

Predator and floral traits change pollinator behaviour, with consequences for plant fitness

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Abstract. 1. Flower-dwelling predators may interfere in plant–pollinator interactions through changes in pollinator behaviour, leading to a reduction in pollination services. Although the context-dependency of tri-trophic interactions is often acknowledged, the relative contribution of predator, pollinator and flower traits in shaping pollinator behaviour has been rarely studied simultaneously.

2. Pollinators' responses to predation risk were evaluated using artificial spiders on *Alstroemeria aurea* flowers in order to experimentally test whether predator traits (colour and size) and nectar availability affect their behaviour and plant fitness.

3. Regarding the predator's traits, both sizes of artificial spiders used were similarly rejected, but spider colour was detected differently. In particular, red and black artificial spiders were more strongly rejected than the rest. In turn, nectar availability increased the time spent in the inflorescences, yet most pollinators rejected flowers with artificial spiders independently of nectar availability. Furthermore, responses to artificial spider colour and nectar availability varied significantly between dipterans and hymenopterans. Finally, artificial spiders reduced seed set and fruit weight of plants owing to changes in the behaviour of the most efficient pollinators.

4. It was shown that poorly studied predator traits such as colour and size may affect pollinator behaviour in different ways among taxa. In addition, feeding necessity in a rich-resource environment was a weaker selecting force than predation risk. Hence, it is argued here that trade-offs between predation and flower reward may arise depending on predator detection, relationship between pollinator and predator size, and resource availability in the ecosystem with consequences for plant fitness.

Key words. Anti-predator behaviour, foraging behaviour, nectar availability, predation risk, visual cues.

Introduction

Predation is one of the most important ecological processes determining population dynamics, and its relevance in pollination biology has been increasingly acknowledged (Romero & Koricheva, 2011). Sit-and-wait predators like spiders, mantids and phymatids are the most common predators found on flowers, and usually have a generalist diet. They may feed on herbivores present in the plants, resulting in a protective behaviour that reduces herbivory damage benefiting the plant (Louda, 1982; Ruhren & Handel, 1999; Romero & Vasconcellos-Neto, 2004). However, their diet can also include flower visitors, which

may result in reduced pollination services and plant reproduction (Suttle, 2003; Dukas, 2005; Arango *et al.*, 2012). A recent meta-analysis showed that when predators interfered in the plant–pollinator mutualism, plant reproductive success decreased by 17% (Romero & Koricheva, 2011). Nonetheless, tri-trophic systems are highly context-dependent and, therefore, the effect of predators on pollinators and plant fitness may vary depending on the system and the ecological context studied (Higginson *et al.*, 2010). Thus, much remains to be learned about the ecological role of flower predators on plants under various scenarios and how they may interfere with plant–pollinator interactions.

The presence of predators makes flowers dangerous foraging sites for pollinators (Chittka *et al.*, 2009). Given the high potential cost of not detecting flower predators, anti-predatory behaviours are strongly selected (Goncalves-Souza *et al.*,

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2008). Visual cues or chemical odours may provide pollinators with reliable information about the presence of predators (Dicke & Grostal, 2001; Goncalves-Souza *et al.*, 2008; Bray & Nieh, 2014). Once predators are detected, pollinators usually decrease their visitation frequency and/or foraging time in a given plant (Dukas & Morse, 2003; Suttle, 2003; Robertson & Maguire, 2005; Romero *et al.*, 2011). However, the extent to which pollinators use these strategies could depend on several factors. First, pollinator traits like taxa and body size are key factors influencing anti-predator behaviours. For instance, lepidopterans and hymenopterans more frequently show avoidance behaviours to predators than do dipterans and coleopterans (Romero *et al.*, 2011, but see Yokoi & Fujisaki, 2009; Brechbühl *et al.*, 2010b). Also, small pollinators tend to display more anti-predator responses than larger ones (Romero *et al.*, 2011), because they are more easily captured than larger ones and, therefore, are more vulnerable to predation (Dukas & Morse, 2003, 2005). Second, pollinator lifestyle (i.e. social versus solitary) may also affect their behaviour (Clark & Dukas, 1994; Rodríguez-Gironés & Bosch, 2012, but see Romero *et al.*, 2011). In particular, the development of anti-predatory behaviour in social insects should presumably be under weaker selection pressure than solitary species. This is because the death of a worker has a relatively low cost to the fitness of the colony (Gadagkar, 1990) in comparison with the death of a solitary bee (Clark & Dukas, 1994). Finally, previous experience can also influence pollinator behaviour (Ings & Chittka, 2009), as several social insects display anti-predatory behaviours after having experienced a predator attack (Jones & Dornhaus, 2011). Nonetheless, pollinator traits alone do not sufficiently explain their behaviour in the presence of floral predators, highlighting the need to consider their partners' traits.

Predator and floral traits may strongly influence pollinator anti-predatory strategies, but their influence and relative contribution in shaping pollinator foraging behaviour has rarely been studied simultaneously (Wang *et al.*, 2013). A few studies have found that, independent of the hunting strategy (i.e. sit-and-wait versus active hunters; Romero *et al.*, 2011), some predator traits such as body size and colour may influence pollinator behaviour. In particular, it was observed that larger praying mantises were more frequently avoided than smaller ones (Bray & Nieh, 2014). In addition, ambush spiders that match their body colour with the colour of their host flower reduce the chances of being detected and, therefore, decrease pollinator avoidance behaviour (Chittka, 2001; They & Casas 2002). They may achieve this either behaviourally by selecting flowers that match their body colour (Chittka, 2001; They & Casas 2002; Heiling *et al.*, 2006) or by actively changing their body colour (Heiling *et al.*, 2005). By contrast, some studies found that spider colour can also attract pollinators (Heiling *et al.*, 2003; Llandres & Rodríguez-Girones, 2011). Specifically, it has been shown that high UV chromatic contrast of the Australian crab spider *Thomisus spectabilis* (Heiling *et al.*, 2003; Heiling & Herberstein, 2004) and of *Misumena vatia* (Greco & Kevan, 1994) can lure pollinators deceptively (Heiling *et al.*, 2005).

On the other hand, floral traits such as flower density, morphology and reward quality, and quantity may also have a critical

influence on pollinator behaviour. In the absence of predators, pollinators often maximize their foraging success by visiting the most rewarding flowers (e.g. with high nectar volume, and/or pollen availability) (Jones, 2010; Jones & Dornhaus, 2011). In contrast, in the presence of predators, it is assumed that pollinators establish a trade-off between the foraging gains and the risk of being preyed upon (Abbott, 2010; Jones, 2010; Wang *et al.*, 2013). For instance, previous studies found that experimentally simulated predation risk caused bees to reject even high rewarding flowers and reduce their foraging activity (Jones & Dornhaus, 2011). However, under similar predation scenarios, Llandres *et al.* (2012) showed that bees increased visitation rate when the reward was high. Therefore, nectar availability can greatly influence pollinator foraging behaviour under diverse predation risk scenarios. Here, we propose that the final behaviour performed by each pollinator will depend on the interaction between several of the traits mentioned (e.g. pollinator taxa and size, predator size and colour, and flower reward availability). Assessing the relative contribution of traits across all the interacting organisms will shed light on the dynamic nature of these tri-trophic interactions.

Artificial models are suitable experimental tools to test how different combinations of predator, pollinator and plant traits may affect pollinator foraging behaviour and, consequently, plant fitness (Goncalves-Souza *et al.*, 2008; Ribas & Raizer, 2013). The aim of this study was to experimentally evaluate, under field conditions, the behaviour of floral visitors of *Alstroemeria aurea* (Alstroemeriaceae) in the presence of artificial spiders and its consequences on plant fitness. We tested whether floral visitors responded differently to artificial spider colour and size and whether this response depended on nectar availability. Several sit-and-wait spiders were observed hunting on *A. aurea*'s flowers in relatively high abundance (25% of the plants had a spider), but manipulation of real spiders was unviable. The most common families observed were Thomisidae and Anyphaenidae (e.g. *Misumenops* sp. and *Sanogasta* sp., respectively), which usually camouflage or hide within flowers, respectively. To answer our questions we made artificial spiders that resembled the real spiders observed in our system. In particular, we addressed the following questions: (i) do pollinators of *A. aurea* detect colour differences and/or size of predators; (ii) does contrasting nectar availability change pollinator decision-making; and (iii) does the presence of artificial spiders indirectly affect plant fitness? The simultaneous evaluation of these traits in pollinators, flowers and predators will contribute to a better mechanistic understanding of the selecting pressures shaping pollinator foraging behaviour and, consequently, plant fitness.

Material and methods

Study site and system

This study was conducted in the upper region of the Challhuaco Valley, Nahuel Huapi National Park, Argentina (41°20'S, 71°19'W), during the summer season of 2015, between January and March. The mean annual temperature is 8.3 °C, with frosts during the autumn and winter, and the annual precipitation varies between 1300 and 1800 mm. *Alstroemeria*



Fig. 1. Inflorescence of *Alstroemeria aurea* with: (a) a *Bombus terrestris* bumblebee collecting nectar, (b) a white small artificial spider and (c) large artificial spider placed in one flower of the inflorescence. [Colour figure can be viewed at wileyonlinelibrary.com].

aurea R. Graham (*Alstroemeriaceae*) or ‘amancay’ is an herbaceous perennial native to the temperate forests of southern South America and grows in the understorey of *Nothofagus pumilio* forest in the study area. This herbaceous plant has vegetative propagation by branching and rhizome fragmentation. Blooming occurs during summer, from December to March. Yellow zygomorphic flowers are arranged in terminal inflorescences usually composed of one to eight protandrous (male) flowers that last from 4 to 5 days in this phase, followed by a transitional 2-day period after which they turn into female flowers that last 3–4 days more (Aizen & Basilio, 1995). During this transitional period the anthers wither, and the style lengthens and begins the exposure of a three-branched stigma. Nectar is the main reward for pollinators and it is released at the bottom of the two inner sepals lined with reddish nectar guides (Aizen & Basilio, 1995). Fruits are explosive capsules containing from one to 25 seeds (Aizen & Raffaele, 1996). This species was mainly pollinated by the native bumblebee *Bombus dalhombii*, but in the last two decades the introduced bumblebees *B. ruderatus* and *B. terrestris* have largely replaced *B. dalhombii*, becoming the most frequent pollinators (Morales *et al.*, 2013) (Fig. 1a). In addition to the bumblebees, this species is visited by a wide range of floral visitors, from less efficient pollinators such as dipterans to nectar robbers.

Artificial spiders consisted of epoxy spheres simulating the spider body, each with two pairs of twisted steel wire imitating the first two pairs of spider legs (Fig. 1b,c). Two different sizes of artificial spiders were constructed (small and large). Small spider models (S) consisted of a 6-mm-diameter sphere (~0.5 g) whereas large models (L) had a 12-mm-diameter sphere (~1.2 g). The size of the small artificial spiders was selected to resemble the size of the largest female crab spiders observed hunting on *A. aurea*. The larger spider models were made to determine whether pollinators detect larger objects as a greater predation risk, since preliminary field observations suggested that real spiders were not perceived by pollinators. Models were placed at the centre of the flower, on one flower within an inflorescence of four to six flowers. All experiments were performed under daylight conditions, between 10.00 and 17.00 hours and under cloudless and sunny conditions when pollinator activity was high (Aizen, 2001). We separately tested the combination of spider colour-size effect from the spider

size-nectar effect on pollinator behaviour, because a full factorial design would have required an elevated number of plants to be observed simultaneously. This would have been difficult in practice, requiring a greater number of observers and adding more bias to our measurements.

Effect of artificial spider colour and size on pollinator behaviour

The effect of spider colour and size on floral visitor behaviour was tested using small and large artificial spiders painted in five different colours (ETERNA, Argentina, acrylic paint; white, green, blue, red, and black). We randomly selected one flower of the inflorescence on which to place the artificial spider, with the sphere in the centre of the flower ensuring that the first pair of legs was visible. After placing the model on one flower, the whole inflorescence was observed over a period of 10 min, at a minimum distance of 2 m to avoid affecting pollinator behaviour. A total of 202 periods of observation, during the flowering season, were performed across all colours and sizes. The identity and abundance of each floral visitor, number of flowers visited and time spent at the inflorescence were recorded. Behavioural responses at the focal flower that hosted the model were classified as: visit (V) when the insect landed on the flower with the spider model and collected nectar, or rejection (R) when the insect overflew the flower with the artificial spider without landing or flew away.

Following the behavioural observations we assessed how spider models might be perceived by pollinators with respect to the flower background; for these we performed both quantitative and qualitative measurements. First, under laboratory conditions, we measured colour reflectance of the flowers and spider models (see methodological details in the Figs S1 and S2 in File S1) followed by estimations of chromatic contrast (Table S1 in File S1), which allowed us to determine whether there was UV reflection in the studied species, and to understand how models on flowers might be perceived through the visual systems of dipteran and hymenopteran. Second, under field conditions, we photographed flowers and spider models to estimate RGB colour curves for each analysed object, using Adobe PHOTOSHOP CS6 (see Fig. S3 in File S1).

Effect of white artificial spider size and flower nectar availability on pollinator behaviour

Spider size and nectar availability were experimentally modified to evaluate the responses of floral visitors. White artificial spiders were used for this experiment because many of the spider species that inhabit the study area are whitish. We randomly selected one flower of the inflorescence to place the artificial spider at the flower centre with the first pair of legs visible. The experimental design consisted of two factors: presence of artificial spider with three levels (C, control without model; S, small spider; and L, large spider) and nectar availability with two levels (with and without nectar), using 53 plants per treatment (i.e. 318 plants in total). Nectar availability in flowers of *A. aurea* was modified manually; we bagged 159 inflorescences with a net the day before the assay to prevent insect visitation and to enhance nectar accumulation without affecting flower microclimatic conditions. The other 159 plants were exposed to floral visitors 24 h before placing the models and removing any remaining nectar with microcapillaries before the assay. Periods of 10 min of observation were simultaneously performed on pairs of experimental inflorescences (i.e. with and without nectar of each spider treatment; C, S, or L). Paired observations allowed us to compare behaviours driven only by different nectar availability scenarios and controlling other possible variables such as the daytime of measurement, microclimatic conditions, sun exposure, and wind intensity. The pairs of plants observed were separated by ~2 m and we recorded the identity and number of floral visitors, time spent in the inflorescence, number of visited flowers and the behavioural response to the artificial spiders (i.e. V or R).

Effect of white artificial spider size and flower nectar availability on plant reproductive success

We used the same experimental design as in the previous experiment (artificial spider and nectar, with three and two levels, respectively) to test the effect of these treatments on plant fitness. For this experiment, a total of 72 plants at the female phase were used, with 12 plants per spider model (C, S, L) and nectar level (with and without). To accomplish this, we marked a different set of plants showing an intermediate stage between the male and female phases (i.e. flowers with wilted anthers and elongated style). To maintain nectar quantity differences between treatments, we visited the marked inflorescences daily and removed nectar or increased it with the addition of two drops of sugar solution (41%), as was appropriate to each treatment. This procedure was repeated in all the experimental plants over 4 days consecutively. To avoid models falling by the effect of wind or rain over the 4 days period, we used in this experiment new lighter artificial spiders permanently adhered with transparent glue (Suprabond Buenos Aires, Argentina) to one flower of the inflorescence. These new models were thinner but with the same section area than previous models to preserve spider size without changing flowers position due to their weight (Fig. S4 in File S1). To control for a possible glue repellent effect on floral visitors, a small drop of glue was also placed in an inflorescence without spider model (C). We did not observe any repellent

effect of the glue (flowers without glue versus glue flowers, $t_{55} = 1.33$, $P = 0.19$). The pistil of the focal flowers with the artificial spider or glue was marked with a thread allowing the identification of the fruit originated by the flower that carried the artificial spider from the rest of the inflorescence. The presence of the artificial spider was the same as duration of the female phase for each inflorescence (no more than 5 days). After this, the flowers withered and the petals and artificial spiders fell. Fruits were collected 30 days after the end of the experiment, allowing appropriate fruit formation before capsule explosion. We recorded the number of fruits per inflorescence (fruit set), their weight after drying them over 72 h at 60 °C, and the number of seeds per fruit (seed set). In addition, the proportion of aborted fruits was recorded to compare the abortion rate between the flowers carrying the artificial spider with those of the same inflorescence, determining the effect of the artificial spider at both flower and inflorescence levels.

Statistical analysis

To test the response of pollinators to different predator colour and size, we evaluated the differences in the mean number of flowers visited and the time spent on each inflorescence across the five artificial spider colours and the two sizes with a three-way factorial ANOVA, using pollinator taxa as an additional factor. In addition, given that artificial spider size was not significant for all dependent variables, we then performed a two-way ANOVA considering only the artificial spider colour and the pollinator taxa as factors. Species richness and floral visitor abundance were also evaluated, with species richness rarefied to control for insect abundance. In the rest of the experiments, the effects of artificial spiders and flower nectar availability on pollinator behaviour and plant fitness were analysed by two- and three-way factorial ANOVAs. The response variables were number of flowers visited, time spent in the inflorescence or flower, pollinator species richness and abundance, fruit set, seed set, and fruit weight. The factors evaluated were nectar (with/without) and artificial spiders (without model, C; small model, S; large model, L); and when appropriate we added pollinator taxa as a factor (Hymenoptera/Diptera or bumblebees/bees/wasps). To determine the effect of artificial spiders at the flower level, within the inflorescence, we evaluated seed set and fruit weight with a nested ANOVA, where the artificial spider treatment (present or absent on the flower) was nested within the inflorescence treatment (with or without artificial spider). In analyses with more than one factor, all interaction terms were included. *Post hoc* tests (Tukey) were performed for significant factors in all analyses. All ANOVA assumptions were achieved. Time spent and number of flowers visited were transformed with the natural logarithm [i.e. $\ln(x + 0.1)$] to attain normality. Finally, χ^2 tests were used to analyse the frequencies of floral visitor rejections to the artificial spiders according to pollinator taxa (e.g. Hymenoptera versus Diptera), artificial spider colour (white/green/blue/red/black), size (small/large), and nectar availability (with/without). Fruit abortion of flowers carrying artificial spiders (small/large) and control flowers (i.e. with glue) were also compared with a χ^2 test.

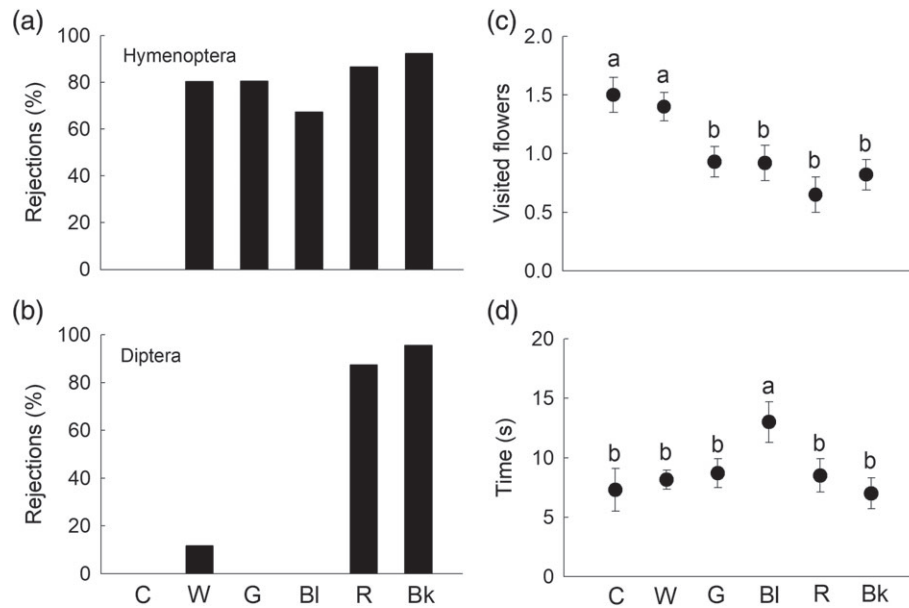


Fig. 2. Behavioural response measured as percentage of rejection of (a) hymenopterans and (b) dipterans to the flowers harboring different artificial spider colours (note that control inflorescences did not register rejections); (c) number of visited flowers per inflorescence (mean \pm SE), and (d) time spent (mean \pm SE) on the inflorescences. Colour treatments are depicted as: C = control, W = white, G = green, BI = blue, R = red and Bk = black. Artificial spiders (small and large spider models were analyzed together). Treatments with the same letter are not significantly different ($P > 0.05$).

Results

Floral visitor assemblage of *Alstroemeria aurea*

A total of 1017 flower-visiting insects, all considered pollinators, were recorded visiting *A. aurea* inflorescences, 59% corresponding to *Bombus terrestris*. In general, hymenopterans were more abundant than dipterans (78.3% vs 20%, respectively), and only a few visits of lepidopterans (0.6%) and coleopterans (1.1%) were observed. The main groups observed within hymenopterans were bumblebees (Apidae), bees (Apidae and Dasymegachilidae), and wasps (Vespidae); whereas within dipterans, nemestrinid flies (Nemestrinidae) and hoverflies (Syrphidae) were the most abundant. Lepidopterans and coleopterans were not included in data analyses because of their low abundance. All visitors observed and analysed in this work are pollinators but with different levels of efficiency.

Effect of artificial spider colour and size on pollinator behaviour

When considering pollinator responses to the flower carrying the model we found that both artificial spider sizes were similarly rejected ($\chi^2_1 = 1.24$, $P = 0.27$); however, rejection rates differed across artificial spider colours ($\chi^2_4 = 42.6$, $P < 0.001$). During 2000 min of observations we recorded 351 behaviour responses, of which 248 were rejections to the artificial spiders. Red and black artificial spiders were the most rejected colours, up to 50% higher than the rest (Fig. 2b). In addition, rejection rate differed among pollinator taxa ($\chi^2_4 = 41.9$, $P < 0.001$). Dipterans landed on flowers with

blue, green and white artificial spiders but often rejected black (95%) and red ones (85%), while hymenopterans similarly avoided all five studied colours (Fig. 2a,b). Spectral reflectance measurements showed no UV reflection from either flowers or spiders, real or artificial (Figs S1 and S2 in File S1). Values of chromatic contrast between artificial spiders and flowers varied with artificial spider colour and pollinator taxa, showing higher contrast values for hymenopterans than for dipterans (Table S1 in File S1). In turn, artificial spiders' RGB spectral curves exhibited different curve pattern with respect to flower curve pattern (Fig. S3 in File S1). In particular, blue, black and red showed a more different pattern with respect to the flowers' curve than did white and green.

The number of visited flowers and the time spent were affected by the artificial spider colour, but not by its size or pollinator taxa involved (Table 1A). As different spider sizes showed similar effects on pollinator behaviour, data were reanalysed without considering the size. In these new analyses where the spider model factor has six levels (control, with no spider, and the five tested colours) both the number of visited flowers and the time spent were, again, strongly affected by the artificial spider colour and pollinator taxa (Table 1B). No significant interaction was observed between taxa and spider colour for any response variable (Table 1B). In general, pollinators visited more flowers in inflorescences without spiders (control) or with white artificial models (Fig. 2c), but spent more time at inflorescences with blue artificial spiders than with any other colour (Fig. 2d). In addition, hymenopterans visited 41% more flowers per inflorescence and spent 5% more time than dipterans. Species richness and pollinator abundance were significantly affected by the artificial spider colour ($F_{5,249} = 20.9$, $P < 0.001$; $F_{5,249} = 20.1$,

Table 1. Analysis of variance considering the effect of artificial spider model colour (white, green, blue, red and black), size (small and large), and the pollinator taxa (Hymenoptera and Diptera) on the number of visited flowers and time spent in inflorescences of *A. aurea*, as response variables.

Source of variation	df	Visited flowers		Time spent	
		F	P	F	P
(A) Model colour	4,440	8.1	<0.001	3.8	0.005
Model size	1,440	1.9	0.16	0.04	0.85
Taxa	1,400	2.5	0.11	1.7	0.19
Interactions		<1	>0.4	<0.7	>0.45
(B) Model colour	5,505	8.4	<0.0001	3.8	0.002
Taxa	1,505	5.0	0.025	4.0	0.04
Colour x Taxa	5,505	0.92	0.47	0.67	0.64

(A) Analysis considering the effect of artificial spider colour and size on response variables. (B) Analysis excluding artificial spider size and comparing the effect of different spider colour and taxa on response variables. *P* values are highlighted in bold letter when significant ($P < 0.05$) or marginally significant ($P < 0.1$).

$P < 0.001$, respectively). Rarefied pollinator richness was higher in control and white artificial spider inflorescences than any other artificial spider colours (Fig. S5 in File S1).

Effect of white artificial spider size and flower nectar availability on pollinator behaviour

At the flower level, pollinators rejected both sizes of white artificial spiders regardless of nectar availability ($\chi^2_3 = 3.56$, $P = 0.31$). A total of 236 responses were registered in 3180 min of observation, of which 162 were rejections. However, hymenopterans showed a higher rejection rate (85%) to artificial spiders than dipterans (12%). Both hymenopterans and dipterans showed similar rejection rates to both sizes of artificial spider ($\chi^2_3 = 1.56$, $P = 0.67$) and this was not influenced by nectar availability ($\chi^2_3 = 1.39$, $P = 0.7$; Fig. 3a,b).

At the inflorescence level, the number of flowers visited per inflorescence was significantly different between dipterans and hymenopterans, but similar between nectar availability and artificial spider scenarios (Table 2A). However, there was a significant interaction between pollinator taxa and artificial spider treatment (Table 2A). Particularly, hymenopterans visited more flowers than dipterans but tended to reduce the number of visited flowers in the presence of the artificial spider, while dipterans did not (Fig. 3c,d). The time spent was significantly higher in inflorescences with nectar, but similar among artificial spider scenarios and between dipterans and hymenopterans, yet we found a significant interaction between the pollinator taxa and nectar availability (Table 2A). Specifically, hymenopterans spent more time than dipterans in inflorescences with high nectar availability (Fig. 3e,f). Furthermore, we found behavioural differences among hymenopterans (Fig. 4) as suggested by the multiple significant interactions between evaluated factors (Table 2B). In particular, bumblebees and bees were the most affected by the presence of artificial spiders, decreasing the number of visited flowers and time spent in the inflorescences,

a pattern only observed in inflorescences with nectar (Table 2B, Fig. 4a,b). Instead, wasps increased the number of visited flowers in the presence of artificial spiders (Fig. 4a), with no changes observed in their visit duration (Fig. 4b). Pollinator richness and abundance were higher in plants with nectar ($F_{1,312} = 14.9$, $P < 0.001$, and $F_{1,312} = 15.9$, $P < 0.0001$, respectively) but were not affected by the artificial spider treatment ($F_{2,312} = 2.3$, $P = 0.10$, and $F_{2,312} = 1.99$, $P = 0.14$, respectively), and no interaction was found for neither variable ($F_{2,312} = 0.08$, $P = 0.92$, and $F_{2,312} = 0.09$, $P = 0.91$, respectively).

Effect of white artificial spider size and flower nectar availability on plant reproductive success

At the flower level, the percentage of aborted fruits was up to three-fold higher in flowers with artificial spiders than in flowers without them ($\chi^2_2 = 8.9$, $P = 0.012$). At the inflorescence level, the presence of artificial spiders affected seed set and fruit weight, but not fruit set (Table 3, Fig. 5). In particular, the seed set and fruit weight of inflorescences with artificial spiders were, respectively, 25% and 15% lower than those without, independent of the spider model size (all $P > 0.30$). Nectar treatment did not affect seed set or fruit weight, but marginally affected fruit set (Table 3), showing higher fruit number in inflorescences without nectar (Fig. 5a). There was a significant interaction between the artificial spider and nectar treatment for seed set (Fig. 5b), but not for fruit weight or fruit set (Table 3). Interestingly, the presence of artificial spiders similarly reduced seed set and fruit weight of all flowers within inflorescences as, according to the nested ANOVA, no differences were found between flowers with artificial spiders and flowers without ($F_{1,219} = 2.1$, $P = 0.14$, and $F_{1,219} = 0.5$, $P = 0.46$, respectively).

Discussion

Floral predators often alter pollinator behaviour; yet, the effect of these predators on their prey and the consequences for the host plants is context dependent. Here, we showed that artificial spiders affected the foraging behaviour of a wide pollinator assemblage, including the most effective pollinators and thus decreasing the reproductive success of *A. aurea*. Our results indicate that artificial spiders are suitable models to test top-down effects on plants through pollinator fear driven by predation risk. However, we found that top-down effects can depend on the interaction between pollinator and predator traits. Pollinator responses to the different colours of artificial spiders were strongly taxon-dependent, with hymenopterans displaying more anti-predatory behaviours than dipterans. As expected, nectar availability increased pollinator arrival and time spent in the inflorescences; however, behaviour responses to nectar also depended on pollinator taxa. Particularly, only hymenopterans increased the arrival and the time spent at high nectar levels, even in risky inflorescences. Yet, nectar availability did not modify the rejection behaviour to flowers carrying artificial spiders. We argue that behavioural differences in pollinators, which may alter host plant fitness, will depend on the combination of traits across all three trophic levels.

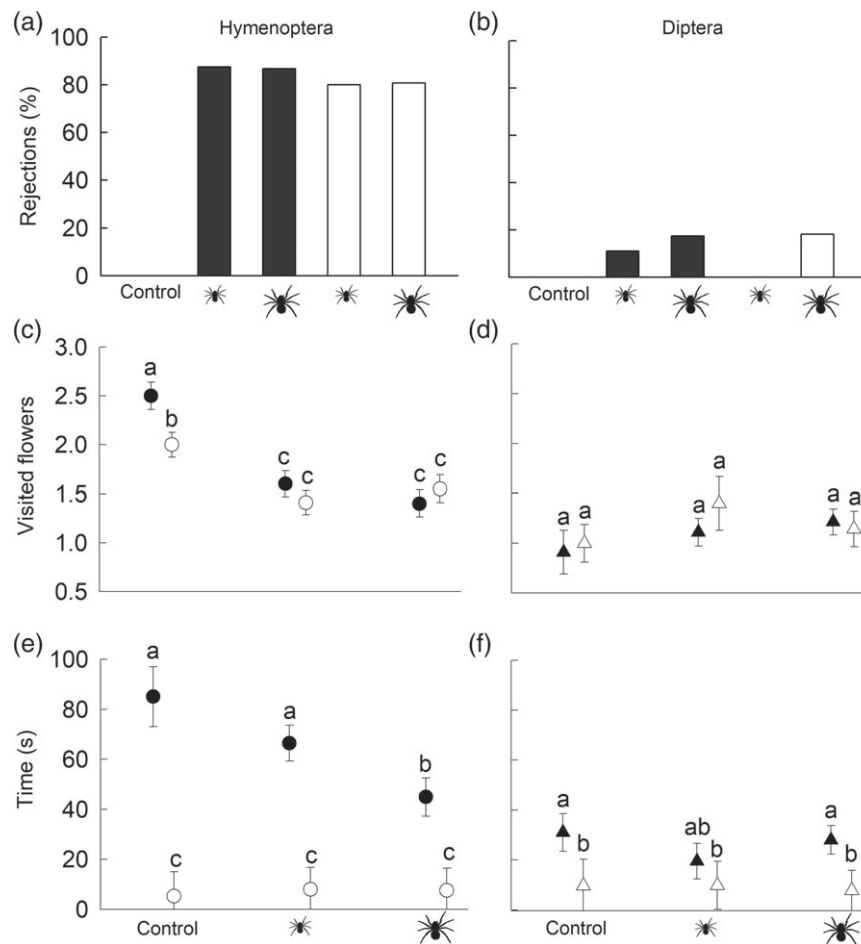


Fig. 3. Behavioural response of hymenopterans (left side) and dipterans (right side) to artificial spiders (control, small and large spider models) on inflorescences with nectar (black dots) and without nectar (white dots). (a,b) Behavioural response expressed as the percentage of rejection (note that control inflorescences did not register rejections), (c,d) number of visited flowers per inflorescence (mean \pm SE); and (e,f) time spent in the inflorescences (mean \pm SE). Treatments with the same letter are not significantly different ($P > 0.05$).

Predator size is a crucial trait that can influence pollinator behaviour, with larger predators eliciting stronger anti-predatory responses (Bray & Nieh, 2014). Yet, our results showed that almost all pollinators similarly rejected both sizes of artificial spiders used, suggesting that both sizes used imply the same risk for the studied pollinator assemblage. Nonetheless, bumblebees, the largest pollinator in the study area, reduced their foraging time when only large models were present. Therefore, at least for bumblebees, large artificial spiders represented a greater threat than smaller ones. Thus, the evaluation of a wider range of predator sizes, taking into account the size variation in pollinator assemblage, needs further investigation. Moreover, a previous study showed that predator size may even interact with other predator traits like body colour. For instance, honeybees landed more frequently on inflorescences with large spider with high UV reflectance (Llandres & Rodriguez-Girones, 2011). Thus, it is likely that pollinators evaluate more than one visual clue when facing predators.

Predator colour is another important visual clue used by pollinators to detect them (Wang *et al.*, 2013). We found that

pollinator rejection behaviour varied among conspicuous artificial spider colours indicating that, despite how pollinators truly see them, artificial spiders were perceived differently. In particular, hymenopterans avoided all spider model colours, whereas dipterans only rejected red and black spider models. Dissimilarities in their visual system may partially explain these behavioural differences between taxa (Briscoe & Chittka, 2001; Morante & Desplan, 2008). The avoidance exclusively observed to red and black artificial spiders by dipterans is possibly owed to a higher contrast with the flowers. Indeed, under dipterans sight, red and black artificial spider models resulted much more contrasting than white and green ones as supported by values of chromatic contrast (Table S1 in File S1). This was also suggested by the RGB curve patterns, where red and black models exhibited a highly different curve with respect to the flower's (Fig. S3 in File S1). Instead, the less rejected colours (e.g. white, green and blue) may be less perceived and possibly more cryptic. Nonetheless, even these less contrasting models, such as green artificial spiders (Table S1 in File S1), were avoided by most hymenopterans, probably because they were close but still above

Table 2. Analysis of variance considering the effect of artificial spider model (control, small and large white artificial spider), nectar availability (with and without) and pollinator taxa on the number of flowers visited and time spent in *A. aurea* inflorescences, as response variables.

Source of variation	df	Visited flowers		Time spent	
		F	P	F	P
(A) (Diptera – Hymenoptera)					
Model	2,502	0.02	0.97	0.10	0.90
Nectar	1,502	0.006	0.94	43	<0.001
Taxa	1,502	5.73	0.017	0.66	0.42
Model × Nectar	2,502	0.33	0.72	0.92	0.4
Model × Taxa	2,502	3.3	0.03	0.99	0.37
Nectar × Taxa	1,502	1.6	0.20	13	<0.001
Model × Nectar × Taxa	2,502	0.5	0.61	2.0	0.1
(B) (Bumblebees - Bees – Wasps)					
Model	2,404	4.3	0.013	0.34	0.71
Nectar	1,404	9.2	0.003	48	<0.001
Taxa	2,404	8.7	<0.001	4.6	0.01
Model × Nectar	2,404	1.6	0.20	3.2	0.04
Model × Taxa	4,404	4.1	0.003	2.2	0.067
Nectar × Taxa	2,404	5.2	0.006	0.95	0.39
Model × Nectar × Taxa	4,404	0.69	0.60	0.87	0.48

(A) ANOVA including factor Taxa with two levels; dipterans and hymenopterans. (B) ANOVA analyzing only hymenopterans (bumblebees, bees and wasps). *P* values are highlighted in bold letter when significant ($P < 0.05$) or marginally significant ($P < 0.1$).

the detection threshold (under hymenopterans sight), suggesting that even nearly cryptic predators may be perceived as threats by some groups. Alternatively, it is also possible that dipterans rely more than hymenopterans on other important cues, like odor, to detect and respond to predator presence (Weiss, 2004). In this way, at long distance, dipterans might not detect less contrasting artificial spiders and in closeness they realize that artificial spiders are not a real threat as other enlightening cues come into play, explaining why dipterans end up landing on those flowers. Independently of the mechanisms, the higher rejection rate of hymenopterans to all artificial spiders agrees with a previous study which showed that hymenopterans are more responsive to predators than dipterans, possibly because an alert signal is triggered by the presence of any strange object (Romero *et al.*, 2011). Yet, our results add to the increasing literature (Yokoi & Fujisaki, 2009; Defrize *et al.*, 2010; Brechbühl *et al.*, 2010a, 2010b) showing that dipterans can also detect and consistently respond to predation risk. In sum, our findings show that dipterans and hymenopterans evaluate predation risk scenarios differently only by varying artificial predators' colours, or their visual perception is different. However, further investigations are needed to understand whether visual cues used by pollinators to respond to flower-predators are driven by contrast or colour differences and, more interestingly, if visual cues assessed vary among pollinators.

Besides predator traits, pollinator foraging behaviour is also highly influenced by the rewards availability in the foraging area. Pollinators usually maximize their foraging success by visiting the most rewarding flowers, yet the presence of predators may jeopardize their foraging choices (Jones & Dornhaus,

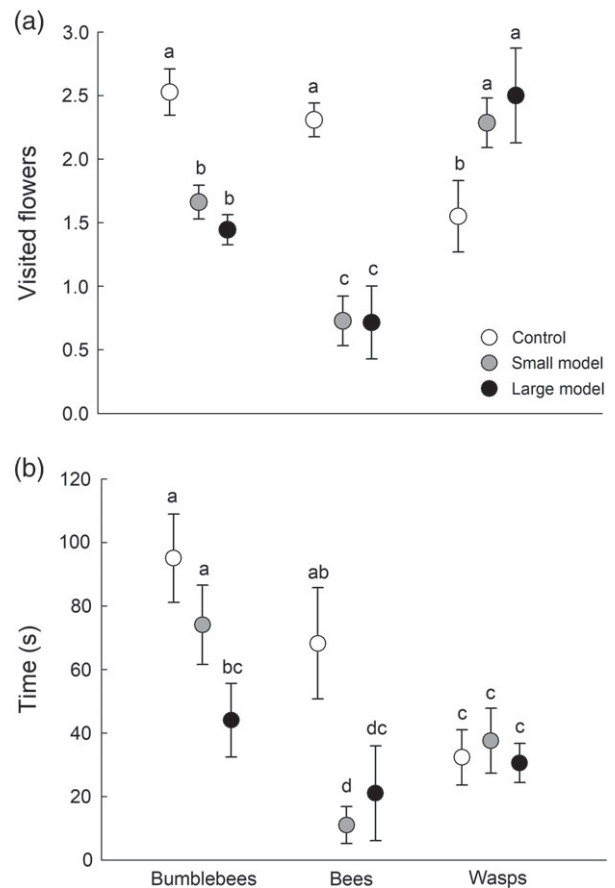


Fig. 4. Behavioural response of main groups of hymenopterans exclusively in inflorescences with nectar expressed as the (a) number of visited flowers and (b) time spent (mean \pm SE) among artificial spider treatments: control, small and large white spider model. Treatments with the same letter are not significantly different ($P > 0.05$).

Table 3. Analysis of variance testing the effect of artificial spider model (control, small and large white artificial spider) and nectar availability (with and without) on the reproductive success of *A. aurea* plants.

Source of variation	Fruit set			Seed set			Fruit weight	
	df	F	P	df	F	P	F	P
Model	2,64	2.1	0.13	2,218	21	<0.0001	12	<0.0001
Nectar	1,64	2.9	0.096	1,218	1.9	0.17	2.3	0.14
Model × Nectar	2,64	1.6	0.20	2,218	3.0	0.05	2.0	0.13

P values are highlighted in bold letter when significant ($P < 0.05$) or marginally significant ($P < 0.1$).

2011). As expected, pollinators increased the foraging activity in inflorescences with higher nectar amounts. Nonetheless, the presence of artificial spiders always made hymenopterans to reject risky flowers, regardless of nectar availability. This result suggests that predation risk may be a stronger selective force than feeding in our study system, similar to the findings in other ecosystems (Llandres *et al.*, 2012), but opposite to some

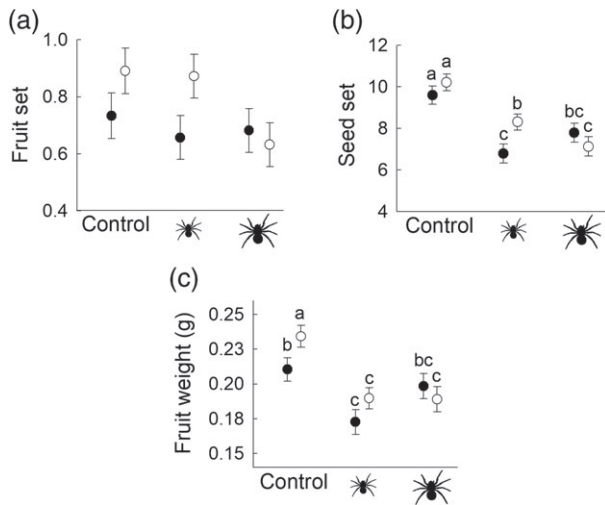


Fig. 5. Reproductive success of *A. aurea* plants (mean \pm SE) with nectar (black circles) and without nectar (white circles) at three spider model scenarios: control, small and large white models. (a) Fruit set, (b) seed set, (c) fruit weight (g). Treatments with the same letter are not significantly different ($P > 0.05$).

theoretical models (Jones, 2010). However, pollinator behaviour is context-dependent and can be subjected to flower abundance and spatial distribution in the foraging area (Fowler *et al.*, 2016). Therefore, the high rejection rate to flowers harboring artificial spiders might be driven by the great surrounding flower abundance and vast spatial distribution of *A. aurea* in the study area, as it occupies most of the forest understory. In this rich-resource context, pollinators might be avoiding all dangerous flowers because of the low cost of finding non-dangerous ones. This suggests that pollinator anti-predatory behaviour might rely on the spatial distribution of flower resources, increasing rejection rates when the cost of finding safe inflorescences is low or inexistent.

Behavioural responses to reward availability and predation risk also resulted highly variable among and within pollinator taxa. All trends previously described were mostly driven by hymenopterans, since dipterans were a minority, visited fewer flowers, spent less time in inflorescences and barely rejected spider models. These foraging differences can be partially explained by the low energy requirements of dipterans, since they do not provide a brood, thus being able to depend on less rewarding flowers (Szymank *et al.*, 2008). In turn, within hymenopterans; bees and bumblebees, reduced flower visitation and foraging time up to 70% in risky inflorescences, even in rewarding inflorescences; whereas *Vespula* wasps increased their visits in the presence of spider models, possibly because they are also aggressive hunters (Dukas, 2005). Since among all these pollinators, bees and bumblebees are the most important for *A. aurea*, the trade-off between maximizing foraging gains and minimizing mortality risk may strongly affect plant reproductive success.

Changes in pollinator behaviour may translate into changes in plant fitness when ambush predators alter the behaviour of the most effective pollinators, limiting pollen movement among plants (Quintero *et al.*, 2015). Here, the presence of artificial

spiders decreased *A. aurea* reproductive success components by reducing 25% the seed set and 15% the fruit weight. Therefore, the non-lethal effects of artificial predators disrupted the plant–pollinator mutualism. This was likely driven by changes in the foraging behaviour of the exotic bumblebee, *B. terrestris*, currently the most abundant and effective pollinator of *A. aurea* (Morales *et al.*, 2013). Furthermore, deviations in bumblebees' behaviour took place at both flower and inflorescence level. On one hand, they always avoided flowers harboring artificial spiders, independently of flowers nectar level, interfering completely with pollination services on these flowers and explaining the increased fruit abortion of those flowers. On the other hand, they also reduced the number of visited flowers and foraging time across the whole inflorescence when artificial spiders were present, and thus, they may have constrained pollen deposition on flowers stigma in comparison with inflorescences free of artificial spiders. Hence, this could explain the seed production decline, and fruits biomass, of all flowers in risky inflorescences. Although nectar availability might influence pollinator behaviour, and therefore plant fitness, we only found a slight tendency of higher fruit production in nectarless inflorescences. This contra-intuitive result may be a consequence of reduced self-pollination through less visitation frequency and foraging time among flowers of the same inflorescence respect to the higher foraging activity that occur within full nectar inflorescences (Harder & Aizen, 2004). Therefore, any potential change in the foraging behaviour of the main pollinator species may determine the selection forces acting on plant reproduction.

In conclusion, artificial spiders affected the foraging behaviour of a wide floral insect assembly with severe consequences on *A. aurea* reproduction success. We showed that poorly studied predator traits like colour may affect pollinator foraging behaviour in different ways among taxa. Interestingly, within a context of rich-resource environment, feeding necessity was a weaker selecting force than predation risk. Consequently, the overall top-down effect of predators on plant fitness could be determined by traits of main pollinators, predators (i.e. colour and size), host plant, and the ecological context (e.g. foraging area). As more scenarios become tested our understanding of the ecological role of flower predators in natural environments will improve.

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the experiments. SSG performed the experiments, analyzed the data, and wrote the first draft which was commented, edited and improved by CQ and MT. There are no disputes over the ownership of the data presented in the manuscript and all contributions have been attributed appropriately.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Supporting information files.

References

- Abbott, K.R. (2010) Background evolution in camouflage systems: A predator-prey/pollinator-flower game. *Journal of Theoretical Biology*, **262**, 662–678.
- Aizen, M.A. & Basilio, A. (1995) Within and among flower sex-phase distribution in *Alstroemeria aurea* (Alstroemeriaceae). *Canadian Journal of Botany*, **73**, 1986–1994.
- Aizen, M.A. & Raffaele, E. (1996) Nectar production and pollination in *Alstroemeria aurea*: responses to level and pattern and flowering shoot defoliation. *Oikos*, **76**, 312–322.
- Aizen, M.A. (2001) Flower sex ratio, pollinator abundance, and the seasonal pollination dynamics of a protandrous plant. *Ecology*, **82**, 127–144.
- Arango, M.A., López-Portillo, J., Parra, V., Hernández-Salazar, L.T., Morales-Mávil, J.E. & Rico-Gray, V. (2012) Effect of the spider *Peucetia viridans* (Oxyopidae) on floral visitors and seed set of *Cnidocolus multibolus* (Euphorbiaceae). *Acta Botanica Mexicana*, **100**, 1–14.
- Bray, A. & Nieh, J. (2014) Non-consumptive predator effects shape honey bee foraging and recruitment dancing. *PLoS One*, **9**, e87459.
- Brechbühl, R., Kropf, C. & Bacher, S. (2010a) Impact of flower-dwelling crab spiders on plant-pollinator mutualisms. *Basic and Applied Ecology*, **11**, 76–82.
- Brechbühl, R., Casas, J. & Bacher, S. (2010b) Ineffective crypsis in a crab spider: a prey community perspective. *Proceedings of the Royal Society B*, **277**, 739–746.
- Briscoe, A.D. & Chittka, L. (2001) The evolution of color vision in insects. *Annual Review of Entomology*, **46**, 471–510.
- Chittka, L. (2001) Camouflage of predatory crab spiders on flowers and the color perception of bees (Aranida: Thomisidae/Hymenoptera: Apidae). *Entomologia Generalis*, **25**, 181–187.
- Chittka, L., Skorupski, P. & Raine, N.E. (2009) Speed–accuracy tradeoffs in animal decision making. *Trends in Ecology and Evolution*, **24**, 400–407.
- Clark, C.W. & Dukas, R. (1994) Balancing foraging and antipredator demands: an advantage of sociality. *The American Naturalist*, **144**, 542–548.
- Defrize, J., Théry, M. & Casas, J. (2010) Background colour matching by a crab spider in the field: a community sensory ecology perspective. *The Journal of Experimental Biology*, **213**, 1425–1435.
- Dicke, M. & Grostal, P. (2001) Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1–23.
- Dukas, R. (2005) Bumble bee predators reduce pollinator density and plant fitness. *Ecology*, **86**, 1401–1406.
- Dukas, R. & Morse, D.H. (2003) Crab spiders affect flower visitation by bees. *Oikos*, **101**, 157–163.
- Dukas, R. & Morse, D.H. (2005) Crab spiders show mixed effects on flower-visiting bees and no effect on plant fitness components. *Ecoscience*, **12**, 244–247.
- Fowler, R.E., Rotheray, E.L. & Goulson, D. (2016) Floral abundance and resource quality influence pollinator choice. *Insect Conservation and Diversity*, **9**, 481–494.
- Gadagkar, R. (1990) Evolution of eusociality—the advantage of assured fitness returns. *Philosophical Transactions of the Royal Society B*, **329**, 17–25.
- Goncalves-Souza, T., Omena, P.M., Souza, J.C. & Romero, G.Q. (2008) Trait-mediated effects on flowers: artificial spiders deceive pollinators and decrease plant fitness. *Ecology*, **89**, 2407–2413.
- Greco, C.F. & Kevan, P.G. (1994) Contrasting patch choosing by anthophilous ambush predators: vegetation and floral cues for decision by a crab spider (*Misumena vatia*) and males and females of an ambush bug (*Phymata americana*). *Canadian Journal of Zoology*, **72**, 1583–1588.
- Harder, L.D. & Aizen, M.A. (2004) The functional significance of synchronous protandry in *Alstroemeria aurea*. *Functional Ecology*, **18**, 467–474.
- Heiling, A.M., Herberstein, M.E. & Chittka, L. (2003) Crab-spiders manipulate flower signals. *Nature*, **421**, 334.
- Heiling, A.M. & Herberstein, M.E. (2004) Predator–prey coevolution: Australian native bees avoid their spider predators. *Proceedings of the Royal Society B*, **271**, S196–S198.
- Heiling, A.M., Chittka, L., Cheng, K. & Herberstein, M.E. (2005) Coloration in crab spiders: substrate choice and prey attraction. *Journal of Experimental Biology*, **208**, 1785–1792.
- Heiling, A.M., Cheng, K. & Herberstein, M.E. (2006) Picking the right spot: crab spiders position themselves on flowers to maximize prey attraction. *Behaviour*, **143**, 957–968.
- Higginson, A.D., Ruxton, G.D. & Skelhorn, J. (2010) The impact of flower-dwelling predators on host plant reproductive success. *Oecologia*, **164**, 411–421.
- Ings, T.C. & Chittka, L. (2009) Predator crypsis enhances behaviourally mediated indirect effects on plants by altering bumblebee foraging preferences. *Proceedings of the Royal Society B*, **276**, 2031–2036.
- Jones, E.I. (2010) Optimal foraging when predation risk increases with patch resources: an analysis of pollinators and ambush predators. *Oikos*, **119**, 835–840.
- Jones, E.I. & Dornhaus, A. (2011) Predation risk makes bees reject rewarding flowers and reduce foraging activity. *Behavioral Ecology and Sociobiology*, **65**, 1505–1511.
- Llandres, A.L. & Rodríguez-Girones, M.A. (2011) Spider movement, UV reflectance and size, but not spider crypsis, affect the response of honeybees to Australian crab spiders. *PLoS One*, **6**, e17136.
- Llandres, A.L., De Mas, E. & Rodríguez-Gironés, M.A. (2012) Response of pollinators to the tradeoff between resource acquisition and predator avoidance. *Oikos*, **121**, 687–696.
- Louda, S.M. (1982) Inflorescence spiders: a cost/benefit analysis for the host plant, *Haplopappus venetus* Blake (Asteraceae). *Oecologia*, **55**, 185–191.
- Morales, C.L., Arbetman, M.P., Cameron, S.A. & Aizen, M.A. (2013) Rapid ecological replacement of a native bumblebee by invasive species. *Frontiers in Ecology and the Environment*, **11**, 529–534.
- Morante, J. & Desplan, C. (2008) The color-vision circuit in the medulla of *Drosophila*. *Current Biology*, **18**, 553–565.
- Quintero, C., Corley, J.C. & Aizen, M.A. (2015) Weak trophic links between a crab-spider and the effective pollinators of a rewardless orchid. *Acta Oecologica*, **62**, 32–39.
- Ribas, A.C.A. & Raizer, J. (2013) Spiders do not affect fruit set in *Byrsonima intermedia* (Malpighiaceae). *Journal of Negative Results*, **10**, 1–5.

- Robertson, I.C. & Maguire, D.K. (2005) Crab spiders deter insect visitations to slickspot peppergrass flowers. *Oikos*, **109**, 577–582.
- Rodríguez-Gironés, M.A. & Bosch, J. (2012) Effects of body size and sociality on the anti-predator behaviour of foraging bees. *Oikos*, **121**, 1473–1482.
- Romero, G.Q. & Koricheva, J. (2011) Contrasting cascade effects of carnivores on plant fitness: a meta-analysis. *Journal of Animal Ecology*, **80**, 696–704.
- Romero, G.Q. & Vasconcellos-Neto, J. (2004) Beneficial effects of flower-dwelling predators on their host plant. *Ecology*, **85**, 446–457.
- Romero, G.Q., Antiqueira, P.A.P. & Koricheva, J. (2011) A meta-analysis of predation risk effects on pollinator behaviour. *PLoS One*, **6**, e20689.
- Ruhren, S. & Handel, S.N. (1999) Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, **119**, 227–230.
- Ssymank, A., Kearns, C.A., Pape, T. & Thompson, F.C. (2008) Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity*, **9**, 86–89.
- Suttle, K.B. (2003) Pollinators as mediators of top-down effects on plants. *Ecology Letters*, **6**, 688–694.
- Thery, M. & Casas, J. (2002) Predator and prey views of spider camouflage e both hunter and hunted fail to notice crab-spiders blending with coloured petals. *Nature*, **415**, 133.
- Wang, M.Y., Ings, T.C., Proulx, M.J. & Chittka, L. (2013) Can bees simultaneously engage in adaptive foraging behavior and attend to cryptic predators? *Animal Behavior*, **86**, 859–866.
- Weiss, M.R. (2004) Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. *Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution* (pp. 171–190) (ed. by L. Chittka and J. D. Thomson), p. 344. Cambridge University Press, Cambridge, UK.
- Yokoi, T. & Fujisaki, K. (2009) Hesitation behaviour of hoverflies *Sphaerophoria* spp. to avoid ambush by crab spiders. *Naturwissenschaften*, **96**, 195–200.

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