Stingless bee interception is not affected by variations in spider silk decoration

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Abstract.   The functional significance of web decorations in orb-web spiders has been an area of intense study for well over a hundred years. Two main hypotheses, (prey attraction and predator avoidance) have had intermittent support and criticism. By varying the decoration pattern, spiders minimize the potential predation costs of constructing a highly visible signal and deter potential prey such as bees from associating decorations with danger. The prey attraction hypothesis implies that as the signal changes, so should the response of the intercepting insects. In this study, I tested the response of bees to varying decoration patterns. I show that stingless bees (*Trigona carbonaria*) respond to the silk decorations of *Argiope keyserlingi* Karsch 1878 in similar ways irrespective of the pattern of decorations. I also demonstrate that the likelihood of prey hitting the capture area is greater than that of hitting the hub area in decorated webs. Since stingless bees respond similarly to different levels of signal strength, I conclude that variation in decorations does not affect prey interception.

Keywords: *Argiope keyserlingi*, *Trigona carbonaria*, decorations, stabilimenta, predator-prey interactions

Orb-web spiders of up to 78 species from 22 genera adorn their webs with extra silk structures called stabilimenta or silk decorations (Herberstein et al. 2000). The functional significance of these structures has been an area of prolonged interest, and there are two main explanations for the evolution and continued presence of silk decorations. The prey attraction hypothesis suggests that since silk decorations reflect light in the ultraviolet part of the light spectrum, in a manner similar to floral nectar guides, flying insects are attracted to the web (Craig & Bernard 1990). The predator avoidance hypothesis suggests that silk decorations are visible to potential predators such as birds and wasps and therefore deter predators either by camouflage or by web protection (Blackledge & Wenzel 2001; Eberhard 2008). Despite a number of studies investigating the function of silk decorations, there is still substantial controversy over their function (Bruce 2006).

While decorations are seen in many species, spiders of the genus *Argiope* occur all over the world and many species in this genus have been studied extensively (Herberstein et al. 2000). These spiders build decorations in the form of bands radiating from the center of the web toward the periphery. These bands vary in length and pattern within individuals as well as across individuals. For example, in the Australian species *Argiope keyserlingi* Karsch 1878, a maximum of four bands is seen, though five or six bands are known to occur rarely (Rao et al. 2007; pers. observ.). Adult *A. keyserlingi* shows the following patterns of decorations: no bands, 1, 2, 3, and 4 bands, while juvenile *Argiope* generally build discoid decorations (Rao et al. 2007). Most studies have focused on variation in decoration between individuals by comparing decorating and non-decorating spiders within the same population; e.g., the Australasian species *Argiope appensa* Walckenaer 1842 (Hauber 1998; Herberstein 2000). While frequency of decoration patterns has been recorded under field conditions (Hauber 1998; Craig et al. 2001; Rao et al. 2007), laboratory tests generally involve a binary choice test (the

Neotropical species *Argiope argentata* (Fabricius 1775) [Craig & Bernard 1990], the Southeast Asian *Argiope versicolor* (Doleschall 1859) [Seah & Li 2002], *A. keyserlingi* [Bruce & Herberstein 2005]). Studies that take into account variation in decorations generally quantify decoration presence, area or length (the Neotropical species *Argiope aurantia* Lucas 1833, *Argiope trifasciata* (Forsskål 1775) [Blackledge 1998], *A. argentata* [Craig et al. 2001], *A. versicolor* [Seah & Li 2002; but see Seah & Li 2001]) and ignore the information inherent in the pattern of the decoration. Furthermore, the majority of studies on the function of silk decorations have been from a visual perspective, with the underlying assumption that the decorations are a signal to visually orienting insects and birds as the primary receivers (Craig & Bernard 1990; Bruce et al. 2005; but see Walter et al. 2008).

Silk decorations are highly visible to insects such as bees. Not only do they reflect light in the UV part of the spectrum (Craig & Bernard 1990), they also form a strong contrast against the mostly dull background (Bruce et al. 2005; Rao et al. 2009), which enhances their visibility. Insects such as bees possess a trichromatic photoreceptor system that allows them to see color. Bees have light receptors sensitive in the UV (300–400 nm), blue (400–500 nm) and green (500–600 nm) spectra of light. Furthermore, the sensitivity of this UV-receptor is significantly higher than the green or blue receptor (Briscoe & Chittka 2001). Bees also have innate preferences for certain colors and patterns, and these preferences have coevolved with flower color and patterns (Biesmeijer et al. 2005). Stingless bees prefer dark centers, radiating stripes and spots (Biesmeijer et al. 2005) and honeybees show an innate preference for the kind of radiating patterns normally seen in flowers (Lehrer et al. 1995). Therefore a spider that displays patterns similar to those found among flowers would benefit by exploiting the sensory biases of its insect prey. Tso et al. (2002) showed that a brightly colored form of the giant wood spider *Nephila maculata* (Fabricus 1793) received more prey that of a melanic form (Tso et al. 2002). Since the spiders and decorated webs are visible and contain flower-like characteristics, it is possible that insects such as bees intercept the web in error.

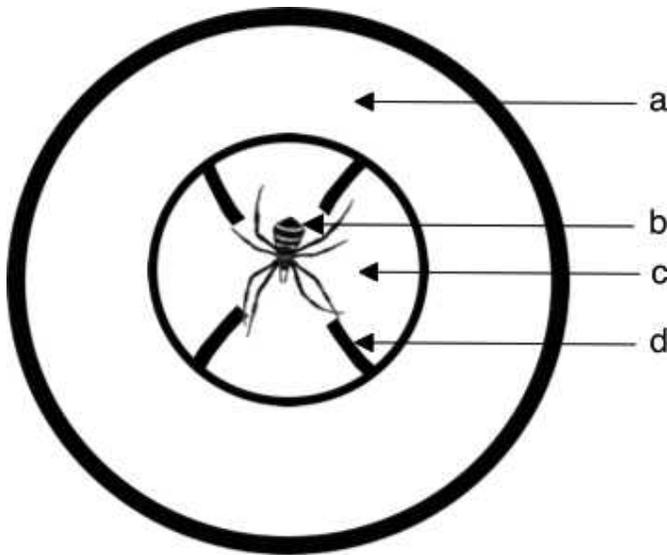[1] Current Address: Inbioteca, Apartado Postal 250, Universidad Veracruzana, Xalapa 91090, Mexico.

Figure 1.—Schematic representation of the web of *Argiope keyserlingi*. a: Capture area, b: Hub area, c: Position of spider, d: Decorations.

Figure 2.—Schematic representation of the experimental setup.

Furthermore, bees that forage 'impulsively' (i.e., make inaccurate decisions quickly) have been shown to benefit in the long run (Burns 2005), and trade-off foraging speed for accuracy (Chittka et al. 2003).

Variation in decoration pattern within a species may be a result of the trade-off between prey attraction and protection from predators (*A. trifasciata*: Blackledge & Wenzel 2001; *A. keyserlingi*: Bruce et al. 2001). There are two main advantages to varying the pattern of decorations: 1) fast learning potential prey, such as bees, are more likely to avoid consistently decorated webs (Craig 1994) and 2) potential predators, such as araneophagic jumping spiders and preying mantids, are prevented from associating decorations with spider prey (*A. keyserlingi*: Bruce et al. 2001; *A. versicolor*: Seah and Li 2001). However, variation in decoration patterns could also be due to a scarcity in silk reserves (*Argiope aetheroides* Yin et al. 1989 [Tso 2004]; *Argiope bruennechi* (Scopoli 1772); *Argiope sector* (Forsskål 1776); *A. keyserlingi* [Walter et al. 2008]) or that decorating behavior has a heritable component (*A, argentata* [Craig et al. 2001]). Most *Argiope* spiders show variation in decoration building, both in frequency of decorations as well as in the patterns of decorations, with the exception of *Argiope picta* L. Koch 1871, which decorates its webs obligately (Bruce & Herberstein 2005). The prey attraction hypothesis predicts an increase in insect interception rates due to the presence of decorations. If the decorations function as a deceptive signal to flower-seeking insects, then the presence of a stronger signal should elicit a greater response. This prediction is supported by the finding that bees trained to certain reward-bearing signals show a receiver bias for exaggerated signals (Naug & Arathi 2007). Furthermore, if the decorations attract insects, then there should be more interception in the hub area than the capture area, since decorations in *Argiope* rarely extend beyond the hub (Eberhard 1990).

In this study I tested the response of potential prey to variation in decoration patterns. Specifically, I asked if stingless bees respond differentia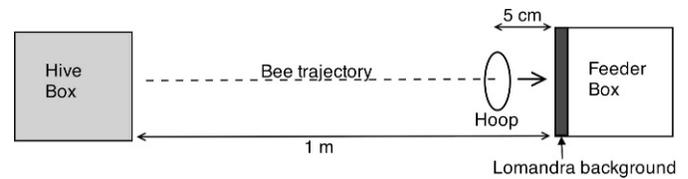lly to this visual signal, which varied in intensity and pattern. I tested if the location of insect interception within the web (i.e., between hub and capture area) differs when insects are presented with a low signal versus a high signal.

METHODS

Study species.—A*rgiope keyserlingi*, also known as the St. Andrew's Cross spider, is an orb-web weaver distributed along the eastern coast of Australia (Levi 1983; Platnick 2009) in a wide variety of habitats, ranging from rainforest margins to urban gardens. It is locally abundant and typically found on long-leaved bushes such as *Lomandra longifolia* and *Pandanus* sp. (Rao et al. 2007). Mature spiders build silk decorations in the form of zigzag deposits of silk (hereafter referred to as 'bands') stretching outward from the center of the web (Fig. 1), while circular silk decorations are typically found only in juveniles' webs. A maximum of four diagonal bands is seen in this species (Rao et al. 2007).

Trigona carbonaria Smith 1863 (Hymenoptera: Apidae) are stingless bees found all along the eastern coast of Australia and occur in the same habitat as *A. keyserlingi*. Typically, these insects nest in hollow logs (Michener 1961). The bees are quite small (body length of worker bees: 3.9–4.3 mm), and average colonies contain a single queen and hundreds of workers (Dollin et al. 1997). Stingless bees of the genus *Trigona* are known to be common prey of *A. argentata* (Craig et al. 2001).

Experiment set up.—A commercially available hive box of *T. carbonaria* (Russell and Janine Zabel Pty. Ltd.) was set up on a table in a semi-enclosed greenhouse located on the campus of Macquarie University in Sydney, Australia. The greenhouse had mesh walls and a transparent plastic roof (VP Structures Pty. Ltd., Australia). Bees were trained to approach a feeder with sugar water (sugar:water = 1:3) that was placed 1 m away from the hive (Fig. 2).

Subsequently, I trained the bees to fly through an empty hoop (28 cm diam.) en route to the feeder. The empty hoop was considered to be the control and a measure of bee activity or hive activity, since the number of bees that forage daily is known to depend on environmental conditions (Heard & Hendrikz 1993). For the experiments, I then swapped the empty hoop with hoops containing female *A. keyserlingi* webs. Spider webs were affixed to hoops by pressing one side of the hoop (with layer of glue) onto a web, and cutting away the extraneous parts. Approaching bees always encountered the web against a background of *Lomandra* photographs, which have similar color properties to those of actual plants (Hoese et al. 2006). Using *Lomandra* photographs rather than real plants as background offers the advantage of having the background as a constant for all experiments, thereby eliminating any effect of plant variation on bee behavior.

There were four treatments based on the signal strength: 1) webs with no decorations (0-band; the weakest signal), 2) webs

Table 1.—Mean ($\pm$ SD, $n = 15$ trails) number of bee interceptions in each of four decoration treatments.

Number of decoration bands	Bee interceptions (mean $\pm$ SD)
0	6.8 $\pm$ 2.01
1	7.2 $\pm$ 3.49
2	8.7 $\pm$ 2.96
4	7.5 $\pm$ 2.50

with one band (1-band), 3) webs with two bands (2-band) and 4) webs with four bands (4-band; the strongest signal). These treatments were chosen to reflect the most common decoration patterns found in the field (Rao et al. 2007). All treatments included spiders of similar body lengths on their webs. Approaching bees were monitored during fifteen 1-min trials for each treatment, and all bees intercepting the web were counted. Bees that did not intercept the web were not counted. The order of the treatments and control was randomized, and there was a delay of at least 10 min between two consecutive treatments. In all treatments, I ensured that the stingless bees flew in a northerly direction and were on their foraging flight, since previous experiments had revealed that bees were most responsive in this context (Rao et al. 2008).

Since the data were normally distributed, I conducted an analysis of variance on the number of bees that hit the web (termed as interceptions and weighted by bee activity), using the signal strength treatments (i.e., the number of decoration bands) as independent variables. I tested for homogeniety of variance using Bartlett's test and normality with the Kolmogorov-Smirnov test. For two of the treatments (webs with 2-bands - low signal and with 4-bands - high signal), I noted the location of interception within the web by recording whether the bees intercepted the hub or the capture area. These two treatments were chosen because the 2-band decoration is one of the most common patterns seen in field conditions, and the 4-band pattern is the maximum number of bands usually seen in this species (Rao et al. 2007). I compared the interception rates between the hub and the capture area using a Mann-Whitney U test.

RESULTS

Signal strength.—The number of decoration bands (0, 1, 2 or 4) did not significantly affect the number of bees intercepted (one way ANOVA, $F_{3,59} = 1.07$, $P = 0.37$; Table 1, Fig. 3).

Interception location.—Bees intercepted the capture area at significantly higher rates than the hub (4-bands; Mann-Whitney U test, $U = 12.5$, $n = 15$, $P < 0.0001$; 2-bands, Mann-Whitney U test, $U = 45$, $n = 15$, $P < 0.01$; Table 2, Fig. 4). In the hub area, more bees intercepted the web in the 2-band treatment than in the 4-band treatment (Mann-Whitney U test, $U = 60.5$, $n = 15$, $P = 0.03$; Fig. 4). However, in the capture area there was no difference in the number of bees intercepting the web across the 4-band or 2-band treatments (Mann-Whitney U test, $U = 108.5$, $n = 15$, $P = 0.88$).

DISCUSSION

Bee interception on spider webs did not change with varying signal strengths, ranging from the 4-band to the 0-band decoration pattern. More bees intercepted the capture area than the hub area in both low signal and high signal treatments.
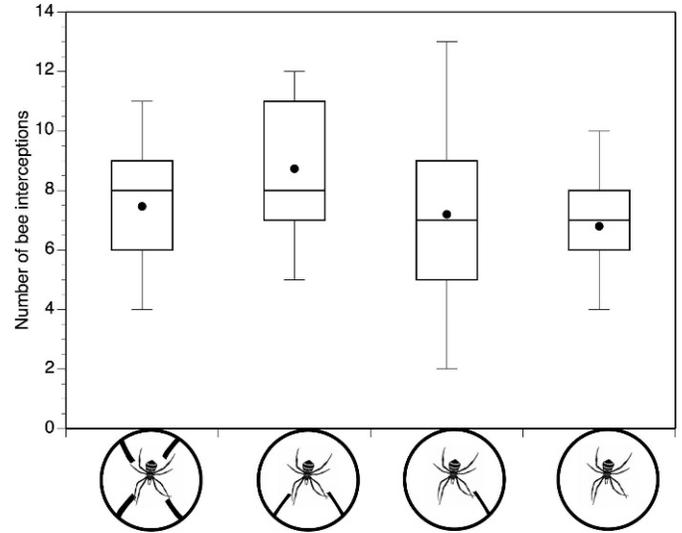

Figure 3.—There was no significant difference in the number of bees intercepting webs with 0, 1, 2, or 4 bands of silk decoration. The figure shows box plots with median (center of box), 25 and 75 percentile (edges of box) and ranges (whiskers); dots in the center represent the means.

The prey attraction hypothesis predicts an increase in the rate of insect interceptions with the presence of decorations, and I therefore expected a proportional increase in interception with an increase in the strength of the signal. In this study, I showed that stingless bees do not discriminate between the different decoration patterns and intercept the webs at similar rates. These results are not in congruence with other studies that have demonstrated a prey-attraction function to silk decorations (*A. argentata*: Craig & Bernard 1990; *A. versicolor*: Li 2005; *Argiope aemula* (Walckenaer 1841): Cheng & Tso 2007). This study adds further support to an earlier study using the same system demonstrating that stingless bees respond more strongly to spiders than to silk decorations (Rao et al. 2008). This implies that *A. keyserlingi* varies its decoration in order to decrease the possibility of learning in bees (*sensu* Craig [1994]). With respect to location of interceptions, more bees intercepted the capture area than the hub with both low and high signal treatments, suggesting that decorations do not attract bees to the hub. However, insect attraction by decorations could be a function of the distance from which the insect sees the web, and the decorations may draw bees in as they go about foraging. Once they get close enough, bees may respond by changing their trajectory towards the periphery. There is some support for this hypothesis by the result that more bees intercepted the hub in the low signal treatment than the high signal treatment

Table 2.—Differences in the mean number of stingless bee interception between different locations (hub or capture area) in 4-band and 2-band treatments (Mann-Whitney U test, $n = 15$).

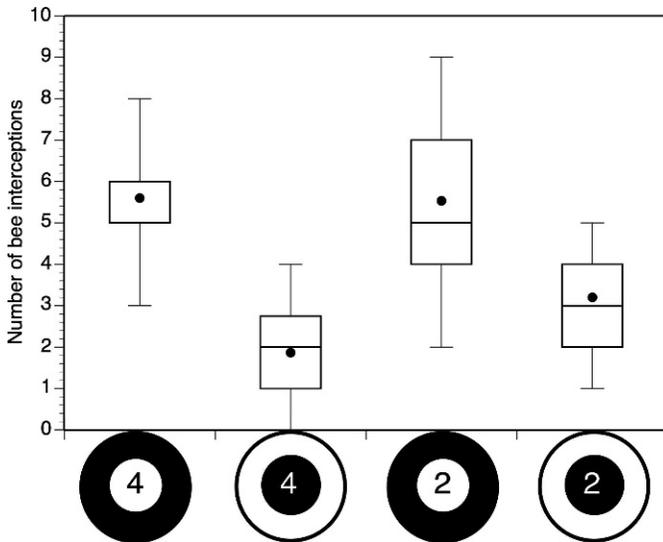| Decoration bands	Hub Area (mean $\pm$ SD)	Capture Area (mean $\pm$ SD)	U	P
4-bands	1.8 $\pm$ 1.5	5.6 $\pm$ 2.1	12.5	< 0.05
2-bands	3.2 $\pm$ 1.5	5.5 $\pm$ 2.3	45.0	< 0.05

Figure 4.—Bees were more likely to intercept the hub than the capture area when corrected for area. Y-axis shows the number of bees that intercepted the web per hub area. Figure shows box plots with median (center of box), 25 and 75 percentile (edges of box) and ranges (whiskers); dots in the center represent the means. For X-axis, inner circle is darkened to depict the hub, outer circle is darkened to depict capture area, and number in center of the circles refers to number of decoration bands.

(Fig. 3), but there was no difference in the number of bees that intercepted the capture area in both treatments. Further experiments should reveal the precise effect of decorations on the trajectories of bee flight.

Most studies that address the effect of decorations on prey generally compare webs with and without decorations by reporting the area or length of decorations, providing little information about the pattern of the decoration. However, the pattern of decorations influences signal strength in two ways. First, there can be an increase in amount of silk used in building decorations without changing the pattern (e.g., by making the bands longer rather than increasing the number of bands). Second, there can be an increase in signal strength by changing the pattern of decoration when more bands are added. In this study, the four treatments represented both a change in the amount of silk incorporated into the decorations as well as a change in the pattern.

Since stingless bees do not respond to variation in decoration pattern and overall signal strength, there may be other explanations for why spiders vary the size and number of decorating bands (Bruce 2006). For example, the decorating frequency displayed by an individual spider is inherited from both parents (Craig et al. 2001). It has been shown to depend on ambient light conditions, with spiders in dim light more likely to build decorations (*A. versicolor*: Seah & Li 2002; *A. keyserlingi*: Herberstein & Fleisch 2003), and silk reserves, with a threshold of silk in the aciniform glands beyond which the spider is less likely to build decorations (Tso 2004).

There are limits to drawing general conclusions from this study. I used a single species of spider and a single species of prey. The patterns of interception seen here may be species-specific, and decorating spiders may be targeting other prey species entirely. Stingless bees have previously been used in

tests of decoration function with mixed results (Craig et al. 2001, Bruce & Herberstein 2005). *Trigona carbonaria* in particular did not respond selectively to different decorations in Y-maze experiments (Bruce & Herberstein 2005), and in another study, *T. carbonaria* responded more explicitly to the presence of the spider than to decorations (Rao et al. 2008). This suggests that *T. carbonaria* may not be susceptible to the visual signal created by web decorations. Further experiments with other model prey species may yield a better understanding of the influence of decoration variation on potential receivers.

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