



Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation

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ARTICLE INFO

Article history:

Received 1 March 2009
Initial acceptance 11 May 2009
Final acceptance 6 October 2009
Available online 16 December 2009
MS. number: A09-00140

Keywords:

Alloyclosa bifurca
orb web
parasitic manipulation of host
parasitoid wasp
Polysphincta clade
Polysphincta gutfreundi

The larva of the ichneumonid wasp *Polysphincta gutfreundi* induces its host, the orb-weaving spider *Alloyclosa bifurca*, to build a highly modified, physically stable orb web, to which the larva then attaches its pupal cocoon, and to add an otherwise unusual linear silk stabilimentum to this web that may camouflage the cocoon. The effects of the larva are apparently due to a chemical product or products that it introduces into the spider. Behavioural modification is gradual, and various behavioural effects arise in a consistent order. If the wasp larva is experimentally removed just before it kills the spider, the spider's behaviour recovers gradually in the reverse order. In addition, a greater delay in removing the larva leads to more pronounced and enduring behavioural changes, so the larval effects may depend on a cumulative or dose-dependent process. Changes in numbers and lengths of radii and numbers of sticky spiral loops could result from correlated larval effects on the reduction in the amount of silk in the spider's sticky spiral silk glands (or a signal thereof), but several other types of behavioural change are probably under separate controls; multiple larval products may be involved. The larvae may affect higher levels of behavioural decisions by spiders that determine overall web 'design', rather than lower levels, such as control of particular behaviour patterns, as may be affected by related wasps. The larva's effects are fine-tuned to details of the host's natural history.

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Some parasitic organisms induce changes in the behaviour of their hosts that favour the reproduction of the parasite rather than that of the host (Moore 2002; Thomas et al. 2005). The mechanisms by which these manipulations are achieved are generally poorly understood. In some cases the parasite elicits host responses by altering properties of the host's nervous system (Helluy & Thomas 2003; Biron et al. 2005, 2006; Lefevre et al. 2007a, b), but the mechanisms by which these alterations affect only a particular subset of the host's behaviour remain to be determined.

Ichneumonid wasp larvae of the *Polysphincta* clade (Gauld & Dubois 2006) (hereafter 'polysphinctine wasps') alter the web-building behaviour of their host spiders late in the penultimate larval instar, inducing the spider to build particularly resistant or protective silk structures that shelter the larva's cocoon from enemies and the elements (Nielsen 1923; Eberhard 2000a, 2001; Gonzaga & Sobczak 2007; Matsumoto & Konishi 2007; Weng & Barrantes 2007; Matsumoto 2008; Gonzaga et al., in press). The behaviour of all spiders with such larvae is altered, and spiders have never been seen to recover and reproduce once behavioural alterations begin (and only very seldom after a wasp lays an egg; see

Gonzaga & Sobczak 2007; Gonzaga et al., in press). Thus by the time the behaviour modification occurs, the spider is sure to die without reproducing, and these behavioural modifications can have evolved only under selection on the wasps, rather than on the spiders.

Web modifications discovered to date in polysphinctine wasps include simple omission of the orb (Gonzaga & Sobczak 2007), construction of a more or less normal, alternative web design (Gonzaga et al., in press), construction of a unique design by selective repression and expression of normal subroutines of orb construction (Eberhard 2001), or other alterations of less certain homologies (Weng & Barrantes 2007; Matsumoto 2008). The behavioural alterations are diverse, but they are consistently adjusted to details of the host's natural history (e.g. durable versus fragile webs, presence or absence of protected retreats) in ways that seem to promote the survival of the wasp's cocoon (Matsumoto 2008, Gonzaga et al., in press).

The behavioural modifications induced by wasp larvae can also aid in understanding the organization of orb web construction behaviour (Eberhard 2000a, 2001). Larval effects offer the opportunity to test the independence of various aspects of orb web construction and design that are otherwise strongly correlated, such as radius length, web area, and numbers of hub loops, radii, and sticky spiral loops.

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The mechanisms by which wasp larvae manipulate their host's behaviour remain unclear, except that they involve a larval product or products injected into the spider (Eberhard 2000b). This study documents changes induced by a polysphinctine wasp, *Polysphincta gutfreundi*, in the web design and construction behaviour of the spider *Alloctylosa bifurca*. Clues to possible mechanisms of the parasite's manipulation of the behaviour of its host come from temporal patterns of behavioural changes and comparisons of various types of altered behaviour.

The life cycle of *P. gutfreundi* (W. G. Eberhard, unpublished data) is similar to that of other polysphinctine species whose life histories have been studied (Nielsen 1923; Eberhard 2000b; Gonzaga & Sobczak 2007; Matsumoto & Konishi 2007; Matsumoto 2008): the adult female briefly paralyzes the host spider on its web, lays an egg on the surface of its abdomen, and leaves. Subsequently the wasp larva feeds for a week or more by sucking the spider's internal fluids through small holes that it makes in the spider's abdominal cuticle and finally induces the spider to build one or more highly modified webs. It then kills the spider, sucks it dry, and attaches its own pupal cocoon to the spider's final web (hereafter the 'cocoon web').

METHODS

Spiders (all mature females) and their parasites were observed and collected near San José, Costa Rica. Unless otherwise specified, captive spiders were induced to build webs on wire hoops that were hung vertically indoors, either by fastening the anchor lines of their webs to the hoops with masking tape or by placing the spiders on previous webs in the hoops. Webs were usually rebuilt every day, and construction behaviour was observed in captivity. Unless noted otherwise, the quantitative measurements presented concerning the process of succumbing to larval effects and then recovering from them used the webs of parasitized spiders, which were inspected daily but were not photographed. The variables measured in these webs were those that most obviously changed and were easily quantified; they included an estimate of the mean radius length ('mean radius length'; the mean length from the centre of the hub to the intersection with the frame for the radii in the four cardinal directions; Fig. 1a), numbers of doubled radii that formed V's at the frame (Fig. 2c, d; 'V radii'; lines attached to eggsac stabilimenta, which often had V shapes in normal orbs, were not counted), the maximum number of sticky spiral loops ('number of sticky spiral loops'), and the presence of a silk stabilimentum (Fig. 1c).

Other webs were coated with cornstarch and photographed. The webs of parasitized spiders were then tweaked and jarred to remove most of the cornstarch from the lines and thus allow the spider to use them in subsequent webs. Additional measurements from photographs included the mean number of recognizable spiral hub loops below and to the two sides (Figs 1b, 2d; 'number of hub loops') and the number of radii that originated on a hub loop (Fig. 1b; 'number of radius origins'; other radii originated in more central portions of the hub).

Larval development was estimated using the degree of visibility of the final instar dorsal 'pseudopods' of the larva, each of which had an apical circle of dark setae. Under the dissecting microscope, these circles were visible through the transparent cuticle of a penultimate instar larva when it was mature and soon to moult (Fig. 3d, inset), but were less visible in penultimate instar larvae that were less mature; their visibility (dark, light, absent) was used to estimate the relative maturity of each penultimate instar larva when it was experimentally removed from its host spider. Larvae were removed from spiders by gently prying and pulling them off with a forceps while the spider sat at the hub of her web.

Spiders were fed *Drosophila* sp. flies until the wasp larva was late in its penultimate instar. Although they were frequently sprayed with water, they were not fed after the larva had been removed (spiders were behaviourally incapable of attacking prey during the first week or so of their recovery, turning towards the prey and shaking the web energetically but not advancing further in the prey attack sequence; e.g. Suter 1978).

Tests of differences in slopes and intercepts of regression lines followed Zar (1996). Most variables were not normally distributed, and Mann–Whitney *U* tests were used unless otherwise specified; means are given ± 1 standard deviation, and the threshold of significance was $P < 0.05$.

RESULTS

Web Modifications with Parasite

Spiders built one to three modified webs on the days immediately before they were killed by the wasp larva. Of 28 spiders, 39% built only one modified web, 50% built two, and 11% built three. Several aspects of web design changed on successive days and provided clues regarding the mechanisms used by the larvae (see Discussion). Comparing the final ('cocoon') web and the penultimate web of 37 spiders that were killed by larvae in captivity, the number of radii and the maximum number of sticky spiral loops both decreased sharply (from 38.0 ± 6.5 to 15.6 ± 5.3 radii, and from 33.1 ± 11.6 to 3.2 ± 6.1 sticky spiral loops; $Z = -6.80$, -6.72 , both $P < 0.001$). The mean radius length also decreased (from 8.2 ± 1.5 to 5.2 ± 1.8 cm; $Z = -3.53$, $P < 0.001$). The spaces between the reduced numbers of sticky spiral loops also appeared to be more irregular (Figs 1a, 2a, b), though no quantitative measurements were made. Nine cocoon webs measured in the field had designs similar to those of the cocoon webs in captivity (14.9 ± 3.3 radii, 0.7 ± 2.0 sticky spiral loops, and mean radius length 3.8 ± 0.6 cm).

The radii in cocoon webs were generally attached to interior frame lines built inside the previous frame lines (Fig. 2a). When the spider built two or three modified webs, the new frames in the successive webs were built inside previous frame lines. Comparing all photographed webs, there were also fewer hub loops (Figs 1b, 2d) in modified webs than in control webs of nonparasitized spiders (Fig. 3a; $Z = 8.94$, $P < 0.0001$). Smaller numbers of radii originated on hub loops (Fig. 1b) in modified webs (Fig. 3c), and the proportion of the radii that originated on hub loops in these webs was smaller (Fig. 3b; $Z = 10.4$, $P < 0.0001$).

In addition, modified webs were more likely than control webs to have linear silk stabilimenta (Fig. 1c). In a sample of 42 captive spiders that built at least one modified web and for which a previous, unmodified web had been measured, linear silk stabilimenta were more common in the modified webs (31% versus 5%) ($\chi^2_1 = 9.23$, $P = 0.0024$). Linear silk stabilimenta were also common in cocoon webs in the field (50% of 10 webs). All of the more than 25 silk stabilimenta seen on modified webs in the lab and the field were linear; none included the silk discs or mats of white stabilimentum silk covering the central portion of the hub that are the most common type of silk stabilimentum on the webs of nonparasitized spiders (74% of 175 and 93% of 86 silk stabilimenta included a disc in two populations of the spider; Eberhard 2003, unpublished data; $\chi^2_1 = 51.9$ for combined data, $P < 0.0001$). One cocoon web in the field had both an eggsac stabilimentum above the hub and a linear silk stabilimentum below. This was striking, because construction of silk stabilimenta is repressed in nonparasitized spiders by the presence of an eggsac stabilimentum (Eberhard 2003), and none of hundreds of webs of nonparasitized

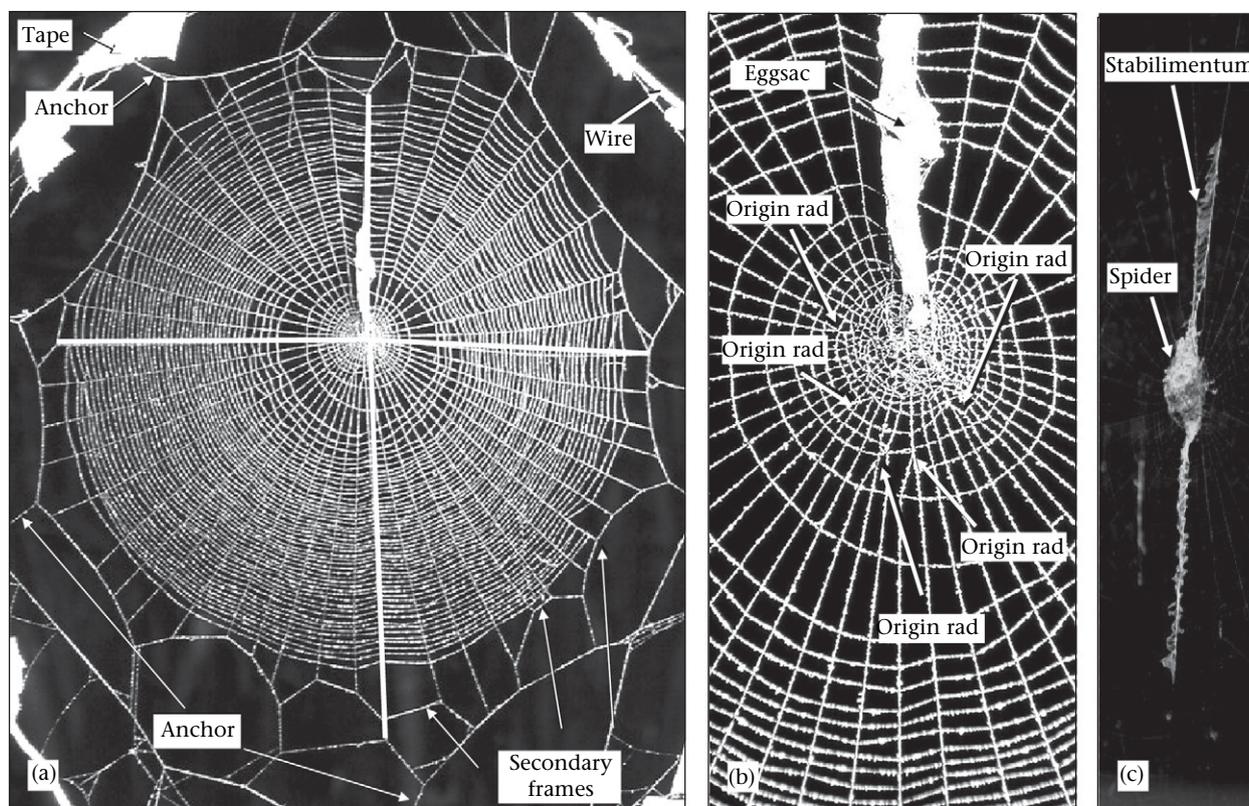


Figure 1. (a) Web, (b) hub and (c) linear silk stabilimentum of a nonparasitized adult female *A. bifurca* (white objects are pieces of masking tape by which the anchor lines of previous webs had been attached to the wire hoop). Thick lines in (a) indicate lengths of radii measured.

spiders with eggsac stabilimenta checked in the field also had a linear silk stabilimentum.

The number of V radii (Fig. 2c, d) also increased in modified orbs. Of 32 cocoon webs, 90.6% had at least one V radius (mean = 2.5 ± 1.8 /web); there no V radii in the immediately preceding webs of the same spiders ($\chi^2_1 = 38.8$, $P < 0.0001$). Expanding this sample to include spiders from which larvae were removed after the spider had built at least one web with larval effects and in which a previous unmodified web by the same spider was observed, there was at least one V radius in 53.8% of 39 modified webs, as opposed to 8.6% in the immediately preceding webs built by the same spiders ($\chi^2_1 = 17.2$, $P < 0.0001$). The mean number of V radii in 71 control, unmodified orbs was 0.056 ± 0.287 . Cocoon webs in the field also had elevated numbers of V radii (mean = 1.1 ± 1.3 , $N = 9$).

Order of Changes

The order in which these changes occurred has implications for the mechanisms by which larvae modify spider behaviour (see Discussion). The overall order of the changes apparently induced by larvae was as follows. Early effects (in modified webs that were followed by one or two additional modified webs) included moderate reductions in the number of radii, the number of loops of sticky spiral, and the area included within the frames and a greater distance between the outer loop of sticky spiral and the frame lines. Later webs showed greater reductions in the numbers and lengths of radii and in the numbers of sticky spiral loops (sticky spirals were often completely absent in cocoon webs; Fig. 2c). The spaces between sticky spiral loops when they occurred were more irregular, and the frequencies of stabilimenta and V radii increased.

The effects on the numbers of radii and sticky spiral loops and mean radius length, as well as on the presence of stabilimenta and V radii were all gradual. In 27 cases in which the spider built two modified webs, the modifications were greater in the second, cocoon web: 14.1 ± 3.8 versus 19.8 ± 6.6 radii in the second versus the first web, 1.7 ± 3.9 versus 9.0 ± 7.1 loops of sticky spiral, 4.40 ± 0.9 cm versus 4.5 ± 1.2 cm mean radius length, 0.59 ± 0.84 versus 0.17 ± 0.49 silk stabilimentum lines per web, and 2.0 ± 1.9 versus 0.7 ± 1.1 V radii per web ($Z = -3.49, -3.50, -2.18, 2.0$ and 2.26 , respectively, $P = 0.0005, 0.0004, 0.029, 0.047$ and 0.024).

Behaviour While Building Webs

The construction behaviour for control webs was similar in most respects to that of other araneid spiders (Eberhard 1982, 1990). The spider apparently always broke and replaced the radius as she returned to the hub during both radius and frame construction (pattern F1 of Eberhard 1982). One behaviour not reported for other orb weavers was to reinforce the stabilimentum suspension lines. The spider moved upwards away from the hub along the eggsac or detritus stabilimentum without breaking or removing any other lines on either the trip upwards or the trip back to the hub; the spider thus laid lines that reinforced the attachment of the sacs to lines at the top of the web. Hub construction began during the last portion of radius construction, after more than half of the radii had been laid. The spider interrupted hub construction to add new radii, and the sites where these additional radii originated could be deduced from the pattern of lines in the hub of the finished web (Fig. 1b).

Construction of modified webs involved some behavioural changes. Very early during construction of a modified web, the

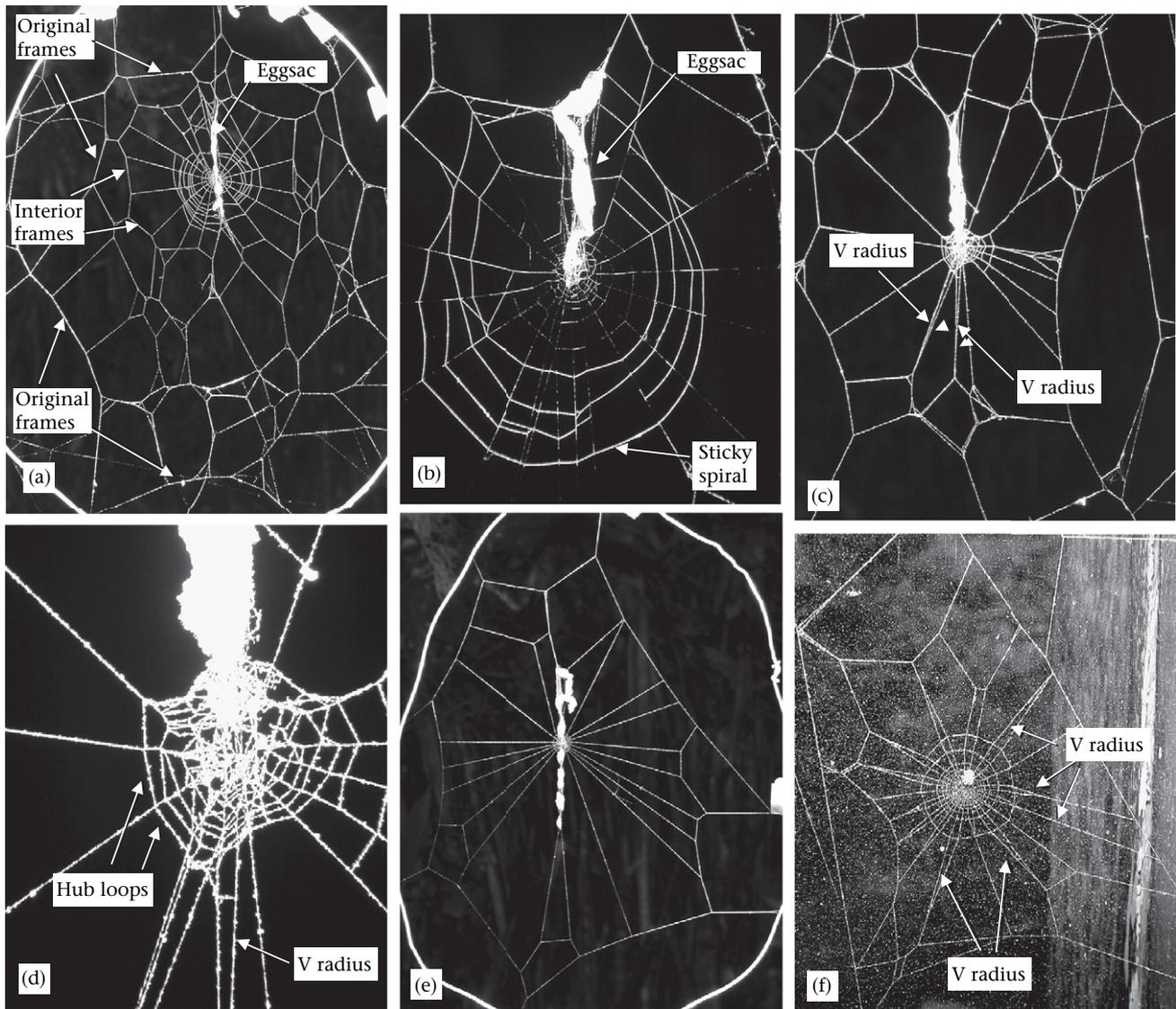


Figure 2. (a–d) Modified webs of parasitized adult female *A. bifurca* illustrating changes induced by larval *P. gutfreundi*. (a) The original frame lines of an earlier orb are longer than the interior frames of the modified orb. (b) Modified web in which all lines were coated with cornstarch and then jarred repeatedly to remove the cornstarch from all but the sticky lines. (c) V radii in a cocoon web. (d) Closeup of the hub of the web in (c) illustrating the lack of radius origins. (e) ‘Resting’ web of a nonparasitized female, which was preceded and followed by normal orbs. (f) Resting web of a mature male in the field near the web of a mature female.

spider sometimes replaced lines by breaking and reeling up previous lines as she walked and laid a new line, as occurs during normal orb web construction. But the spiders sometimes also added new lines without breaking previous lines. Some behaviour patterns during the early stages were not easily classified (again, as occurs in the construction of normal orbs), but recognizable radius construction and frame construction eventually became frequent. In contrast to normal orb construction, new radial lines were usually doubled, with both the new radial line laid on the trip away from the hub towards the frame and the line laid on the way back to the hub being left intact. Usually the second line lay alongside the first (F4 of Eberhard 1982), but in some cases the second line was attached at a different point at the hub and was thus slightly separated from the first line, forming a narrow V (F3 of Eberhard 1982) that could be distinguished in the finished web (Fig. 2c, d). In two cases a spider mixed V radius construction and typical F1 radius construction while building a single modified web. All spiders building modified webs made at least one trip up along the eggsac stabilimentum and above, adding a line during both the

outwards and the return trip. No further addition of radii was seen once hub construction began; this observation is in accord with the analyses of photos of finished webs described above, which showed that such late radii were rare.

Spiders building modified webs also often reinforced frame lines that were already in place. For instance, during the construction of an interior frame line (Fig. 2a) the spider often added lines to more distant frame lines and to anchor lines, moving all the way to the wire frame; during such a trip the spider added her drag line to the line along which she was walking on both the outwards and the return trip and did not break and reel lines. Frame reinforcement of this sort was not seen in control web construction and is not typical of orb weavers in general (Eberhard 1982; Foelix 1996).

Recovery after the Larva was Removed

When the larva was experimentally removed after it had induced the spider to produce one or more altered webs, but before

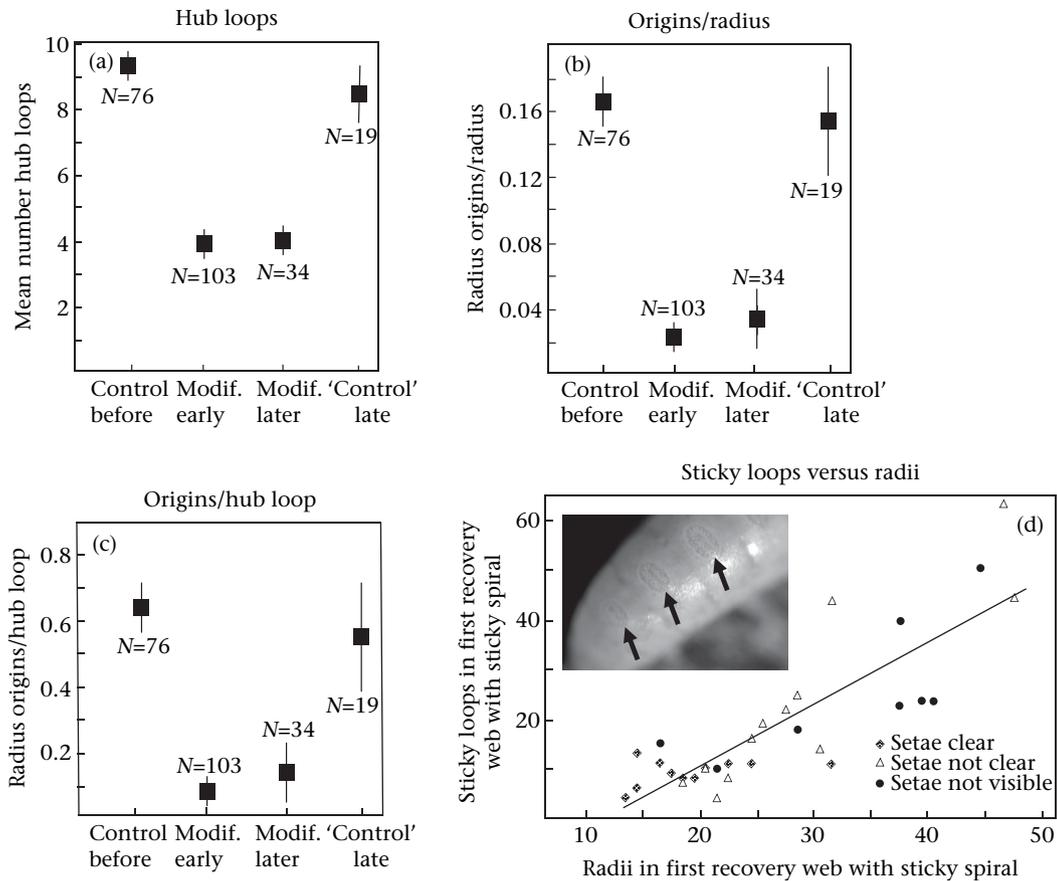


Figure 3. Comparisons from photographs between control webs of nonparasitized spiders, 'early' modified webs (first and second modified webs spun while the larva was on the spider), 'later' modified webs (generally the first and in some cases also the second sticky spiral recovery web), and the final 'control' webs (the last web photographed for each spider that had made at least 10 days of sticky spiral recovery webs). Sample sizes vary because not all webs of all spiders were photographed. The variables compared are (a) the numbers of hub loops, (b) the numbers of radius origins/radius, and (c) the numbers of radius origins/hub loop (vertical lines represent 95% confidence intervals). In (a–c) all pairwise comparisons were highly significant ($P < 0.001$) except those between early and late modified webs and between control and 'final control' webs. In (d), differences in the strength of the effects of a penultimate larva on the numbers of radii and sticky spirals are compared for cases in which the larvae showed different degrees of maturity when they were removed (maturity was judged by the distinctiveness of the circles of setae of the last instar visible through the cuticle of the penultimate instar; arrows in inset). Larvae that were more mature produced significantly more severe effects in the first sticky spiral recovery web. The numbers of radii (mean = 16.9 ± 5.3) and sticky spiral loops (mean = 9.3 ± 2.6) differed significantly between the most mature larvae and the intermediate (respective means 26.2 ± 9.4 , 23.2 ± 18.5) or the immature larvae (30.8 ± 10.0 , 25.6 ± 13.5). Respective Z values for radii: $Z = -2.80$, -2.68 , $P = 0.005$, 0.007 ; for sticky spirals: $Z = -2.03$, -3.18 , $P = 0.042$, 0.001 .

the larva had moulted to the final instar and killed the spider, the spider gradually recovered during the following 1–2 weeks, eventually making more or less normal webs. A sample of 42 spiders that had produced at least one web that completely lacked sticky spiral lines, either immediately before the larva was removed or in the first web she made afterwards, was used to document the course of recovery. The first postremoval web that included sticky spiral silk was built 4.69 ± 1.63 days after the larva was removed (range 1–8, median 5). During the period between removal of the larva and production of the first web with sticky silk ('early recovery webs'), there was no significant change in the number of radii, the radius length, or stabilimentum construction, whereas the number of V radii increased towards the end of this period (Fig. 4b, c). In the webs built after sticky spiral lines had reappeared ('sticky spiral recovery webs'), the numbers of radii and sticky spiral loops and the lengths of the radii gradually increased (Fig. 4a).

Various aspects of building behaviour returned to control values at different rates in sticky spiral recovery webs. V radius construction declined slowly (Fig. 5a), whereas stabilimentum construction decreased more abruptly, reaching normal values by the third day (Fig. 5b). The changes in the numbers of radii and sticky spiral loops and in the mean radius length were more prolonged; they had nearly reached the control values but were all still

rising on the 13th day (Fig. 4a). Analyses of photographed webs showed that variables related to hub loops also recovered gradually (Fig. 3a–c). In a subsample of 12 spiders for which there was a photo of the first web lacking a sticky spiral, and of a late recovery web (an average of 17.8 ± 7.7 days later), the number of radii originating on the hub loops increased from 0.25 ± 0.45 to 4.42 ± 3.42 ($Z = -3.64$, $P = 0.0003$), the number of hub loops from 6.50 ± 1.60 to 7.90 ± 1.73 ($Z = -2.02$, $P = 0.043$), and the proportion of the radii that originated on a hub loop from 0.026 ± 0.048 to 0.26 ± 0.16 ($Z = -3.58$, $P = 0.0003$). These changes all occurred despite the lack of feeding during the entire postremoval period. Spiders occasionally showed brief relapses; for instance, the sticky spiral was absent in one web after the first sticky spiral recovery web in 16 of 71 spiders.

The estimated degree of maturity of the larva when it was removed had a significant effect on the delay before the spider first began to produce a sticky spiral again. For 14 mature penultimate instar larvae (dark setae), the mean number of days was 5.0 ± 2.0 (range 0–8), for 10 intermediate larvae (light setae) the mean was 1.2 ± 1.9 (range 0–5), and for 7 younger larvae in (no setae visible) the mean was 0.4 ± 0.5 days (range 0–1; $P = 0.0013$ and 0.0010 comparing larvae with dark setae with those with light setae and no setae, respectively; $P = 0.39$ comparing larvae with light and no

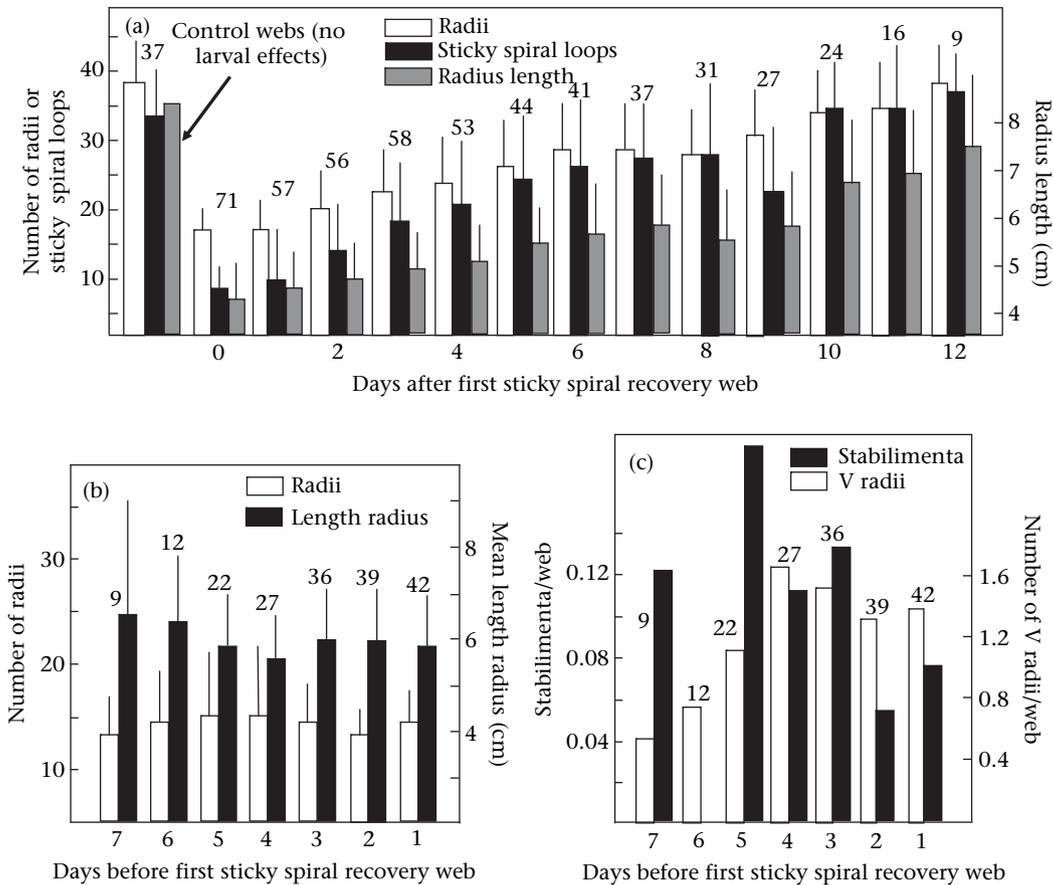


Figure 4. The time course of changes induced by larva. (a) Gradual increases in numbers of radii, sticky spiral loops, and average radius length in successive sticky spiral recovery webs. (b) Lack of change in numbers of radii and radius lengths in early recovery webs (built during period in which no sticky silk was produced). (c) Lack of consistent change in stabilimenta and V radii in early recovery webs. In (b) and (c) the first web in which sticky spirals were present after the larva was removed was on day 0. Sample sizes are given above bars; they are smaller towards the left because some spiders built only a few webs before beginning to build sticky spirals.

setae). Greater larval maturity also correlated with more severe reductions in the numbers of radii and sticky spiral loops in the first sticky spiral recovery web after the larva was removed (Fig. 3d).

Some aspects of the overall designs of recovery webs resembled those of control orbs, whereas others were different, providing insights into how web traits are controlled (see Discussion). The relationship between the number of radii and the number of sticky spiral loops in sticky spiral recovery webs differed only slightly from that in control webs (Fig. 6a). The regression lines had similar

slopes (1.26 and 1.12, respectively), and the sticky spiral recovery webs were simply displaced towards lower numbers of radii and sticky spiral loops. In contrast, the relationships between the number of radius origins and the number of hub loops (Fig. 6b), between the number of radius origins on hub loops and the number of radii (Fig. 6c), and between the number of hub loops and the number of radii (Fig. 6d) were different in early recovery webs. The relationship between the number of radii and the radius length in all sticky spiral recovery webs (Fig. 7) was also different.

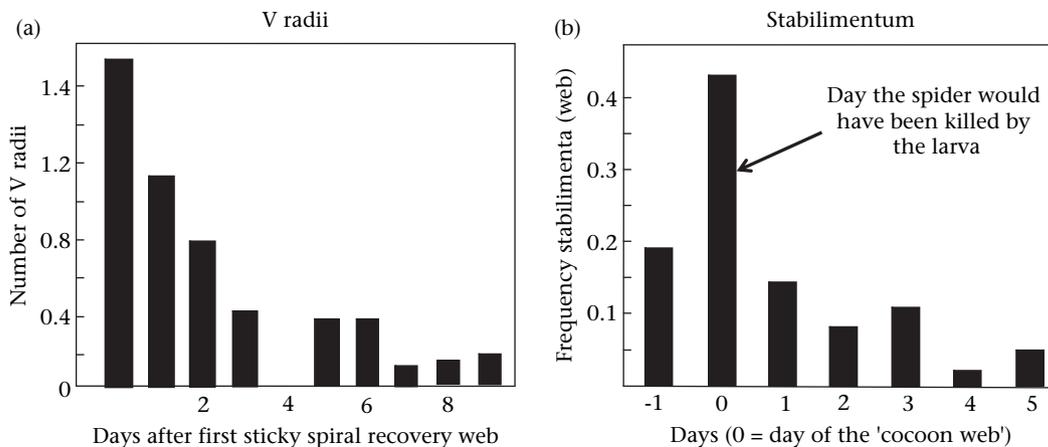


Figure 5. Time course of recovery in sticky spiral recovery webs from changes induced by larvae in (a) V radii and (b) stabilimenta.

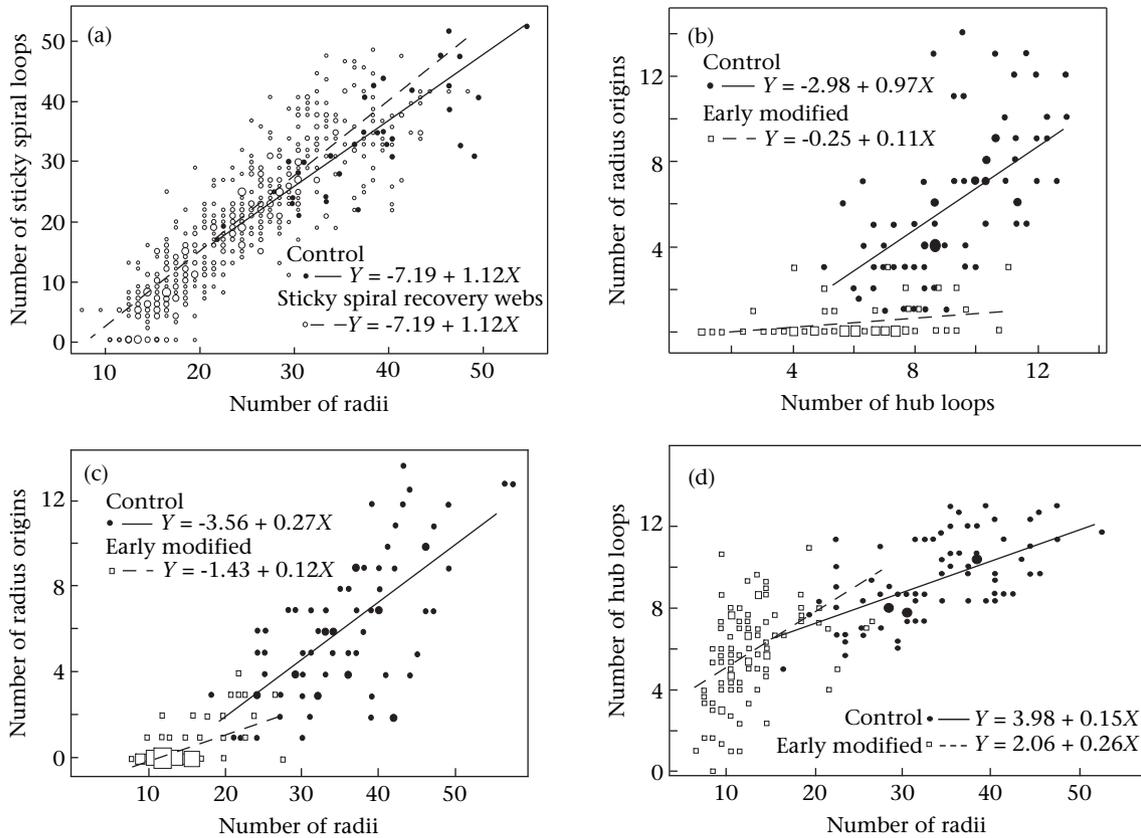


Figure 6. Relations between web variables in control and modified webs of mature female *A. bifurca*. (a) Nonparasitized spiders (control) compared with all sticky spiral recovery webs and (b–d) nonparasitized spiders (control) and all modified webs prior to the first web with sticky spiral lines. Larger symbols indicate multiple webs with the same values. The slopes and intercepts of the control and modified webs differed significantly in all cases ($P < 0.01$).

DISCUSSION

Possible Functions of Modified Behaviour

Several modifications induced by the wasp larvae, including shorter radii, a doubling of the number of lines in each radius (some of which resulted in V radii), and reinforced frame and anchor lines during radius construction, probably improved the physical stability of the web and may thus improve survival of the wasp

pupa. Inhibition of sticky spiral production may also improve web stability by reducing the likelihood that the web will accumulate weighty debris. The sticky spiral also constitutes a substantial fraction of the material in an orb, so by repressing sticky spiral production, the larva causes this material to be retained in the spider’s body from where it can then be ingested.

The increased production of linear silk stabilimenta in cocoon webs may also favour the wasp’s survival. Stabilimentum production might seem to be slightly disadvantageous to the wasp, because the silk in the stabilimentum is unavailable to the larva when it consumes the spider. Other evidence suggests, however, that silk stabilimenta in *A. bifurca* function as camouflage (Eberhard 2003), and a linear silk stabilimentum could help camouflage the linear outline of the wasp’s cocoon. Cocoon webs had only linear silk stabilimenta and never the disc-shaped stabilimentum design that is much more common on the orbs of nonparasitized spiders (Eberhard 2003) but which would not camouflage the cocoon as effectively. This is the first report of effects on stabilimentum production by a polysphinctine wasp. Along with the larva’s induction of the strong radii and frame and anchor lines of the orbs of *A. bifurca* to support the cocoon web, these changes represent still another example of a polysphinctine wasp that has adaptively adjusted its effects on host behaviour to the natural histories of its host.

Higher- versus Lower-level Effects of Larvae

The most extensive previous study of the effects of polysphinctine wasp larvae on spider behaviour concluded that the larva of *Hymenoepimecis argyraphaga* manipulate the host spider

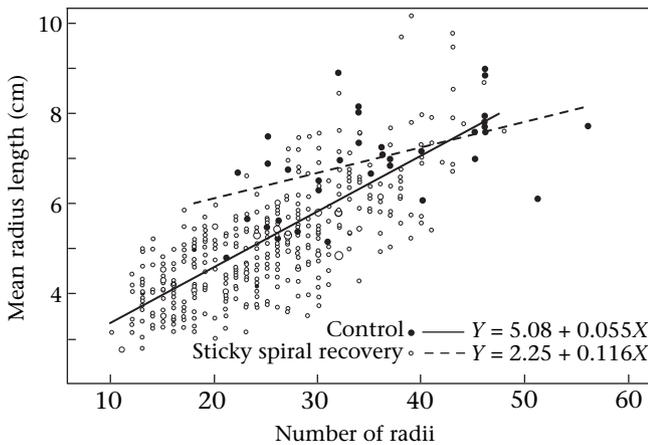


Figure 7. Similar but not identical relationships between the numbers of radii and the mean radius lengths in control and sticky spiral recovery webs (slopes differ, $P < 0.001$).

behaviour at a low level in the hierarchy of organization of individual behaviour patterns, inducing the spider to repeat one subroutine of construction of frame lines in orbs and repressing all other routines (Eberhard 2001). In contrast, several aspects of the overall designs of modified webs in this study suggest that the larva of *P. gutfreundi* did not affect one aspect of the web independent of others, but rather affected the spider at higher levels of behavioural decisions that determine overall designs of webs. The correlations between numbers of radii and sticky spiral loops (Fig. 6a) and, to a lesser extent, between the number of radii and the mean radius length were similar to those of control webs (Fig. 7), so these variables were thus not altered independently by the larva. One exception was that correlations involving hub loops and radii originating on hub loops differed in modified and control webs (Fig. 6b–d). This difference may be a result of normal hub construction consisting of two phases (Eberhard 1990; Zschokke 1996). The radii in modified webs may correspond to early radii, and the larva's effects may be to repress the construction of later radii.

Understanding the larva's effects depends on understanding how the spider's construction behaviour is organized. The different amounts of time needed for the spider to recover from the larva's effects on construction of silk stabilimenta and V radii, as opposed to radius length and the numbers of radii and sticky spiral loops (Figs 4a, 5a, b), imply that these behaviour patterns are under at least semi-independent control. On the other hand, the linkage between the number and length of radii and the number of sticky spiral loops (Fig. 4a) may result from a single mechanism: reduced sticky spiral silk. Experimental manipulation of the reserves of sticky silk in the glands of two species of orb weavers resulted in simultaneous changes in the numbers of both radii and sticky spiral loops (Eberhard 1988).

The modified webs elicited by *P. gutfreundi* resembled the resting webs spun by nonparasitized *A. bifurca*, raising the possibility that larvae were simply triggering the decision to build an alternative design already programmed in the spider. Cocoon webs were not, however, identical to either type of resting web. They resembled the resting or moulting webs of nonparasitized *A. bifurca* (Fig. 2e; similar resting webs also occur in the related genus *Cyclosa*, Zschokke & Bolzern 2007; and in *Argiope argentata*, G. Barrantes & W. G. Eberhard, unpublished data) in that the resting webs also lacked sticky spirals. But the resting webs of *A. bifurca* had longer radii than cocoon webs (mean 6.9 ± 1.3 cm, $N = 16$, versus 5.2 ± 1.8 cm, $N = 14$; $Z = 2.28$, $P = 0.023$), fewer hub loops (mean 2.7 ± 1.3 , $N = 7$, versus 5.7 ± 2.3 , $N = 105$; $P < 0.0001$), and were less likely to have V radii (6.2% of 16 versus 90.6% of 32, $\chi^2_1 = 32.4$, $P < 0.00001$), stabilimenta (0% of 17 versus 27.6% of 29; $\chi^2_1 = 5.68$, $P = 0.017$), or interior frames (Fig. 2e).

Cocoon webs also resembled the resting webs made by two mature male *A. bifurca* (Fig. 2f) in having short radii with multiple V radii. But the male webs apparently differed (the sample was too small for statistical tests of significance) in lacking linear silk stabilimenta, having relatively larger hubs, having a larger fraction of V radii, and lacking numerous interior frame lines. The modular elements of orbs and their associated behaviour patterns lend themselves to reorganization and recombination in novel configurations, as often occurs during the evolution of behaviour (West-Eberhard 2003).

Possible Dose-dependent Effects of Larvae

The alterations in the spider's web caused by the wasp larva were all reversed when the larva was experimentally removed shortly before it killed the spider. The process of succumbing to the larva's effects was gradual, as was that of recovering from them, but

recovery was much slower, lasting up to about 14 days as opposed to usually only 1–2 days to succumb. The web traits that were affected earliest as the spider succumbed (reduced numbers of radii and sticky spiral loops, reduced radius length) were the slowest to recover when the larva was removed. This pattern suggests that the severity of the larva's effects may depend on the concentration of a psychotropic substance or substances injected by the larva into the spider. Perhaps the psychotropic manipulative material is transferred gradually and degraded or deactivated gradually when the larva is removed.

This concentration hypothesis is supported by several types of evidence. Larval *P. gutfreundi* have direct access to blood in the spider's abdomen, but not to her central nervous system, which is in the spider's cephalothorax; the larva did not otherwise penetrate the spider's body. The larva's midgut undergoes complex secretory modifications during the period when the spider's behaviour is altered (W. G. Eberhard & B. A. Huber, unpublished data). And the effects on webs were more severe if the larva was nearer to its final moult when it was removed (and thus may have transferred a greater quantity into the host; Fig. 3d).

It is not obvious how to reconcile the larva's multiple adaptive and at least partially independent effects on the spider's behaviour with this apparently simple pattern of behavioural changes that suggests a dosage-dependent response. The specificity of the larva's effects would seem to be entirely due to chemical properties, as the larva cannot improve specificity by injecting its products into certain parts of the host, as do the adult females of some other parasitoids (Gal et al. 2005). One extreme hypothesis, that a different larval product is used to modify each independent aspect of the spider's behaviour, seems unlikely (though not ruled out definitively), because the products would have to become inactivated during recovery in the reverse order in which they were injected.

The opposite extreme hypothesis, that a single, multipotent larval product acts as a neuromodulator or neurohormone, could explain the inverse order of changes if different behaviour patterns were affected by different concentrations of the larval product. Some insects show multiple behavioural responses to single neuromodulator (e.g. the effect of octopamine on multiple aspects of foraging in honeybees; Schulz & Robinson 1999; Barron et al. 2007). Such diverse responses could all be controlled by a single master switch mechanism that coordinates the expression of an entire set of coadapted behaviour patterns, each of which conceivably has a slightly different response threshold. But this master switch hypothesis is unlikely to explain the effects of the larval wasp on *A. bifurca* spiders. This is because, in contrast to cases such as the neuromodulator effect in honeybees, the design properties of the spider's nervous system have surely not evolved to facilitate the suite of behavioural manipulations produced by a wasp larva by providing a master switch mechanism. For example, there is no reason to suppose that selection on the spider has favoured linking the control of linear silk stabilimentum construction (as opposed to disc stabilimenta or lack of stabilimenta) to control of the amount of sticky spiral silk or to doubling the radii. It does not seem likely that the special mix of effects documented here, which is adaptive from the point of view of the larva, would be produced by concentration changes in only a single product. One further (as yet entirely speculative) possibility is that the mature female wasp facilitates particular larval effects during the multiple stabs she delivers to the spider's thorax with her ovipositor after the spider has already been parasitized (W. G. Eberhard, unpublished data). Perhaps specific portions of the spider's nervous system are rendered sensitive to the products that are later introduced by the wasp larva. In sum, it is not certain whether more than one specific response-modulating larval product is involved.

Acknowledgments

I thank Ian Gauld, Paul Hanson, and Herbert W. Levi for kindly identifying the wasps and spiders; Federico Bolaños and Jeremy Niven for help with statistics and literature; and M. J. West-Eberhard for comments on the manuscript. Financial support was provided by the Smithsonian Tropical Research Institute and the Universidad de Costa Rica.

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