The evolution of cryptic spider silk: a behavioral test

Todd A. Blackledge and John W. Wenzel
Department of Entomology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA

Phylogenetic patterns of change in spider silk coloration provide insight into the selective pressures directing evolution of silks. Trends toward evolution of silks with low reflectance of ultraviolet (UV) light suggest that reduced UV reflectance may be an adaptation to reduce visibility of webs to insect prey. However, a test of the visibility of primitive and derived spider silks is lacking. Several genera of orb-weaving spiders include conspicuous designs of silk, called "stabilimenta," at the center of their webs. Due to their large size, stabilimenta present signals that insects can use to avoid webs. Unlike other silks in the orb web, which reflect little UV light, evolutionarily derived stabilimentum silk retains a bright UV reflectance. But, unlike primitive silks, stabilimentum silk also reflects large amounts of blue and green light. We compared the visibility of primitive tarantula silks and derived stabilimentum silks to insects by using the ability of honey bees to learn to forage at targets of spider silk. We found that the unique spectral properties of stabilimentum silk render it cryptic to insects and that primitive silks are more visible to bees. Our findings support a hypothesis that the coloration of stabilimentum silk is an adaptation to reduce the ability of insects to avoid webs and that ancient biases in the color vision of insects have acted upon the evolution of spider silk coloration through sensory drive. But our findings question the emphasis on UV reflectance alone for visibility of spider silks to insects. *Key words:* color vision, orb web, sensory drive, spiders, stabilimenta, ultraviolet light. *[Behav Ecol 11:142–145 (2000)]*

The function of signals in animal communication is determined as much by their effective reception as by the information they contain. Variation in the sensory physiologies of organisms affects the efficiency with which they can perceive and process signals. This leads to sensory biases which can act upon the evolution of signals through selection for signal conspicuousness or processing, rather than information content, in a process called "sensory drive" (Endler, 1992; Guilford and Dawkins, 1991; Ryan and Rand, 1993). Recently, sensory drive has been identified as a particularly important mechanism of evolution for conspicuous sexual signals (Basolo, 1990; McClintock and Uetz, 1996; Proctor, 1992; Ryan and Rand, 1990), and it is thought to be a major force leading to the evolution of signal diversity (Basolo and Endler, 1998; Endler, 1992; Ryan, 1998).

Little is known about the role of sensory drive in the evolution of other forms of animal communication, particularly between predators and prey (Endler, 1991; Fleishman, 1992). How insects perceive silk in spider webs provides a system to study the effect of sensory drive in an interspecific context. Phylogenetic comparison of the color properties of spider silks demonstrates that many evolutionarily derived silks have lower ultraviolet (UV) reflectance than do primitive silks (Bond and Opell, 1998; Craig and Bernard, 1990; Craig et al., 1994). Craig et al. (1994) suggested that reduced reflection of UV light by silks enhances prey capture of spiders by reducing the visibility of webs to insects. Because reduced UV reflectance is associated with an increase in species diversity, it has also been hypothesized to be a key innovation contributing to the radiation of orb-weaving spiders (Bond and Opell, 1998).

Yet many orb webs with otherwise low UV reflectance contain conspicuous designs of silk called "stabilimenta" (Figure 1). These linear or spiral swaths of silk are added to the centers of orb webs by many diurnal spiders and are unusual in

that they have a bright reflectance across all wavelengths of light visible to insects, including UV (Craig and Bernard, 1990; Blackledge TA, Rowe MP, unpublished data). The function of stabilimenta is a contentious issue (Craig and Bernard, 1990; Eberhard, 1990; Edmunds, 1986), and the debate centers upon two hypotheses (Blackledge, 1998b; Blackledge and Wenzel, 1999). Stabilimenta may be a predator defense, distorting or camouflaging the outline of spiders (Eberhard, 1973; Schoener and Spiller, 1992), advertising presence of sticky webs (Blackledge and Wenzel, 1999; Eisner and Nowicki, 1983; Horton, 1980; Kerr, 1993), or distracting attackers (Schoener and Spiller, 1992; Tolbert, 1975). Alternatively, stabilimenta might act as a prey attraction for insects (Craig and Bernard, 1990; Hauber, 1998; Tso, 1996, 1998).

The prey attraction hypothesis argues that reflection of UV light makes spider silk more conspicuous to insects (Craig and Bernard, 1990). In primitive silks, conspicuous silk decreases effectiveness of webs. However, stabilimentum silk, found in webs with silks that otherwise reflect little UV light, provides an attractive signal to pollinating insects by mimicking flowers or open sky (Craig and Bernard, 1990; Craig et al., 1994). In contrast, Blackledge (1998a) suggested that stabilimentum silk is cryptic to many insects because objects with a white + UV reflectance (i.e., bright but flat spectra), such as stabilimentum silk, are perceived to be the same color as natural backgrounds of soil and foliage by many insects (Chittka et al., 1994; Kevan et al., 1996). This is in contrast to primitive spider silk, which has a UV reflective peak, making it conspicuous to insects. Therefore, a direct comparison of the visibility of primitive silk and stabilimentum spider silk to insects is necessary to understand fully the implications of the evolution of the coloration of spider silks. In this study, we compared the visibility of primitive tarantula silks (*Pterinochilus* sp.) and derived stabilimentum silks (*Argiope aurantia*) to honey bees (*Apis mellifera*). Bees are a common prey of *Argiope* (Blackledge and Wenzel, 1999) and share many aspects of their visual system with other insect prey of spiders (Blackledge, 1998a).

METHODS

We used the ability of bees to learn to forage at targets of spider silk, rewarded with sucrose, as an index of the visibility
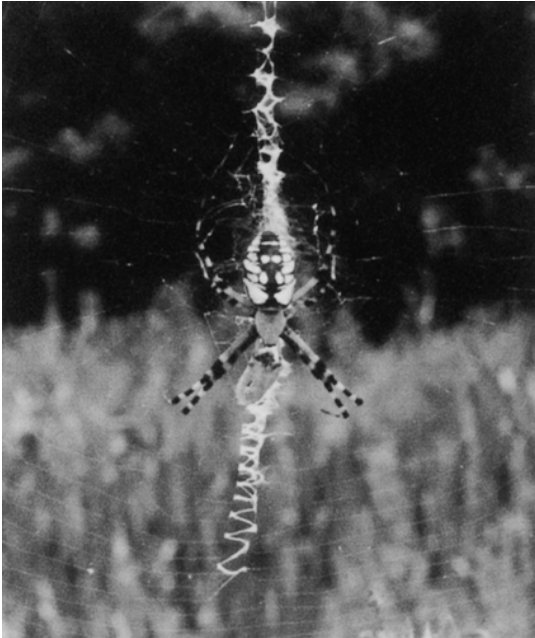

Figure 1
Female *Argiope aurantia* in a web containing a stabilimentum. These designs typically are built in the nonsticky center of the web, where spiders wait when foraging. The silk is a bright white to humans and also reflects large amounts of ultraviolet light.

Figure 2
Discrimination of primitive and stabilimentum spider silk targets versus blank targets. Bees that chose the rewarded target more often than expected by chance are denoted by asterisks (binomial test comparing success of each bee against 50% rewarded choices, $p < .05$). Six of 10 bees trained to primitive silk showed significant learning, but 0 of 10 bees trained to stabilimentum silk showed significant learning. These two distributions are significantly different (chi-square test, df = 1, $p < .001$). Sign-negative bees ($n = 4$) were trained to rewarded blank targets versus unrewarded stabilimentum silk targets and demonstrated that lack of learning was due to crypsis of the stabilimentum silk rather than to simple avoidance of stabilimentum silk.

of those silks. Individual bees were initially trained to forage at two stations, 1 m apart. Each station consisted of two 3 × 4 cm artificial plexiglass flowers, 15 cm apart. The flowers were placed at approximately the same height as the top of the grass in the field where the experiment took place. Microliter syringes were used to inject 2 μl rewards of 1.5 M sucrose solution into the tubular bases of the flowers. Bees were trained to make a single choice between the two flowers at a station before that station was covered by a wooden board, forcing bees to fly to the other station. Thus, in a single bout of foraging, a bee would make approximately 15–35 choices at the two stations before it returned to the hive. Bees were individually marked and were only used during the day on which they were trained. A visit to a flower was scored if a bee completely entered the 3-cm high tubular base of the flower. The experiment was conducted in a 3.8 × 2.3 × 2.0 m nylon screen enclosure, in the field, and the bee hives were located in an adjacent, identical enclosure. Therefore, bees had little access to real flowers and were essentially naive foragers.

Once a bee was trained to the artificial flowers, usually within a single foraging bout, it was randomly assigned to a group trained to either primitive silk ($n = 10$) or stabilimentum silk ($n = 10$) targets. Targets were constructed by wrapping silk around 2-cm diameter wire hoops until an opaque disk was formed. Primitive silk targets were made from silk from captive raised specimens of the tarantula *Pterinochilus* sp., and stabilimentum silk targets were constructed by wrapping approximately 15 stabilimenta, built by mature female *Argiope aurantia,* around a single target. Then targets were suspended off the front of all flowers. At each station, one target was a blank, consisting of only the wire hoop itself, and the other hoop was a silk target. Bees were again allowed a single choice at a station before it was covered, but were only rewarded when they chose flowers with the silk targets. Thus, the ability of bees to learn to forage at the rewarded flowers was a function of the visibility of the silk targets, against a background of grass, compared to the blank targets. After each choice, the
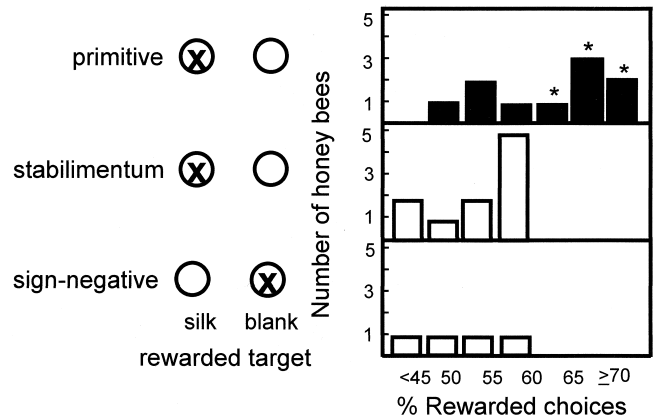
flowers at a station were switched with a random probability of 0.5 to prevent bees from using spatial cues in their learning (Orth and Waddington, 1997). Bees were allowed 6 bouts of foraging over a single day, for a total of about 100–200 choices per bee.

RESULTS

We computed the percent rewarded choices for each bee using all choices made after the first time a bee encountered both rewarded and unrewarded flowers. Bees trained to primitive tarantula silks made more correct (rewarded) choices than did bees trained to stabilimentum silk (Figure 2; Mann-Whitney U test, $U = 79$, $p < .025$). Six of 10 bees showed significant learning when primitive silks were associated with rewards (binomial test comparing success of each bee against 50% rewarded choices, $p < .05$), whereas 0 of 10 bees trained to rewarded stabilimentum silk showed significant learning (binomial test comparing success of each bee against 50% rewarded choices, $p < .05$). The number of bees showing learning versus those that did not was significantly different between the two treatments (chi-square test, df = 1, $p < .001$). Learning was generally quick. Four of the six bees that showed significant learning (to primitive silk) remained at or above 60% rewarded choices after only their 10th choice. Only one of the 6 required more than 100 choices before achieving that same level of success. Bees rarely touched the silk or blank targets during the experiment (once or twice maximum), indicating that their choices were made primarily on visual criteria.

We controlled for the possibility that insects evolved to avoid stabilimentum silk specifically by training additional bees ($n = 4$) in a sign-negative experiment where the blank target was rewarded and the stabilimentum target was unrewarded. Even when stabilimentum silk was associated with the unrewarded stimulus, honey bees failed to discriminate stabilimentum silk from a blank target (Figure 2), and this distribution was significantly different from bees trained to primitive spider silks (Mann-Whitney U test, $U = 36$, $p < .025$).

DISCUSSION

Our study is the first direct comparison of the visibility of primitive and derived spider silks to insects and supports the general hypothesis of evolution of spider silks toward low visibility. Our behavioral assay demonstrates that stabilimentum silk is less visible than primitive spider silk to bees foraging in a natural environment. Previous studies have indicated the importance of low visibility of webs for prey capture (Craig, 1986, 1988; Craig and Freeman, 1991; Rypstra, 1982) and suggested that the color of spider silks has evolved to decrease visibility of webs to insects (Bond and Opell, 1998; Craig et al., 1994). But these studies misplace emphasis on patterns of evolution of UV reflectance alone. We found that visibility of spider silk can only be assessed by considering the spectral reflectance of that silk across all wavelengths of light perceived by insects and how that contrasts with the background (Blackledge, 1998a). Both primitive and stabilimentum silks reflect large amounts of UV light (Craig and Bernard, 1990; Blackledge TA, Rowe MP, unpublished data). But primitive tarantula silks have a UV reflective peak that contrasts with the flat reflectance spectrum of soil and foliage. The spectrum of stabilimentum silk includes strong blue and green reflectance, as well as UV, resulting in a flat spectrum, similar to that of soil and foliage (Blackledge, 1998a).

Our findings are inconsistent with the hypothesis that stabilimenta attract insect prey. We found that stabilimentum silk is neither attractive to nor highly visible to bees. Instead, our study supports the finding of Blackledge and Wenzel (1999) that spiders include stabilimentum in their webs at the cost of prey capture, because cryptic coloration would reduce the ability of prey to use stabilimenta to avoid webs. Our findings are also consistent with the predator defense hypothesis. Stabilimenta are still clearly visible to vertebrate predators of spiders such as birds or lizards (Figure 1; Blackledge, 1998a; Blackledge and Wenzel, 1999; Horton, 1980; Schoener and Spiller, 1992). Furthermore, the cryptic properties of stabilimentum silk do not preclude their function as physical barriers or camouflage against insect predators of spiders such as sphecid or pompilid wasps. These functions may even be enhanced if the low visibility of stabilimentum silk prevents wasp predators from reliably using stabilimenta as signals to locate spider prey.

The cryptic nature of stabilimentum silk is particularly interesting because it indicates that sensory drive may account for the evolution of spider silk coloration. Insects can use stabilimenta to avoid webs, costing spiders prey (Blackledge and Wenzel, 1999). Yet the cryptic properties of the silk used to make stabilimenta likely reduces this cost to spiders. The color perception of insects, which interprets stabilimenta as cryptic, appears to be based on an ancient bauplan, and is perhaps ancestral for arthropoda (Chittka, 1996). Stabilimenta are by definition a feature of orb webs, although the aciniform glands used to spin the silk are present in other spiders. The origin of orb-weaving spiders is unknown, but they are thought to be at least pre-Jurassic (Coddington, 1990), although the earliest fossils are early Cretaceous (Selden, 1989). Therefore, the sensory bias in the insect visual system, which causes stabilimentum silk to be cryptic to insects, was in place before the evolution of stabilimenta and thus likely acted as a selective agent on the spectral properties of spider silk.

The evolution of spider silk coloration through sensory drive is unusual in two respects. Previously described examples of sensory drive acting on signal evolution are largely confined to sexual signals. In the context of sexual selection, sensory drive has usually been found to lead to increased conspicuousness rather than crypsis of signals. Thus, our research demonstrates the broader applicability of sensory drive to signals evolving under natural selection. But it also cautions that such evolution can have the novel effect of selecting for a signal that is cryptic to one class of receivers, such as insect prey, but not to others, such as vertebrates (Endler, 1991).

Many new questions arise. What are the adaptive consequences of color for other types of spider silks? The sticky capture silk of a typical orb web reflects little UV light (Craig et al. 1994), making it blue-green colored and therefore conspicuously colored to insects (Blackledge, 1998a). This suggests that decreased UV reflectance of these silks may not be an adaptation to reduce visibility to insects. Instead, sticky silk threads are so thin that they may not subtend a sufficient visual angle for insects to perceive their color (Giurfa and Vorobyev, 1997; Giurfa et al., 1995). Perhaps the color properties of such derived silks are imperceptible to insects and are a nonadaptive by-product of changes in their biochemical or physical properties associated with silk stickiness. Also, orb-weaving spiders produce at least seven different types of silks from a similar number of glands (Foelix, 1996). We cannot fully understand interactions between the evolution of silk coloration and insect vision until we better understand the homologies between different silks. For instance, prey-wrapping silks are produced from the same gland as stabilimentum silks (Foelix, 1996); perhaps that silk is also cryptic, allowing spiders to camouflage captured prey in their webs.

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