Interacciones entre arañas cangrejo y polinizadores: estrategias de caza de las arañas cangrejo y estrategias antidepredatorias de los polinizadores

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Interacciones entre arañas cangrejo y polinizadores: estrategias de caza de las arañas cangrejo y estrategias antidepredatorias de los polinizadores

Memoria presentada por Ana López Llandres para optar a Grado de Doctora por la Universidad de Granada

La Doctoranda

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Granada, enero 2011

Dr. Miguel Ángel Rodríguez-Gironés Arbolí, Científico Titular de la Estación Experimental de Zonas Áridas-CSIC

CERTIFICA

Que los trabajos de investigación realizados en la Memoria de Tesis Doctoral: "Interacciones entre arañas cangrejo y polinizadores: estrategias de caza de las arañas cangrejo y estrategias antidepredatorias de los polinizadores", son aptos para ser presentados por la Lda. Ana López Llandres ante el Tribunal que en su día se designe, para aspirar al Grado de Doctora en Ciencias Biológicas por la Universidad de Granada.

Y para que así conste, en cumplimiento de las disposiciones vigentes extendemos el presente certificado a 11 de enero de 2011 en Almería.

VºBº Director

Dr. Miguel Ángel Rodríguez-Gironés Arbolí

A mis padres Juan Pedro y Blanca,
y a mis cuatro abuelos

A mi gran pequeño Ori

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RESUMEN

RESUMEN

La mayoría de los polinizadores son animales que van buscando comida y sus pautas de movimiento están gobernadas por las mismas reglas que las que siguen otros animales forrajeadores. Dos de los principales factores que afectan a las estrategias de forrajeo de los animales son la distribución de recursos y el riesgo de depredación. Sin embargo, aunque el efecto de la distribución de recursos ha sido estudiado en numerosos trabajos, el efecto del riesgo de depredación ha sido ignorado tradicionalmente por el hecho de que parecía demasiado infrecuente como para afectar al comportamiento de los polinizadores. No obstante, en la última década numerosos estudios han demostrado que los polinizadores pueden presentar una marcada respuesta antidepredatoria, y esto puede afectar al éxito reproductivo de las planta, teniendo como consecuencia un efecto indirecto en el mutualismo planta-polinizador.

El objetivo de la presente tesis doctoral es profundizar en el conocimiento de la función y los mecanismos del comportamiento antidepredatorio de los polinizadores y del comportamiento de caza de las arañas cangrejo, entendiendo cómo contribuyen estos comportamientos al éxito reproductivo de cada especie y estudiando los mecanismos que están detrás de los mismos.

En el capítulo I estudiamos la respuesta de los polinizadores, *Apis mellifera* y *Eristalis tenax*, a la interacción entre riesgo de depredación impuesto por las arañas cangrejo y disponibilidad de néctar. Encontramos que los sírfidos y las abejas respondieron ante variaciones en la cantidad de recurso y el riesgo de depredación de manera completamente diferente a nivel de parche. Los polinizadores más susceptibles, *A. mellifera*, evitaron los parches peligrosos especialmente si tenían pocos recursos. Sin embargo, los polinizadores menos susceptibles, *E. tenax*, visitaron más frecuentemente los parches pobres y peligrosos. A nivel de flor sin embargo, ambos polinizadores presentaron una respuesta similar y evitaron las flores con araña. Además, las inflorescencias recibieron tantas visitas de abejas en los parches peligrosos y ricos como en los parches seguros y pobres. Estos resultados demuestran que la escala espacial podría determinar el efecto de los depredadores en las interacciones planta-polinizador y sugieren que a nivel evolutivo, un mecanismo por el que las flores regularmente

asociadas con arañas cangrejo podrían atraer más polinizadores sería incrementando la cantidad de néctar que producen.

En el capítulo II estudiamos la relación causa-efecto entre coloración y condición en arañas cangrejo australianas, *Thomisus spectabilis*, recolectadas en el campo en dos años consecutivos y en el laboratorio estudiamos si las arañas responden cambiando el color frente a distintos regímenes de comida y de color de fondo. Encontramos que las arañas recolectadas en el 2008 reflejaron más UV que las recolectadas en el 2009. Los resultados de la relación entre coloración y condición demostraron que, en el 2008 hubo una relación positiva entre condición y reflectancia UV, lo que no sucedió para las arañas recolectadas en el año 2009. Por otro lado, en el laboratorio la dieta afecta la condición, pero no la cantidad de UV que refleja *T. spectabilis*. Estos resultados sugieren que al presentar mayor reflectancia en el UV, las arañas cangrejo australianas presentan una ventaja a la hora de capturar a sus presas y están en mejor condición que las arañas que no reflejan UV.

En el capítulo III estudiamos la variación en la coloración en distintas poblaciones de arañas cangrejo australianas, *Diaea evanida* y *Thomisus spectabilis*, en el campo así como la respuesta de distintas presas nativas, *Trigona carbonaria* y *Austroplebeia australis*, ante variación en la coloración de *D. evanida* y *T. spectabilis* respectivamente. Encontramos que las abejas de la especie *T. carbonaria* no mostraron ninguna preferencia ante variaciones en la coloración de arañas de la especie *D. evanida*. Sin embargo, las abejas de la especie *A. australis* mostraron mayor preferencia por arañas menos contrastantes de la especie *T. spectabilis*. Además encontramos gran variación en la cantidad de UV reflejada en el campo por las arañas cangrejo australianas de ambas especies, lo que sugiere que la cantidad de UV que las arañas reflejen en el campo podría ser explicada por la disponibilidad de presas de distintas especies en un momento y lugar determinados.

En el capítulo IV estudiamos el papel del camuflaje, el movimiento, la reflectancia UV y el tamaño de las arañas cangrejo australianas *Thomisus spectabilis* en la tasa de visitas de las abejas de la miel y el éxito de captura de las arañas. Ni el contraste cromático ni el acromático que las arañas presentaron fueron suficientes para que las abejas presentaran una respuesta antidepredatoria. Sin embargo el movimiento de las arañas, su

tamaño y su reflectancia UV determinaron la tasa a la que las abejas visitaron las flores con araña. Estos resultados sugieren que sólo las arañas cangrejo australianas que son grandes "engañan" a sus presas reflejando UV, y resaltan la importancia de otras señales que provocan una respuesta antidepredatoria en las abejas de la miel.

En el capítulo V estudiamos si las abejas sociales, *Apis mellifera*, y solitarias, *Nomia strigata*, marcan con señales químicas las flores en las que han sido atacadas (en las que simulamos un ataque) para avisar a sus conespecíficos del peligro de forrajear en esa flor. Encontramos que aunque las abejas solitarias respondieron de manera similar ante las flores experimentales (donde simulamos el forcejeo) y las flores control, las abejas sociales respondieron de manera muy diferente: a pesar de que las abejas se aproximaron a ambas flores a la misma tasa, tras la aproximación la probabilidad de que las abejas se posaran en la flor era mucho mayor para flores control y la probabilidad de que las abejas rechazaran la flor era mucho mayor si la flor era experimental. Estos resultados apoyan la idea de que un rasgo de las historias de vida de distintas especies de abejas, la sociabilidad, está asociado con la evolución de las señales de alarma.

Los resultados de la presente tesis demuestran que las arañas cangrejo tienen un efecto importante en el comportamiento de los polinizadores que visitan las flores (e incluso los parches) donde se albergan. Estos efectos pueden tener consecuencias a nivel ecológico y evolutivo en el comportamiento antidepredatorio de los polinizadores y este a su vez puede tener consecuencias en las estrategias de caza de las arañas. Además, los resultados de esta tesis revelan posibles efectos resultantes de la interacción araña cangrejo-polinizador en las redes de polinización y en las plantas que albergan los depredadores asociados a flores. Dada la estrecha relación entre los polinizadores y las plantas, cualquier efecto en el comportamiento de los polinizadores podrá afectar, tanto a la estructura de las redes de polinización, al determinar qué especie de polinizador interacciona con qué planta, como al éxito reproductivo las plantas que sean polinizadas por esos visitantes florales. Futuros trabajos deberían estudiar esta posibilidad con más detalle.

INTRODUCCIÓN GENERAL

INTRODUCCIÓN

La estrategia reproductiva de una especie comprende un conjunto de procesos asociados a la producción de descendencia (Thompson 1975). En el caso de las plantas, la viabilidad de la mayoría de sus poblaciones depende, en última instancia, de la producción de semillas, y producir una semilla requiere de la polinización (Doust 1989). Entre el 70 y el 90% de las 250.000 especies estimadas de angiospermas son polinizadas por animales, y de ellas, 67% son exclusivamente polinizadas por insectos (Buchmann & Nabhan 1996; Kearns et al. 1998). El comportamiento de forrajeo de los visitantes florales es uno de los principales determinantes de las pautas reproductivas de las plantas cuyas flores son visitadas por animales. Cierto que, además del comportamiento de los polinizadores, hay muchos factores que determinan tanto el éxito en la transferencia de polen entre las flores – por ejemplo la complementariedad de los rasgos morfológicos de polinizadores y flores (Campbell et al. 1996), como la fertilización del óvulo – por ejemplo los sistemas de incompatibilidad (Barrett 1998). Aún así, en ausencia de polinización por viento, no habrá polen que viaje de una flor a otra a no ser que un polinizador elija posarse en esas dos flores en el orden correcto. Por lo tanto, los polinizadores son esenciales para el funcionamiento de la mayoría de los ecosistemas terrestres, y el conocimiento adecuado su comportamiento de forrajeo es esencial para comprender las relaciones planta-polinizador y para poder entender y gestionar de manera apropiada los recursos naturales.

Además, el comportamiento de los polinizadores determina el flujo de genes entre las plantas, lo que a su vez determina la depresión por endogamia a la que estas plantas están sujetas (Schoen 1982; Linhart et al. 1987). Aunque hay especies en las que las plantas que presentan auto-polinización tienen menor depresión por endogamia que las plantas que presentan polinización cruzada (Fisher 1941; Lloyd 1979), en la mayoría de los casos la viabilidad de la progenie aumenta con la distancia que recorre el polen, al menos para distancias cortas e intermedias (Richards 1986; Johannsson et al. 1998). Además, un mayor flujo de genes implica una mayor variabilidad genética en la población y por tanto una mayor facilidad de adaptarse mediante selección natural a nuevos cambios (Morran et al. 2009). Por lo tanto, en la medida en que las estrategias

de forrajeo de los polinizadores afectan la estructura genética de las poblaciones de plantas, determinan también su trayectoria evolutiva.

El mutualismo planta-polinizador ha despertado gran interés para biólogos desde hace ya más de un siglo. Ya Darwin (1859) señaló su potencial importancia para estudiar los procesos de selección natural cuando escribió "…Así puedo comprender cómo una flor y una abeja pudieron lentamente -ya sea simultáneamente, o una después de otra- modificarse y adaptarse entre sí del modo más perfecto, mediante la conservación continuada de todos los individuos que presentasen ligeras desviaciones de estructura mutuamente favorables" . Desde entonces las relaciones entre plantas y polinizadores han sido un tema central en biología y muchos autores las han citado como ejemplo de evolución mediada por interacciones bióticas (Grant 1949; Van Der Pijl 1961; Fægri & Van Der Pijl 1979; Proctor et al. 1996)

En sus inicios, la ecología de la polinización estudiaba sistemas simples que a menudo consistían en pares aislados de especies de plantas y polinizadores (Vanderpijl 1961; Baker & Hurd 1968). Un ejemplo clásico de coevolución entre pares de especies sería el caso de la orquidea de Madagascar, *Angraecum* sesquipedale, que debido a su gran logitud de corola es polinizada por una especie de polilla, *Xanthopan morgani praedicta*, que tiene una gran longuitud de probóscide para alcanzar el néctar de la flor (Wasserthal 1997). En este caso, existe coevolución entre la longitud de corola de las flores y longitud de probóscide de los polinizadores de tal manera que ambas partes han desarrollado mejores adaptaciones para dispersar el polen (en el caso de las flores) y tomar néctar (en el caso de los polinizadores) en respuesta la una de la otra (Darwin 1862; Nilsson et al. 1985; para otros resultados ver Wasserthal 1997; Rodríguez-Gironés & Llandres 2008). Aunque el estudio de sistemas aislados de pares de especies de plantas y polinizadores ayudó a delimitar preguntas concretas y a estudiar sistemas simples en una disciplina aún por entonces joven, también contribuyó a la idea de que deberíamos esperar relaciones ecológicas y evolutivas estrechas entre esos pares de especies de planta y polinizador.

Posteriormente, la comprensión de que la generalización ecológica en las redes de polinización, donde cada especie de flor es visitada por un gran número de polinizadores de distintas especies (Feinsinger 1983; Roubik 1992), era la norma en

lugar de la excepción, llevó a algunos autores a proponer la idea de que las redes de polinización estaban dominadas por una gran heterogeneidad espacio-temporal en las presiones selectivas. Esto llevó a pensar que la asociación entre plantas y polinizadores era mucho menos estrecha de lo que anteriormente se pensaba (Herrera 1996; Waser et al. 1996). Esta visión promovió una aproximación de conjunto de las redes de polinización, donde las interacciones de plantas y polinizadores son estudiadas como piezas de un puzle ecológico mucho más complejo. En particular, numerosos estudios empezaron a considerar la red de polinización en su conjunto (Jordano 1987; Memmott 1999) y la relación ente las interacciones planta-polinizador y planta-herbívoro (Strauss 1997; Gómez 2005). Como consecuencia, los resultados de la interacción mutualista planta-polinizador se ven alterados por otras posibles interacciones con otras especies: estos grupos de especies coevolucionarán entre sí, ya que las acciones que lleve a cabo una de las partes tendrán influencia en la interacción entre las otras dos partes, de tal manera que esto provocará a su vez un efecto en la primera parte, y esa retroalimentación podrá actuar a escala ecológica y evolutiva (Bronstein & Barbosa 2002; Møller 2008).

La mayoría de los polinizadores son animales que van buscando comida [aunque hay excepciones que incluyen animales buscando pareja (Schiestl et al. 1999; Schiestl 2005) o lugares donde ovopositar (Thompson & Pellmyr 1992)] y sus pautas de movimiento están gobernadas por las mismas reglas que las que siguen otros animales forrajeadores. Dos de los principales factores que afectan a las estrategias de forrajeo de los animales son la distribución de recursos (Stephens & Krebs 1987) y el riesgo de depredación (Lima & Dill 1990). Sin embargo, aunque el efecto de la distribución de recursos ha sido estudiado en numerosos trabajos (Baker & Hurd 1968; Pyke et al. 1977; Pyke 1978; Pyke 1979; Pleasants & Zimmerman 1979; Pleasants 1981; Real 1981; Real et al. 1982), el efecto del riesgo de depredación ha sido ignorado tradicionalmente por el hecho de que parecía demasiado infrecuente como para afectar al comportamiento de los polinizadores (Pyke 1979; Miller & Gass 1985). No obstante, en la última década numerosos estudios han demostrado que los polinizadores pueden presentar una marcada respuesta antidepredatoria (Dukas 2001; Dukas & Morse 2003; Heiling & Herberstein 2004; Dukas 2005; Dukas & Morse 2005; Reader et al. 2006; Ings & Chittka 2008; Brechbühl et al. 2010a), y esto puede afectar al éxito reproductivo de las planta (Muñoz & Arroyo 2004; Dukas 2005; Gonçalves-Souza et al. 2008), teniendo

como consecuencia un efecto indirecto en el mutualismo planta-polinizador: si el riesgo de depredación es suficiente como para inducir cambios en el comportamiento de los polinizadores, los depredadores podría imponer presiones selectivas sobre los rasgos florales de las plantas. El área de investigación del grupo de trabajo en el que esta tesis se enmarca estudia la evolución de las interacciones planta-polinizador. Dentro de este marco, la presente tesis se centra las interacciones entre los depredadores de los polinizadores (arañas cangrejo) y los polinizadores. El efecto de estas interacciones sobre el mutualismo planta-polinizador está siendo estudiado en paralelo por otros miembros del grupo y no será presentado en esta tesis (ver también trabajos teóricos de Jones 2010; Abbott 2010; Higginson et al. 2010).

Según Tinbergen (1963), para poder entender el comportamiento de un animal debemos responder a preguntas que se engloben en una de las siguientes cuatro categorías: preguntas sobre el **desarrollo** o la ontogenia**,** sobre los **mecanismos**, sobre las **funciones** o adaptaciones y sobre la **evolución** de ese comportamiento. Las dos primeras nos permiten conocer las causas próximas que explican ese comportamiento y las dos últimas nos permiten conocer las causas últimas o evolutivas. Hay varios trabajos que responden a preguntas sobre la función del comportamiento antidepredatorio de los polinizadores. Desde el punto de vista funcional tanto la evitación del depredador como la eficiencia del forrajeo podrían ayudar a explicar el comportamiento de los polinizadores. Existen numerosos estudios que han demostrado que los polinizadores evitan flores (Dukas 2001; Heiling & Herberstein 2004; Herberstein et al. 2009), parches (Dukas & Morse 2003; Muñoz & Arroyo 2004) e incluso áreas enteras (Dukas 2005) donde el riesgo de depredación es alto, lo que conlleva una disminución en la tasa de mortalidad de esos polinizadores. Además, existe un estudio de laboratorio que demuestra que el comportamiento antidepredatorio de los abejorros de la especie *Bombus occidentalis* depende de las reservas energéticas de la colmena (Cartar 1991). En este estudio demostraron que a medida que las reservas energéticas de la colmena disminuían, los abejorros aceptaban con mayor probabilidad enfrentarse a un determinado riesgo de depredación cuando forrajeaban (Cartar 1991). Asimismo, de acuerdo con un modelo desarrollado por Clark & Dukas (1994), la respuesta antidepredatoria de las abejas ante una amenaza de un depredador dependerá de su grado de sociabilidad. De acuerdo con este modelo las abejas solitarias deberían iniciar la respuesta de escape más a menudo y a una distancia mayor con respecto al

depredador en comparación con las abejas sociales, ya que para una abeja solitaria la muerte implica la pérdida de todo su éxito reproductivo (si aún no ha puesto huevos que puedan emerger) mientras que para la social sólo implica la pérdida de una de las abejas obreras que alimentan a las crías de la colmena (Clark & Dukas 1994).

Pese al creciente número de publicaciones que estudian la función del comportamiento antidepredatorio de los polinizadores, son pocos los trabajos que investigan sus causas, desarrollo y evolución. No obstante, entre las causas que determinan el comportamiento antidepredatorio, varios estudios han demostrado que las señales visuales y olfativas pueden jugar un papel importante (Dukas 2001; Reader et al. 2006; Abbott 2006; Gonçalves-Souza et al. 2008; Ings & Chittka 2008; Brechbühl et al. 2010b). Asimismo, aunque la bibliografía es más escasa, el **desarrollo** a través del efecto del aprendizaje individual también parece jugar un papel importante en el comportamiento antidepredatorio de algunas especies de abejas y abejorros (Dukas 2001; Ings & Chittka 2008; Ings & Chittka 2009).

Sistema arañas cangrejo-polinizador-flor

Aunque algunos trabajos analizan el efecto de depredadores como lagartijas y avispas sobre el comportamiento de los polinizadores y las interacciones planta-polinizador (Muñoz & Arroyo 2004; Dukas 2005), la mayoría de los estudios han considerado la interacción araña cangrejo-polinizador-flor. Algunas especies de arañas cangrejo (Thomisidae) son depredadores que siguen la estrategia de "sentarse y esperar" utilizando flores como plataformas de caza. Es decir, estas arañas tienden una emboscada a sus presas mientras las esperan escondidas en las flores (Oxford & Gillespie 1998; Thery & Casas 2002; Dukas & Morse 2003; Morse 2007). Para profundizar en el conocimiento del comportamiento antidepredatorio de los polinizadores, el sistema araña cangrejo-polinizador-flor es excelente, ya que, en comparación con otros depredadores que cazan al vuelo (como por ejemplo pájaros o avispas lobo de las abejas), y que por tanto no están asociados con ningún tipo de planta o flor, las arañas cangrejo están totalmente asociadas a flores, ya que atacan a los polinizadores mientras éstos están consumiendo los recursos de las flores (Morse 2007). Aunque no se ha descrito que las plantas tengan una asociación coevolutiva con las arañas cangrejo, hay algunos estudios que han demostrado que las arañas cangrejo

pueden interferir con los polinizadores y provocar como consecuencia un descenso en el éxito reproductivo de las plantas (Suttle 2003; Gonçalves-Souza et al. 2008). Sin embargo, otros estudios no han encontrado efectos significativos de la presencia de arañas cangrejo en el éxito reproductivo de las plantas (Dukas & Morse 2005; Brechbühl et al. 2010b) y existe un estudio que ha encontrado efectos positivos de las arañas cangrejo en las plantas mediados por la depredación de insectos fitófagos (Romero & Vasconcellos-Neto 2004).

Para poder entender el efecto de las arañas cangrejo en el comportamiento de los polinizadores, y como consecuencia su potencial efecto indirecto en el éxito reproductivo de las plantas, habría que estudiar en detalle las estrategias antidepredatorias de los polinizadores ante la presencia de arañas cangrejo. Asimismo, la asociación entre polinizadores y arañas cangrejo es tan estrecha que para poder entender el comportamiento de una de las partes necesitamos entender la otra. Por lo tanto, es necesario estudiar tanto el comportamiento de los polinizadores frente a las arañas como el de las arañas frente a esos polinizadores.

FUNCIÓN

Función del comportamiento antidepredatorio en los polinizadores

La principal función del comportamiento antidepredatorio de un animal es aumentar su probabilidad de supervivencia (Dill 1987; Lima & Dill 1990). Por otro lado, la relación positiva entre la cantidad de recursos obtenidos y el éxito reproductivo es particularmente directa y fácil de medir en algunas especies de polinizadores (e.g Seeley 1985; Pelletier & Mcneil 2003; Bosch & Kemp 2004). Por lo tanto, los polinizadores idealmente deberían comportarse de manera que maximicen la tasa de ingesta y minimicen el riesgo de depredación al mismo tiempo para maximizar su éxito reproductivo. Sin embargo, con bastante frecuencia las estrategias que maximizan la tasa de ingesta de un animal, como por ejemplo salir a forrajear y ser más conspicuo, están asociadas a un mayor riesgo de depredación que las estrategias que consiguen menor cantidad de recursos, como por ejemplo esconderse y quedase inmóvil (Lima & Dill 1990). Para que un animal consiga maximizar su éxito reproductivo necesita llegar a un equilibrio entre conseguir recursos y evitar ser depredado, respondiendo de manera simultánea ante variaciones en el riesgo de depredación y en la disponibilidad de

recursos (revisado en Gilliam & Fraser 1987; Lima & Dill 1990; Brown & Kotler 2004). Sin embargo, hasta la fecha, no existen estudios que determinen cómo se comportan los polinizadores frente a variaciones asociadas a la cantidad de recurso y al riesgo de depredación simultáneamente, ya que se ha estudiado por separado el comportamiento de los polinizadores frente a la variación en la cantidad de recursos y en el riesgo de depredación (e. g. Real & Rathcke 1991; Dukas & Morse 2003). Podría esperarse, por tanto, que los polinizadores estén dispuestos a enfrentarse a mayores niveles de depredación si la cantidad de recursos que esperan obtener de visitar los parches peligrosos es alta (ver, por ejemplo, Nonacs & Dill (1990) para experimentos realizados con colonias de hormigas de la especie *Lasius pallitarsis*, y Butler et al. (2005) para experimentos realizados con el pinzón vulgar *Fringilla coelebs*).

El cómo los polinizadores se enfrenten a variaciones en la cantidad de recurso y en el riesgo de depredación dependerá además de las estrategias de vida de los mismos. Existen modelos teóricos que predicen que el comportamiento antidepredatorio óptimo de las abejas depende de si éstas son sociales o solitarias (Clark & Dukas 1994, Rodríguez-Gironés & Bosh, en revisión). Sin embargo, hasta la fecha sólo existe un experimento de laboratorio con abejorros sociales, *Bombus occidentalis,* en el que encontraron que a medida que las reservas energéticas de la colmena disminuían, los abejorros aceptaban con mayor probabilidad enfrentarse a un determinado riesgo de depredación cuando forrajeaban (Cartar 1991).

Es conocido que las abejas sociales son capaces de comunicarse la presencia de un depredador en distintos contextos (Wittmann 1985; Millor et al. 1999; Breed et al. 2004; Abbott & Dukas 2009; Nieh 2010). A nivel de flor algunos estudios han sugerido que las abejas usan señales químicas de alarma para avisar a sus hermanas de la colmena sobre la presencia de un depredador (Dukas 2001; Reader et al. 2006; Abbott 2006). Sin embargo estos estudios no han conseguido aislar la señal de alarma al usar abejas muertas o su olor. Podría esperarse por tanto que las abejas sociales "marquen" aquellas flores donde han sufrido un ataque con estas señales químicas de alarma y sin embargo esto no pase en el caso de abejas solitarias.

Función de las estrategias de caza en las arañas cangrejo

Los depredadores han evolucionado estrategias de caza que tienden a maximizar el éxito de captura de sus presas y, a su vez, las presas han evolucionado estrategias antidepredatorias que tienden a minimizar el riesgo de ser cazadas por sus depredadores potenciales, lo que conduce a una carrera de armamentos coevolutiva entre depredador y presa (Dawkins & Krebs 1979). Por lo tanto para poder entender el comportamiento antidepredatorio de los polinizadores es indispensable estudiar la función de las distintas estrategias de caza de sus depredadores, las arañas cangrejo.

A pesar de que los depredadores que siguen la estrategia de "sentarse y esperar" no buscan activamente sus presas, es bien conocido que estos depredadores son capaces de usar distintas estrategias para aumentar su tasa de captura. Algunos ejemplos incluirían la elección parches más provechosos (Metcalfe et al. 1997), el mostrar una coloración críptica para evitar ser detectados por las presas (Cott 1957) o el engañar a las presas atrayéndolas (Eberhard 1977), entre otros. Las hembras de algunas especies de arañas cangrejo son capaces de cambiar el color de su cuerpo a lo largo de varios días según el color de la flor en la que se encuentren (Gabritschevsky 1927; Oxford & Gillespie 1998; Thery & Casas 2002; Morse 2007). Además, algunos estudios han demostrado que las arañas de un color eligen preferentemente flores de su mismo color para forrajear (Weigel 1941; Heiling et al. 2005b). Estos estudios apoyan la **hipótesis de la cripsis** en arañas cangrejo, según la cual la habilidad de cambiar de color en estas arañas se ha seleccionado porque los visitantes florales son menos capaces de detectar una araña del mismo color que la flor en la que se encuentra que una araña de color muy contrastante con el de la flor (Oxford & Gillespie 1998; Thery & Casas 2002; Morse 2007).

Sorprendentemente, algunas especies de arañas cangrejo australianas son capaces de explotar el sistema sensorial de sus presas para atraerlas (Heiling et al. 2003; Herberstein et al. 2009). Estudios realizados con arañas cangrejo australianas han demostrado que, para estas arañas, cuanto mayor es el contraste de color entre la araña y la flor, más cantidad de abejas atrae la flor con araña en comparación con una flor control (Heiling et al. 2003; Heiling et al. 2005a; Herberstein et al. 2009). Estas arañas reflejan el color ultravioleta (UV), lo que sugiere que las arañas cangrejo australianas engañan a sus presas con colores que lo polinizadores asocian con comida. Esta idea se conoce como **hipótesis de atracción de presas** (Herberstein et al. 2009).

Hasta la fecha, la hipótesis de la cripsis en arañas cangrejo sólo ha sido puesta a prueba en un estudio de campo en el que, contrariamente a lo que se pensaba, encontraron que el contraste de color en la araña cangrejo europea *Misumena vatia* no juega un papel importante en el camuflaje frente a sus presas (Brechbühl et al. 2010a). .Para poder confirmar estos resultados sería necesario realizar más estudios que determinen el papel del camuflaje en las arañas cangrejo con respecto a sus presas.

La respuesta de las abejas europeas, *Apis mellifera,* frente a arañas cangrejo australianas ha dado resultados similares para distintas especies de araña cangrejo Australianas que reflejan UV: las abejas prefieren posarse en flores con araña en comparación con flores sin araña (Herberstein et al. 2009). Sin embargo esta preferencia desaparece cuando se bloquea la reflectancia UV de las arañas usando crema solar, lo que indica que es la reflectancia UV la que determina la preferencia de las abejas (Heiling et al. 2005a). No obstante, para poder determinar si reflejar UV es beneficioso para estas arañas habría que estudiar directamente la relación entre reflectancia y éxito de caza. Además, la reflectancia UV en las arañas cangrejo australianas varía enormemente entre individuos de la misma y distintas especie e incluso en el mismo individuo, que, dependiendo de las condiciones, refleja más o menos ultravioleta y aún no se sabe bien en qué medida ocurre esta variación en el campo en diferentes poblaciones de arañas ni hay estudios que determinen el comportamiento de las presas ante esta variación en la coloración de las arañas, lo que dificulta la interpretación del papel evolutivo de la reflectancia UV en las arañas cangrejo australianas.

MECANISMOS

Mecanismos de los polinizadores

El procesamiento de la información de color por el sistema visual de las abejas sigue distintas vías dependiendo del ángulo visual subtendido por el objeto: cuando el ángulo es grande las abejas usan el contraste de color o contraste cromático para discriminar entre el objeto (la araña) y su fondo (la flor). Cuando el ángulo es pequeño, en cambio, las abejas usan el contraste en verde o contraste acromático (Giurfa et al. 1996). En la práctica esto significa que el contraste de color es relevante cuando las abejas están a menos de 5-10 cm de las flores, a distancias más grandes las abejas sólo perciben el

contraste en verde. Por lo tanto, para poder estudiar el papel de las estrategias de caza de las arañas cangrejo y su efecto en el comportamiento de las abejas parece necesario tener en cuenta el sistema visual de las abejas.

Mecanismos visuales: estrategias de caza de las arañas cangrejo.

A pesar de que se ha estudiado el comportamiento de las abejas de la miel, *Apis mellifera*, frente a distintas especies de arañas cangrejo australianas que reflejan UV (Herberstein et al. 2009), hasta la fecha sólo existe un experimento en el que estudiaron la respuesta de una especie de abeja nativa australiana, *Austroplebeia australis,* frente a la arañas cangrejo australiana, *Thomisus spectabilis*. En este estudio encontraron que, al igual que las abejas de la miel, las abejas nativas son también más atraídas por flores con araña, acercándose más frecuentemente a flores con araña que a flores sin araña. Sin embargo, a la hora de posarse eligen posarse en flores sin araña más frecuentemente que en flores con araña (Heiling & Herberstein 2004). Estos resultados sugieren que en la coevolución entre abejas nativas y arañas cangrejo, las abejas han desarrollado una respuesta antidepredatoria. En cambio, las abejas europeas, que han sido introducidas en Australia hace sólo unos 200 años (Hopkins 1886), aún no han tenido la oportunidad de desarrollar una respuesta antidepredatoria frente las arañas australianas que reflejan UV (Heiling & Herberstein 2004). Cabe destacar que las arañas cangrejo europeas, con las que las abejas de la miel sí que han compartido una historia evolutiva, no reflejan UV o reflejan muy poca cantidad en el campo, y hay experimentos de laboratorio que demuestran que las abejas de la miel o ignoran o muestran una respuesta de evitación frente a las arañas europeas pero nunca se sienten atraídas por ellas (Herberstein et al. 2009; Brechbühl et al. 2010a). Sin embargo el hecho de que sólo se haya estudiado la respuesta de las abejas nativas frente a arañas nativas australianas en una especie de abeja nativa y en una especie de araña australiana dificulta la extrapolación de la idea de que las abejas australianas han evolucionado una estrategia antidepredatoria frente a sus depredadores nativos.

Como se ha mencionado anteriormente, hasta la fecha la hipótesis de la cripsis en arañas cangrejo sólo ha sido puesta a prueba en una especie de araña cangrejo europea *Misumena vatia* (Brechbühl et al. 2010a). Sin embargo, en este estudio los experimentadores eligieron los tratamientos en función de cómo percibían ellos el

contraste entre arañas y flores, sin tener en cuenta que el sistema visual de los polinizadores es muy distinto al de los humanos y que los polinizadores pueden no percibir lo que para nosotros es altamente contrastante. Para poner a prueba la hipótesis del camuflaje es necesario tener en cuenta el sistema visual de las abejas a la hora de determinar los distintos tratamientos, pues sólo así podemos estar seguros de que las arañas del tratamiento "críptico" presentan realmente un menor contraste con la flor, según lo perciben las abejas, que las arañas del tratamiento "contrastante".

Mecanismos olfativos: señales de alarma en abejas

Es bien conocido que las abejas sociales utilizan señales olfativas para comunicarse a la hora de desarrollar distintas tareas, como por ejemplo organizar a los miembros de la colmena para la realización de las distintas actividades (Free 1987), coordinar la actividad de forrajeo (Goulson 2003; Nieh 2004; Thom et al. 2007), o para señalizar sobre la presencia de un depredador cerca de la colmena (Wittmann 1985; Millor et al. 1999). Además de comunicarse sobre la presencia de un depredador cerca de la colmena, las abejas sociales son capaces de comunicarse sobre la presencia de un depredador en otros contextos. Un estudio reciente ha demostrado que después de sufrir un ataque en una determinada fuente de alimento, las abejas de la miel son capaces de incorporar un feedback negativo en la danza de sus hermanas de la colmena que previene el reclutamiento de nuevos individuos a la fuente de alimento donde las abejas han sido previamente atacadas (Nieh 2010). Asimismo, mientas forrajean en las flores, se ha sugerido que las abejas también son capaces de comunicarse la presencia de un depredador usando señales olfativas: los abejorros y abejas de la miel evitan flores que contienen o una abejorro muerto recientemente o su olor, lo que sugiere que las feromonas de alarma emitidas por un abejorro muerto podrían ser una señal olfativa que provoque el comportamiento antidepredadorio en conespecíficos (Dukas 2001; Reader et al. 2006; Abbott 2006). Sin embargo con estos estudios no queda claro si las abejas sociales están respondiendo a señales de alarma o a factores asociados con abejas estresadas, heridas o muertas.

Mecanismos de las arañas cangrejo

Algunos experimentos de laboratorio han demostrado que las arañas cangrejo de la especie *Misumena vatia* son capaces de cambiar el color de su cuerpo en función del

color del fondo en el que se encuentran (Packard 1905; Gabritschevsky 1927; Thery 2007). Sin embargo aún no se ha realizado ningún estudio que determine el mecanismo a través de cual las arañas cangrejo australianas con capaces de reflejar UV y cambiar el color de su cuerpo con respecto al sistema visual de sus presas. Manipulando el régimen de comida y el color de fondo donde las arañas se encuentran podríamos determinar si el reflejar UV en estas arañas depende del fondo de color, del régimen de comida o de la interacción entre ambos. Además, con este experimento y con datos de campo de reflectancia UV y condición para estas arañas podríamos determinar si es realmente el estado de inanición lo que produce la reflectancia UV o viceversa, lo que nos permitiría resolver la relación de causalidad, si la hubiera, entre coloración y condición para estas arañas.

DESARROLLO

La habilidad de un animal para detectar a un depredador puede ser innata o aprendida. Existen crecientes estudios que sugieren que el repertorio de estrategias antidepredatorias en invertebrados puede depender de su experiencia pasada frente a depredadores (Chivers et al. 1996; Rochette et al. 1998; Wisenden & Millard 2001). Según estos estudios, el aprendizaje puede ayudar a los animales a "refinar" su respuesta antidepredatoria en determinados ambientes.

Papel del desarrollo en el comportamiento antidepredatorio de los polinizadores

Existen muy pocos estudios que hayan tenido en cuenta el papel del aprendizaje en las estrategias antidepredatorias de los polinizadores. A pesar de ello, los resultados de estos estudios demuestran que el aprendizaje individual puede tener un efecto importante en el comportamiento antidepredatorio de algunas especies de abejas y abejorros (Dukas 2001; Ings & Chittka 2008; Ings & Chittka 2009). En estos estudios los autores encontraron que las abejas y abejorros aprenden a evitar aquellas flores donde han sufrido previamente un ataque(Dukas 2001), las flores que contienen arañas (Ings & Chittka 2008; Ings & Chittka 2009) e incluso las flores que no tienen depredadores pero que son del mismo color que las flores donde han sufrido un ataque (Ings & Chittka 2009) .

Papel del desarrollo en el comportamiento de caza de las arañas cangrejo

Respecto al desarrollo del comportamiento de caza de las arañas frente a sus polinizadores, Morse (2000) demostró que el papel de la experiencia puede determinar el uso de flores para forrajear en las arañas cangrejo de la especie *Misumena vatia*. Sus resultados demostraron que la experiencia previa de estas arañas cuando forrajeaban en una determinada especie de flor podía modificar su comportamiento en la elección de flores donde forrajear a continuación y esto afectó al éxito de captura de las arañas.

Por lo tanto, a pesar de que la bibliografía sobre el papel del desarrollo en el comportamiento de los polinizadores frente a las arañas y en el comportamiento de las arañas frente a los polinizadores es escasa, los estudios publicados en este área indican que la experiencia a través del aprendizaje puede jugar un papel importante en los sistemas araña cangrejo-polinizador.

EVOLUCIÓN

Evolución del comportamiento antidepredatorio en polinizadores

De acuerdo con la regla de Hamilton (1964a; 1964b), al detectar a un depredador potencial, un individuo se beneficiará de alertar a los miembros del grupo si el coste asociado al señalizador, C, es menor que el beneficio acumulado para los miembros del grupo, B_i, descontando por parentesco genético entre receptor y emisor, r_i.

$$C < \Sigma r_i \cdot B_i,$$

donde el sumatorio del término de la derecha se realiza para todos los miembros del grupo.

Uno de los principales costes de las señales de alarma es que aumenta la probabilidad de detección, atrayendo la atención de depredadores. Pero este coste desaparece cuando el señalizador está siendo atacado. Cuando esto sucede, el coste de producir una señal de alarma es mínimo y la regla de Hamilton se satisface fácilmente si $r > 0$. Cuando son atacados, muchos insectos emiten señales de alarma que son usadas como señales de advertencia por sus conespecíficos para huir del peligro (Aldrich et al. 1991; Hardie & Minks 1999; Wyatt 2003). La regla de Hamilton se cumplirá a menudo en insectos eusociales, subsociales y clónicos, ya que se caracterizan por su alto valor de r y B: las

víctimas suelen estar próximas a otros individuos, a los que suelen estar emparentados (alto valor de *r*) y que se beneficiarán de la señal de alarma (alto valor de *B*). Por lo tanto deberíamos esperar que las señales de alarma evolucionen en abejas sociales con reclutamiento que suelen forrajear próximas a otros individuos de su misma colmena y sin embargo no lo hagan en abejas solitarias caracterizadas por su bajo valor de *r* y *B*. Sin embargo hasta la fecha no existe ningún estudio que considere cómo un rasgo de las historias de vida de las abejas, la sociabilidad, afecta a la evolución de las estrategias de evitación de los depredadores, emisión y respuesta ante señales de alarma.

Evolución de las estrategias de caza en arañas cangrejo

Hasta la fecha todas las especies de arañas cangrejo australianas a las que se les ha medido el color reflejan UV y sin embargo ninguna de las cinco especies de arañas cangrejo europeas que se han medido lo hacen (Herberstein et al. 2009). Estos datos revelan que la reflectancia UV en arañas cangrejo es más común en Australia que en Europa. Sin embargo, para confirmar que la reflectancia UV en arañas cangrejo es un rasgo que sólo ocurre en Australia, sería necesario hacer mediciones de más especies de arañas cangrejo australianas y europeas y realizar una filogenia del grupo para determinar si la reflectancia UV en arañas cangrejo australianas es sinapomórfica y derivada de un antecesor común o si, por el contrario, ha evolucionado de manera independiente varias veces. Aunque en la presente tesis este objetivo no se aborda, el estudio de la reflectancia UV en arañas cangrejo europeas y australianas está siendo realizado por el grupo de trabajo en el que se enmarca esta tesis.

A pesar de que el papel de la evolución en los sistemas araña cangrejo-polinizador podría jugar un papel determinante en el comportamiento antidepredatorio de los polinizadores frente a las arañas cangrejo y en el comportamiento de caza de las arañas frente a los polinizadores, en la actualidad no existe ningún trabajo publicado en este área de estudio.

JUSTIFICACIÓN Y OBJETIVOS

La presente tesis pretende profundizar en el conocimiento de la función y los mecanismos que están detrás del comportamiento antidepredatorio de los polinizadores y del comportamiento de caza de las arañas cangrejo, entendiendo cómo contribuye ese comportamiento a la supervivencia y, potencialmente, a la reproducción del animal en cuestión y los mecanismos que están detrás del mismo. A pesar de que el estudio del desarrollo y la evolución de esos comportamientos son objeto de estudio del grupo de trabajo donde esta tesis se enmarca, la presente tesis no se centra en el estudio de los mismos.

Respuesta de los polinizadores ante variaciones en la cantidad de recursos y el riesgo de depredación (capítulo I)

A pesar de que se ha estudiado el efecto de los depredadores asociados a flores (Dukas & Morse 2003; Suttle 2003; Reader et al. 2006) y de la cantidad de recursos (Pleasants 1981; Real & Rathcke 1991; Makino & Sakai 2007) sobre el comportamiento de los polinizadores por separado, hasta la fecha no existe ningún experimento que estudie el comportamiento de los polinizadores ante variaciones en la disponibilidad de néctar y en el riesgo de depredación de manera simultánea. Cabría pensar que, al igual que otros animales (revisado en Gilliam & Fraser 1987; Lima & Dill 1990; Brown & Kotler 2004), los polinizadores aceptarían enfrentarse a mayores niveles de depredación cuando explotan parches ricos en néctar que cuando explotan parches pobres en néctar. Uno de los objetivos de esta tesis es estudiar la respuesta de los polinizadores a la interacción entre riesgo de depredación y disponibilidad de néctar (**capítulo I**). Para llevar a cabo este objetivo realizamos un experimento en el que asignamos parches de flores a los siguientes tratamientos: con arañas y con néctar añadido, con arañas y sin néctar añadido, sin arañas y con néctar añadido y sin arañas y sin néctar añadido. Para ello añadimos de 3 a 7 arañas cangrejo de las especies *Thomisus onustus* y *Synaema globosum* a los parches con araña y retiramos todas las arañas cangrejo de los parches sin araña. Posteriormente registramos el número de polinizadores y el número de visitas por polinizador en los distintos grupos de polinizadores más abundantes (abejas de la miel, *Apis mellifera*, y sírfidos, *Eristalis tenax*) a cada parche. Para determinar la

susceptibilidad de depredación de abejas y sírfidos, en aquellos parches con araña anotamos durante el periodo de observación el número de abejas y sírfidos atacados y capturados por las arañas, así como la identidad de las presas de aquellas arañas que estaban comiendo al llegar a observar el parche. Además, para tener una idea de lo que ocurre a nivel de flor, para cada visita de abejas y sírfidos a flores con araña anotamos la respuesta de la araña tras la visita del polinizador y el comportamiento del polinizador tras la respuesta de la araña.

Relación causa-efecto entre coloración y condición en arañas cangrejo australianas y respuesta de las abejas nativas frente a arañas cangrejo australianas (capítulos II y III)

Básandonos en la hipótesis de atracción de presas en arañas cangrejo australianas, predeciríamos que si reflejar UV implica atraer más presas y no tiene ningún coste asociado para las arañas, todas las arañas reflejarían UV. Sin embargo, si el reflejar UV implica un coste para las arañas, como por ejemplo un mayor riesgo de depredación [algunos depredadores de arañas, como pájaros y avispas, ven el UV (Peitsch et al. 1992; Maier 1992)], esperaríamos que sólo las arañas más hambrientas, para las que el riesgo de morir de hambre fuera alto, reflejaran UV para atraer presas y en cambio las arañas más saciadas siguieran una estrategia de reflejar menos UV y ser menos conspicuas para sus depredadores. Para resolver la relación, si la hubiera, de causalidad entre reflectancia UV y condición de las arañas cangrejo y para determinar si las arañas son capaces de modular su reflectancia UV en función de su régimen de comida (**capítulo II**), analizamos la relación entre condición y coloración para la araña cangrejo australiana *Thomisus spectabilis* usando datos de campo de arañas recolectadas en la misma población en dos años consecutivos (2008 y 2009), y, en el laboratorio, estudiamos si las arañas responden cambiando el color (modulando la reflectancia UV) frente a distintos regímenes de comida (alto y bajo) y de color de fondo (fondo blanco con alta reflectancia UV y fondo amarillo con baja reflectancia UV). El experimento se llevó a cabo en un invernadero cuyo revestimiento era permeable al paso de la luz de longitudes de onda entre 300 y 700 nm. Estos datos fueron analizados teniendo en consideración el sistema visual de las presas potenciales de las arañas cangrejo.

Para ver si es generalizable la hipótesis de que, a diferencia de las abejas europeas, las abejas australianas presentan un comportamiento antidepredatorio frente a las arañas

cangrejo australianas (**capítulo III**), realizamos un experimento de elección de flores en el que le ofrecimos a abejas nativas australianas de la especie *Trigona carbonaria* dos flores: una con una araña cangrejo de la especie *Diaea evanida* y otra sin araña. Durante el experimento anotamos el número de abejas que se aproximaron a las dos flores y el primer aterrizaje en una de las flores. El mismo procedimiento lo repetimos cubriendo las flores con un plástico transparente para eliminar las pistas olfativas. Para determinar la variación en la coloración en distintas poblaciones de arañas cangrejo en el campo así como la respuesta de distintas presas nativas ante parte de esa variación (**capítulo III**), hicimos un estudio en el que cuantificamos el color de dos especies de arañas cangrejo, *Thomisus spectabilis* y *Diaea evanida*, recolectadas en el campo y determinamos la relación entre el porcentaje de aproximaciones a flores con araña y la variación en la coloración de las arañas para nuestro experimento con *Diaea evanida* y *Trigona carbonaria* y para un experimento publicado con *Thomisus spectabilis* y *Austroplebeia australis* (Heiling & Herberstein 2004). Estos datos fueron analizados teniendo en consideración el sistema visual de las presas potenciales de las arañas cangrejo.

Papel del camuflaje, el movimiento, el UV y el tamaño de las arañas cangrejo australianas en la tasa de visitas de las abejas de la miel y el éxito de captura de las arañas (capítulo IV).

Hasta la fecha, la hipótesis de la cripsis en arañas cangrejo sólo ha sido puesta a prueba en un estudio de campo en el que, contrariamente a lo que se pensaba, encontraron que el contraste de color en la araña cangrejo europea *Misumena vatia* no juega un papel importante en el camuflaje frente a sus presas (Brechbühl et al. 2010a). Sin embargo en este estudio los experimentadores eligieron los tratamientos en función de cómo se percibía el contraste de color entre arañas y flores para el ojo humano, sin tener en cuenta que el sistema visual de los polinizadores es muy distinto al de los humanos y que los polinizadores pueden no percibir lo que para nosotros es altamente contrastante o percibir aquello que es indetectable para nosotros, como por ejemplo el color UV. Asimismo, aunque se sabe que las arañas cangrejo australianas eligen preferentemente flores de su color para forrajear (Heiling et al. 2005b), aún no se ha realizado ningún estudio en el que se determine el papel del camuflaje en estas arañas que reflejan UV con respecto a las presas que "engañan".

Para determinar el papel del camuflaje en las arañas cangrejo australianas con respecto a sus presas (**capítulo IV**), realizamos un experimento de campo en el que estudiamos el efecto del camuflaje entre la araña cangrejo de la especie *Thomisus spectabilis* y la abeja de la miel *Apis mellifera*. En el experimento estudiamos la respuesta de las abejas ante la presencia de arañas cangrejo amarillas y blancas situadas en flores de la especie *Bidens alba* (margaritas blancas con el centro amarillo). Asimismo, también estudiamos la respuesta de las abejas frente a arañas en las que manipulamos el color del cuerpo pintando de azul sus patas delanteras o su abdomen, haciéndolas así altamente detectables tanto cromáticamente como acromáticamente. Además de la respuesta de las abejas frente a las arañas en los distintos tratamientos, también estudiamos el éxito de captura de las arañas. Estos datos fueron analizados teniendo en consideración el sistema visual de las presas potenciales de las arañas cangrejo.

<u>¿Marcan las abejas sociales y solitarias las flores peligrosas? (capítulo V)</u>

A pesar de que se ha sugerido que las abejas sociales son capaces de comunicarse la presencia de un depredador a nivel de flor usando señales químicas, aún no existe ningún estudio que determine si las abejas realmente usan señales químicas para marcar la presencia de depredadores. Según la regla de Hamilton (1964a; 1964b), deberíamos esperar que las señales de alarma evolucionen en abejas sociales pero no en abejas solitarias. Por ello uno de los objetivos de esta tesis se centra en estudiar si las abejas sociales y solitarias usan señales químicas para marcar la presencia de depredadores Para determinar si las abejas sociales y solitarias responden a señales olfativas de alarma emitidas por conespecíficos recientemente atacados en flores (**capítulo V**) realizamos un experimento en el que determinamos si la abeja social, *Apis mellifera*, y la abeja solitaria, *Nomia strigata*, usan señales químicas de alarma liberadas por conespecíficos que han sido recientemente atacados para detectar flores potencialmente peligrosas. Para ello contamos el número de abejas sociales y solitarias que visitaron y rechazaron flores donde habíamos simulado un ataque de un depredador atrapando a una abeja con unas pinzas y lo comparamos con el número de abejas que visitaron y rechazaron flores control.

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Capítulo I

Susceptibility to attacks determines how pollinators trade off predation risk for foraging success

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Abstract

Although the behaviour of animals facing the conflicting demands of increasing foraging success and decreasing predation risk has been studied in many taxa, in the context of pollination the response to food availability and predation risk have only been studied in isolation. We compared visit rates of hoverflies and honeybees to 40 *Chrysanthemum segetum* patches in which we manipulated the patch level of predation risk (with and without crab spiders) and of nectar availability (rich and poor patches) using a full factorial design. Pollinators responded differently to the trade-off between food reward and predation risk: honeybees preferred rich safe patches and avoided poor risky patches while the number of hoverflies was highest at poor risky patches. Because honeybees were more susceptible to predation than hoverflies, our results confirm theoretical predictions according to which, in the presence of competition for resources, less susceptible pollinators should concentrate their foraging effort on riskier resources. Crab spiders had a negative indirect effect on the rate at which inflorescences were visited by honeybees. This effect was mediated through changes in the foraging strategy of honeybees, and could be reversed by increasing nectar productivity of inflorescences.

Submitted

Introduction

While early work in plant-pollinator interactions focused on pair-wise interactions, recent work has shown that plant-pollinator interactions are embedded within communities that are comprised of multi-species interactions (e.g. Jordano 1987; Bascompte et al. 2003; Ings et al. 2009). Because predators can determine the number, types, abundances and spatial patterns of species that co-occur in natural communities (Connell 1971; Power et al. 1985; Croll et al. 2005; Larson & Paine 2007), they could play an important role in structuring plant-pollinator interactions (Suttle 2003; Muñoz & Arroyo 2004; Dukas 2005). Nevertheless, the interplay between predation risk and resource availability has so far been neglected in the context of plant-pollinator interactions.

Foragers must often trade-off between maximizing the rate of resource harvesting and minimizing predation risk. When animals must choose between foraging at rich patches where they are exposed to high levels of predation risk or poor patches where they are exposed to low levels of predation risk, in general they select the rich-risky patches if the difference in intake rate that they can expect is high or the difference in predation risk is low (see Gilliam & Fraser 1987; Dill 1987; Lima & Dill 1990; Brown & Kotler 2004 for reviews). For example, Nonacs and Dill (1990) found that when they exposed *Lasius pallitarsis* ant colonies to patches that differed in food quality and mortality risk, the ants foraged at the risky patches only when the difference in intake rate between risky-rich and safe-poor patches was large enough that the increase in colony growth rate compensated for mortality losses. Butler et al. (2005) found a similar pattern when studying the response of chaffinches, *Fringilla coelebs*, choosing between safe and risky patches that varied in the density of seeds they contained: the birds switched from safe to risky patches when seed density in risky patches was between two and four times greater than in safe patches.

In the context of pollination responses to resource availability (see e.g. Pleasants 1981; Real & Rathcke 1991; Makino & Sakai 2007) and predation risk (Dukas & Morse 2003; Suttle 2003; Dukas 2005) have been studied in isolation. Because of their strong, direct links between resource acquisition and fitness, pollinators have long been used as a model system to test predictions from optimal foraging theory (e.g. Pyke 1979; Heinrich 1979; Dreisig 1995). The effect of predators on pollinator behaviour, on the other hand, was long neglected on the assumption that predation is too infrequent to affect the foraging strategy of pollinators (Pyke

1979; Miller & Gass 1985). Nevertheless, a number of studies over the last decade have shown that predators can affect the foraging strategy of pollinators at the inflorescence, plant and patch levels (Dukas 2001; Dukas & Morse 2003; Muñoz & Arroyo 2004; Dukas 2005). These studies have shown that, through their non-consumptive effects on pollinator behaviour (Dukas & Morse 2003; Gonçalves-Souza et al. 2008), predators may have top-down effects on plant fitness and even affect the structure of the plant-pollinator community (Suttle 2003; Gonçalves-Souza et al. 2008).

The response of pollinators to the interplay between resource availability and predation risk has ecological and evolutionary implications. When predators are relatively sedentary and flowers or inflorescences long-lived (Morse 2007), resources will tend to accumulate in areas where predation risk is high if pollinators avoid them. Will the accumulation of resources tempt pollinators back into predator-rich patches? A similar question can be raised at the evolutionary time scale. Predators can show strong preferences for some host plant species (Morse 2000; Morse 2007). Any plant species that recurrently experiences low reproductive success because it is used as hunting platform by ambush predators might be selected to increase reward production – at least if pollinators are willing to increase their exposure to predation risk in order to increase the rate at which they acquire resources. The purpose of this paper is hence to study how pollinators trade off foraging efficiency for avoidance of predation risk. To tease apart the role of predators ambushing at flowers (hereafter referred to as ambush predators) and resource availability from other floral traits, instead of comparing visit rates at flower species naturally differing in nectar production and the frequency with which they harbour ambush predators, we compared visit rates at flowers of a single species manipulated to differ in their level of predation risk and resource availability. In particular, this experiment allows us to answer the following questions:

- How do pollinators trade off between intake rate and avoidance of predation risk?
- If pollinators avoid predator-rich areas, could inflorescences recover their attractiveness increasing their rate of nectar production?
- Do ambush predators affect plant-pollinator interactions through their direct effects on pollinator densities or through their indirect effects on pollinator behaviour?

The purpose of this study is not so much to learn how spatial heterogeneity in resource availability and predation risk affects plant-pollinator interactions in a particular community, as

to understand the factors affecting the foraging strategies of pollinators. This insight can then be incorporated into models and used to understand the ecological assemblage and evolutionary trajectories of pollination networks (Rodríguez-Gironés & Santamaría 2005; Rodríguez-Gironés & Santamaría 2010).

Materials and methods

Study site and species

We conducted our experiment in May 2007 in an abandoned crop field at "la Raña" (39º 41' 51" N, 5º 27' 55"W) within "Las Villuercas-Ibores" region in Extremadura, south-western Spain. The most common flowering plants at our study site were *Chrysanthemum segetum* L. (Asteraceae), *Ornithopus compressus* L. (Papilonaceae), *Anthemis sp*. (Asteraceae), *Hedypnois cretica* L. (Asteraceae), *Leontodon taraxacoides* Mérat (Asteraceae), *Echium plantagineum* L. (Boraginaceae), *Silene gallica* L. (Caryophyllaceae) and *Calendula arvensis* L. (Asteraceae). We selected the field site because of the abundance of *C. segetum* inflorescences: they are commonly used by crab spiders as hunting platform and are visited by a large number of nectar- and pollen-collecting insects, including Hymenoptera, Diptera, Coleoptera and Lepidoptera. In what follows, we consider only the interactions between honeybees, *Apis mellifera* L. (Apidae), hoverflies, *Eristalis tenax* L. (Syrphidae), hereafter "hoverflies" and *C. segetum*, as they were the only ones sufficiently common for statistical analysis.

We used adult females of two crab spider species, *Thomisus onustus* Walckenaer and *Synema globosum* Fabricius (Thomisidae), as ambush predators in our experiment. Crab spiders are sit-and-wait ambush predators and use their enlarged powerful raptorial front legs to capture their prey (Morse 2007). *T. onustus* and *S. globosum* prey mainly on bees and flies and were locally abundant in our field site.

Experimental treatments

We selected 40 1x1 m^2 patches with high density of *C. segetum* for the experiment and mowed a 1.5 m wide strip of vegetation around each patch to decrease the number of crab spiders leaving the patch by bridging (Corcobado et al. 2010). We grouped patches in ten blocks of four nearby patches each and allocated patches at random to the following treatments: rich-risky patches, poor-risky patches, rich-safe patches and poor-safe patches, with one patch of each type per block. On 06–May-2007 we counted and removed all the spiders we found from the 40 patches and we also counted the number of pollinators per patch at four times of day:

09:30, 11:30, 15:30 and 17:30. We counted the number of *C. segetum* inflorescences per patch on 6, 13, 20 and 29-May-2007 and 01–Jun-2007.

On 07-May-2007 we added seven *T. onustus* and three *S. globosum* females to each risky patch. To counteract the effects of spider displacements, we removed all spiders we found in safe patches during the experiment and we added crab spiders to risky patches whenever we found less than three individuals during the observations.

For the nectar treatment we added 50 μl of 15% (w/w) sucrose solution to 40 haphazardly selected *C. segetum* inflorescences (not harbouring spiders) in each rich patch twice per day, starting at 09:00 and 14:00. We used low concentration nectar because of the speed at which water evaporated from the exposed droplets. We chose the number of inflorescences to which we added sucrose solution and the amount of sucrose added per inflorescence so as to double nectar availability in rich and poor patches (see results). Sucrose solution was added with a repeater micropipette on the disc of inflorescence heads. To control for possible effects of manipulation, we followed the same procedure (albeit with an empty pipette) in poor patches. We selected the order in which nectar was added to patches each day at random, with the following constraints: patches within a block were visited sequentially, the 20 patches that were visited first at 09:00 were visited last at 14:00, and patches that were visited first one morning were visited last the following morning.

We observed each patch for 15 minutes per day. We counted the number of pollinators present in the patch upon arrival of the observer and we recorded the number of insects visiting the patch and the number of inflorescences visited by each insect during the observation period. We also recorded the number of spiders in the patch and how many of them were consuming prey at the start of the observations. Finally, we recorded the number of spider attacks and prey captures. Twenty patches were observed between 10:00 and 14:00, and the remaining 20 after 15:00. In each half of the day we observed the 20 patches where nectar had been more recently added. The experiment was discontinued during rainy days.

Strength of manipulations

The strength of the predation-risk manipulation was determined comparing the number of crab spiders present in risk and safe patches during the experiment with their natural density, which was estimated from the number of crab spiders we encountered on patches before the start of the experiment.

To assess the strength of the nectar enrichment manipulation, we assumed that nectar production rate by *C. segetum* is similar to that of *C. coronarium* in Greece, where each disk

floret produces 0.01 µl of 47% (w/w) nectar per day (Petanidou & Smets 1995). Because there are approximately 300 disk florets per inflorescence in *C. segetum* (Howarth & Williams 1972), nectar productivity must be about 3 µl per inflorescence. Nectar productivity per patch was therefore estimated as the number of *C. segetum* inflorescences times 3 µl of 47% (w/w) per inflorescence. We used an ANOVA to test whether rich and poor patches differed in the number of inflorescences (averaged over the five counts) and hence in the amount of resources they produced.

Effect of predation risk and resource availability – patch level

For each patch, we calculated the average number of pollinators (honeybees and hoverflies) visiting each patch, the average number of inflorescences visited by pollinator within the patch and the average number of open inflorescences observed between 15 and 19-May-2007. (Averages were thus based on five observations per patch.) These average values were entered into a mixed effects model to determine the effect of treatment and inflorescence abundance on pollinator behaviour. The model included nectar presence (poor vs. rich patches), spider presence (safe vs. risky patches) and their interaction as fixed factors, block (10 levels) as random factor and number of inflorescences per patch as a covariate. The dependent variables were the number of pollinators (honeybees and hoverflies) and the average number of inflorescences visited per pollinator within the patch. To achieve homogeneity of variances, we log transformed the number of pollinators and square-root transformed the number of inflorescences that each pollinator visited. Interactions between the number of inflorescences per patch and treatment (resource availability and/or predation risk) are not reported because they were never statistically significant and models including these interactions always lead to increases in the AIC value greater than two units (Akaike 1973).

Effect of predation risk and resource availability – inflorescence level

We first analysed the effect of predation risk and resource availability on the average number of visits that inflorescences received. For each patch, we divided the total number of visits recorded during the observations (averaged over 15 to 19-May-2007) by the number of inflorescences in the patch, thus obtaining the average number of visits per inflorescence. We analysed separately the data for honeybees and hoverflies, using mixed effects models that included nectar presence (poor vs. rich patches), spider presence (safe vs. risky patches) and their interaction as fixed factors and block (10 levels) as random factor. We used Box-Cox

transformations to achieve homogeneity of variances, with $\lambda = 0.35$ for the honeybees and $\lambda = 0.5$ (equivalent to the square-root transformation) for the hoverflies.

We then focused on the response of pollinators to the presence of predators on the inflorescences they approached. To determine whether pollinator species and patch resource availability affected the probability of landing on predator-harbouring inflorescences we used a generalized linear mixed effects model with a binomial distribution and identity link function. For each inflorescence visited in risky patches throughout the period of observations, the type of inflorescence chosen (with or without predator) was used as dependent variable in the analysis. Resource availability (rich vs. poor patches) and pollinator species (honeybees vs. hoverflies) were the fixed factors, block (10 levels) was used as random factor and the proportion of inflorescences harbouring spiders was used as covariate. If pollinators chose inflorescences at random, irrespective of the presence of spiders, there should be a linear relationship, with slope of one, between the proportion of inflorescences harbouring spiders and the probability of choosing a spider-harbouring inflorescence. If pollinators avoided inflorescences with spiders, the probability of choosing a spider-harbouring inflorescence might increase with the proportion of inflorescences harbouring spiders, but the slope of the relationship would be smaller than one. To test whether pollinators avoided spiders, we performed a Wald's Z test on the slope (Dobson & Barnett 2008), the null hypothesis being slope = 1.

Effect of spider encounters on patch departure

Whenever a pollinator landed on a spider-harbouring inflorescence, we scored the response of the spider according to one of the following categories: indifference if the spider did not respond to the arrival of a pollinator, approach if the spider oriented and moved in the direction of the pollinator, strike if the spider attempted to capture the pollinator with its forelegs and failed to contact the pollinator, struggle if the spider enclosed the pollinator with its forelegs but the pollinator managed to escape and kill if the spider managed to capture the pollinator. If the pollinator was not killed, we further recorded whether the next inflorescence it visited was within the same patch. We used a generalised linear mixed effect model with binomial distribution to determine the factors affecting patch departure (stay vs. leave patch). The model included spider response (still, approach, strike and struggle), resource availability (rich vs. poor patches) and pollinator species (honeybees vs. hoverflies) as fixed factors and block (10 levels) as random factor.

Susceptibility to predation

We compared the susceptibility to predation of honeybees and hoverflies in two ways. First we used Fisher's exact test to compare (1) the proportion of honeybees and hoverflies that were attacked by spiders after landing on spider-harbouring inflorescences, and (2) from the attacked individuals, the proportion that were actually captured. In this analysis, we only included those pollinators that landed on spider-harbouring inflorescences while we were observing the patch. In a second analysis, we estimated susceptibility to predation as the number of honeybees or hoverflies that were being consumed by spiders when we arrived to a patch, normalised by the rate at which pollinators of the corresponding species visited the patch. (For each patch, we obtained a single value averaging over all observations.) This surrogate of susceptibility was then compared (honeybees vs. hoverflies) with a Wilcoxon matched-pairs test.

Unless otherwise specified, all results are reported as average ± SD, where averages refer to least squares means.

Results

Strength of manipulations

Because *C. segetum* inflorescences in our patches received over 99% of honeybee and hoverfly visits to experimental patches during the observations, as a first approximation we can ignore other plant species when estimating resource availability per patch. There were no significant differences ($F_{1,38} = 0.128$, $P = 0.721$) between the number of *C. segetum* inflorescences at rich (175.4±65.5) and poor patches (182.3±55.5). At 3 µl of nectar per inflorescence, the average patch offered 537 µl of nectar. Given that the density of 15% and 47% sucrose solution is 1.06 and 1.22 $g \cdot cm^{-3}$, respectively, the 50 µl of 15% sucrose solution that we added to 40 inflorescences per rich patch correspond to 554 µl of 47% nectar, implying that we essentially doubled the amount of nectar available at rich patches. On average, we added nectar to 22% of *C. segetum* inflorescences.

There were 0.014±0.012 crab spiders per inflorescence before the manipulation, with a range of 0 to 6 spiders per patch. During the observations, the number of spiders per inflorescence in risky patches was 0.023±0.009, with 0 to 7 spiders per patch. Therefore the distribution of the number of crab spiders per patch had similar ranges in risky and un-manipulated patches, although the average spider density in risky patches was 65% higher than the natural density in

the area. We found and removed only 5 spiders from the safe patches throughout the experiment, so safe patches were essentially predator-free.

Although resource availability and predation risk affected the average number of pollinators visiting patches (see below), they did not affect the range of visitors we encountered. Before the onset of the experimental manipulation, the number of visitors we encountered upon arrival to a patch was between 0 and 5 for honeybees and between 0 and 3 for hoverflies. During the application of experimental treatments, the number of visitors we encountered upon arrival to patches was between 0 and 4 for both honeybees and hoverflies – so pollinator activity at experimental patches was well within natural levels.

Effect of predation risk and resource availability – patch level

Patches with more inflorescences attracted more honeybees (slope = 0.002, SE = 0.0007, $F_{1,26}$ = 7.06, P = 0.013). There was a statistically significant interaction between resource availability and predation risk on the number of honeybees visiting patches ($F_{1,9}$ = 5.10, P = 0.050). This interaction stems from the finding that, although honeybees preferred rich to poor patches and safe to risky patches, honeybees preference for rich patches was higher in safe than in risky patches: the number of honeybees visiting rich-safe patches was more than double than the number of honeybees visiting poor-safe patches, while the number of honeybees visiting rich-risky patches was only 50% higher than the number visiting poor-risky patches (Fig. 1a).

The number of inflorescences that honeybees visited before leaving a patch increased with the number of inflorescences in the patch (slope = 0.004, SE = 0.001, $F_{1,26}$ = 9.79, P = 0.004). Honeybees visited more inflorescences per patch in safe than in risky patches ($F_{1,9}$ = 19.62, P = 0.002); they also visited more inflorescences per patch in rich than in poor patches ($F_{1,9}$ = 22.26, P = 0.001). The effect of the interaction between resource availability and predation risk on the number of inflorescences that honeybees visited per patch was not statistically significant ($F_{1,9}$ = 1.79, P = 0.21; Fig. 1b).

The number of hoverflies visiting patches increased with the number of inflorescences in the patch (slope = 0.002, SE = 0.0005, $F_{1,26}$ = 12.42, P = 0.002). The interaction between resource availability and predation risk had a statistically significant effect on the number of hoverflies visiting patches ($F_{1,9}$ = 10.16, P = 0.011). Although the numbers of hoverflies visiting rich-safe and poor-safe patches was similar, more hoverflies visited poor-risky than rich-risky patches. The number of hoverflies visiting patches was therefore smallest at rich-risky and highest at poor-risky patches (Fig. 2a).

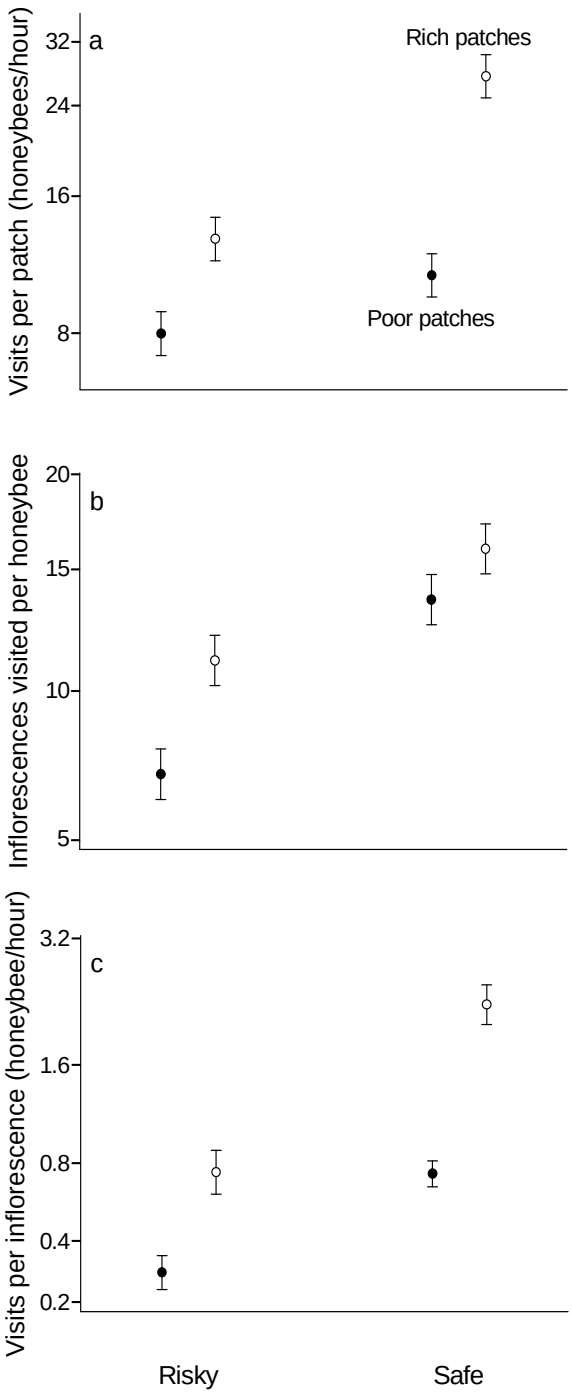

Figure 1 *a) Average rate (hour^{-1}) at which honeybees, Apis mellifera, visited patches; b) average number of inflorescences that individual honeybee visited before leaving the patch, and c) rate (hour^{-1}) at which the average inflorescence was visited by honeybees. Circles represent least-squared means ± standard errors for the four treatments (rich and poor, safe and risky patches; 10 replicas).*

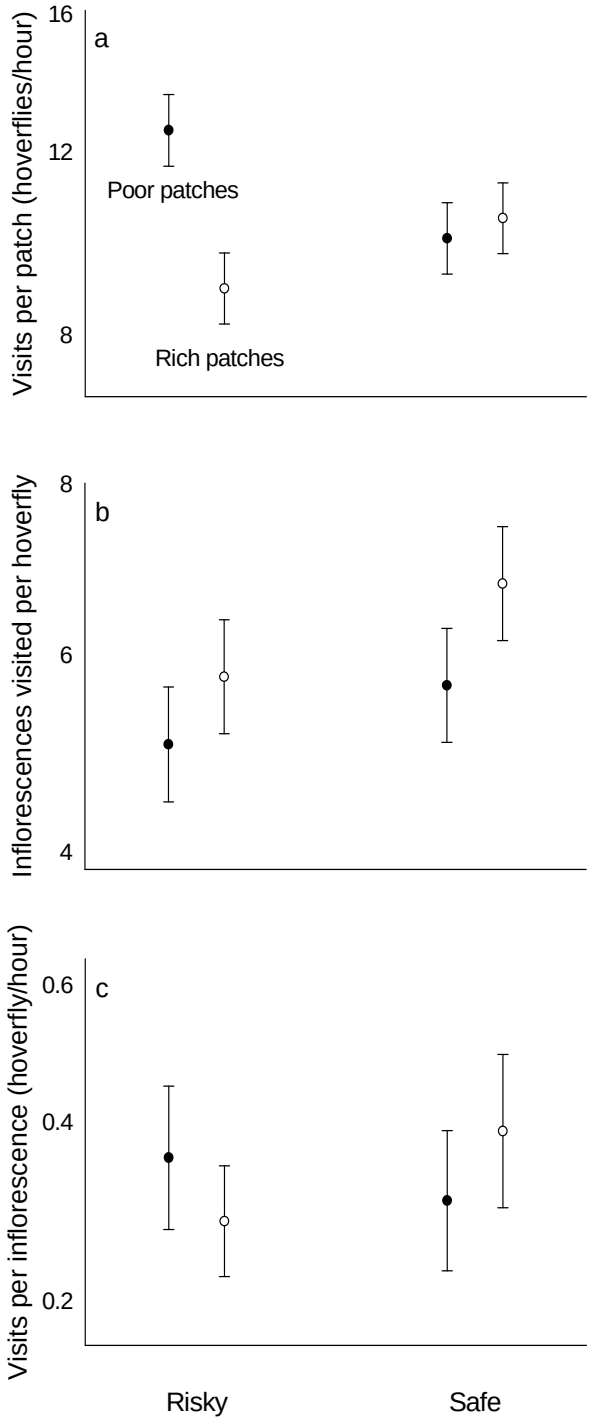

Figure 2 *a) Average rate (hour^{-1}) at which hoverflies, Eristalis tenax, visited patches; b) average number of inflorescences that individual hoverflies visited before leaving the patch, and c) rate (hour^{-1}) at which the average inflorescence was visited by hoverflies. Circles represent least-squared means ± standard errors for the four treatments (rich and poor, safe and risky patches; 10 replicas).*

None of the factors studied had a clear effect on the number of inflorescences that hoverflies visited per patch. Hoverflies tended to visit more inflorescences in patches where inflorescences were more abundant, but this trend was not statistically significant (slope = 0.002, SE = 0.001, $F_{1,26}$ = 3.43, P = 0.075). Likewise, although the average number of inflorescences that hoverflies visited per patch was higher in rich than in poor patches, in safe than in risky patches, the effects of resource availability ($F_{1,9}$ = 2.87, P = 0.12) and predation risk ($F_{1,9}$ = 4.03, P = 0.076) did not reach statistical significance. The interaction between resource availability and predation risk had no discernable effects on the number of inflorescences that hoverflies visited per patch ($F_{1,9}$ = 0.05, P = 0.82).

Effect of predation risk and resource availability – inflorescence level

Both the number of honeybees visiting patches and the number of inflorescences that each honeybee visited per patch were greater in rich than poor patches, in safe than risky patches (Figs 1 a, b). As a result, there were statistically significant effects of resource availability ($F_{1,9}$ = 54.19, P = 0.00004) and predation risk ($F_{1,9}$ = 74.93, P = 0.00001), but not of their interaction ($F_{1,9}$ = 3.28, P = 0.10), on the rate at which inflorescences were visited by honeybees. Note that inflorescences in risky-poor patches received less than half the number of honeybee visits per unit time than inflorescences in safe-poor patches, but inflorescences in risky-rich patches received as many honeybee visits as in safe-poor patches (Fig. 1c).

The pattern was different for hoverflies, as the number of inflorescences visited per hoverfly was lowest in the patches that received the greatest number of hoverfly visitors (Figs 2 a, b). This combination resulted in inflorescences receiving similar rates of hoverfly visits in all patch types (Fig. 2c). Neither resource availability ($F_{1,9}$ = 0.02, P = 0.90), predation risk ($F_{1,9}$ = 0.29, P = 0.60) or their interaction ($F_{1,9}$ = 2.16, P = 0.18) had statistically significant effects on the rate at which inflorescences were visited by hoverflies.

We now focus on those inflorescences where spiders were hunting. The probability that visitors to risky patches landed on spider-harbouring inflorescences was not significantly affected by patch type (rich or poor), pollinator species (honeybee or hoverfly) or their interaction (all $P \geq 0.2$). However, due to the small number of visits to inflorescences with spiders (61 out of 8081 pollinator visits) the test has relatively little power and the null hypotheses must be retained with caution. Despite the low proportion of visits to spider-harbouring inflorescences, the probability of landing on a spider-harbouring inflorescence increased with the proportion of inflorescences within a patch which harboured spiders (P <

0.0001). The slope of this relationship, 0.332 (SE 0.06), was significantly smaller than one (W = -11.13, $P < 0.001$), indicating that both honeybees and hoverflies avoided spider-harbouring inflorescences. Avoidance of spider-harbouring inflorescences becomes also apparent when we note that the proportion of inflorescences harbouring spiders in risky patches, 0.02, was greater than the proportion of visits to spider-harbouring inflorescences in risky patches, 0.0075. If honeybees and hoverflies were selecting inflorescences at random, the probability that they selected 61 or fewer spider-harbouring inflorescences out of 8081 landings would be $5.6 \cdot 10^{-20}$ (binomial test).

Effect of spider encounters on patch departure

The tendency of honeybees and hoverflies to leave the patch following a non-lethal encounter with a spider increased as the response of the spider escalated from indifference through approach and strike to struggle (Fig. 3). The effect of spider response on the probability of leaving the patch was highly significant (deviance = 21.10, df = 3, $P = 0.0001$). All honeybees and hoverflies remained in the patch after encountering a spider that did not react to their landing, and left the patch after a struggle with a crab spider. On the other hand, neither visitor species (honeybee vs. hoverfly) nor patch type (rich vs. poor patches) had statistically significant effects on the probability of leaving the patch upon an encounter with a spider (species: deviance = 0.72, df = 1, $P = 0.40$; resource availability: deviance = 0.85, df = 1 $P = 0.36$).

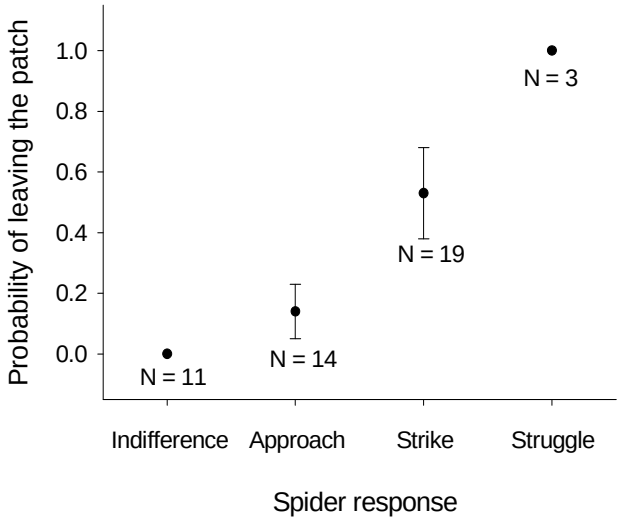

Fig. 3 *Proportion of pollinators leaving the patch after a non-lethal encounter with a crab spider, plotted against the response of the spider. Error bars represent standard errors, and sample sizes are indicated for each group.*

Susceptibility to predation

Over 13 days of observations, we recorded 33 honeybees and 28 hoverflies landing on inflorescences harbouring crab spiders. Of these, 20 honeybees (60.61%) and 16 hoverflies (57.14%) were attacked by the spider. Spiders were therefore equally likely to attack honeybees and hoverflies (Fisher's exact test, two-tailed: $P = 0.80$). Of the 20 honeybees attacked, 9 (45%) were killed, while only 4 (25%) hoverflies were captured by spiders. Although the difference in susceptibility was not statistically significant (Fisher's exact test, two-tailed: $P = 0.30$), the probability of detecting a significant difference with our sample size would be very low. Even if the observed capture frequencies represented the real susceptibility to predation of honeybees and hoverflies, over 80 honeybees and 80 hoverflies would have to be attacked before the probability of detecting a significant difference in success rate reached 50% (as calculated from 2000 Monte Carlo simulations for each sample size). A more powerful test of susceptibility to predation is therefore obtained comparing the number of honeybees and hoverflies that spiders were eating when we arrived to the patches, normalised by the visit rate of the corresponding species. When we compared those prey that spiders were consuming at the start of the observations, the proportion of visiting pollinators captured by spiders was higher for honeybees (0.15 ± 0.12) than for hoverflies (0.04 ± 0.03), the difference being significant according to the Wilcoxon matched-pair test ($Z = 3.88$, $P < 0.001$, N=20).

Each patch was observed during 15 minutes per day. The number of honeybees and hoverflies captured per day over all our patches can therefore be estimated from the number of observations, dividing them by the number of observation days (13) and multiplying by the number of 15-minute intervals per foraging day (40 if we assume ten hours of foraging activity per day). This leads to an estimated value of 27.7 honeybees and 12.3 hoverflies captured per day in our patches.

Discussion

To the best of our knowledge, this is the first study of how pollinators trade off intake rate and predation risk. It allows us to answer the three questions we raised at the beginning of the study. (1) Honeybees and hoverflies responded to the trade-off between predation risk and foraging success, albeit in completely different ways. The most susceptible pollinators, honeybees, avoided risky patches, particularly if their profitability was low (Fig. 1a), while less susceptible hoverflies visited most often low-quality risky patches (Fig. 2a). (2) The presence of ambush predators affected the rate at which individual inflorescences were visited by

honeybees. When controlling for resource availability, honeybee visit rates (number of visits per inflorescence per unit time) in safe patches were more than double than the rates in risky patches (Fig. 1c). Nevertheless, inflorescences in risky-rich patches received as many honeybee visits per unit time as inflorescences in safe-poor patches. Thus, while ambush predators make inflorescences less attractive to honeybees, inflorescences can recover their attractiveness increasing nectar production rate. (3) More hoverflies visited risky than safe patches. This observation cannot possibly result from a direct effect of spiders on hoverfly density at risky patches. The number of honeybees visiting risky patches was smaller than the number of honeybees visiting safe patches. While a direct effect of crab spider on honeybee density would predict this trend, the magnitude of the effect, particularly in rich patches, is incompatible with the rate at which crab spiders removed honeybees from the population. We must conclude that crab spiders exerted an indirect effect on plant-pollinator interactions, mediated by changes in the foraging behaviour of pollinators.

Different patterns at different spatial scales

At the flower level, honeybees and hoverflies showed similar responses. Honeybees and hoverflies avoided spider-harbouring inflorescences. We found no significant differences between species in the probability of landing on spider-harbouring inflorescences while foraging in risky patches. Furthermore, the rates observed for honeybees (33/4,405 = 0.0075) and hoverflies (28/3,676 = 0.0076) were so similar that any statistically significant difference that could be detected increasing sample size would most likely be biologically irrelevant. Honeybees and hoverflies also reacted similarly to non-lethal encounters with crab spiders. They tended to remain in the patch if the crab spider responded weakly to their presence, and to leave the patch after an attack (Fig. 3). The two species therefore exhibited strong anti-predator behaviour at the inflorescence level, and at this spatial scale the anti-predator response was not affected by resource availability in the patch. Despite these similarities, when we analysed the foraging strategies of honeybees and hoverflies at the patch level we found striking differences in the number of individuals visiting patches (Figs. 1a and 2a). Between-species differences in patch-level response can have their origin in mechanistic and functional differences.

Patch choice: mechanisms

Honeybees are central-place foragers. Numerous observations on marked bees indicate that workers concentrate their foraging effort on a restricted area that they revisit trip after trip, even though each trip may include visits to inflorescences not belonging to the bee's core

territory (e.g Ribbands 1949; Free 1966). Honeybees have an efficient communication system allowing workers to recruit nest-mates to rich patches (von Frisch 1967). At the same time, honeybees that have been attacked at a food source can prevent recruitment to that source (Nieh 2010), and individual workers learn to avoid areas where they have been attacked (Abramson 1986; Dukas 2001). Through the processes of recruitment and learning, rich and safe patches will be included in the foraging territories of more honeybees than poor and risky patches. Because honeybees concentrate their foraging effort on relatively small territories, the number of honeybees observed in a patch will be related to the number of honeybees that include the patch within their foraging territory.

Much less is known about the foraging ecology of hoverflies. Males acquire mating territories and are therefore residential (Wellington & Fitzpatrick 1981). If males avoided spider-harbouring inflorescences and left patches upon attack by crab spiders, male territories would concentrate in safe patches. However, we rarely observed hoverflies behaving territorially. Most of our observations concerned foraging individuals that arrived to the patch and left it after visiting a few inflorescences. Because the flight pattern of foraging *E. tenax* is characterised by a strong directionality (Gilbert 1983), non-territorial hoverflies are likely to wander through their environment without forming special attachments to any particular location. If this is the case, hoverflies will have little or no information concerning the quality of the patches they approach. The number of hoverflies arriving to a patch must therefore be a function of the attractiveness of the patch, as assessed from whatever information hoverflies can obtain at a distance. Because resource availability and abundance of crab spiders cannot be detected at a distance, if hoverflies have no information concerning the patches they approach they must rely on other cues to select patches. Hoverflies showed a preference for patches with more *C. segetum* inflorescences – a trait that can be perceived from afar. They may also have used the presence of other pollinators as a cue to assess the suitability of patches (Morse 1981). Nevertheless, if hoverflies decided which patches to visit solely on the basis of inflorescence density and number of honeybees present in the patch, there would be a negative correlation, across treatments, between the number of honeybees and hoverflies visiting patches. The absence of such correlation (compare figures 1 and 2) implies that hoverflies include additional information in the patch-selection rules. Studies with individually marked hoverflies will be required to confirm whether hoverflies have foraging territories.

Functional considerations: predator avoidance

From a functional point of view, honeybees may be avoiding risky patches because of their higher susceptibility to predation. Although there were no obvious differences in the ability of honeybees and hoverflies to detect and avoid spider-harbouring inflorescences, honeybees were more vulnerable to predation than hoverflies once they landed on a spider-harbouring inflorescence. Schmalhofer (2001) suggested that the low representation of syrphids on the diet of *Misumenoides formosipes*, relative to honeybees, might be due to the clumsiness of honeybees (Fritz & Morse 1985) and the extreme agility and speed of syrphids (Barth 1991). Whatever the reason for the difference in susceptibility to predation between honeybees and hoverflies, susceptibility to predation is known to affect the predator-avoidance response of pollinators. For example, Dukas and Morse (2003) reported that while small and easily handled pollinators like *B. ternarius* and *A. mellifera* avoided crab spiders (*Misumena vatia*), larger pollinators like *B. terricola* and *B. vagans* did not show any anti-predatory response (see also Dukas & Morse 2005). Gonçalves-Souza et al. (2008) also found that not all pollinator species responded equally to the presence of an artificial crab spider sitting on *Rubus rosifolius* flowers: while hymenopterans tended to avoid flowers harbouring the artificial crab spider, lepidopterans did not show such a response. These authors suggest that the absence of predator-avoidance mechanisms in butterflies could be due to their unpalatability. Indeed, we have observed in the field crab spiders eschewing butterflies after grabbing them with their forelegs. According to these and our study, pollinators suffering a low predation risk will show a weak anti-predatory response, while pollinators that are more vulnerable to predation will show stronger anti-predator behaviour.

Functional considerations: foraging efficiency

Grand and Dill (1999) consider how two species of foragers should allocate their foraging effort between a rich-risky and a poor-safe environment. Assuming that each individual adopts a fitness-maximising strategy, and in the absence of interference competition, they conclude that resource competition will lead susceptible foragers to exploit the poor-safe habitat and less-susceptible foragers to exploit the rich-risky habitat. This prediction agrees with our results, when we compare the number of honeybees and hoverflies at poor-safe and rich-risky patches. Exploitation competition is known to play an important role in pollinator communities. To cite some examples, where *Bombus appositus* and *B. flavifrons* competed for the nectar produced by *Delphinium barbeyi* and *Aconitum colombianum*, *B. appositus* concentrated its foraging effort on flowers of *D. barbeyi* and *B. flavifrons* on flowers of *A.*

colombianum, but when one species was temporarily removed, the remaining bumblebee species increased visitation to the other flower species (Inouye 1978). In an experiment with marked bumblebees, Thomson et al. (1987) found that when some bees were removed, remaining bees shifted their foraging activity towards the removal areas, thus increasing their foraging efficiency. Likewise, competition with honeybees forced *B. occidentalis* colonies to change their foraging strategy, allocating a greater fraction of their foragers from pollen to nectar collection (Thomson 2004). Nevertheless, it is unclear whether hoverflies and honeybees compete through the exploitation of resources or some form of territoriality: hoverflies, *Melanostoma mellinum*, foraged preferentially on flowers and patches where bumblebees, *B. terricola* and *B. vagans*, had been excluded, and avoided returning to flowers from which they had been displaced by bumblebees (Morse 1981). It is therefore possible that, in our experiment, hoverflies were not choosing risky patches to maximise their fitness. They may be simply excluded from rich-safe patches by honeybees. It is important to elucidate the mechanisms of resource competition between pollinator groups if we are to understand how pollination networks are structured.

Other than competition for resources, honeybees and hoverflies may be reacting differently to our experimental treatments because they have different requirements. Honeybees must collect enough resources to sustain the growth of the colony during spring and summer, bringing enough pollen and nectar to feed non-foraging workers and developing larvae, and to keep the colony alive over the fall and winter (Seeley 1985). Hoverflies, on the other hand, require only resources for their own needs (including egg production, but not larval growth). This life-history difference means that, to make ends meet, hoverflies can exploit resources where the average rate of gain is relatively low, while bees require much richer resources. Indeed, bumblebees rarely visit flowers where their average rate of gain is less than 0.02 W (Heinrich 1975), while hoverflies accept resources with net energy intake rate of about 0.01 W (Gilbert 1983). If the productivity of *C. segetum* patches is just above the threshold for productive honeybee exploitation, a small increase in predation risk may suffice to tip the balance between exploitation and neglect. By itself, however, it does not explain why hoverflies visited poor-risky patches at a higher rate than poor-safe and rich-safe patches. A combination of several factors (use of information, avoidance of competition and low energetic requirements) may be required to explain the complex pattern of patch use by hoverflies.

Plant perspective

Ambush predators can have positive and negative effects on the reproductive success of the plants they use as hunting platforms (Suttle 2003; Romero & Vasconcellos-Neto 2004; Gonçalves-Souza et al. 2008). When pollination limits the reproductive success of the plant, plants with ambush predators are likely to experience a decrease in seed set. Because ambush predators like crab spiders preferentially adopt certain flowers and inflorescences as hunting platforms (Morse 2007), it has been suggested that they can affect the population dynamics of their host plants (Suttle 2003). At the evolutionary time scale, however, our results suggest a mechanism through which flower species regularly associated with ambush predators could attract pollinators despite the increased predation risk: increasing reward production. Nectar availability in rich patches was roughly double than in natural patches. Because the proportion of *C. segetum* inflorescences to which we added nectar was on average 0.22, and given that bees are risk-averse foragers (in the sense that they prefer to visit patches where all flowers have similar amounts of nectar rather than patches with the same average amount of nectar per flower but higher inter-flower variance; Real 1981; Waddington et al. 1981; Real et al. 1982), rich patches, as perceived by pollinators, were less than "twice as good" as poor patches. This increase in nectar availability was sufficient to compensate for the presence of predators: inflorescences in safe-poor and risky-rich patches received similar amounts of honeybee visits (Fig. 1c).

Because hoverflies and honeybees strongly avoided spider-harbouring inflorescences, and promptly left them when spiders attacked them, spider-harbouring inflorescences were likely to produce few seeds and export little pollen. Predators, however, also affected visitation rate to inflorescences in their neighbourhood. The spatial scale at which predators and resource availability affect pollinator behaviour must be included in any ecological or evolutionary analysis of how predators affect plant-pollinator interactions. This is because the reproductive success of an individual plant will not only depend of its phenotypic traits, but also on the traits of its neighbours.

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Capítulo II

Temporal and individual variation in Australian crab spider UV-colouration: the link between colour, condition and background.

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Abstract

Sit-and-wait predators have evolved several traits that increase the probability of encountering prey, including lures that attract prey. Crab spiders (Thomisidae) are sit-and-wait predators known by their ability to match the background colouration. However, some crab spiders that ambush on flowers use a different strategy to camouflage. They are UV-reflective, creating a contrast against UV-absorbing flowers that is attractive for pollinators. However, there is considerable variation in the amount of UV reflected between individuals. The aim of this study was to investigate variation in UV-reflectance with respect to previous foraging success and background colouration. In the field, we predicted that because highly UV *Thomisus spectabilis* individuals had attracted more prey they would be in a better body condition than UV-dull individuals. We found that in 2008 spiders were, overall, more UV-reflective than in 2009. Interestingly, in 2008 spider UV and overall colour contrast were positively correlated with spider condition, but not in 2009. In order to distinguish between the causality responsible for these patterns, we experimentally manipulated satiation level of spiders and the background colour they were exposed to. In the laboratory spiders increased their UV-reflectance regardless of their food intake or background colouration. This suggests that UV-reflectance is not caused by condition or background matching, but is up regulated by individuals to attract pollinators. We believe that the observed seasonal variation in conspicuousness/UV-reflectance could be the result of plasticity in response to predators and/or prey.

Submitted

Introduction

Even though sit-and-wait predators do not actively search for their food, several strategies have evolved that may increase their probability of capturing prey. Examples include the selection of profitable patches (Metcalfe et al. 1997; Janetos 2004; Scharf & Ovadia 2006), displaying cryptic and disruptive colouration to avoid detection by prey (Cott 1957) and the building of traps (Shear 2004). In addition to these somewhat passive strategies, many sit-and-wait predators employ tactics that actively attract their prey. Prey attraction evolved in many different taxa and often exploits prey signals used in sexual interactions or when searching for food. For instance, the bolas spider *Mastophora dizzydeani* releases a chemical that mimics the pheromones of female moths, which attracts male moths of the species *Spodoptera frugiperda* to its sticky ball trap (Eberhard 1977; Eberhard 1980). Exploiting a non-sexual response, the common death adder *Acanthophis antarcticus* captures lizards by waving a conspicuous worm-like caudal lure that incites a predatory response on their prey (Nelson et al. 2010).

Several species of crab spiders (Thomisidae) are sit-and-wait predators that exploit the interaction between plants and insects by sitting on flowers to ambush pollinating insects (Morse 2007). Their colouration usually resembles the colour of the flowers they are sitting on (Chittka 2001; Thery & Casas 2002; Thery et al. 2005; Morse 2007). Furthermore, crab spiders, like the European *Misumena vatia* and *Thomisus onustus*, can adjust their body colour to match the flower colour background, which makes these crab spiders less detectable by prey such as honeybees (Packard 1905; Gabritschevsky 1927; Thery & Casas 2002; Thery 2007).

However camouflage is not the only strategy used by crab spiders. Some crab spiders seem to be highly conspicuous to their prey, rather than blend into the background. These spiders are UV-reflective (they appear white to humans), which, when viewed through honeybee eyes, creates a strong contrast against the UV-absorbing flower (Heiling et al. 2005). Instead of deterring prey by increasing their visibility, these spiders are attractive to pollinators (Heiling et al. 2003). When honeybees had to choose between flowers with and without the contrasting UV-reflective Australian crab spider *Thomisus spectabilis*, they were more likely to land on flowers occupied by their predators (Heiling et al. 2003). When the same experiment was performed with the non-UV-reflective European *Misumena vatia, Xysticus sp.* and *Synaema globosum* the honeybees were generally repelled by spider harbouring flowers (Herberstein et al. 2009). Moreover, when the level of UV reflected by *Thomisus spectabilis* was

experimentally eliminated, honeybees actively avoided flowers occupied by crab spiders (Heiling et al. 2005).

Despite the apparent benefit of UV-reflection for crab spiders, there is a high level of intra- and inter-individual variation in the amount of UV reflected by spiders (Llandres et al. 2010). Based on the use of colour to attract prey by crab spiders we predict that individuals that reflect more UV-light, and consequently form a stronger colour contrast against a UV-absorbing flower background, will capture more prey and, as a result, will be in a better condition than individuals that are less UV-reflective. Such data may indicate the strength of a relationship between two variables, but it does not indicate causality. Only manipulating the feeding level of spiders can reveal if it is in fact satiation that causes UV-reflectance or vice-versa. On the other hand, *Thomisus spectabilis* can attract pollinators based on the strength of its UV-contrast, hence, we expect food-limited spiders to increase conspicuousness and attract more prey. If greater visibility however entails costs, such as greater risks of predation, we expect satiated spiders to reduce the UV-contrast. In addition to adjusting their colour in response to their satiation level, we also expect crab spiders to adjust their colouration accordingly to the background they are sitting on, as this will affect their visibility to approaching prey.

Here we address these outstanding questions by analysing the relationship between the colouration of the spider *Thomisus spectabilis* and their condition using field data from two years and, in the laboratory, testing how this predator responds to different feeding regimes and background colours. We modelled these colour data into the visual system of the crab spider's prey, the *Apis mellifera* photoreceptor response.

Methods

Reflectance spectra measurements

For the measurement of the reflectance spectra of organisms and objects we measured the samples using an optical fibre probe (Ocean Optics Inc, Dunedin, U.S.A.) connected to a spectrometer (USB2000, Ocean Optics Inc, Dunedin, U.S.A.) and to a light source (PX-2 light source, Ocean Optics Inc, Dunedin, U.S.A.). The probe was positioned at 45° above the samples. The reference spectrum was taken using the WS-1 Diffuse Reflectance Standard

(Ocean Optics Inc, Dunedin, U.S.A.; >98% reflectance from 250 to 1500 nm). The dark spectrum was taken from the black velvet used as background to the measurements. We took five spectral measurements from each organism/object.

Calculation of bee's photoreceptor excitation and colour contrasts

We evaluated how the spiders and flowers are perceived by potential prey, the honeybee, *Apis mellifera*, by calculating photoreceptor excitations and colour contrasts using the colour hexagon model (Chittka 1992; Chittka 1996). First, the relative quantum catch of each bee photoreceptor, P, was calculated by:

$$(1)\ P = R\int_{300}^{700} I_S(\lambda)\ S(\lambda)\ D(\lambda)\ d\lambda$$

where $I_S(\lambda)$ is the reflectance calculated from the spiders flowers; $S(\lambda)$ is the spectral sensitivity function of each bee photoreceptor; $D(\lambda)$ is the illuminant spectrum CIE D65 (provided in Chittka & Kevan 2005); and R is the sensitivity factor, calculated by:

$$(2)\ R = \frac{1}{\int_{300}^{700} I_B(\lambda)\ S(\lambda)\ D(\lambda)\ d\lambda}$$

where $I_B(\lambda)$ is the reflectance of the environmental background. For the environmental background we used the leaf spectrum provided by Chittka & Kevan (2005).

The excitation of each bee photoreceptor, E_{UV}, E_{Blue}, E_{Green}, was calculated from the relative quantum catch of the photoreceptors, P:

$$(3)\ E = \frac{P}{P+1}$$

The E_{UV}, E_{Blue} and E_{Green} for each spider and flower were calculated using the average of the excitation values calculated from the five reflectance spectra taken for each spider and flower. These values were used to calculate coordinates in the bee colour hexagon (Chittka et al. 1992; Chittka 1996):

$$(4)\ x = \sqrt{3}/2(E_{Green} - E_{UV})$$

$$(5)\ y = E_{Blue} - 0.5(E_{Green} + E_{UV})$$

Then, the colour contrast was calculated by the Euclidian distance between the spiders and the flower in the colour hexagon:

$$(6)\ \Delta St = \sqrt{(x_{spider} - x_{flower})^2 + (y_{spider} - y_{flower})^2}$$

where x and y are the coordinates of the hexagon calculated by formulae (4) and (5).

Variation of spider colouration

We collected females of *Thomisus spectabilis* Doleschall, 1859 (Thomisidae) spiders sitting on flowers of white daisies *Bidens alba* var. *radiata* (Asteraceae) in Airlie Beach, Queensland, Australia, in May 2009 (n = 42). We also used part of some published data from reflectance spectra of *T. spectabilis* (n = 67) collected in the same site and flowers in April 2008 (Llandres et al. 2010). In the laboratory we weighed the spiders and measured the length of their first leg tibia-patella. For analyses, we only considered spiders whose tibia-patella length exceeded 2.00 mm, because spiders smaller than that are too small for an accurate colour measurement. We collected 13 white *B. alba* daisies in 2009 and used data from eight white *B. alba* daisies collected in 2008 (Llandres et al. 2010) from the same sites where we collected the spiders. Because there was no difference in flower colour between years (Table 1) we pooled the colour data of flowers collected in 2008 and 2009 to generate a flower model against which to calculate colour contrast between spiders and flowers. We measured the light reflectance of the dorsal side of the spider abdomen and flowers using the methodology described above. We compared the spider colour (honeybee photoreceptor excitation values and colour contrast against the flower), weight and leg length, and the flower colour (honeybee photoreceptor excitation values) between years using a t-test.

			T-test (df = 19)	
	2008 (n = 8)	2009 (n = 13)	t	p
E_{UV}	0.42 ± 0.03	0.43 ± 0.03	0.78	0.44
E_{blue}	0.80 ± 0.01	0.80 ± 0.01	0.54	0.60
E_{green}	0.77 ± 0.01	0.78 ± 0.01	1.51	0.15

Table 1. *Excitations values for each of the honeybee's photoreceptors (E_{UV}, E_{Blue}, E_{Green}) generated by the reflectance spectrum of white daisy (Bidens alba) flowers where crab spiders (Thomisus spectabilis) sit to ambush pollinators.*

Relationship between spider colouration and spider body condition

We used the residuals of the regression log(weight) vs. log(tibia-patella leg length) as an index of spider condition (Jakob et al. 1996). To test if spider colouration is related to their condition, we calculated separately for each year the regression between the honeybee's photoreceptors excitation values and colour contrast values, as independent variables, and spider condition, as the dependent variable. We used a Bonferroni correction ($\alpha < 0.0125$) to account for the four regressions performed using the same data set.

Effect of spider condition and background colouration on spider UV-colouration

In order to understand how *Thomisus spectabilis* varied their colour in response to feeding regimes, background colour and the interaction of these variables, we submitted females to two treatments in a factorial design for 30 days. The spiders were randomly placed in containers with different colour backgrounds (UV-bright, white to human eyes and UV-dull, yellow to human eyes). The spiders in each of the colour treatments were further subjected to one of two feeding regimes (high prey and low prey). Originally the experimental design was balanced, however due to the death of three spiders and the exclusion of two spiders that laid eggs, one that moulted and two spiders that went missing, the sample sizes became unbalanced, as follows: low feeding regime in white UV-bright containers (n = 10), high feeding regime and white UV-bright containers (n = 7), low feeding regime and yellow UV-dull containers (n = 8) and high feeding regime and yellow UV-dull containers (n = 7).

The low feeding regime consisted of one housefly (*Musca domestica*) 15 days after the beginning of the experiment whereas the high feeding regime consisted of two houseflies per week during the first two weeks and eight houseflies per week during the last two weeks. The containers were made of colour cardboards (11 × 11 × 11 cm size). The top of the containers was covered with plastic cling wrap (Glad Wrap®) that allows the transmission of all wavelengths between 300-700 nm. The reflectance spectra of the colour containers are shown in the results section (Fig. 4 a). The experiment was conducted in a glasshouse with controlled temperature (night: 16; day: 25 °C; 12:12h cycle) and perspex panels that allowed the passage of all wavelengths between 300-700 nm (Heiling & Herberstein 2004).

We weighed the spiders, measured the first leg tibia-patella length and collected the reflectance spectra of spiders' abdomen before and after the application of our treatments. We calculated

the UV photoreceptor excitation value in the *Apis mellifera* (E_{UV}) vision using methodology described earlier.

First we tested the effectiveness of the feeding treatment by analysing the variation in weight of spiders before and after the experiments using a General Linear Model (GLM) with repeated measurements. The dependent variables were weight before and after the treatments. The date of the weight measurements (before and after treatments) entered in the model as the within subjects factor and background colouration and feeding treatment as between subjects factors. Secondly we tested the effect of the treatments on the E_{UV} of the spiders also using a GLM with repeated measures. The dependent variables were E_{UV} before and after the treatments. The date of the E_{UV} measurements (before and after treatments) entered in the model as the within subjects factor and background colouration and feeding treatment as between subjects factors.

Results

Variation of spider colouration between years

The reflectance spectrum of field collected *Thomisus spectabilis* was different between years. In 2008 the spiders reflected more light between 300 nm and 400 nm than in 2009 (Fig. 1). As a consequence, the spider colouration as perceived by the honeybee was also different between years (Table 2). The colour contrast of the spiders against the flower background was 0.09 units higher in 2008 than 2009 (Table 2). This contrast seems to be caused by differences mainly in the E_{UV}, as this variable was 0.10 units higher in 2008 than in 2009. On the other hand, E_{Blue} and E_{Green} had very similar values in both years, although the average difference of only 0.02 units in E_{Blue} was statistically significant (Table 2). Even though spiders in 2009 were slightly heavier and had longer leg lengths than in 2008 these differences were not statistically significant (Table 2).

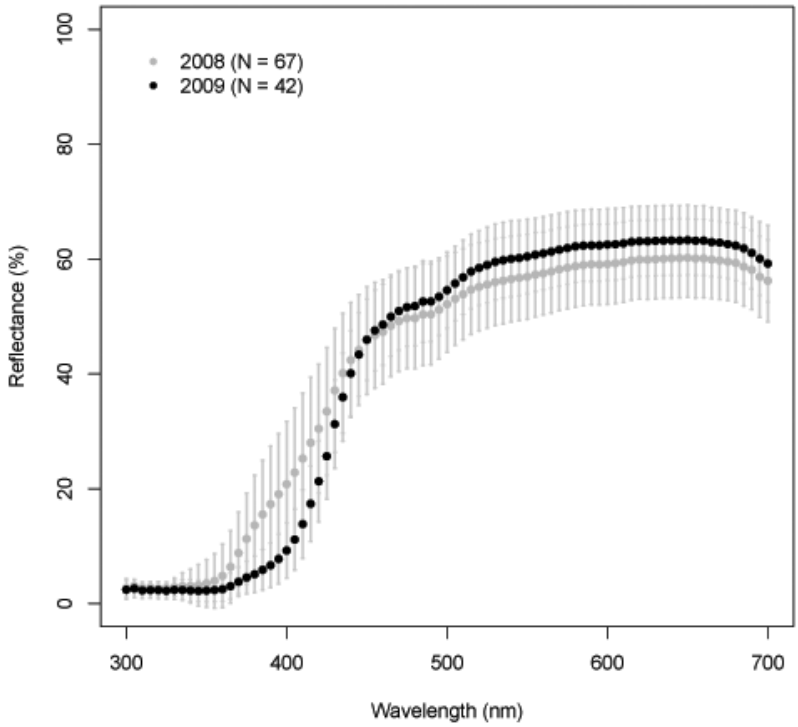

Fig. 1. *Average reflectance spectra of the crab spiders (Thomisus spectabilis) collected in 2008 (n = 67) and 2009 (n = 42). Error bars represent standard deviations.*

	2008	2009	T-test (df = 107)	
	(n = 67)	(n = 42)	t	p
E_{UV}	0.66 ± 0.09	0.56 ± 0.08	5.92	< 0.01
E_{blue}	0.83 ± 0.04	0.81 ± 0.03	2.04	0.04
E_{green}	0.80 ± 0.02	0.81 ± 0.02	1.42	0.16
Colour contrast	0.21 ± 0.07	0.12 ± 0.06	7.48	< 0.01
Weight (g)	0.115 ± 0.100	0.134 ± 0.086	0.98	0.33
Leg length (mm)	3.65 ± 0.98	3.81 ± 0.96	0.81	0.42

Table 2. *Excitations values for each of the honeybee's photoreceptors (E_{UV}, E_{Blue}, E_{Green}) and colour contrast generated by the crab spiders (Thomisus spectabilis) reflectance spectrum, and weight and tibia-patella leg length from spiders collected in 2008 and 2009. Values were compared using a t-test.*

Relationship between spider colouration and spider body condition

Variation in the leg length explained more than 85% of the variation in weight in both years (Fig. 2). The residuals of those regressions, used as an index of the spider condition, suggest that the condition of the spiders was more variable in 2008 than in 2009, but the difference is not statistically significant (Levene's Test: $F_{1, 107} = 3.00$, $P = 0.09$). The minimal residual value in both years was -0.19 but the maximal residual value was 0.48 in 2008 and 0.24 in 2009.

In the analysis of the spider condition and spider colouration there was no relationship between E_{Blue} and E_{Green} and spider condition in either 2008 or 2009 (Fig. 3). However, in 2008 there were positive significant relationships between E_{UV} and colour contrast and spider body condition (Fig. 3), but these relationships were absent in 2009 (Fig. 3).

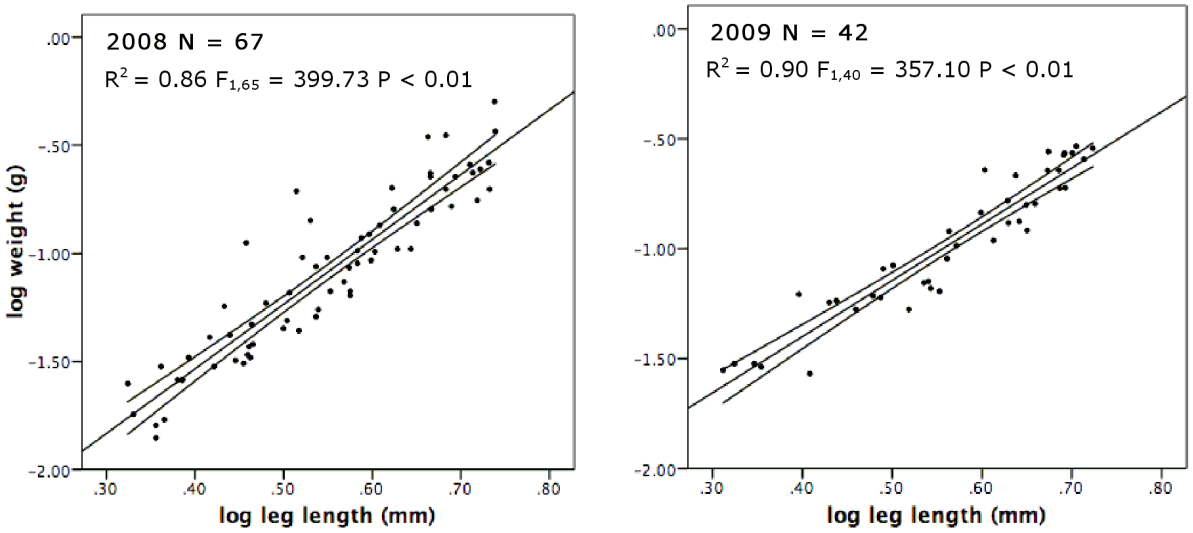

Fig. 2. *Linear regression analyses of the weight against first leg tibia-patella length from crab spiders collected in 2008 (n = 67) and 2009 (n = 42). Values have been log transformed. Curves below and above regression line represent 95% confidence intervals.*

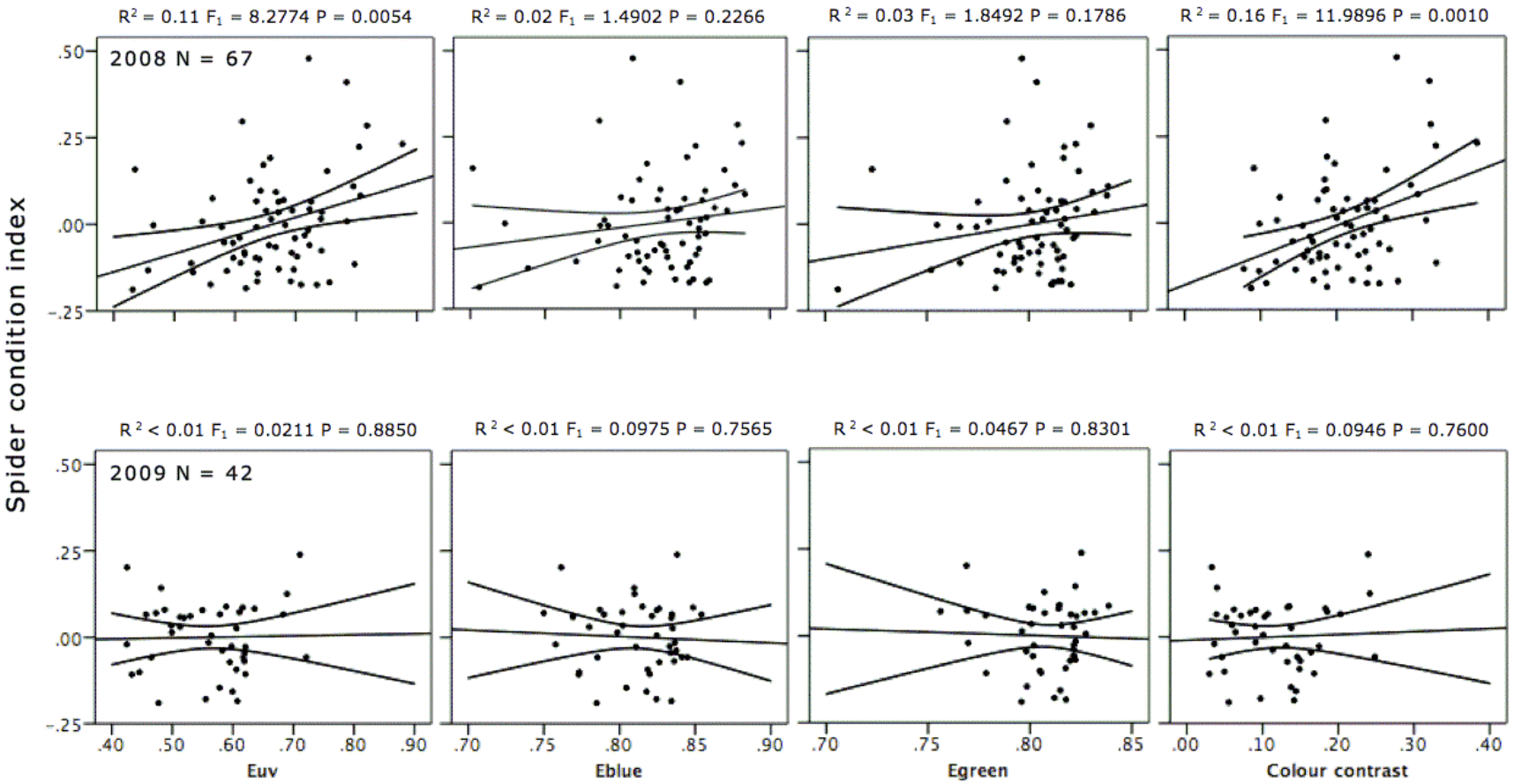

Fig. 3. *Linear regression between the spider condition index and the calculated colour contrast and excitations values for each of the honeybee's photoreceptors (E_{UV}, E_{blue}, E_{green}). Spider condition was estimated from the residuals of the regression analyses of log(weight) vs. log(first leg tibia-patella length) by year. Curves below and above regression line represent 95% confidence intervals. We used a Bonferroni correction on the significance level ($\alpha < 0.0125$).*

Effect of spider condition and background colouration on the spider UV colouration

The difference in weight gain between high and low feeding treatments was statistically significant (repeated measures GLM: $F_{1,28} = 92.22$, $P < 0.01$). The weight of spiders in the low feeding treatment decreased by 6% during the 30 day treatment whereas the weight of the spider in the high feeding treatment increased by 61% on average (Table 3). However, weight gain was not affected by background colour ($F_{1,28} = 3.01$, $P = 0.10$) or the interaction between these two variables ($F_{1,28} = 1.55$, $P = 0.22$).

		Low		High	
		White UV-bright (n = 10)	Yellow UV-dull (n = 8)	White UV-bright (n = 7)	Yellow UV-dull (n = 7)
before	Weight (g)	0.178 ± 0.103	0.100 ± 0.022	0.131 ± 0.082	0.137 ± 0.075
	Leg length (mm)	4.40 ± 0.84	3.90 ± 0.48	3.87 ± 0.83	4.34 ± 0.80
after	Weight (g)	0.168 ± 0.088	0.095 ± 0.023	0.195 ± 0.098	0.226 ± 0.070
	Leg length (mm)	4.45 ± 0.68	3.89 ± 0.48	4.08 ± 0.69	4.30 ± 0.76

Table 3. *Weight and leg length of crab spiders (Thomisus spectabilis) before and after they were submitted to two different feeding treatments (low and high) and two colour backgrounds (white UV-bright and yellow UV-dull).*

Overall during this experiment all spiders significantly increased the E_{UV} regardless of the treatment applied (Table 4; Fig. 4 b). There was no significant effect of background colour (white UV-bright and yellow UV-dull) or the interaction between the colour background and the feeding treatment (low and high feeding regime) on the E_{UV} but there was a significant effect of the feeding treatment on E_{UV} (Table 4).

Although we randomly allocated spiders into the treatments, after the exclusion of several spiders from the experiment (see methods), we ended up with a marginally significant difference in E_{UV} between the low and high feeding regime at the beginning of the experiment (Fig. 4 b). While we found a significant effect of the feeding treatment on E_{UV} (Table 4), this is largely due to the difference in E_{UV} between the treatments at the start of the experiment as both groups had almost exactly the same E_{UV} at the end of the experiment (Fig. 4 b).

Source	df	F	P
Date	1	98.58	**< 0.01**
Date* Feeding Treatment	1	4.69	**0.04**
Date * Colour Treatment	1	2.10	0.16
Date * Feeding treatment * colour treatment	1	0.53	0.47
Error(Date)	28		

Table 4. *Repeated measures linear model testing the effect of feeding treatment and background colouration on the excitation value of the UV photoreceptor (E_{UV}) of the honeybee produced by the Thomisus spectabilis body colouration.*

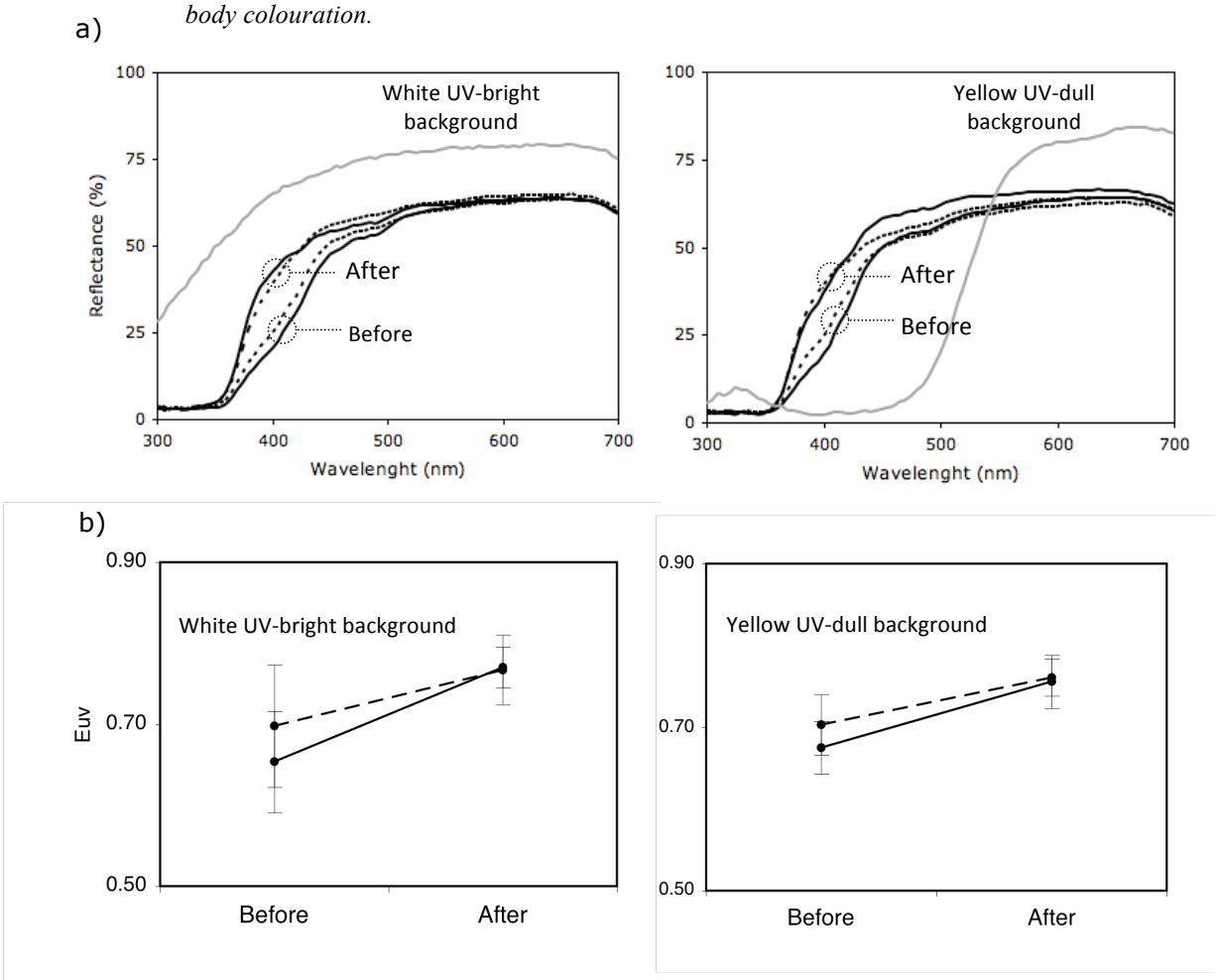

Fig. 4. *(a) Average reflectance spectrum and (b) calculated excitations values for the honeybee's UV photoreceptor (E_{UV}) of crab spiders before and after they were submitted to two different colour backgrounds and two feeding regimes in a factorial design. The graphs on the left show spiders on white UV-bright background and graph on the right show spiders on the yellow UV-dull background. Dashed lines represent spider in the low feeding regime and black lines represent spiders in the high feeding regime. The reflectance spectra of the backgrounds are indicated by their respective names. Error bars were omitted in (a). Error bars represent standard deviations in (b).*

Discussion

We have found temporal and individual variation in spider reflectance especially in the UV-region of the spectrum (between 300 and 400 nm). As a consequence when modelled into the honeybee eye, the spiders varied in the overall colour contrast against the UV-dull flowers mostly because of differences in the excitation of the honeybee UV photoreceptor. On average in 2008 the spiders were more UV-reflective and created a greater colour contrast than in 2009. Colour contrast can be seen as a gradient of difficulty to discriminate between two colours. The lower the value the more difficult the task. In behavioural experiments honeybees can discriminate targets that differ as little as 0.05 units in their colour space (Dyer & Chittka 2004). Furthermore, the lower the colour contrast the longer honeybees take to learn to discriminate between two targets and the longer the bees take to make a choice between targets (Dyer & Chittka 2004). Moreover, the ability to discriminate between two different colours is likely to be lower in a natural foraging condition, where honeybees are subjected to distracting factors (Spaethe et al. 2006). In 2009 ten spiders fell below the 0.05 units of colour contrast, but none did so in 2008. On the other hand, in 2008, 17 spiders had a colour contrast above 0.25, but none in 2009. Thus, our results suggest that from a honeybee perspective, in 2009 more spiders were adopting a strategy of low conspicuousness, whereas in 2008 more spiders were adopting a strategy of higher colour contrast and thus greater visibility.

When we analysed the relationship between colour and body condition we found, in 2008, that E_{UV} and the overall colour contrast were positively correlated with spider body condition. However, in 2009, when the spiders were less UV reflective and had less overall chromatic contrast, the correlation was absent. The relationship between high E_{UV} contrast and body condition, together with previous experiments (Heiling et al. 2003; Heiling et al. 2005; Herberstein et al. 2009; Bhaskara et al. 2009), suggests that greater conspicuousness, achieved by a higher E_{UV} contrast, is advantageous for these spiders. Therefore why would some crab spiders adopt a potentially less efficient foraging strategy of low conspicuousness?

Prey often change their foraging behaviour, reducing their food intake, when predators are present. Common anti-predatory responses include a reduction in foraging time and the selection of less risky foraging patches (Lima 1998). However, predator pressure can also directly affect prey colouration. For instance, the fiddler crab *Uca vomeris* can change their carapace from a bright to a dull colour over the course of a few minutes. Colonies highly

exposed to bird predators have, on average, a duller colouration than less exposed colonies and there is evidence that individuals reduce their conspicuousness if the danger of predation is experimentally increased (Hemmi et al. 2006). Similar to fiddler crabs, crab spiders can change their body colour over several days (Gabritschevsky 1927; Schmalhofer 2000; Thery 2007). Therefore the difference in spider colouration between years quantified in our study may indicate that spiders are adjusting their body colouration in response to variation in predation pressure. Insects, such as wasps, and birds, the most likely predators of crab spiders, are able to perceive UV-light (Foelix 1996; Briscoe & Chittka 2001; Hart 2001; Morse 2007) and thus white UV-bright spiders may suffer a higher risk of predation due to an increased conspicuousness whereas white UV-dull spiders may reduce the probability of detection of predators by matching the white UV-dull flower.

Alternatively, spiders could be adjusting their colouration in accordance to the behaviour of the most common prey. Different species of pollinators respond differently to the presence of crab spiders (Dukas & Morse 2003; Brechbühl et al. 2010a; Brechbühl et al. 2010b) and some prey species are attracted to the high UV-contrast but not others (Heiling et al. 2003; Heiling & Herberstein 2004; Herberstein et al. 2009; Llandres et al. 2010). In this scenario one would expect that in 2008 the most common prey were those attracted to UV-bright spiders, but the common prey in 2009 were not. For example, honeybees are attracted and land more frequently in flowers harbouring UV-bright spiders (Herberstein et al. 2009), but Australian native bees are less likely to land on such flowers (Heiling & Herberstein 2004; Llandres et al. 2010). If in 2008 the most common prey were honeybees, the best strategy would be to invest in high UV-reflectance, whereas if in 2009 the most common prey were native bees, the most efficient strategy would be to reduce conspicuousness.

In our experiment on the effect of background colouration and food intake on the spiders' UV colouration we found that the spiders have the ability to increase UV-reflectance independently of the food intake and do not necessarily adjust it in accordance with the background or satiation. Rather, spiders in all treatments increased their UV-reflectance throughout the experiment. We also found an effect of the food treatment on the rate of increase of E_{UV}. Despite of that, this difference cannot be interpreted as a biologically significant effect of food intake on the rate of increase because the E_{UV} achieved at end of the experiment was the same in both groups. It is possible that, given more time, spiders in the high food intake would continue to increase their E_{UV} at a higher rate and surpass the low food intake spiders. However

our results do not allow drawing any conclusion in this regard.

The reason for the overall increase in E_{UV} regardless of the treatments could be that spiders were isolated from predators. In absence of predator cues, they may have adopted a high contrast strategy in order to increase prey capture. Contrary to our study, the crab spider *Misumena vatia* did change their colouration to match the colouration of white and yellow backgrounds (Packard 1905; Gabritschevsky 1927; Thery 2007). However, *M. vatia* is not UV-reflective and cannot lure pollinators. Thus one can predict that the best strategy for this species is to always reduce conspicuousness, whereas for *T. spectabilis* it could be advantageous to increase conspicuousness in certain circumstances to lure prey via UV-reflectance. However this interpretation cannot explain why *T. spectabilis* did not change colour with respect to the background treatments in our experiment. It may be that our experimental set up was too artificial and did not elicit a natural response. For instance, *M. vatia* did not change colouration in certain experimental conditions (Packard 1905; Gabritschevsky 1927; Thery 2007), and Thery (2007) specifically reports an unpublished study where *M. vatia* when submitted to different backgrounds in a greenhouse did not change their colouration. Thery (2007) suggests that the lack of colour change was caused by the low light intensity inside the greenhouse. Nevertheless, in our study *T. spectabilis* did change colouration by overall increasing UV-reflectance, and thus the same explanation cannot be directly applied to our experiment.

Finally, our experiment also helps to resolve the causation of the relationship between body condition and UV reflectance found in the field. The fact that at the end of the experiment the spiders had different body conditions but almost exactly the same E_{UV} shows that it is not the body condition that causes the difference in UV reflectance, and suggests that high UV-reflectance results in greater foraging success and hence greater body condition.

In conclusion, our results lend support to the view that the colour contrast created by UV-reflection in Australian crab spiders increases the spiders' foraging success. However, the relationship between satiation, background colour and spider colour seems to be quite complex. It may additionally depend on predator cues or the composition of the prey population resulting in the observed temporal variation in spider colour.

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Capítulo III

The effect of colour variation in predators on the behaviour of pollinators: Australian crab spiders and native bees

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Abstract

1. Australian crab spiders exploit the plant-pollinator mutualism by reflecting UV light that attracts pollinators to the flowers where they sit. However, spider UV-reflection seems to vary broadly within and between individuals and species and, we are still lacking any comparative studies of prey and/or predator behaviour towards spider colour variation.

2. Here we looked at the natural variation in the colouration of two species of Australian crab spiders, *Thomisus spectabilis* and *Diaea evanida*, collected from the field. Furthermore, we examined how two species of native bees responded to variation in colour contrast generated by spiders sitting in flowers compared to vacant flowers. We used data from a bee choice experiment with *D. evanida* spiders and *Trigona carbonaria* bees and also published data on *T. spectabilis* spiders and *Austroplebeia australis* bees.

3. In the field both spider species were always achromatically (from a distance) undetectable but chromatically (at closer range) detectable for bees. Experimentally, we showed species specific differences in bee behaviour towards particular spider colour variation: *T. carbonaria* bees did not show any preference for any colour contrasts generated by *D. evanida* spiders but, *A. australis* bees were more likely to reject flowers with more contrasting *T. spectabilis* spiders.

4. Our study suggests that some of the spider colour variation that we encounter in the field may be partly explained by the spider's ability to adjust the reflectance properties of its colour relative to the behaviour of the species of prey available.

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Introduction

The signal communication between plants and their pollinators often occurs in such a way that both the transmitter and the receiver benefit from the signal produced. However, in several instances third party organisms exploit this interaction. Despite the controversy about the effects of nectar robbers in plant-pollinator mutualism, they are a classical example of how a third organism exploits this interaction (Maloof & Inouye 2000). Nectar robbers usually exploit plant-pollinator mutualism by piercing a hole in the corolla of the flower and drinking nectar without contacting the pollen or stigma. Although the selective impact of nectar robbing on flower morphology has mostly been ignored (Maloof & Inouye 2000), recent studies demonstrated that nectar robbers preferentially exploit flowers with long corolla tubes, reducing their reproductive success (Galen & Cuba 2001; Castro et al. 2008; Navarro & Medel 2009). Moreover, Ornelas et al. (2007) and Gomez et al. (2008) showed that there is a correlated evolution between nectar production and corolla tube length, which led some authors to suggest that the preference of nectar robbers for longer flowers could be explained by the higher nectar content of these flowers (Navarro & Medel 2009).

There are further examples of plant-pollinator exploitation in spiders (Araneae), which have evolved several interesting forms of prey deception that lure prey with colours that resemble pollinator's food rewards. Under the right light conditions the orb-web spider *Nephila clavipes* produces webs with yellow silk that represents a stimulus for *Trigona fluviventris* bees searching for food: bees are more frequently attracted to yellow than to white webs under bright light and are less able to learn to avoid yellow webs (Craig et al. 1996). Furthermore, although there is still debate about the function of silk decorations in *Argiope* orb-web spiders, over the past decade several studies showed that these spiders also exploit the visual system of their prey by creating UV-reflective silk decorations that attract pollinators searching for food (Bruce et al. 2001 ; Li et al. 2004; Bruce et al. 2005; Li 2005; Cheng & Tso 2007; Blamires et al. 2008; Tan et al. 2010). In addition, studies investigating the function of body colour markings in orb-web spiders demonstrated that spiders significantly reduced their foraging success when those colour markings were experimentally altered (Hauber 2002; Tso et al. 2006; Chuang et al. 2007; Tso et al. 2007; Bush et al. 2008; Chuang et al. 2008). All these recent findings support the idea that conspicuous body colouration in these spiders lure their prey.

In a similar way, several Australian crab spiders exploit the plant-pollinator mutualism by attracting and ambushing pollinators on flowers (Heiling et al. 2003; Heiling & Herberstein 2004): they produce UV-reflective body colours that attract prey to the flowers they occupy. However, different species of pollinators react in a different fashion to Australian UV-contrasting spiders. European bees, *Apis mellifera,* approached and landed more on *Chrysanthemum frutescens* and *Cosmos sp.* flowers harbouring a *Thomisus spectabilis* and *Diaea evanida* spiders, respectively, compared to vacant flowers (Heiling et al. 2003; Herberstein et al. 2009). Similarly, the Australian native bee, *Austroplebeia australis* was also more likely to approach but less likely to land on *C. frutescens* flowers harbouring a *T. spectabilis* spider compared to vacant flowers (Heiling & Herberstein 2004). These studies suggest that in the co-evolution between Australian native bees and crab spiders, the bees have evolved an anti-predatory response. The European honeybees, on the other hand, were introduced into Australia in 1822 (Hopkins 1886) and feral colonies became widespread by 1860 (Laurie 1886). Therefore honeybees have not had the opportunity to evolve a response to the deceptive Australian crab spider. To date, the response of native bees to the presence of native crab spiders has only been tested with one species of Australian bees and one species of Australian crab spider, which makes it difficult to extrapolate the suggestion that Australian bees have evolved an anti-predatory response towards crab spiders to native pollinators more generally.

It is difficult to interpret variation in pollinator response to crab spiders, because the colour signal produced by crab spiders is a plastic trait, and spiders change their colour over several days (Oxford & Gillespie 1998). For example, *Misumena vatia* spiders turn from white to yellow in 10-25 days on artificially coloured backgrounds, and the reversed change can take 4-6 days (Gabritschevsky 1927; Schmalhofer 2000). Other studies have also reported similar colour changes for *Misumenoides formosipes* and *Thomisus onustus* crab spiders (Heckel 1891; Gertsch 1939). Australian *Thomisus spectabilis* and *Diaea evanida* crab spiders can also change their body colour over several days between two colour morphs (UV-bright white morphs and UV-dull yellow morphs; Llandres, pers. obs). Although spider UV-reflection seems to vary broadly within and between individuals and species (Herberstein et al. 2009), we still do not know to what extent this variation occurs in natural populations of spiders. By quantifying colour variation in spider natural populations as well as the response of different species of pollinators to the individual variation in UV-reflection of different species of crab

spiders, we will be able to identify the ultimate mechanisms that maintain this colour trait in Australian crab spiders.

We currently know that different species of pollinators respond differently to the presence of a crab spider (Dukas & Morse 2003; Robertson & Maguire 2005; Dukas & Morse 2005; Gonçalves-Souza et al. 2008; Brechbühl et al. 2010). Therefore, including a community approach, in which crab spider's background colour matching is explored from the perspective of several main receivers in the field (community sensory ecology perspective), is necessary to understand this crab spider-flower visitor interactions (Brechbühl et al., 2010; Defrize et al., 2010). Furthermore, we also know that there is considerable variability in Australian crab spider colouration that creates high levels of variation in their visibility to prey and predators (Herberstein et al. 2009). Therefore, in order to disentangle these two factors, we looked at the natural variation in the coloration of Australian *Thomisus spectabilis* (Doleschall) and *Diaea evanida* (L. Koch) crab spiders and at the response of native pollinators to some of this variation.

Materials and Methods

Spectrometer calculations

We measured the spectral reflectance (300 to 700 nm) of crab spiders and flowers using an optic fibre probe (Ocean Optics Inc, Dunedin, U.S.A.) connected to a USB 2000 spectrometer (Ocean Optics Inc, Dunedin, U.S.A.). The USB 2000 spectrometer was connected to the PX-2 light source (Ocean Optics Inc, Dunedin, U.S.A.) and attached to a PC running OOIBase 32 spectrometer software (Ocean Optics Inc, Dunedin, U.S.A.).

We took five samples of each spider and flower and averaged them to calculate the photoreceptor excitation values (E) for the photoreceptors (ultraviolet, blue and green) of honeybees (for methods see Chittka, 1992). We used honeybees (*Apis melifera*) as a visual model because the spectral sensitivity functions of Australian native bees are not known (note that for other bee species, such as bumblebees, the spectral sensitivities of the three receptor classes are very similar to the honeybees' receptors (Peitsch et al. 1992). The E_{uv}, E_{blue} and E_{green} values describe the excitations by the UV, blue and green photoreceptors and we used them to calculate the colour loci of spiders and their flower background in the bee colour hexagon. Then, we estimated the chromatic contrast between each pair of spider and flower by

the Euclidean distance between the colour loci of the spider and the flowers in the colour hexagon (Chittka 1992). Honeybees only use chromatic contrast for objects at short distances and they use the green photoreceptor (achromatic contrast) to discriminate an object from long distance (i.e. objects that subtend a small visual angle) (Giurfa et al. 1996; Spaethe et al. 2001). Hence, we also calculated achromatic contrast between honeybees and their background as the difference between the value of the green photoreceptor when excited by the spider and the value of the green photoreceptor when excited by the flower. Values greater than zero indicated that spiders were brighter than flower and values lower than zero indicated that flowers were brighter than spiders. In order to describe the excitation of UV and blue photoreceptors in the bees' retina we also calculate the specific contrast for these bee photoreceptors using the same method.

Field data

We collected female crab spiders *Thomisus spectabilis* (Thomisidae) (n = 79) from *Bidens alba* Linn. (Asteraceae) white daisies and female *Diaea evanida* (Thomisidae) (n = 95) sitting on *Cosmos sp*. (Asteraceae) yellow daisies. *Thomisus spectabilis* spiders and *B. alba* flowers were collected in the surrounding areas of Airlie Beach, Queensland (Australia), in April 2008 and *D. evanida* spiders and *Cosmos sp.* flowers were collected in suburban areas of Sydney, New South Wales (Australia), from February to March 2008. A total of eight flowers of each species were collected at random. We used a total of 8 flowers of each species because data collected for the reflectance properties of each flower species, *Cosmos sp*. (N=95) and *B. alba* (N=111) flowers, have shown that the colour variation in these particular flower species is quite low (the mean±SD values for the E_{uv}, E_{blue} and E_{green} were 0.01±0.01, 0.07±0.03 and 0.70±0.04 respectively for *Cosmos sp.* flowers and 0.42±0.05, 0.80±0.03 and 0.77±0.03 respectively for *Bidens alba* flowers).

We calculated E_{uv}, E_{blue} and E_{green} values of the flowers and spiders as viewed by the honeybee using the methodology described before (Chittka 1992). For the eight flowers of each species of plants we measured the reflectance and averaged the eight E values of each plant species to obtain a natural mean background spectrum for each flower species. With the mean background spectrum we calculated the colour contrast and also the UV, blue and green contrast to determine how each colour region contributes to the overall colour contrast created by the spider against a flower. Once caught, we maintained spiders in plastic containers with the flowers on which they were sitting in the field until we took their colour measurements

within 5 days after spider collection. Previous experience has shown that this period in not enough to generate significant colour change in these spider species (Llandres, pers. obs).
To compare the excitation values (E) of the honeybee UV, blue and green photoreceptors between spiders and flowers collected in the field we used a t-test comparison for each of the three photoreceptors of honeybees and for each spider vs. flower species comparison. We used a false discovery rate adjustment ($\alpha < 0.027$) to account for the three non-independent calculations for the three photoreceptors of honeybees for each spider vs. flower species comparison (Benjamini & Hochberg 1995). We opted for the false discovery rate adjusted alpha instead of the Bonferroni adjustment because the Bonferroni adjustment has been shown repeatedly to be overly conservative (Benjamini et al. 2001; Narum 2006).

Bee choice experiment

The bee choice experiment was carried out on the campus grounds of Macquarie University, Sydney, in March 2008. We used the Australian crab spider *Diaea evanida*, and *Cosmos sp* flower species collected from the surrounding areas of Sydney in February 2008 and the Australian native bee *Trigona carbonaria* Smith (Apidae). The spiders used for the experiment were maintained in plastic containers in the laboratory against a constant dark background. They were fed with houseflies (*Musca domestica*) every week and watered daily. The native bees were maintained in an outdoor hive on campus and, trained to visit a nectar feeder (30% sucrose solution), which consisted of a plastic jar (4 cm in diameter) placed upside down on a plastic lid. The days that the experiment was carried out the feeder was replaced by the experimental trials and between each trial the feeder was offered to the native bees again.
The experiment consisted of giving native bees a choice between two flowers, one of them occupied by a spider and the other without a spider. The spiders were anaesthetised with carbon dioxide and placed on a randomly selected flower of the pair. The spiders were placed on the petals in a way that resembled their natural hunting position. The flowers were placed on a black plastic lid and each pair of lids was positioned against a black background with a distance of 10 cm between the flower centres. The flower petals were cut to equalise the diameter of the flowers and their centre discs diameter were similar in size to ensure that the decision of the bees was not influenced by differences in flower traits. Each daisy and crab spider was used only once.
The experiment was carried out including olfactory cues (N= 37 choice trials) and excluding them (N= 35 choice trials). To exclude olfactory cues we covered the plastic lids where the

flowers were placed with as see-through plastic foil (Glad Wrap®). The plastic foil is evenly permeable (<10% reduction) to all wavelengths of light between 300-700 nm (Heiling et al. 2003). We observed the number of native bees that approached the flowers within a distance of 4 cm during a period of 4 minutes and also observed the first bee that contacted one of the flower pair. After each day of the experiment we measured the spectral reflectance of spiders and flowers and calculated UV, blue, green and colour contrast for each pair of spider and flower.

To confirm that the two flowers from each trial offered to native bees were similar in colour, we used a paired t-test to compare the E_{UV}, E_{blue} and E_{green} of both petals and central disc between flowers with spider and vacant flowers. We further compared the number of bee approaches between spider-harbouring flowers and spider-free flowers using a matched paired t-test. Bee contact (first bee to contact the flower) was analysed with an independent binomial test. We used independent test for each comparison between flower with and without spider for the experiment with and without smell. We used a false discovery rate adjustment ($\alpha < 0.027$) to account for the three non-independent calculations of the three photoreceptors of honeybees for flower petals and central discs comparisons between flowers with spider vs. vacant flower.

Response of native bees to variation in spider contrasts

For these analyses, we used data from our bee choice experiment with *D. evanida* spiders and *T. carbonaria* native bees (hereafter Exp. 1) and also published data (Heiling & Herberstein 2004) from an experiment with *T. spectabilis* spiders and *Austroplebeia australis* Friese (Apidae) native bees (hereafter Exp. 2). In these analyses we considered only the data of native bees approaching flowers when olfactory cues were included. We did this because there were too few landings for analysis and because native bees did not seem to respond well to the presence of the plastic foil in the experiment where odour was removed (see also Heiling & Herberstein 2004). In order to test how native bees responded to the variation in colour contrast generated by the spiders against the flowers we used a regression analysis with the percentage of approaches to the flower with spider as the dependent variable. E_{UV}, E_{blue}, E_{green} and colour contrast generated by spiders against flowers were used as the independent variables.

Eight independent analyses were carried out for the data of the two bee choice experiments (four for each experiment). We performed an independent regression for all the independent variables (E_{UV}, E_{blue}, E_{green} and colour contrast) of each of the two experiments because all the

independent variables were highly correlated with each other. We used a false discovery rate adjustment ($\alpha < 0.024$) to account for the 4 regressions that we performed for each experiment. To account for the non-normality of the residuals of our dependent variables we used Monte Carlo procedures to calculate empirical p-values of all the regressions (Davison & Hinkley 2006). A total of 1999 Monte Carlo simulations were run using PopTools, version 3.0.6 (Hood 2008) in Microsoft Excel 2007. Furthermore, we performed two independent Partial Least Square (PLS) regression analysis (Carrascal et al. 2009), one for each of the two experiments, including percentage of approaches to the flower with spider as the dependent variable and the UV, blue and green contrast as the independent variables. PLS analyses are especially useful when the predictor variables are highly correlated because this type of analysis allows us to include all the independent variables in a single PLS regression analysis to determine the weight with which each independent variable contribute to explain the dependent variable (for a detailed explanation see Carrascal et al. 2009).

Results

Field data

The average values of the overall colour contrast created by *Thomisus spectabilis* spiders against *B. alba* flowers and *Diaea evanida* spiders against *Cosmos sp*. flowers are above the value of 0.05 (Table 1 and Fig. 1), the theoretical detection threshold of honeybees (Thery & Casas 2002). The overall colour contrast values are a product of individual values in the UV, blue and green contrasts. Average UV, blue and green contrasts for *D. evanida* spiders found on *Cosmos sp.* flowers and for *T. spectabilis* found on *B. alba* flowers are shown in Table 1.
There was a significant difference in the excitation values between *D. evanida* spiders and *Cosmos sp.* flowers for the UV and blue photoreceptor ($P < 0.001$, Table 1a) but not for the green photoreceptor ($P = 0.3$, Table 1a). The difference in the photoreceptor excitation values between *T. spectabilis* spiders and *B. alba* flowers was only significant for the UV photoreceptor ($P < 0.001$; Table 1b).

a)	Spider (n=95)	Flower (n=8)	Contrast	t_{101}	*P*
UV	0.13±0.09	0.01±0.00	0.12±0.09	3.752	**<0.001**
blue	0.38±0.12	0.06±0.01	0.32±0.11	7.634	**<0.001**
green	0.69±0.05	0.71±0.01	-0.02±0.04	-1.041	0.300
Overall colour			0.31±0.10		
b)	Spider (n=79)	Flower (n=8)	contrast	t_{85}	*P*
UV	0.65±0.09	0.42±0.02	0.23±0.09	6.982	**<0.001**
blue	0.82±0.04	0.79±0.01	0.03±0.04	2.165	0.033
green	0.79±0.03	0.77±0.01	0.02±0.03	1.550	0.124
Overall colour			0.21±0.07		

Table 1. *Results of the t-test comparing excitation values (mean±SE) of the honeybee's UV, blue and green photoreceptor between crab spiders and flowers from the field. We compared the E values between (a)* Diaea evanida *spider and* Cosmos sp *flower species and between (b)* Thomisus spectabilis *spider and* Bidens alba *flower species. Data of the mean (±SE) individual UV, blue and green contrast as well as the overall colour contrast that spiders generate against flowers of each species are also shown. We used a false discovery rate adjustment on the significance level ($\alpha < 0.027$). Values in boldface are significant.*

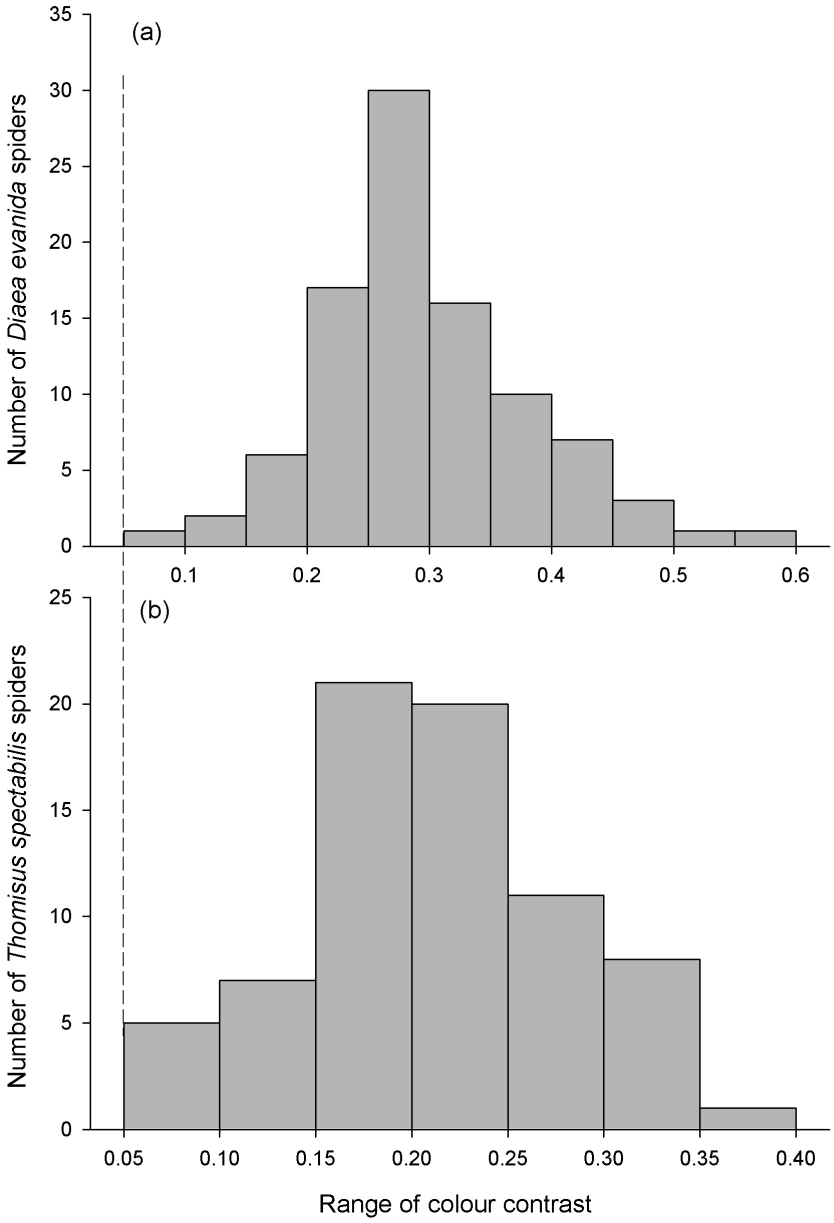

Fig. 1. *Frequency histograms for the colour contrasts that (a)* Diaea evanida *crab spiders generated against* Cosmos sp. *flowers and (b)* Thomisus spectabilis *crab spiders generated against* Bidens alba *flowers collected from the field. Dashed line indicates the threshold for colour contrast detection calculated for honeybees.*

Bee choice experiment

The excitation values of the flower petals and central disc between flowers with and without a spider did not show a significant difference on the UV, blue or on the green photoreceptor excitation values for the flowers used for both experiments, with and without smell (Table 2).

a) With odour	n	Flower without spider (Mean±SD)	Flower with spider (Mean±SD)	t_{72}	*P*
PETALS					
E uv	37	0.01±0.01	0.01±0.01	1.229	0.222
E blue	37	0.08±0.04	0.07±0.04	1.287	0.202
Egreen	37	0.70±0.01	0.70±0.01	-1.182	0.241
CENTRE					
E uv	37	0.01±0.01	0.01±0.01	0.604	0.547
E blue	37	0.06±0.04	0.06±0.04	0.221	0.825
Egreen	37	0.53±0.06	0.54±0.07	-0.782	0.436
b) without odour	n	Flower without spider (Mean±SD)	Flower with spider (Mean±SD)	t_{68}	*P*
PETALS					
E uv	35	0.01±0.01	0.01±0.01	0.987	0.326
E blue	35	0.08±0.04	0.07±0.04	1.076	0.285
Egreen	35	0.70±0.01	0.70±0.02	-1.030	0.306
CENTRE					
E uv	35	0.01±0.01	0.00±0.01	0.873	0.385
E blue	35	0.07±0.04	0.06±0.05	0.564	0.574
Egreen	35	0.53±0.07	0.54±0.07	-0.312	0.755

Table 2. *Results of the t-test comparisons for the UV, blue and green excitation values for the bee's photoreceptor between flowers with spiders and vacant flowers for the experiment (a) with smell (n=37) and (b) without smell (n=35). We compared petals and central disc (centre) of the flowers using a false discovery rate adjustment on the significance level ($\alpha < 0.027$).*

Despite the fact that both flowers (with and without spider) from each trial were similar in colour, our results show that in the experiment including olfactory cues native bees approached

flowers with spider more often compared to vacant flowers (P = 0.033, Fig. 2). However, when the smell was excluded native bees approached flowers randomly (P = 0.523, Fig. 2). Native bees showed a non-significant tendency to contact vacant flowers more frequently than spider-harbouring flowers (P = 0.062 for experiment with olfactory cues and *P* = 0.055 for the experiment without olfactory cues, Fig. 2).

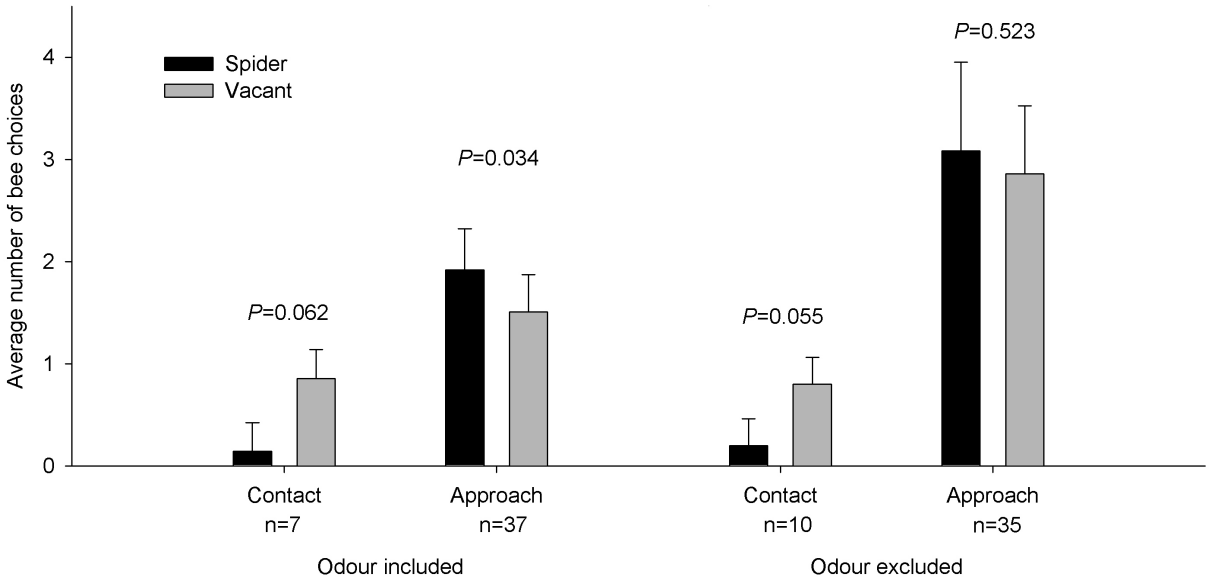

Fig. 2. *Average number of Australian* Trigona carbonaria *native bees (+95% CI), making contact with flowers and average number of the same bee species (+95% CI) coming closer than 4 cm to flowers harbouring* Diaea evanida *crab spiders (black bars) and flowers without crab spiders (grey bars) for both experiments including and excluding olfactory cues. The number of trials included in the analyses for each experiment is represented by n.*

Response of native bees to variation in spider colour contrasts

The mean (±SD) UV, blue and green individual contrasts that spiders generated against flowers were 0.31±0.22, 0.46±0.18 and 0.03±0.03 respectively for *D. evanida* spiders and *Cosmos sp.* flowers used for Exp. 1 and 0.15±0.03, 0.01±0.00 and 0.00±0.00 respectively for *T. spectabilis* spiders and *C. frutescens* flowers used for Exp. 2.

The regressions showed non-significant relationships between percentages of *T. carbonaria* bee approaches and overall colour contrast as well as individual UV, blue and green contrasts of *Diaea evanida* spiders (all $P \geq 0.540$, Table 3a). In contrast, in *A. australis* bees, the percentage of approaches to flowers with spider decreased significantly with individual UV

contrast of *T. spectabilis* (P = 0.021 Fig. 3b). There were marginally significant negative relationships for the overall contrast and also for the individual blue and green contrast of the spiders (Table 3b and Fig. 3a, c and d).

a) Response of *T. carbonaria* bees towards *D. evanida* spider contrasts				
Independent variable	d.f.	R^2	F	P
Colour contrast	1	0.009	0.332	0.561
UV contrast	1	0.009	0.346	0.580
Blue Contrast	1	0.003	0.134	0.703
Green Contrast	1	0.011	0.378	0.540
b) Response of *A. australis* bees towards *T. spectabilis* spider contrasts				
Independent variable	d.f.	R^2	F	P
Colour contrast	1	0.141	4.545	0.040
UV contrast	1	0.162	5.226	**0.021**
Blue Contrast	1	0.132	4.237	0.048
Green Contrast	1	0.131	4.320	0.053

Table 3. *Results of the simple linear regression models to test the relationship between UV, blue, green and colour contrast generated by spiders and (a) percentage of* Trigona carbonaria *bee approaches to flowers harbouring a* Diaea evanida *spider (n=37) and (b) percentage of* Austroplebeia australis *bee approaches to flowers harbouring a* Thomisus spectabilis *spider (n=30). Only data from the experiment in which the odour was included were analysed. We used a false discovery rate adjustment on the significance level ($\alpha < 0.024$) to account for the 4 regressions that we performed for each experiment. Values in boldface are significant.*

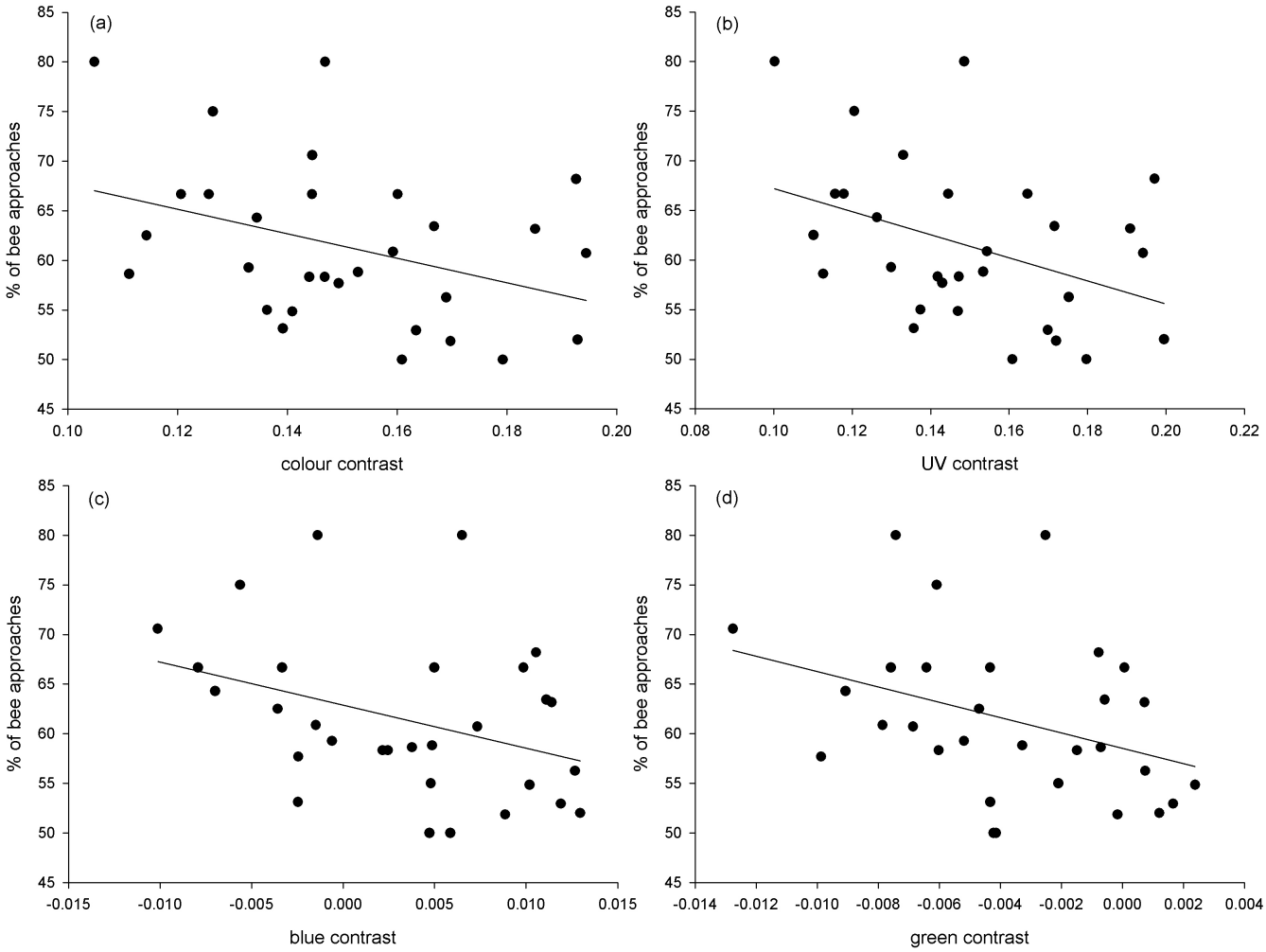

Fig. 3. *Linear regression between percentage of* Austroplebeia australis *bee approaches to flowers occupied by a* Thomisus spectabilis *crab spider and (a) Colour, (b) UV, (c) blue and (d) green contrast between the spider and the flower.*

The PLS regression analyses showed that for the Exp. 1 all the components accounted for a very marginal proportion of the explained variance (all ≤ 5%, Table 4). However, for the Exp. 2 the PLS showed that the first component accounted for a major proportion of the explained variance (18%, Table 4), while the second and third components accounted for a marginal proportion of the explained variance (1% and 2% respectively, Table 4). The second and the third components work with the residual not explained by the first component, but, since their contribution was marginal, only the first component was considered to interpret the results. The meaning of the components can be interpreted considering the weights attained by each variable. The addition of the square of the weights within each component sums to one. Knowing this, if we focus on component one, the square weights with which each individual contrast contribute to explain the behaviour of *A. australis* bees were 0.37, 0.31 and 0.31 for

the UV, blue and green contrast respectively. This means that, for example, the UV contrast explained a 37% of the total variance explained by the PLS regression model.

a)	W Component 1	W Component 2	W Component 3
UV contrast	-0.63	-0.11	-0.68
Blue contrast	-0.39	0.19	0.72
Green contrast	0.66	0.97	-0.07
R^2	0.04	0.04	0.05
b)	W Component 1	W Component 2	W Component 3
UV contrast	-0.61	-0.71	-0.22
Blue contrast	-0.55	0.69	0.75
Green contrast	-0.56	0.08	-0.62
R^2	0.18	0.01	0.02

Table 4. *Results of the Partial Least square (PLS) regression analyses carried out with the percentage of bee approaches to flowers with a spider as the dependent variable and the UV, blue and green contrast as the independent variables for (a)* Trigona carbonaria *bee approaches to flowers harbouring a* Diaea evanida *spiders (n=37) and (b)* Austroplebeia australis *bee approaches to flowers harbouring a* Thomisus spectabilis *spiders (n=30). Only data from the experiment in which the odour was included were analysed. W component 1, 2 and 3 are the weights of each variable in the first, second and third components. A high* W^2 *value indicates that the independent variable explained a high proportion of the total variance explained by the PLS regression model.*

Discussion

Our study supports the idea that, unlike introduced pollinators (Heiling et al. 2003; Herberstein et al. 2009), Australian native bees are able to detect and avoid flowers harbouring crab spiders despite the fact that they are initially attracted to them. Our results showed that *T. carbonaria* native bees approached more but landed less on spider-harbouring flowers when odour was included. When we excluded olfactory cues native bees did not approach more frequently but were more likely to land on spider-free flowers. We suspect that the methods we used to

exclude the odour may have affected native bee behaviour because bees seemed to be highly attracted to the plastic used to cover the flowers and they performed several inspection flights towards both flowers. Despite of that, we cannot rule out the possibility of an odour based predator recognition mechanism in addition to the colour component. In fact, it is very likely that pollinating insects use more than one of these components to recognise and avoid their predators. Certainly, in a field study Reader et al. (2006) showed that *Apis mellifera* bees responded to olfactory cues indicating the recent presence of a crab spider.

We found species specific differences in bee behaviour towards particular spider colour variation. *Trigona carbonaria* native bees did not show any preference for any of the individual colour contrasts generated by *D. evanida* spiders but, *A. australis* native bees showed a negative preference for flowers with more contrasting *T. spectabilis* spiders. It remains unclear why both bee species reacted differently to the extent of spider colour contrast, but we can think of three possibilities: firstly, there might be differences in the photoreceptors' spectral sensitivities between *Apis mellifera* and the two bee species used for this study; secondly, there may be differences in the visual system between *T. carboniaria* and *A. australis* bee species and thirdly, the range of contrasts generated by both spider species used for the experiments was quite different and may have affected native bee behaviour. Further research is needed to confirm any interpretation of the differences in the behaviour of the two species of native bees presented in our study. According with our result, several studies reported that different species of prey reacted differently towards predatory cues (Sullivan et al. 2004; e.g. Lloyd et al. 2009). For example, Lloyd et al. (2009) showed that three species of skinks (*Carlia rostralis, Carlia storri* and *Carlia rubrigularis*) reacted differently towards olfactory cues of a potential predator (*Vanarus tristis* goannas): although *C. rostralis* and *C. storri* skinks avoided the scent of the predator, *C. rubrigularis* did not show any avoidance behaviour towards predator olfactory cues.

In addition, our results showed that despite the negative preference for more UV-contrasting spiders, all the individual photoreceptor contrasts that *T. spectabilis* spiders generated against the flowers partly explained the behaviour of the *A. australis* native bees. Moreover, the PLS analysis showed that the three individual contrasts (UV, blue and green) contributed almost equally to the response of *A. australis* behaviour towards *T. spectabilis* spiders. Other studies performed with the exotic bee *Apis mellifera* have demonstrated that UV coloration in Australian crab spiders is the driving force that determines honeybee attraction towards *T.*

spectabilis spiders (Heiling et al. 2003; Heiling et al. 2005; Herberstein et al. 2009). In our study, however, we did not find that crab spider UV colouration was particularly important compared to other colours.

Taking into account our field data when examining how the overall colour contrast between spider and flower was created, both species presented a different pattern: in *D. evanida* spiders, differences in the UV and blue photoreceptor excitation values between flowers and spiders were crucial in generating overall contrast, whereas in *T. spectabilis* only the difference in UV photoreceptor excitation value contributed significantly to the overall colour contrast. In both spider species, the green photoreceptor excitation values were not different between spider and flower. Honeybees use the green photoreceptor (achromatic contrast) to discriminate objects from a long distance (i.e. objects that cover a small visual angle in the retina) (Giurfa et al. 1996; Spaethe et al. 2001). Our results indicate that, although Australian crab spiders are highly conspicuous when bees use their chromatic contrast to detect objects from a short distance, at a long distance they match the E_{green} excitation of the flowers, which makes them highly camouflaged from their prey's perspective (Thery et al. 2005).

Considering our field data, the preference of *Austroplebeia australis* bees for low UV contrasting *T. spectabilis* spiders (Exp. 2) seems counterintuitive since most of the *T. spectabilis* spiders found in the field generated higher UV contrast values than those preferred by *A. australis* bees. This leads us to the following question: why are most spiders reflecting more UV in the field than the amount of UV that generates a more preferred contrast for certain species of native bees? We think that the answer to this question may lie in the availability of prey species. To date, it has only been suggested that variation in UV of Australian crab spider coloration could be the result of a trade-off between attracting prey and avoiding predators (Herberstein et al. 2009). Supporting this hypothesis, in a recent experiment Fan et al. (2009) found that the colouration pattern in the orb-web spider *Nephila pilipes* is the result of a trade-off between visually attracting prey and avoiding predators. In the case of crab spiders, because the more UV reflective spiders are, the more conspicuous they will be for both potential prey and predators (Heiling et al. 2005), high UV-reflective spiders may be more successful in the absence of predators than less UV-reflective spiders (Herberstein et al. 2009). However, our study highlights that some of this variation may indicate a colour strategy that matches the colour preferences or responses of the most abundant prey. Indeed, several studies have shown that different species of pollinators respond differently to the presence of a crab

spider (Dukas & Morse 2003; Robertson & Maguire 2005; Dukas & Morse 2005; Gonçalves-Souza et al. 2008; Brechbühl et al. 2010). It, therefore, seems reasonable to assume that crab spiders present different strategies that increase foraging success according to the availability of prey species locally present. Accordingly, if the *T. spectabilis* spiders collected in the field for the present study were exposed mainly to *Apis mellifera* bees, it is parsimonious that most spiders generated a high UV-contrast against the flowers, because high contrasting spiders would be more successful in attracting honeybees than low contrasting spiders (Heiling et al. 2005).

We propose that like other spiders, crab spiders may have evolved a foraging behaviour that exploits the colour cues that insects seek while searching for food (Craig & Bernard 1990; Craig et al. 1996; Tso 1996; Herberstein et al. 2000; Bruce et al. 2001; Bruce et al. 2005). The variation in UV coloration of these spiders in different locations and at different times of the year might reflect the frequency of the most abundant prey and their species specific colour response. Thus, the ability to up or down regulate UV would enable spiders to exploit whatever populations of prey are locally abundant. Some studies in animal body colouration showed that phenotypic plasticity in colouration allows animals to adjust their colour in response to specific types of predators (Hanlon et al. 1999; Templeton & Shriner 2004; Stuart-Fox et al. 2006; Stuart-Fox et al. 2008). For example, dwarf chameleons differently adjusted their colouration in response to two predator species that differed in their visual capabilities (Stuart-Fox et al. 2006; Stuart-Fox et al. 2008). Following the same reasoning predators, and especially stationary predators, that are able to adjust their coloration to attract locally abundant species of prey are likely to increase their foraging performance. This, in turn, might help us to explain why different foraging strategies can be maintained in different populations of predators.

We believe that the selective advantage of exploiting different types of prey might have been one of the major forces influencing the evolution of UV coloration in Australian crab spiders and can explain the existing species specific variation in UV coloration as well as variation within different individuals of the same species. Moreover, our study highlights the importance of considering other colours than just UV to understand why crab spiders attract or deter certain species of prey. Our study also highlights the importance of studying background matching in the field from a community sensory ecology perspective (Defrize et al. 2010). Since each prey species has evolved specific visual abilities and behavioural responses to the same stimulus, by understanding crab spider background colour matching from the perspective

of several main receivers in the field we will be able to better understand the function of background colour matching in these particular generalist predators.

Acknowledgements

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Capítulo IV

Spider movement, UV reflectance and size, but not spider crypsis, affect the response of honeybees to Australian crab spiders

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Abstract

According to the crypsis hypothesis, the ability of female crab spiders to change body colour and match the colour of flowers has been selected because flower visitors are less likely to detect spiders that match the colour of the flowers used as hunting platform. However, recent findings suggest that spider crypsis plays a minor role in predator detection and some studies even showed that pollinators can become attracted to flowers harbouring Australian crab spider when the UV contrast between spider and flower increases. Here we studied the response of *Apis mellifera* honeybees to the presence of white or yellow *Thomisus spectabilis* Australian crab spiders sitting on *Bidens alba* inflorescences and also the response of honeybees to crab spiders that we made easily detectable painting blue their forelimbs or abdomen. To account for the visual systems of crab spider's prey, we measured the reflectance properties of the spiders and inflorescences used for the experiments. We found that honeybees did not respond to the degree of matching between spiders and inflorescences (either chromatic or achromatic contrast): they responded similarly to white and yellow spiders, to control and painted spiders. However spider UV reflection, spider size and spider movement determined honeybee behaviour: the probability that honeybees landed on spider-harbouring inflorescences was greatest when the spiders were large and had high UV reflectance or when spiders were small and reflected little UV, and honeybees were more likely to reject inflorescences if spiders moved as the bee approached the inflorescence. Our study suggests that only the large, but not the small Australian crab spiders deceive their preys by reflecting UV light, and highlights the importance of other cues that elicited an anti-predator response in honeybees.

Submitted

Introduction

Predators have evolved a wide variety of strategies to capture their prey. Among these strategies, the sit and wait tactic consists on remaining stationary and attacking approaching prey (Anderson & Karasov 1981; Olive 1982) and it is commonly found in insects, arachnids, amphibians, lizards and snakes, among other animal groups (Morse 1986; Shafir & Roughgarden 1998; Hatle & Salazar 2001; Yong 2003; Clark 2004). Despite the fact that animals that present this strategy do not actively search for their food, they have evolved several tactics that can increase their chances of capturing incoming prey. To cite some examples, sit and wait predators are often under selective pressure to select profitable hunting sites (Metcalfe et al. 1997; Scharf & Ovadia 2006; Morse 2007), to present cryptic coloration to avoid being detected by their prey (Cott 1957) or to attract their prey by luring them (Eberhard 1977; Nelson et al. 2010).

Many crab spiders (Thomisidae) specialise in ambushing pollinators on flowers. In several species, adult females can change their body colour to match the colour of the flowers on which they sit (Gabritschevsky 1927; Oxford & Gillespie 1998; Thery & Casas 2002; Morse 2007). Moreover, some studies report that crab spiders settled preferentially on flowers that matched their body colour: yellow crab spiders selected preferentially yellow flowers and white crab spiders tended to sit on white flowers to forage (Weigel 1941; Heiling et al. 2005b). All these studies support the crypsis hypothesis in crab spiders, according to which the ability to change body colour to match the colour of flowers has been selected in crab spiders because flower visitors are less likely to detect spiders when they match the colour of the flower used as hunting platform (Oxford & Gillespie 1998; Thery & Casas 2002; Morse 2007).

Some studies show indeed that pollinators use visual cues to assess the presence of predators on flowers while foraging (Gonçalves-Souza et al. 2008; Llandres et al. 2010; Brechbühl et al. 2010b). Different bee species, like *Apis mellifera* and *Trigona* sp., avoided *Rubus rosifolius* flowers containing artificial crab spiders (Gonçalves-Souza et al. 2008). When flowers contained objects resembling different morphological traits of spiders (abdomen or forelimbs), bees avoided objects resembling spider forelimbs (Gonçalves-Souza et al. 2008). Likewise, solitary bees and hover flies avoided *Anthemis tinctoria* flowers containing a pinned dried *Xysticus* sp. crab spider

(Brechbühl et al. 2010b). Different species of pollinators, however, reacted differently towards spider harbouring flowers. While some species avoided flowers with spiders, others showed indifference towards them (Brechbühl et al. 2010b). Furthermore, at least in some systems spider colour matching with the background plays at best a minor role in predator detection (Brechbühl et al. 2010a).

Even more surprising is the finding that some pollinators can become attracted to spider-harbouring flowers when the colour contrast between spider and flower increases (Herberstein et al. 2009). Australian crab spiders reflect more UV-light than their flowers, and are therefore conspicuous to bees (Herberstein et al. 2009). Nevertheless, in the green house bees were attracted to UV-reflecting spiders, suggesting that Australian spiders lure prey with colours that pollinators associate with food rewards (Heiling et al. 2003; Herberstein et al. 2009). European bees, *Apis mellifera,* approached and landed more on inflorescences with UV-reflecting crab spiders than on vacant inflorescences (Heiling et al. 2003; Herberstein et al. 2009). This preference disappeared when UV reflection was prevented applying a UV-absorber to crab spiders, indicating that UV reflection mediates bee preference (Heiling et al. 2005a). Australian native bees, *Austroplebeia australis* and *Trigona carbonaria* were also more likely to approach inflorescences harbouring UV-reflecting *Thomisus spectabilis* than vacant inflorescences, but they landed preferentially on vacant inflorescences (Heiling & Herberstein 2004; Llandres et al. 2010). These studies suggest that in the co-evolution between Australian native bees and crab spiders, the bees have evolved an anti-predatory response. In contrast, the European honeybees, introduced into Australia in 1822 (Hopkins 1886), have not had the opportunity to evolve a response to the deceptive Australian crab spider.

The aim of this study was to determine, under field conditions, the effect of colour matching on the interaction between the Australian crab spider *Thomisus spectabilis* and the European honeybee *Apis mellifera*. We studied the response of honeybees to the presence of white or yellow crab spiders sitting on *Bidens alba* inflorescences (white daisies with yellow centres) and also the response of honeybees to crab spiders that we made easily detectable by painting blue the spider's forelimbs or abdomen. Honeybees responded similarly to white and yellow spiders, to control and painted spiders, regardless of the morphological trait of the spider painted blue. However spider UV

reflection, spider size and spider movement affected honeybee behaviour: honeybees were more likely to land on spider-harbouring inflorescences when the spiders were large and had high UV reflectance or when spiders were small and reflected little UV, than when spiders had other trait combinations. In addition, honeybees were more likely to reject inflorescences if spiders moved as the bee approached the inflorescence. Finally, spider hunting success was affected by spider size, but not by the colour attributes of the spider.

Materials and Methods

Ethics statement

Animal ethics permits for invertebrates are not required in Australia, nevertheless our field work protocol adheres to the ASAB ethics guidelines (http://asab.nottingham.ac.uk/ethics/guidelines.php), whereby we minimized the impact on individuals and populations by using the least disruptive technique. As all field work was completed in non-protected areas, no specific collection permits were required.

Study area and species

We run the experiments in May and June 2009, at roadside patches of daisies, *Bidens alba*, in the vicinity of Cannonvale (Queensland, Australia). We conducted the observations in six patches, distant at least one kilometre from each other. *Bidens alba* has white inflorescences with yellow centres (Fig. 1C), it was one of the dominant flowering species in our study site and it was commonly used by crab spiders as hunting platform. In our field sites *B. alba* inflorescences were mainly visited by honeybees, *Apis mellifera*. Our model predator was *Thomisus spectabilis*. We used white and yellow adult and sub-adult females (Fig. 1 A and B). The colour signal produced by these spiders is a plastic trait, spiders can change between white and yellow colour over several days (Gabritschevsky 1927; Oxford & Gillespie 1998; for other species of crab spiders see Morse 2007). We collected white spiders from *B. alba* patches and yellow spiders from *Sphagneticola trilobata* patches. We kept spiders in plastic containers, feeding them with honeybees every week and watering them daily.

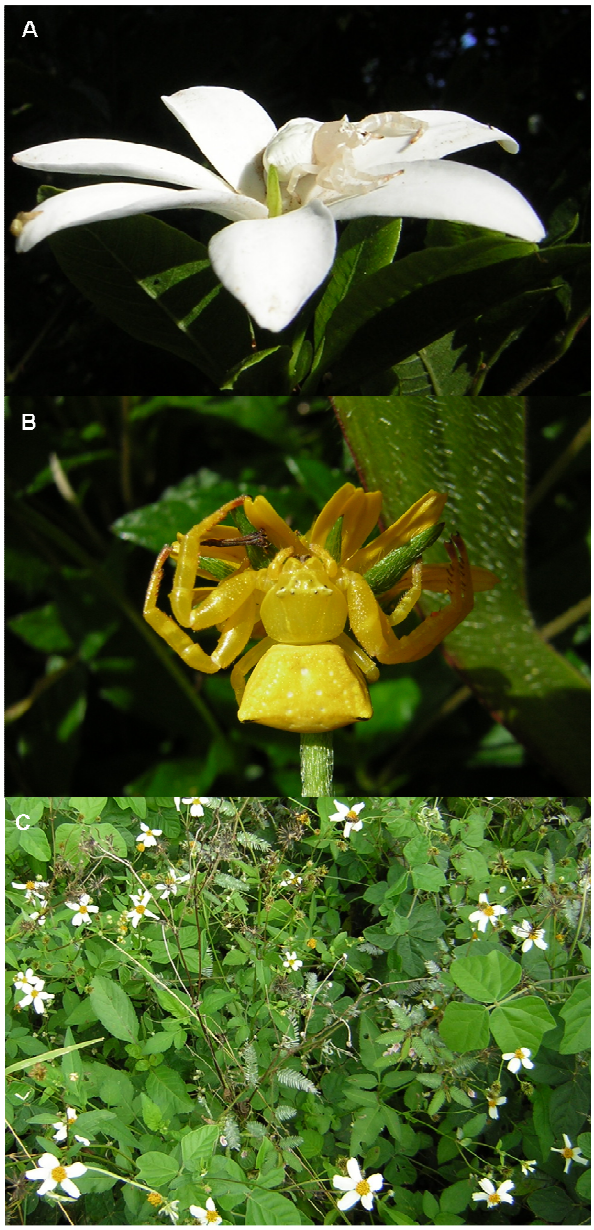

Figure 1. Spiders and inflorescences used in Experiment 1. *(A) A white female Thomisus spectabilis crab spiders sitting on a white flower, (B) a yellow female Thomisus spectabilis crab spiders sitting on a yellow inflorescence and (C) a Bidens alba patch.*

Each day we measured with a hand-held calliper the tibia-patella length and prosoma width of the spiders used that day and the reflectance spectra of spiders (dorsal side of abdomen) and inflorescences (upper side of inner and outer florets). Because tibia-patella length and prosoma width were highly correlated ($P < 0.0001$, $F_1 = 1053.02$, $R^2 = 0.906$, $N = 111$), we used prosoma width as a measure of spider size in all analyses.

Spider and inflorescences colour measurements

Spiders and inflorescence samples were analysed with an Ocean Optics USB4000 spectrometer using a fibre-optic probe connected to a black probe holder to exclude ambient light at an angle of 45º to the surfaces to measure (spiders or inflorescences). All the measurements were taken in the dark. The USB4000 spectrometer was connected to the PX-2 light source and attached to a PC running OOSpectra Suite spectrometer software. Reflectance data (300-700 nm) were generated relative to a white standard (Ocean Optics WS-1) and a black standard (black tape used as background to the measurements). For each sample, 10 spectra were averaged to reduce noise from the spectrometer with an integration period of 250 ms. We took in total three samples of each spider and inflorescence and averaged them to calculate the excitation values (E) that spiders and inflorescences would produce on the different photoreceptors (ultraviolet, blue and green) of honeybees following the methodology described below.

Calculation of bee's photoreceptor excitation values (E values)

We evaluated how the spiders and inflorescences are perceived by *Apis mellifera* bees by calculating photoreceptor excitations and colour contrasts using the colour hexagon model (Chittka 1992; Chittka 1996). The relative amount of light (quantum catch) absorbed by each bee photoreceptor, P_i, where i stands for UV, Blue or Green, was calculated by the formula:

$$P_i = R_i \int_{300}^{700} I_S(\lambda)\ S_i(\lambda)\ D(\lambda)\ d\lambda \qquad (1)$$

where $I_S(\lambda)$ is the spectral reflectance calculated from the spiders or inflorescences; $S_i(\lambda)$ is the spectral sensitivity function of bee photoreceptor i and $D(\lambda)$ is the illuminating daylight spectrum for which norm-function D65 is employed for open habitats (provided in Chittka & Kevan 2005). In equation (1) R_i is the sensitivity factor, determined by the equation:

$$R_i = \frac{1}{\int_{300}^{700} I_B(\lambda)\ S_i(\lambda)\ D(\lambda)\ d\lambda} \qquad (2)$$

where $I_B(\lambda)$ is the reflectance of the environmental background to which receptors are adapted. Note that in most conditions under which bees view flowers, the background will be green foliage, therefore, for the environmental background we used the green leaf spectrum provided by Chittka & Kevan (2005).

The excitation of each bee photoreceptor, E_{UV}, E_{Blue}, E_{Green}, was calculated from the relative quantum catch of the photoreceptors, P_i:

$$E_i = \frac{P_i}{P_i + 1} \tag{3}$$

As mentioned before, the E_{UV}, E_{Blue} and E_{Green} for each spider and inflorescence were calculated using the average of the excitation values calculated from the three reflectance spectra taken for each spider and inflorescence.

Calculation of colour contrast

We used the E_{uv}, E_{blue} and E_{green} values to calculate the colour loci of spiders and their flower background in the bee colour hexagon and estimated the chromatic contrast between each pair of spider and inflorescence by the Euclidean distance between their colour loci in the bee colour hexagon. For doing this E_{uv}, E_{blue} and E_{green} were used to calculate coordinates in the bee colour hexagon (Chittka et al. 1992; Chittka 1996):

$$x = \sqrt{3}(E_{Green} - E_{UV})/2 \tag{4}$$

$$y = E_{Blue} - 0.5(E_{Green} + E_{UV}) \tag{5}$$

Then, the colour contrast was calculated by the Euclidian distance between the spiders and the inflorescences in the colour hexagon:

$$\Delta St = \sqrt{(x_{\text{spider}} - x_{\text{inflorescence}})^2 + (y_{\text{spider}} - y_{\text{inflorescence}})^2} \tag{6}$$

where x and y are the coordinates of the hexagon calculated by equations (4) and (5).

The processing of colour information by the visual system of honeybees follows different pathways depending on the angle subtended by the visual target: when the angle is large (greater than 15º), honeybees use colour contrast to discriminate between

an object and its background, but when the angle is small they use green contrast. Hence honeybees only use chromatic contrast (colour contrast, equation 6) to discriminate an object at short distances and they use the green photoreceptor (achromatic contrast) to discriminate an object from long distance (Giurfa et al. 1996). In practice, this means that for our experiments colour contrast became relevant when bees were approximately less than 5-10 cm from inflorescences: According to Giurfa et al. (1996), the relationship between the radius of an object (r) and the distance at which the object can be detected if it offers colour contrast with the background, d, is:

$$\tan(15^{o}/2) = r/d \qquad (7)$$

Detection distances of 5 and 10 cm therefore correspond to stimuli with radio 0.7 and 1.3 cm, respectively. An effective diameter between 1.3 and 2.6 cm is reasonable for *T. spectabilis* (average prosoma width ± SD = 3.60 ± 0.70).

To account for "long distance" detection, we also calculated green contrast between spiders and inflorescences as the excitation difference in the green photoreceptor between the target, spider, and the background, inflorescence. In order to describe the excitation of UV and blue photoreceptors we also calculate the specific contrast for these bee photoreceptors using the same method. Moreover, spider UV reflection has been shown to be a key factor determining the interaction between Australian crab spiders and honeybees (Heiling et al. 2003; Heiling et al. 2005a; Herberstein et al. 2009), thus, we further computed the percentage of light reflected by each spider in the UV range (300-400 nm), %UV, as an absolute-value of UV reflectance, independent of assumed properties of the receiver visual system.

Experiment 1: effect of natural spider colour on honeybee behaviour

In Experiment 1 we studied the response of honeybees to the presence of white or yellow crab spiders, *T. spectabilis*, on white daisies with yellow centres, *B. alba* (Fig. 1). In each trial we selected three nearby *B. alba* inflorescences and placed a crab spider female on one of them. We waited for the spider to adopt a hunting attitude and recorded spider behaviour (attacks and bee captures) and honeybee visits to the three

inflorescences for the following 90 minutes. We defined spider behaviours as follows: attack if the spider attempted to capture the bee with its forelegs, and capture if the spider managed to capture and kill the bee. We considered that a bee visited an inflorescence when it landed on it. When spiders captured a prey, we removed it from their chelicerae with forceps and continued the observations. We completed 34 trials with white and 36 with yellow spiders, conducting observations in sunny days, between 09.00 and 15.30, when honeybee activity was high. We used each daisy and crab spider only once. If, during the observations, a spider tried to escape from the inflorescence where we had placed it, we excluded it from the experiment and started another trial with a new spider. To determine if we excluded some particularly unsuccessful hunters, we compared the excitation photoreceptor values E_{uv}, E_{blue} and E_{green} and the colour/green contrasts calculated for yellow (N = 10) and white (N = 14) excluded spiders with the same values calculated for yellow (N = 26) and white (N = 17) spiders that successfully captured a honeybee during the experiment with independent t-tests.

We define as a "struggle" an event in which a crab spider embraces a honeybee with its forelimbs, regardless of whether the embrace ends in a successful capture or not. To determine whether the rate of honeybee visits to spider inflorescences decreased after a struggle, we performed the following analysis. We divided each trial in two temporal blocks: "early" and "late". For trials in which we observed a struggle (N = 43), we considered as early observations from the start of the trial to the struggle, and as late observations from the struggle to the end of the trial. For trials without struggle (N = 15), early and late refer to the first and second half of the trial, respectively. For each temporal block, we calculated the rate of honeybee visits (number of visits per minute) to the spider inflorescence. We then compared these visit rates with a repeated-measures ANOVA. The dependent variable was the rate of honeybee visits to spider inflorescence, temporal block (early and late) entered in the model as the within subject factor, and struggle ("yes" if there was a struggle, "no" if there was no struggle) entered as the between subject factor.

To ascertain the factors affecting bee choice, we fitted a series of generalised linear models to the data and used Akaike Information Criterion, AIC, to select the most parsimonious model (Akaike 1973). The null model assumed that honeybees visited spider harbouring inflorescences and control inflorescences with the same probability, i.e. the model assumed that the probability of visiting a spider harbouring inflorescence

was p=1/3 regardless of spider size or colour. The second simplest model assumed that spider presence affected honeybee choice, independently of any specific spider trait. The rest of models included several factors that could also affect bee choice: spider colour, size and %UV, and four indexes of colour matching between spider and inflorescence (both inner and outer florets): UV contrast (E_{uv}(spider) – E_{uv}(inflorescence)), blue contrast (E_b(spider) – E_b(inflorescence)), green contrast (E_g(spider) – E_g(inflorescence)) and colour contrast between inflorescence and spider. When several explanatory variables were correlated, we run alternative models with one or the other variable, but we did not include correlated explanatory variables in a single model.

For each analysis, we report in detail the most parsimonious model (the model with the lowest AIC value) and comment the differences with those models within two AIC units – when there were any such models. We used likelihood ratio tests to determine whether those factors remaining in the most parsimonious model had statistically significant effects on the probability that honeybees landed on spider-harbouring inflorescences (Dobson & Barnett 2008). The likelihood ratio test computes the deviance between two nested models. If the independent variables included in the more complex model, but not in the simpler model, have no explanatory value, then the deviance is expected to have a χ^2distribution, with as many degrees of freedom as extra parameters has the more complex model. All models assumed a binomial distribution of visits to spider-harbouring inflorescences. Thus, if *m* bees visited the patch during a trial the probability that *n* of them visited the spider-harbouring inflorescence would be given by the binomial distribution,

$$P(n \mid m) = \binom{m}{n} \cdot \pi^n \cdot (1-\pi)^{m-n},$$

where the probability that an individual bee landed on the spider-harbouring inflorescence, π, is given by the fitted statistical model. We repeated the fitting procedure with different link functions (identity, logit, probit and cloglog) to select the best-fitting relationship between independent and dependent variables. Link functions had minor effects on AIC values and did not affect the variables included in the most parsimonious model. We therefore only report the results of the best-fitting link function.

We used a similar procedure to determine the factors affecting the hunting success of spiders. The dependent variable (hunting success) had again a binomial distribution. To control for possible effects of pollinator activity, on top of the explanatory variables described above we included for these analyses the number of bees visiting the patch.

Experiment 2: effect of artificial spider colour on honeybee behaviour

To study the reaction of honeybees towards easily detectable predators we painted some spiders with a dark-blue permanent marker. Furthermore, because it has been claimed that honeybees have a higher tendency to avoid flowers with traits resembling the shape of spider forelimbs than flowers with traits resembling the body of spiders (Gonçalves-Souza et al. 2008), we painted in blue the forelimbs of some *T. spectabilis* females and the dorsal side of the abdomen of other females (Fig. 2). We randomly allocated white *T. spectabilis* females to one of the following treatment (37 females per treatment): "forelimbs", "abdomen" and "control", and painted in blue the two first pairs of legs, the dorsal side of the abdomen and the ventral side of the abdomen, respectively. The ventral side of the abdomen of crab spiders is not visible to approaching bees, but painting it served as a control for the manipulation (which could affect the behaviour of spiders) and ensured that all spiders provided the same olfactory cues. Using these three treatments rather than white and yellow spiders, we run an experiment similar to Experiment 1. In Experiment 2, however, trials lasted only 45 minutes and were discontinued when spiders struggled with a landing bee.

Figure 2. Blue painted spiders used in Experiment 2. *(A) a Thomisus spectabilis female with the forelimbs painted on blue and (B) a Thomisus spectabilis female with the dorsal part of the abdomen painted on blue.*

To determine the factors affecting honeybee choice, we used Akaike Information Criterion, AIC, to select the most parsimonious model as explained above. As with Experiment 1, the base model assumed that honeybees visited spider harbouring inflorescences and control inflorescences with the same probability, i.e. p=1/3 for spider harbouring inflorescences. The second simplest model assumed that spider presence affected honeybee choice independently of any specific spider trait. The rest of models included some factors that could also affect bee choice: we only included treatment and spider size as possible explanatory variables in the statistical models for Experiment 2.

To determine the factors affecting the hunting success of spiders for Experiment 2, we included treatment and spider size as explanatory variables in the statistical models. The dependent variable (hunting success) had a binomial distribution. As in Experiment 1, to control for effects of pollinator activity, we added the number of bees visiting the patch as an explanatory variable in these analyses.

Experiment 3: effect of blue spots on honeybee behaviour

To determine whether honeybees were attracted to objects presenting the blue colour that we used to paint the spiders of Experiment 2 we performed a series of observations (N = 41 trials) in which we selected four inflorescences, roughly forming a square 20-30 cm in side. We painted a blue spot on each external floret, forming roughly a circle, on two inflorescences (blue inflorescences) and the calyx of the other two (control inflorescences) to control for possible effects of ink smell. We then waited for honeybees to visit the four inflorescences ten times and noted how many of the visits had occurred on blue inflorescences. The number of times that 0, 1… 10 blue inflorescences were visited was compared to the number expected if honeybees were equally likely to visit blue and control inflorescences (binomial distribution, ten trials, p = 1-p = 0.5) using a χ^2 test. Because the probability that blue inflorescences received 0-3 or 7-10 inflorescences was very small, and given that the χ^2 test is unreliable if the expected number of observations in some cells of the contingency table is smaller than five, to ensure that expected values were greater than five in each cell we pooled observations corresponding to 0-3 and 7-10 blue inflorescences visited.

Experiment 4: effect of spider movement on honeybee behaviour

We placed white *T. spectabilis* females (N = 29) on *B. alba* inflorescences, waited until they adopted a hunting attitude and recorded their behaviour with a video camera during 30 minutes. When honeybees landed on the spider inflorescence and spiders prepared to strike an attack, we gently brushed bees away to prevent captures. For all approaching honeybees, we recorded whether they landed on the spider inflorescence or rejected it. We considered that a bee rejected an inflorescence when it approached the inflorescence, hovered for a few video frames in front of it (sometimes touching it with its forelegs) and left without landing. We observed every honeybee approach frame by frame and noted the position of the spider (above or below the inflorescence) and whether it moved from the time when the honeybee entered the image until it landed or rejected the flower. We used generalised linear models to determine whether spider position and movement affected the response of the bee. The dependent variable of each model was the response of the bees (binomial error distribution: bees could either land on the inflorescence, 1, or reject it, 0), the explanatory variables were spider position (above or below the inflorescences) and spider movement ("yes" if they moved before the bee landed or "no" if the spider remained still). Spider identity was included in all models as a random factor.

Unless otherwise specified, all results are reported as average ± SE.

Results

Experiment 1: effect of natural spider colour on honeybee behaviour

Although, on the honeybee colour hexagon, there was substantial overlap between the colour loci of white spiders and outer florets of *B. alba* and between the colour loci of yellow spiders and inner florets (Fig. 3), there was variability in the loci of spiders and inflorescences and colour matching between individual spiders and the inflorescences they used as hunting platforms was generally poor. Colour contrast was 0.14 ± 0.01 (mean ± SE, colour hexagon units) between white spiders and white outer florets and 0.17 ± 0.01 between yellow spiders and yellow inner florets. Both values were therefore higher than the 0.05 threshold considered necessary for colour discrimination in honeybees (Thery & Casas 2002). Colour contrasts between white spiders and yellow florets (0.32 ± 0.01) and between yellow spiders and white florets (0.27 ± 0.01) were even easier to discriminate by the visual system of honeybees.

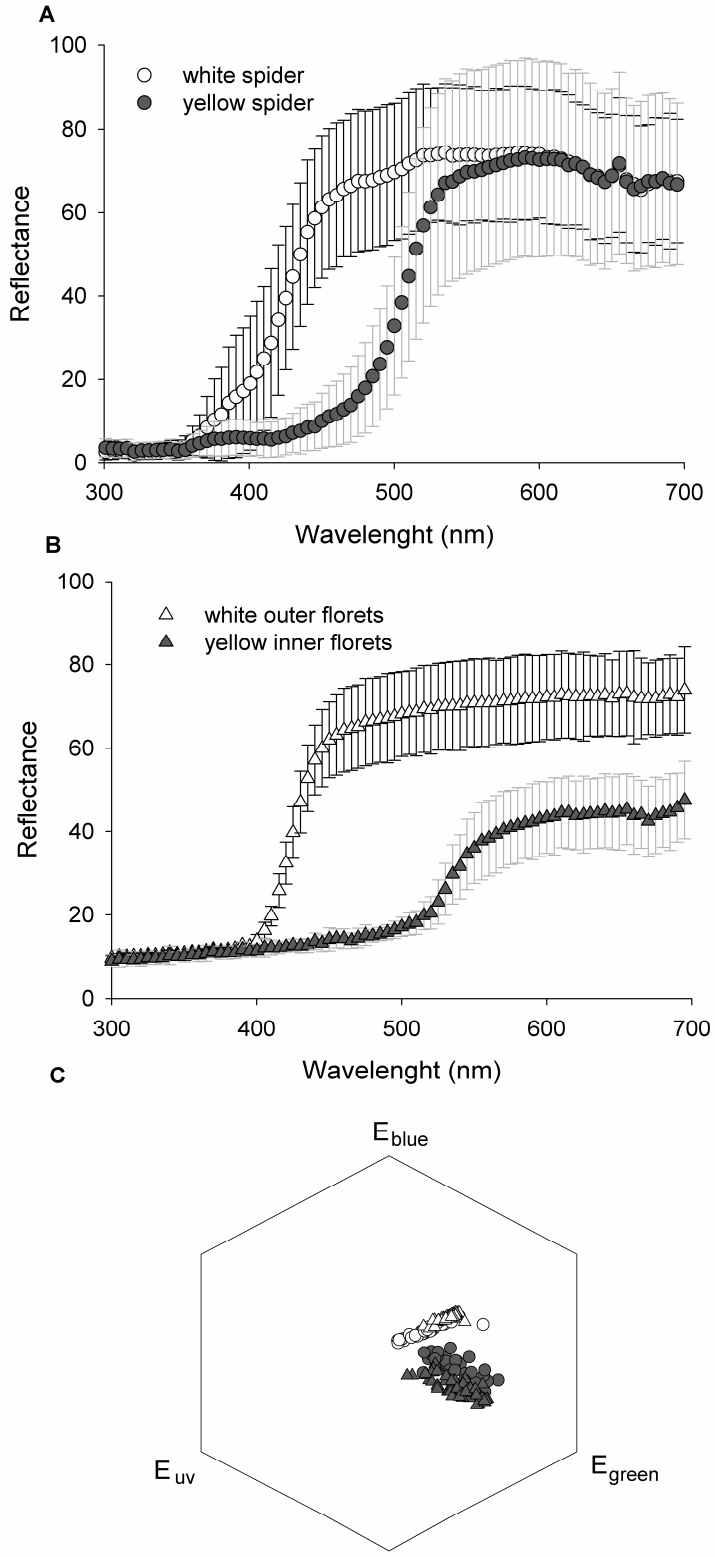

Figure 3. Reflectance spectra of inflorescences and spiders (Experiment 1). *Reflectance spectra of (A) yellow (grey circles N = 36) and white (white circles N = 34) Thomisus spectabilis females, (B) white outer florets (white triangles N = 34) and yellow inner florets (grey triangles N = 36) of Bidens alba inflorescences. Error bars in panel (A) and (B) represent standard deviations. Panel C illustrates the colour loci of all spiders and inflorescences in the colour hexagon of honeybees calculated for white spiders (white circles N = 34), white outer florets (white triangles N = 34), yellow spiders (grey circles N = 36) and yellow inner florets (grey triangles N = 36).*

However, in terms of green contrast, both white (0.02 ± 0.01) and yellow (-0.01 ± 0.01) spiders were virtually indistinguishable from the white outer florets of *B. alba* inflorescences, but contrasted sharply (white: 0.27 ± 0.01, yellow: 0.24 ± 0.02) with the yellow inner florets. Taken together, these results imply that honeybees could not discriminate white or yellow spiders against the white florets of *B. alba* (where they commonly sit to hunt) when they were at large distances (more than 5-10 cm away), but they could detect the presence of the spider at closer distance, regardless of the colour of the spider or its background.

The spiders we excluded had similar colour to those we used: neither the excitation photoreceptor values E_{uv}, E_{blue} and E_{green} nor the colour/green contrasts calculated for yellow and white excluded spiders differed from the same values calculated for yellow and white spiders that successfully captured a honeybee during the experiment (all $P > 0.10$).

The effect of presence or absence of a struggle on the rate at which bees visited spider inflorescences was not significant ($F_{1,55} = 0.55$, $P = 0.46$), however, there was a significant effect of time in the trial (early vs. late) ($F_{1,55} = 14.46$, $P < 0.001$) and the interaction between time in the trial and presence or absence of a struggle on the rate at which bees visited spider inflorescences ($F_{1,55} = 11.92$, $P = 0.001$). In trials with a struggle, the average rate of honeybee visits to spider inflorescences was 0.278 (± 0.04) visits per minute before the struggle and decreased to 0.005 (± 0.001) afterwards: only 11 honeybees visited a spider inflorescence after the spider struggled with another honeybee. In contrast, in trials without struggle the average rate of honeybee visits to spider inflorescences was 0.163 (± 0.03) visits per minute in the first half of the observations and 0.176 (± 0.03) in the second half. This result suggests that, during a struggle with a crab spider, honeybees released chemical information that elicited an avoidance response from approaching honeybees. For this reason, to ascertain the factors affecting bee choice, we only analyse honeybee visits up to and including the first struggle.

The null model assumed that honeybees visited spider inflorescences with a probability of 1/3. The model that assumed that the probability of honeybees visiting spider inflorescences was independent of spider attributes, but not necessarily equal to 1/3, provided only a slightly better fit to the data (deviance = 2.92, df = 1, P = 0.08). According to this model, the probability of honeybees visiting spider inflorescences was

0.30. Overall, therefore, there was a modest (and not statistically significant) rejection of spider inflorescences.

The probability that honeybees landed on spider inflorescences, however, was not independent of spider attributes. According to the most parsimonious model, the probability that a bee selected the spider-harbouring inflorescence for landing was

$$\pi = 0.74 - 0.13*\textit{spider size} + 0.12*\textit{UV} + 0.14*\textit{Gci} + 0.049*\textit{Spider size}*\textit{UV} - 0.21*\textit{UV}*\textit{Gci},$$

where *spider size* refers to spider prosoma width (in mm), *UV* to %UV reflectance of spiders and *Gci* to the green contrast generated by spiders against the inner florets of their inflorescence. The second best supported model (with a difference of less than two units in its AIC from the first model) was the model that included spider size, % UV, Gci and the double interactions between spider size and %UV, spider size and Gci, and %UV and Gci. Nevertheless, of the variables retained in both models only spider size (deviance = 7.511, df = 1, P = 0.006) and the interaction between spider size and %UV reflectance (deviance = 8.61, df = 1, P = 0.003) significantly affected honeybee behaviour. The probability that honeybees landed on spider-harbouring inflorescences was greatest when the spiders were large and had high UV reflectance or when spiders were small and reflected little UV, and smallest when spiders were small and had high UV or large and reflected little UV (Fig. 4). Neither Gci (deviance = 1.26, df = 1, P = 0.26), % UV (deviance = 0.44, df = 1, P = 0.51), nor the interaction between Gci and UV (deviance = 2.87, df = 1, P = 0.10), nor between spider size and Gci (deviance = 0.01, df = 1, P = 0.90) had statistically significant effects on honeybee behaviour.

Although honeybees responded similarly to the presence of white and yellow spiders, both spider colour and spider size affected the probability that a spider successfully captured a bee during the observations. The model retained to explain hunting success was:

$$\text{logit}(\pi) = -9.39 + 5.85*\textit{spider colour} + 3.25*\textit{spider size} - 2.30*\textit{spider colour}*\textit{spider size}$$

Both spider size (deviance = 14.16, df = 1, P < 0.001) and colour (deviance = 5.59, df = 1, P = 0.018) had statistically significant effects on hunting success. Although hunting success increased with body size for both white and yellow spiders, the difference was more noticeable for yellow than for white spiders (deviance for the interaction term = 4.60, df = 1, P = 0.032). Thus, 20 out of 20 yellow spiders with prosoma width greater

than 3.44 mm successfully captured a honeybee during the observations, while only 14 out of 20 white spiders of similar size succeeded at capturing a honeybee (Fig. 5).

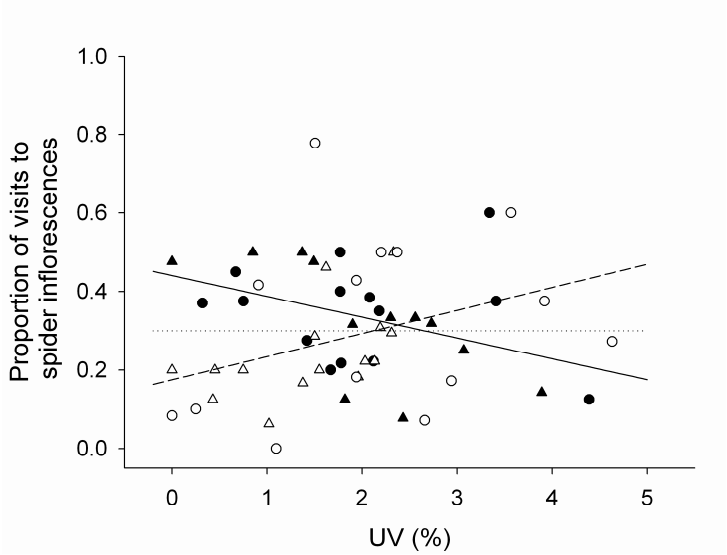

Figure 4. Effect of spider UV and spider size on honeybee behaviour (Experiment 1)*. Proportion of honeybee visits to spider inflorescences vs spider UV reflectance considering only those trials that received more than four honeybee visits to the patch. Trials with less than five visits were removed because the statistical model gives relatively little weight to trials with few honeybee visits. Black symbols represent small spiders (prosoma width < 3.44 mm) and white symbols represent large spiders (prosoma width > 3.44 mm). The value of 3.44 mm represents the median value of spider prosoma's width for trials that received more than four honeybee visits to the patch. Triangles represent yellow spiders and circles represent white spiders. Regression lines between proportion of honeybee visits to spider inflorescences and spider UV reflectance for small (solid line) and large (dashed line) spiders are given in the figure, together with the expected proportion of visits to spider inflorescences if honeybees treated all inflorescences alike (p = 1/3; dotted line).*

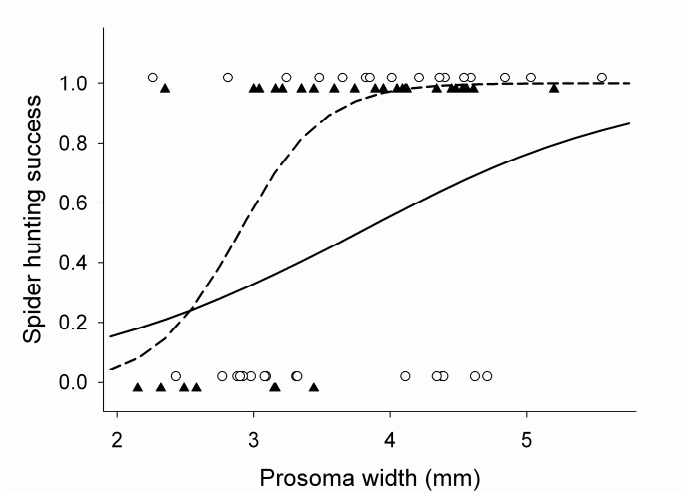

Figure 5. Effect of spider colour and size on spider hunting success (Experiment 1). *Spider hunting success vs spider size for white spiders (white circles) and yellow spiders (black triangles). Lines represent fitted values of capture probability for white (solid line) and yellow (dashed line) spiders.*

Experiment 2: effect of artificial spider colour on honeybee behaviour

As we have seen, green contrast of white and yellow spiders against the white outer florets of *B. alba* was insufficient for honeybee detection at more than 5-10 cm. The spider manipulation of Experiment 2 achieved high colour contrast (white outer florets 0.35 ± 0.01, yellow inner florets 0.59 ± 0.02) and green contrast (white outer florets -0.40 ± 0.02, yellow inner florets -0.14 ± 0.03) between the blue-painted spider traits and the inflorescences used as hunting platforms. Therefore, Experiment 2 ensured that spiders were easily detectable by honeybees at all distances (Fig. 6).

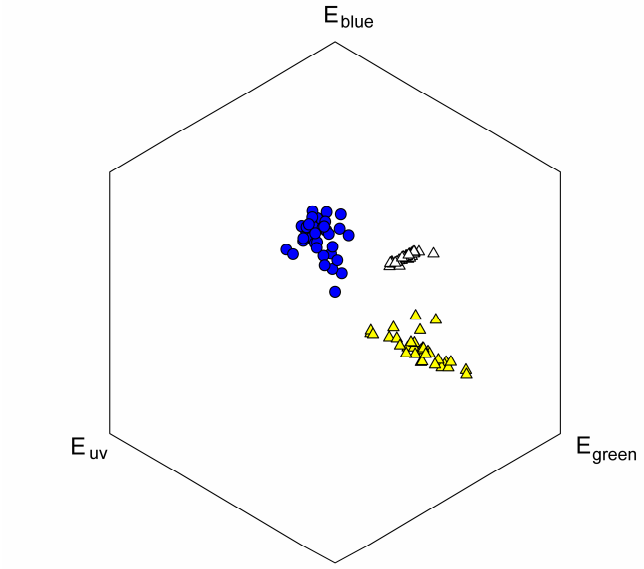

Figure 6. Colour loci of blue-painted spiders in the colour hexagon of honeybees (Experiment 2). *Colour loci in the colour hexagon of honeybees calculated for blue-painted spiders (blue circles N = 37), white outer florets (white triangles N = 37) and yellow inner florets (yellow triangles N = 37).*

There was a significant effect of spider presence on honeybee behaviour (deviance = 5.81, df = 1, P = 0.01). According to this result, honeybees landed on spider-harbouring inflorescences with a probability of 0.30, which was slightly lower than 1/3, therefore experiment 2 also shows that honeybees were slightly repelled by inflorescences with spiders. Only spider size (mm) remained in the most parsimonious model, according to which the probability that honeybees visiting the path landed on the spider-harbouring inflorescence, π, was

$$\text{cloglog}(\pi) = -0.44 - 0.17 * \textit{spider size}$$

Larger spiders therefore elicited stronger avoidance responses than smaller spiders (deviance = 5.26, df = 1, P = 0.02; Fig. 7). Treatment (deviance = 0.48, df = 1, P = 0.78) did not appear in the most parsimonious model (ΔAIC = 3.70). Size also affected the

probability that spiders hunted a bee during the observations: large spiders posed a stronger risk for honeybees than small ones because the probability of hunting a bee greatly increased with spider size (deviance = 28.00, df = 1, $P < 0.001$), but treatment (deviance = 0.81, df = 2, P = 0.66) did not enter the most parsimonious model ($\Delta AIC = 3.21$, Fig. 8), which was

$\text{logit}(\pi) = -6.12 + 1.90 * \textit{spider size}$

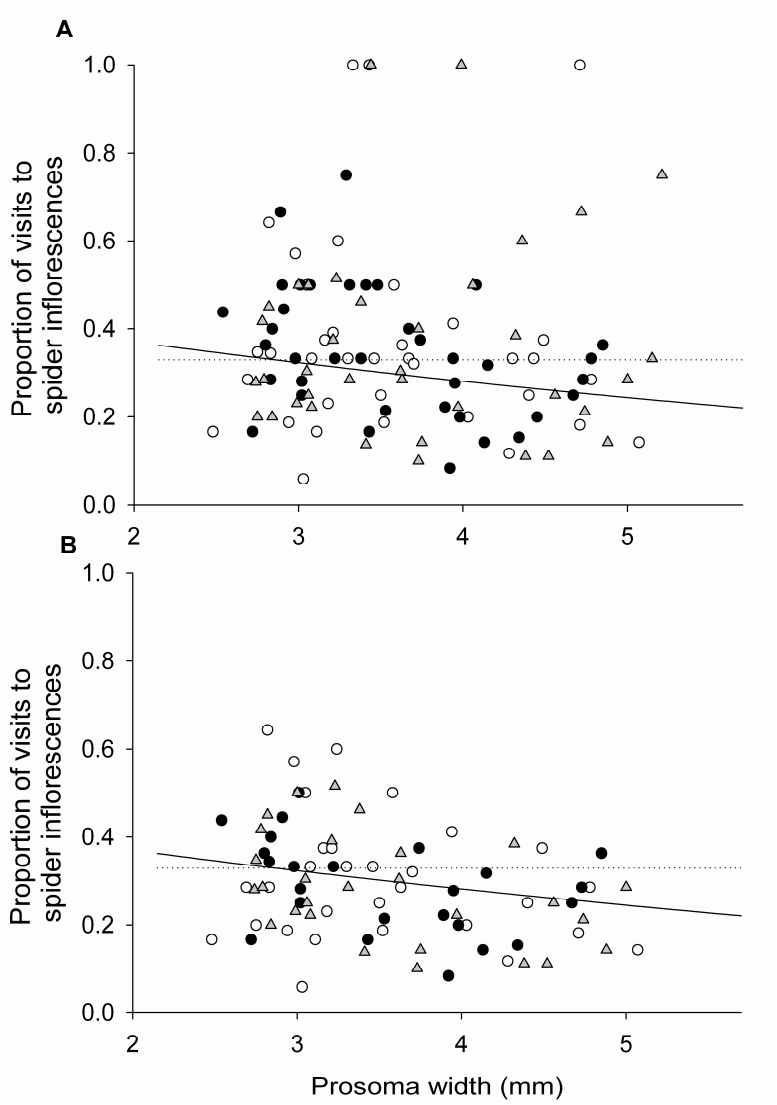

Figure 7. Effect of spider size on honeybee behaviour (Experiment 2). *Proportion of honeybee visits to spider inflorescences vs spider size (A) considering all the trials conducted in the experiment and (B) considering only those trials that received more than six honeybee visits to the patch. Black circles represent spiders with the dorsal part of the abdomen painted on blue, grey triangles represent spiders with the forelimbs painted on blue and white circles represent control spiders. Solid lines represent fitted probability of landing on spider harbouring inflorescences. Although the relationship between probability of landing on spider inflorescences and spider size is not apparent in panel (A), the statistical model gives relatively little weight to trials with few honeybee visits removed to produce (B). Dotted lines represent the expected value if honeybees treated all inflorescences alike (p = 1/3).*

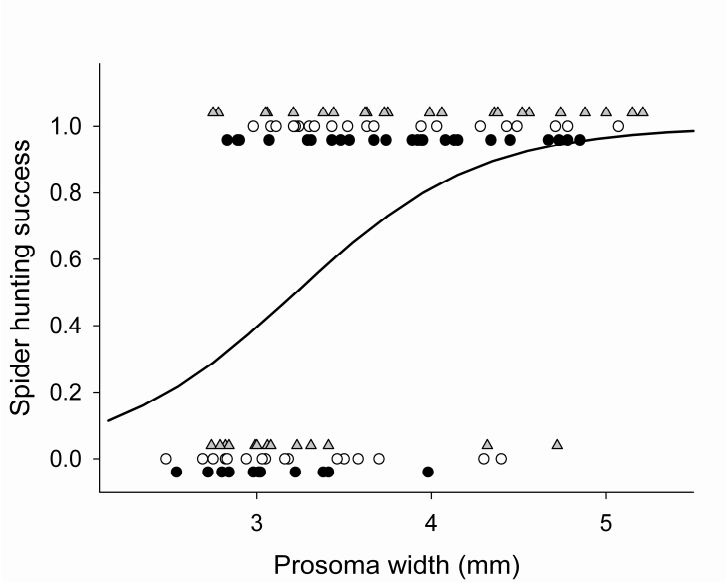

Figure 8. Effect of spider size on spider hunting success (Experiment 2). *Spider hunting success vs spider size for spiders with the dorsal part of the abdomen (black circles), forelimbs (grey triangles) and ventral part of the abdomen (control treatment) (white circles) painted on blue. The line represents the fitted value of the probability of capturing a honeybee.*

Experiment 3: effect of blue spots on honeybee behaviour

Although the results of Experiment 2 suggest that visual cues played a minor role in the predator avoidance response of honeybees, an alternative interpretation is possible. It could be argued that blue markings made spiders easier to detect, but failed to elicit an avoidance response because honeybees had a tendency to approach inflorescences with blue markings. If this were the case we should expect honeybees to be generally more attracted to objects containing blue markings compared to objects without blue markings. However the results of Experiment 3 showed that the frequency with which honeybees visited blue-painted inflorescences was not different than expected by chance ($\chi^2 = 6.85$, df = 4, P = 0.14, Fig. 9).

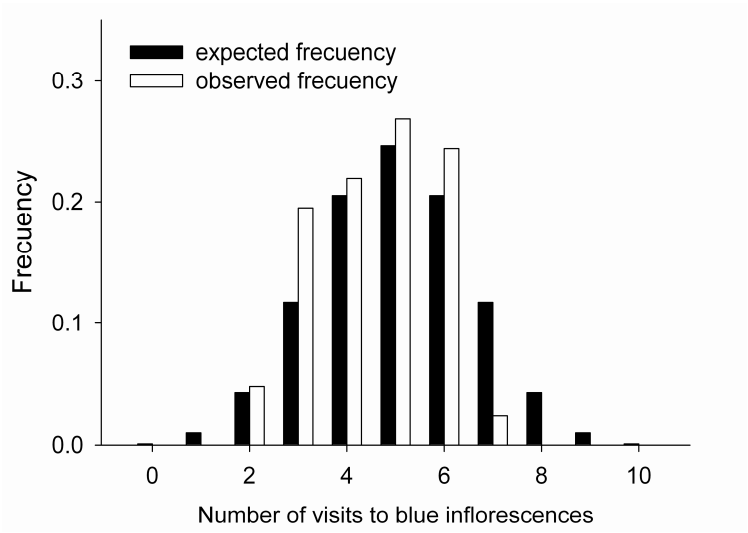

Figure 9. Effect of blue spots on honeybee behaviour (Experiment 3). *Histograms showing (A) the expected frequency of the number of honeybee visits to blue inflorescences when honeybees are equally like to visit control and blue inflorescences (black bars) and (B) observed frequency for the number of honeybee visits to blue inflorescences in Experiment 3 (white bars).*

Experiment 4: effect of spider movement on honeybee behaviour

Spider movement (deviance = 42.64, df = 1, P < 0.001) but not spider position (deviance = 0.95, df = 1, P = 0.32) affected the probability that a bee selected the spider-harbouring inflorescence for landing. Honeybees were more likely to avoid spider-harbouring inflorescences if spiders moved during their approach rather than remaining still, and the aversive effect of spider movement was more pronounced when spiders were below the inflorescence than when they waited above it (deviance for the interaction term = 5.25, df = 1, P = 0.02; Fig. 10).

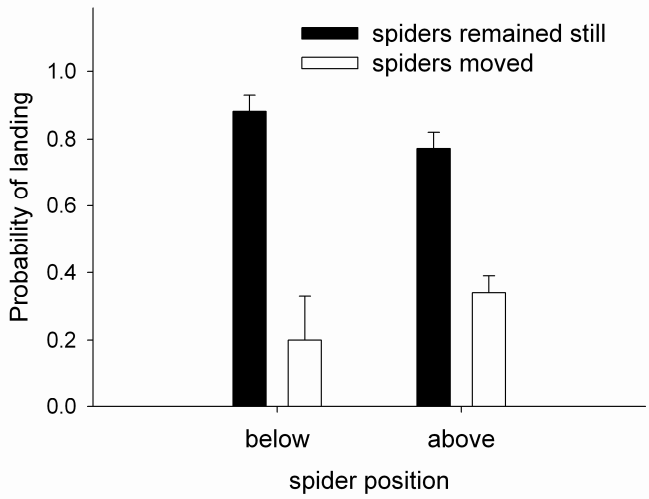

Figure 10. Effect of spider movement on honeybee behaviour (Experiment 4)*. Effect of spider movement and position on the probability (± SE) that honeybees landed on spider harbouring inflorescences.*

Discussion

The rate at which honeybees visited spider-harbouring inflorescences was not affected by the colour contrast between spiders and inflorescences, or the contrast between specific spider traits and the inflorescences. In Experiment 1 honeybees landed as often on inflorescences containing a white spider as on inflorescences containing a yellow spider. In Experiment 2 they did not discriminate between control spiders and spiders with their forelimbs or abdomen painted blue. Therefore, neither the high chromatic contrast which spiders generated against inflorescences in Experiment 1, nor the high chromatic and achromatic contrast that blue-painted spiders generated against inflorescences in Experiment 2 were sufficient to elicit a strong anti-predatory response in honeybees. These results, however, do not imply that honeybees did not respond to the presence of spiders on inflorescences: spider body size, UV reflectance and spider movement affected the rate at which honeybees visited spider inflorescences and, overall, there was a modest rejection of spider-harbouring inflorescences, which reached statistical significance in Experiment 2. According to our results, honeybees were more likely to land on spider-harbouring inflorescences when the spiders were large and had high UV reflectance or when spiders were small and reflected little UV, while other spider trait combinations elicited stronger avoidance responses. Likewise, honeybees were more likely to reject inflorescences if spiders moved as the bee approached the inflorescence than if spiders remained still.

Our results confirm a recent study which reported that colour matching between *Misumena vatia* and their flowers affected neither pollinator flower choice or spider hunting success (Brechbühl et al. 2010a). Although the authors of this study did not measure the reflectance properties of spiders and flowers, making it difficult to assess the extent to which spiders were conspicuous to pollinators (Brechbühl et al. 2010a), we have shown that their findings remain valid when we control for the visual systems of pollinators: neither the colour contrast nor the green contrast that spiders generated against inflorescences affected honeybee response towards risky flowers. Despite the fact that our and Brechbühl *et al.* (2010a) study showed that spider crypsis plays a minor role in predator detection for pollinators, most of the yellow spiders that we collected in the field were collected from yellow daisies (*Sphagneticola trilobata*) and most of the white spiders were collected from white daisies (*Bidens alba*), which suggests that background colour matching may play an important role in crab spiders.

One possible explanation of this finding is that crab spiders use background colour matching in response to their predators instead of their prey, but in the absence of data this possibility must be treated with caution.

Our study provides partial confirmation, under field conditions, of previous studies which suggested that Australian crab spiders exploit the plant-pollinator mutualism by creating a high UV contrast that makes flowers highly attractive for potential visitors (Heiling et al. 2003; Heiling et al. 2005a; Herberstein et al. 2009). While, in our experiments, honeybees were less deterred by large spiders with high UV reflectance than by large spiders with low UV reflectance, UV reflectance only mitigated the avoidance response, without transforming aversion into attraction. Previous work used anesthetized crab spiders and we used active spiders. Because honeybees were more likely to reject inflorescences if spiders moved as the bee approached the inflorescence than if spiders remained still, the fact that anesthetized spiders do not move may help explain the difference between our and previous results. Because previous studies had used relatively large (0.09 to 0.17 g, corresponding to 3.42-4.10 mm in prosoma width in our data set) (Heiling et al. 2003; Heiling et al. 2005a; Herberstein et al. 2009) and immobilized spiders, they had only detected the positive effect of UV reflectance on bee attraction (see Fig. 4). Our study shows that the UV prey "attraction" hypothesis holds for large but not for small spiders.

Because honeybees have shared an evolutionary history with crab spiders that reflect little UV (Herberstein et al. 2009), it is not entirely surprising that, in the absence of UV reflection, honeybees avoid large (and dangerous) spiders but disregard the presence of small (and relatively innocuous) spiders. Spiders with naturally low levels of UV reflection in Experiment 1, and blue-painted spiders in Experiment 2, generated negative UV contrast against their inflorescences, not unlike those recorded for European crab spiders (see UV contrast in *Synaema globosum*, *Misumena vatia*, *Xysticus* sp. and *Thomisus onustus* from Herberstein *et al.* (2009)). Alternatively, it is also possible that in the absence of UV reflectance honeybees avoided large but no small spiders simply because larger spiders were easier to detect. Interestingly, although honeybees could potentially behave flexibly in response to different degrees of predation threat, this behaviour only held when spiders were low UV-reflective. Higher UV-reflective spiders received, in contrast, more visits if they were large and dangerous

– supporting the idea that the European honeybees have not had the opportunity to evolve a response to the deceptive UV reflective Australian crab spider.

Heiling *et al.* (2005b) reported that honeybees were attracted to inflorescences containing a white *T. spectabilis* female and were slightly repelled by inflorescences containing yellow *T. spectabilis* females. The apparent discrepancy between their and our results disappears if we note that Heiling et al. used large and immobilized spiders for their experiments, and that the yellow females they used reflected little UV light while their white females were highly UV reflective (Heiling et al. 2005b). It is probably UV reflectance, and not colour (white/yellow) per se, that was responsible for the different behaviour of honeybees in their study.

If honeybees responded differently to spiders with low and high UV reflectance, it is important to point out that UV reflectance had no effect on spider hunting success. Large spiders were very successful at capturing bees and seemed to need little help of UV reflectance to capture their prey: 21 out of 39 spiders with a prosoma width larger than 3.50 mm successfully captured a honeybee within the first 15 minutes of observation. Few small spiders managed to capture bees, and given that honeybees avoided small, UV-reflecting spiders, it is difficult to imagine how UV reflectance might improve their hunting success. Thus, although UV reflectance could be beneficial in terms of hunting success when prey are scarce or when crab spiders prey on pollinators other than honeybees, our study provides little evidence that UV reflectance has evolved because UV reflecting spiders have higher intake rates.

An argument similar to the one sketched above suggests that colour matching does affect hunting success: in Experiment 1, yellow spiders were more likely to capture bees than white spiders (Fig. 5). However, we find it unlikely that the difference was due to the colour of the spiders. First of all, honeybees responded similarly to white and yellow spiders, possibly because colour contrast and green contrast were similar for both morphs. Rather, the difference may reside in the motivation of both spider groups. While running Experiment 1 we were impressed by the fact that white spiders appeared sluggish and less eager to capture bees than yellow spiders – although we realise that this is a subjective impression and difficult to quantify. Although the relationship between body mass and body size was similar for white and yellow spiders (data not shown), white and yellow spiders might be in different nutritional state: we collected

white spiders from *Bidens alba* inflorescences that were commonly visited by honeybees and, therefore, honeybees may have been the main prey of white spiders. In contrast, yellow spiders were collected from *Sphagneticola trilobata* inflorescences which were hardly visited by any bee at the time of collection. Indeed, white spiders were commonly collected while feeding on honeybees, whereas yellow spiders were collected with other prey items such as crickets. It is, hence, possible that yellow spiders were more motivated to catch honeybees than white spiders because honeybees were a more valuable reward for them.

There is a final caveat concerning the generality of our results. We found that colour matching did not affect the response of honeybees to spider inflorescences and that, before the spider struggled with a honeybee, the anti-predator response of honeybees was modest at best (Figs. 4 and 7). While these results confirm those of a recent study (Brechbühl et al. 2010a), it should be pointed out that a number of previous studies report strong anti-predator responses of honeybees (e.g. Dukas & Morse 2003; Reader et al. 2006). Why do honeybees avoid crab spiders in some contexts but not in others? Honeybees seem to rely on different cues to detect predators. We have seen that size and movement affected the probability that honeybees avoided crab spiders (Fig. 10) and that honeybees appeared to avoid a chemical cue emitted by the recently attacked bee. Other studies report different mechanisms (Dukas 2001; Reader et al. 2006; Higginson et al. 2007), and of particular relevance may be the role of learning (Dukas 2001; Ings & Chittka 2008), as it could help explain variability between ecological contexts.

In conclusion, the degree of matching between spiders and flowers (either chromatic or achromatic contrast) and the presence of any morphological trait of the spider painted blue did not influence honeybee behaviour when choosing a flower to visit, but honeybees slightly avoided spider inflorescence, and the probability of avoidance depended on spider size, spider UV reflection and spider movement. However, although spider movement helped pollinators to show anti-predator behaviour, honeybees were more likely to avoid larger (and riskier) spiders compared to smaller (and less risky) ones only when they were not UV-reflective or reflected very little amount of UV. In contrast, UV-reflective spiders attracted more prey as spider size increased. Moreover, we found that large spiders received more honeybee visits as they increased their UV reflection and the opposite occurred for small spiders. Our study, therefore, supports the

idea that Australian crab spiders deceive their preys by reflecting UV colouration only for large but not for small spiders, and highlights the importance of other cues that elicited an anti-predator response in honeybees. It is worth mentioning that, to date, studies investigating the effect of UV reflection in Australian crab spiders have found that UV reflection helps spiders to attract European honeybees to the flowers where they sit (Heiling et al. 2003; Heiling et al. 2005a; Herberstein et al. 2009), but it does not help spiders to attract Australian native bees (Heiling & Herberstein 2004; Llandres et al. 2010). Although, so far, it has been proposed that this result could be explained by the fact that in the co-evolution between crab spiders and bees, native bees have evolved an anti-predatory response towards UV reflective Australian crab spiders, an alternative plausible explanation is that the introduction of European honeybees to Australia (honeybees were introduced in Australia approximately 200 years ago (Hopkins 1886)) has released the selection of certain spider traits, like UV reflection, that are currently present in natural populations.

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Capítulo V

Social bees, *Apis mellifera*, but not solitary bees, *Nomia strigata*, used alarm signals to mark dangerous inflorescences

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Abstract

Social bees are known to avoid inflorescences marked with dead conspecifics or their smell. Although this result suggests that social bees mark with alarm signals dangerous flowers to warn conspecifics, the avoidance response could also be triggered by substances the release of which was not selected for its signalling value. To discriminate between these two options we note that the evolution of alarm signals is predicted in social bees, but not in solitary bees, while both social and solitary bees are expected to react to non-signalling cues associated with predation risk. We simulated dangerous inflorescences waiting for a bee to land on them and pinching it with forceps, and compared the rate at which bees visited these experimental inflorescences and unmanipulated control inflorescences. We conducted the experiment with the eusocial honeybee, *Apis mellifera*, and a solitary bee, *Nomia strigata*. Whereas *N. strigata* treated similarly control and experimental inflorescences, honeybees strongly rejected inflorescences where we had simulated a predation attempt. Our results show that only the social bee species marks dangerous inflorescences with alarm cues, suggesting that the release of the alarm cue has been selected for its signalling value.

Introduction

Predators constitute an ubiquitous selective pressure to which different species have responded evolving a wide variety of morphological and behavioural adaptations (Lima & Dill 1990; Barbosa & Castellanos 2005). The evolutionary response of a particular species to the threat of predation is determined by a diverse array of factors, including the biotic and abiotic characteristics of the environment, physical and phylogenetic constraints. In particular, there is a complex bidirectional relationship between anti-predator responses and life histories. On the one hand, several life history traits (e.g. early maturation, sociality) can evolve as anti-predator responses (Hamilton 1971; Werner & Gilliam 1984; Inman & Krebs 1987; Vulinec 1990). On the other hand, different life histories can favour different anti-predator strategies (Elgar 1989; Clark & Dukas 1994; le Roux et al. 2009). In this paper we study how a life-history trait, sociality, affects the evolution of a predator-avoidance strategy, release of and response to alarm cues.

Animals rely on cues to detect their predators and avoid fatal encounters (Lima & Dill 1990). Such cues can belong to different sensory domains, such as visual, auditory or chemical (Chivers & Smith 1998; Barbosa & Castellanos 2005). They can be produced by the predator itself, or by its prey (Kats & Dill 1998; Wyatt 2003; Barbosa & Castellanos 2005). Prey-produced alarm cues can, at least in principle, be divided in two groups, according to whether their release is merely a by-product of the predation attempt (i.e. body fluids escaped through skin injuries) or has been selected for its signalling value (Chivers & Smith 1998; Kats & Dill 1998; Wyatt 2003). The active release of alarm cues can most easily evolve in group-living animals (Seyfarth et al. 1980; Gyger et al. 1988; Arakaki 1989; Chapman et al. 1990; Aldrich et al. 1991; Evans et al. 1993; Hardie & Minks 1999; Wyatt 2003). According to Hamilton's rule (Hamilton 1964a; Hamilton 1964b), upon detection of a potential predator an individual will benefit from alerting group members if the cost to the sender, C, is smaller than the cumulative benefit to group members, B_i, discounted by the appropriate coefficients of relatedness, r_i:

$$C < \Sigma r_i \cdot B_i, \qquad (1)$$

where the sum in the right-hand side is conducted over all group members.

One of the main costs of producing alarm signals is that the sender risks increasing the probability of detection, attracting the attention of the incoming predator. Nevertheless, Equation 1 seems to be satisfied in many eusocial, subsocial and clonal insects, characterised by high values of *r* and *B*: victims are often surrounded by other individuals, which can benefit from the alarm cue (high *B*), and these individuals are often kin (high *r*). Indeed, many insects release, when attacked, chemical alarm cues that are used as warning signals and help conspecifics flee from danger (Arakaki 1989; Aldrich et al. 1991; Hardie & Minks 1999). For example, when caught by a predator the larvae of the subsocial lace bug *Gargaphia solani* emit an alarm pheromone that makes nearby nymphs stop feeding and run (Aldrich et al. 1991). On the other hand, Equation 1 is less likely to be satisfied in solitary insects. In these species, potential senders are unlikely to be surrounded by individuals that can benefit from receiving the signal (low *B*), and receivers, if they exist, need not be related to the sender (low *r*). We should therefore expect the evolution of alarm cues in social, but not in solitary insects (Wyatt 2003).

Social bees can communicate the presence of a dangerous predator in different contexts. When a predator is close to the hive honeybees and stingless bees release alarm pheromones that provoke other bees to engage in colony defence by attacking the intruder (Wittmann 1985; Roubik et al. 1987; Schmidt 1998; Breed et al. 2004; Schorkopf et al. 2009). Honeybees are less likely to perform the waggle dance and they engage in fewer waggle runs at the hive after visiting an artificial source of nectar harbouring a recently killed conespecific, than after visiting a similar source of nectar without dead bee (Abbott & Dukas 2009). And following an attack at a food source, honeybees can incorporate negative feedback signals in the waggle dance of their nest-mates to prevent the recruitment of new foragers to the sources where they have been attacked (Nieh 2010). At the inflorescence level, bees can respond to olfactory cues that may indicate the presence of a dangerous predator: bumblebees avoided inflorescences containing either a freshly killed bumblebee or its smell, suggesting that alarm pheromones released by recently killed bees might be an important olfactory cue that elicits avoidance response in their conspecifics (see also Dukas 2001; Reader et al. 2006 for honeybees; Abbott 2006). Likewise, honey bees, *Apis mellifera*, showed a strong avoidance response towards inflorescences where a crab spider, *Thomisus spectabilis*, had previously struggled with another honey bee (Llandres & Rodríguez-Gironés, chapter IV).

The evolution of chemical alarm cues at the inflorescence level should be most likely in bee species with developed recruitment systems. We predict that after being attacked at an inflorescence, highly eusocial species that communicate the location and distance to food sources, like some species of stingless bees and honeybees, will mark the inflorescence with alarm cues to warn conspecific foragers (who will be most likely colony members recruited to that location) of the potential danger. However, we should not expect solitary bees to mark dangerous inflorescences with alarm cues. Therefore, the aim of this study is to determine whether a social bee, *Apis mellifera*, and a solitary bee, *Nomia strigata*, release chemical alarm cues when attacked to assist conspecifics in the detection of potentially dangerous inflorescences. For this propose, we compared the number of *A. mellifera* and *N. strigata* bees visiting and rejecting control inflorescences and inflorescences where we simulated a predator attack by pinching a bee with forceps. We found that only the social species avoided inflorescences where a conspecific had been attacked.

Material and Methods

Study site and species

We conducted Experiment 1 in May and June 2009, with honeybees, *A. mellifera*, foraging at roadside patches of *Bidens alba* in the vicinity of Cannonvale (Queensland, Australia). We used six patches, distant at least one kilometre from each other, for the observations. We performed Experiment 2 in July 2010, with solitary bees, *N. strigata*, foraging at a population of *Melastoma malabathricum* in MacRitchie Reservoir Park, Singapore.

Experimental procedure

For each trial we selected and marked one *B. alba* or *M. malabathricum* inflorescence and assigned it the experimental or control treatment in pseudo-random order: treatment was allocated randomly to odd inflorescences, and even inflorescences were allocated to whatever treatment had not been used for the previous observation. For the experimental treatment, we waited until a bee landed on the selected inflorescence and carefully pinched it with forceps, holding the bee on the inflorescence from two to ten seconds. Upon marking the inflorescence (control group) or releasing the bee (experimental group) we started recording the number of bees approaching and visiting the focal inflorescence. We distinguished two bee responses: visits and rejections. We considered that a bee visited an inflorescence when it landed on it, and that the bee rejected the inflorescence when it approached it, hovered for a few seconds in

front of it (sometimes touching it with its forelegs) and then left without landing. Approaches were the sum of visits and rejections. In Experiment 1 most trials lasted 30 minutes (whit a mean±SD of 24±8.9 minutes), although a parallel experiment occasionally forced us to terminate trials sooner. In Experiment 2, because bees visited inflorescences at a higher rate, trials lasted only 10 minutes. We conducted 35 replicates in Experiment 1, and in Experiment 2 we performed 49 control and 48 experimental trials. All trials were conducted during sunny weather, at the peak time of bee activity.

Statistical Analyses

We compared the duration of observations for control and experimental inflorescences using a t test (Experiment 1).

We used generalized linear models with quasi-Poisson (Experiment 1, where we had significant overdispersion) or Poisson (Experiment 2) error distribution and log link function to determine whether bees approached control and experimental inflorescences at the same rate. The models included the number of bee approaches to inflorescences as dependent variable, and treatment (experimental vs. control) as the independent variable. In Experiment 1, we used the logarithm of trial duration as offset to control for variability in observation time.

Upon approach, bees could either visit or reject the inflorescence. To determine whether the probability that bees visited inflorescences after approaching them was the same for control and experimental treatments, we used generalized linear models with binomial error distribution and logit link function. In these analyses, the dependent variable was the set {number of visits, number of approaches} and the independent variable was treatment (experimental vs. control).

We used likelihood ratio tests to assess whether treatment had a statistically significant effect on bee response (Dobson & Barnett 2008). All results are reported as average ± 95% confidence interval.

Results

Experiment 1

There was no effect of treatment on the duration of observations ($P > 0.7$). The difference between the rates at which honeybees approached control (0.11 ± 0.03 inflorescences per minute) and experimental (0.12 ± 0.03 inflorescences per minute) *B. alba* inflorescences was not statistically significant (deviance = 0.40, df = 1, $P = 0.580$). Following an approach,

however, the probability that honeybees visited experimental inflorescences (0.01 ± 0.01) was much lower than the probability that they visited control inflorescences (0.94 ± 0.05). This difference was highly significant (deviance = 220.66, df = 1, P < 0.001). In summary, honeybees approached at the same rate control and experimental inflorescences, but following an approach they rejected inflorescences where we had simulated a predation attempt and visited control inflorescences (Figure 1).

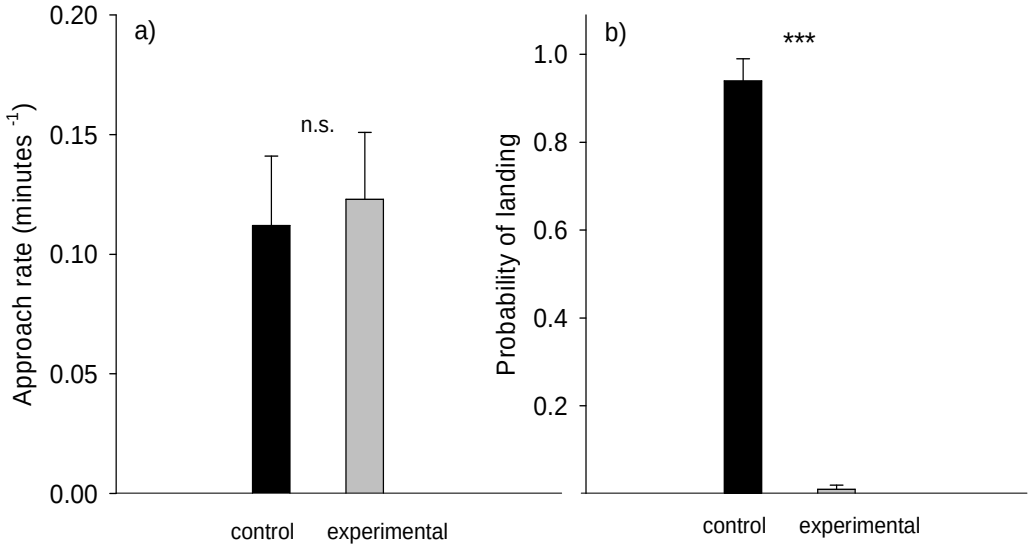

Fig. 1. a) Average approach rate and b) average probability of landing after an approach of Apis mellifera *bees on control (black bars) and experimental (grey bars)* Bidens alba *inflorescences. Error bars represent 95% confidence intervals. *** refers to P < 0.001,* ***n.s.*** *refers to non-significant comparisons.*

Experiment 2

Nomia strigata bees approached control *M. malabathricum* inflorescences at a rate of 0.290 (± 0.045) inflorescences per minute and experimental inflorescences at a rate of 0.320 (± 0.064) inflorescences per minute. The difference was not statistically significant (deviance = 0.77, df = 1, P = 0.380). Moreover, following an approach, the probability that *N. strigata* bees visited control inflorescences (0.75 ± 0.07) was similar to the probability that they visited experimental inflorescences (0.70 ± 0.07). This difference was not statistically significant (deviance = 1.00, df = 1, P = 0.314). Therefore, the rate at which *N. strigata* bees approached inflorescences and the probability that they landed on them were independent of the inflorescence treatment (Figure 2).

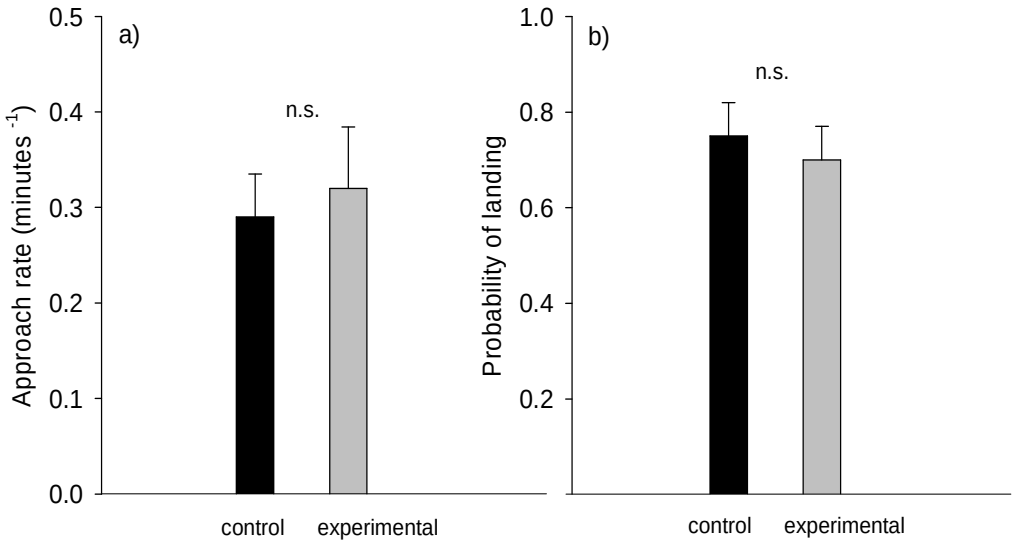

Fig. 2. a) Average approach rate and b) average probability of landing after an approach of Nomia strigata *bees on control (black bars) and experimental (grey bars)* Melastoma malabathricum *inflorescences. Error bars represent 95% confidence intervals. n.s. refers to non-significant comparisons.*

Discussion

Our results provide clear evidence that a social bee, *A. mellifera,* but not a solitary bee, *N. strigata,* released chemical alarm cues when attacked to warn conspecifics of the presence of danger at specific inflorescences, thus confirming our original hypothesis. Whereas the solitary bee *N. strigata* treated similarly control and experimental inflorescences (Figure 2), treatment had a strong effect on the response of the social bee *A. mellifera* to the inflorescences they approached: despite the fact that honeybees approached both inflorescence types at the same rate, following an approach there was a high probability that honeybees landed on control inflorescences, while they rejected most inflorescences where we had simulated a predation attempt (Figure 1). From a total of 105 honeybees that approached experimental inflorescences, 104 flew away and only one landed on the inflorescence.

A number of previous studies have shown that social bees avoid cues associated with dead conspecifics. For example, bumblebees avoided inflorescences containing either a freshly killed bumblebee or its smell (Abbott 2006) and inflorescences treated with extract of conspecific body parts (Stout et al. 1998). Furthermore, Dukas (2001) offered 20 *A. mellifera* honeybees a choice between an artificial nectar source with a dead bee (killed by pressing it gently inside a test tube) and a control feeder, and found that 19 of the 20 bees sampled chose

to land on the control feeder. These experiments are consistent with the hypothesis that social bees, upon encountering a predator, emit an alarm signal to warn conspecifics. However, because experimental inflorescences were marked with dead, and often crushed bees, conspecifics might be reacting to the presence of substances the release of which has not been selected for its signalling value. Indeed, there are several species of animals, such as cockroaches, isopoda, caterpillars and springtails among others, that use fatty acids released from body fluids of conspecifics to recognize and avoid their predators (Rollo et al. 1994; Nilsson & Bengtsson 2004; Yao et al. 2009). Moreover, some animals when injured by predators passively release fluids that induce an alarm response in neighbouring individuals of the same and other species. For example, fishes, sea urchins, sea snails, crustaceans and other aquatic animals respond alarmed to chemicals released passively from injured conspecifics (Snyder & Snyder 1970; Smith 1992; Jacobsen & Stabell 2004; Fleming et al. 2007; Shabani et al. 2008). Crayfish, *Orconectes virilis*, responded similarly to cues from injured conspecifics, sympatric heterospecifics and novel heterospecifics (Pecor *et al.* 2010). Likewise, exposure to cues released by damaged individuals of their own or other species triggered a predator-avoidance response in the freshwater gastropod *Lymnaea stagnalis*, a response that was strongest to cues from sympatric species (Dalesman *et al.* 2007).

In our experiments, bees were neither killed nor injured. (All bees left the area flying as soon as we released them.) Honeybees could therefore not be responding to the presence of a substance that had escaped the body of the attacked bees through punctures of their exoskeleton. Rather, honeybees must have responded to a substance released by attacked bees. The release of this substance may be a simple by-product of stress metabolism, or it may have been selected for its signalling value. If, as a by-product of their metabolism, bees released some volatile when attacked, both solitary and social bees would be selected to avoid inflorescences marked with such substance. If anything, we would expect solitary bees to respond more strongly than social bees to the presence of cues associated with danger: essentially, this is because the loss of a bee has a relatively minor impact on the reproductive output of a honeybee colony, but a very strong impact on the reproductive output of a solitary bee (Clark & Dukas 1994, Rodríguez-Gironés & Bosch, unpublished manuscript). Indeed, at our study sites honeybees ignored the presence of ambush predators when selecting inflorescences for landing (Llandres & Rodríguez-Gironés, chapter IV), while *N. strigata* strongly avoided inflorescences with ambush predators (unpublished results). It follows that

the release of the substance to which honeybees responded must have been selected for its signalling value.

Alternatively, honeybees could be responding to "footprints" – scent marks deposited by the attacked bee during its normal foraging activity. It is known that honeybees, bumblebees and some species of solitary bees avoid visiting inflorescences that have been recently depleted by a bee of the same or a different species (Giurfa & Nunez 1992; Giurfa 1993; Stout et al. 1998; Gawleta et al. 2005; Wilms & Eltz 2008). When simulating the predation attempt we held bees on the inflorescences during two to 10 seconds, so that experimental inflorescences might have contained stronger "footprints" than control inflorescences. However, data from another experiment showed that honeybees foraging on *B. alba* spent from 0.4 to 20.75 seconds per inflorescence, with an average (± SD) of 6.70 (± 5.83) seconds (unpublished data). Moreover, we never observed honeybees rejecting inflorescences following visits lasting 10 second or more. Thus, we can safely conclude that honeybees were not rejecting experimental inflorescences because attacked bees had left unusually strong scent marks on them.

Finally, although we should not expect solitary bees to release chemical alarm cues that indicate the presence of a dangerous predator at inflorescences, they would certainly benefit from responding to alarm cues emitted by other naturally sympatric species. Interspecific recognition of alarm cues among animals through "eavesdropping" allows potential prey to gain information about nearby predators, and has been reported in other taxa (Dalesman et al. 2007; Morales et al. 2008; Ito & Mori 2010). In the case of bees, we are only aware of one study which reported the behaviour of a solitary bee, *Eucera notata*, and *A. mellifera* to the presence of a dead honeybee (Reader *et al.* 2006). Surprisingly, while honeybees avoided inflorescences containing dead conspecifics, *E. notata* bees showed indifference towards the dead honeybees (Reader *et al.* 2006). Although, in principle, we should expect *E. notata* to avoid dead honeybees, the corpses spent several hours in the freezer before they were used in the experiment, and it is therefore likely that they emitted no smell when presented to *E. notata* bees. The avoidance response of honeybees can be attributed to the avoidance of competition instead of the avoidance of a predator. Indeed, Somers (2004) interpreted avoidance of a freeze-killed bee as evidence of competition avoidance and Abbott (2006) showed that when bumblebees were given a choice between an inflorescence with a crush-killed bee and an inflorescence with a freeze-killed bee, they where less likely to land on the inflorescence with

the crush-killed bee than on the inflorescence with the freeze-killed bee. This result also indicates that a freeze-killed bee was not considered as a dangerous stimulus by the bees.

In conclusion, our study provides compelling evidence that *A. mellifera*, but not *N. strigata*, use alarm signals to mark dangerous inflorescences where they have been attacked. A comparative study, including several solitary, primitively social and eusocial bee species, must be used to determine the generality of our findings, and the extent to which sociality is associated with the use of alarm signals to mark dangerous inflorescences.

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DISCUSIÓN INTEGRADORA

RESULTADOS Y DISCUSIÓN

Volviendo al marco teórico que desarrollamos en la introducción de la presente tesis doctoral, los resultados de esta tesis nos han permitido esencialmente profundizar en el conocimiento de la **función** y las **causas** del comportamiento antidepredatorio de los polinizadores y de las estrategias de caza de las arañas cangrejo, entendiendo cómo contribuyen estos comportamientos al éxito reproductivo de cada especie y estudiando los mecanismos que están detrás de los mismos. Además, los resultados de la presente tesis dejan abiertas varias preguntas que nos permitirían conocer, al menos en parte, el papel del **desarrollo** y la **evolución** en el comportamiento antidepredatorio de los polinizadores.

En general, los resultados de esta tesis demuestran que las especies de polinizadores estudiados, abejas de distintas especies y sírfidos, muestran una respuesta antidepredatoria bastante buena frente a flores con arañas cangrejo. Los capítulos I y III de esta tesis demuestran que los sírfidos, *Eristalis tenax*, y las abejas sociales, *Apis mellifera* y *Trigona carbonaria*, evitan visitar las flores que presentan arañas cangrejo. Sin embargo, en el caso de *Apis mellifera*, aunque los resultados del capítulo I, muestran que las abejas de la miel pueden presentar un comportamiento antidepredatorio muy bueno frente a las arañas cangrejo, tanto a nivel de flor como a nivel de parche (evitando visitar flores y, en general, parches enteros con arañas), en el experimento presentado en el capítulo IV la respuesta antidepredatoria de las abejas de la miel a nivel de flor fue, en el mejor de los casos, bastante modesta, ya que ni el gran contraste cromático que presentaban las arañas blancas y amarillas en flores de *Bidens alba* ni el gran contraste cromático y acromático que presentaban las arañas pintadas de azul fueron suficientes para que las abejas presentaran una respuesta antidepredatoria eficiente.

La misma dualidad se ha encontrado en otros trabajos. Algunos de estos trabajos encuentran que las abejas de la miel presentan una clara respuesta antidepredatoria frente a flores y parches enteros con arañas cangrejo (Dukas 2001; Dukas & Morse 2003; Dukas & Morse 2005; Reader et al. 2006) y, sin embargo, otros estudios encuentran que *A. mellifera* muestra un comportamiento antidepredatorio bastante

modesto frente a flores con araña (Brechbühl et al. 2010a; Brechbühl et al. 2010b) ¿Por qué las abejas de la miel evitan las arañas cangrejo en unos contextos pero no en otros? Las abejas utilizan distintas fuentes de información para evitar depredadores. De acuerdo a los resultados de esta tesis el tamaño de la araña (capítulo IV), su color (capítulo IV), el movimiento de la araña (capítulo IV) y el olor a señal de alarma (capítulo V) pueden afectar a la estrategia antidepredatoria de las abejas y podrían estar explicando la diferencia entre los distintos resultados. Además de estos mecanismos, nosotros creemos que el efecto del **desarrollo** del comportamiento antidepredatorio a través del aprendizaje individual en estas abejas podría explicar la diferencia en los resultados en distintos contextos. Si bien estudios de laboratorio han demostrado que las abejas y abejorros son capaces de aprender a evitar a sus depredadores (Dukas 2001; Ings & Chittka 2008; Ings & Chittka 2009), aún queda por demostrar cómo de frecuente es este fenómeno en la naturaleza. Por lo tanto, sería necesario realizar más estudios en este campo para confirmar que el papel del aprendizaje puede explicar las inconsistencias existentes entre los distintos estudios.

Los resultados de la presente tesis desvelan algunos de los mecanismos que utilizan las abejas para detectar flores potencialmente peligrosas. Los resultados del capítulo IV demuestran que las abejas utilizan **mecanismos visuales** para detectar a sus depredadores: el movimiento de las arañas, su tamaño y su reflectancia UV determinaron la tasa a la que las abejas de la miel visitaron las flores con araña. De acuerdo con estos resultados la probabilidad de que una abeja se posara en flores con araña fue mayor cuando las arañas eran más grandes y reflejaban más UV o cuando eran pequeñas y reflejaban poco UV y sin embargo esa probabilidad disminuía cuando las arañas eran grandes sin UV o pequeñas con UV. Además, la probabilidad de que las abejas rechazaran flores con araña fue mayor cuando las arañas se movieron mientras las abejas se aproximaban a la flor que cuando las arañas permanecieron quietas.

Además de los mecanismos visuales, algunos estudios sugieren que los **mecanismos olfativos** podrían ser relevantes a la hora de determinar el comportamiento de los polinizadores frente a flores peligrosas. Heiling & Herberstein et al. (2004) encontraron que las abejas australianas de la especie *Austroplebeia australis* evitaron flores con arañas cangrejo australianas de la especie *Thomisus spectabilis* en presencia de señales olfativas y sin embargo se posaron con la misma frecuencia en flores con y sin araña en

ausencia de estas señales olfativas. Reader et al. (2006) encontraron que las abejas de la miel, *A. mellifera*, respondieron a factores olfativos que indicaban la presencia reciente de una araña cangrejo evitando aquellas flores en las que habían colocado una araña cangrejo inmediatamente antes de ofrecérsela a las abejas. Asimismo, un estudio reciente demuestra que las abejas solitarias de la especie *Nomia strigata* examinan más aquellas flores con olor a su depredador, la hormiga tejedera *Oecophylla smaragdina*, que flores control o flores con olor a hormigas no depredadoras, *Polyrachis sp.* (Gonzálvez & Rodríguez-Gironés, datos no publicados). Por lo tanto, estos estudios sugieren que, además de los mecanismos visuales, los mecanismos olfativos podrían estar jugando un papel determinante en las estrategias antidepredatorias de distintas especies de polinizadores.

Los mecanismos olfativos, además, pueden jugar un papel importante en la comunicación entre los polinizadores. A nivel de flor algunos estudios han sugerido que las abejas usan señales químicas de alarma para avisar a sus hermanas de la colmena sobre la presencia de un depredador (Dukas 2001; Reader et al. 2006; Abbott 2006). Sin embargo estos estudios no han conseguido demostrar que las abejas responden a una señal de alarma, ya que usan como estímulo abejas muertas o su olor. Los resultados del capítulo V de esta tesis revelan que el olor a señal de alarma es un factor determinante de la estrategia antidepredatoria de las abejas de la miel. Los resultados de este capítulo muestran que aunque la abeja solitaria, *Nomia strigata*, respondió de manera similar ante las flores experimentales (donde simulamos un ataque) y las flores control, la abeja social, *A. mellifera*, respondió de manera muy diferente: a pesar de que las abejas se aproximaron a ambas flores a la misma tasa, tras la aproximación la probabilidad de que las abejas se posaran en la flor era mucho mayor para flores control y la probabilidad de que las abejas rechazaran la flor era mucho mayor si la flor era experimental. Estos resultados demuestran que las abejas sociales marcan las flores donde han sufrido un ataque con señales químicas de alarma y esto les permite comunicarse sobre el peligro de forrajear en esa flor. Asimismo, un estudio reciente ha demostrado que después de sufrir un ataque en una determinada fuente de alimento, las abejas de la miel son capaces de comunicarse el peligro incorporando un feedback negativo en la danza de sus hermanas de la colmena que previene el reclutamiento de nuevos individuos a la fuente de alimento donde las abejas han sido previamente atacadas (Nieh 2010). Estos

resultados, por lo tanto, demuestran la importancia del papel de la comunicación en las estrategias antidepredatorias de las abejas.

De acuerdo a los resultados del capítulo I de esta tesis los sírfidos, *Eristalis tenax*, y las abejas, *Apis mellifera*, respondieron ante variaciones en la cantidad de recurso y el riesgo de depredación de manera completamente diferente a nivel de parche. Los polinizadores más susceptibles, *A. mellifera*, evitaron los parches peligrosos, especialmente si tenían pocos recursos, mientras que los polinizadores menos susceptibles, *E. tenax*, visitaron más frecuentemente los parches pobres y peligrosos. Sin embargo, a nivel de flor ambas especies de polinizadores, abejas y sírfidos, presentaron una respuesta similar, ya que ambas especies evitaron flores con arañas independientemente de que los parches tuvieran o no néctar añadido y se comportaron de manera similar después de un encuentro no letal con una araña cangrejo en los parches peligrosos. Las abejas de la miel presentan un buen sistema de comunicación que las permite reclutar nuevos individuos de la colmena a una determinada fuente de alimento (von Frisch 1967) y también, como hemos mencionado anteriormente, son capaces de comunicarse el peligro de forrajear en una fuente de alimento donde han sido previamente atacadas (Nieh 2010), disminuyendo así el reclutamiento de nuevos individuos de la colmena a la localización peligrosa. Además, las abejas son capaces de aprender a evitar flores y áreas dónde han sido previamente atacadas (Abramson 1986; Dukas 2001). Por lo tanto, el reclutamiento y el aprendizaje podrían haber jugado un papel determinante a la hora de hacer que las abejas incluyan los parches ricos y seguros en sus áreas de forrajeo pero no los parches pobres y peligrosos. Esto podría explicar por qué, además de presentar una estrategia antidepredatoria bastante buena a nivel de flor, las abejas presentan una estrategia antidepredatoria eficiente a nivel de parche que los sírfidos no son capaces de desarrollar. Desde el punto de vista **funcional,** tanto la evitación del depredador como la eficiencia de forrajeo podrían estar determinando el patrón de forrajeo observado de abejas y sírfidos a nivel de parche. Las abejas podrían estar evitando los parches peligrosos debido a su mayor susceptibilidad de depredación. Existen varios estudios que han reportado que mientras algunas especies de polinizadores presentan una respuesta de evitación bastante fuerte frente a la presencia de arañas cangrejo, otras especies responden de manera mucho más débil o incluso ignoran a los depredadores (Dukas & Morse 2003; Gonçalves-Souza et al. 2008; Brechbühl et al. 2010a). Por otro lado, Grand & Dill (1999) estudiaron el uso óptimo

del hábitat cuando dos especies compiten por recursos en dos tipos de hábitats: uno rico y peligroso y otro pobre y seguro. De acuerdo con este modelo la especie que presente mayor mortalidad tenderá a forrajear en el hábitat seguro y pobre (Grand & Dill 1999). Nuestros resultados apoyan la predicción de este estudio cuando comparamos el número de abejas y sírfidos en parches ricos y seguros y en parches pobres y peligrosos. Estos resultados son relevantes ya que, en general, no son sólo las flores con arañas las que recibirán menos visitas de abejas de la miel: la presencia de depredadores también puede afectar a las flores vecinas. Dado que, en muchos ambientes, el éxito reproductivo de las plantas aumenta con el número de visitas de polinizadores que reciben (e.g. Herrera 1987; Fishbein & Venable 1996) es importante tener en cuenta la escala espacial a la que los depredadores afectan el comportamiento de los polinizadores: ésta podría determinar el efecto de los depredadores en las interacciones planta-polinizador. En la medida en que el fenotipo de una planta afecta la probabilidad de que albergue depredadores, el éxito reproductivo de una planta no sólo dependerá de sus rasgos fenotípicos, sino que también dependerá de los rasgos de sus plantas vecinas.

Por otro lado, otro de los resultados relevantes del capítulo I desde el punto de vista de la función del comportamiento antidepredatorio de los polinizadores, demuestra que, a igualdad de riesgo impuesto por las arañas, las abejas prefieren los parches con más recursos (la misma tendencia aparece también para los sírfidos si nos centramos en el número de flores visitadas en el parche por cada sírfido). Esto implica que los polinizadores están dispuestos a afrontar mayores niveles de depredación cuando explotan parches ricos en recursos. En relación con este resultado, un experimento de laboratorio realizado con abejorros de la especie *Bombus occidentalis*, demostró que a medida que las reservas energéticas de la colmena disminuían, los abejorros aceptaban con mayor probabilidad enfrentarse a un determinado riesgo de depredación cuando forrajeaban (Cartar 1991). Por lo tanto la evaluación de la riqueza de un determinado parche dependerá del estado energético de la colmena y de los recursos ofrecidos por el ambiente. Si, por ejemplo, el ambiente ofrece muy pocos recursos las abejas podrían enfrentarse a mayores niveles de depredación que si el ambiente ofrece muchos recursos y esto también podría ser una explicación potencial de por qué las abejas de la miel utilizadas para el experimento del capítulo IV no mostraron una estrategia antidepredatoria eficiente frente a flores con araña, ya que el experimento fue realizado

en el comienzo de la época seca, cuando los recursos de las abejas empiezan a ser más escasos en la zona de estudio.

Respecto a la **función** de las estrategias de caza de las arañas cangrejo, los resultados del capítulo IV de la presente tesis no apoyan la hipótesis de la cripsis en arañas cangrejo: en nuestros experimentos las abejas *A. mellifera* se posaron con la misma frecuencia tanto en flores de *Bidens alba* que contenían una araña blanca como en flores que contenían una araña amarilla, y tampoco discriminaron entre las arañas a las que les pintamos de azul el abdomen o las patas delanteras y las arañas control. Además, la frecuencia de visitas a flores control fue muy similar a la de las flores con araña. Estos resultados sugieren que el contraste de color que presentan las arañas cangrejo no juega un papel importante en el camuflaje frente a sus presas, idea que prevalece desde hace casi un siglo (Gabritschevsky 1927). Aunque la falta de respuesta a las arañas en nuestro experimento pudiera achacarse a factores como una baja disponibilidad de recursos en el ambiente, no debemos olvidar que Brechbühl et al. (2010a) obtuvieron los mismos resultados, y que ningún experimento hasta la fecha muestra que las arañas aumenten su éxito de caza al adoptar el color de las flores en que se albergan. Además, los resultados de los capítulos II y IV confirman resultados de estudios previos, que sugieren que las arañas cangrejo australianas explotan el mutualismo planta-polinizador creando un alto contraste ultravioleta que atrae a sus presas (Herberstein et al. 2009). Según los resultados del capítulo II encontramos variación temporal y entre individuos en el color reflejado por las arañas cangrejo de la especie *Thomisus spectabilis* recolectadas en el año 2008 y 2009, sobre todo en la región del espectro del UV (de 300 a 400 nm). De media, las arañas recolectadas en el 2008 reflejaron más UV que las recolectadas en el 2009. Además, los resultados de la relación entre coloración y condición demostraron que en el 2008 hubo una relación positiva entre condición y reflectancia UV, lo que no sucedió para las arañas recolectadas en el año 2009. Por otro lado, en el laboratorio la dieta afectó la condición, pero no la cantidad de UV que reflejaba *T. spectabilis*. Estos resultados, junto con otros experimentos (Herberstein et al. 2009), sugieren que al presentar mayor contraste de color mediado a través de la reflectancia en el UV, las arañas cangrejo australianas que reflejan gran cantidad de UV obtienen una ventaja a la hora de capturar a sus presas y están en mejor condición que las arañas que no reflejan UV.

Resultados del capítulo IV demuestran que la probabilidad de que una abeja se posara en flores con araña fue mayor cuando las arañas eran grandes y reflejaban mucho UV o cuando eran pequeñas y reflejaban poco UV que cuando las arañas eran grandes y no reflejaban UV o cuando eran pequeñas y reflejaban mucho UV. Estos resultados confirman parcialmente, en condiciones de campo, que aquellas arañas que reflejan más UV son capaces de atraer más abejas de la miel que las arañas que no lo reflejan, confirmando también la idea de que las arañas cangrejo australianas explotan el mutualismo planta-polinizador reflejando UV para atraer a sus presas, pero sólo para arañas grandes. Sin embargo nosotros creemos que hay que ser cautelosos a la hora de interpretar estos resultados. Es importante puntualizar que aunque las abejas de la miel respondieron de manera diferente ante arañas con distinto grado de reflectancia UV (capítulo IV), el UV no influyó en el éxito de captura de las arañas. Las arañas grandes tuvieron un gran éxito de caza independientemente del UV que reflejaron. De las arañas pequeñas, sin embargo, pocas consiguieron cazar una abeja, y dado que las abejas evitaban aquellas arañas pequeñas cuanto más ultravioleta tuvieran, es difícil imaginar cómo el UV podría mejorar su éxito de captura.

Además, resultados de los capítulo II y III de esta tesis demuestran que las arañas cangrejo australianas de la especie *Thomisus spectabilis* y *Diaea evanida* presentan una gran variabilidad en la cantidad de UV que reflejan en el campo. ¿Por qué si el reflejar UV es beneficioso hay arañas que no lo reflejan? De acuerdo a los resultados del capítulo III, las abejas nativas australianas de la especie *T. carbonaria* no mostraron ninguna preferencia por ningún contraste de color de las arañas cangrejo de la especie *D. evanida* y las abejas nativas de la especie *A. australis* mostraron mayor preferencia por arañas menos contrastantes de la especie *T. spectabilis*. Por lo tanto, la cantidad de UV que las arañas reflejen en el campo podría ser explicada por la disponibilidad de presas de distintas especies en un momento y lugar determinados. Si la mayoría de presas disponibles para las arañas son abejas de la especie *A. australis* parece razonable que las arañas adopten una estrategia de reflejar poco UV. Sin embargo, quedan por determinar los mecanismos que podrían usar las arañas para ajustar la reflectancia de UV a la disponibilidad de presas. Alternativamente, además de las presas, los depredadores también podrían estar influyendo en el color que presentan las arañas cangrejo en el campo (Heiling et al. 2005). Si el reflejar UV implica a su vez mayor riesgo de depredación [algunos depredadores potenciales de las arañas cangrejo, como

son las avispas y también los pájaros, son capaces de ver el UV (Peitsch et al. 1992; Maier 1992)], las arañas que reflejan menos UV podrían estar invirtiendo en una estrategia de ser menos conspicuas en respuesta a presiones selectivas impuestas por sus depredadores. Por lo tanto aquellas arañas con más UV podrían sufrir mayor riesgo de depredación que las arañas que reflejen menos ultravioleta y esto, junto con la presión selectivas impuestas por sus presas, podría explicar por qué existe variación en la coloración UV de estas arañas en el campo.

Con respecto a los **mecanismos** de las estrategias de caza de las arañas cangrejo, los resultados del capítulo II de esta tesis no nos permiten determinar los mecanismos a través de los cuales las arañas cangrejo australianas con capaces de reflejar UV y cambiar el color de su cuerpo. A lo largo del experimento de laboratorio realizado en el capítulo II de esta tesis las arañas incrementaron la cantidad de UV reflejado independientemente de los tratamientos de régimen de comida y del color del fondo al que fueron sometidas. Con estos resultados podemos descartar que el color de fondo y la cantidad de comida sean los factores responsables de la reflectancia UV en arañas cangrejo australianas. Al final del experimento las arañas presentaron distinta condición y el mismo UV, por lo que los mecanismos por los que las arañas reflejan UV siguen siendo a día de hoy desconocidos. Descartamos, al menos, la posibilidad de que la condición en que se encuentra una araña sea directamente responsable de su coloración.

Aunque la presente tesis se ha enfocado esencialmente en aspectos de función/mecanismos de las estrategias de caza de las arañas cangrejo y de las estrategias antidepredatorias de los polinizadores, los resultados desvelan posibles preguntas que nos permitirían conocer el papel del desarrollo y la evolución en el comportamiento antidepredatorio de los polinizadores. La importancia del desarrollo del comportamiento antidepredatorio de los polinizadores a través del aprendizaje individual ya ha sido considerada al principio de esta discusión. Respecto a la **evolución** del comportamiento antidepredatorio de los polinizadores, los resultados del capítulo V sugieren que un rasgo de las historias de vida de las abejas, la sociabilidad, podría estar asociado a la evolución del uso de señales de alarma para marcar flores peligrosas en las abejas. Estos resultados demuestran que las abejas sociales, *A. mellifera*, pero no las solitarias, *N. strigata*, usan señales químicas de alarma para marcar flores peligrosas donde han sido previamente atacadas. Sin embargo, para conocer la evolución de este comportamiento

antidepredatorio a nivel de flor mediado por las señales de alarma en abejas y para demostrar la generalidad de estos resultados, estudiando el grado en el que la sociabilidad está asociada al uso de señales de alarma a nivel de flor en abejas, sería necesario realizar un estudio comparativo incluyendo varias especies de abejas solitarias, de abejas sociales primitivas y de abejas eusociales.

Por último, aunque, como ya se ha mencionado, la presente tesis se ha centrado en el estudio de las interacciones entre las arañas cangrejo y los polinizadores, dada la estrecha relación entre los polinizadores y las plantas, cualquier efecto en el comportamiento de los polinizadores podrá afectar, a través del mutualismo planta-polinizador, a las plantas que sean polinizadas por los visitantes florales. Los resultados de esta tesis revelan posibles efectos resultantes de la interacción araña cangrejo-polinizador en las plantas que albergan los depredadores asociados a flores. Uno de los resultados relevantes del capítulo I de esta tesis demuestran que aunque la tasa de visitas de las abejas (numero de visitas por inflorescencia por unidad de tiempo) en los parches seguros fue más del doble que en los parches peligrosos con la misma disponibilidad de néctar, la atracción del néctar añadido hizo que las inflorescencias en los parches peligrosos y ricos recibieran tantas visitas como en los parches seguros y pobres. Este resultado sugiere que a nivel evolutivo, los depredadores asociados a flores podrían ejercer presiones selectivas en las plantas que son colonizadas de manera recurrente por los mismos. Un mecanismo por el que las flores de especies que están regularmente asociadas con arañas cangrejo podrían atraer más polinizadores (compensando por la pérdida de visitas de polinizadores que supone presentar depredadores asociados a flores) sería incrementando la cantidad de néctar que producen. Si, como estos resultados demuestran, los polinizadores se exponen a mayores niveles de depredación cuando explotan parches ricos en néctar, cabría esperar que aquellas especies de plantas que sean colonizadas de manera recurrente por arañas cangrejo, y que, por tanto, sufran menor éxito reproductivo, sufrirán una presión selectiva para incrementar la cantidad de néctar que producen y así compensar la pérdida de visitas de sus polinizadores.

Los resultados del capítulo I sugieren además que los depredadores asociados a flores, a través de su efecto en el comportamiento de los polinizadores, podrían potencialmente afectar la estructura de las redes de polinización y el éxito reproductivo de las plantas (Dukas & Morse 2003; Suttle 2003; Dukas 2005; Robertson & Maguire 2005). Las

redes de polinización son un subconjunto de redes tróficas más amplias y deberían, por lo tanto, estar sujetas a las mismas constricciones a las que están sujetas las redes tróficas. La estructura de las redes tróficas está parcialmente determinada por el comportamiento de forrajeo y el comportamiento antidepredatorio de los animales que componen esa red (Schmitz et al. 2008). Por lo tanto sería esencial considerar las estrategias adaptativas de forrajeo de los polinizadores para entender la prevalencia y la fuerza de los efectos de las interacciones de unas especies sobre otras en las redes de polinización. Apoyando esta idea un estudio reciente ha demostrado que la presencia de la hormiga tejedora, *Oecophylla smaragdina*, en las flores de *Melastoma malabathricum* determina la tasa de visitas de dos especies de polinizadores: el polinizador más eficiente, *Xylocopa latipes*, es el menos vulnerable a ser depredado por las hormigas y visita mayoritariamente flores con hormiga. Sin embargo, el polinizador menos eficiente, *Nomia strigata*, es más vulnerable a ser depredador por las hormigas y evita posarse en flores que contienen hormigas (Gonzálvez et al., resultados no publicados). Estos resultados junto con resultados de esta tesis sugieren, por tanto, que los depredadores asociados a flores podrían jugar un papel importante a la hora de determinar la estructura de las redes de polinización – posibilidad que deberá ser estudiada con más detalle.

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CONCLUSIONES

1. Los sírfidos, *Eristalis tenax*, y las abejas, *Apis mellifera*, respondieron ante variaciones en la cantidad de recurso y el riesgo de depredación de manera completamente diferente a nivel de parche. Los polinizadores más susceptibles a la depredación, las abejas, evitaron los parches peligrosos especialmente si tenían pocos recursos, mientras que los polinizadores menos susceptibles, los sírfidos, visitaron más frecuentemente los parches pobres y peligrosos.

2. A nivel de flor, sin embargo, sírfidos y abejas presentaron una respuesta similar: ambas especies evitaron flores con araña, no respondieron ante variaciones en la cantidad de néctar en el parche y se comportaron de manera similar después de un encuentro no letal con una araña cangrejo.

3. La tasa de visitas de las abejas en los parches seguros fue más del doble que en los parches peligrosos que ofrecían la misma cantidad de recursos. Sin embargo, como consecuencia de la preferencia de las abejas por los parches con más néctar, las inflorescencias recibieron tantas visitas de abejas en los parches peligrosos y ricos como en los parches seguros y pobres. Estos resultados sugieren que a nivel evolutivo, un mecanismo por el que las flores regularmente asociadas con arañas cangrejo podrían atraer más polinizadores sería incrementando la cantidad de néctar que producen.

4. De media, las arañas de la especie *Thomisus spectabilis* recolectadas en el 2008 reflejaron más UV que las arañas recolectadas en el año 2009. Además, en el 2008 hubo una relación positiva entre condición y reflectancia UV, lo que no sucedió para las arañas recolectadas en el año 2009. Por otro lado, en el laboratorio la dieta afecta la condición, pero no la cantidad de UV que refleja *Thomisus spectabilis*. Estos resultados sugieren que al presentar mayor reflectancia en el UV, las arañas cangrejo australianas presentan una ventaja a la hora de capturar a sus presas y están en mejor condición que las arañas que no reflejan UV.

5. Las dos especies de abejas nativas australianas se comportaron de manera distinta frente a variaciones en la coloración de las arañas cangrejo utilizadas para los dos experimentos: las abejas *Trigona carbonaria* no mostraron ninguna preferencia ante variaciones en la coloración de arañas de *Diaea evanida*. Sin embargo, las abejas *Austroplebeia australis* mostraron mayor preferencia por arañas menos contrastantes de *Thomisus spectabilis*. Estos resultados, junto con resultados de la cantidad de UV reflejada en el campo por las arañas cangrejo australianas de ambas especies, sugieren que la cantidad de UV que las arañas reflejen en el campo podría ser explicada por la disponibilidad de presas.

6. Las abejas de la miel no respondieron al contraste de color (ni cromático ni acromático) entre arañas e inflorescencias. Sin embargo el movimiento de las arañas, su tamaño y su reflectancia UV determinaron la tasa a la que las abejas visitaron las flores con araña. Estos resultados sugieren que sólo las arañas cangrejo australianas que son grandes "engañan" a sus presas reflejando UV, y resaltan la importancia de otras señales que provocan una respuesta antidepredatoria en las abejas de la miel.

7. Las abejas sociales, *Apis mellifera*, pero no las abejas solitarias, *Nomia strigata*, emitieron señales químicas de alarma al ser atacadas en flores para prevenir a conespecíficos del peligro de forrajear en esa flor. Estos resultados apoyan la idea de que un rasgo de las historias de vida de distintas especies de abejas, la sociabilidad, está asociado con la evolución de las señales de alarma.

CONCLUSIONS

1. Honeybees, *Apis mellifera*, and hoverflies, *Eristalis tenax*, responded to the trade-off between predation risk and foraging success, albeit in completely different ways at the patch level. The most susceptible pollinators, honeybees, avoided risky patches, particularly if their profitability was low, while less susceptible hoverflies visited most often low-quality risky patches.

2. At the flower level, honeybees and hoverflies showed similar responses: they avoided spider-harbouring inflorescences regardless of nectar availability at the patch level and they reacted similarly to non-lethal encounters with crab spiders.

3. Honeybee visit rates (number of visits per inflorescence per unit time) in safe patches were more than double than the rates in risky patches that offered the same amount of resources. However, as a consequence of honeybee preference for more rewarding patches, inflorescences in risky-rich patches received as many honeybee visits per unit time as inflorescences in safe-poor patches. These results suggest that, at the evolutionary time scale, a mechanism through which flower species regularly associated with ambush predators could attract pollinators would be increasing reward production.

4. We found that in 2008 *Thomisus spectabilis* spiders were, overall, more UV-reflective than in 2009. Interestingly, in 2008 spider UV and overall colour contrast were positively correlated with spider condition, but not in 2009. On the other hand, in the laboratory spiders increased their UV-reflectance regardless of their food intake or background colouration. This suggests that greater conspicuousness, achieved by a higher E_{UV} contrast, is advantageous for these spiders because it helps them to attract pollinators and, as a result, they achieve a better condition than less conspicuous spiders.

5. The bee choice experiments with *Trigona carbonaria* and *Austroplebeia australis* native Australian bees showed species differences in bee behaviour towards particular spider colour variation: *Trigona carbonaria* bees did not

show any preference for any colour contrasts generated by *Diaea evanida* spiders, but *Austroplebeia australis* bees were more likely to reject flowers with more contrasting *Thomisus spectabilis* spiders. This, together with data of the reflectance properties of crab spider colouration collected from the field, suggests that some of the spider colour variation that we encounter in the field may be partly explained by the spider's ability to adjust the reflectance properties of its colour relative to the behaviour of the species of prey available.

6. Honeybees did not respond to the degree of matching between spiders and inflorescences (either chromatic or achromatic contrast). However spider UV reflection, spider size and spider movement affected honeybee behaviour. These results suggest that only the large, but not the small Australian crab spiders deceive their prey by reflecting UV light, and highlight the importance of other cues that elicited an anti-predator response in honeybees.

7. The social bee, *Apis mellifera,* but not the solitary bee, *Nomia strigata,* released chemical alarm cues when attacked to warn conspecifics of the presence of danger at specific inflorescences. This result lends support to the view that a life-history trait, sociality, affects the evolution of a predator-avoidance strategy, release of and response to alarm cues.