SHORT COMMUNICATION

The effect of regurgitated digestive fluid on the spider's own legs in *Philoponella vicina* (Araneae: Uloboridae)

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Abstract. Philoponella vicina O. Pickard-Cambridge 1899 rests on its orb web in a cryptic posture with its legs folded against its body. While feeding, the spider coats the entire prey with digestive fluid and changes its posture, spreading its anterior legs wide. We tested whether this change in leg position may function to protect against damage to its legs from its own digestive fluid. When we touched detached legs I with prey packages wetted with digestive fluid, more setae fell from the legs than when we applied tap water in a similar manner. In addition, intersegmental membranes were damaged by digestive fluid, but not by water. This and other uloborids may thus break their cryptic postures while feeding in order to avoid damage from their own digestive enzymes.

Keywords: Cryptic posture, membrane degradation, setae

The feeding process of uloborids is unusual in several respects. Since these spiders lack cheliceral venom glands, they wrap their prey with large amounts of silk, forming rounded, compact, compressed packages. The spider then repeatedly wets the entire surface of the prey with regurgitated fluid containing digestive enzymes and does not masticate the prey with its chelicerae (Eberhard et al. 2006a). The digestive enzymes must penetrate the prey without the benefit of holes made by the spider while injecting venom or masticating the prey, as occurs in other spiders. The enzymes apparently gain access to the prey's interior by digesting prey membranes, because digested prey showed extensive, membrane-specific damage (Eberhard et al. 2006 a, b). As with other spiders, uloborids have multiple setae around the mouth that presumably function to filter the liquid they ingest (Foelix 2010). Uloborid spiders cover the entire prey package with digestive fluid while feeding, while most spiders wet only the portion close to their chelicerae (Weng et al. 2006).

Philoponella vicina O. Pickard-Cambridge 1899 and other uloborids rest on their webs in various constrained cryptic postures that vary in different genera, but have the common effect of obscuring the outlines of their anterior legs (Opell & Eberhard 1984). In Philoponella, the distal portions of legs I are folded ventrally tight against the body, with their metatarsi and tarsi close to the sternum. This posture is thought to provide protection against visually orienting predators (Opell & Eberhard 1984). This species and other uloborid spiders (i.e., Uloborus trilineatus, U. diversus) break their cryptic postures when feeding, spreading their anterior legs apart (Fig. 1) (Weng et al. 2006; W. Eberhard unpublished results). Weng and coworkers (2006) hypothesized that this spread-leg posture functions to avoid damage to the spider's front legs from its own digestive enzymes.

We used adult female *P. vicina* (length: 5–10 mm), which build approximately horizontal orb webs in sheltered sites in tropical forests and forest edges where they feed on several types of prey (Fincke 1981, Eberhard et al. 2006a), to evaluate the effect of their regurgitated digestive fluid on their own legs. We thus tested the hypothesis that these spiders break their cryptic postures and expose themselves to increased predation in order to avoid possible damage while feeding.

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We collected mature female *Philoponella vicina* in a patch of secondary forest on the campus of the Universidad de Costa Rica in San José, Costa Rica. We induced 15 spiders to build their webs indoors on wire hoops (approximately 20 °C, 80% relative humidity) in order to feed them and to obtain digestive fluids. We sacrificed 13 other mature females by freezing them.

For each experiment, we detached both legs I of a spider and placed them on a glass slide inside a humid chamber (a Petri dish containing cotton soaked in water). These humid conditions slowed the desiccation rate of the regurgitated fluid; otherwise, when it was extracted and exposed to air, it dried in a few seconds. One of the legs (the "experimental" leg) received regurgitated digestive fluid, while the other (the "control" leg) received tap water. To obtain digestive fluid, we fed prey (wild Drosophila flies or Tetragonisca stingless bees) to spiders in their webs. We pulled the prey from the spider's grasp with a pair of forceps after the spider had wrapped the prey and wet it with digestive fluid (the prey package changed from opaque white to translucent and shiny) (Eberhard et al. 2006b). We removed the prey while the spider was regurgitating and rotating the package to wet it. This behavior precedes ingestion, which begins when the spider stops rotating its wetted prey. We applied digestive fluid (or water) from five different prey packages to each leg. Each package was touched to the leg at five different spots, sufficient to wet the entire surface of the leg as the liquid dispersed. The touches were gentle, and the entire surface of the leg became wet, but it was not possible to be sure that equal amounts of water and digestive liquid were applied. No region of the leg was touched preferentially. Each application lasted for about 30 s, until the liquid evaporated.

To control for the possibility that setae were lost from the leg due to the mechanical stress produced when we touched the legs with prey packages, we used packages of prey taken from spiders before they had regurgitated and which we wet with water. We alternated the legs receiving treatments (experimental, control, experimental, etc.). As it was necessary to wait for spiders to wrap and begin to feed on new prey, the lapse of time between repeats of the treatments was not uniform, but on the order of 10 min.

We then observed the two legs under a dissection microscope at 100X. Counts of fallen setae were made in the white ring on tibia I, where individual setae and their sockets were easy to distinguish. All intersegmental membranes were checked for damage, such as holes or complete separation of leg segments (Fig. 2b). We calculated the area of this white ring for one side of the leg, using a calibrated ocular

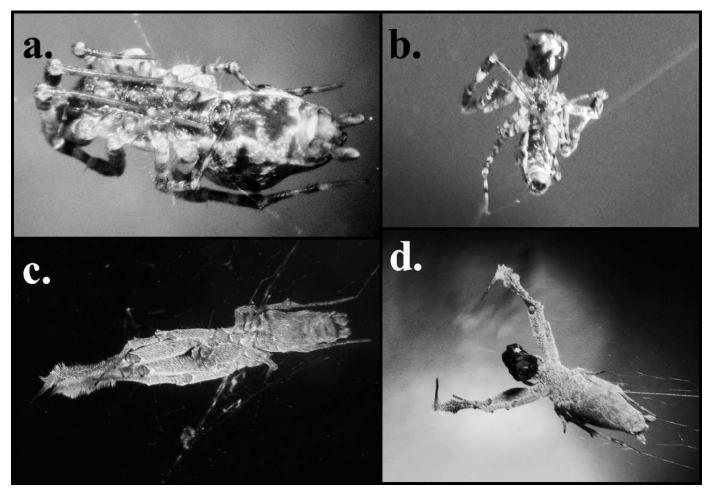


Figure 1.—Ventral views of *Philoponella vicina* on its web while a) resting and b) feeding. Ventral views of *Uloborus trilineatus* while c) resting and d) feeding. Note that the anterior legs are spread while feeding in both species.

micrometer with a resolution of $0.5~\mu m$. To calculate the area, we multiplied the width of the leg times the length of the white ring. We counted the number of intact tibial setae in that area and the number of pores from which setae had fallen (Fig. 2a).

We calculated the proportion of setae that were lost per area in each treatment. We used proportions to avoid possible effects of individual variation. We compared the proportions with a Wilcoxon

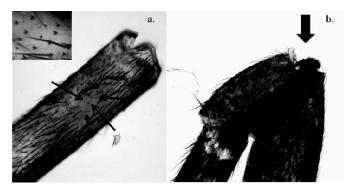


Figure 2.—a) Pores left by fallen setae (arrows) on the tibia of leg I of *Philoponella vicina* resulting from the application of its own digestive fluid (detail of pores at upper left); b) Damage (arrow) to the membrane at the femur-patella articulation of leg I that had been treated with regurgitated digestive fluid.

test. All means are reported with one standard deviation. Voucher specimens are housed in the Museo de Zoología (USJ) at the Universidad de Costa Rica.

The mean white area of leg I exposed to the digestive fluid treatment was $160 \pm 42.3~\mu\text{m}^2$ (n=18), and the mean setae density was 0.31 ± 0.06 setae / μm^2 . More setae were missing following the digestive fluid treatment than following the tap water treatment (Z=2.69; P=0.007; Fig. 3) (the treatment variances were equal - Levene's F=2.04; gl=12; P=0.23; Fig. 3). Six of 13 legs exposed to the regurgitated fluid had damage in at least one joint membrane (Fig. 2b), but no legs exposed to tap water showed any deterioration. The femur-patella joint was the most frequently damaged, with six legs affected; four legs were damaged at the tibia-metatarsus joint, and four legs at the patella-tibia joint.

Thus the legs of *P. vicina* are susceptible to injury from the digestive enzymes that the spider applies to its prey. Some setae also fell out with the tap water treatment, probably due to the mechanical effect of our applying the wetted prey package to the legs. The sharpest negative effect of the regurgitated digestive fluid was the damage to the joint membranes, which only occurred when regurgitated fluid was applied. This effect would probably be severely damaging to a living spider.

It is likely that the change in resting posture during feeding functions to prevent contact of the spider's legs with the regurgitated liquid that covers the prey package. This could be especially important, because these spiders spend up to several hours feeding on a given prey (Eberhard et al. 2006a) and because their digestive

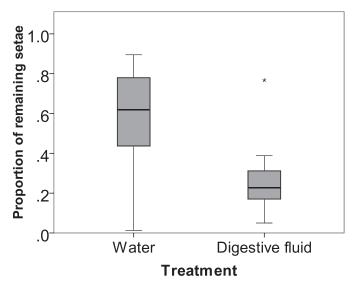


Figure 3.—Proportion (median \pm percentiles and range) of setae that were missing from tibia I of *Philoponella vicina* after applying its own digestive fluid (left) or tap water (right) (Z = 2.69, P = 0.007).

fluid has a low surface tension, which aids it in wetting and digesting the prey (Weng et al. 2006).

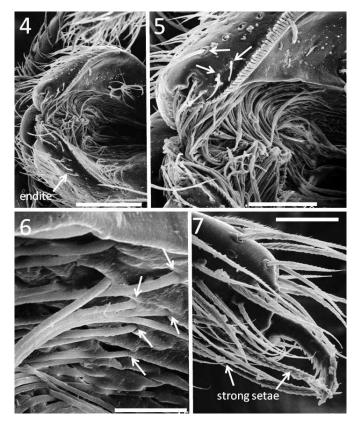
These considerations bring into focus additional feeding problems for uoborids that we will only mention briefly. The setae near the spider's mouth differ from those on other parts of the body in not having conspicuous sockets at their bases (Figs. 4-6). Perhaps this design serves to avoid membrane damage from digestive fluids. The fact that these setae are intact (Figs. 4-6), despite having been repeatedly exposed to digestive fluid during the spider's lifetime, makes it clear that they are not damaged by digestive fluids. In contrast, the mouth setae of an araneid, Argiope argentata, that envenomates and then masticates its prey and thus may have less need to digest prey membranes, differ from those of P. vicina in having clear sockets. In addition, the palps of P. vicina, which manipulate the wet prey during regurgitation, are provided with long, robust setae at their tips (Fig. 7), perhaps to help prevent digestive fluids from contacting the rest of the palp when the spider rotates the prey while feeding. Further observations and comparisons with other species will be needed to test these hypotheses.

ACKNOWLEDGMENTS

We thank M. Mardones for help feeding spiders and M. Vargas for help with the SEM. F. Bolaños and M. Araya provided guidance with statistical issues. We are grateful to two anonymous reviewers, whose useful comments improved the quality of this manuscript.

LITERATURE CITED

Eberhard, W.G., G. Barrantes & J.-L. Weng. 2006a. Tie them up tight: wrapping by *Philloponella vicina* breaks, compress and sometimes kill their prey. Naturwissenschaften 93:251–254.



Figures 4–7.—SEM images of setae near the mouth and on the palp of *Philoponella vicina*. Arrows in Figure 5 mark "typical" setal bases with clear sockets on the distal edges of the endites. Arrows in Figure 6 mark the less distinct sockets at the bases of setae on the dorsal surfaces of the endites near the mouth. Arrows in Figure 7 mark the two robust setae near the tarsal claw, which were observed to repeatedly contact the prey package as it was rotated during feeding. Scale lines are, respectively, 150, 60, 15, and 43 μ long.

Eberhard, W.G., G. Barrantes & J.-L. Weng. 2006b. The mystery of how spiders extract food without masticating prey. Bulletin of the British Arachnological Society 13:372–376.

Fincke, O.M. 1981. An association between two Neotropical spiders (Araneae: Uloboridae y Tengellidae). Biotropica 13:301–307.

Foelix, R.F. 2010. Biology of Spiders. Third edition. Oxford University Press, New York.

Opell, B.D. & W.G. Eberhard. 1984. Resting postures of orb-weaving uloborid spiders (Araneae: Uloboridae). Journal of Arachnology 11:369–373.

Weng, J.-L., G. Barrantes & W.G. Eberhard. 2006. Feeding by *Philoponella vicina* (Araneae: Uloboridae) and how uloborid spiders lost their venom glands. Canadian Journal of Zoology 84:1752–1762.

Manuscript received 3 April 2011, revised 3 December 2011.