have direct effects on plant pollination success, damaging flowers during nectar consumption (Galen & Geib 2007), and indirect effects, mediated by changes in pollinator behaviour. These indirect effects can be negative (Ness 2006) or positive (Altshuler 1999) for plant reproduction. As a result, ants have imposed strong evolutionary pressures on plant traits. Indeed, it is well known that many flowers produce ant repellents at the time of anthesis (Willmer & Stone 1997; Raine *et al.* 2002). These ant repellents have generally been assumed to minimise exploitation (Ghazoul 2001; Junker & Bluthgen 2008) and interference (Willmer & Stone 1997; Raine *et al.* 2002; Junker *et al.* 2007) competition between ants and pollinators.

Exploitation and interference competition need not be the only mechanisms through which ants affect plant-pollinator interactions. Although ants are not normally considered flower-dwelling ambush predators, we argue here that this vision may need to be revised – at least in the tropics. *Oecophylla smaragdina* (Fabricius) (weaver ants; possibly several cryptic species) are ubiquitous in any habitat with trees from Sri Lanka and India, through S China, SE Asia, and Melanesia to N Australia (Crozier *et al.* 2010). They are numerically co-dominant in natural ecosystems ranging from Australian tropical savannah (Arnan *et al.* 2011) to lowland rainforest in New Guinea (Klimes *et al.* 2011) and Borneo (Davidson *et al.* 2007), and they are abundant enough to be effective in controlling pests in a wide range of tree crops (Crozier *et al.* 2010). Colonies defend huge, three-dimensional territories, and the major workers (the only caste outside the nests) are aggressive generalist predators that can affect plant-pollinator interactions. For example, *O. smaragdina* workers repel pollinators from *Nephelium lappaceum* (Tsuji *et al.* 2004) and pollen thieves from *Melastoma malabathricum* (Gonzálvez *et al.* 2013), while they are active hunters of fig wasps (Ranganathan & Borges 2009). Although the negative effect of ants on pollinator visit rate has previously been attributed to the territorial behaviour of the ants (Tsuji *et al.* 2004; Gonzálvez *et al.* 2013), *O. smaragdina* ants are also known to raid bee nests (Seeley 1983), making it likely that weaver ants would use flowers as hunting platforms.

To evaluate the possibility that *O. smaragdina* plays an important role in structuring plant-pollinator interactions in SE Asia and tropical Australia, we checked for the presence of weaver ants on a wide range of flowers within their habitat, and observed their interactions with flower-visiting insects. Rather than focusing on specific taxa or a particular location, we made wide-ranging observations in different localities over the complete geographic range of the ant species, from southern China (April-August 2011; Northern limit) and Sri Lanka (July 2006; W limit) through SE Asia (February-July 2010) and into NE Australia (April 2008 and May 2009; SE limit). We looked for plant individuals satisfying the following conditions: they were in bloom, they were patrolled by *O. smaragdina*, and they received pollinators. Plant individuals without *O. smaragdina* were not included in the sample because it is impossible to decide whether weaver ants would use those flowers as hunting platforms if given the chance, while plants that received no pollinators in a 15-minute observation period were excluded because the absence of ants at flowers could simply be explained in terms of economic profitability. Because our aim was to assess the use of flowers as hunting platforms by *O. smaragdina* ants, we did not census the local abundance of plants and ants, or the proportion of plants and flowers harbouring ants. Individual plants were observed for 15 minutes and we typically observed 3-5 individuals per species, depending on availability – although for some species, when plants of small size grew in clumps, the number of individuals observed was much higher. Due to the relatively low sampling effort per plant species, it is important to note that we are only reporting true positives, not true negatives – i.e., further work might show that *O. smaragdina* ants hunt pollinators at flowers where we failed to observe them. The results of our observations are summarised in Table 1.

Except in Singapore, where most of our observations were performed in suburban areas, *O. smaragdina* ants were very abundant; far more abundant than all other ambush predators combined. Although we did not quantify the proportion of flowers patrolled by weaver ants systematically throughout our survey, several observations suggest that these are generally high. In rural Singapore (late March, 2010), 27 ± 5% (mean ± s.e.m.) of *M. malabathricum* flowers were patrolled by *O. smaragdina* ants (Gonzálvez *et al.*

2013) and a *Xanthostemon chrysanthus* tree harbouring an *O. smaragdina* colony had over 60% of inflorescences patrolled by ants, with an average of 3.7 ants per occupied inflorescence. Moreover, while weaver ants patrolling *Turnera ulmifolia* at a suburban garden in Sri Lanka were present at only 1% of the flowers, they captured *Trigona* bees at 28% of the flowers they occupied.

Overall, we observed *O. smaragdina* ants at plants of 48 species in 32 families that were in bloom and attracting pollinators at the time of the observations. In 31 of the 48 plant species, we observed ants patrolling flowers. We observed ants attacking flower visitors at all these 31 species (successfully capturing bees and other visitors at 15 of them; Fig. 1) and drinking nectar at only 3 species: *Heliconia psittacorum* L.f., *Costus woodsonii* and *C. speciosus* (Koen. ex Retz) Sm. In six of the plant species where ants did not patrol flowers, ant repellents may have precluded them from doing so: in these species, ants were attacking approaching bees from branches, and in three of them we observed successful captures (*Cinnamomum iners*, *Syzygium cumini* and *Mallotus barbatus*). It is also worth noting that in seven of the species where we observed successful captures from flowers (*Ficus semicordata*, *Turnera ulmifolia*, *Calliandra emarginata*, *Mallotus barbatus*, *Asystasia gangetica*, *Bidens alba* and *Musa acuminata*), and three of the species where we observed unsuccessful attacks (*Catharanthus roseus*, *Clerodendrum* sp. and *Costus woodsonii*), the observations were made in the secondary, hunting, territory of the ants – making it highly unlikely that the ants were simply defending their territory. Cooperative hunting tactics, with nearby individuals rapidly coming to the aid of the ant that first attacks the bee, allow *O. smaragdina* to capture pollinators considerably larger than themselves (Wojtusiak *et al.* 1995). We observed *O. smaragdina* capturing *Apis cerana* from eight plant species, *A. mellifera* from *Bidens alba*, and *Nomia strigata* from *Melastoma malabathricum* (Table 1).

Table 1. *Plants in bloom where we observed Oecophylla smaragdina ants. The table indicates whether ants patrolled flowers and their reaction towards flower visitors. In the ant response column, cells are left empty when we observed no ants at flowers, while NBV indicates that bees did not visit ant-harbouring flowers (although they visited nearby flowers of the same plant) and NR indicates that ants did not respond to the arrival of flower visitors. Species are sorted in alphabetical order within families.*

Family	**Plant species**	**Locality**	**Ants at flowers**	**Flower visitors**	**Ant response**
Acanthaceae	*Asystasia gangetica* (L.) T. Anderson	Bin[a]	Yes	*Apis cerana*	Captures
Acanthaceae	*Thunbergia grandiflora* Roxb.	XTBG[b]	Yes	*Xylocopa* sp.	
Anacardiaceae	Unidentified species	XTBG[b]	No	*Trigona* sp	Attacks (from branches)
Apocynaceae	*Catharanthus roseus* (L.) G. Don	Bin[a]	Yes	*Trigona* sp.	Attacks
Arecaceae	*Adonidia merrillii* (Becc.) Becc.	WCP[c]	Yes	*A. cerana*	Captures
Arecaceae	*Archontophoenix alexandrae* (F. Muell.) H. Wendl & Drude	Kuch[d]	Yes	*Trigona* sp.; *A. dorsata*	Captures (*Trigona*)
Asteraceae	*Bidens alba* (L.) DC.	AB[e]	Yes	*A. mellifera*	Captures
Asteraceae	*Sphagneticola trilobata* (L.) Pruski	Kuranda[f]	No	*A. mellifera*; butterflies	
Bignoniaceae	*Tecoma stans* (L.) Juss.ex Kunth	YK[g]	No	*Trigona* sp.	

Costaceae	*Costus woodsonii* Maas	KRP[h]	Yes	Flies	Attacks
Cucurbitaceae	*Momordica charantia* L.	Cairns[i]	No	*Xylocopa* sp.; *Trigona* sp.; *A. mellifera*	
Dilleniaceae	*Dillenia suffruticosa* (Griff. ex Hook. f. & Thomson) Martelli	SBW[j]	Yes	*Xylocopa* sp., *A. cerana,* small flies	Attacks
Euphorbiaceae	*Mallotus barbatus* Müll. Arg.	XTBG[b]	No	*Trigona* sp; *A. cerana*	Captures (from branches)
Fabaceae	*Andira inermis* (W. Wright) Kunth ex DC.	Kuch[d]	No	*Trigona* sp.; *A. dorsata*	
Fabaceae	*Bauhinia blakeana* Dunn.	Kuch[d]	Yes	*Trigona* sp.	Captures
Fabaceae	*Bauhinia variegata* L.	XTBG[b]	Yes	*Xylocopa* sp.	Attacks
Fabaceae	*Caesalpinia pulcherrima* (L.) Sw.	KRP[h]	Yes	*Trigona* sp.	NR
Fabaceae	*Callerya atropurpurea* (Wall.) Schot	McRR[k]	Yes	*Xylocopa latipes*; hornets, lycaenids	Attacks
Fabaceae	*Callerya* sp.	XTBG[b]	No	Halictid bees	
Fabaceae	*Calliandra emarginata* (Humb.& Bonpl. ex Willd.) Benth.	NUS[m]	Yes	*A. cerana*	Captures

Fabaceae	*Cassia auriculata* L.	XTBG[b]	Yes	*Xylocopa* sp.	Attacks
Fabaceae	*Cassia fistula* L.	WCP[c], XTBG[b]	Yes	*A. cerana*; *Xylocopa* sp.	Attacks
Fabaceae	*Erythrina crista-galli* L.	WCP[c]	Yes	*A. cerana*	Captures
Fagaceae	*Castanopsis indica* (Roxb.) Miq.	XTBG[b]	No	Flies, small bees	Attacks (from branches)
Gentianaceae	*Fagraea fragrans* Roxb.	WCP[c]	No	*A. cerana*	NBV
Goodeniaceae	*Scaevola taccada* (Gaertn.) Roxb.	Bin[a]	Yes	*Trigona* sp.; *Xylocopa* sp.; Halictidae; flies	Captures (*Trigona* and halictidae)
Lamiaceae	*Callicarpa glabrifolia* S. Atkins	Kuch[d]	Yes	*Trigona* sp.	Attacks
Lamiaceae	*Clerodendrum* sp.	Kuch[d]	Yes	*Trigona* sp.	Attacks
Lamiaceae	*Gmelina asiatica* L.	XTBG[b]	Yes	*Xylocopa* sp.	NBV
Lamiaceae	*Orthosiphon aristatus* (Bl.) Miq.	JCU[l]	No	*Trigona* sp.	
Lauraceae	*Cinnamomum iners* Reinw.	Kuch[d]	No	*Trigona* sp.	Captures (from branches)
Lecythidaceae	*Couroupita guianensis* Aubl.	WCP[c]	No	*A. cerana*	Attacks from branches

Lythraceae	*Lagerstroemia speciosa* (L.) Pers.	WCP[c]	Yes	*A. cerana*; *Xylocopa* sp.	NR
Melastomataceae	*Melastoma malabathricum* L.	McRR[k]	Yes	*Nomia strigata*; *Xylocopa* sp.; *Amegilla* sp.	Attacks and captures (*N. strigata*)
Moraceae	*Ficus semicordata* Buch. Ham. ex Sm.	XTBG[b]	Yes	Fig-wasps	Captures
Musaceae	*Musa acuminata* Colla	XTBG[b]	Yes	*A. cerana*	Captures
Myrtaceae	*Syzygium campanulatum* Korth.	KRP[h]	No	Small bees, beetles, flies and butterflies	
Myrtaceae	*Syzygium cumini* (L.) Skeels	Kuch[d]	No	*Trigona* sp.	Captures (from branches)
Myrtaceae	*Xanthostemon chrysanthus* (F. Muell.) Benth.	NUS[m]	Yes	*A. cerana*	Captures
Nyctaginaceae	*Bougainvillea* sp.	Kuch[d]	No	*Trigona* sp.	
Passifloraceae	*Turnera ulmifolia* L.	Bin[a], Kegalle[n]	Yes	*Trigona* sp.; *A. cerana*	Captures (*Trigona*, *A. cerana*)
Piperaceae	*Piper umbellatum* L.	XTBG[b]	No	Flies, *Apis cerana*, *Apis florea*	
Pittosporaceae	*Pittosporum tobira* (Thunb.) W.T. Aiton	XTBG[b]	No	*Apis* sp.	
Rubiaceae	*Ixora chinensis*	XTBG[b]	Yes	Butterflies; Syrphid	

	Lam.			flies	
Rubiaceae	*Ixora* sp.	Kuch[d]	Yes	*Trigona* sp.	NR
Rubiaceae	*Morinda angustifolia* (Roxb.) Hook.	XTBG[b]	Yes	Flies	Attacks
Solanaceae	*Solanum trilobatum* L.	XTBG[b]	Yes	*Xylocopa* sp.	Attacks
Vitaceae	*Cissus hastata* Miq.	Kuch[d]	Yes	*Trigona* sp.	Attacks

a Bintan, Indonesia

b Xishuangbanna Tropical Botanical Garden, Yunnan Province, China

c West Coast Park, Singapore

d Kuching, Borneo, Malaysia

e Airlie Beach, Queensland, Australia

f Kuranda, Queensland, Australia

g Yorkeys knob, Queensland, Australia

h Kent Ridge Park, Singapore

i Cairns, Queensland, Australia

j Sungei Buloh Wetland Reserve, Singapore

k MacRitchie Reservoir, Singapore

l James Cook University Campus, Queensland, Australia.

m National University of Singapore campus, Singapore

n Kegalle, Sri Lanka

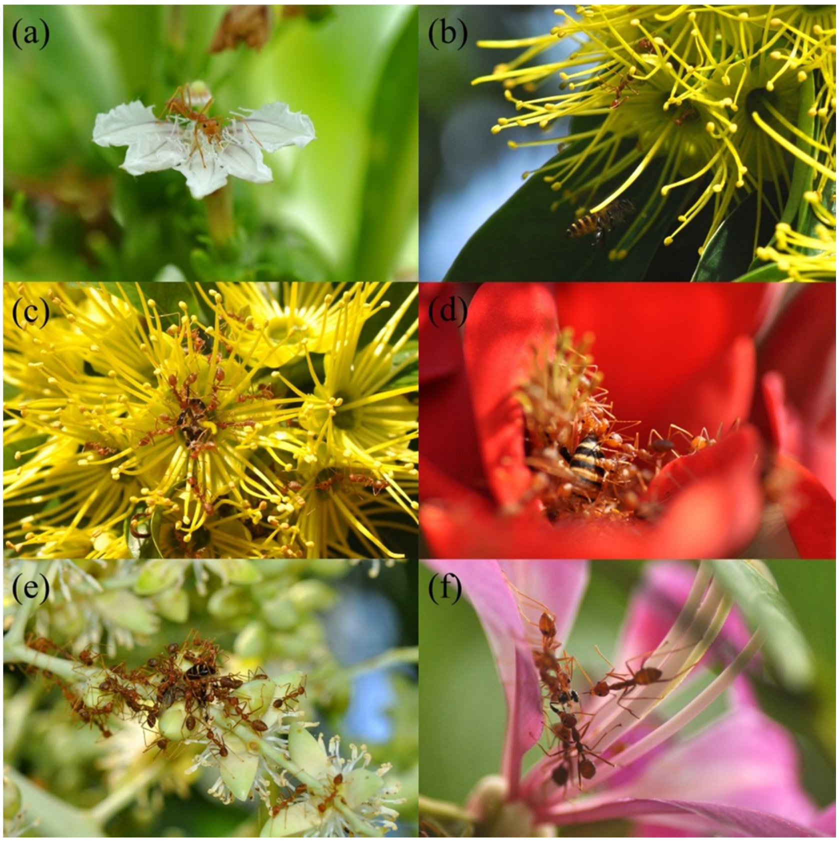

Fig 1. *(a) Weaver ant waiting for floral visitors on a Scaevola taccada flower. (b)* Weaver ants ambushing Apis cerana on *Xanthostemon chrysanthus flowers. (c-e) Successful captures of A. cerana on (c) Xanthostemon chrysanthus, (d) Erythrina crista-galli and (e) Archontophoenix alexandrae flowers. (f) Trigona bee captured by weaver ants on Bauhinia blakeana flowers.*

It is clear from Table 1 that *O. smaragdina* ants make widespread use of flowers as hunting platforms. Because they are ubiquitous and mobile (Crozier *et al*. 2010), they have the potential to affect the relationships between plants and their pollinators at both ecological and evolutionary time scales, as predicted by recent models (Suttle 2003; Abbott 2010; Higginson *et al.* 2010; Jones 2010; Rodríguez-Gironés 2012). Although the system has received little attention, published data and the observations conducted for this study suggest that pollinators avoid *O. smaragdina* ants, showing behavioural responses similar to those used to avoid other ambush predators (Tsuji *et al.* 2004; Gonzálvez *et al.* 2013).

We now turn to the effect of weaver ants on their host plants. Depending on the costs and benefits that flower-patrolling ants impose on the plants, flowers patrolled by weaver ants could have higher or lower reproductive success than ant-free flowers. Pollinator effectiveness depends on the number of pollen grains removed and/or deposited per flower visit, which together with pollen quality determine per-visit pollinator effectiveness, and the rate at which pollinators visit flowers. Ants can affect both terms of the equation. Consider first per-visit effectiveness. Interference competition between bee species can increase the rate of between-plant movements and therefore enhance fruit and seed set (Greenleaf & Kremen 2006; Carvalheiro *et al.* 2011). Likewise, it has been suggested that aggressive ants at flowers may reduce the number of flowers that bees visit per plant, favouring outcrossing and increasing seed set per bee visit (Altshuler 1999). In this way, *O. smaragdina* could affect the per-visit effectiveness of pollinators. Ants can also increase or decrease pollinator visit rates. Thus, *O. smaragdina* ants reduce the pollination success of *Nephelium lappaceum* because pollinators avoid plants with ant nests (Tsuji *et al.* 2004). In the case of *M. malabathricum*, however, the pollinators actually prefer plants with ant nests, which have higher fruit set and seed set than plants without ant nests (Gonzálvez *et al.* 2013). This preference is mediated by the effect of weaver ants on pollen thieves: although the flowers of *M. malabathricum* attract a wide array of visiting bees, they are pollinated almost exclusively by large carpenter bees, *Xylocopa* spp. (Gross 1993). *Oecophylla smaragdina* ants deter small bees, which remove pollen but are poor at transferring and depositing it from and into other flowers. Carpenter bees, which are too big to be affected by ant attacks, experience reduced interspecific exploitation competition at plants with ant nests and, as a result, concentrate their foraging effort on these plants. Interestingly, *M. malabathricum* flowers attract *O. smaragdina* ants during anthesis with a so-far unidentified cue (Gonzálvez *et al*. 2013).

The effect of *O. smaragdina* ants on the pollination of *M. malabathricum* flowers has wider implications. It is often assumed that flowers produce ant-repellent substances to prevent ants from consuming their nectar (Junker & Bluthgen 2008) or from scaring their pollinators (Willmer & Stone 1997). In the latter scenario, production of ant repellents would only make sense when pollinators are at risk from ant attacks. We can therefore predict that the evolution of ant repellents is more likely when flowers are pollinated mainly by susceptible, small insects (such as flies, butterflies or small to medium-sized bees) than when they are pollinated by large animals (including birds and bats, but also large bees), safe from ant attacks.

As well as affecting the ecological and evolutionary trajectory of specific plant species, *O. smaragdina* ants might affect the structure of entire communities. Plant-pollinator networks are the result of ecological processes operating at different temporal and spatial scales. Trait complementarity and exploitation barriers play a key role during network build-up (Santamaría & Rodríguez-Gironés 2007). Under trait complementarity, the similarity between the reward that the plant has to offer and the resource that the pollinator seeks determines whether species pairs interact. Barriers, on the other hand, are plant phenotypic traits that prevent certain pollinator species from accessing the reward. In a sense, *O. smaragdina* ants behave as indirect exploitation barriers: only pollinators with a low susceptibility to predation (e.g. those with large body sizes) will exploit flowers commonly associated with predatory ants. The balance between exploitation and complementarity among the processes that operate during network build-up affects the topology of the ensuing network. In particular, stronger barriers lead to more nested communities (Santamaría & Rodríguez-Gironés 2007). We can therefore expect that the nestedness of plant-pollinator networks will increase when *O. smaragdina* ants are present. The presence of ants could also increase the modularity and decrease the connectivity of the community, because susceptible pollinators will specialise on ant-free flowers to reduce predation risk and non-susceptible pollinators will concentrate on ant-harbouring flowers to reduce intra-specific competition for resources (Gonzálvez *et al*. 2013). In turn, these topological properties (nestedness, connectivity and modularity) are known to affect the ecological properties of the network, such as its robustness to external perturbations (Bascompte 2009).

We predict that other ant species will ambush pollinators at flowers in tropical Africa and the Neotropics, where other arboreal ants occupy the ecological niche filled by *O. smaragdina* in SE Asia. In particular, a very similar species in the same genus, *O. longinoda*, occupies the same habitats and ecological niche in a broad band across equatorial Africa (Wojtusiak *et al.* 1995). In many plant species, flowers are frequently visited by pollinators, and it may be faster and easier to capture insects by ambushing at flowers than searching through the vegetation. We should therefore expect predatory ants to use many flowers as hunting platforms. Indeed, the production of ant repellents by flowers of African (Willmer & Stone 1997) and American (Raine *et al.* 2002) plant species suggests that ants interfere with the pollination process in these continents. Just as in SE Asia, we should expect flowers to produce ant repellents if they rely mainly on small bees for their pollination, but not if their most effective pollinators are not susceptible to predation by weaver ants. As a result, at the landscape level there will be a mosaic of flowers with and without ant repellents, with and without predatory ants, and this heterogeneity should affect the foraging behaviour of pollinators, the structure of pollination networks and the reproductive success of plants.

The structure and stability of ecological communities cannot be understood from knowledge of a single type of interactions, such as predator-prey or plant-pollinator interactions, but from the complex interplay of them all (Melián *et al.* 2009). In particular, it has been suggested that the stability of communities is strongly dependent on the proportion of mutualistic interactions (Mougi & Kondoh 2012). *Oecophylla smaragdina* is a keystone predator species, very abundant and broadly distributed throughout tropical SE Asia and NE Australia (Crozier *et al.* 2010). As we have documented, these ants often use flowers to ambush foraging bees. The presence of ants at flowers, by altering the foraging behaviour of bees, can affect the relationship of a plant with its flower visitors, modulating the evolution of flower traits (Gonzálvez *et al.* 2013). Due to the abundance of ants, however, we hypothesise that the topology of plant-pollinator networks will differ between communities where ants are present and absent, and that flowers will follow different evolutionary pathways depending on whether their main pollinators are susceptible or not to the attack of *O. smaragdina* ants. To check these predictions, it will be important not only to carry out more studies of ant impacts on plant-pollinator pairs, but also to conduct community-level studies, using the comparative method to trace the evolution of ant repellents across plant lineages, and comparing the topology of pollinator networks in localities with and without *O. smaragdina* ants. Furthermore, to assess the generality of our observations, future work must evaluate whether African and American ant species use flowers as hunting platforms in the way that *O. smaragdina* ants do.

Acknowledgements

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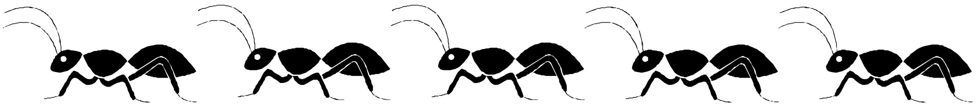
CAPÍTULO 2



Predators induce structural changes in mutualistic networks

Francisco G. Gonzálvez [1]*, Chen J.[2] & Miguel A. Rodríguez-Gironés [1]

Abstract

Mutualistic networks have been traditionally studied as independent entities within ecosystems. The impact that other interactions, such as parasitism or predation, may have on network topological properties has been neglected for logistic reasons. Because it has been suggested that predators could affect the structure of pollination network, here we present the first experimental exploration of the role of predators in shaping pollination networks. We built eight artificial plant communities, permitting predatory weaver ants, *Oecophylla smaragdina*, to visit flowers at four communities, and recorded the diversity and abundance of floral visitors at communities with and without ants. The presence of predatory ants caused alterations in the size and topological properties of networks, such as web asymmetry or nestedness, through the behavioural changes they induce on floral visitors. Predation risk should be considered a determining factor in structuring mutualistic networks where predators are abundant.

Introduction

Network theory provides a useful tool to represent, characterize and compare the complexity of biotic interactions in biological communities. Network theory also assists to uncover the ecological and evolutionary processes behind the dynamics of ecological interactions by breaking down their entangled architecture (Bascompte & Jordano 2007; Mougi & Kondoh 2012). In the study of ecological networks, researchers have traditionally focused on identifying topological properties of ecological webs (Dunne *et al.* 2002; Ings *et al.* 2009), and over the last decade multiple approaches have been developed to study the particular case of mutualistic interactions. This body of research has detected that mutualistic networks share a number of common properties, such as (1) heterogeneous distribution of the number of interactions per species (Jordano *et al.* 2003), (2) nested organization of the interaction matrix, that is, if a species interacts with species A, it has a high probability of interacting with species that are more generalists than A (Bascompte *et al.* 2003) and (3) high asymmetry, so that if a certain species depends strongly on another, this later species most often depends weakly on the former (Bascompte *et al.* 2006).

For logistic reasons, mutualistic networks have traditionally been studied as if they were independent entities within their ecosystems. Indeed, the influence of other interactions, such as predation or parasitism, on topological properties and evolutionary processes of mutualistic networks has been typically disregarded (see Melian *et al.* (2009) for an exception). And yet, it is widely assumed that predators play a key role in structuring food webs (McPeek 1998), and it has been suggested that ambush predators can play an essential role on the ecological and evolutionary trajectory of plant-animal mutualisms and that they can affect the topology of pollination networks (Galen & Cuba 2001; Goncalves-Souza *et al.* 2008; Llandres *et al.* 2012; Rodríguez-Gironés *et al.* 2013).

Despite these suggestions, no quantitative studies to date investigate the effect of predators on plant-pollinator interactions at the community level.

There is evidence that ambush predators induce behavioural changes in foraging pollinators (Dukas 2001; Dukas & Morse 2003; Heiling & Herberstein 2004), even though these results are not always consistent (Brechbuhl *et al.* 2010; Llandres & Rodriguez-Girones 2011), and that they can affect the reproductive success of their host plants (Suttle 2003). Nevertheless, most research on the effect of ambush predators on plant-pollinator interactions has focused on crab spiders – the most common ambush predator in temperate meadows – and it is unclear whether crab spiders can affect the structure of pollination networks (Llandres *et al.* 2012). In tropical areas, however, predation pressures could be strong enough to affect the structure of pollination networks. In particular, *Oecophylla smaragdina*, one of the most abundant predators in Tropical Asia, ambushes at flowers to hunt pollinators (Rodríguez-Gironés *et al.* 2013) and its presence affects the flower choice of bees (Gonzálvez & Rodríguez-Gironés 2013; Gonzálvez *et al.* 2013). Here, for the first time, we conducted an experimental study on the effect of predators, *O. smaragdina*, on the structuring of mutualistic plant-pollinator networks.

Materials and methods

Study site and species

The study was carried out at Xishuangbanna Tropical Botanical Garden (XTBG), Yunnan province, China, from May to June 2011. To conduct the experiment we first created eight artificial communities by selecting six plant species grown in greenhouses at XTBG. The choice of plant species was based on a priori field observations to ensure that the communities included species with different levels of generalisation. We selected *Impatiens balsamina* and *Perilla frutescens* as the most generalist plant species, *Clerodendrum thomsoniae* and *Salvia splendens* as plants of medium level of specialization and *Beloperone guttata* and *Ixora chinensis* as the most specialist species (Fig. 1). The flowers of these plant species did not produce ant-repellent substances (personal observations). Each community was composed of three potted individuals of each species, having a total of 18 plants. We distributed plant individuals in rectangular arrays (3 X 6) fulfilling Sudoku conditions. Communities were randomly distributed and spaced at least 100 meters apart. Of the eight communities, four were located next to *Oecophylla smaragdina* nests, allowing workers to patrol freely the whole community. The other four communities were placed far from weaver ant colonies, putting some glue (tanglefoot) at the base of flowerpots to prevent ants from colonising the plants.

Experimental procedure

During the first week we conditioned weaver ants to visit flowers by putting small pieces of meat on them. After this period of training they frequently patrolled flowers searching for resources. Should the baits have a direct effect on pollinators, the same treatment was applied in ant-free communities as a control. Observations started on the second week, in the absence of baiting. We conducted four 15-minute pollinator censuses per individual plant, having a total of three hours of observations per plant species in each community. During censuses we recorded and identified all visitors that touched the sexual organs of flowers, as well as the number of visits made by each floral visitor.

Fig. 1. *(a) Oecophylla smaragdina patrolling on a Perilla frutescens inflorescence. (b) Impatiens balsamina flower being visited by Ceratina sp. (subsg. Ceratinidia). (c) Papilio memmon collecting nectar from Salvia splendens flowers. (d) Clerodendrum thomsoniae inflorescence. Weaver ant on Ixora chinensis (e) and Beloperone guttata (f) flowers.*

Topological properties of pollination webs

For each web we calculated a number of parameters that characterize the topology of mutualistic networks. Each community was composed of *P* plant species (notice that *P* is a constant number through the whole experiment, *P* = 6) and *A* floral visitor species.

We estimated web size as total number of potential interactions, $W= A*P$, and determined the total number of interactions (L) and visits (V) recorded. The degree of connectivity of networks was evaluated through their connectivity, $C=100*(L/W)$, which is the percentage of all possible interactions that take place (Jordano 1987). We also calculated web asymmetry, $D = (A-P)/(A+P)$, defined as the balance between plants and floral visitor numbers (Bluthgen *et al.* 2007) and nestedness by applying NODF (Almeida-Neto *et al.* 2008; Almeida-Neto & Ulrich 2011). Because we fully quantified the frequency of the interactions NODF calculations were based on quantitative data. Finally, we estimated the global clustering coefficient for each community to assess the degree to which nodes tend to cluster together (Opsahl 2012).

Statistical analysis

All parameters were estimated with the statistical software R 2.12.2 (R Development Core Team 2010), using libraries Bipartite and tnet, (Dormann *et al.* 2008; Opsahl 2009). We used t-tests to compare parameter values from communities with and without weaver ants. Unless otherwise specified, results are reported as mean ± standard deviation.

Results

Over the whole experiment we recorded 46 insect species visiting the flowers of the six selected plant species. However, the number of flower visitor species, A, was, on average, 1.4 times higher at ant-free communities (20.75 ± 2.87) than at ant-harbouring communities (14.50 ± 0.57), the difference being statistically significant (t=4.26, df=6, p<0.01) (Fig.2a). Because each artificial community was composed of the same six plant species, web size, *W*, depended exclusively on the number of flower visitor species, being 87 ± 3.46 and 124.5 ± 17.23 for communities with and without ants, respectively. While we recorded 36.25 ± 10.30 types of interactions (Fig. 2b) between flowers and visitors *(L)* and 6.13 ± 3.82 visits per plant and per hour *(V)* (Fig. 2c) at ant-free communities, these numbers decreased to 23.25 ± 0.5 and 3.63 ± 2.08, respectively, at ant-harbouring communities. Both differences were statistically significant (*L*: t=3.11, df=6, p<0.05; *V*: t=3.37, df=6, p<0.05).

Despite the differences in the number of visitor species between communities with and without ant colonies, network connectivity was, on average, very similar in both kinds of communities (26.97 ± 1.25% and 28.80 ± 4.75% in communities with and without ants, respectively; t=0.74, df=6, p=0.48). Because the number of plant species was the same for all communities, this result implies that the number of plant species visited per pollinator was similar at ant-free and ant-harbouring communities.

Web asymmetry was higher at ant-free communities (0.54 ± 0.04 on average) than at ant-harbouring communities (0.41 ± 0.01), the difference being statistically significant (t=5.97; df=6, p<0.001) (Fig.2d). In addition, communities without weaver ants were more nested (14.46 ± 3.49) than communities patrolled by ants (4.89 ± 1.16) (t=2.60; df=6, p<0.05) (Fig.2e).

Communities with and without ants also diverged when considering global clustering coefficients: while at ant-free communities the proportion of species directly linked to a focal species was 0.68 ± 0.17, this proportion decreased to 0.26 ± 0.04 at ant-harbouring communities (t=4.52, df=6, p<0.01) (Fig.2f)

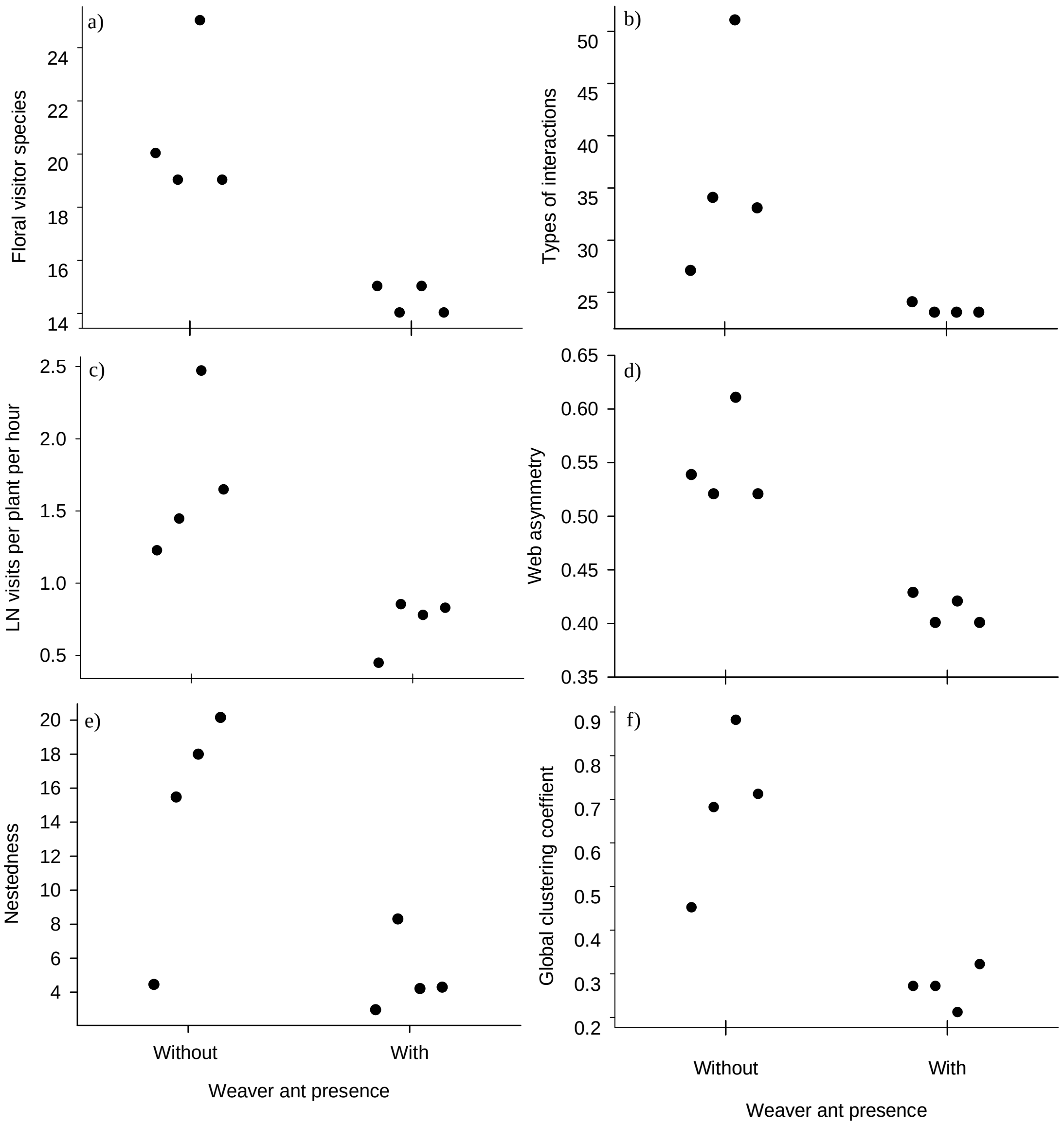


Fig 2. *Network topological properties from communities with and without ants: a) floral visitor species, b) types of interactions, c) ln visits per hour per plant, d) web asymmetry, e) nestedness, and f) global clustering coefficient.*

Discussion

Our results show that predators caused variation in the size and topology of plant-pollinator interaction networks through the behavioural changes they induce in floral visitors. Pollinators were more abundant (more visits per plant and per hour) and more diverse (more species per community) in ant-free than in ant-harbouring communities. If this result was to be expected, as vulnerable pollinators will most likely concentrate their foraging effort in predator-free patches, the effect of weaver ants on network topology has deeper ecological and evolutionary consequences: weaver ants affected the web asymmetry, clustering coefficient and nestedness of the communities they colonised.

As previous work suggested (Dukas 2001; Dukas & Morse 2003; Heiling & Herberstein 2004; Dukas 2005) predation risk had strong effects on the foraging choices of floral visitor: the presence of predatory ants decreased the diversity and abundance of plant-pollinator interactions. It is known, for example, that Trigona (Tsuji *et al.* 2004) and middle-sized solitary bees (Gonzálvez *et al.* 2013) reduce their visitation rates at plants where they detect the presence of the weaver ant *Oecophylla smaragdina*. And, although the effect of predatory ants on the foraging behaviour of pollinators has been reported only at the plant level, pollinators are known to avoid patches where crab spiders are abundant (Dukas 2001; Dukas & Morse 2003; Dukas 2005). Our results, however, go a step further, revealing that pollinators exploit preferentially those predator-free communities within the ecosystem, so that the presence of predators modifies the abundance and distribution patterns of floral visitors-plant interactions, possibly affecting the ecological and evolutionary trajectories of plant-pollinator networks. For example, the decrease in pollinator diversity forced plants in communities with ants to increase their level of specialisation, even though, on average, pollinators at ant-harbouring communities had similar levels of generalisation as pollinators of ant-free communities. The presence or absent of weaver ants at plant communities also affected the clustering of networks. Links in networks built on ant-harbouring communities were less clustered than in networks without predation risk. These differences in the clustering coefficient may affect network vulnerability. In this way, the loss of keystone links in networks from communities without predatory ants could have larger effects than networks from communities with ants.

Networks from ant-free communities showed higher nestedness than networks obtained when predators were present in the community. That is, mutualistic networks built at plant communities with ants are less cohesive than those originated at ant-free communities (Bascompte *et al.* 2003), increasing effective interspecific competition and reducing the number of coexisting species (Bastolla *et al.* 2009). The quantitative version of the NODF nestedness index incorporates two components. In a nested community we expect that, if species X interacts with species Y, X will also interact with species more generalist than Y. Moreover, the number of X individuals interacting Y individuals will be smaller than the number of X individuals interacting with more generalist species (Almeida-Neto & Ulrich 2011). Why, thus, does the presence of predatory ants decrease nestedness? Once again the different behavioural responses of floral visitors to predation risk appear to be the key to understand alterations in network structure. Specifically, in communities with weaver ants nestedness decreases because some floral visitors ceased foraging at generalist flowers to visit specialist species. As a result, predation risk reduces the interaction frequency of some generalist species.

It should be noted that, in this experiment, the effect of ambush predators on the topology of pollination networks is mediated through the indirect effect of predation risk on pollinator behaviour, and not directly through an effect of predators on pollinator abundance. Indeed, due to the small size and relative proximity of our experimental communities, they all shared the same pool of possible visitors, and the pattern we observe reflects the foraging choices of different pollinator species. In undisturbed rain forest, however, weaver ants may be continuously distributed over large continuous expanses (personal observation), and could contribute to the regulation of pollinator population densities (Rodriguez-Girones 2012).

This is the first experimental demonstration that the presence of ambush predators can affect the structure of plant-pollinator interaction networks. By affecting even the most basic topological properties of mutualistic networks, predation plays an important role shaping their ecological and evolutionary processes. These networks, which have been traditionally studied as an independent entity (see Melian *et al.* (2009) for an exception), should be considered as an integral part within the ecosystem, taking into account the effect of the remaining biotic interactions on the dynamics of the mutualisms. Without considering the influence that predation risk may have on structuring networks, particularly in those ecosystems where predators are highly abundant, the estimates of some network parameters such number and diversity of interactions, web asymmetry and even nestedness might be highly biased. Although in our particular case we have focused on the effect of a predator on network topology, it seems likely that other symbiotic relationships, such parasitism or commensalism, may also affect structural patterns in mutualistic networks directing the evolution of ecological communities.

Acknowledgments

We thank Jordi Bascompte for helpful suggestions. This work was supported by the Ministerio de Ciencia e Innovación/FEDER (projects CGL2007-63223/BOS and CGL2010-16795 to MARG) and CSIC (studentship JAE-Pre_08_01008 to FGG).

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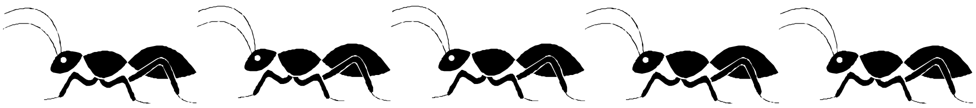
CAPÍTULO 3



Flowers attract weaver ants which deter less effective pollinators

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Abstract

1. Many flowers produce ant-repellent substances that prevent ants from discouraging pollinator visits. When a flower's most effective pollinator is unaffected by predatory ants, however, flowers should benefit from the presence of ants that deter less effective pollinators from consuming resources.
2. Behavioral assays revealed that *Melastoma malabathricum* flowers, pollinated by large carpenter bees, *Xylocopa* spp., produce ant attractants that recruit weaver ants, *Oecophylla smaragdina*.
3. The presence of ants was associated with an increase in the reproductive success of *M. malabathricum* flowers. This outcome likely resulted from the filtering effect of ants on the community of flower visitors: ants deter less effective pollinators and attract *Xylocopa* bees through an indirect effect on resource depletion.
4. *Synthesis*. Although plant–pollinator interactions are classified as mutualisms, not all flower visitors are effective pollinators, and some can be parasites or conditional parasites. As a result, predators that deter flower visitors can have positive or negative effects on plant fitness, depending on whether they deter all visitors or a subset of them, and on the relative effectiveness of deterred and undeterred visitors.

Introduction

Arboreal ants can benefit their host plant through the consumption of herbivorous insects. Plants have developed an array of strategies, such as providing ants with food or shelter, to attract them (Janzen 1966) – leading to tight mutualistic relationships between both taxonomic groups (Davidson, Snelling et al. 1989, Fiala, Grunsky et al. 1994, Pringle, Dirzo et al. 2011). Ants, however, are likely to attack all insects visiting the plant, and their presence risks deterring pollinators from visiting flowers (Willmer and Stone 1997, Tsuji, Hasyim et al. 2004, Willmer, Nuttman et al. 2009), potentially decreasing the plant's reproductive success. To counteract this negative effect, some plant species produce ant-repellent substances during the flower's fertile period (Willmer and Stone 1997, Ghazoul 2001, Raine, Willmer et al. 2002, Junker, Chung et al. 2007, Willmer, Nuttman et al. 2009), ensuring pollination without losing the protection of ants. Ants are not alone in interfering between plants and their pollinators. Other predators, notably crab spiders, ambush visiting insects at flowers (Morse 2007), affecting the foraging choices of pollinators (Dukas 2001, Dukas and Morse 2003) and sometimes reducing plant reproductive success. It has been suggested, however, that plants may benefit from the presence of ants on flowers or other ambush predators. This would be the case if their negative effect (reduction in pollinator visits) is compensated by the protection they offer by removing florivorous insects and seed predators (Higginson, Ruxton et al. 2010).

We further predict that plants could benefit from ants ambushing at flowers if they selectively deter ineffective pollinators, and that this benefit could promote floral traits that raise the probability of ant recruitment at flowers. To test whether flowers attract ants which deter ineffective pollinators, thus increasing the plant's reproductive success, we conducted a number of experiments and observations on the interaction between weaver ants, *Oecophylla smaragdina* Fabricius, the tropical shrub *Melastoma malabathricum* L. and its flower visitors. Our aim was accomplished by performing the following steps: (i) we first studied the association between plant quality and weaver ant presence, (ii) we then quantified bee visit rates at plants with and without weaver ant nests, (iii) we assessed the pollination effectiveness of the different flower visitors, (iv) we examined the association between weaver ant presence and fruit and seed set of the host plant, and (v) we tested whether weaver ants were attracted to flowers. Finally, to study the mechanisms responsible for the bee foraging choices, (vi) we developed an optimal-foraging model and compared its predictions with observed patterns.

Materials and methods

The study was carried out at MacRitchie Reservoir in the Central Catchment Nature Reserve, Singapore, from April to June 2010. Weaver ants are extremely aggressive generalist predators that build their nests with the living leaves of a broad range of tree and shrub species. In our study site, they colonized about half of the *M. malabathricum* plants, where we often observed them patrolling flowers or tending aphids at their base. The nectarless flowers of *M. malabathricum* have an inner and outer whorl of stamens, apically poricidal, and attract bees able to extract pollen by sonication. This species is a self-compatible shrub but pollen vectors are required to effect fruit set (Gross 1993). Flowers are visited by a wide array of insects, but due to the gap between anthers and stigma only large bees seem likely to effect pollination. In our study population, the most common visitors to *M. malabathricum* flowers were two carpenter bees, *Xylocopa latipes* Drury and *X. confusa* Pérez, and a smaller solitary bee, *Nomia strigata* Fabricius: out of the 436 visits to unmanipulated *M. malabathricum* flowers that we recorded (see Effect of weaver ant presence on bee visits), 432 (99.1%) were by *Xylocopa* and *Nomia* bees. We therefore restrict our study to *Xylocopa* and *Nomia* bees, ignoring in what follows the possible effect of infrequent visitors such as *Amegilla zonata*, *Ceratina* spp. or *Lasioglossum* spp.

Colonization by weaver ants and plant quality

We selected and tagged 25 *M. malabathricum* plants with weaver ant nests and 25 plants without nests. If a trait differed between colonised and uncolonised plants, the difference could result from the presence of ants at some plants, or from some other factor, which affected the trait under study and the probability of ant colonisation. To estimate the possibility that ant-colonised plants had higher fitness because ants selected high-quality plants to build their nests, we assessed plant quality through height, number of flowers per plant and day (since most *M. malabathricum* flowers last one single day; Gross 1993) and pollen production per flower. Stamens from bagged, unvisited flowers were kept in 70% ethanol and the number of pollen grains produced was determined by counting under a microscope (Olympus BHT-BH2) as detailed in Luo, Zhang & Renner (2008). We used t tests to determine whether plants with and without weaver ant nests differed in height, number of flowers or pollen production.

Association between weaver ant presence and bee visits

We observed bee visit rates at the 50 tagged plants. Each plant was observed four times, in pseudo-random order (i.e., no plant was observed twice before all other plants had been observed at least once). At each observation, we selected 4 flowers and recorded the number of times that they were visited by *Xylocopa* and *Nomia* bees over a 10 minute period. The number of *Xylocopa* and *Nomia* visits per plant was averaged over the four observation periods. Averages were log transformed to achieve homogeneity of variances and analysed with a repeated-measures ANOVA, having plant as subject, bee species as within-subject repeated measures, and ant presence as a categorical factor. A preliminary analysis distinguished between *X. latipes* and *X. confusa* visits. We found no differences between the two *Xylocopa* species. To increase the power of the tests comparing *Xylocopa* and *Nomia*, which was the main purpose of the study, and given that we observed no obvious differences in their time of activity or behaviour, in this and subsequent analyses we pooled data from the two *Xylocopa* species.

Pollination effectiveness of Nomia and Xylocopa bees

We evaluated the pollination effectiveness of *Nomia* and *Xylocopa* bees by measuring pollen removal, as well as fruit and seed set of flowers with known visitation histories. To study pollen removal we bagged flowers before opening. Upon unbagging the flowers, we removed an internal and an external stamen to assess pollen production (see Luo, Zhang et al. 2008), observed the flowers and, after a known number of bee visits, removed all remaining stamens to determine pollen removal rates. The number of pollen grains remaining after a known number of visits was determined as explained above (see Luo, Zhang & Renner 2008). The amount of pollen left in anthers was fitted to a Generalized Linear Model (GLM) with normal distribution and log link function, having the number of *Xylocopa* and *Nomia* visits as continuous covariates, anther origin (internal or external stamen) as a categorical variable, and the two first-order interactions between number of visits and anther origin. Statistical significance was assessed through likelihood ratio tests for this and subsequent GLM analyses. In these analyses, we selected the combination of error distribution and link function that provided the best fit (lowest AIC; Dormann, Gruber et al. 2008) to our data.

We studied the effect of bee visits on fruit and per-fruit seed set by following the same set of bagged flowers until fruit ripening. For each mature fruit, we estimated seed production by counting the number of viable (white) seeds for one carpel and multiplying by five. The relationship between the probability of fruiting and the number of bee visits was analysed with a GLM with binomial error distribution and complementary log-log link function ($\log(-\log(1-\pi))$). The relationship between seed production per fruit and the number of bee visits was analysed with a GLM with gamma distribution and power link function. In both cases, models included the number of *Xylocopa* and *Nomia* visits as separate, continuous covariates.

Association between weaver ant presence and fruit and seed set

We marked 12 flowers per plant on 25 plants with ant colonies and 25 without ant colonies to determine the relationship between ant presence and fruit set and both per-fruit and per-plant seed set, although 16 marked flowers were lost due to branches breaking during storms in plants with ant nests. We estimated fruit set by following flowers until fruit ripening. Per-fruit seed set was calculated by counting the number of viable seeds for one carpel and multiplying by five. Per-plant seed set was estimated by multiplying, for each plant, mean per-fruit seed set by the number of flowers produced by the plant and the proportion of marked flowers that produced a fruit. This is an estimate of the number of seeds produced per plant each day. The relationship between the probability of fruiting and ant presence was analysed with a GLM with binomial distribution and logit link function. Estimated per-fruit and per-plant seed sets of plants with and without weaver ants were compared with GLMs, introducing plant as a random factor in the former analysis.

Attraction test

To test whether *M. malabathricum* flowers produce ant-attracting cues, we wiped one half of a 14-cm diameter Petri-dish with a newly opened flower and the other half with a two-day old withering flower (Ghazoul 2001). We collected weaver ants from nests without *M. malabathricum* in their foraging territories and we set an individual ant in the centre of the dish, recording over five minutes the amount of time spent on each half. The experiment was done using 20 replicates (a different plant and ant colony was used for each replicate). To confirm that the preference for the “new flower” side was due to an ant-attracting cue in new flowers, rather than to the presence of ant-repellent substances in withering flowers, we repeated the test using *M. malabathricum* leaves and twigs instead of new flowers. Because residuals were normally distributed for each data set, we used paired t-tests to check whether ants spent more time on one side of the Petri dish or the other.

All analyses were carried out using STATISTICA version 10 (StatSoft 2011) except for those testing the relationship between seed set and ant presence, which were conducted on R version 2.8.0. (R Development Core Team 2010).

Effects of trophic competition on bee visits

The pattern of bee visit rates that we observed suggested that *Nomia* bees were visiting mainly flowers where predation risk was lowest and that *Xylocopa* bees were avoiding competition for resources. To assess the likelihood of this interpretation, we developed an optimality model to predict the frequency of *Xylocopa* visits to flowers at plants with and without weaver ant nests. The model was parameterised with data collected in this study and assumed that each *Xylocopa* bee foraged in such a way as to maximise her pollen intake rate, taking into account how other bees were behaving. Model predictions were then compared with the rates at which *Xylocopa* bees visited flowers at plants with and without ant nests.

Results

Colonization by weaver ants and plant quality

Height was similar for *M. malabathricum* plants with and without ant nests (159.56 ± 41.90, 163.44 ± 50.24 cm. respectively; $t_{48} = -0.29$, $P = 0.38$), showing that ants did not select large (or small) plants for their nests. The number of flowers opening per day was also similar at plants with and without ants (7 ± 2.56, 6.8 ± 2.98 respectively; $t_{48} = -0.25$, $P = 0.80$), indicating that ants did not select plants with more flowers either. We found no differences ($t_{48} = -0.54$, $P = 0.58$) in the number of pollen grains produced by external stamens of flowers in plants with (119,732.00 ± 16,507.57) and without (116,251.55 ± 21,264.92) ant nests. Pollen production in internal stamens of plants with (92,004.00 ± 11,781.57) and without (89,276.00 ± 9,408.34) ant nests was also similar ($t_{48} = -0.90$, $P = 0.37$). It follows that ants did not select to build their nests in the most (or least) productive plants.

Association between weaver ant presence and bee visits

While the average rate at which bees visited flowers was similar at plants with and without ant nests ($F_{1,48} = 0.15$, $P = 0.70$) (Fig 1), there was a strong interaction ($F_{1,48} = 32.75$, $P < 0.0001$) between plant type and bee species: small *Nomia* bees concentrated their foraging effort on ant-free plants and large *Xylocopa* bees preferentially exploited flowers on ant-harbouring plants (Fig 1). When exploiting ant-harbouring plants, *Nomia* bees spent several seconds inspecting flowers, seldom landing on ant-harbouring flowers and being readily captured or chased away by the ants whenever they did so. *Xylocopa* bees, on the other hand, were unaffected by the presence of ants and ignored their attacks, occasionally flying off to the next flower with an ant hanging from their legs.

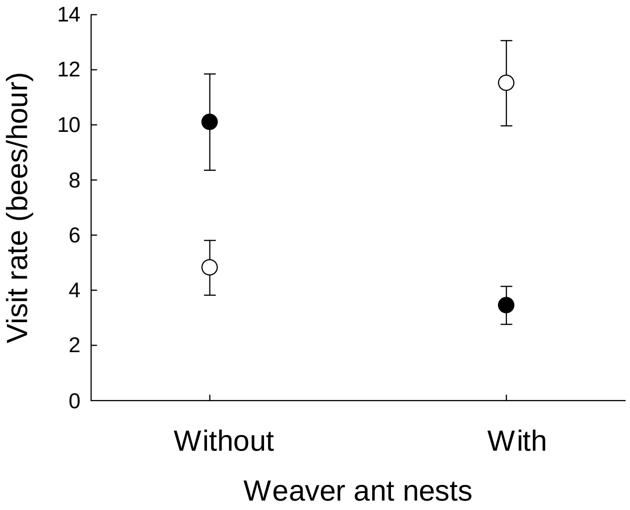

Fig 1. *Visit rates by Nomia (black circles) and Xylocopa (empty circles) bees at plants with and without weaver ant nests. Error bars represent standard errors.*

Pollination effectiveness of Nomia and Xylocopa bees

The amount of pollen left in anthers decreased exponentially with the number of *Xylocopa* (χ^2_1 = 250.39, P < 0.0001) and *Nomia* (χ^2_1 = 287.62, P < 0.0001) visits (Fig 2). Pollen removal was also affected by anther type (χ^2_1 = 68.69, P < 0.0001) but not by the interactions between anther type and the number of either *Xylocopa* (χ^2_1 = 3.07, P = 0.079) or *Nomia* (χ^2_1 = 0.30, P = 0.584) visits. Both species extracted comparable amounts of pollen per visit (confidence intervals of regression coefficients for *Xylocopa* and *Nomia* visits: [-0.64,-0.46] and [-0.78,-0.56], respectively).

Despite the efficiency of *Nomia* bees at collecting pollen, however, they only fertilized flowers when they accidentally landed on the stigmas. Out of 185 flowers visited exclusively by *Nomia* bees, only 11 (5.9%) produced fruits, while 53.9% of the 206 flowers visited by *Xylocopa* bees set fruit (G-test for the difference between flowers with and without *Xylocopa* visits: G = 114.75, P < 0.0001). When the analysis was restricted to flowers received a single bee visit, 27 out of 55 flowers visited only by a *Xylocopa* bee set fruit, while only 5 out of 64 flowers visited by a single *Nomia* bee set fruit (G = 24.87, P < 0.0001). Moreover, for those flowers receiving several *Xylocopa* visits, the probability of setting fruit increased with the number of visits (χ^2_1 = 109.66, P < 0.0001) (Fig 3) while the effect of increasing numbers of *Nomia* visits was not statistically significant (χ^2_1 = 0.62, P = 0.431).

When we concentrated on those flowers that had actually set fruit, however, the number of viable seeds produced per fruit (per-fruit seed set) was not affected significantly by the number of *Nomia* (slope: mean ± Std. Error. -1.27 ± 13.62; χ^2_1 = 0.007, P = 0.93) or *Xylocopa* (slope: mean ± Std. Error. 28.33 ± 21.26; χ^2_1 = 2.34, P = 0.12) visits — although there was a tendency for per-fruit seed set to increase in fruits with more than three *Xylocopa* visits: per-fruit seed set was 363.90 ± 35.25 when there were three or fewer *Xylocopa* visits and 469.22 ± 35.25 when there were more than three visits ($F_{1,67}$ = 2.23, *P* = 0.14). As a result, per-fruit seed set was similar for flowers that had been successfully fertilized by *Nomia* (mean ± Std. Dev.: 457.12 ± 274.44; N = 8) and *Xylocopa* (398.30 ± 291.69; N = 61).

To recap, although *Nomia* and *Xylocopa* bees removed similar amounts of pollen per visit, *Xylocopa* bees were much more likely to fertilise flowers. Nevertheless, once a flower had been successfully fertilised, seed set did not depend on the number of visits it had received or the species of the bee that had fertilised the flower.

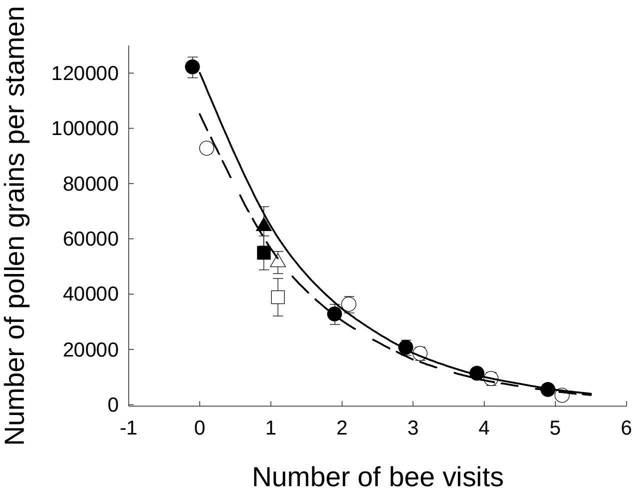

Fig 2. *Pollen removal. Exponential decrease in the number of pollen grains left in outer (black symbols) and inner (empty symbols) anthers with increasing numbers of bee visits. For the case of a single bee visit, we distinguish between the number of pollen grains remaining after a Nomia (squares) or Xylocopa (triangles) visit. For more than one visit, different combinations are pooled for clarity. Error bars are standard errors. Lines represent the fitted regressions (solid line external anthers, dotted line internal anthers) for the most parsimonious model.*

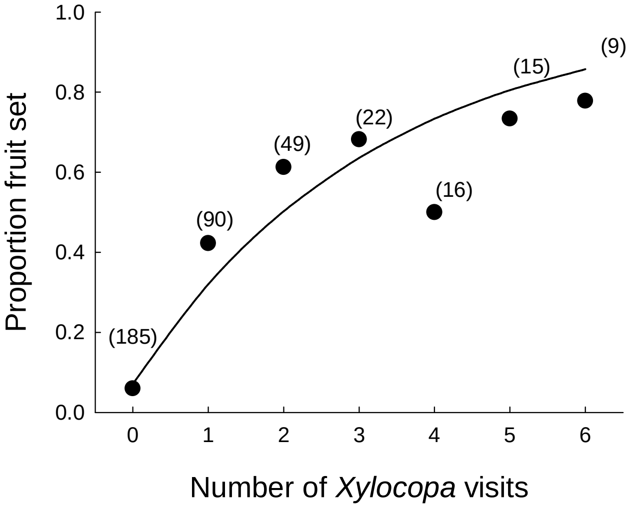

Fig 3. *Increase in proportion fruit set with the number of Xylocopa visits. Proportion of flowers producing mature fruits vs. the number of Xylocopa visits. Numbers in brackets are sample sizes. Line represents the fitted regression for the model with lowest AIC, log(1-fruit set) = -0.074 - 0.312·Xylocopa visits.*

Association between weaver ant presence and fruit and seed set

Because ant-harbouring plants received more visits from the most effective pollinators, *Xylocopa* bees, and ant-free plants were visited mainly by the least effective pollinators, *Nomia* bees, fruit set, per-fruit seed set and per-plant seed set were higher at plants with ant nests. Only 40% (N = 300) of marked flowers in ant-free plants set fruit, while fruit set increased to 65% (N = 284) in plants with ant nests ($\chi^2_1 = 38.46$, $P < 0.0001$) (Fig.4). In the same way, fruits at ant-free plants produced significantly fewer seeds than fruits at ant-harbouring plants ($F_{1,48} = 31.50$, $P < 0.0001$; mean ± Std. Dev.: 731.2 ± 191.1 and 1194.0 ± 297.3, respectively) (Fig.4). Note that per-fruit seed set in unbagged flowers was double that in bagged flowers, probably because unbagged flowers received more bee visits than bagged ones. This difference suggests that per-fruit seed set increases with the number of *Xylocopa* visits and that the trend detected with bagged flowers (per-fruit seed set was higher when flowers received more than three *Xylocopa* visits, although the difference was not statistically significant) would have become significant if we had left some flowers exposed for a longer time. Because plants with and without ant nests produced similar numbers of flowers, while fruit set and per-fruit seed set were higher at plants with ant nests, per-plant seed set was 2.7 times higher at plants with weaver ant nests (5281.70 ± 555.11) than at plants without nests (1941.13 ± 253.20; $F_{1,48} = 30.79$, $P < 0.0001$) (Fig.4).

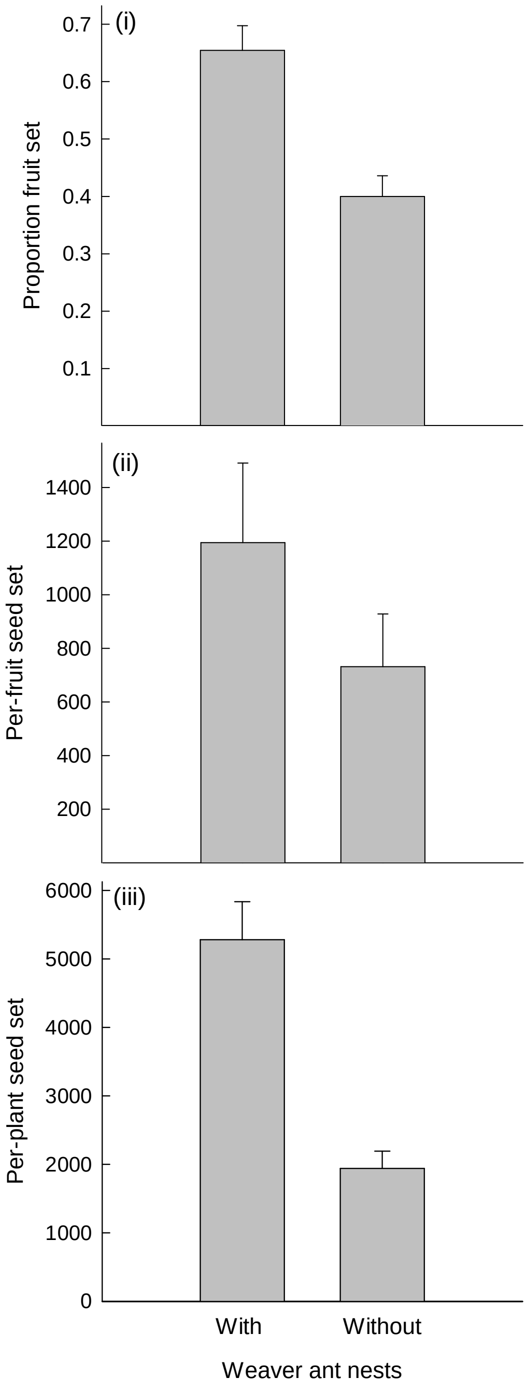

Fig 4. *Effect of weaver ant presence on (i) proportion fruit set, (ii) per-fruit seed set and (iii) per-plant seed set. (i) Proportion of flowers producing mature fruits at plants with and without weaver ant nests. (ii) Number of viable seeds per fruit produced at plants with and without weaver ant nests. (iii) Estimated number of viable seeds per plant produced in a day at plants with and without weaver ant nests. Error bars are standard errors.*

Attraction test

Ants spent significantly more time on the “new flower” than on the “withering flower” half of the dish (t_{19} = -3.53, *P* = 0.002) (Fig 5). When we repeated the test using *M. malabathricum* leaves and twigs instead of new flowers, ants spent similar amounts of time on both sides of the dish (t_{19} = 0.30, *P* = 0.77 and t_{19} = -0.18, *P* = 0.86, respectively) (Fig.5), confirming that the preference for the “new flower” side was due to an ant-attracting cue in new flowers, rather than to the presence of ant-repellent substances in withering flowers.

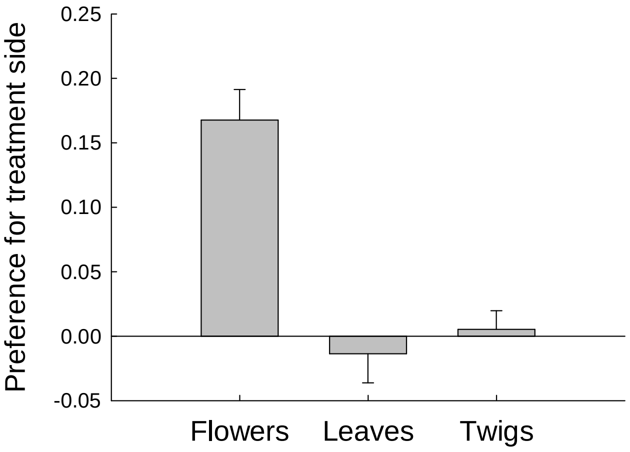

Fig 5. *Attraction of weaver ants to Melastoma flowers. Ant preference for the half of the Petri dish wiped with new flowers, leaves or twigs. For simplicity, data are plotted as preference for the treatment side (i.e. newly-open flower, leaf or twig) relative to the control side (withering flower). Preference = (time (in secs) spent on treatment side – 150)/150. This preference index ranges from -1 if ants spent all the time in the control side to +1 if they spent all the time in the treatment side. Error bars are standard errors.*

Effects of trophic competition on bee visits

As we have seen, *Nomia* bees preferentially visited flowers at ant-free plants, and *Xylocopa* bees preferentially visited flowers at ant-harbouring plants. The behaviour of *Nomia* bees can be explained as an anti-predator response. But how do we explain the preference of *Xylocopa* bees for ant-harbouring plants? Because weaver ants pose little threat to the large *Xylocopa* bees, which ignored the presence of ants at flowers and their attacks (see results), we hypothesise that *Xylocopa* bees were simply avoiding inter-specific competition, foraging at those plants where pollen depletion was less severe. To assess the viability of this hypothesis, we calculated the optimal foraging strategy of *Xylocopa* bees and compared it with the strategy we observed.

The foraging strategy of a bee exposed to the risk of predation is affected by factors such as the proportion of flowers harbouring predators, the ability of bees to detect the presence of predators and their probability of being captured upon landing on a predator-harbouring flower (Clark and Dukas 1994 Rodríguez-Gironés & Bosch 2012). Because most of these parameters are unknown for the *Nomia*-weaver ant system, we cannot determine the extent to which *Nomia* bees were following their optimal strategy. For *Xylocopa* bees, on the other hand, we can assume that there was a negligible predation risk and calculate their optimal foraging strategy, given how *Nomia* bees were behaving.

If *Xylocopa* bees were optimal foragers, the proportion of *Xylocopa* visits to flowers of plants with and without ant nests would be such that the expected pollen intake rate per flower of a *Xylocopa* bee would be independent of the plant type it exploited (R Development Core Team 2010). If we assume that handling times are equal at flowers of plants with and without weaver ant nests (the validity of this assumption will be checked below), the equality of intake rates translates into equality of resources found per flower. In other words, if *Xylocopa* bees were optimal foragers, pollen removal would follow the same pattern in flowers of plants with and without ant nests. Because the amount of pollen removed per visit by *Nomia* and *Xylocopa* bees was similar, pollen depletion depended solely on the total rate at which flowers were visited, regardless of the bee species that was visiting the flowers. Thus, the condition for optimal foraging is simply that the rate at which flowers were visited, combining *Xylocopa* and *Nomia* visits, was the same for flowers of plants with and without ant nests.

Flowers of plants with and without ant nests received an average of 3.45 and 10.09 *Nomia* visits per hour, respectively (Fig. 1). If we denote by ν_0 and ν_1 the rate of *Xylocopa* visits to flowers at plants without and with ant nests, then the optimal-foraging condition becomes

$$10.09 + \nu_0 = 3.45 + \nu_1 \qquad \text{(A1)}$$

Equation A1 does not allow us to predict optimal visit rates in absolute terms: the actual rate at which *Xylocopa* bees are expected to visit flowers depends on many factors, such as the number of bees and flowers, the distance between bee nests and *M. malabathricum* plants, and the availability of alternative resources. Nevertheless, equation (A1) predicts the relationship between *Xylocopa* visit rates to flowers of plants with and without weaver ant nests:

$$\nu_1 - \nu_0 = 6.64 \qquad \text{(A2)}$$

In our observations, we found (Fig. 1) $\nu_1 = 11.51 \pm 1.55$ and $\nu_0 = 4.81 \pm 0.99$ visits per hour, so that $\nu_1 - \nu_0 = 6.70$ – in perfect agreement with the predicted difference of 6.64 visits per hours.

Equations A1 and A2 rely on the assumption that *Xylocopa* handling times were similar for flowers at plants with and without weaver ant nests. To check this assumption, we videotaped 68 *Xylocopa* visits (34 at plants with ant nests and 34 at plants without ant nests) and counted the number of frames from landing on the flower to departure. *Xylocopa* handling times (mean ± Std. Dev.) were 1.20 ± 0.33 s for flowers at plants with ant nests and 1.16 ± 0.17 s in plants without ant nests. This difference was not statistically significant ($t_{66} = 0.29$, $P = 0.77$), confirming the validity of the model's assumption.

Discussion

The presence of weaver ant nests was associated with an increase in the reproductive success of *M. malabathricum* shrubs: fruit set, per-fruit seed set and per-plant seed set were higher at plants with than without nests. This was most likely the indirect effect of changes in small-bee behaviour in response to predation risk by ants. Small bees were easily captured by ants and avoided plants with ant nests, quickly depleting resources at ant-free shrubs. Larger *Xylocopa* bees, safe from predation by weaver ants, responded to interspecific competition by concentrating their foraging effort at ant-harbouring plants. Because *Xylocopa* bees were by far the most effective pollinator of *M. malabathricum* flowers, the combination of the anti-predator response of *Nomia* bees and competition avoidance of *Xylocopa* bees was associated with a higher reproductive success of ant-harbouring plants. Although we did not quantify pollen flow, given that both *Nomia* and *Xylocopa* bees removed similar amounts of pollen and that the probability of pollen transfer from bees to flower stigmas was much higher for *Xylocopa* bees (*Nomia* bees seldom fertilized flowers), male reproductive success must also have been higher for plants with weaver ant nests than for plants without nests.

In principle, the higher reproductive success of *M. malabathricum* plants with ant nests could be causally unrelated to the presence of ants. For instance, ants might be building their nests in more vigorous plants, or on those growing on more fertile soil. This explanation, however, seems unlikely. Plant density was very high in our population, and ant-colonised plants were interspersed between the ant-free plants. If abiotic factors were affecting plant growth and ant colonisation, the heterogeneity of such factors must take place in a very small spatial scale. Most important, plants with and without ant nests had similar sizes, numbers of flowers and pollen production rates, making it unlikely that ants were selecting plants with traits correlated with higher fecundity.

Bee behaviour

A number of studies have shown that social and solitary bees are able to avoid predator-harbouring flowers, plants, and patches (Briscoe and Chittka 2001, Dukas 2001, Dukas and Morse 2003, Dukas 2005). While it is not presently clear whether bees learn to associate some areas with predators and avoid foraging at them, or whether they detect and avoid predator-harbouring flowers, avoidance of ant-harbouring plants by *Nomia* bees fits well with existing literature. Why, however, were *Xylocopa* bees attracted to ant-harbouring plants? We suggest that *Xylocopa* bees were simply maximising pollen intake rate, and preferred plants where inter-specific competition was lowest. The tight fit of optimal-foraging model predictions to the data supports this interpretation. As for the mechanism allowing *Xylocopa* bees to concentrate their foraging effort in plants with weaver ant nests, we know little about their foraging strategies, but comparison with other bee species may shed some light on the issue. Bees can track changes in the spatial distribution of resources (Fiala, Krebs et al. 1996, McPeek 1998, Pagel 1999, Devy and Davidar 2006, Bascompte and Jordano 2007). Bumblebees, for instance, change their foraging territories when competitors exploiting neighbouring patches are experimentally removed (Kelley and Magurran 2003). If *Xylocopa* bees have similar cognitive abilities, it is not surprising that they concentrate their foraging effort where competition with *Nomia* bees is lowest. In this respect, it should be noted that resource partitioning is facilitated by the slow turnover rate of ant-harbouring plants: ant nests can remain in the same plants for months (personal observations)

Evolution of ant attraction

Through its geographical range, *M. malabathricum* flowers are visited by a diverse array of small bees. However, due to the large gap between their anthers and stigma, *Xylocopa* bees seem to be their main pollinator. In contrast, small bees behave as less effective pollinators: they provide *M. malabathricum* with a weak pollination service in the absence of effective pollinators, but when *Xylocopa* bees are abundant they decrease its pollination success. This happens because small bees, which seldom fertilise flowers, make them less attractive to the most effective pollinators, reducing the rate at which they visit flowers. Even worse: small bees sometimes scavenge pollen from stigmas in the late morning (Gross and Mackay 1998). Because weaver ants and *M. malabathricum* have almost identical distributional ranges and the two species have co-existed for at least one million years (Renner and Meyer 2001, Azuma, Ogata et al. 2006), it seems likely that the same ecological play presented in this paper, incorporating the interactions between flowers, ambush predators, pollinators and less effective pollinators, has been repeated generation after generation (with minor variations, perhaps, in the identity of the secondary characters, the less effective pollinators), applying a selective pressure on *M. malabathricum* to encourage weaver ants to patrol their flowers.

Possibly as a result of these selective pressures, *M. malabathricum* produces some unidentified cues (not necessarily volatile substances) that entice *O. smaragdina* ants to patrol flowers during anthesis. Although future work will be required to evaluate whether the production of these cues is a genuine adaptation, this possibility receives support from the fact that, of all flowers tested so far, *M. malabathricum* is only the second one reported to produce ant-attracting cues in fertile flowers. Out of 64 plant species tested so far (Willmer and Stone 1997, Ghazoul 2001, Raine, Willmer et al. 2002, Ness 2006, Junker, Chung et al. 2007, Agarwal and Rastogi 2008, Junker and Bluthgen 2008, Opsahl 2009, Willmer, Nuttman et al. 2009), production of ant-repellent substances has been reported in 73% of the species studied. Attraction of ants to flower odours has only been reported twice. In the first case volatiles produced by *Luffa cylindrica* flowers repel large ant species, but attract the tiny nectar-feeding *Tapinoma melanocephalum* (Agarwal and Rastogi 2008). Nevertheless, because the *T. melanocephalum* individuals used for the experiments regularly consumed nectar at *L. cylindrica* flowers, it is unclear whether flowers produce substances to attract them. It seems just as likely that *T. melanocephalum* ants had learnt to associate flower odours with the presence of nectar. In the second case reported the alpine orchid *Chamorchis alpine* is pollinated by ants and uses floral scents to attract them (Opsahl 2009). Here, however, we report for the first time that floral substances may play a new role by attracting predatory ants to flowers to deter ineffective floral visitors. Attraction of ambush predators to flowers can be seen as an indirect means of resource concealment, which may have evolved because predators deter ineffective pollinators, leading to a reduction in the rate of resource depletion that further attracts more effective pollinators (Jordano, Bascompte et al. 2003).

More importantly, our results stress the ecological lability of both plant–animal and animal–animal interactions – which may switch between mutualistic and antagonistic depending on their ecological context. When small bees are the only visitors of *M. malabathricum* flowers they provide a certain pollination service (mutualistic interaction); however, in the presence of effective pollinators small bees act as thieves, removing pollen and making flowers less attractive to the most-effective pollinator (antagonist interaction) (Hargreaves, Harder et al. 2009). Conversely, ants that may reduce the reproductive success of plants by deterring small pollinators (antagonist interaction) enhance plants' fitness when more effective pollinators, unaffected by their predatory habits, are present (mutualistic interaction). Our system is, to our knowledge, the first one in which all actors in the play are broad generalists, yet their interaction may have resulted in the evolution of an unlikely floral trait —floral attractants for predatory ants.

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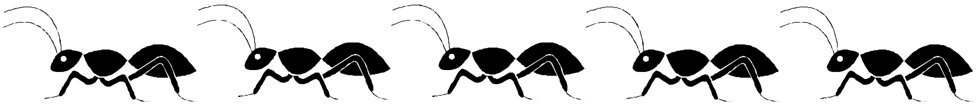
CAPÍTULO 4



Variability in bee abundance may affect the evolutionary trajectory of tritrophic interactions

Francisco G. Gonzálvez, Richard T. Corlett and Miguel A. Rodríguez-Gironés

Abstract

Plant-pollinator interactions are subject to heterogeneous selective forces when the pollinator community composition varies through space and time. Here we suggest that interactions between plants, pollinators and predators may also exhibit a spatiotemporal mosaic of selective pressures. To test this hypothesis we examined how temporal variability in bee abundance affected the relationship between the predatory ant, *Oecophylla smaragdina*, and the reproductive success of *Melastoma malabathricum* plants. A previous study reported that the reproductive success was greater at plants with than without ant nests. The effect was mediated through the behaviour of foraging bees: small bees, which are ineffective pollinators, avoided plants with ants where they were exposed to predation risk, and large bees, the most effective pollinators, concentrated their foraging on plants with ants, where resource competition was lowest. Two years later, when bee density was lower, small bees still avoided ant-harbouring plants, but there were so few of them that they had little effect on resource depletion and large bees visited plants with and without ants at similar rates. As a result, plant reproductive success was similar at plants with and without weaver ant nests, and lower than when bees were abundant. Tritrophic interactions may therefore show variability in the magnitude and direction of selective pressures.

Introduction

Natural selection is the end product of numerous heterogeneous processes. As a result, the magnitude and direction of selective pressures often change in space and time (Thompson 2005, Siepielski, DiBattista et al. 2009). This variability has important evolutionary implications. Spatial heterogeneity in selective pressures, for example, can lead to local adaptation, whereby different phenotypes are adapted to their respective environments (Kingsolver, Diamond et al. 2012). Spatiotemporal heterogeneity is particularly clear in plant-animal mutualistic networks (Herrera 1988). If, for example, several pollinator species exert different selective pressures on their mutualistic plant species, spatiotemporal variability in the composition of the pollinator assemblage will normally result in variation in net selective pressures on the plant (Herrera 1988, Horvitz and Schemske 1990). In southeastern Spain, for example, *Lavandula latifolia* is subject to a spatiotemporal mosaic of selective pressures due to the variability in the size and composition of its pollinator assemblage (Herrera 1988).

On the other hand, during the last decade researchers have recognized that other trophic levels, such as herbivores, predators and parasitoids, constitute a determining force in the evolutionary trajectory of pollinator-plant interactions (Dukas and Morse 2003, Suttle 2003, Heiling and Herberstein 2004, Reader, Higginson et al. 2006, Rodríguez-Gironés, Gonzálvez et al. 2013). Some studies conducted with ambush predators such as crab spiders in temperate meadows and weaver ants in tropical forests show that the presence of predators can modulate the nature of the pollinator-plant mutualism: by modifying pollinator foraging behaviour, predators exert indirect effects on the fitness of the plants that harbour them (Suttle 2003, Goncalves-Souza, Omena et al. 2008, Gonzálvez, Santamaría et al. 2013). Although spatiotemporal differences in plant fitness have been tested in locations with different degrees of predation risk (Suttle 2003, Dukas 2005, Gonzálvez, Santamaría et al. 2013), in most of these studies it is implicitly assumed that selective pressures on predator-harbouring plants remain invariable when the activity of predators is constant. However, because pollinator-plant interactions show a mosaic in selective pressures when the pollinator community composition varies through space and time, we predict that tritrophic interaction also result in heterogeneous selective pressures.

To test if tritrophic interactions exhibit a mosaic of selective pressures, we focus on how the strength of plant-pollinator-predator interactions changes through time. We use as study system the triple association between the aggressive weaver ant, *Oecophylla smaragdina*, its host plant, *Melastoma malabathricum* and its flower visitors. A study conducted in 2010 revealed that the flowers of *M. malabathricum* produce substances that attract predatory ants (Gonzálvez, Santamaría et al. 2013). Ants deterred small *Nomia strigata* bees, which were ineffective pollinators, and attracted the most effective pollinators, *Xylocopa* bees, through an indirect effect on resource depletion (Gonzálvez, Santamaría et al. 2013). As a result, ant-harbouring plants had higher reproductive success than ant-free plants (Gonzálvez, Santamaría et al. 2013). In 2012 bee density in the same location was remarkably lower. We therefore decided to replicate the 2010 study, in order to determine whether the effect of tritrophic interaction on plant fitness depended of bee abundance we decided to replicate the original experiment. In particular, we addressed the following questions: (1) does the response of foraging bees to ant presence depend on bee abundance? (2) Is the effect of ant presence on plant reproductive success independent of bee abundance?

Materials and methods

The new study was conducted from March to April 2012 at the same population, in MacRitchie Reservoir in the Central Catchment Nature Reserve, Singapore. Weaver ants, *Oecophylla smaragdina*, are extremely aggressive predatory ants that build their nests in a broad range of trees and shrubs by gluing leaves together with larval silk (Crozier, Newey et al. 2010). In our field site about half of the tropical shrub, *Melastoma malabathricum*, acted as host plant for the weaver ants (Gonzálvez, Santamaría et al. 2013). Ants patrolled the entire plant including flowers, where they are frequently observed. *M. malabathricum* flowers do not produce nectar, but produce pollen in two whorls of apically poricidal stamens and the pollen is collected by bees able to sonicate the anthers (Gross 1993, Gonzálvez, Santamaría et al. 2013). The most frequent pollinators in our study site were the large *Xylocopa* bees, being the most effective pollinators of *M. malabathricum* and the small solitary bees, *Nomia strigata*, which were less effective pollinators (Gonzálvez, Santamaría et al. 2013).

Relationship between weaver ant presence and bee visits

We first selected and tagged 20 *M. malabathricum* plants with weaver ant nests and 20 plants without nests. We could not use the same set of individual plants used for the 2010 study (Gonzálvez, Santamaría et al. 2013), because some plants had died and other plants had changed their status (colonized/not colonized by weaver ants) . We observed bee visit rate four times in each plant in pseudo-random order: all plants had been observed n times before any plant was observed n+1 times. At each observation we chose four flowers and recorded over 10 minutes the number of times that *Xylocopa* and *Nomia* bees visited them.

Relationship between weaver ant presence and fruit and seed set

On the 20 plants with weaver ant nests and 20 without nest we marked 10 randomly selected flowers per plant to determine fruit set and seed set per fruit. Fruit set (the probability of fruiting) was determined following fruits until ripening or abortion. Seed set per fruit was estimated collecting all marked fruits that ripened, counting the number of viable seeds in a randomly chosen carpel and multiplying this number by five.

Temporal variation

We compared bee visit rates, fruit set and seed set per fruit between 2010 and 2012. All parameters recorded in 2010 were determined as described above except for the number of replicates. While in 2012 we tagged 20 plants with and 20 without weaver ant nests and 10 flowers per plant to assess fruit set, two year earlier we marked 25 plants with and 25 without ant nests and 12 flowers per plant.

Statistical analyses

To have a straightforward comparison between the 2010 and 2012 datasets, we first conducted on the 2012 dataset the analyses performed on the 2010 dataset. In a second step we analysed the data from both studies together.

To determine the relationship between weaver ant presence and bee visits in 2012 we first averaged the number of *Xylocopa* and *Nomia* visits per plant over the four observation periods. These averages were log-transformed and analysed with a repeated-measures ANOVA, having plant as subject, bee species as within-subject repeated measures, and ant presence as categorical factor. To assess whether the effect of ant presence on bee visit rates differed between 2010 and 2012 we conducted the same analysis with the data from the two studies, including year as categorical factor.

We applied a generalized linear model (GLZ) with binomial distribution and Logit link function to analyse the relationship between the probability of fruiting and weaver ant presence in 2012. The relationship between seed set per fruit and weaver ant presence in 2012 was tested with a general linear model (GLM), considering plant as a random factor. To determine whether ants had consistent effects on fruit set and seed set per fruit, we conducted further analyses on the combined data from 2010 and 2012, adding year as categorical factor, *Xylocopa* visit rate as continuous covariate and the interactions between ant presence, year and *Xylocopa* visit rate. With these analyses, other than comparing the data from both years, we could determine whether variability in *Xylocopa* visit rates within a group of plants (same year and ant colonization status) could account for variability in reproductive success. Except for the addition of the new explanatory variables, the models had the same structure as those for the 2012 dataset. Starting from the full models, we systematically searched for the most parsimonious model, removing non-significant terms (first higher order interactions, then lower order interactions and finally main effects) and selecting the model with the lowest AIC (Akaike 1973).

For the GLZ analyses, we used type II log-likelihood ratio tests to assess statistical significance. All analyses were conducted using R. 2.15.0 (R Development Core Team 2010).

Results

Relationship between weaver ant presence and bee visits

In 2012, *Nomia* bees were seldom observed at *Melastoma* flowers, and the rate at which they visited flowers was more than 11 times smaller than the rate at which *Xylocopa* bees visited them (Fig 1). This difference was highly statistically significant ($F_{1,38}=22.95$; $p<0.0001$). Although the few *Nomia* bees that we observed visited only ant-free plants (not a single *Nomia* bee was seen visiting flowers at ant-harbouring plants), the number of *Xylocopa* visits per hour was similar at plants with and without weaver ant nests (Fig 1). As a result, neither the presence of ants ($F_{1,38}=1.67$ $p=0.20$) or its interaction with bee species ($F_{1,38}=1.01$, $p=0.32$) had statistically significant effects on the average rate at which bees visited flowers.

The data from 2012 contrasted markedly with those obtained two years earlier, when *Xylocopa* bees preferentially exploited flowers on ant-harbouring plants as a result of an indirect effect of resource depletion by *Nomia* bees (Gonzálvez, Santamaría et al. 2013). When we included in the same analysis the number of visits in both years, we found that bees foraged at the same rate at plants with and without weaver ant nests (ant presence: $F_{1,86}=0.11$, $p=0.73$; Fig.1). However, in 2012 the rate at which bees visited *M. malabathricum* flowers was 3.46 times smaller than the rate observed in 2010 (year: $F_{1,86}=11.84$, $P<0.001$). In addition, there was a significant effect of the interaction between the presence of weaver ants and bee species on the overall visit rate (including both years, ant presence x bee species: $F_{1,86}=24.07$, $p<0.0001$). That is, while *Nomia* bees concentrated their foraging effort on ant-free plants, *Xylocopa* bees foraged mainly at ant-harbouring plants.

We found no significant effect of the interaction between ant presence and year on visit rates ($F_{1,86}$ = 0.05, p=0.81) or of the interaction between bee species and year, although in this later case the interaction came close to significance ($F_{1,86}$ = 3.38, p=0.06). Conversely, the interaction between the presence of ants, bee species and year had highly significant effects on visit rates ($F_{1,86}$ = 10.50, p=0.001). Thus, when bees were abundant and competition for resources was strong (2010), small *Nomia* bees foraged mainly at safe ant-free plants and large *Xylocopa* bees foraged at ant-harbouring flowers to avoid resource competition. When, on the other hand, *Nomia* bees were scarce, they had little effect on resource depletion and *Xylocopa* bees exploited all flowers equally (Fig. 1).

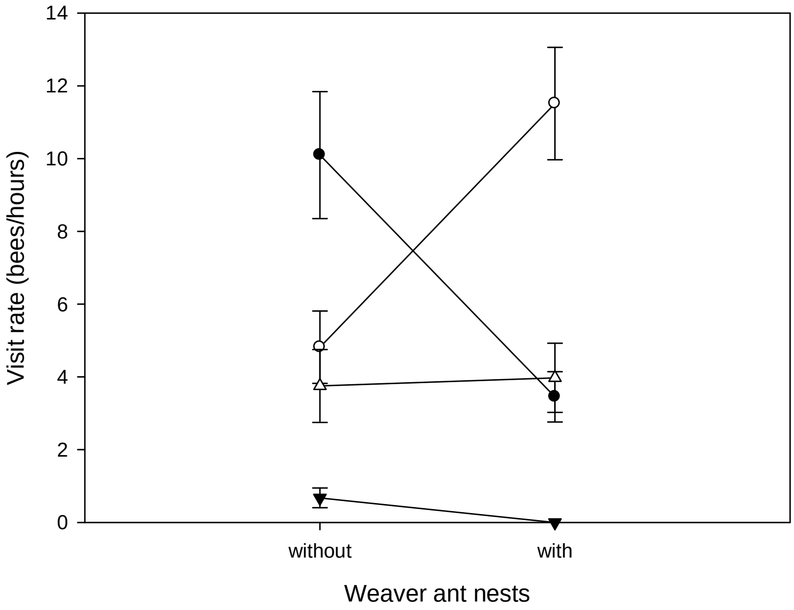


Fig 1. *Visit rates by Nomia (blank symbols) and Xylocopa (empty symbols) in 2010 (circles) and 2012 (triangles) at plants with and without weaver ant nests. Error bars represent standard errors.*

Relationship between weaver ant presence and fruit and seed set

In 2012 *Xylocopa* bees, the most effective pollinator, visited at similar rates plants with and without weaver ant nests (Fig.1). As a result, the proportion of flowers that produced mature fruits was similar in plants with (24.53%) and without (21.88%) weaver ant nests (χ^2=0.26, df=1, p=0.60). Likewise, ant-harboring plants produced comparable numbers of seeds per fruit (378.23 ± 27.40) than plants without ants (359.34 ± 38.06; $F_{1.24}$=0.05, p=0.81; Fig.2).

In contrast, in 2010 we found that fruit set and per fruit seed set were higher at ant-harbouring plants (Gonzálvez, Santamaría et al. 2013). In the comparison of the two studies, fruit set and seed set followed rather similar patterns. In the case of fruit set, the most parsimonious model included the three explanatory variables (year, ant presence and *Xylocopa* visit rate) as well as the interactions between year and the other two variables. Fruit set was more than double in 2010 than in 2012 (Fig. 2a), the differences being statistically significant (year: χ^2=61.01, df=1, p<0.0001). Overall, reproductive success was higher for plants with than without ant nests (ant presence: χ^2=20.57, df=1, p<0.0001), and plants that received more *Xylocopa* visits were more likely to produce fruits (*Xylocopa*: χ^2=9.10, df=1, p=0.003). As we have seen, however, in 2012 fruit set was very similar for plants with and without ant nests, while in 2010 there were clear differences between plants with and without nests (Fig. 2a). Not surprisingly, then, we found a statistically significant effect of the interaction between ant presence and year on fruit set (year x ant presence: χ^2=7.49, df=1, p=0.006). Finally, fruit set increased faster with *Xylocopa* visit rate on 2012, when pollinator limitation was stronger, than on 2010 (year x *Xylocopa*: χ^2=12.31, df=1, p=0.0005).

The general pattern was similar for the seed set (Fig. 2b). Although in this case the most parsimonious model was the full model, including the three explanatory variables (year, ant presence and *Xylocopa* visit rate) and all their interactions, neither the three-way interaction between the explanatory variables ($F_{1,63}$=0.01, p=0.91) or the two-way interaction between *Xylocopa* visit rate and year ($F_{1,63}$=1.44, p=0.23) affected seed set. Seed set was higher in 2010 than in 2012 (year: $F_{1,63}$=65.67, p<0.0001) and, overall, it was also higher at plants with than without ants (ant presence: $F_{1,63}$=23.00, p<0.0001), although the effect of ants on seed set was apparent only during 2010 (ant presences x year interaction: $F_{1,63}$=7.79, p=0.007; Fig. 2b). After controlling for ant presence and year, seed set increased with the rate of *Xylocopa* visits ($F_{1,63}$=29.30, p<0.000), an effect that was mainly present at plants with ant nests (ant presence x *Xylocopa* visit rate interaction: $F_{1,63}$=3.70, p=0.059).

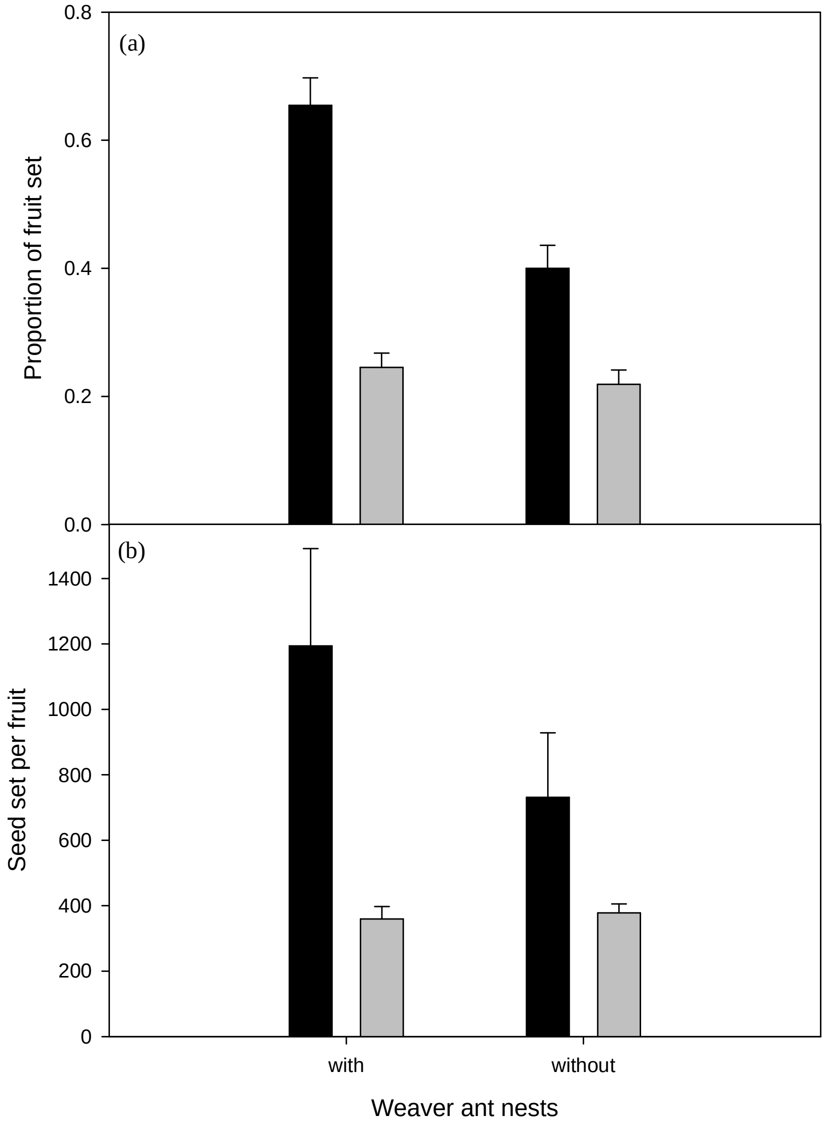


Fig 2. *Effect of weaver ant presence on (a) the proportion of fruit set and (b) seed set per fruit in 2010 (black bars) and 2012 (grey bars). (a) Proportion of flowers producing mature fruits at plants with and without weaver ant nests. (b) Number of viable seed per fruit produce at plants with and without weaver ant nests.*

Discussion

Our results show that temporal variation in the pollinator community modifies the selective pressures that weaver ants exert on their host plants. In 2010 the presence of ants on flowers deterred *Nomia* bees and attracted the most effective pollinator, *Xylocopa* bees. However, two years later *Xylocopa* bees visited plants with and without ant nests similarly often - although *Nomia* bees still foraged mainly at plants without ants, there were so few of them that they hardly affected resource availability. As a consequence, in 2010 fruit set and per-fruit seed set were higher at plants with ant nests, whereas two years later plants with and without nests had similar reproductive success.

Temporal variation in bee behaviour in response to predatory ants

The results of this study support our interpretation that the response of the system is driven by two processes: predator avoidance of *Nomia* bees and intake rate maximization of *Xylocopa* bees. *Nomia* bees avoided plants with ant nests in 2010 and 2012. However, although the relative effect of ants on *Nomia* visit rates was at least as high in 2012 as in 2010, the absolute difference was much higher in 2010. Indeed, if during 2012 we never observed *Nomia* bees visiting plants with ants, visit rates at ant-free plants were also very low (Fig. 1). Thus, pollen consumption by *Nomia* bees during the 2012 season was probably negligible. In contrast, two years earlier *Nomia* bees consumed about half the pollen produced by *Melastoma* plants in our study population (Gonzálvez, Santamaría et al. 2013). As a consequence, if *Xylocopa* bees were to forage at those plants where resource depletion was lower, they should select plants with ants when *Nomia* bees are abundant (2010 data), but they should be indifferent between plants with and without ants in the relative absence of *Nomia* competitors (2012 data).

Temporal variations in selective pressures on the host plant

Our results showed that temporal variation in abundance and composition of the pollinator community leads to temporal variation in plant reproductive success. Bee abundance affected plant reproductive success in two different ways, a direct and an indirect one. First, bee abundance has a direct effect on plant fitness because *Melastoma* is pollinator-limited, and its reproductive success increases with the frequency of bee visits, particularly in the case of the most effective pollinators, *Xylocopa* bees. This was true even after controlling for the overall effect of year and ant presence (Figs. 3 and 4). Second, bee abundance determines the strength of resource competition and the extent to which the most effective pollinator preferentially visited ant-harbouring plants. This second indirect effect was mediated through the presence of predatory ants at some plants. It may not affect the average reproductive success of *M. malabathricum* plants, but affected the variance of this fitness component. As a result, both the mean (direct effect) and variance (indirect effect) of plant reproductive success, measured as fruit set and seed set per fruit, were much higher in 2010, when bee abundance was higher, than in 2012 (Fig 2).

If the link between bee abundance and mean plant reproductive success has mainly ecological implications (i.e., it can affect the competitive ability of *M. malabathricum* in the community), the relationship between bee abundance and the variance of reproductive success has evolutionary consequences. Thus, in years of high bee abundance plant traits favoring colonization by ants will tend to be selected for, given that ant-colonized plants achieve the highest fruit set and per-fruit seed set when bees are abundant. However, when bees are scarce ant presence does not induce resource partition, plants with and without ant nest are visited equally often by *Xylocopa* bees, and traits promoting ant colonization will be selectively neutral. Although this argument ignores other costs and benefits of the ant-plant association (defense against herbivores, reduction in photosynthetic potential…), taking these into account will modify the average magnitude of the selective pressures on traits promoting colonization by ants, but will not change the fact that the magnitude of these selective pressures varies with bee abundance.

Although we have not examined spatial variations in selective pressures promoted by the presence of weaver ants on *M. malabathricum*, we would expect to find differences throughout its distributional range if, for example, the set of pollinators which visit *M. malabathricum* flowers varies geographically. Spatial variations in the identity of pollinators between genetically isolated populations may originate local adaptations in response to the presence of weaver ants by changing the magnitude and direction of the selective pressures on their host plant. In fact, it has been reported that in some areas of North Queensland (Australia) *Trigona carbonaria* is the most common visitor of *M. malabathricum* flowers (Gross 1993). Although, in this population, the pollination effectiveness of the different groups and the effects of ants on pollinator behaviour have not been determined, it seems likely that weaver ants have different effects on plant reproductive success than in our study population.

Temporal variations in bee populations and their implications for tritrophic interactions

Melastoma malabathricum is a perennial shrub (Gross 1993), and therefore the fitness of an individual is linked to its lifelong reproductive success. Temporal variability is then, in a sense, averaged out, and the fitness of ant-colonized and ant-free plants will be related to the time-averaged reproductive success of plants with and without ant nests, respectively. Overall, plants with ant nests had a higher reproductive success than plants without nests, suggesting that there is a net selective pressure on plant traits enhancing colonization by ants and patrolling of flowers. Although this is consistent with the finding that *M. malabathricum* flowers attract weaver ants (Gonzálvez, Santamaría et al. 2013), it is important to point out that a proper calculation of the net selective pressure on ant attractants should be based on much longer time series, and it should take into account other possible costs and benefits of ant colonization.

Fluctuations over time in bee diversity and abundance within populations have been commonly associated to natural factors such as climatic changes and variations on the availability of food resources (Wolda 1978). In fact, climatic variability is one of the factors that may have affected bee abundance in our community, the beginning of 2010 being much drier than the average year (National Environment Agency. Singapore Government 2010). Nowadays, phenomena associated with human activities, such as habitat fragmentation, agriculture and grazing, the use of pesticides and herbicides and the introduction of non-native species, have increasingly stronger effects on pollinator abundance and diversity (Rortais, Arnold et al. 2005, Hendrickx, Maelfait et al. 2007, Stout and Morales 2009, Winfree, Aguilar et al. 2009). Many of these factors tend to decrease the number of pollinators and, thus, to reduce plant reproductive success. However, although the effects of changes in land use on pollinator-plant interactions have been widely reported, their effect on plant-pollinator-predator interactions is, to date, unknown. From this study, however, we may infer that a decrease in bee number in disrupted habitats not only will affect directly plant reproductive success by reducing fruit set and seed set but also will have an impact on plant fitness by affecting the structure of communities as consequence of changes in resource competition and predation risk. In short, global environmental change may modify tritrophic interactions between plants, bees and their predators leading to a homogenization in selective forces and a loss of biotic interactions.

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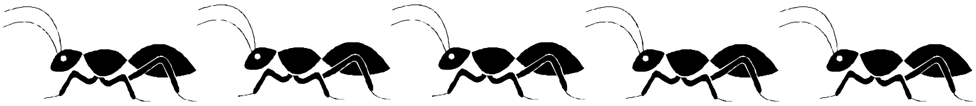
CAPÍTULO 5



Seeing is believing: Information content and behavioural response to visual and chemical cues

Francisco G. Gonzálvez and Miguel A. Rodríguez-Gironés

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Abstract

Predator avoidance and foraging often pose conflicting demands. Animals can decrease mortality risk searching for predators, but searching decreases foraging time and hence intake. We used this principle to investigate how prey should use information to detect, assess and respond to predation risk from an optimal foraging perspective. A mathematical model showed that solitary bees should increase flower examination time in response to predator cues and that the rate of false alarms should be negatively correlated with the relative value of the flower explored. The predatory ant, *Oecophylla smaragdina*, and the harmless ant, *Polyrhachis dives,* differ in the profile of volatiles they emit and in their visual appearance. As predicted, the solitary bee *Nomia strigata* spent more time examining virgin flowers in presence of predator cues than in their absence. Furthermore, the proportion of flowers rejected decreased from morning to noon, as the relative value of virgin flowers increased. In addition, bees responded differently to visual and chemical cues. While chemical cues induced bees to search around flowers, bees detecting visual cues hovered in front of them. These strategies may allow prey to identify the nature of visual cues and to locate the source of chemical cues.

Introduction

Foraging animals can reduce mortality using sensory information to evaluate predation risk and reducing exposure to predators (Lima and Dill 1990). Predator avoidance has important ecological and evolutionary consequences, as it can affect the pattern of interactions within communities (Peacor and Werner 2001) and determine the strength of trophic cascades through indirect behavioural effects (Gonzálvez, Santamaría et al. 2013). Nonetheless, research on this topic presents important biases and some aspects of how animals use sensory information to assess predation risk have been relatively neglected. Thus, although information about the presence of predators is received through several sensory channels, mostly in form of visual and chemical cues (Kelley and Magurran 2003), most studies of anti-predator decision making consider a single sensory modality (Dicke and Grostal 2001). Besides, research on how animals use different sensory modalities to assess predation risk has focused mainly on the threat-sensitivity (graded response in relation to the magnitude of risk) (Helfman 1989) and sensory-compensation (compensation for poor information in one sensory modality by increasing reliance on another sense) (Hartman and Abrahams 2000) hypotheses – which revolve around the ideas that redundancy increases the reliability of cues, and that the efficiency of cues is habitat-specific.

If it is true that information loss during transfer depends on the sensory channel combination, a more important difference is that visual and chemical cues convey different information (Smith and Belk 2001). And yet, little effort has been devoted to study the information content of predator-related cues and the extent to which different cues trigger different behavioural responses – rather than responses of different intensity. Although visual cues have higher resolution in time and space (Kats and Dill 1998, Brown and Cowan 2000), for many taxa they are also more ambiguous than chemical cues (Brown and Magnavacca 2003). In particular, animals with poor visual resolution may find it difficult to discriminate between predatory and harmless species on the basis of visual cues alone. We should therefore expect prey to respond differently, and not just at different intensities, to the presence of visual and chemical cues: if the nature of the cue is ambiguous we would expect prey to seek confirmation of potential threats, while if the ambiguity resides in the spatiotemporal origin of the cue, prey should try to locate the predator.

Solitary bees constitute an ideal system to study the mechanisms of predator avoidance. Bees use chemical and visual information during foraging (Chittka and Thomson 2001). Particularly in solitary bees, there is a very tight link between foraging efficiency, predator avoidance and fitness (Rodriguez-Girones and Bosch 2012). As a result, bees have evolved predator-avoidance strategies (Dukas 2001, Dukas and Morse 2003, Heiling and Herberstein 2004, Dukas 2005, Brechbühl 2009, Gonzálvez, Santamaría et al. 2013) and respond to the trade-off between minimising predation risk and maximising foraging efficiency (Ings and Chittka 2008). Furthermore, it is known that chemical information plays an important role in risk assessment: the response of bees to ambushing crab spiders changes when chemical cues are removed (Heiling, Herberstein et al. 2003), and social bees release alarm pheromones to mark flowers where a predator is hidden (Dukas 2001, Llandres, Gonzálvez et al. 2013). Finally, it is easy to study bees in their natural habitat without interfering with their foraging activity. Studying predator avoidance in the field - rather than in the lab - is important because animals can perceive the laboratory environment as very dangerous, a perception that can affect the outcome of experiments (Magurran, Irving et al. 1996, Hartman and Abrahams 2000).

While empirical studies on the use of different sensory modalities to assess predation risk have focused on the issues of additivity and sensory compensation, theoretical investigations of anti-predator behaviour normally look at the relationship between predation risk and optimal behaviour (Brown, Laundre et al. 1999). Models typically assume that prey have perfect information of predation risk, and ignore the process of risk assessment (Lima and Steury 2005). In this paper we concentrate on how prey use predator-related visual and chemical cues to detect, assess and respond to predation risk. To integrate our work into an optimal foraging perspective, we first developed a mathematical model to determine how environmental parameters affect the time that bees should spend examining flowers prior to deciding whether to land on them. We then examined in the field the behaviour of female solitary bees, *Nomia strigata*, visiting flowers with and without visual and chemical cues from predatory, *Oecophylla smaragdina*, and harmless, *Polyrhachis dives*, ants. We had previously confirmed, using gas chromatography-mass spectrometry (GC-MS), that the two ant species differed in the profile of cuticular volatiles they emitted. If bees were able to discriminate between the two ant species, they should treat *P. dives* flowers as safe flowers. Otherwise, they should treat them as dangerous flowers. The model predicted that bees should spend more time examining flowers that they perceive as riskier and that the probability of false alarms should decrease from early morning to noon as resources become depleted. As for the nature of the response, chemical cues indicate that a predator is, or has been, near the flower (Smith and Belk 2001). Chemical cues should therefore induce bees to search for predators. Visual cues, on the other hand, indicate that the flower is occupied by another individual. In response to visual cues, bees should attempt to determine whether the flower occupant is dangerous or harmless.

Material and methods

Model description

In this section we offer a verbal description of the model. A detailed mathematical treatment is provided as appendix I. The aim of the model is to calculate the predator-avoidance strategy that maximises the expected fitness of female solitary bees. We first develop a basic model to study the optimal relationship between examination time and environmental parameters, ignoring the effect of distinct predator cues. We then extend the basic model in two directions: introducing predator cues and variability in the reward offered by different flowers.

The basic model assumes that, upon approaching a flower, bees can spend a certain time examining it prior to deciding whether to land or move on and search for a new flower. Flower examination decreases the uncertainty concerning the presence or absence of a predator at the flower, and given enough time a bee could be certain that the flower does, or does not, harbour a predator. However, a bee that spends a long time examining each flower will visit few flowers per day – there is a trade-off between increasing intake rate and decreasing predation risk – so the optimal strategy will normally consist of collecting only partial information. We model information acquisition as a Bayesian-like process. Upon approaching a flower, bees have a prior expectation that the flower harbours a predator. This prior expectation depends on the abundance of predators in the environment. Information is updated during flower examination, leading to a posterior expectation that the examined flower harbours a predator. Because examination is a stochastic process, the posterior expectation is not fully determined by the presence or absence of a predator at the flower. Rather, it is a random variable, and its mean and variance depend on whether the flower harbours a predator, and on the amount of time invested in examining the flower. Longer times lead to more accurate expectations. Finally, the predator avoidance strategy of bees, in this cue-free basic model, is defined by two parameters: flower examination time and a rejection criterion – essentially, a value of the perceived level of threat above which bees abandon flowers without landing.

Ignoring other sources of mortality and resource limitation, the expected fitness of a female solitary bee will be the number of eggs she can provision before dying (Rodriguez-Girones and Bosch 2012). We assume that bees have a fixed lifespan, so that the number of eggs they can lay, if they manage to avoid predation, is determined by the time required to provision eggs. The two components of the predator avoidance strategy affects this time. Lower rejection criteria lead to higher number of false alarms (rejected safe flowers), and hence higher provisioning times because bees must visit more flowers to provision an egg. The effect of examination time on provisioning, however, time is more subtle: longer examination times tend to increase provisioning time, because bees spend more time per flower, but they can decrease the number of false alarms, shortening provisioning times. At the same time, however, increasing the examination time and lowering the rejection criterion increase the probability of surviving to rear an offspring. Expected fitness is an increasing function of this probability of surviving, and a decreasing function of the time required to provision an egg (Rodriguez-Girones 2012). The model is restricted to solitary bees because the relationship between foraging efficiency, survival and fitness differs between social and solitary bees (Clark and Dukas 1994, Rodriguez-Girones and Bosch 2012). We consider two extensions of the basic model.

First, we study how bees should respond to the detection of cues (Appendix I b). For simplicity, we assume that cue detection is an immediate, all-or-none process.

Furthermore, we consider a single cue type, ignoring any difference in the information that visual and chemical cues may convey. An explicit treatment of these differences would require the development of a cognitive model and is beyond the scope of this paper. Bees may detect predator cues at safe and dangerous flowers, although the probability of detecting them is greater at dangerous flowers. Thus, bees may detect visual cues at safe flowers if a harmless insect walks on the petals, and they can detect chemical cues if a predator is, or has recently been, in the neighbourhood of the flower they approach. Likewise, bees may fail to detect cues when approaching dangerous flowers. The predator may be hidden in the underside of the flower, and the wind may blow volatiles away from the approaching bee. Within this framework, we derive the optimal response of bees when the probability of detecting a cue is $0.5 + \rho$ if the flower harbours a predator, and $0.5 - \rho$ otherwise. Note that the parameter ρ is tightly linked to the reliability of the cue: when $\rho = 0$, cues convey no information. On the other hand, when $\rho = 0.5$, cues become maximally informative.

Finally, we extend the basic model to study the optimal strategy of bees when they encounter rich and poor flowers, differing in the amount of resources they offer – in the absence of predator cues. We assume that bees can discriminate between rich and poor flowers once they are sufficiently close to them, although bees cannot discriminate between rich and poor flowers at a distance (Goulson, Chapman et al. 2001), so that they encounter flowers at random (that is: bees cannot specialise in visiting only rich flowers). In this situation, the foraging strategy of bees consists of four parameters: two examination times and two rejection criteria (one pair of parameters for each flower type, rich and poor). As in the previous case, these parameters determine the probabilities of landing at safe and dangerous flowers, and from these probabilities we can calculate average time required to provision an egg, and the probability of surviving through egg provisioning. From these quantities, we can easily compute expected fitness (Appendix I c).

The biology of our model species, *N. strigata*, is not known with sufficient detail to estimate the parameters involved in the model. For this reason, we can only use our model to make qualitative predictions (Appendix I d). Although we cannot experimentally measure the rejection threshold of bees, we can measure examination times and proportion of false alarms, and compare them to model predictions. Thus, we can evaluate how examination times and proportion of false alarms should change with the overall predation risk, with the conspicuousness of predators, or the number of flowers that bees must exploit in order to provision an egg.

Study site and species

We tested the model comparing the behaviour of solitary bees, *Nomia strigata*, exploiting nectarless *Melastoma malabathricum* flowers with and without cues associated to the presence of two ant species: the predatory weaver ant, *Oecophylla smaragdina*, and the harmless *Polyrhachis dives*. We run two independent experiments to study the effect of chemical and visual cues. We selected this system because small bees avoid rambutan trees (*Nephelium lappaceum*) with *O. smaragdina* but no with *P. dives* ants (Tsuji, Hasyim et al. 2004) and, in particular, *N. strigata* bees exhibit strong antipredator behaviour when exploiting flowers at *M. malabathricum* plants harbouring *O. smaragdina* nests (Gonzálvez, Santamaría et al. 2013). We carried out all experimental work at MacRitchie Reservoir in the Central Catchment Nature Reserve, Singapore, from early June to late July 2010 and within a geographical range of 100 m. Because *N. strigata* seldom forages at plants with weaver ant nests (Gonzálvez, Santamaría et al. 2013), all observations were conducted at ant-free plants on sunny, windless days during the peak activity period of *N. strigata* (8:30 - 12:00). *N. strigata* visit rate at ant-free plants was on average 10.2 bees h^{-1} per flower. Weaver ants were the only predators that we observed attacking *N. strigata* bees in our study site.

Effect of chemical cues

GC-MS analysis revealed that the two ant species differed in the volatile mixtures they emitted (Appendix II and Figure S1), offering bees the possibility to use chemical information to discriminate between them and detect predatory ants.

We bagged flowers before anthesis to prevent pollen removal by foraging bees and scent contamination by bees or patrolling ants (Stout and Goulson 2001). Using forceps we placed a living ant (*O. smaragdina* or *P. dives*, depending on the treatment) inside each bag. The ant was free to patrol the flower surface for at least one hour. When it was time to use a flower for the observations, we removed both bag and ant, cut off the floral stem, fastened it with clips to an ant-free shrub and videotaped the arrival of the first *N. strigata* bee to the flower. Flowers in the control treatment were subject to the same manipulation, except that no ant was introduced in the bag. Each treatment was replicated 50 times. Flowers and ants were used only once (i.e., we recorded a single bee visit per flower), and flowers were allocated to treatment sequentially (one flower per treatment), to prevent correlations between time and treatment. Although bees were not marked, to minimise the probability of using the same bee several times we conducted observations at spatially separate plants. We observed one flower at a time.

Effect of visual cues

We used dead ants as visual cues. Ants (*O. smaragdina* or *P. dives*, depending on the treatment) were frozen to death and kept for two days in a well-ventilated room before the observations. GC-MS analysis confirmed that dead ants did not differ in the cuticular volatiles they emitted (Appendix II and Figure S2), so that bees could not use chemical cues to discriminate between them. On the other hand, humans can easily discriminate between the two species by sight. Body size and colour are the most conspicuous differences: *O. smaragdina* ants (mean ± standard deviation: 9.50±0.32 mm, $n = 20$) were longer than *P. dives* ants (5.60±0.26 mm, $n = 20$), and this difference was a reliable indicator of ant species (t test: $t_{38} = 41.65$, $p < 0.0001$). *O. smaragdina* ants were also more brownish than *P. dives* ants (Figure S3). In principle, then, bees could use visual cues, such as body size, to discriminate between the two ant species.

We bagged ant-free flowers as explained above. When it was time to use a flower we removed its bag, cut off the floral stem, fastened it with clips to an ant-free shrub, glued a dead ant with a drop of Loctite Super Glue to the middle of a haphazardly selected petal of the flower and recorded the arrival of the first *N. strigata* bee to the flower. Control flowers had a drop of glue but no dead ant. Each treatment was replicated 50 times.

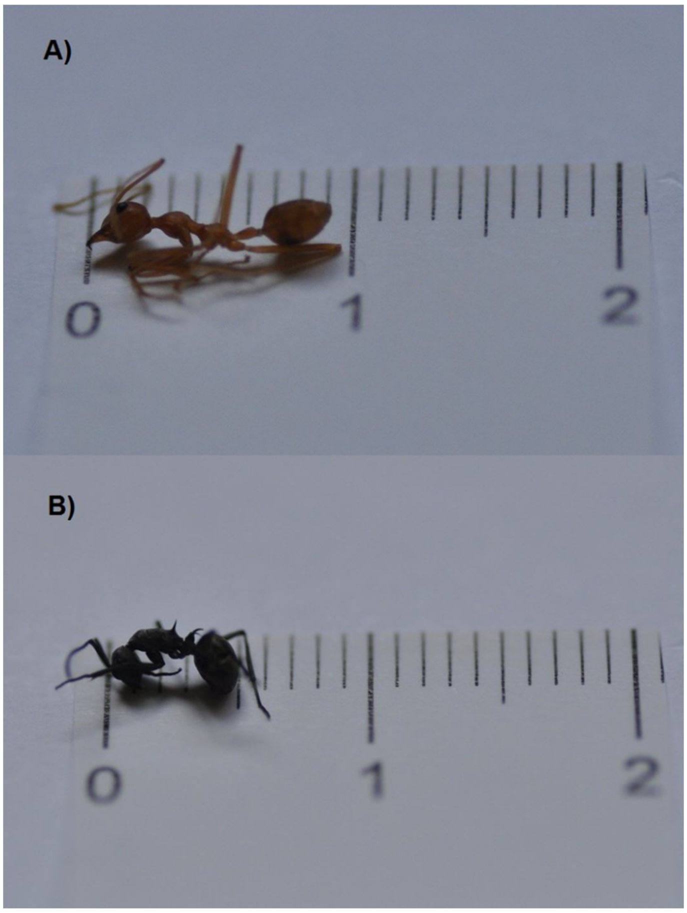

Fig S3. *Picture showing the body size of (A) a O. smaragdina, and (B) P. dives. Note the size and colour differences. O. smaragina is longer and more brownish than P. dives.*

Video analysis

We recorded the arrival of the first bee with a Sony DCR-SR4 Handycam camcorder. Using Picture Motion Browser, we extracted the following information from each video: examination time, and presence or absence of hovering, exploration and rejection. Examination time was quantified as the time elapsed since bees first approached within approximately 5 cm of flowers until they either landed on them or left them to search for another flower. We selected 5 cm as a standard distance from flowers because it was the approximate length at which bees halted their approach flight. Nevertheless, because the initial approach of bees to flowers was very fast, changing this distance would lead to very minor changes in examination time. We adopted the following operational definitions of hovering, exploration and rejection. A bee hovered when she performed side-to-side scans (Ings, Wang et al. 2012) in front of ants/petals during at least one second. A bee explored a flower when she approached it within 5 cm and then performed flights (not necessarily within 5 cm of the flower) under and/or around the flower. Flower approaches ended when the bee "landed" on the flower to exploit its pollen or "rejected" the flower and moved on to another one. Examination time was a continuous variable, while hovering, exploration and rejection were binomial variables (i.e., we scored whether bees did or did not perform these behaviours).

Statistical analyses

We used generalised linear models (GLM) to study the effect of time of day, cue type (visual *vs*. chemical), ant species (*O. smaragdina*, *P. dives* or no ant – control) and the interaction between cue type and ant species on the following response variables: examination time, probability of rejecting flowers, probability of hovering and probability of exploring flowers. For the examination times, the GLM had gamma distribution and inverse link function, while for the probabilities they had binomial distribution and logit link function. Unless otherwise specified, analyses were performed using R 2.15.0 (R Development Core Team 2010). We applied type II log likelihood ratio (LR) tests – function Anova (model, type = "II", test.statistic = "LR") – to calculate significance levels and used planned contrasts – function contrast(model, list1, list2) – to make pairwise comparisons between groups.

To compare examination times prior to the rejection or acceptance of flowers with dead *O. smaragdina* ants, we performed a general linear model on log-transformed data, using bee decision (rejection *vs.* acceptance) as fixed factor and time of day as covariate.

In four out of six experimental groups, all bees eventually landed on the flower they approached, preventing the algorithm implemented by R to converge. For this particular test, we therefore calculated model likelihood as explained by (Barnett and Dobson 2008), set the probabilities of rejection equal to 0 in the four groups where all flowers were accepted and estimated the remaining parameters using the Solver command of Microsoft Excel 2010. As in previous cases, we used likelihood ratio tests to calculate significance levels. Data are available in the electronic supplemental material (Table S1).

Results

Model results

The optimal values of the examination time and rejection threshold increased with the proportion of dangerous flowers, and examination time was greater for cryptic than for conspicuous predators (Fig. 1a, b). We can therefore use examination time as an indicator of risk assessment: examination times should increase with the level of threat perceived by bees. The proportion of false alarms (the proportion of safe flowers that bees reject after examination) attained a maximum for intermediate values of the proportion of dangerous flowers and was also greater for cryptic than conspicuous predators (Fig. 1c).

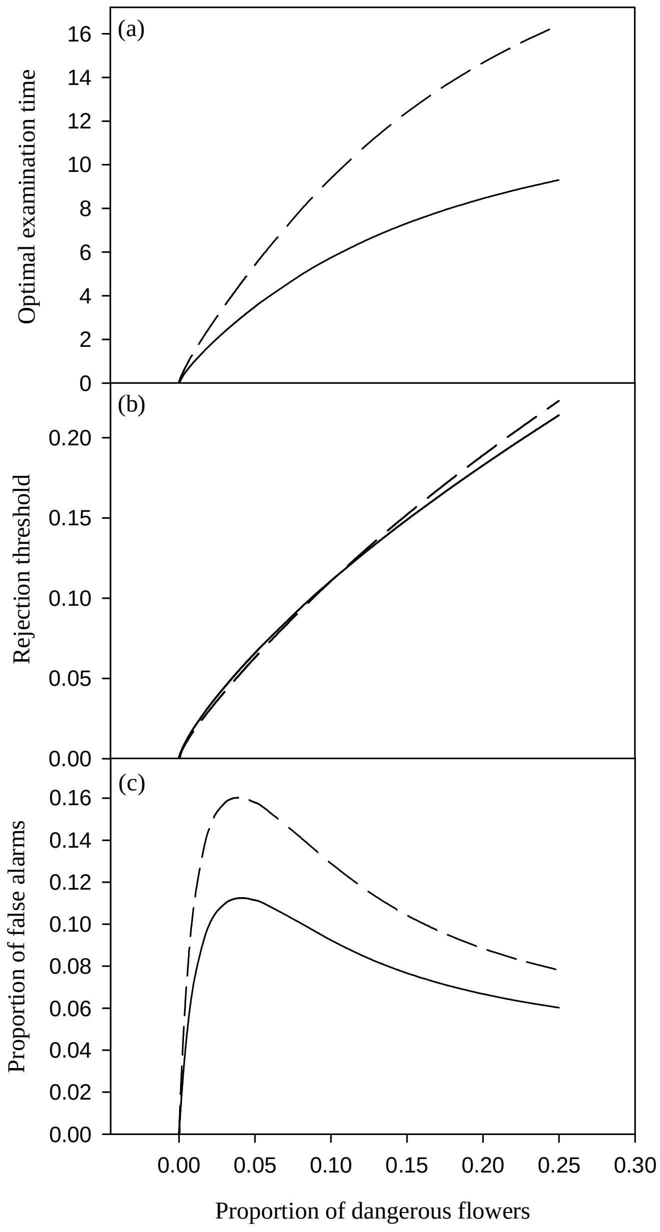

Fig 1. *Effect of predator abundance and conspicuousness. Optimal examination time, in seconds (a), rejection threshold (b) and proportion of false alarms (c) for different abundances of conspicuous (k_s = 0.1, k_d = 0.5; solid line) and cryptic (k_s = 0.05, k_d = 0.25; dashed line) predators.*

Surprisingly, the optimal predator-avoidance response was essentially independent of the number of flowers that bees must exploit to provision an offspring, although expected fitness increased as the number of flowers that must be exploited decreased (data not shown). These results imply that bees should behave similarly in rich environments, with a high level of reward per flower, and poor environments, where many more flowers must be exploited to provision an offspring. This, however, does not mean that bees should pay no attention to the reward offered by flowers, as shown by the predictions of the model for heterogeneous environments. When, within one environment, flowers differed in the reward they offered, optimal examination times and rejection thresholds were relatively similar at both flower types. For the particular example we considered, examination times were typically 10% longer at more rewarding flowers, and rejection thresholds about 20% greater (Fig. 2a, b). The rate of false alarms, however, was almost three times greater at poor than at rich flowers (Fig. 2c).

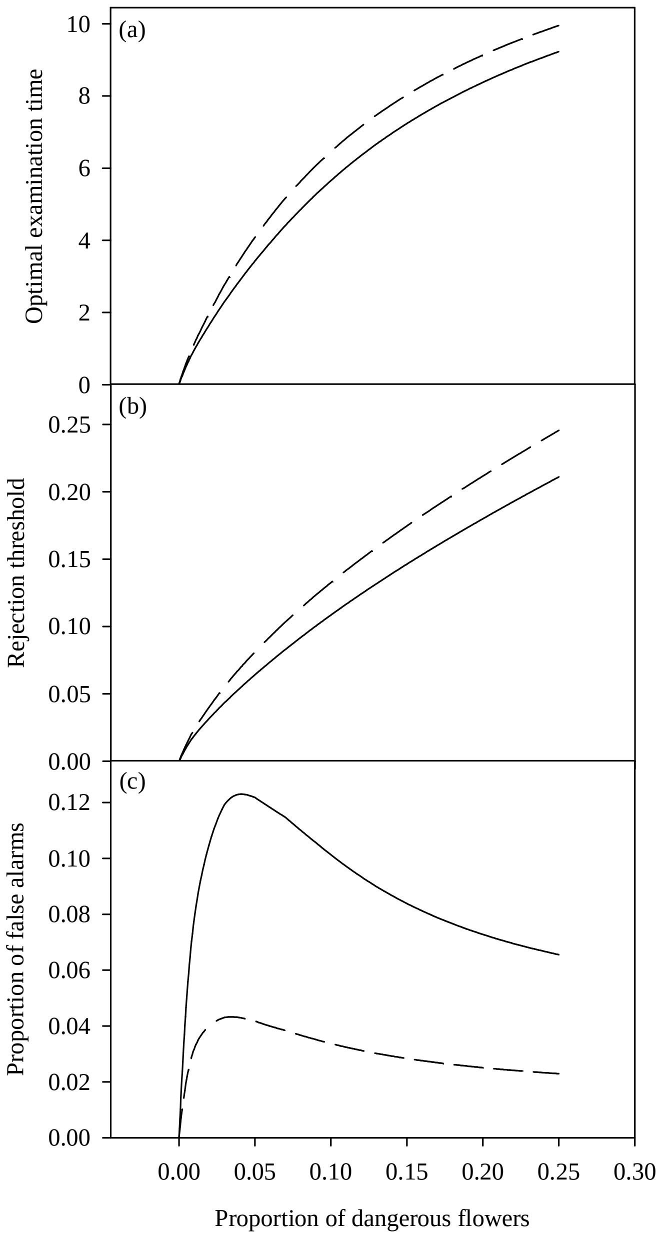

Fig 2. *Effect of variability in reward. Optimal examination time, in seconds (a), rejection threshold (b) and proportion of false alarms (c) for poor (solid line) and rich (dashed line) flowers in a heterogeneous environment.*

When the probabilities that safe and dangerous flowers were associated with predator cues were given by 0.5 - ρ and 0.5 + ρ, respectively, the value of ρ determined the conditional probability that a flower where cues had, or had not, been detected harboured a predator. When ρ increased, the probability that a flower was dangerous decreased if no cues were detected, and increased if cues were detected. As a result, the optimal examination time increased with ρ at flowers where cues were detected and decreased at flower where no cues were detected (Fig. 3).

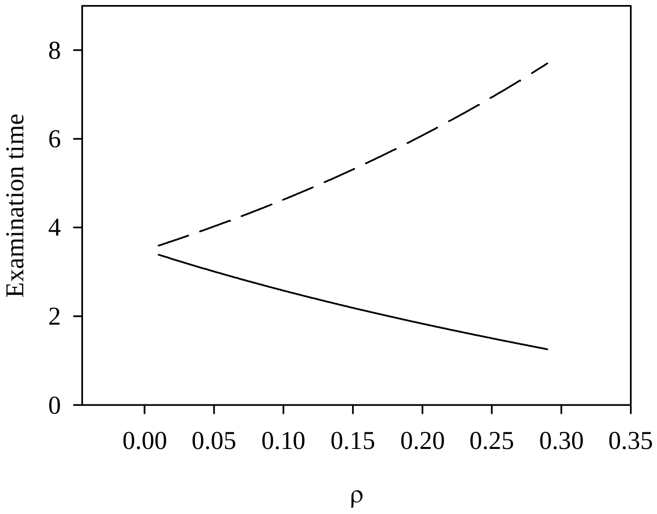

Fig 3. *Expected effect of cue detection. Examination time at flowers where predator cues are detected (dashed line), and where no cue is detected (solid line) as a function of □. Parameter values as for conspicuous predators in Fig. 1.*

Experimental results

*Examination times***.** We recorded the approach of *N. strigata* bees to unvisited *M. malabathricum* flowers with a dead ant glued to a petal (visual cue) or to flowers impregnated with the smell of ants (chemical cue). Time of day had no effect on examination time (LR: $\chi^2_1 = 0.97$, $p = 0.32$), but there was a significant effect of the interaction between ant species and cue type on examination time (LR: $\chi^2_2 = 7.14$, p =

0.028): examination times were longer at flowers with *O. smaragdina* cues than at control flowers or flowers with *P. dives* cues, but examination times at control flowers and flowers with *P. dives* cues only differed for visual cues (Fig. 4). Indeed, planned contrasts showed that examination times were longer when bees approached flowers with *O. smaragdina* cues than when they approached flowers with *P. dives* cues, regardless of whether the flowers contained visual ($t_{294} = 3.54$, $p = 0.0005$) or chemical ($t_{294} = 5.57$, $p < 0.0001$) cues. Furthermore, examination times at flowers with *P. dives* visual cues were longer than at control flowers ($t_{294} = 3.22$, $p = 0.0014$), but there was no difference in examination times at control flowers and flowers with *P. dives* chemical cues ($t_{294} = 0.14$, $p = 0.89$).

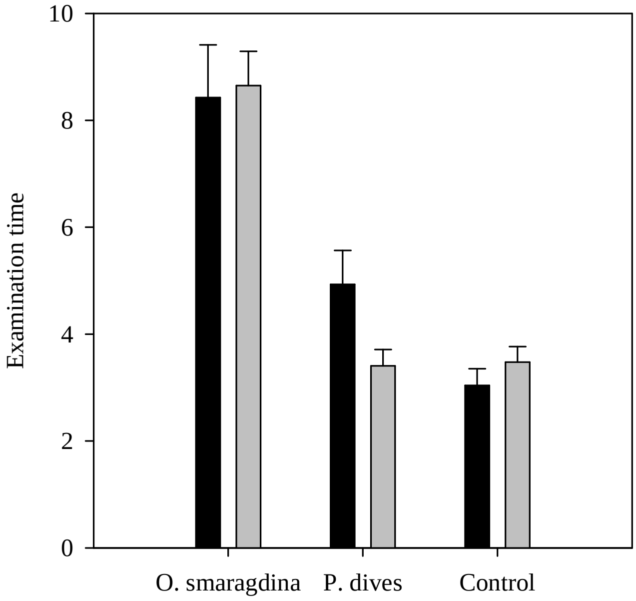

Fig 4. *Average examination times (s) of Nomia bees at flowers with visual (black bars) and chemical (grey bars) cues from O. smaragdina and P. dives ants and control flowers. Error bars represent 95% confidence intervals.*

*Nature of behavioural response***.** The probability that *Nomia* bees explored flowers was independent of time of day (LR: $\chi^2_1 = 0.13$, $p = 0.72$), but was affected by the interaction between ant species and cue type (LR: $\chi^2_2 = 19.00$, $p < 0.0001$; Fig. 5a): the probability of exploring flowers was similar when bees encountered *O. smaragdina* or *P. dives* visual cues ($t_{294} < 0.01$, $p > 0.99$), but in the presence of chemical cues the probability of exploring flowers was much higher if the cues were associated with *O. smaragdina* than with *P. dives* ants ($t_{294} = 5.39$, $p < 0.0001$). The probability of exploring was similar for bees encountering control flowers or flowers with *P. dives* chemical cues ($t_{294} = -0.96$, $p = 0.34$), but bees encountering flowers with *P. dives* visual cues were more likely to explore than bees encountering control flowers ($t_{294} = 2.61$, $p = 0.01$). Finally, when bees encountered flowers with *O. smaragdina* cues, they were more likely to explore them if flowers had chemical than if they had visual cues ($t_{294} = -2.28$, $p = 0.02$).

We turn now to the hovering response. Bees were more likely to hover early in the morning than towards noon (LR: $\chi^2_1 = 4.66$, $p = 0.03$), and there was a significant effect of the interaction between cue type and ant species on the probability of hovering (LR: $\chi^2_2 = 7.45$, $p = 0.02$; Fig. 5b). This interaction stems from a much greater difference in the probability of hovering between visual and chemical *O. smaragdina* cues ($t_{294} = 4.00$, $p = 0.0001$) than between visual and chemical *P. dives* cues ($t_{294} = 1.21$, $p = 0.2276$). Furthermore, bees were more likely to hover in front of a dead *O. smaragdina* than *P. dives* ant ($t_{294} = 3.47$, $p = 0.0006$), while the probability of hovering in front of a flower with chemical cues from either species was as low as for control flowers (*O. smaragdina vs. P. dives*: $t_{294} = 0.35$, $p = 0.73$; *P. dives vs*. control: $t_{294} = 0.39$, $p = 0.70$)

False alarms. All bees approaching flowers with chemical cues eventually landed on them (Fig. 5c). For bees in the visual-cue treatment, all bees accepted control flowers, but one bee (out of 50: 2%) rejected a flower with a dead *P. dives* ant, and the number of bees rejecting flowers was greatest (9 out of 50: 18%) for flowers with a dead *O. smaragdina* (Fig. 5c). Both cue type (LR: $\chi^2_1 = 17.65$, $p < 0.0001$) and ant species (LR: $\chi^2_2 = 16.07$, $p = 0.0003$) had statistically significant effects on the probability that bees rejected flowers, but the effect of their interaction was not significant (LR $\chi^2_2 < 0.01$, $p > 0.99$). The probability that bees rejected flowers was greatest in the early morning and decreased as the morning progressed, the effect of time of day being statistically significant (LR: $\chi^2_1 = 5.08$, $p = 0.024$).

When flowers had a dead *O. smaragdina* ant, examination times were shorter for bees that rejected the flower (4.97 ± 4.38 s.) than for bees that eventually landed on the flower (9.18 ± 7.22 s.), a difference that was statistically significant ($F_{1,47} = 6.76$, $p = 0.012$).

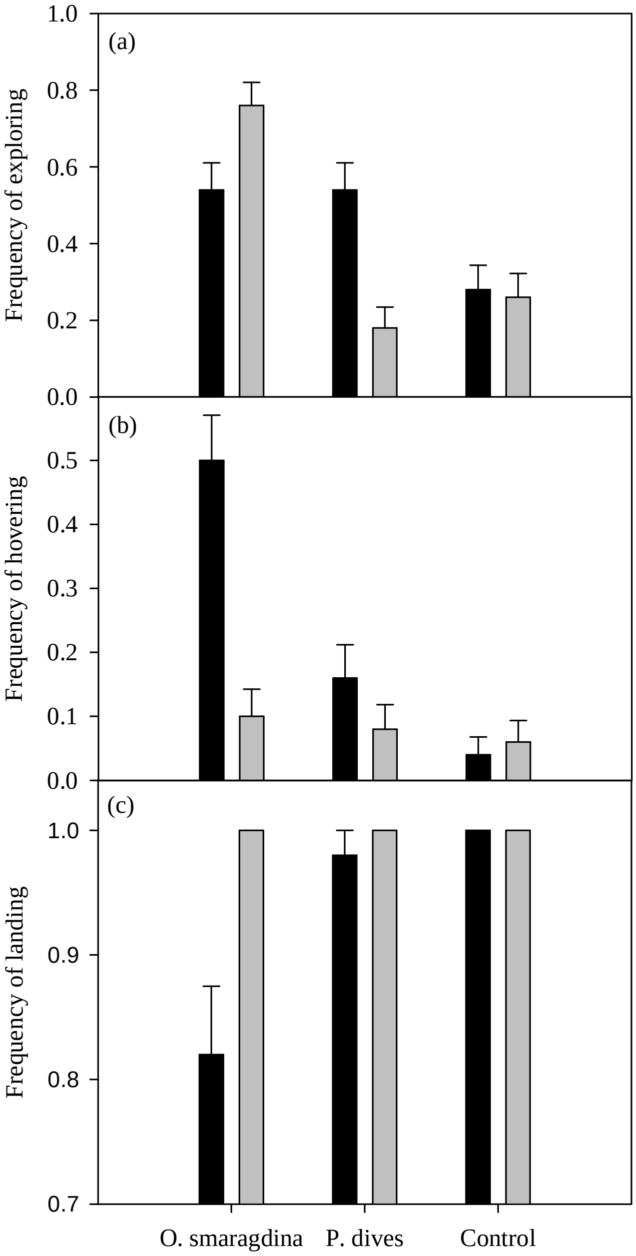

Fig 5. *Frequency of bees (a) exploring under flowers and/or performing fast movements around them, (b) hovering in front of flowers and (c) landing on flowers with visual (black bars) and chemical (grey bars) cues from O. smaragdina and P. dives ants and control flowers. Error bars represent 95% confidence intervals.*

Discussion

Rather than asking whether the response of prey species to different predator cues is additive, or depends on environmental condition, our experiments were designed to test whether the nature of the response depends on the information conveyed by the cues. As predicted, we found that bees spent more time examining flowers with cues from the predatory ant, *O. smaragdina*, than flowers without cues or with cues from a harmless ant, *P. dives*. Besides, bees engaged in different behaviours in response to visual and chemical cues: bees hovered in front of visual cues and explored flowers where they had detected a chemical cue. Presumably, these responses helped bees to determine whether visual cues corresponded to a predator and to locate the source of the chemical cues.

The model we have presented can help us understand a number of results, such as the increase in examination time in response to predator cues and the decrease in the rate of false alarms from early morning towards noon. Optimal examination time increases with the expectation, previous to examination, that the flower harbours a predator (Fig. 1). In the absence of cues, this expectation is simply the proportion of flowers harbouring predators in the patch or population. When cues are detected, however, the expectation that the flower harbours a predator exceeds the proportion of predator-harbouring flowers, triggering an increase in optimal exploration time (Fig. 3). The relationship between time of day and proportion of false alarms is less intuitive. While experiments were conducted on bagged, virgin flowers with a full pollen crop, the amount of pollen available at open flowers decreased exponentially with time of day (Gonzálvez, Santamaría et al. 2013). Early in the morning, when flowers open, bees forage in a homogeneous, rich environment. However, as resources become depleted, the environment becomes more and more heterogeneous, with experimental flowers offering more resources than the average flower. Because, in heterogeneous environments, the frequency of false alarms should be greater for poor than for rich flowers (Fig. 2), the probability of false alarms should decrease from early morning to noon, as the relative value of experimental flowers increases. It should be noted, however, that we cannot conclude from our observation that the decrease in the rate of false alarms was due to an increase in the relative value of previously unvisited flowers. Additional work would be required to confirm this mechanistic link.

The model also predicted results from previous experiments, such as the increase in exploration time, and in the rate of false alarms, when predators are cryptic (Ings and Chittka 2008). The model, however, failed to explain other results, and these failures are as informative as its successes. The model did not predict that visual and chemical cues should trigger different response types. In contrast, it predicted an increase in examination time, from morning to noon, which was not observed. These failures underline the need to develop dynamic cognitive models of predator avoidance. Thus, the model assumed that flower examination time would be pre-determined upon arrival to a flower. In practice, it makes more sense to abandon a flower as soon as a predator has been unambiguously detected. If we are to include this feature into a model, however, the model should explicitly address the dynamic nature of information acquisition. Likewise, our model did not incorporate differences in information content from different cue types, or the possibility of choosing among several behaviours in response to perceived cues. It could therefore not predict that visual and chemical cues would trigger different responses. A cognitive model is better suited to incorporate these nuances. A cognitive model would also allow the incorporation of features such as learning and memory, which have been shown to play a role in predator avoidance (Ings and Chittka 2008, Ferrari, Vrtelova et al. 2012, Ings, Wang et al. 2012). If applying a cognitive model to explain the behaviour of insects may, at first, seem odd, we should keep in mind that insects are capable of performing complex cognitive tasks, such as rule learning and categorisation (Giurfa, Zhang et al. 2001) or numerosity (Dacke and Srinivasan 2008, Carazo, Font et al. 2009). Indeed, it has been argued that brain size is more likely to be correlated with the degree of detail and precision of perception than with cognitive capacity (Chittka and Niven 2009).

In our experiments, bees responded differently to visual and chemical cues associated with predatory ants. Although bees increased the time they spent examining flowers prior to landing in response to both cue types (Fig. 4), the behaviours they engaged during flower examination depended on the type of cue perceived. Upon detection of a chemical cue, most bees engaged in exploratory behaviour, flying around and under the flower (Fig. 5a). Because chemical cues have little spatiotemporal resolution (Smith and Belk 2001), detection of these cues does not necessarily imply that the flower harbours a predator – the predator may be in a nearby flower or may be already gone. It makes therefore sense to determine whether the predator represents a threat before abandoning the flower. Interestingly, chemical cues never led to flower rejection – bees only rejected flowers when dead ants were present. Upon detection of visual cues, however, some bees engaged in exploration and others hovered in front of the dead ant (Fig. 5b). The side-to-side scans performed during hovering probably helped bees fixate the image, contributing to the discrimination between predatory and harmless ants (Ings, Wang et al. 2012).

A key step of threat assessment is the discrimination between predatory and harmless species (Mathis and Vincent 2000). Certain cues can be used to identify as predators individuals belonging to different taxonomic groups. Thus, a volatile in the urine of most mammalian carnivore predators triggers predator-avoidance responses in mice (Ferrero, Lemon et al. 2011). When predatory and harmless species are closely related, however, discrimination between them may be more difficult. For instance, wall lizards, *Podarcis muralis*, presented with visual and chemical cues were unable to discriminate between predatory and harmless snakes when entering a refuge (Amo, Lopez et al. 2006). In this respect, we can conclude that chemical cues provide reliable information about predator identity: *N. strigata* bees responded to chemical cues from the predatory *O. smaragdina* ant, but not from the harmless *P. dives* ants (Figs. 4 and 5). Given the distinctness of the bouquet of cuticular volatiles from the two species (Fig. S1) and the highly developed scent-recognition system of bees (Chittka and Thomson 2001), this difference was to be expected. Visual cues, however, were less specific: visual cues from harmless ants triggered a predator-avoidance response, similar in nature, although weaker, to the response triggered by predatory ant visual cues (Figs. 4 and 5). Once again, this result is consistent with the limited spatial resolution and image-forming capabilities of the bee's visual system (Srinivasan and Lehrer 1988).

Although the use of chemical information about risk by terrestrial insects has been questioned (Kats and Dill 1998), our results add to the growing evidence that chemical cues play a key role in predator avoidance by terrestrial insects. For instance, it has been reported that chemical cues from *O. smaragdina* deter fruit flies from ovipositing in mangoes on which ants have patrolled (Van Mele, Vayssieres et al. 2009), and that social bees mark with alarm signals flowers where they have been attacked by a predator (Llandres, Gonzálvez et al. 2013). Visual cues also trigger predator avoidance in bees. Bumblebees, *Bombus terrestris*, avoid flowers where cryptic predators lie in ambush (Ings and Chittka 2008), and it has been shown that bumblebees learn to avoid specific shapes, rather than colour contrasts (Ings, Wang et al. 2012). Likewise, honeybees, *Apis mellifera*, use visual cues to detect predators. Although the exact features that they use to identify predators are unknown, size, colour contrast and movement affect the probability that honeybees detected cryptic crab spiders (Llandres and Rodriguez-Girones 2011). In our experiment, *N. strigata* bees responded differently to the presence of dead *O. smaragdina* and *P. dives* ants. These ants differ in a number of traits, size being the most conspicuous one. Bees may have used body size to discriminate between predatory and harmless species, but it is also possible that size acts as a non-specific feature, much as the volatile in the urine of carnivores (Ferrero, Lemon et al. 2011), and that bigger visual stimuli, of any kind, are perceived as more threatening.

Predator avoidance has important ecological and evolutionary consequences. Predators affect the structure of food webs and ecological communities, and the indirect effects of predators, mediated by the predator avoidance responses of their prey, are at least as strong as the direct effects, mediated by prey consumption (Peacor and Werner 2001, Gonzálvez, Santamaría et al. 2013). To understand these indirect effects it is important to study the behavioural response of prey to predator cues. In the system we study, the increase in the time that *N. strigata* bees spend examining flowers upon detection of *O. smaragdina* cues explains why the foraging efficiency of *N. strigata* bees is higher at ant-free than at ant-harbouring plants. This difference in foraging efficiency eventually explains why large *Xylocopa* bees, which are not susceptible to predation from *O. smaragdina* ants, preferentially forage at ant-harbouring *M. malabathricum* plants. The preference of *Xylocopa* bees, in turn, explains the higher reproductive success of plants with *O. smaragdina* nests (Gonzálvez, Santamaría et al. 2013).

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Appendix S1. The model

a) Generalities

For reasons that will be clear below, the present model applies only to female solitary bees. We first study the optimal relationship between examination time and environmental parameters, ignoring the effect of distinct predator cues. We assume that, upon approaching a flower, bees can spend a certain time examining it prior to deciding whether to land or move on and search for a new flower. Flower examination decreases the uncertainty concerning the presence or absence of a predator at the flower.

Ignoring other sources of mortality and resource limitation, the expected fitness of a solitary bee will be the number of eggs she can provision before dying (Rodriguez-Girones and Bosch 2012). We assume that bees are time limited: it is time, and not physiology or resources, what limits the number of eggs that bees can provision. If the maximum number of eggs that a bee can provision is *M*, and we denote by *S* the probability that a bee survives the time required to provision an offspring, then the average number of eggs that a bee will lay, *W*, is (Rodriguez-Girones 2012)

$$W = \frac{S}{1-S} \cdot \left(1 - S^M\right) \tag{A1}$$

Equation A1 represents the fitness of a female solitary bee. For social bees, fitness is related to the number of reproductive individuals produced by the colony (most female workers never lay eggs), and for male bees fitness is related to the number of females with which they copulate.

We will assume that the value of *M* is limited by a finite season. Specifically, if the maximum lifespan of a bee is *D* days, and she can forage for a time *T* per day, then the maximum number of offspring the bee can possibly rear is determined by the average foraging time required to provision an egg, τ. The relationship between M and τ is

$$M = D \cdot T / \tau \tag{A2}$$

If we denote average travel time between flowers, examination time and handling time by t_t, t_{ex} and t_h, respectively, then the time required to provision an offspring is (Rodriguez-Girones 2012)

$$\tau = [t_t + t_{ex} + (1\text{-}r) \cdot L_s \cdot t_h] \cdot N / [(1\text{-}r) \cdot L_s], \tag{A3}$$

where r is the proportion of predator-harbouring flowers, L_s the probability of landing on a safe (predator-free) flower after examination, which will be computed below, and N the number of flowers that a bee must harvest to provision an egg. Therefore,

$$M = D \cdot T \cdot (1\text{-}r) \cdot L_s / \{[t_t + t_{ex} + (1\text{-}r) \cdot L_s \cdot t_h] \cdot N\} \tag{A4}$$

To compute fitness we need an expression for the probability that a bee survives to rear an offspring, S. If we denote by p the probability that the bee is attacked when visiting a flower, and by σ the probability that an attack results in the capture and death of the bee (referred to as susceptibility to predation), then S is given by (Rodriguez-Girones 2012)

$$S = \left[\frac{1 - p}{1 - p \cdot (1 - \sigma)} \right]^N \tag{A5}$$

Finally, to derive the relationship between examination time and expected fitness we need to know how the probability that the bee is attacked during a flower visit, p, and the probability of landing on a safe flower, L_s, depend on examination time, t_{ex}. The prior probability that the flower harbours a predator is the proportion of flowers in the environment that harbour predators, r. Following flower examination, the perceived likelihood that the examined flower harbours a predator, $r'(t_{ex})$, will normally differ from the prior. On average, when bees explore dangerous (i.e. predator-harbouring) flowers, $r'(t_{ex}) > r$, and the expected value of $r'(t_{ex})$ approaches 1 asymptotically as t_{ex} increases. Likewise, when bees explore safe (i.e. predator-free) flowers, $r'(t_{ex}) < r$, and the expected value of $r'(t_{ex})$ approaches 0 asymptotically as t_{ex} increases. We denote by $\langle r'(t_{ex}|safe)\rangle$ and $\langle r'(t_{ex}|dangerous)\rangle$ the expected value of the posterior perceived probabilities that safe and dangerous flowers, respectively, harbour predators. In what follows we assume that

$$\langle r'(t_{ex}|safe)\rangle = r\cdot e^{-k_s\cdot t_{ex}} \tag{A6}$$

and

$$\langle r'(t_{ex}|dangerous)\rangle = r + (1-r)\cdot\left(1-e^{-k_d\cdot t_{ex}}\right), \tag{A7}$$

where the parameters k_s and k_d are related to the speed at which information is acquired. Although examination leads, on average, to a gain in information and the posterior probabilities when t_{ex} approaches infinity tend to 0 and 1 for safe and dangerous flowers, respectively, we must acknowledge that examinations is a noisy process. Thus, in any particular flower visit the posterior perceived probability may have any value between 0 and 1. A useful way to model this situation is to assume that perceived probabilities have a beta distribution with parameters α and β ($\alpha > 0$, $\beta > 0$), with probability density function

$$f(r'|\alpha,\beta) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\cdot\Gamma(\beta)}\cdot r'^{\alpha-1}\cdot(1-r')^{\beta-1} \tag{A8}$$

on the support $0 < x < 1$. To reduce the number of free parameters, we will set throughout $\alpha = 2$, so that the expected value of r' is (DeGroot 1975)

$$\langle r' \rangle = \frac{2}{2+\beta} \tag{A9}$$

Within this framework, the predator-avoidance strategy of bees is defined by two parameters: an examination time, t_{ex}, and a rejection threshold r^* so that flowers are rejected if $r'(t_{ex}) > r^*$ and accepted otherwise. Once these two parameters are known, the probability of suffering an attack (p in equation A5) is simply the probability that the bee approaches a dangerous flower and, following examination, her perceived probability that the flower harbours a predator is $r'(t_{ex}) < r^*$. Therefore,

$$p = r \cdot \int_0^{r^*} \frac{\Gamma(\alpha + \beta_d)}{\Gamma(\alpha) \cdot \Gamma(\beta_d)} \cdot s^{\alpha-1} \cdot (1-s)^{\beta_d - 1} \cdot ds \tag{A10}$$

with

$$\beta_d = \frac{2 \cdot \left[1 - \langle r'(t_{ex} | dangerous) \rangle\right]}{\langle r'(t_{ex} | dangerous) \rangle} \tag{A11}$$

The probability of landing on and exploiting the resources of a safe flower, L_s, is computed in a similar fashion. L_s is simply the probability that, after exploring a safe flower, the bee's perceived probability that the flower harbours a predator is $r'(t_{ex}) < r^*$. Hence,

$$L_s = \int_0^{r^*} \frac{\Gamma(\alpha + \beta_s)}{\Gamma(\alpha) \cdot \Gamma(\beta_s)} \cdot s^{\alpha-1} \cdot (1-s)^{\beta_s - 1} \cdot ds \tag{A12}$$

with

$$\beta_s = \frac{2 \cdot \left[1 - \left\langle r'\left(t_{ex} \middle| safe\right)\right\rangle\right]}{\left\langle r'\left(t_{ex} \middle| safe\right)\right\rangle} \qquad \text{(A13)}$$

Equations (A1-A13) can be used to calculate the expected fitness of any predator-avoidance strategy – combination of t_{ex} and r^*. The optimal predator-avoidance strategy is then obtained numerically. (The calculations can be performed with a spreadsheet.)

b) Optimal response to cues

To study how bees should respond to different cues, we consider the simple situation where, upon approach to a flower and before examining it, bees may detect a single type of cue. (For simplicity, we ignore variability in cue intensity: cues are detected or not, but detected cues do not differ in intensity. In real situations, cue intensity is likely to play a role in predator avoidance.) We assume that bees may detect cues at safe and dangerous flowers, although the probability of detecting them is greater at dangerous flowers. Let us denote by s_c and d_c the probabilities that a bee is in information state c upon approaching a safe and dangerous flower, respectively. The variable c specifies whether the cue has been detected. It takes values of 1 if the cue is detected, and 0 otherwise. Information state c can be used to refine the probability that a given flower is dangerous prior to examination. Using Bayes theorem of conditional probability (DeGroot 1975), the probability that an approaching flower harbours a predator, given that it produces information state c, is

$$r_c = \frac{r \cdot d_c}{(1 - r) \cdot s_c + r \cdot d_c} \qquad \text{(A14)}$$

The optimal strategy of bees is obtained as explained above, using r_c as prior in the calculations.

Note that the information content of the cues depends on the difference between the s_c and the d_c. To explore how bees should respond to predator cues, we consider the following situation,

$$S = (s_0, s_1) = (0.5 + \rho, 0.5 - \rho) \tag{A15}$$

$$D = (d_0, d_1) = (0.5 - \rho, 0.5 + \rho) \tag{A16}$$

and calculate the optimal strategy as a function of ρ. When $\rho \sim 0$, vectors S and D do not differ, and bees cannot use cues to estimate the probability that the flower they approach harbours a predator. On the other hand, when $\rho \sim 0.5$, the cues become maximally informative and no examination is required to discriminate safe and dangerous flowers.

c) Predator avoidance in heterogeneous environments

We now consider the situation where flowers differ in the reward they offer bees. For simplicity, we study the particular case where a proportion v_1 of flowers offer reward ε_1 and proportion $v_2 = 1 - v_1$ of flowers offer reward $\varepsilon_2 > \varepsilon_1$. Handling times are $t_{h,1}$ and $t_{h,2}$ for flowers of types 1 and 2, respectively. We assume that bees cannot discriminate between the two flower types at a distance, so that they approach flowers at random, irrespectively of the reward they offer, but can detect the level of reward upon arrival, so that they can respond differently two the two flower types, examining flowers of type i (i =1 or 2) for time $t_{ex,i}$ and rejecting them if the perceived probability that the flower harbours a predator is greater than r^*_i. In this case, the predator avoidance strategy consist of a set of four parameters, ($t_{ex,1}$, r^*_1, $t_{ex,2}$, r^*_2), which together determine the expected fitness of foraging bees, as specified below.

We first note that, knowing the foraging strategy of a bee, the probability that the bee is attacked when visiting a flower of type i, p_i, and the probability of landing at safe flowers of type i, $L_{s,i}$, are given by equations (A10) and (A12), using $t_{ex,i}$ and r^*_i for the calculations. To calculate the relationship between foraging strategy and expected fitness, we use the mean-field approximation. In essence, this means that if a bee visits 100 flowers, we assume that exactly $100 \cdot \nu_1$ of them will offer reward ε_1 – thus ignoring sampling noise. Within this approximation, the probability that a bee survives provisioning one offspring, S, is given by

$$S = \left[\frac{1 - p_1}{1 - p_1 \cdot (1 - \sigma)} \right]^{n_1} \cdot \left[\frac{1 - p_2}{1 - p_2 \cdot (1 - \sigma)} \right]^{n_2} \tag{A17}$$

where n_1 and n_2 are the number of flowers offering reward ε_1 and ε_2 exploited. Assuming that the total provisioning per offspring, $n_1 \cdot \varepsilon_1 + n_2 \cdot \varepsilon_2$ must be equal to N (the number of exploited flowers in the simpler model), then

$$n_i = \frac{\nu_i \cdot L_{s,i}}{\varepsilon_1 \cdot \nu_1 \cdot L_{s,1} + \varepsilon_2 \cdot \nu_2 \cdot L_{s,2}} \cdot N \tag{A18}$$

Finally, to compute expected fitness we must derive the time required, on average, to provision an offspring, τ. This is

$$\tau = \frac{t_t + \nu_1 \cdot t_{ex,1} + \nu_2 \cdot t_{ex,2} + (1 - r) \cdot (\nu_1 \cdot L_{s,1} \cdot t_{h,1} + \nu_2 \cdot L_{s,2} \cdot t_{h,2})}{(1 - r) \cdot [\varepsilon_1 \cdot \nu_1 \cdot L_{s,1} + \varepsilon_2 \cdot \nu_2 \cdot L_{s,2}]} \cdot N \tag{A19}$$

Substituting this value of t in equation (A2) we obtain the maximum number of offspring that a bee can produce if she survives predation, M, and using this value of M and the survival S calculated in equation (A17) we obtain the expected fitness using equation (A1). As in the previous case, the optimal predator-avoidance strategy can be obtained numerically.

d) Deriving categorical predictions

To derive categorical predictions, we study the behaviour of the model when all parameters are kept fixed, except for one. For the purpose of illustration, we consider the following situation as baseline: bees must harvest the resources from $N = 1000$ flowers to provision an offspring, their susceptibility is $\sigma = 0.1$, their foraging time (product $D \cdot T$) is limited to 100 hours, average travel time between flowers is $t_t = 2$ s and average handling time $t_h = 4$ s, the speed of information acquisition is determined by $k_s = 0.1$ and $k_d = 0.5$ and the proportion of dangerous flowers is $r = 0.05$.

To study how overall predation risk affects the predator-avoidance strategy, we calculate the optimal strategy for different proportions of dangerous flowers, r. Once we have the optimal strategy, we can investigate the consequences of following it. For instance, bees will not exploit all safe flowers they encounter (equation A12). Following examination of safe flowers, the perceived probability that the flower harbours a predator will occasionally be greater than the rejection threshold. In these instances, bees will move on in search of new flowers. The proportion of false alarms (incorrectly rejected safe flowers) is simply $1 - L_s$, and is readily calculated from equation (A12) once the optimal strategy is derived through numerical maximisation. The same procedure can be used to investigate the effect of other parameters, such as the number of flowers that must be exploited to provision an offspring, N. Another parameter that has not been mentioned so far but is implicit into the model and therefore can be studied following the same methodology is the conspicuousness of the predator. Indeed, conspicuous predators will be easier to identify than cryptic ones, and therefore low values of k_s and k_d will correspond to cryptic predators, while large values of these parameters will correspond to conspicuous predators.

We derived predictions for the following model parameters: the bee must exploit $N = 1000$ flowers to provision an egg, her probability of succumbing to a predator attack is $= 0.1$, her foraging budget is constraint to $D \cdot T = 100$ hours, and average travel and handling times per flower are 2 and 4 s, respectively. For conspicuous predators, rate of information acquisition during flower examination is determined by $k_s = 0.1$ and $k_d = 0.5$, at safe and dangerous flowers, respectively. For cryptic predators, these parameters take values $k_s = 0.05$ and $k_d = 0.25$. Finally, in the heterogeneous environment we assume that bees must obtain 1000 reward units to provision an egg, 95% of the flowers they encounter offer one reward unit (poor flowers) and the remaining 5% offer two reward units (rich flowers).

Appendix S2. Cuticular compound extraction and analysis

Cuticular compounds were extracted from living and dead ants ($n = 4$ per treatment). Living ants were anesthetized by placing them for 15 seconds in a freezer. Dead ants were frozen to death and aired for two days prior to the extraction of cuticular compounds.

Cuticular compounds were extracted by immersing samples in hexane for 5 min. The extracts were analysed by gas chromatography (GC) and mass spectrometry (MS). The

GC-MS instrumentation (Agilent HP6890GC/5973MS) was equipped with a HP-5MS column injection (30 m, ID 0.25 mm, film thickness 0.25 µm) with helium as carrier gas. Ionization was by electron impact (70eV, source temperature 230ºC). From each sample, 2 µl were injected (split with a 10:1 ratio) with injection temperature at 250ºC. The GC temperature programme began with injection at 60ºC, increased at rate of 3ºC min^{-1} up to 80ºC, then rose at 5ºC min^{-1} to 260ºC and was held at this temperature for 50 min. Ant volatile compounds were identified by comparing their mass spectra with Wiley7n.1 libraries.

Whereas the hydrocarbon n-undecane, an alkane hydrocarbon characteristic of formicine species used as an alert signal (Holldobler and Wilson 1990), was the most abundant volatile in living ants of both species, the peak of abundance of this compound was higher in *O. smaragdina* than in *P. dives* (mean ± standard error: $2.33\cdot10^6\pm0.82\cdot10^6$ and $1.04\cdot10^6 \pm 0.66\cdot10^6$ respectively; t test for independent samples: $t_6 = 2.45$, $p < 0.05$). Heneicosane and tricosane, both major volatiles in *O. smaragdina*, were absent in *P. dives* ants. The two ant species therefore differed in the volatile mixtures they emitted (Fig. S1, electronic supplementary material), offering bees the possibility to use chemical information to distinguish between them and detect predatory ants. Profile comparisons between living and dead ants further demonstrated that dead ants did not emit cuticular volatiles (Fig. S2).

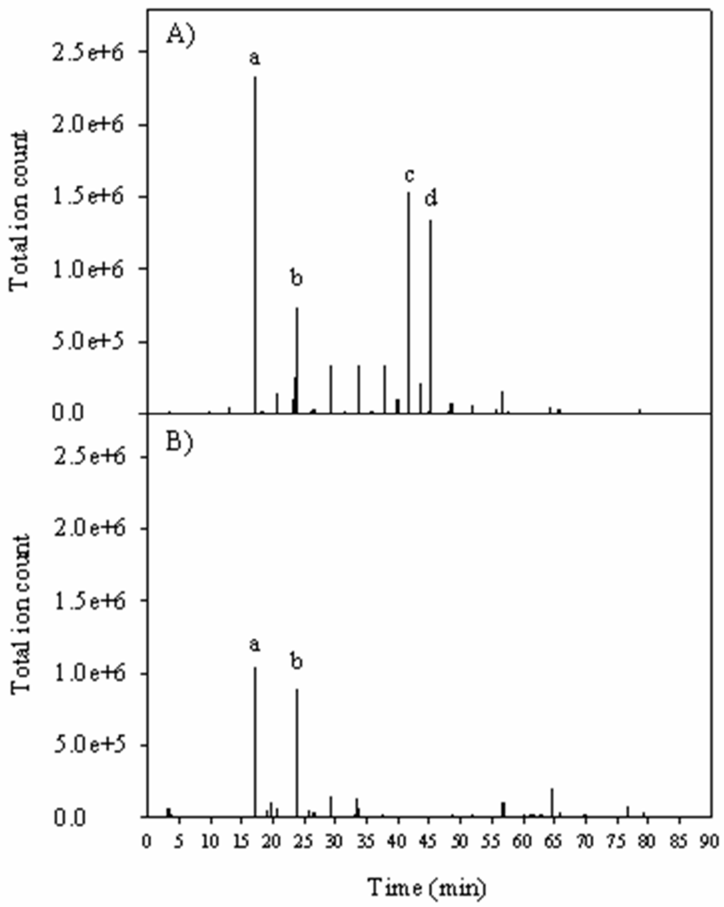

Fig S1. *Chromatogram of (A) a living O. smaragdina, and (B) a living P. dives. Labelled peaks are (a) undecane, (b) tridecane, (c) heneicosane and (d) tricosane.*

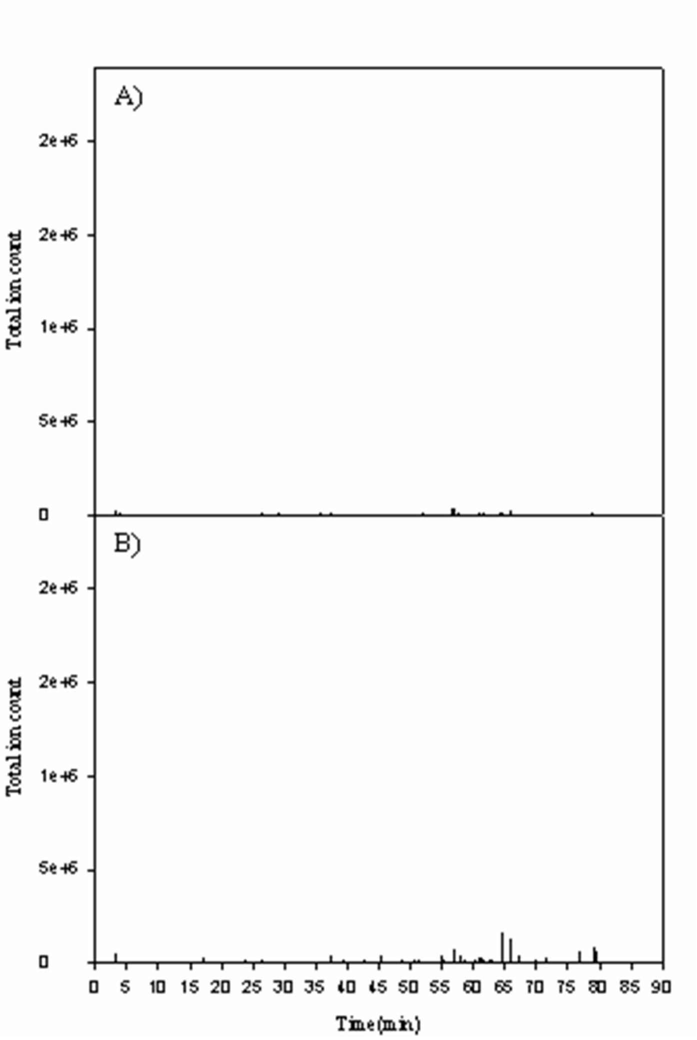

Fig S2. *Chromatogram of (A) a dead O. smaragdina, and (B) a dead P. dives.*

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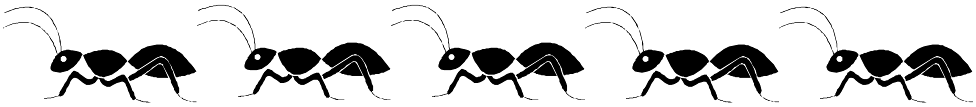
DISCUSIÓN INTEGRADORA



RESULTADOS Y DISCUSIÓN

En general, los resultados de esta tesis demuestran que la hormiga tejedora, *Oecophylla smaragdina*, al actuar como depredador emboscado en flores, desempeña un papel fundamental en la evolución de los mutualismos planta-polinizador en el Sudeste Asiático. La presencia de la hormiga tejedora sobre flores puede actuar moldeando las estrategias antipredatorias de los visitantes florales y repercutiendo en el éxito reproductivo de las plantas que las albergan. Estos cambios comportamentales en los visitantes florales no sólo tienen implicaciones evolutivas en un sistema de planta en particular, sino que además, pueden modificar las propiedades más básicas de las redes de polinización.

La hormiga tejedora como depredador emboscado en flores

Las hormigas pueden inducir cambios comportamentales en los polinizadores, impidiendo el acoplamiento de los mutualismos planta–polinizador. Esto se produce fundamentalmente por competencia directa por el néctar entre la hormiga y el visitante floral (Lach 2008) o por depredación (Junker, Chung et al. 2007, Willmer, Nuttman et al. 2009), siendo en este último caso donde localizamos a la hormiga tejedora, tal y como lo demuestran nuestras observaciones en campo (capítulo 1). Más concretamente, de las 48 especies de plantas que observamos con hormiga tejedora recibiendo la visita de visitantes florales, en 31 especies los visitantes fueron atacados por las hormigas (con capturas exitosas en 15 especies de plantas). Por tanto, los visitantes florales que coexisten con la hormiga tejedora están expuestos a un riesgo de depredación durante sus tareas de forrajeo y pueden disminuir su tasa de visita a plantas con hormiga. *Trigona* spp., por ejemplo, visita con menor frecuencia plantas de la especie *Nephelium* *lappaceum* cuando tienen nidos de hormiga tejedora (Tsuji, Hasyim et al. 2004). Igualmente, la abeja solitaria *Nomia strigata* evita forrajear en *Melastoma malabathricum* con nidos de hormiga (capítulo 3). De hecho, este comportamiento de evasión es bastante común cuando los polinizadores localizan depredadores emboscados en flores. Así, estudios llevados a cabo con arañas cangrejo muestran que los polinizadores, por lo general, exhiben una respuesta antipredatoria de evitación muy similar a la que exhiben frente a la hormiga tejedora (Dukas and Morse 2003, Reader, Higginson et al. 2006).

La hormiga tejedora y su efecto sobre las redes de polinización

Varios modelos teóricos (Suttle 2003, Higginson, Ruxton et al. 2010, Rodriguez-Girones 2012) predicen que la hormiga tejedora tiene el potencial de afectar las relaciones entre plantas y polinizadores a escalas ecológicas y evolutivas debido a su abundancia, ubicuidad y gran movilidad. El capítulo 2 de la presente tesis confirma experimentalmente la influencia de la hormiga tejedora sobre las interacciones planta-polinizador al inducir cambios en el tamaño y la estructura de las redes de polinización. Concretamente aquellas comunidades de plantas en las que la hormiga tejedora estuvo presente mostraron una disminución en el número de interacciones planta-polinizador. Este resultado apoya la idea de que el riesgo de depredación disminuye la diversidad y abundancia de las interacciones planta-polinizador (Dukas 2001, Dukas and Morse 2003, Heiling and Herberstein 2004). Además, las redes de comunidades con hormiga mostraron menor asimetría, coeficiente de agrupamiento, y anidamiento. De esta manera, redes construidas a partir de comunidades con hormigas exhibieron una mayor vulnerabilidad a las perturbaciones que redes de comunidades sin hormigas. Además, las redes de comunidades con hormiga, al tener un menor anidamiento, fueron menos cohesivas que aquellas originadas en comunidades sin hormiga (Bascompte, Jordano et al. 2003). Esta diferencia en el anidamiento entre comunidades probablemente se deba a que los polinizadores cesaron de forrajear plantas más generalistas para visitar las más especialistas. Como consecuencia, la frecuencia de interacción entre especies más generalistas disminuyó. En este capítulo se demuestra experimentalmente por primera vez en la literatura que los depredadores pueden tener un efecto indirecto sobre la estructura de las redes de polinización, mediado por el comportamiento antipredatorio de los polinizadores. Estos resultados evidencian la importancia de considerar la depredación como un factor determinante en la topología de las redes planta-polinizador.

La hormiga tejedora y su efecto sobre el éxito reproductivo de las plantas

El papel de la hormiga tejedora como depredador emboscado en flores no sólo tiene un efecto a nivel de las redes de polinización, sino que además, afecta a la trayectoria evolutiva de interacciones planta-polinizador particulares. A lo largo de la literatura científica se ha postulado que, por lo general, la presencia de hormigas depredadoras repercute negativamente sobre la polinización de la planta que las alberga (Willmer, Nuttman et al. 2009), y que las plantas, para contrarrestar el efecto negativo que las hormigas ejercen sobre el mutualismo planta-polinizador, desarrollan en sus flores repelentes de hormiga (Willmer and Stone 1997, Raine, Willmer et al. 2002, Willmer, Nuttman et al. 2009). Sin embargo, los resultados del capítulo 3 demuestran que el efecto (positivo o negativo) que tienen las hormigas predadoras sobre la interacción planta-polinizador depende del contexto ecológico. En nuestro sistema de estudio en particular, la hormiga tejedora es atraída por las flores de *Melastoma malabathricum*, permitiendo el acoplamiento planta-polinizador y ahuyentando visitantes florales poco efectivos. *M. malabathricum* fue visitada fundamentalmente por dos tipos de polinizador: las grandes abejas carpintero del género *Xylocopa* y las abejas solitarias *Nomia strigata*– cuyo tamaño es parecido al de la abeja de la miel, *Apis mellifera*. Mientras que las primeras fueron polinizadores efectivos y, además, invulnerables al ataque de las hormigas, las segundas participaron insignificantemente en la reproducción de la planta y evitaron forrajear en plantas con nidos de hormiga. De esta manera, *Nomia strigata* explotó preferentemente plantas sin hormiga, consumiendo los recursos disponibles. A su vez, *Xylocopa sp*, libre de la depredación por la hormiga, respondió a la competición interespecífica concentrando su esfuerzo de forrajeo en plantas con nidos de hormiga. Como resultado, las plantas patrulladas por la hormiga tejedora mostraron un mayor éxito reproductivo que las plantas sin hormigas.

Análogamente al papel que desempeña la hormiga sobre el mutualismo planta-polinizador en bosques tropicales, en climas templados, las arañas cangrejo también pueden tener un efecto de cascada trófica sobre las plantas que las albergan. Mientras que algunos estudios han demostrado que las arañas cangrejo pueden modificar el comportamiento de los polinizadores teniendo repercusiones negativas sobre el éxito reproductivo de las plantas (Suttle 2003, Goncalves-Souza, Omena et al. 2008), otros estudios no han encontrado una relación significativa entre la presencia de la araña cangrejo y el éxito reproductivo de la planta (Dukas and Morse 2005, Brechbuhl, Kropf et al. 2010). Además, las arañas cangrejo también pueden tener efectos positivos en las plantas mediados por la depredación de insectos fitófagos (Romero and Vasconcellos-Neto 2004). Sin embargo, a diferencia de la hormiga tejedora, las arañas cangrejo son poco abundantes y móviles en los hábitats donde se presentan y, aunque pueden llegar a afectar al éxito reproductivo de las plantas que las albergan, es poco probable que su presencia tenga algún efecto sobre las poblaciones de plantas y las interacciones planta-polinizador a nivel de comunidad. No obstante, la divergencia de resultados en el éxito reproductivo de las plantas que albergan a arañas cangrejo y la hormiga tejedora secundan la idea de que el efecto de los depredadores emboscados en flores sobre el mutualismo planta-polinizador depende fundamentalmente del contexto ecológico. Además, apoyando esta idea, un estudio reciente ha demostrado que flores polinizadas por abejas de pequeño tamaño y vulnerables al ataque de las hormigas mostraron más frecuentemente repelentes de hormiga que flores polinizadas por las grandes e invulnerables abejas *Xylocopa* (Gonzálvez et al. datos no publicados).

Variación temporal en la interacción hormiga tejedora-planta-polinizador

Es conocido que las interacciones planta-polinizador muestran un mosaico de presiones selectivas cuando la composición de la comunidad de polinizadores varía a través del tiempo y el espacio (Herrera 1988). Cuando consideramos interacciones tritróficas, sin embargo, desconocemos si existe una heterogeneidad espaciotemporal en la magnitud y dirección de sus presiones selectivas. En el capítulo 4 abordamos esta cuestión centrándonos en cómo cambia a través del tiempo la fuerza de la interacción entre plantas, polinizadores y hormigas. Los resultados de este capítulo muestran que la variación temporal en la abundancia de polinizadores modificó las presiones selectivas que las hormigas ejercieron sobre sus plantas hospedadoras. Mientras que en 2010 encontramos una partición del recurso en la comunidad de polinizadores debido a que *Nomia strigata* concentraba sus esfuerzos de forrajeo en plantas sin nidos de hormiga y *Xylocopa sp* visitaba principalmente plantas patrulladas por la hormiga tejedora, en

2012, cuando la abundancia de abejas fue significativamente menor, *Xylocopa* visitó de manera similar plantas con y sin hormigas. Aunque este último año *N. strigata* también forrajeó principalmente en plantas sin hormiga, su número fue tan escaso que apenas afectó a la disponibilidad de recursos. Como resultado, en 2010 el éxito reproductivo de plantas con hormiga fue mayor que en plantas sin hormiga, mientras que dos años más tarde el éxito reproductivo fue similar en ambos tipos de plantas. Por tanto, la relación entre la abundancia de las abejas y la variación en el éxito reproductivo de la planta podría tener consecuencias evolutivas y afectar principalmente a aquellos rasgos de la planta que favorecen la colonización por las hormigas. De tal manera que en aquellos años en los que la abundancia de abejas sea alta, se seleccionarían aquellos rasgos que aumentan la presencia de hormigas predadoras en la planta mientas que en los que el número de abejas sea menor la selección de los rasgos que promueven la colonización de las hormigas sería neutral.

Aunque en esta tesis doctoral no hemos analizado las variaciones espaciales de las presiones selectivas que la hormiga tejedora ejerce sobre sus plantas hospedadoras, cabría esperar que ocurriesen cuando el conjunto de visitantes florales variase a lo largo de la distribución geográfica de la planta hospedadora. De manera que cuando en poblaciones de plantas genéticamente aisladas la hormiga tejedora ejerce un efecto en el éxito reproductivo de la planta, ya sea positivo o negativo dependiendo del contexto ecológico, la planta puede llegar a desarrollar adaptaciones locales en respuesta a la presencia de la hormiga tejedora. En el caso de *Melastoma malabathricum*, por ejemplo, se ha descrito que en el norte de Queensland (Australia) el visitante floral más común es una pequeña abeja social, *Trigona carbonaria*, con un efecto negativo en su éxito reproductivo. Futuros trabajos deberían examinar cómo varían espacialmente las interacciones tritróficas entre hormiga-planta-polinizador y las consecuencias ecológicas y evolutivas a nivel local en el mutualismo planta-polinizador.

La hormiga tejedora y su efecto sobre el comportamiento antipredatorio de los polinizadores

La presencia de la hormiga tejedora en flores no sólo va a tener implicaciones en la trayectoria evolutiva de las plantas, sino que además, los rasgos de los polinizadores también son susceptibles al cambio evolutivo. Cuando un determinado polinizador es vulnerable a la presencia de la hormiga tejedora, dicho polinizador deberá desarrollar ciertas estrategias antipredadoras que le permitan disminuir el riesgo de depredación al realizar sus tareas de forrajeo. Los resultados del capítulo 5 muestran que la abeja solitaria *Nomia strigata* pasa más tiempo examinando flores con señales procedentes de la hormiga tejedora que flores sin señales o con señales de la hormiga no depredadora *Polyrhachis dives*. Además, *N. strigata* tuvo una diferente respuesta comportamental a la presencia de señales químicas y visuales procedentes de la hormiga tejedora. Al detectar señales químicas *N. strigata* respondió volando alrededor o debajo de la flor. Cuando localizó las señales visuales la abeja, por otro lado, realizó vuelos estáticos y movimientos en zig-zag enfrente de la hormiga muerta. Debido a que las señales químicas tienen baja resolución espaciotemporal, la detección de estas señales no implica que la hormiga se encuentre en la flor. La presencia de estas señales podría advertir que la hormiga se encuentra próxima o ha dejado la flor recientemente indicando, simplemente, la posibilidad de un riesgo de depredación para la abeja. De hecho, es conocido que la presencia sobre mangos de señales químicas procedentes de la hormiga tejedora es una señal de riesgo lo suficiente potente como para que las moscas de la fruta no ovipositen sobre los mangos (Van Mele, Vayssieres et al. 2009). Además, mientras que la existencia de señales visuales conduce a la abeja a un rechazo de la flor, la presencia de señales químicas solo actúa como señal de alarma pero nunca como señal de rechazo. En este sentido, es conocido que muchos polinizadores evitan forrajear en flores donde detectan señales visuales asociadas a predadores. *Bombus terrestris*, por ejemplo, evita flores donde hay predadores crípticos emboscados (Ings and Chittka 2008). Por otro lado, en nuestro experimento, las señales visuales probablemente contribuyeron a la discriminación entre la hormiga tejedora y la hormiga *P. dives*. Aunque las características exactas que utilizan la mayoría de polinizadores para identificar a los predadores son desconocidas, las abejas de la miel, *Apis mellifera*, emplean rasgos tales como el contraste de color, tamaño y/o movimiento para detectar arañas cangrejo crípticas (Llandres and Rodriguez-Girones 2011). A diferencia de las arañas cangrejo, en nuestro caso, las hormigas fundamentalmente difieren en el tamaño corporal. Aunque éste no es un rasgo específico de las especies de hormiga estudiadas, *N. strigata* posiblemente usó el tamaño corporal de las hormigas para diferenciar entre hormigas depredadoras y no depredadoras. En el futuro, otros trabajos deberían evaluar la respuesta comportamental de las abejas a la presencia conjunta de señales químicas y visuales e incluso a hormigas vivas. En este último caso se espera que el movimiento de las hormigas desempeñe un papel fundamental en el rechazo de la flor por la abeja (observaciones personales) como ocurre en el caso de las arañas cangrejo (Llandres and Rodriguez-Girones 2011). Además, conocer qué compuestos específicos de la hormiga tejedora desencadenan el comportamiento antipredatorio en las abejas sería un gran avance en el entendimiento de la evolución química de las interacciones predador-presa.

Las hormigas como moldeadoras de los mutualismos planta-polinizador

Mientras que esta tesis ha profundizado en las consecuencias que tiene la presencia de la hormiga tejedora, *Oecophylla smaragdina*, en la evolución de las interacciones planta-polinizador en bosques tropicales del Sudeste Asiático la existencia en bosques tropicales y subtropicales africanos de otra especie de hormiga tejedora del mismo género, *O. longinoda*, con un comportamiento y hábitat similar al de la hormiga tejedora asiática, hace presuponer que pueda desempeñar un papel semejante al de *O. smaragdina*. No menos predecible sería la influencia de otras especies de hormigas arbóreas sobre las redes de polinización en bosques tropicales africanos y neotropicales. En estos bosques muchas especies de hormiga que nidifican en los árboles han coevolucionado durante millones de años con sus plantas hospedadoras de manera que las hormigas ofrecen protección frente el ataque de los herbívoros. Muchas de estas plantas, para contrarrestar los efectos negativos que tiene la presencia de hormigas en sus flores, han desarrollado barreras físicas y químicas permitiendo un adecuado acoplamiento planta-polinizador (Raine, Willmer et al. 2002). Es fácil predecir, por tanto, que las hormigas han ejercido presiones selectivas suficientemente potentes sobre los mutualismos planta-polinizador como para afectar su trayectoria evolutiva. Sin embargo, futuros trabajos son necesarios para evaluar qué contexto ecológico y mecanismos han permitido el desarrollo y evolución de las interacciones planta-polinizador-hormiga en estos bosques tropicales y subtropicales.

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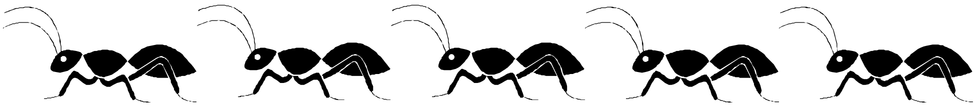
CONCLUSIONES/CONCLUSIONS



CONCLUSIONES

1. Al contrario que las arañas cangrejo, principales depredadores emboscados en flores en prados de climas templados, la hormiga tejedora *Oecophylla smaragdina*, debido a su abundancia, ubicuidad y gran movilidad, constituye un sistema modelo ideal para estudiar el efecto de los depredadores sobre las trayectorias ecológicas y evolutivas de los mutualismos planta-polinizador. A lo largo de los bosques tropicales del SE Asiático, la hormiga tejedora usa flores de un amplio rango de especies vegetales como plataforma de caza de visitantes florales. La presencia de la hormiga tejedora sobre las flores puede afectar al comportamiento de forrajeo de los polinizadores y al éxito reproductivo de las plantas que las albergan.
2. En aquellas comunidades donde los depredadores sean abundantes es importante tener en cuenta el efecto de los depredadores sobre la estructura de la red de polinización para no sesgar la estima de los parámetros. La presencia de la hormiga tejedora en comunidades vegetales causó alteraciones en el tamaño y propiedades topológicas de la red planta-polinizador, tales como la asimetría de la red, el coeficiente de agrupamiento y el anidamiento. Estos efectos indirectos que las hormigas ocasionaron sobre la estructura de la red fueron mediados por el comportamiento antipredatorio de los polinizadores.
3. Cuando la densidad de visitantes florales de la especie de planta *Melastoma malabathricum* es suficientemente elevada, las plantas que albergan a la hormiga tejedora tienen un éxito reproductivo mayor que las plantas sin hormiga. *M. malabathricum*, polinizada por las grandes abejas carpintero del género *Xylocopa sp.*, atrae a la hormiga tejedora a sus flores. La presencia de la hormiga tejedora en las flores disuade solamente a polinizadores menos efectivos

generando una partición del recurso entre el polinizador *Xylocopa* y los polinizadores menos efectivos.

4. El efecto de las hormigas depredadoras sobre el éxito reproductivo de las plantas depende fundamentalmente del contexto ecológico. La presencia de depredadores que disuaden visitantes florales pueden tener efectos positivos o negativos en el éxito reproductivo de las plantas dependiendo de si las hormigas disuaden a todos los visitantes o a un subconjunto de ellos, así como de la eficacia relativa de cada uno de los visitantes florales.
5. La interacción hormiga tejedora-planta-polinizador tiene variabilidad en la magnitud y dirección de las presiones selectivas a través del tiempo. Mientras que en 2010 el éxito reproductivo de las plantas *Melastoma malabathricum* con nidos de hormiga fue mayor que el de las plantas sin hormiga como consecuencia de una partición del recurso mediada por la presencia de la hormiga tejedora, dos años más tarde, cuando la densidad de visitantes florales fue menor, plantas con y sin hormiga tuvieron un éxito reproductivo similar. Esto se produjo debido a que, a pesar de que los polinizadores menos eficaces aun forrajearon principalmente en plantas sin hormiga, su número fue tan bajo que no tuvo efecto sobre la disminución del recurso. Como consecuencia, la tasa de visita del polinizador más eficaz, *Xylocopa sp*, fue semejante en plantas con y sin hormiga.
6. Las abejas solitarias deberían incrementar el tiempo de examen de las flores en respuesta a señales procedentes de depredadores. Nuestros resultados muestran que la abeja solitaria, *Nomia strigata*, pasó más tiempo examinando flores con señales de hormiga tejedora que flores con señales una especie de hormiga más dócil, *Polyrhachis dives*, y flores sin señales.

7. Las abejas solitarias podrían emplear una respuesta comportamental como estrategia para identificar la naturaleza de las señales visuales y localizar el origen de las señales químicas procedentes de la hormiga tejedora. Las abejas solitarias respondieron de manera diferente a las señales químicas y visuales asociadas a la hormiga tejedora. Mientras que las señales químicas promovieron movimientos exploratorios de la abeja alrededor de la flor, las señales visuales generaron en la abeja vuelos estáticos enfrente de la flor. Además, las abejas solo rechazaron las flores en presencia de señales visuales y nunca con señales químicas procedentes de la hormiga tejedora.

CONCLUSIONS

1. Unlike crab spiders -- the most common flower-dwelling ambush predators in temperate meadows -- the weaver ant *Oecophylla smaragdina* constitutes an ideal model system to study the effect of predators on the ecological and evolutionary trajectories of the mutualisms plant-pollinator, due to its abundance, ubiquity and mobility. Weaver ants use flowers of a wide range of plant species, throughout the tropical forests of SE Asia, as a hunting platform of the incoming visitors. The presence of weaver ants on flowers may affect the foraging behaviour of pollinators and the reproductive success of their host plants.
2. In communities where predators are abundant it is important to consider their effect on the structure of the pollination network to avoid biasing the estimation of the topological parameters. Weaver ants affected the size of a plant-pollinator interaction network, as well as topological parameters such as web asymmetry, clustering coefficient and nestedness. Ants indirectly affected network topology, through the behavioural changes they induced on floral visitors.
3. When the density of flower visitors of the plant species *Melastoma malabathricum* was high and resource competition intense, ant-harbouring plants showed higher reproductive success than ant-free plants. *M. malabathricum*, pollinated by the large carpenter bees of the genus *Xylocopa sp*, attracts weaver ants to its flowers. The presence of weaver ants on flowers deters less effective pollinators and attracts *Xylocopa* bees through an indirect effect on resource depletion.

4. The effect of predatory ants on the reproductive success of the plants depends on the ecological context. The presence of predators that deter flower visitors can have positive or negative effects on plant fitness, depending on whether they deter all visitors or a subset of them, and on the relative effectiveness of deterred and undeterred visitors.
5. The interaction between weaver ants, pollinators and plants shows variability in the magnitude and direction of selective pressures through time. While in 2010 the reproductive success of *Melastoma malabathricum* was higher at plants with ant nests than at plants without nests, two years later, when the density of flower visitors was lower, plants with and without ants had similar reproductive success. This is due to the fact that, although *Nomia* bees still foraged mainly at plants without ants, there were so few of them that they hardly affected resource availability. As a result, the visit rate of the most effective pollinator, *Xylocopa sp*, was similar at plants with and without ants.
6. Bees are expected to increase flower examination time in response to predator cues. The solitary bee, *Nomia strigata*, spent more time examining virgin flowers in presence of predator cues than flowers with cues from the harmless ant, *Polyrhachis dives*, or flowers without cues.

   Solitary bees may use a behavioural response as strategy to identify the nature of visual cues and to locate the source of chemical cues. The solitary bees responded differently to visual and chemical cues from weaver ants. While chemical cues induced bees to search around flowers, bees detecting visual cues hovered in front of them. In addition, the bees accepted all flowers with chemical cues and only rejected flowers with visual cues.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ANDAMAN SEA
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