

SHORT COMMUNICATION

Zombie spiders and ecdysone: manipulation of *Allocyclosa bifurca* (Araneae: Araneidae) behavior by a parasitic wasp

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Abstract. In previous studies, the “cocoon” webs built by spiders of the genera *Cyclosa* Menge, 1866 and *Allocyclosa* Levi, 1999 under the influence of *Polysphincta* spp. wasps resembled molting webs built by unparasitized spiders; this behavioral manipulation was associated with increased concentrations of the molting hormone ecdysone. The present study documents an additional aspect of the cocoon webs of *Allocyclosa bifurca* (McCook, 1887) built under the influence of the wasp *Polysphincta gutfreundi* Gauld, 1991. Molting webs were more likely to have an associated barrier web than were prey capture orbs; and cocoon webs were even more likely to have barrier webs. The similarity between molting and cocoon webs accords with previous indications of ecdysone use by the wasps. The accentuation of molting web traits in the cocoon webs (also seen in other species) implies that the wasps manipulate the spiders using mechanisms other than simply replicating hormonal stimuli involved in normal molting.

Keywords: behavioral manipulation, molting hormone, Ichneumonidae

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Parasitic organisms often induce changes in the behavior of their host organisms, and evidence has gradually accumulated that it is common that these changes represent adaptations that improve the chances of survival of the parasite (Hughes et al. 2012). The specific mechanisms utilized by parasites to exercise these manipulations are by and large still poorly understood. Larval parasitoid ichneumonid wasps in the *Polysphincta* complex induce their host spiders to build special “cocoon webs” that protect the wasp’s cocoon (Korenko et al. 2014, 2018; Gonzaga et al. 2017). Several types of evidence indicate that the mechanism utilized by the wasps to achieve this manipulation involves increasing the concentration of ecdysone in the spider’s body, a change normally associated with preparation for molting (the “ecdysone hypothesis”) (Kloss et al. 2017; Eberhard & Gonzaga 2019). Molting in spiders is associated with increased ecdysone titers just before the molt (Krishnakumaran & Schneiderman 1970; Bonaric & De Reggi 1977; Bonaric 1988). Increased titers of ecdysone are also associated with several related processes, including ovarian development, vitellogenesis, pheromone production and sexual receptivity (Trabalon et al. 1992, 1998, 2005; Pourié & Trabalon 2003).

One type of evidence favoring the ecdysone hypothesis is the resemblance between the “molting webs” built by unparasitized *Allocyclosa bifurca* (McCook, 1887) spiders just before they molted, and the “cocoon webs” they built under the influence of a *Polysphincta gutfreundi* wasp larva just before the larva killed the spider and built its cocoon. Both molting and cocoon webs, when compared with prey capture orbs built in captivity, had fewer radii, shorter radii, fewer sticky spiral loops, and more reinforced “V-type” radii (Eberhard & Gonzaga 2019). Molting and cocoon webs were not identical, however, as cocoon webs had especially large numbers of “V” radii and were less likely to include portions of old prey capture webs built previously (Eberhard & Gonzaga 2019).

This evidence was incomplete in one respect, however, because the webs in captivity were all built on planar wire hoops. While the orb webs of *A. bifurca* are often built at sites where all available attachment sites are close to the same plane (“planar sites”, such as slightly irregular walls, windows, or (occasionally) irregularities in the bark of upright tree trunks), other *A. bifurca* web sites offer abundant attachment sites in three dimensions (“3-D sites”). These include the

large, stiff leaves of *Agave* spp. and other similar plants whose leaves are arranged in rosette arrays. Some webs of *A. bifurca* at such 3-D sites have a small tangle of lines (a “barrier web”) on one side of the orb (Fig. 1). Barrier webs never occurred at planar sites.

The objective of this note is to correct the omission of barrier webs from the previous study. The central question concerns the frequencies of barrier webs in prey capture, molting, and cocoon webs at 3-D sites. Assuming that barrier webs function to protect the spider from enemies, as is generally thought (summarized in Eberhard 2020), then barrier webs were expected to be more common in molting than prey capture webs. And, in turn, if barrier webs are more common in molting webs, then the ecdysone hypothesis predicts that they should also be more common in cocoon webs.

Spiders were observed weekly in San Pedro de Montes de Oca (el. 1100 m) (9° 56′ 13.18″ N, 84° 03′ 06.59″ W) and (for a shorter period) near San Antonio de Escazu (el. 1325–1350m) (9° 53′ 20.09″ N, 84° 8′ 17.90″ W) in the Central Valley of Costa Rica during the early rainy season (May–July) of 2019. For each web, I noted the developmental stage of the spider (adult or penultimate instar; these are the stages that are parasitized by the wasps; they will be called “mature females” hereafter; mature males are much smaller than females in this species, so all of these individuals were undoubtedly female) or earlier instars (called “immatures” hereafter); the presence or absence of a wasp larva on the spider’s abdomen; whether the web was a prey capture orb (with a sticky spiral), a resting or molting web (distinctly smaller area, lacking sticky spiral lines (hereafter called “molting webs”); see Eberhard & Gonzaga 2019), or a cocoon web (small area, lacking sticky spiral lines, and at the center either a wasp cocoon or a dead spider being fed upon by a last instar wasp larva); and whether or not there was a barrier web adjacent to the web. In most cases, I noted whether the barrier web was on the same or the opposite side of the web as the spider resting at the hub (Fig. 1).

Each cocoon web was marked with a small drop of yellow paint (nail polish) at the top of the stabilimentum to avoid counting it a second time on a subsequent visit. Similarly, I checked whether barrier webs lasted from one week to the next and might thus sometimes have been recounted. I marked one or a few lines in each barrier web with dots of pink nail polish, and then checked each

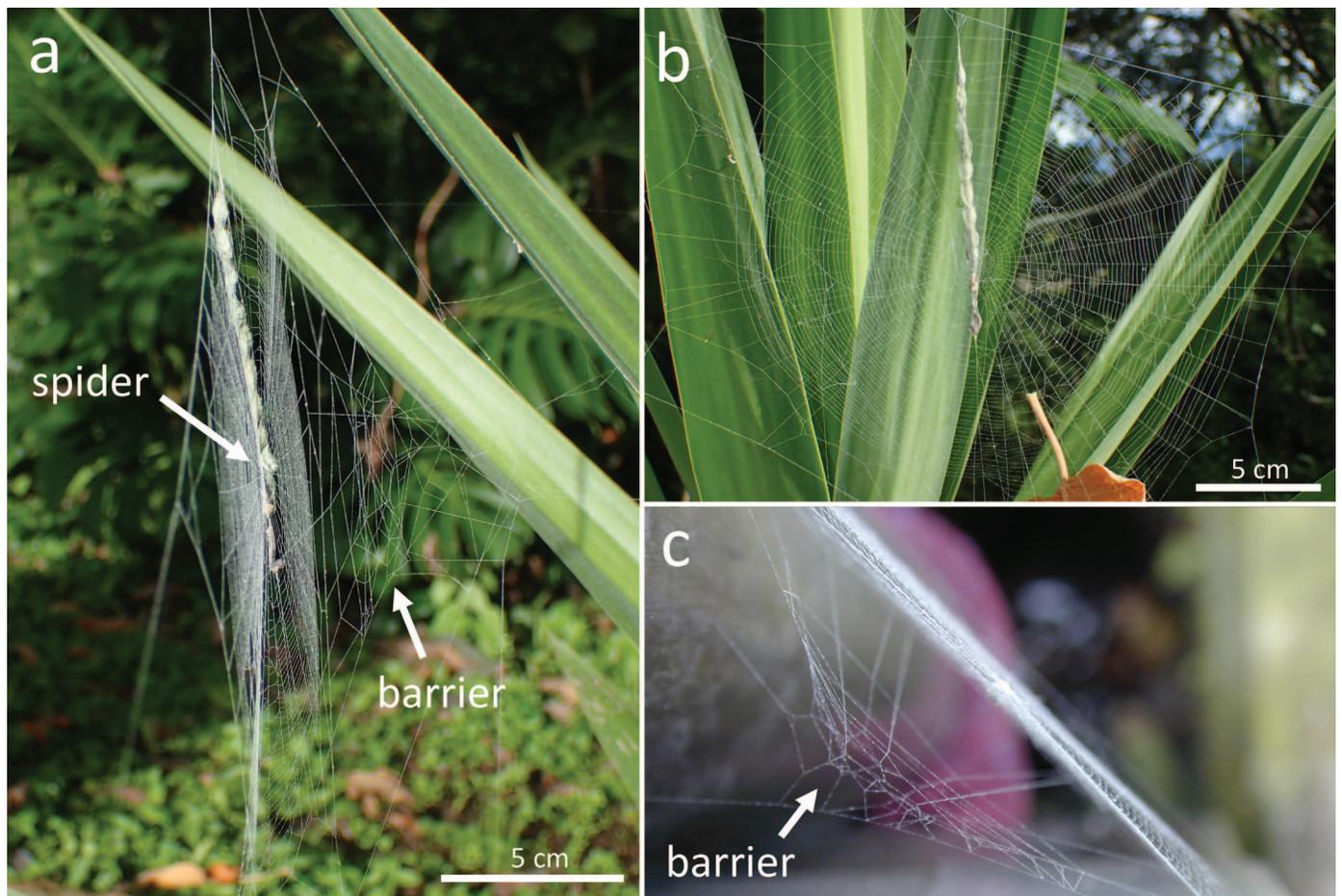


Figure 1.—Prey capture orb web of a mature female *Allocyclosa bifurca* with a barrier web on the same side of the orb as the spider, seen from the side (A), perpendicular to the orb (B), and from directly above (C) (a different web). The view from above illustrates the approximately radial and spiral organization seen in some barrier webs.

barrier web discovered on subsequent dates for these marks. Extensive experience with *A. bifurca* in the field and captivity (Eberhard 2003, 2010; Eberhard & Gonzaga 2019) has shown that their orb webs are generally renewed every day or two, and seldom (if ever) last an entire week, so recounting the same orbs was not deemed a problem. The spiders themselves were not marked, and the same individual spiders were probably observed repeatedly for several weeks.

Of a total of 777 webs of mature females and immatures, most (87.1%) were prey capture webs; 7.7% were molting webs; and 5.1% were cocoon webs. Among 677 prey capture webs, the fraction that had a barrier web was 21.7%; this fraction was 38.3% among 60 molting webs, and 75.0% among 40 cocoon webs. The frequencies of barrier webs differed significantly; cocoon webs had more barriers than either prey capture ($\chi^2 = 57.7$, $df = 1$, $P = 3.1 \times 10^{-14}$) or molting webs ($\chi^2 = 13.0$, $df = 1$, $P = 0.00032$); molting webs had more barriers than prey capture webs ($\chi^2 = 8.58$, $df = 1$, $P = 0.0034$).

The maximum numbers of mature female and immature spiders seen on a given day were 37 and 52. On these “maximum” days, the fraction of prey capture orbs with barriers was 29.7% for mature females, and 25.0% for immatures. These frequencies were similar to the total frequency of prey capture webs that had barrier webs (21.7% of 677 webs), suggesting that repeated observations of some spiders gave representative frequencies of barrier webs.

A total of 148 barrier webs associated with prey and molting webs were marked; only 5.4% of these marks were sighted on any

subsequent visit. Barrier webs showed very strong tendencies to be on the same side of the orb as the spider in both prey capture webs (96.0% of 149 webs) and molting webs (95.2% of 21 webs) (both $P < 0.0001$ with χ^2).

Two predictions of the hypothesis that barrier webs provide protection for spiders on their webs were confirmed: barrier webs were more frequent with molting than with prey capture webs; and barrier webs tended to be on the same side of the orb as the spider. Further support for this hypothesis came from a direct observation of an interaction with a *P. gutfreundi* wasp. A female *P. gutfreundi* that had been hovering in a gentle wind flew toward an unparasitized mature female *A. bifurca* resting at her hub and encountered the barrier web and landed there; the moment the wasp touched the barrier web, the spider dropped to the bottom of her orb and remained motionless. Within 5 s the wasp flew off and did not return. Even though barrier webs did not always defend spiders in this way, either in this species (Eberhard 2018) or in others (Gonzaga & Sobczak 2007), there is nevertheless substantial evidence that barrier webs do provide some defense for spiders on their webs (summarized in Eberhard 2020); even a partial defense against wasps could favor barrier web construction.

The ecdysone hypothesis was supported by the fact that cocoon webs resembled molting webs in being more frequently accompanied by barrier webs. Thus the presence of a barrier web can be added to the other aspects of cocoon webs that resemble molting webs (fewer radii, shorter radii, fewer sticky spiral loops, and more reinforced “V-

type” radii) (Eberhard & Gonzaga 2019). The especially high frequency of barrier webs associated with cocoon webs compared with molting webs (75% vs. 38.3%) also resembles differences found in other aspects of cocoon webs in this and other species, in that cocoon webs showed an accentuated degree difference compared with capture webs (Eberhard & Gonzaga 2019). Whether this accentuation stems from simple differences in hormone concentrations (e.g., higher ecdysone concentration when building cocoon webs) or from some other difference (e.g., additional signaling molecules) is not known.

One possible weakness of this study was that some barrier webs may have been counted more than once. Nevertheless, the relatively low frequency with which marked barrier lines were sighted on subsequent visits (5.4% of 148 webs) indicates that barrier webs do not generally last long (at least during the rainy season), and that re-sighting was relatively rare.

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