

Picking the right spot: crab spiders position themselves on flowers to maximize prey attraction

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(Accepted: 12 June 2006)

Summary

In plant-pollinator interactions, pollinating insects provide reproductive service to plants and receive food rewards. Flowers advertise the presence of nectar or pollen through various characteristics, including visual displays. In daisies (*Chrysanthemum frutescens*), the center of the inflorescence appears as a UV-absorbing bull's-eye that attracts pollinators, for example honeybees. *Thomisus spectabilis* crab spiders occupy daisies and prey on honeybees. They typically position themselves on the lingulate florets of daisies and create a color contrast that deceives honeybees. Honeybees prefer daisies with a *T. spectabilis* on the lingulate florets to vacant daisies. In contrast, when offered the choice between a vacant daisy and a daisy whose center was covered by a *T. spectabilis*, honeybees preferred the vacant daisy. Similarly, honeybees were deterred by daisies whose center was covered by lingulate daisy florets making a rectangle about the size of a *T. spectabilis*. Covering the lingulate florets of daisies by a rectangle of lingulate daisy florets, however, neither attracted nor repelled honeybees. Honeybees seem to rely on the visibility of the daisy center to locate food reward and, by positioning themselves on the lingulate florets of daisies, *T. spectabilis* exploit these sensory biases of prey.

Keywords: *Thomisus spectabilis*, *Apis mellifera*, flower signal, communication, signal exploitation.

Introduction

The interaction of plants and pollinators is mutually beneficial: plants receive a reproductive service, while the pollinators receive a food reward in

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the form of nectar and/or pollen (e.g., Harder et al., 2001). Plants advertise the presence of rewards by various cues, such as flower size and flower color (Chittka & Kevan, 2005). Moreover, visual features of flowers contrast against the background, whether it is background vegetation (Chittka et al., 1994; Endler, 1999) or parts of the flower (Lunau, 2000). Such spots, lines, and blotches of contrasting color on the petals of inflorescences signal pollen or nectar rewards (Lunau, 2000). In addition to the shape of such nectar guides (Medel et al., 2003), nectar guide coloration *per se* affects pollinator behavior (Heuschen et al., 2005). In radially symmetric inflorescences such as daisies (Asteraceae), the center of the flowers has the appearance of a bull's-eye, which contains dark ultraviolet-absorbing pigments (flavones) that contrast against the lingulate florets of the daisy (Lunau, 2000; Gronquist et al., 2001) and increase the attractiveness of an inflorescence from a distance (Free, 1970).

The evolution of signals affects the evolution of receiver characteristics and *vice versa* (Endler & Basolo, 1998). Receiver characteristics that can respond to selection include the perception and processing of the signal and the behavior of the receiver (Endler, 1999). Receiver characteristics are often exploited by third parties at a cost to the signaler or the receiver (Zuk & Kolluru, 1998; Haynes & Yeorgan, 1999). A well known example of such sensory exploitation is brood parasitism by cuckoos (*Cuculus canorus*), where the begging calls produced by cuckoo nestlings elicit parental care in birds of other species (Kilner et al., 1999).

In plants, crab spiders such as *Thomisus spectabilis*, can exploit sensory signals mediated between plants and their pollinators to attract prey. These spiders ambush pollinators on flowers, and select flowers based on cues that also attract insects (Greco & Kevan, 1994; Chien & Morse, 1998), such as honeybees (Heiling et al., 2004). Female *T. spectabilis* can change their body color between white and yellow (Heiling et al., 2005b). To the UV-perceiving eyes of a bee, the UV-reflecting white spiders against less UV-reflecting lingulate florets of inflorescences generate a strong color contrast that lures honeybees deceptively (Heiling et al., 2003; Heiling et al., 2005b). *T. spectabilis* are commonly found on radially symmetric inflorescences, such as daisies (*Chrysanthemum frutescens*), and are almost always found on the peripheral lingulate florets of the inflorescence, leaving its center with the plant's reproductive organs visible to oncoming pollinating insects. In our recent research, we offered spiders the choice between differently colored

flowers, and found that *T. spectabilis* avoided occupying floral backgrounds on which they generate a color contrast that deters honeybees. Instead, they select floral background colors according to their own coloration to optimize their signaling effect to oncoming pollinators/prey (Heiling et al., 2005b). Our results parallel studies which have revealed that birds prefer positions at which the prevailing light conditions maximize the signaling effect of their coloration during mating (Endler & Théry, 1996; Gomez & Théry, 2004). Similarly, crab spiders, which are restricted to a small prey capture arena such as an inflorescence, might position themselves in a way that optimizes their signaling effect. Here, we experimentally investigate this idea by examining the effect of spider position within their capture arena on the response of honeybees. We predict that while spiders naturally positioned on the lingulate florets of daisies will deceive honeybees (Heiling et al., 2003), spiders atypically positioned in the center of inflorescences will repel prey from the spider occupied inflorescences.

Material and methods

We collected *Thomisus spectabilis* crab spiders in Brisbane, Australia, and transferred them into the laboratory of Macquarie University, Sydney, Australia. The spiders were kept on a 12:12 hours light cycle, with the temperature ranging from 20 to 25°C. They were fed live crickets (*Acheta domestica*) and *Drosophila* flies every week and watered daily. In our experiments, we used white *T. spectabilis* that reflect light in the ultraviolet range of the spectrum (see Figure 1).

Honeybees, *Apis mellifera* (Apidae), which were introduced into Australia from Europe about 200 years ago, were kept in an outdoors hive at the University. We trained them to visit a feeding station, providing a 25% sucrose solution. The feeding station was removed and the honeybees were offered the choice between two stimuli, a manipulated inflorescence and an unmanipulated inflorescence. We used *Chrysanthemum frutescens* (Asteraceae) daisies with white lingulate florets and an UV-absorbing yellow center (see Figure 1). These are not native to Australia, but are a natural substrate of *T. spectabilis* and attract honeybees (Wignall et al., 2006).

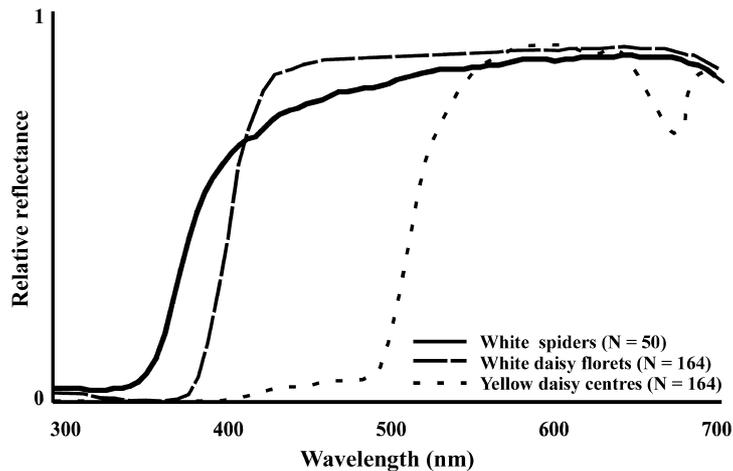


Figure 1. Median spectral reflectance of white *Thomisus spectabilis* (data taken from Heiling et al., 2005b) and the white lingulate florets and the yellow centers of *Chrysanthemum frutescens* (data taken from Heiling et al., 2004), measured over the range from 300 nm to 700 nm.

Stimuli

Experiment 1. – To investigate whether a crab spider unnaturally positioned in the center of the daisy affects honeybee behavior, we offered honeybees the choice between a vacant *C. frutescens* and a daisy with the center occupied by an adult female *T. spectabilis* ($N = 38$). The spiders were anaesthetized with carbon dioxide before being placed into the daisy center, to eliminate any influence of spider behavior on the choice of honeybees (Figure 2A). We compared these data to the results of a previous experiment (Heiling et al., 2003) in which we tested (using the same procedures) the behavior of honeybees when confronted with spiders positioned on the lingulate florets of a white daisy (Figure 2B).

Experiment 2. – To investigate whether spider reflectance and/or body shape is crucial for repelling honeybees, or whether any covering of the daisy center would repel honeybees, we offered them the choice between an unmanipulated *C. frutescens* and a daisy with the center covered by lingulate florets ($N = 27$; Figure 2C). This covering, forming a rectangle, consisted of three freshly cut pieces of lingulate florets from randomly selected daisies arranged parallel in the center of the inflorescences. Each covering was used only once in the experiment and its size corresponded to the average size



Figure 2. Stimuli used in the experiments to test honeybee response to the presence of *T. spectabilis* (A, B) and lingulate florets (C, D) in the center or on the lingulate florets of *C. frutescens*.

of adult female *T. spectabilis* (length: mean \pm SD = 10.19 mm \pm 0.579, $N = 63$; width of the ophistosoma: mean \pm SD = 8.342 mm \pm 0.446, $N = 38$).

Experiment 3. – In this control experiment, we gave honeybees the choice between a vacant daisy and a daisy whose lingulate florets were covered in part by a rectangle formed from randomly selected lingulate daisy florets. The size and shape of this covering was the same as in Experiment 2 (Figure 2D). This experiment revealed whether the addition of lingulate florets without covering the daisy center affects the behavior of honeybees ($N = 21$).

Experimental procedure

The stimuli were placed in black plastic lids (diameter = 4 cm) and covered using Glad Wrap™, which is a clear wrap foil that consists of polypropylene. The foil is permeable to all wavelengths of light above 300 nm, with less than 5% attenuation. The stimuli were covered, as flower odors are known to affect honeybee choice (Pelz et al., 1997; Laska et al., 1999; Heiling et al., 2004). Daisies were assigned randomly to a pair and each pair of daisies was horizontally positioned on a rectangle (18 × 13 cm) of black cardboard, with a distance of 8 cm between the daisy centers. We recorded the first visit (landing) of a honeybee on either of the two daisies and then removed the bee from the population. That is, each subject bee was only tested once.

The spiders used in Experiment 1 were measured (body length and width of the opisthosoma) to the nearest 0.1 mm and weighed to the nearest 0.1 mg. Moreover, we measured the center diameters of the daisies used in Experiments 1 and 2, as the size ratio of the daisy center to an object covering the center might influence its signaling effect. Spiders, daisies and lingulate floret coverings were used only once in the experiments, the lids were cleaned and the backgrounds renewed after each trial.

Results

The mean body mass of *Thomisus spectabilis* presented in the center of daisies did not differ from the mass of spiders used in a former study (Heiling et al., 2003) that tested for the effect of spider presence on the lingulate florets of daisies on the response of honeybees (mean ± SD = 0.134 g ± 0.036, $t_{61} = -0.12$, $p = 0.91$). Moreover, the center diameters of daisies did not differ between the experiments that tested for the effect of spiders and petal cuttings in the daisy center (mean ± SD = 10.346 ± 0.524, $t_{63} = 0.17$, $p = 0.87$).

The presence of *T. spectabilis* in the center and on the lingulate florets of daisies clearly affected the response by honeybees (Figure 3). In 26 out of 38 cases in which the center of the daisy was occupied by a crab spider, honeybees landed on the vacant daisy (two-tailed binomial $p = 0.03$). This means that the presence of *T. spectabilis* in the center of daisies clearly deterred honeybees. In contrast, when confronted with a vacant daisy and a daisy

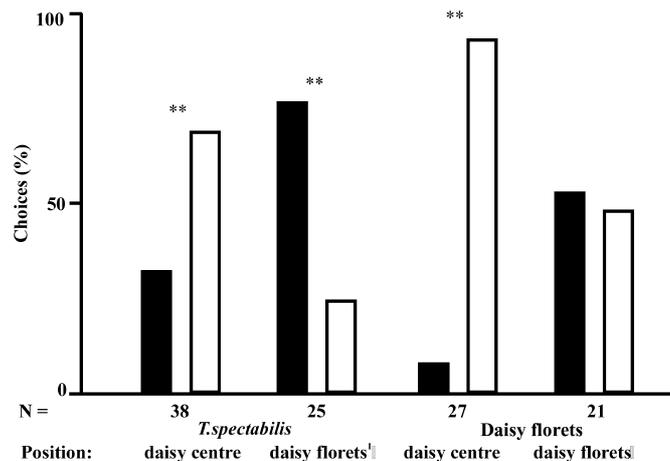


Figure 3. The percent of choice (first landing) of honeybees when confronted with a manipulated (black blocks) and a vacant *C. frutescens* (white blocks). Four different paired choice tests are shown, with the appearance of the manipulated inflorescences altered by the presence of *T. spectabilis* or floret cuttings on the lingulate florets or in the center of inflorescences. When the center was covered either by a spider or by lingulate florets, the inflorescence was rejected by honeybees. ** $p < 0.01$. ¹Results are taken from Heiling et al. (2003).

with the lingulate florets occupied by a crab spider, honeybees preferred the spider-occupied daisy, choosing the vacant daisy in only 6 out of 25 cases, (two-tailed binomial $p = 0.01$; data taken from Heiling et al., 2003).

When lingulate florets covered the center of the daisy, the vacant daisy was again more likely to be chosen by honeybees (25 out of 27 cases, two-tailed binomial $p < 0.0001$). In contrast, daisies whose lingulate florets were covered by floret cuttings were chosen about equally often as vacant daisies (11 out of 21 cases; two-tailed binomial $p = 1.0$).

Discussion

The position of predatory crab spiders *Thomisus spectabilis* on the daisy *Chrysanthemum frutescens* clearly influenced the decision of approaching honeybees to enter a daisy. Female spiders atypically positioned in the center of daisies deterred honeybees from daisies. In this case, vacant daisies were preferred over daisies occupied by spiders. These results add to a previous study (Heiling et al., 2003) that revealed an attractive effect of *T. spectabilis*

that are naturally positioned on the lingulate florets of daisies. Our experiments further revealed that covering the center of daisies by a rectangle of lingulate daisy florets corresponding to the size of spiders also deterred honeybees from daisies. In contrast, covering the lingulate florets of daisies by a rectangle of lingulate florets had neither an attractive nor a deterrent effect on the choice of honeybees.

In our study, the UV-absorbing daisy centers (Figure 1) displayed the presence of reward. The deterring effect of a covered center indicates that, for locating reward, honeybees rely on the visibility of the reproductive organs of the inflorescence. When the center of the daisy was covered, honeybees were deterred. One reason for the deterred effect might be that a covered center makes it more difficult for the bee to access the reward. The honeybees used in our study were not naïve and might have experienced daisies as a natural food reward. Thus, an alternative explanation for the deterrent effect of daisies with the center occupied by crab spiders might be that these inflorescences did not visually resemble natural daisies. Although learning effects might explain why bees rejected daisies with a center covered by crab spiders, they do not explain why crab spiders occupying the lingulate florets of daisies attract honeybees. This deceptive effect of crab spiders on the lingulate florets of daisies was obviously the result of sensory biases of honeybees, rather than learned.

We attribute the deterrent effect to the centers being covered. Some alternative explanations may be entertained, but we find these unlikely. First, differences in olfactory cues are unlikely to have played any role. That is because the covering of transparent plastic foil blocked all olfactory cues emanating from daisies and spiders. Second, the UV-absorbing center of daisies (Figure 1) was not completely covered, leaving a small area surrounding the spiders or lingulate florets visible to approaching honeybees (see Figure 2). In general, visual signals are perceived if they contrast against background color (Endler, 1993; Spaethe et al., 2001). It may be argued that the degree of color contrast in the center of the daisy was the crucial factor in repelling bees. This hypothesis should predict a larger deterrent effect of a spider on the daisy center than lingulate florets on the daisy center. The spider reflects strongly in the UV range, much stronger than the daisy (Heiling et al., 2005b, Figure 1). Furthermore, Figure 2 in the present paper shows that the spider leaves more of a gap than the lingulate florets, thus making the contrast more apparent to approaching bees. The results (Figure 3), however, indicate that

the lingulate florets had a numerically (but not statistically) stronger deterrent effect, refuting this contrast hypothesis.

In terms of prey capture, it is clearly beneficial for spiders to occupy the lingulate florets of daisies and avoid covering the daisy center. However, spider positioning on inflorescences might also affect the risk of predation. Natural enemies of spiders include predators such as birds (Gunnarsson, 1998; Gruner, 2005) and wasps (Toft & Rees, 1998; Culin & Robertson, 2003). Behavioral strategies of sit-and-wait predatory spiders for avoiding predation include minimizing the length of the escape route or hiding (Henderson & Elgar, 1999; Heiling, 2004). When disturbed, *T. spectabilis* quickly move out of the field of view beneath the lingulate florets of daisies (Heiling, pers. obs.). By positioning themselves on the peripheral parts of the inflorescence they minimize their escape route. Consequently, the natural positioning of *T. spectabilis* might be beneficial for avoiding predators as well as capturing prey.

Not all crab spiders, however, sit on the lingulate florets of inflorescences. In contrast to adult *T. spectabilis*, juveniles often occupy the center of daisies (Heiling, pers. obs.). Small juveniles might emit a weaker signal and thus not deter honeybees even if part of the inflorescence center is invisible. The size-dependent differences of positioning on inflorescences between adult and juvenile *T. spectabilis* may also be due to morphological characteristics. Juveniles have shorter fangs than adults and due to their shorter striking range they may have a greater chance of grabbing their prey when staying in the center of the daisy, where reward-seeking insects typically land. This means that by staying in the center they may have a greater chance of approaching prey in time. Alternatively, juveniles staying in the center of the daisy may starve or be predated and the only spiders that stay alive until they reach adulthood are those with a preference for the periphery of daisies. Investigating these ideas, however, will need further research. In other species such as the closely related *T. onustus*, adult crab spiders typically position themselves in the center of daisies (*Leucanthemum vulgare*, Théry et al., 2005), while *Misumena vatia*, observed on rough chervils (*Chaerophyllum temulum*) and groundsels (*Senecio vernalis*), position themselves on all parts of the inflorescences (Chittka, 2001). Both species prey on honeybees and are well camouflaged from the view of honeybees and bird predators, with *T. onustus* being cryptic only on the center of the inflorescence (Chittka, 2001; Théry et al., 2005). The camouflage of *T. onustus* and *M. vatia* means

in all likelihood that their covering the inflorescence center neither deters prey nor attracts predators. Female *T. spectabilis* use an opposite signaling strategy, appearing conspicuous to honeybees and attracting prey to the inflorescence by leaving its center visible to approaching prey and at the same time creating an attractive color contrast against the floral background (Heiling et al., 2003, 2005a, b). The cost of such a strategy may be visibility to predators such as birds (Heiling et al., 2005a). Differences across species in the how they position themselves on inflorescences are thus understandable in light of differences in visual signaling strategies.

Acknowledgements

We thank Lars Chittka for comments on the manuscript and Mark Mirza for providing photographs. This work was supported by Macquarie University and the Australian Research Council (DP0449673).

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