Feeding by *Philoponella vicina* (Araneae, Uloboridae) and how uloborid spiders lost their venom glands

J.-L. Weng, G. Barrantes, and W.G. Eberhard

Abstract: Feeding by uloborid spiders is unusual in several respects: cheliceral venom glands are absent; prey wrapping is extensive (up to several hundred metres of silk line) and severely compresses the prey; the spider's mouthparts usually never touch the prey; and the entire surface of the prey is covered with digestive fluid. This paper presents observations on *Philoponella vicina* O. Pickard-Cambridge, 1899, which provide possible causal links between these traits. The spider begins ingesting soon after it wets the prey, gaining access to the prey's interior through a broken cuticle that was broken during wrapping and by digestion of the prey's membranes. The more abundant of the two types of wrapping lines is also digested, but the remaining shroud of wrapping silk is dense and filters digested prey particles. Robust setae on the palpal tarsus and the spread position of the anterior legs during feeding probably protect the spider from contact with the digestive fluid. Spiders extracted about 65% of the wet contents of the prey, but feeding was slow and involved substantial water evaporation. We propose that selection in uloborid ancestors to recover wrapping silk led to increased wetting of the prey's surface and that compressive wrapping facilitated this wetting. These traits could have led to loss of the now superfluous cheliceral poison glands.

Résumé : L'alimentation des araignées de la famille des uloboridés est exceptionnelle à plusieurs égards : il n'y a pas de glandes à venin sur les chélicères; les proies sont fortement emballées (avec jusqu'à plusieurs centaines de mètres de fil de soie) et très comprimées; les pièces buccales de l'araignée ne touchent généralement jamais la proie; la surface entière de la proie est recouverte de liquide digestif. Nous présentons des observations faites sur *Philoponella vicina* O. Pickard-Cambridge, 1899 qui montrent des liens de causalité possibles entre ces caractéristiques. L'araignée commence l'ingestion de la proie peu après l'avoir humectée et elle accède à l'intérieur de la proie par le bris de la cuticule durant l'emballement et par la digestion des membranes de la proie. Le type de fil d'emballage le plus commun est aussi digéré, mais l'enveloppe restante de soie d'emballage est dense et elle filtre les particules digérées de la proie. De fortes soies sur le tarse du palpe et la position ouverte des pattes antérieures durant l'alimentation protègent probablement l'araignée du contact avec le liquide digestif. Les araignées extraient environ 65 % du contenu humide de la proie, mais le processus d'alimentation est lent et il se produit une importante évaporation d'eau. Nous émettons l'hypothèse selon laquelle, chez les ancêtres des uloboridés, la sélection de la récupération de la soie d'emballage a conduit à un humectage accru de la surface de la proie et que la compression de la proie pendant l'emballage facilite cet humectage. Ces caractéristiques peuvent avoir conduit à la perte des glandes à venin des chélicères alors devenues superflues.

[Traduit par la Rédaction]

Introduction

Spiders have long been known to feed by regurgitating digestive fluid onto their prey and then sucking up the nutrient-laden broth (Bertkau 1885 in Bartels 1930; Bartels 1930; Zimmermann 1934; Comstock 1948; Kaestner 1968; Collatz 1987; Foelix 1996; Eberhard et al. 2006*b*). The regurgitated fluid, which presumably comes largely from the midgut (Kaestner 1968), is very rich in proteins (about 10 times richer than vertebrate duodenal or pancreatic juice) and is diluted when it enters the prey (Collatz 1987). Spiders suck liquid from their prey by using the strong muscular sucking stomach to increase the volume of the foregut. The ingested food is probably nearly completely liquid, as thick brushes of setae in the mouth cavity and a second filter in the pharynx (the "palate plate") strain out particles as small as ~1 μm (Foelix 1996).

Several aspects of feeding are unusual in the family Uloboridae. These spiders lack cheliceral poison glands (Opell 1979) (the possibility mentioned by Opell (1988) that they have poison glands in the midgut has apparently never been investigated); they wrap prey with apparently excessive amounts of silk (up to hundreds of metres/prey — Lubin 1986; Opell 1988; Eberhard et al. 2006*a*, 2006*b*); wrapping times are approximately two orders of magnitude greater than in other orb weavers in the family Araneidae (Tillinghast and Townly 1994; Eberhard et al. 2006*a*); and they

Received 5 April 2006. Accepted 20 August 2006. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 9 January 2007.

J.-L. Weng[1,2] and G. Barrantes. Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica.
W.G. Eberhard. Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica; Smithsonian Tropical Research Institute.

[1]Corresponding author (e-mail: jweng@ksu.edu).
[2]Present address: Department of Entomology, Kansas State University, 123 West Waters Hall, Manhattan, KS 66506-4004, USA.

 doi:10.1139/Z06-149

wet the entire surface of the prey rather than only the area near their own mouthparts, which is typical of other spiders (Lubin 1986, Opell 1988; Eberhard et al. 2006*b*). This paper describes further details of feeding in the uloborid *Philoponella vicina* O. Pickard-Cambridge, 1899, a species that builds typical orb webs at tropical forested sites (Eberhard et al. 1993; Fincke 1981) and that provide causal links between these traits. Our findings suggest a hypothesis concerning how uloborids evolved their unusual method of feeding and lost their poison glands.

Materials and methods

Mature female spiders collected at 1300–1500 m near San Antonio de Escazú, San José Province, Costa Rica, were observed attacking prey and feeding at room temperature in captivity under a dissecting microscope. Prey attack and feeding behavior were filmed on more than 20 different spiders using a digital video camera (30 frames/s) with close-up lenses; closer views of prey wrapping and feeding were also recorded under the microscope. Wrapped prey packages of *P. vicina* were removed from spiders at different stages of feeding by seizing the package with forceps or, more often, by inducing the spider to drop it (e.g., by capturing the spider briefly in a small vial). Discarded prey packages were collected from below webs. The pH of digestive liquid was determined by pressing a piece of pH paper (colorpHast pH indicator strips, pH 0–14; Merck KGaA, Darmstadt, Germany) to a prey package immediately after the spider had started wetting it ($n = 6$). Droplets of digestive fluid were also collected ($n = 4$) using a fine capillary tube that had been heated and drawn down to a small diameter (0.06–0.07 mm); the tip was touched to a newly wet prey package so that the liquid entered the tube by capillarity. Prey packages ($n > 50$) were carefully torn open under a dissecting microscope and photographed there or photographed after they had been transferred to a compound microscope. Digital photographs were labeled using Microsoft PowerPoint and the contrast enhanced using Adobe Photoshop. Prey packages were also examined under the scanning electron microscope (SEM) (Hitachi S-2360 N) after being air-dried and then sputter-coated with gold–palladium. Mean values are given with ±1 SD. Voucher specimens will be deposited in the Museum of Comparative Zoology, Cambridge, Massachusetts.

Wrapping silk composition and its responses to digestive fluids were studied by inducing spiders to spin sheets of wrapping silk in which the lines were out of contact with the prey. This was done by giving the spider a *Drosophila* Fallén, 1823 prey, which had been impaled on a 11 mm long human hair (~100 μm in diameter) (Eberhard et al. 2006*a*). In wrapping such prey ($n > 10$), the spider always bent the hair into a loop and produced a sheet of wrapping silk across the loop that was isolated from the surface of the prey. Each impaled fly was given to a spider for wrapping and was removed before the spider regurgitated onto it. Digestive fluid collected using a fine capillary tube was immediately applied to a sheet of wrapping silk ($n = 2$) and then placed in a humid chamber for 40 min to examine its digestive effects. We also tested the effect of general proteases in fresh pineapple juice on sheets of wrapping silk ($n = 5$). A droplet (~5 μL) of fresh pineapple juice was placed on the sheet of silk and a drop of distilled water was used as a control on another part of the same sheet.

To determine the effect of wrapping and digestive fluids on prey survival, we provided a series of three worker ants (*Crematogaster* Lund, 1831), one every other day, to each of 11 adult female spiders in captivity. One ant was removed from the spider's grasp as soon as she finished wrapping it (as she was transferring it to her palps to begin regurgitation); the package was left for 10 min and then the shroud was carefully opened to check if the ant was alive. The wrapped ant in the second trial was also removed just before the spider regurgitated digestive fluid on it and was immediately submerged in distilled water. It was shaken gently in the water to assure that it was wetted. After 10 min, the shroud was removed to check for survival of the ant. The third wrapped ant was removed 2 min after the spider began to wet the package with digestive fluid, placed in a humid chamber for 8 min, and then the shroud was removed. The order of treatments was alternated among the spiders. The ant was considered dead if it did not begin to move within 30 min after the shroud was removed.

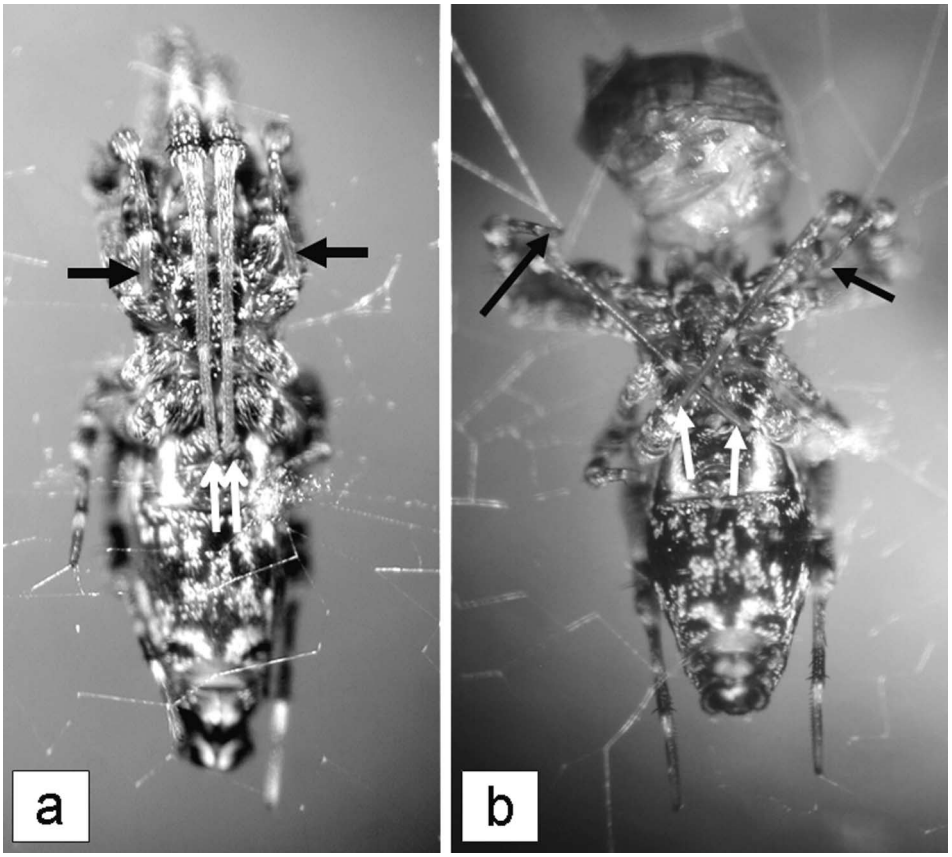
Diameters of lines were measured in images from the SEM. Relative surface tensions of digestive fluid and water were compared by placing droplets of equal volume on clean glass slides at room temperature. The spinnerets and spigots from which wrapping lines emerged were determined by killing two spiders with boiling water while they were wrapping prey, fixing them in 70% ethanol, and then dehydrating them and coating them for examination with the SEM (above).

To estimate food uptake and water loss during feeding, spiders and prey were weighed before and after feeding ($n = 12$). Wet mass of spiders were determined to the nearest 0.1 mg on a Cahn 7500 electrobalance.

Results

Spiders wrapped their prey extensively in silk before feeding (see Eberhard et al. 2006*a*). During the final burst of wrapping, a mass of clear liquid appeared on the distal anterior surface of the chelicerae (and perhaps also on the endites) of four spiders observed wrapping under a dissecting microscope. Feeding always began as soon as wrapping ended. The spider transferred the prey package from her legs II and III to her palps and chelicerae and immediately began to rotate the package rapidly. Her anterior legs were spread, and remained spread, for the entire time that she fed (Figs. 1*a*, 1*b*). Her tarsi I remained folded ventrally near her sternum, while her tarsi II grasped the web. She regurgitated clear liquid while turning the prey, spreading it over the surface of the package. This initial wetting behavior lasted for 1–20 min, depending on prey size. By the time the spider first stopped turning the prey package, the entire surface of the package was wet. The shroud of wrapping silk, which had been white and had nearly completely obscured the prey, became more or less transparent in most places. The least transparent portions were those that were elevated from the prey's surface, where air pockets were apparently still present under the shroud. Most portions of the prey were thus readily visible, revealing damage owing to

Fig. 1. Crouching positions of the uloborid spider *Philoponella vicina* at the hub of the web during the day, (*a*) without prey and (*b*) in the spread-leg position while feeding. The white arrows indicate the tarsi of legs I, which are folded against the sternum and are out of contact with the web, and the black arrows indicate the tarsi of legs II, which hold the web. The length of the spider (cephalothorax and abdomen) is approximately 0.5 cm.



wrapping such as collapsed compound eyes in *Drosophila* prey (Eberhard et al. 2006*a*).

Following initial wetting, the spider rotated the prey only intermittently. During rotation, her fangs repeatedly opened and grasped the prey package, probably helping to rotate it. They usually did not penetrate the shroud (Eberhard et al. 2006*b*); however, in rare cases (2 in >50), large prey packages had holes where the spider had fed. The chelicerae have teeth that may have produced such holes (Fig. 2*c*). The palps also moved during rotation of the prey package, nearly always contacting its surface with only the claw and some of the robust setae near the tip of the tarsus (Figs. 2*a*, 2*b*). Periodically the spider stopped rotating the package and fed for up to >20 min. Observations of six spiders under a dissecting microscope showed that the spider cyclically regurgitated abruptly and then ingested fluid slowly, on the order of once every 30 s (Eberhard et al. 2006*b*). Her chelicerae and mouth area usually failed to contact the prey directly, contacting only the shroud covering the prey (Eberhard et al. 2006*b*), except when the shroud was broken.

By observing the small particles of red visual pigments in the eyes of flies such as *Drosophila* sp., we were able to follow the movements of prey tissues during feeding. Red particles began to accumulate on the spider's endites within <30 s after she first regurgitated and then ingested fluid near a collapsed portion of a compound eye. Apparently the fly's cuticle was broken where the compound eye had collapsed, which allowed material from inside the prey to pass through the prey cuticle and shroud and to reach the spider's mouth; here particles were filtered out and accumulated on her endites. The in-and-out flow of liquid during feeding was illustrated by movements of the red particles on the spider's endites that were often carried away from her mouth during subsequent regurgitations of digestive fluid and then returned as she sucked.

An additional level of filtering was suggested by the mat of very fine particles present on the inner surface of the silk shroud of one discarded prey package that was teased open after the spider had finished feeding (Fig. 3*a*). The particles in this mat were outside the prey's cuticle, so this material had presumably been degraded by the spider's digestive fluid and had left the prey's body, but was then filtered by the shroud.

Properties of the digestive fluid

The pH of a drop of the clear regurgitated digestive fluid collected from newly wetted prey packages from each of three spiders was 10. The liquid contained a substantial amount of dissolved material. When a drop of regurgitated fluid was placed on a glass slide, it left a semi-transparent "crust" after it dried. Similarly, each of five prey packages taken from spiders just after wetting began and examined with the SEM had patches covered with a fine-grained solid where the liquid had been applied by the spider (Fig. 4*c*).

Fig. 2. The distal portion of the tarsus of (*a*, *b*) the palp and (*c*) the chelicera of a mature female *P. vicina*. In *a*, both a terminal claw and several robust dark setae project from near the tip of the palp. In *b*, these setae and the claw are the only portions of the palp that contact a large prey package during feeding. In *c*, teeth are present on both segments of the chelicera (anterior view).

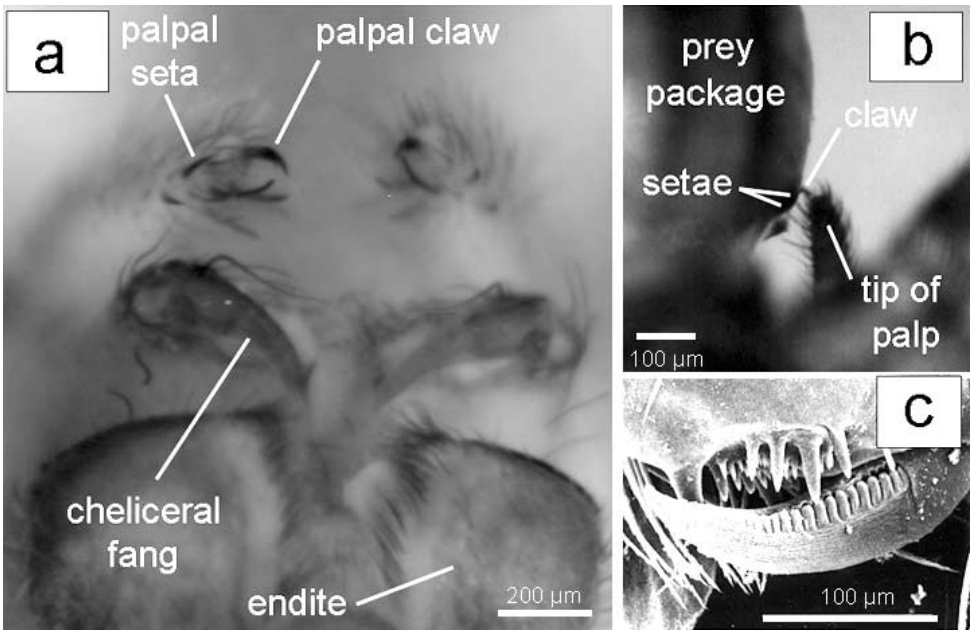


Fig. 3. Effects of digestion. (*a*) A mat of fine-grained detritus was revealed on the inner surface of a shroud that was teased open after the prey package had been discarded after *P. vicina* had fed on it. There were impressions in this mat of the prey's compound eye; a broken piece of eye cuticle is visible in the lower portion of the micrograph. There were also loose setae that had apparently been freed from their sockets when their basal membranes were digested (see also *c*). (*b*) Portions of a prey project through the shroud of a prey package that was discarded by a spider after feeding on it. The shroud conforms tightly to the contours of the prey; a compound eye is visible at the upper left corner of the micrograph. (*c*) The femur–tibia articulation of a fly discarded by a spider after feeding (shroud teased open) in which most setae have fallen from their sockets, and with intact setae that also lack their basal membranes scattered nearby. (*d*) The basal tip of a disarticulated femur of a prey discarded after feeding by *P. vicina* completely lacks the intersegmental membrane.

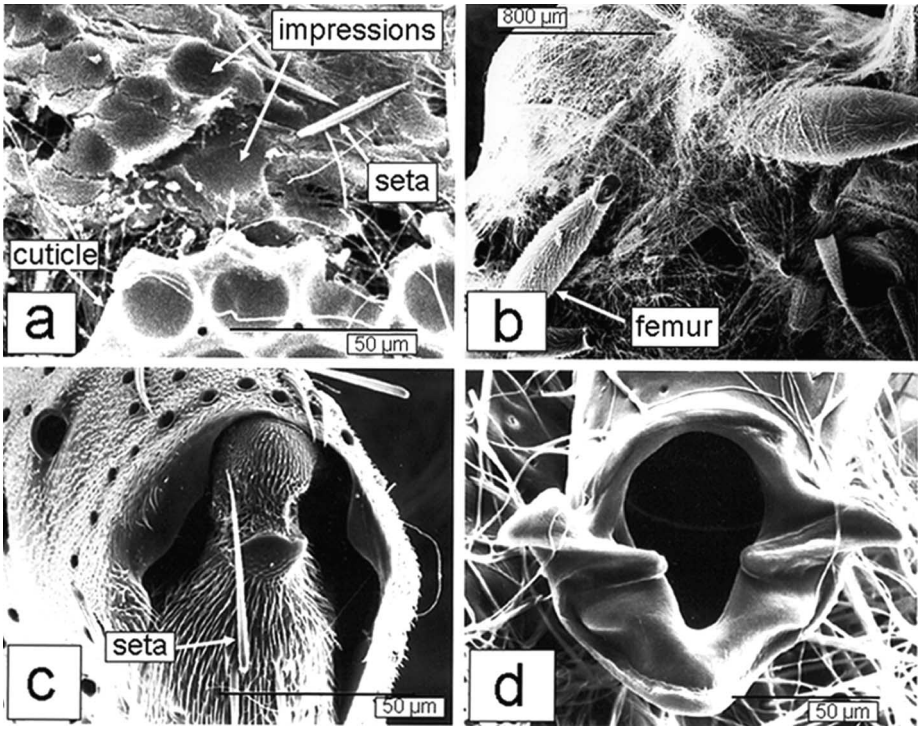
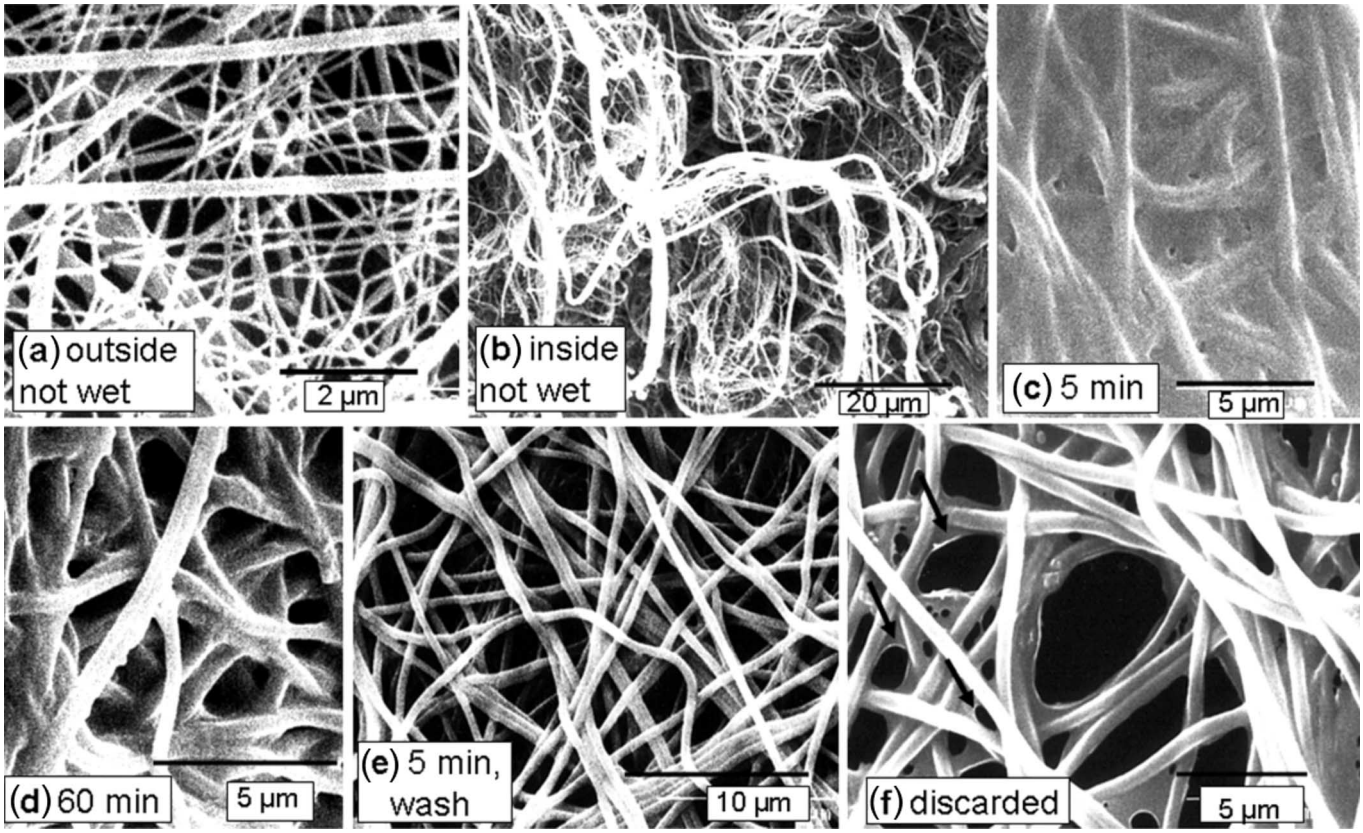


Fig. 4. Prey-package silk from *P. vicina* that was air-dried after different treatments. (*a*) The exterior of a prey package that had not been wetted with digestive fluid has both thick and thin lines; (*b*) the interior of a prey package that had not been wetted also has thick and thin lines; (*c*) the external surface of a prey package removed from a spider 5 min after it was first wetted was covered with fluid containing much dissolved material; (*d*) the external surface of prey package removed from a spider 60 min after it was first wetted was covered with fluid containing much less dissolved material; (*e*) the external surface of a prey package removed from a spider 5 min after it was first wetted, and which was then washed in water for 1 min to remove the dissolved material; and (*f*) the exterior of prey package discarded by a spider after feeding showing (arrows) small residues of dissolved material in interstices of the silk shroud.



Washing in water removed this material in three additional packages (Fig. 4*e*). The concentration of this material was apparently gradually reduced later in feeding (Fig. 4*d*), and when the prey was discarded, only vestiges were visible in eight such prey examined with the SEM (arrows in Fig. 4*f*).

The surface tension of the digestive fluid was apparently lower than that of water. A 0.15 mL drop of digestive fluid collected from the surface of a prey bundle spread readily into a 1.7 mm^2 puddle when placed on a glass slide, whereas the same volume of distilled water spread to only a 0.27 mm^2 puddle and remained more distinctly rounded at its edges. In addition, an unwetted prey bundle taken from a spider during wrapping and then placed in tap water was not immediately wetted, which is in contrast with the seemingly instantaneous wetting of the silk shroud when the spider regurgitated. The unwetted bundle floated on the water surface and did not stay submerged when forced down. In contrast with the rapid change to transparency when a prey package was wetted by the spider, the shroud only gradually became transparent over the space of several minutes.

The wrapping silk of *P. vicina* included lines with two different ranges of diameters from two types of aciniform spigots (Figs. 5*a*, 5*b*). Silk lines in prey packages that were removed before the spider wet them had both thick lines with diameters of about 0.5–0.6 µm (presumably the type-B aciniform lines of Kovoor and Peters 1988) and large numbers of thinner lines with diameters of about 0.1–0.2 µm (presumably type-A aciniform of Kovoor and Peters 1988). Both types of lines were present on both the inner and the outer surfaces of a shroud that had not been wetted by the spider (Figs. 4*a*, 4*b*). In contrast, the silk lines in the shrouds of eight discarded prey packages were almost exclusively thick lines (Figs. 4*e*, 4*f*). Thus, the smaller diameter lines were removed during feeding. This digestion may occur relatively quickly. When a prey package was removed after only 5 min of exposure to the digestive fluid and was washed in water to remove the solid residue from the digestive fluid, no small diameter lines were visible (Fig. 4*e*).

Experimental applications of a droplet of digestive fluid for 40 min to each of two sheets of wrapping silk in a humid environment caused a gradual reduction in the lines (Fig. 6*b*). Similar applications of tap water had no apparent effect (Fig. 6*a*), whereas the application of pineapple juice also caused strong reductions in the lines. Examination under SEM of the edges of areas destroyed by digestive fluid showed few thin lines and a concentration of intact thick lines (Figs. 6*c*, 6*d*), suggesting selective removal of the thin lines.

The fluid regurgitated by the spider apparently digested prey membranes. Prey dissected from discarded prey packages were always somewhat disarticulated (Fig. 3*b*) and scat-

Fig. 5. Spinnerets of a *P. vicina* that was killed while wrapping a prey: (*a*) posterior median spinneret and (*b*) posterior lateral spinneret. Silk lines of small and large diameters (presumably type-A and type-B aciniform lines, respectively) emerge from small (arrows in *b*) and large (arrow in *a*) diameter spigots.

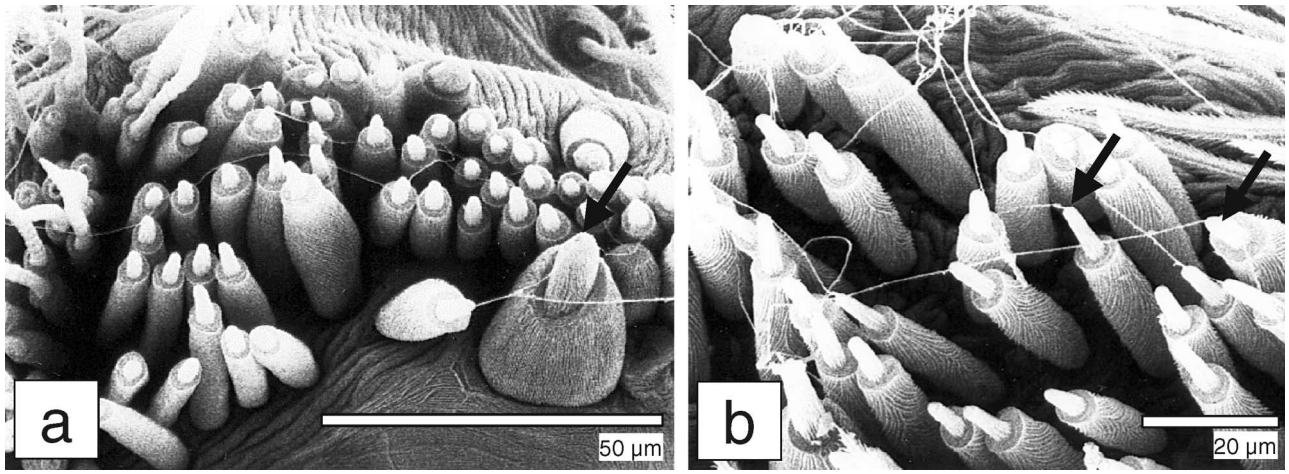


Fig. 6. Results of experimental treatments exposing a sheet of wrapping silk of *P. vicina* to (*a*) water and (*b–d*) spider digestive fluid for 40 min, then washing in water. (*a*) The lines near the hair, where a drop of water was placed, remained intact; (*b*) lines near the hair, where the drop of spider digestive fluid was placed, were nearly completely gone; (*c*) a closeup of the sheet in *b* shows multiple breaks in thin, type-A lines; and (*d*) at the lower edge of a large hole in the sheet (for a magnified view see *b*) is a multi-stranded cable of thick, unbroken, type-B lines.

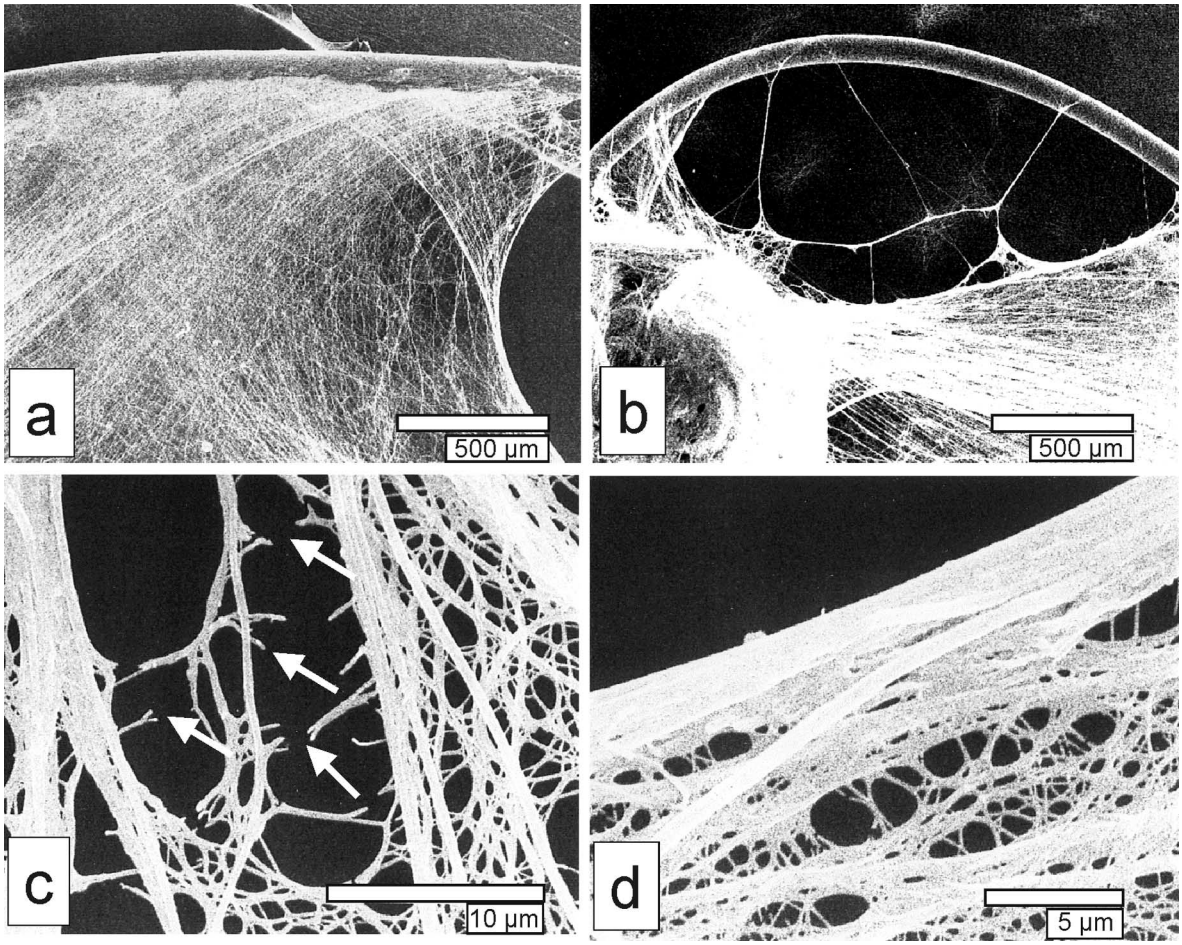
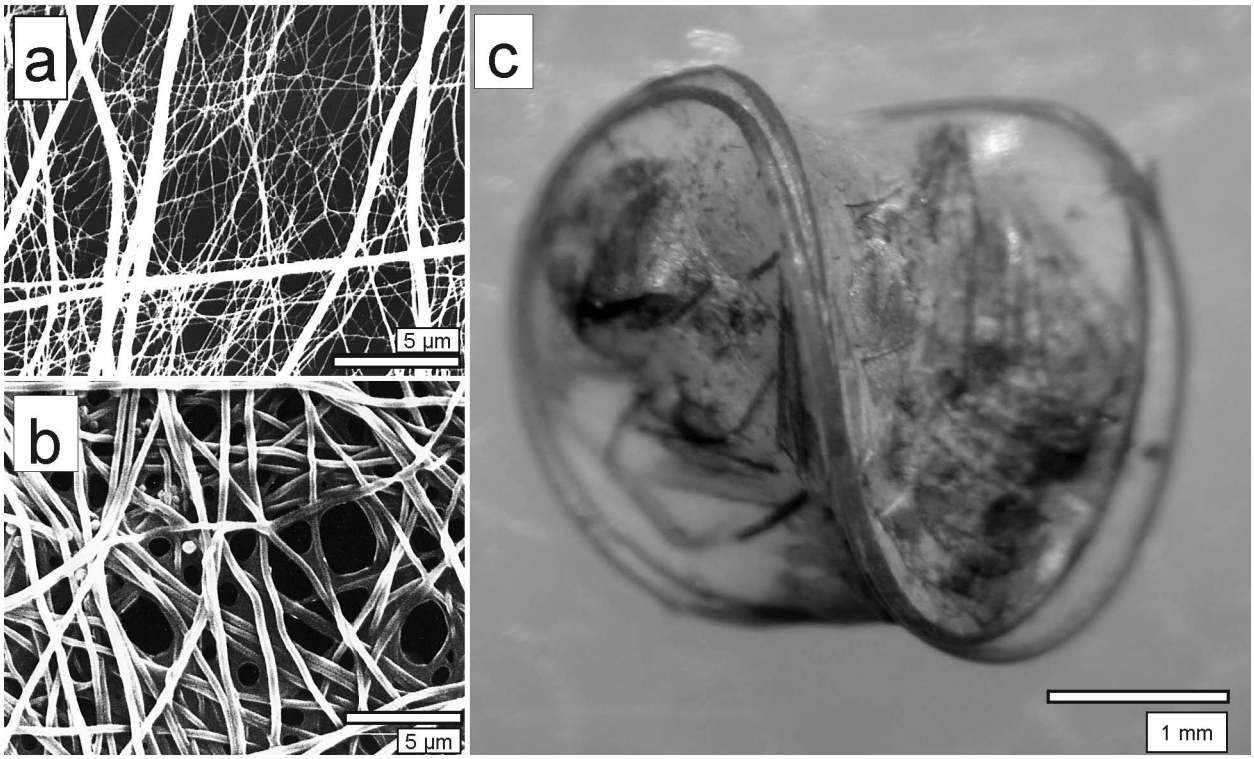


Fig. 7. Wrapping silk of *Uloborus* spider. (*a*) Silk lines of a prey package that was removed from the spider before she wetted it. (*b*) Silk lines of a prey package that was discarded by the spider after feeding on it. (*c*) A 1.1 cm hair that was inserted through a *Drosophila* fly and bent by wrapping silk applied by the spider.



tered setae that had separated from the prey adhered to the inner surface of the shroud. The ends of disarticulated segments of legs from discarded prey packages that were examined in the SEM completely lacked intersegmental membranes (Fig. 3*d*). In addition, the free seta scattered on the inner surface of the shroud also lacked their basal membranes (Fig. 3*c*).

The digestive fluid also apparently caused the death of the prey, which had survived being wrapped. Most of the ants in packages that were removed from the spider after being wetted with digestive fluid were dead (91%), whereas only 18% of unwetted ants and 27% of ants wetted with water were dead ($\chi^2_{[2]} = 13.9$, $p = 0.0009$).

Spinnerets and spigots that produced wrapping lines

Wrapping lines were found emerging from two sizes of spigots on the posterior median and posterior lateral spinnerets (Figs. 5*a*, 5*b*). The morphology, numbers, and locations of these spigots corresponded closely with those for type-A and type-B aciniform gland spigots in the genus *Polenecia* Lehtinen, 1967 (Kovoor and Peters 1988).

Food uptake and water loss

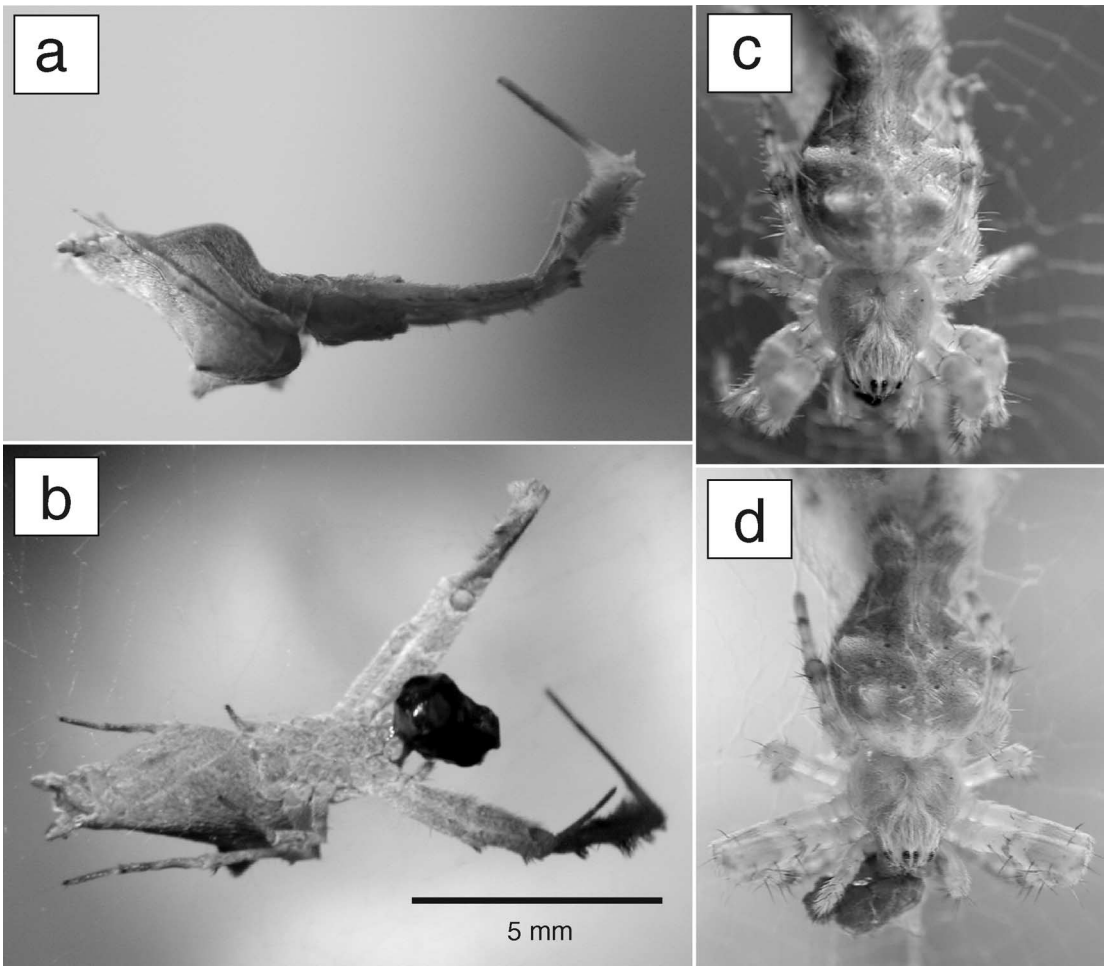
The combined wet mass of the spider and her prey was typically greater before feeding than after, suggesting that water was lost during feeding on a variety of prey (flies, moths, parasitic wasps, a termite). The wet mass (mean ± 1 SD) of the spider and the prey prior to capture were 11.1 ± 2.5 and 2.8 ± 1.2 mg, respectively (sum = 13.9 ± 2.7 mg, $n = 12$). Soon after the spider had fed and the prey had been discarded, their corresponding mass were 12.9 ± 2.5 and 0.4 ± 0.3 mg (sum = 13.3 ± 2.6 mg), respectively. The mass gain by the spider was 1.8 ± 1.0 mg, about 64% of the prey's original wet mass. The combined mass of the spider plus the prey was reduced, on average, by 0.6 mg after feeding. This represented an average loss of 25% ± 23% of the prey's wet mass, presumably owing to evaporation of water.

Comparisons with other spiders

Another uloborid, genus *Uloborus* Latreille, 1806, also wrapped *Drosophila* prey for many minutes and strongly compressed the prey package: a 11 mm hair through the fly was bent even more sharply back on itself than hairs wrapped by *P. vicina* (Fig. 7*c*). The shroud covering a prey that had been wrapped but not fed on also contained lines with two different classes of diameters — approximately 0.3–0.4 µm and 0.04–0.05 µm (Fig. 7*a*). The shroud covering a prey that had been fed on and discarded by the spider contained only the thicker diameter lines (Fig. 7*b*), indicating that this genus also produces multiple diameter wrapping lines and that smaller diameter lines are later digested. Feeding spiders spread their anterior legs (Fig. 8*b*), which were otherwise kept pressed together during the day (Fig. 8*a*; also Opell and Eberhard 1983).

An unidentified species of the deinopid genus *Deinopis* MacLeay, 1839 (voucher specimen FN21-133B) wrapped a large cockroach (approximately 50%–80% of the mass of the spider) for only 2–4 min and then began to feed without wetting the prey package (except, presumably, the area near the mouth). Thus, this species lacked both the extensive wrapping and the wetting of the entire prey package that are typical of uloborids.

Fig. 8. A mature female *Uloborus* spider at the hub of her web (*a*) holds her anterior legs together when without prey during the day, but (*b*) spreads them while feeding. Similarly, a mature female *Allocyclosa bifuca* (*c*) folds her anterior legs tightly against her body when she lacks prey during the day, but (*d*) alters her stance while feeding. Scale bar applies to all parts of the figure.



The wrapping silk of the araneid *Allocyclosa bifurca* (McCook, 1887) included both thick and thin lines, as well as masses of apparent liquid (Figs. 9*a*, 9*b*). Large holes in the shrouds of discarded prey in some areas where the spider had fed (Fig. 9*c*), and the nearly complete absence of wrapping lines in the masticated packages of smaller prey (Fig. 9*d*), indicate that the spiders digested both thick and thin wrapping lines while feeding. Similar holes also occurred in shrouds of the prey of another araneid, *Argiope argentata* (Fabricius, 1775) (Tillinghast and Kavanagh 1977). Pineapple juice also digested holes in a *A. bifurca* shroud.

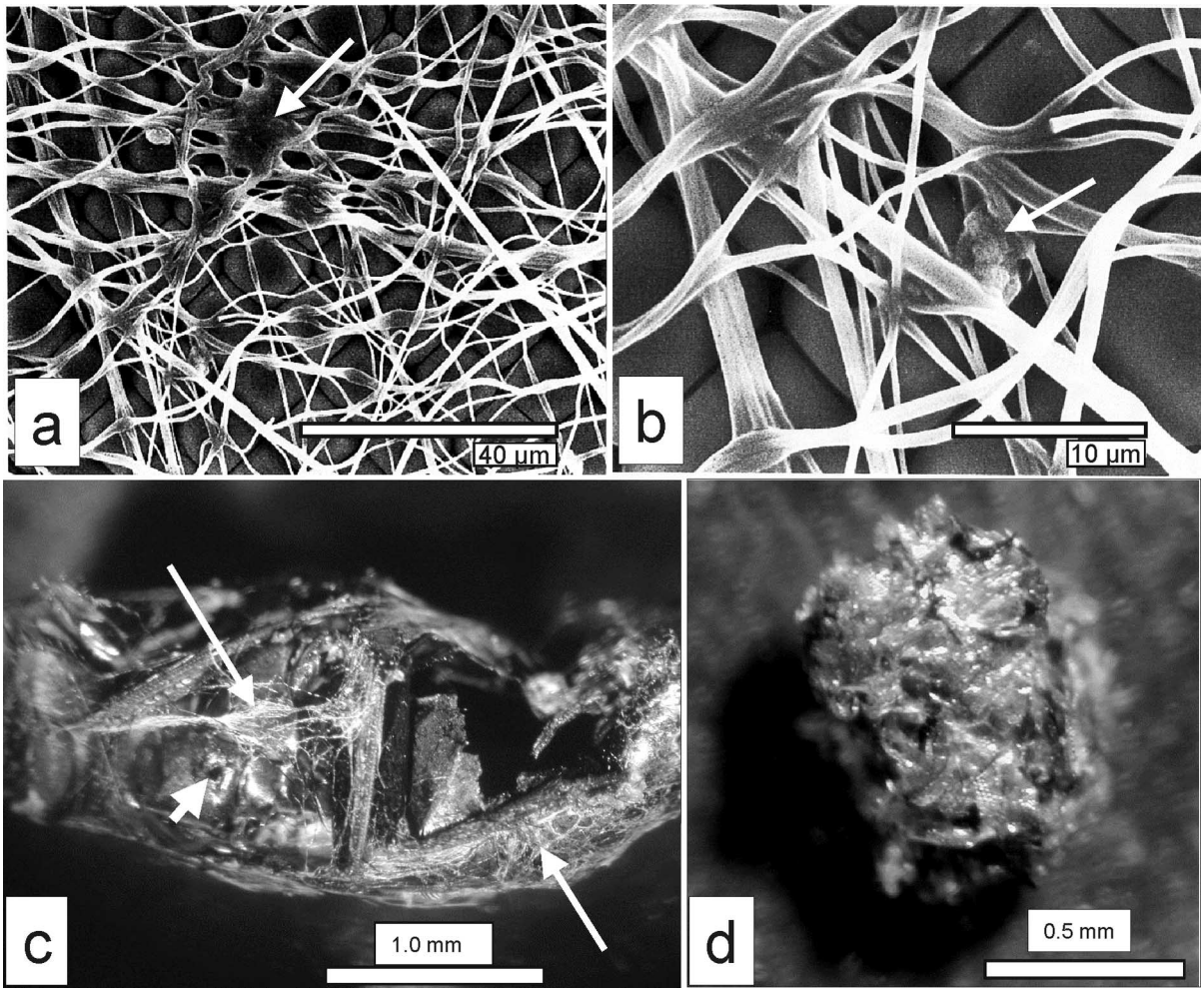
When acalyptrate or muscoid flies were given to each of six different mature female *A. bifurca* with wet mass of 30.3 ± 6.6 to 35.4 ± 7.5 mg (mean ± 1 SD), the mass of the prey decreased from 9.35 ± 3.7 to 1.5 ± 0.8 mg. Of this decrease in prey mass, the spider gained 5.1 ± 2.2 mg, or about 54% of the prey's mass, and 3.0 ± 2.3 mg (approximately 30% of the prey's original mass) was lost, presumably by evaporation.

Discussion

The digestive fluid of *P. vicina* resembles that of other spiders in being alkaline (Pickford 1942; Herrero and Odell 1988). It also resembles that of *Pisaura mirabilis* (Clerck, 1757) (Nitzsche 1988) in being relatively concentrated and leaving a heavy residue when it evaporates (Fig. 4*c*). The fluid apparently digests the prey's membranes, its internal tissues, and the thin type-A aciniform lines of the spider's wrapping silk. The low surface tension of the digestive fluid of *P. vicina* (compared with that of water) probably helps spiders extract nutrients from isolated portions of the prey (e.g., inside legs segments) without masticating them (Eberhard et al. 2006*b*). The digestive fluid also kills prey that survive the compression produced by wrapping lines, confirming Opell's (1988) predictions. Prey may be killed because the fluid contains a poison, or because its wetting action causes them to suffocate.

It is not clear how the spider's own membranes were protected from being degraded by her digestive fluid. However, the robust, long setae near the tip of the pedipalp may reduce possible damage from sustained contact with the wet surface of the prey package during the long feeding period (Fig. 2*b*). Spreading the anterior legs while feeding presumably also functions to avoid damage from wetting, by keeping the legs out of contact with the digestive fluid on the prey package. The endites and chelicerae of other groups of spiders also contact digestive fluid during feeding, but without apparent damage (Eberhard et al. 2006*b*).

Fig. 9. Wrapping silk of *Allocyclosa bifurca*. (*a*, *b*) In the shroud over the compound eye of a prey that was removed from the spider before she began to feed are thick and thin lines and masses of apparent liquid (arrow in *b*). Many lines have multiple strands. (*c*) The lines in the shroud on a discarded prey (a calliphorid fly) are intact over much of the prey's surface (long arrows) but are gone at the two sites where the spider had fed — the anterior portion of the abdomen (large hole on right) and on the thoracic pleuron (small hole marked with a short arrow on left). (*d*) The remains of a small prey (*Drosophila* sp.) discarded after the spider had fed consist of only small fragments. There is also almost no vestige of the shroud of silk that was applied during the attack wrapping.



Why do uloborid spiders wrap their prey so extensively? It is probably costly in several respects. Wrapping obviously represents a substantial investment of time, energy, and materials. The dense shroud probably also has a sponge effect, retaining liquids (Opell 1979), and retention of digestive liquid will increase the amount that the spider must regurgitate in order for a given amount to reach the prey. It will also decrease the fraction of the liquid in the prey that the spider is able to recover by sucking, because spiders never squeezed the shroud in any way while sucking. Thus, contrary to Opell (1979), a sponge effect seems likely to be disadvantageous rather than advantageous, unless the greater concentration of nutrients resulting from evaporation somehow increases digestive efficiency (B.D. Opell, personal communication (2005)).

One possible advantage of the extensive prey wrapping of uloborids is that it compresses the prey into a compact package with a reduced surface area, and thus facilitates the unusual (unique?) uloborid feeding technique of wetting the entire surface of the prey with digestive fluid (Eberhard et al. 2006*a*). A second possible positive effect is that the silk shroud filters out much of the solid material, which accumulates as a thick mat of fine detritus on its inner surface (Fig. 3*a*). The dense setae around the mouth and the palate-plate filter indicate that, for as yet unknown reasons, ingestion of solid particles is disadvantageous for spiders in general. Presumably these advantages of extensive wrapping outweigh the costs in *P. vicina*.

Thus, the reduced surface area of the prey package that results from extensive compressive wrapping makes it possible to cover the package with a smaller amount of digestive fluid. This leaves unanswered, however, the question of why uloborids wet their prey this way. This feeding technique is presumably derived rather than ancestral, as we know of no other spider group in which it occurs (Eberhard et al. 2006*b*), and our brief observations tentatively suggest that it is absent in the sister family Deinopidae (further observations of other types of prey are needed). Wetting the entire prey likely has at least two costs. Uloborids spread their anterior legs while feeding on all prey, which constitutes a partial breaking of crypsis, revealing two legs (Figs. 1*b*, 8*b*). Some araneids that masticate their prey also assume postures that

reduce leg contact with larger prey (Figs. 8*c*, 8*d*), and thus also disrupt crypsis. Wetting, at least of small prey that would have otherwise been masticated, may have the disadvantage of increasing the susceptibility of uloborids to predators.

Wetting the entire surface is also likely to result in water loss. Under the moderately humid indoor conditions of this study, an average of approximately 25% of the prey's wet mass was lost during feeding. This number is only a crude estimate of losses in nature, because water loss probably varies with both different web sites and prey. The humidity at web sites in nature is surely (at least during the wet season) higher than that under which we made our observations. We also used a mixture of prey which was undoubtedly different from that in nature (9 orders were represented in a sample of 46 prey collected from spiders feeding in the field; Eberhard et al. 2006*a*). The thick shroud of silk must further increase water loss, by increasing the surface area of the prey package that is exposed to evaporation.

To a first approximation, however, water loss and food extraction seem similar to those for the araneid spider *A. bifurca*. The approximately 25% water loss to evaporation in *P. vicina* was similar to the approximately 30% water loss in *A. bifuca*, as was the percentage of the prey's original mass that was gained by the spider (64% compared with 55%, respectively). These are only preliminary data, as the quantity of food that is extracted from a given prey varies widely, even intraspecifically (Turnbull 1962; data in dry mass of prey), and it is probably affected by several variables, such as the relative sizes of the spider and its prey, as well as the thickness of the prey's cuticle. More detailed comparisons must await further studies.

The water loss that results from wetting the entire surface of the prey package may not be critical in *P. vicina* (perhaps the spiders obtain sufficient water from their prey?). Some species of the genera *Philoponella* and *Uloborus* inhabit dry habitats in the Sonoran desert (Muma and Gertsch 1964), and *Zosis geniculata* (Olivier, 1789) and *Octonoba octonaria* (Muma, 1945) occur at sheltered sites (e.g., inside buildings) where rain never falls (Lent and de Oliveira 1961; Opell 1979; W.G. Eberhard, unpublished observations of *O. octonaria* in a grain elevator in Kansas).

Histochemically, the type-A glands of the uloborid *Polenecia* differ from type-B glands (Kovoor and Peters 1988); the proximal portion of the type-A gland produces a protein that lacks the amino, reducing, and carboxyl groups that occur in the product of type-B glands (and also the distal portion of type-A glands). The two types also differ morphologically, as the cells lining the lumen are taller in type-B glands than in type-A glands (Kovoor 1987). Kovoor and Peters (1988) found only type-A glands in four other uloborid genera (*Uloborus*, *Zosis* Walckenaer, 1842, *Hyptiotes* Walckenaer, 1837, and *Miagrammopes* O. Pickard-Cambridge, 1870). The pair of larger aciniform spigots on the median spinnerets of *Uloborus* and *Hyptiotes* species, which probably correspond to the similar pair of larger spigots that produce the thick wrapping lines (type B) in *P. vicina* (Figs. 5*a*, 5*b*), were associated with glands that were type B with respect to both morphology and histochemistry (Kovoor and Peters 1988). Nevertheless, the present study documents two types of silk with respect to diameters and susceptibility to digestion in both *Philoponella* and *Uloborus*. *Philoponella* is thought to be more closely related to *Zosis* and *Uloborus* than to *Polenecia* (Opell 1979).

Digestion of aciniform type-A lines (also seen in *Uloborus* sp.) reduces but does not eliminate the estimated material costs of wrapping. If one assumes that 10 type-A wrapping lines are produced for every type-B line (Figs. 5*a*, 5*b*), and that their respective diameters are 0.15 and 0.55 μm (Eberhard et al. 2006*a*), then type-A lines represents about 40% of the volume of silk in the shroud. These numbers are only approximations, but they indicate that the recovery of type-A lines by the spider could represent appreciable savings.

Perhaps the explanation for these apparent inconsistencies is that there are further histochemical differences between type-A and type-B glands that result in different susceptibilities to general proteases but that were not revealed by the staining techniques used by Kovoor and Peters (1988). Both type-A and type-B aciniform glands (distinguished using morphological and histochemical criteria) occur in several araneine genera (*Argiope* Audouin, 1826, *Araneus* Clerck, 1757, *Cyrtophora* Simon, 1864, *Cyclosa* Menge, 1866, *Eriophora* Simon, 1864, and *Nemoscolus* Simon, 1895) (summarized in Kovoor 1987; Kovoor and Peters 1988) and in lines of different diameters occur in the wrapping silk of *A. bifurca* (Figs. 9*a*, 9*b*). In related groups, such as Nephilidae, Tetragnathidae, Metinae, Linyphiidae, and Theridiidae, only type-B glands are present (Kovoor 1987; Kovoor and Peters 1988). Multiple diameters, which differ by up to a factor of 3–4, occur in the wrapping lines of the distantly related pisaurid, *P. mirabilis* (Fig. 19*c* in Nitzsche 1988).

Evolutionary loss of venom glands: the "wrapping hypothesis"

Our observations suggest the hypothesis that three unique uloborid traits related to feeding (wetting the entire prey package, compacting the prey with extensive wrapping, and the lack of cheliceral poison glands) are evolutionarily related. *Menneus* Simon, 1876 and *Deinopis* MacLeay, 1839, in the sister family Deinopidae, attack wrap their prey (Akerman 1926; Robinson and Robinson 1971; J. Coddington, personal communication (2005)), as do many araneids (Robinson et al. 1969; Robinson and Olizarri 1971), which are thought to be related to these dinopoids (Coddington 2005). However, prey are not wrapped and compressed extensively in either group as they are in uloborids. As in araneids, deinopids possess venom and bite their prey. This combination of evidence favors the hypothesis that spiders ancestral to the family Uloboridae attack wrapped their prey and that venom glands were probably lost in the uloborid line after wrapping had evolved. Wrapping in moderate amounts of silk may have evolved to facilitate immobilization, transport, or handling (Eberhard 1967; Robinson et al. 1969). Subsequent selection to recover some of this silk could have favored more extensive wetting the prey package during or after feeding. Digestive liquid probably accumulates on the outer surface of the prey near the mouthparts in most spiders (Eberhard et al. 2006*b*), thus making recovery of wrapping silk in this area feasible. In fact, partial recovery of wrapping silk is evidenced by the holes in the shrouds in the area where the spider regurgitated as it fed in the araneids *Allocyclosa* and *Argiope* (Fig. 9*c*) and also in the pisaurid *P. mirabilis* (Nitzsche 1988). Changes to increase the

digestibility of type-A wrapping lines might also have been favored. Digestible wrapping lines may be ancestral because they also occur in araneoids (Griswold et al. 1998).

More extensive wetting of the prey could favor more extensive wrapping, by reducing its cost in terms of lost silk. More wrapping, in turn, could result in greater compaction of the prey if the wrapping lines were laid under tension and were at all adhesive and extensible (Eberhard et al. 2006*a*), and this could make wetting even more advantageous by reducing the surface area to be wetted. Preliminary filtering of prey contents by the shroud might confer an additional advantage to more extensive wrapping. Then if, as it seems likely, greater compression and general wetting with digestive fluid could kill prey, selection favoring efficiency in protein synthesis and use could have led to the loss of cheliceral poison glands.

Acknowledgements

We thank Yael Lubin and Brent Opell for useful ideas and discussions regarding spiders, Jon Coddington for information on the genus *Deinopis*, and the Smithsonian Tropical Research Institute and the Universidad de Costa Rica for financial support.

References

Akerman, C. 1926. On the spider, *Menneus camelus* Pocock, which constructs a moth catching expanding snare. Ann. Natal Mus. **5**: 411–422.

Bartels, M. 1930. Ueber den Fressmechanismus und den chemischen Sinn einiger Netzspinnen. Rev. Suisse Zool. **37**: 1–41.

Coddington, J.A. 2005. Phylogeny and classification of spiders. *In* Spiders of North America: an identification manual. *Edited by* D. Ubick, P. Paquin, P.E. Cushing, and V. Roth. American Arachnological Society. pp. 18–24.

Collatz, K.-G. 1987. Structure and function of the digestive tract. *In* Ecophysiology of spiders. *Edited by* W. Nentwig. Springer-Verlag, Berlin. pp. 229–239.

Comstock, J.H. 1948. The spider book. *Revised and edited by* W.J. Gertsch. Comstock Publishing Associates, Ithaca, N.Y.

Eberhard, W.G. 1967. Attack behavior of diguetid spiders, and the origin of prey wrapping in spiders. Psyche (Stuttg.), **74**: 173–181.

Eberhard, W.G., Platnick, N.I., and Schuh, R.T. 1993. The natural history and behavior of web parasites of the spider *Tengella radiata*: the spider *Mysmenopsis tengellacompa* sp. n. (Mysmenidae) and the bug *Lipokophila eberhardi* sp. n. (Plokiophilidae). Am. Mus. Novit. **3065**: 1–17.

Eberhard, W.G., Barrantes, G., and Weng, J.-L. 2006*a*. Tie them up tight: *Philoponella vicina* spiders mangle and kill their prey by wrapping. Naturwissenschaften, **93**: 251–254. doi:10.1007/s00114-006-0094-1. PMID:16544123.

Eberhard, W.G., Barrantes, G., and Weng, J.-L. 2006*b*. Feeding by *Philoponella vicina* (Araneae, Uloboridae) and the mystery of how spiders extract food from prey without masticating them. Bull. Br. Arachnol. Soc. In press.

Fincke, O. 1981. An association between two Neotropical spiders (Araneae: Uloboridae and Tengellidae). Biotropica, **13**: 301–307. doi:10.2307/2387809.

Foelix, R. 1996. Biology of spiders. Harvard University Press, Cambridge, Mass.

Griswold, C.E., Coddington, J.A., Hormiga, G., and Scharf, N. 1998. Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopioidea, Araneoidea). Zool. J. Linn. Soc. **123**: 1–99. doi:10.1006/zjls.1997.0125.

Herrero, M.V., and Odell, G.V. 1988. Algunas caracteristicas de los venenos y secreciones digestivas de *Aphonopelma seemanni* y *Sphaerobothria hoffmanni* (Araneae: Theraphodidae) de Costa Rica. Rev. Biol. Trop. **36**: 241–246. PMID:3238077.

Kaestner, A. 1968. Invertebrate zoology. II. Arthropod relatives, Chelicerates, Myriapoda. Translated and adapted by H.W. Levi and L.R. Levi. Wiley Interscience, New York.

Kovoor, J. 1987. Comparative structure and histochemistry of silk-producing organs in arachnids. *In* Ecophysiology of spiders. *Edited by* W. Nentwig. Springer-Verlag, Berlin. pp. 160–186.

Kovoor, J., and Peters, H.M. 1988. The spinning apparatus of *Polenecia producta* (Araneae, Uloboridae): structure and histochemistry. Zoomorphology (Berl.), **108**: 47–59. doi:10.1007/BF00312214.

Lent, H., and de Oliveira, S.J. 1961. The tropicopolitan spider "*Ulborus geniculatus*" (Olivier), a new predator of the cigarette beetle in warehouses; possibility of its use in biological control. Rev. Bras. Biol. **21**: 351–358.

Lubin, Y.D. 1986. Web building and prey capture in the Uloboridae. *In* Spiders, webs, behavior and evolution. *Edited by* W.A. Shear. Stanford University Press, Stanford, Calif. pp. 132–171.

Muma, M.H., and Gertsch, W.J. 1964. The spider family Uloboridae in North America north of Mexico. Am. Mus. Novit. **2196**: 1–43.

Nitzsche, R.O.M. 1988. "Brautgeschenk" und Umspinnen der Beute bei *Pisaura mirabilis*, *Dolomedes fimbriatus* und *Thaumasia uncata* (Arachnida, Araneida, Pisauridae). Verh. Natwiss. Ver. Hambg. **30**: 353–393.

Opell, B.D. 1979. Revision of the genera and tropical American species of the spider family Uloboridae. Bull. Mus. Comp. Zool. **148**: 443–549.

Opell, B.D. 1988. Prey handling and food extraction by the triangle-web spider *Hyptiotes cavatus* (Uloboridae). J. Arachnol. **16**: 272–274.

Opell, B.D., and Eberhard, W.G. 1983. Resting postures of orb-weaving uloborid spiders. J. Arachnol. **11**: 369–376.

Pickford, G.E. 1942. Studies on the digestive enzymes of spiders. Trans. Conn. Acad. Arts Sci. **35**: 33–72.

Robinson, M.H., and Olizarri, J. 1971. Units of behavior and complex sequences in the predatory behavior of *Argiope argentata* (Fabricius) (Araneae: Araneidae). Smithson. Contrib. Zool. **65**: 1–36.

Robinson, M.H., and Robinson, B. 1971. the predatory behavior of the ogre-faced spider *Dinopis longipes* F. Cambridge. Am. Midl. Nat. **85**: 85–96. doi:10.2307/2423914.

Robinson, M.H., Mirick, H., and Turner, O. 1969. The predatory behavior of some araneid spiders and the origin of immobilization wrapping. Psyche (Stuttg.), **76**: 487–501.

Tillinghast, E., and Kavanagh, E.J. 1977. The alkaline proteases of *Argiope* and their possible role in web digestions. J. Exp. Zool. **202**: 213–222. doi:10.1002/jez.1402020210.

Tillinghast, E., and Townly, M. 1994. Silk glands of araneid spiders. *In* Silk polymers: materials science and biotechnology. *Edited by* D. Kaplan, W.W. Adams, B.L. Farmer, and C. Vincy. ACS Symposium Series No. 544, American Chemical Society, Oxford University Press, Oxford. pp. 29–44.

Turnbull, A.L. 1962. Quantitative studies of the food of *Linyphia triangularis* Clerck (Araneae: Linyphiidae). Can. Entomol. **94**: 1233–1249.

Zimmermann, E.W. 1934. Unterschungen über den Bau des Mundhohlendaches der Gewebespinnen. Rev. Suisse Zool. **41**: 149–176.