Correlations between leg positions and spaces between sticky lines in the orbs of *Micrathena duodecimspinosa* (Araneae: Araneidae)

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Summary

Although orb web construction behaviour is relatively well studied, there are few studies of the mechanisms with which behavioural decisions are executed, in terms of where the spider grasps lines and attaches them to each other. Video analyses were used here to show that the distance from the previous sticky loop at which the araneid spider *Micrathena duodecimspinosa* gripped the radius with her leg oIV during sticky spiral construction varied according to the spider's position in her web with respect to both gravity and the edge of the web. This grasping site, in turn, was correlated with the site on the radius where the spider attached the sticky line. The spacing between loops of sticky spiral, a functionally important aspect of orb design, was thus determined in part, though not completely, by the site at which leg oIV grasped the radius at the moment of attachment.

Introduction

There is a long tradition of careful studies of orb web construction behaviour, going back to the pioneering naturalists Henri Fabre (1912) and R. W. G. Hingston (1920), and there have been many subsequent studies (major studies and general reviews include those of Wiehle 1927; Peters 1939, 1954; Witt *et al*. 1968; Eberhard 1972, 1982; Vollrath 1992; Zschokke & Vollrath 1995a,b; Kuntner *et al*. 2008; Herberstein & Heiling 1999; and Herberstein & Tso 2011). Orb construction behaviour has been more thoroughly studied than perhaps any other type of spider behaviour. Direct observations and experiments have demonstrated that certain stimuli affect decisions during orb construction, such as where to lay radii and where to attach sticky lines to these radii. Nevertheless, there is an unfilled gap regarding the body positions and movements that result in the execution of these decisions. Improved understanding of such mechanism questions will be crucial to a more complete understanding of the behavioural process of orb web construction, and of its evolution.

Placement of the sticky spiral has attracted special attention as it is highly repetitive (and thus easily studied), and it is also functionally very important in determining an orb's ability to capture prey (Eberhard 1986, Blackledge *et al*. 2009). Previous studies demonstrated that the spaces between loops of sticky spirals in orbs are influenced by various stimuli. Some 'reference' stimuli are sensed anew at each encounter with a radius: the site where the inner loop of sticky spiral is attached to the radius (IL site); the distance between the outer loop of temporary spiral and the inner loop of sticky spiral (TS-IL distance), and the difference between the current TS-IL distance and the TS-IL distance on the immediately preceding radius or radii (Hingston 1920; Eberhard & Hesselberg in press). Other, preliminary 'settings' stimuli may act to modulate the spider's responses to reference stimuli. For instance, the relationship between the amount of sticky silk in the spider's glands and the area covered by the radii and frame lines of the web influences how far attachments should be from the corresponding IL sites. Several other possible preliminary settings variables correlate with the distance from the IL reference point (sticky spiral spacing): the direction of gravity (Le Guelte 1966; Vollrath 1986, 1988), the distance from the hub (LeGuelte 1966), the length of the spider's legs (Vollrath 1987), the degree of hunger and the spider's weight (Christianson *et al*. 1962; Herberstein & Heiling 1999), and the amount of silk available (Reed *et al*. 1969; Eberhard 1988; Crews & Opell 2006). It has not been clear, however, how these stimuli are translated into changes in sticky spiral spacing, in terms of the positions of the spider's body and legs at the moment of the attachment. Mechanisms of this sort are the subject of this study.

The advent of cheap, portable, video-recording equipment has made behavioural analyses possible at finer scales of time and morphology than were previously feasible. Although studies of some types of arachnid behaviour have exploited these opportunities and examined heretofore inaccessible details in, for example, sexual behaviour (Peretti *et al*. 2006; Aisenberg & Barrantes 2010) and attacks on prey (Barrantes & Weng 2006), video recordings have only rarely been used to study orb construction behaviour. Even the most sophisticated of these studies (Zschokke 1993, 1996, 2000; Zschokke & Vollrath 1995a,b) involved recordings made at a distance rather than close up, and thus did not allow resolution of fine behavioural details.

Illumination of behavioural details can be useful in several ways, including clarification of the order in which decisions are made, and the mechanisms by which particular decisions are executed. The present study utilizes video recordings of the araneid *Micrathena duodecimspinosa* to examine details of leg positions during sticky spiral construction, and shows that variations in these details are correlated with variations in the spaces between sticky spiral lines.

Methods

Webs of adult female *M. duodecimspinosa* were photographed in the field near San Antonio de Escazú, San José Province, Costa Rica (1320 m a.s.l.) after being coated lightly with cornstarch. The digital photographs were analysed using ImageJ software. The construction behaviour of five mature female *M. duodecimspinosa* was videotaped in close-up views with ambient light in the field (spiders generally built after dawn) with a hand-held SONY DCR-TRV50 digital camera equipped with +6 close-up lenses. Absolute scales were not available in the video recordings because distances and angles of view were not constant, but I could determine quite precisely whether the spaces between loops of sticky spiral on adjacent radii increased or decreased by using a caliper to compare distances on the computer screen. Behavioural details during construction could thus be associated with changes in sticky spiral spacing.

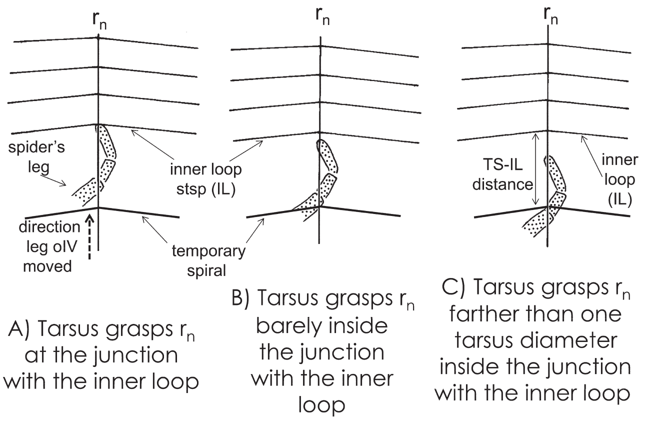

Fig. 1: Schematic drawings illustrating terms used in the text and showing sites grasped by tarsus oIV just prior to attachment of the sticky spiral: at the junction with inner loop (A); slightly inward of the junction (less than the diameter of the tarsus from the junction) (B); farther inward than the diameter of the tarsus (C).

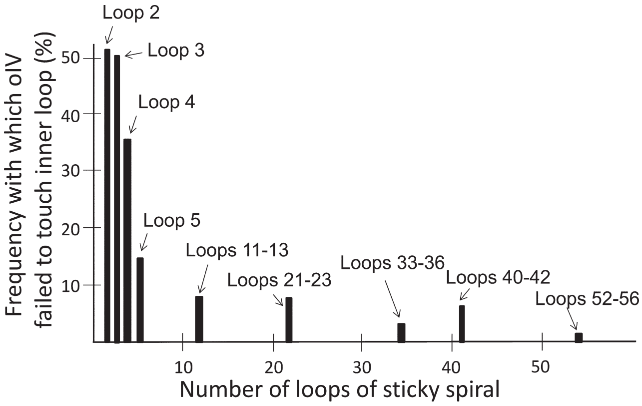

Fig. 2: The frequency with which tarsus oIV failed to contact the inner loop of sticky spiral during sticky spiral construction decreased as a mature female *M. duodecimspinosa* moved inward from the edge of an orb.

Measurements from photographs of different webs were standardized before being combined for analysis by dividing all values from a given web by the median for that web, in order to reduce variation due to variables such as individual spider size, silk gland reserves, and building site. Thus, a value of 1.0 indicated that an observed value was equal to the median for that web. Voucher specimens of *M. duodecimspinosa*, identified by H. W. Levi, have been deposited in the Museum of Comparative Zoology, Harvard University, and the Museo de Zoología of the Escuela de Biología, Universidad de Costa Rica.

Results

General aspects of sticky spiral construction resembled those of other araneids (Eberhard 1982). After finishing radius, hub, and temporary spiral construction, the spider always started building the sticky spiral near the edge of the web and gradually spiraled inward, using the temporary spiral lines (especially in the outer portion of the orb) as bridges between radii. Each time she encountered a radius, the spider moved outward along it (away from the hub) until she contacted the innermost line of sticky spiral that she had already laid (henceforth the inner loop; see Fig. 1); she then turned and attached the sticky line that she was producing to the radius. To do this, she grasped the radius on either side of the attachment point with the tarsi of her legs oIII and oIV (outer legs III and IV, on the side of her body farthest from the hub of the orb), and touched her spinnerets to the line between these tarsi. She then moved back inward on the radius, along the temporary spiral to the next radius, and outward along this line. Spiders worked very rapidly: near the hub, where the rate of attachments was highest, the spider made more than one attachment per second.

Correlation between exploratory movements of leg oIV, the site where it grasped r_n, and the sticky spiral space

The information available to a spider regarding the positions of lines already in place in her web (especially the reference stimuli mentioned above) probably depends on small details of the movements of her legs (see Discussion), so careful descriptions of leg movements are important in determining which cues may be available to the spider. Many details of sticky spiral construction behaviour in video recordings of five *M. duodecimspinosa* spiders were quite uniform. The spider first grasped the next radius to which an attachment would be made (r_n in Fig. 1) with leg oIII (this leg followed leg oII to grasp this radius—see Eberhard 1987), and then she brought her leg oIV forward to contact r_n and extended it outward (Fig. 1). Apparently the tarsus (or perhaps sometimes the metatarsus) of leg oIV slid along the radius as the leg was extended (it was not possible to verify directly that it maintained contact with the radius). The movement of oIV outward along r_n toward the inner loop was relatively slow, usually taking 0.03–0.06 s. Usually, this apparent searching movement continued until the dorsal surface of tarsus oIV touched the inner loop. Immediately following contact, the tarsal claw grasped the radius at or near the junction with the inner loop. The spider then brought the line to her spinnerets and attached the sticky spiral to the radius a few tenths of a second later. The site of attachment was approximately midway between the sites gripped by legs oIII and oIV.

Thus, there appear to be three decisions regarding sites on r_n: where to grasp the radius with leg oIII; where to grasp the radius with oIV (the oIV grasping site); and where to attach the sticky line relative to tarsi oIII and oIV (the attachment site). Given the close and consistent temporal association between contact with the inner loop during the apparent searching movement and grasping the radius with oIV, the information available to the spider to decide where tarsus oIV should grasp the radius with respect to the inner loop was probably affected by details of the searching movement of leg oIV. This effect was confirmed by further analyses. In some cases, the spider failed to extend her leg oIV far enough to touch the inner loop before she grasped the radius; she thus could not have used the site of the previous sticky spiral loop to determine the site of sticky spiral attachment in these cases. Overall, contact did not occur preceding at least 13.7% of 1015 attachments in one closely analysed video record (contact may have also been lacking in a few other cases in which the angle of view did

Change in site grasped by oIV on adjacent radii	Changes in sticky spiral space on successive radii					
	+	=	-	N	χ^2	p
At junction (a) → farther inside (c)	43	6	4	53	32.4	<0.0001
At junction (a) → slightly inside (b)	12	4	7	23	1.3	ns
Slightly inside (b) → farther inside (c)	6	5	4	15	0.4	ns
Slightly inside (b) → at junction (a)	2	9	15	26	9.9	<0.01
Farther inside (c) → slightly inside (b)	3	4	8	15	2.3	ns
Farther inside (c) → at junction (a)	3	15	33	51	25.0	<0.0001

Table 1: Relationship between changes in the sites where a mature female *M. duodecimspinosa* grasped the radius with her leg oIV and changes in the sticky spiral space on successive radii in a video recording of sticky spiral construction. Sites at which she grasped the radius were classified in three categories (a, b, c) corresponding to Figure 1; changes in sticky spiral spacing were scored as either an increase (+), a decrease (-) or no change (=) in the space between loops on successive radii. The expected values for the χ^2 analysis were calculated on the basis of the number of sticky spiral spaces (N) which showed changes (an increase or a decrease).

not permit confident discrimination). Failures to contact the inner loop were much more common in the two outermost loops of sticky spiral in this web, and then fell off sharply (Fig. 2).

In 100% of the cases in which leg oIV failed to contact the inner loop, the site grasped by oIV was inward from (on the hub side of) the junction with the inner loop (positions B or C in Fig. 1). In contrast, when contact was made in a sample of 1174 cases in one web, the grasping site was right at the junction with the inner loop of sticky spiral in 47.3% (Fig. 1a), slightly inward of this junction (less than the diameter of the tarsus inward of this junction in 14.3% (Fig. 1b), and even farther inward from the junction in 38.2% (Fig. 1c) (χ^2 = 111.0, df = 1, p < 0.0001 comparing grasping at vs. inside the inner loop in cases in which contact was made or was not made).

The oIV grasping site also varied according to the spider's position in her web. Grasping at the junction (A in Fig. 1) was less common in the upper 90° of the orb (Fig. 3A), but equally frequent in the lower 90° and the sides of the web. It was also approximately twice as common near the hub as it was near the edge of the web (Fig. 3B).

Spaces between loops of sticky spiral

The grasping site of oIV on r_n in turn correlated with variations in where the sticky spiral was attached to this radius. In order to determine whether the site where leg IV grasped r_n correlated with where the sticky spiral was attached (and thus with the space between loops), I held the effects of the spider's position in her web nearly constant by comparing sequential attachments to adjacent radii. When there was a change from one radius to the next in the oIV grasping site with respect to the junction with the inner loop, I checked whether the resulting sticky spiral space increased or decreased. I found (Table 1) that large increases in the distance of the grasping site from the inner loop were associated with increases in the space between sticky spiral loops (first line in Table 1), and large decreases were associated with reductions in the space (last line in Table 1); smaller changes in the site of grasping were associated with smaller, often insignificant differences. At this level, however, the likelihood that these changes in spaces would occur was not affected by whether or not tarsus oIV contacted the inner loop on the second of the two radii (χ^2 = 1.25, df = 1, p >> 0.05).

In summary, differences in the exploratory behaviour of leg oIV were correlated with differences in the sites grasped

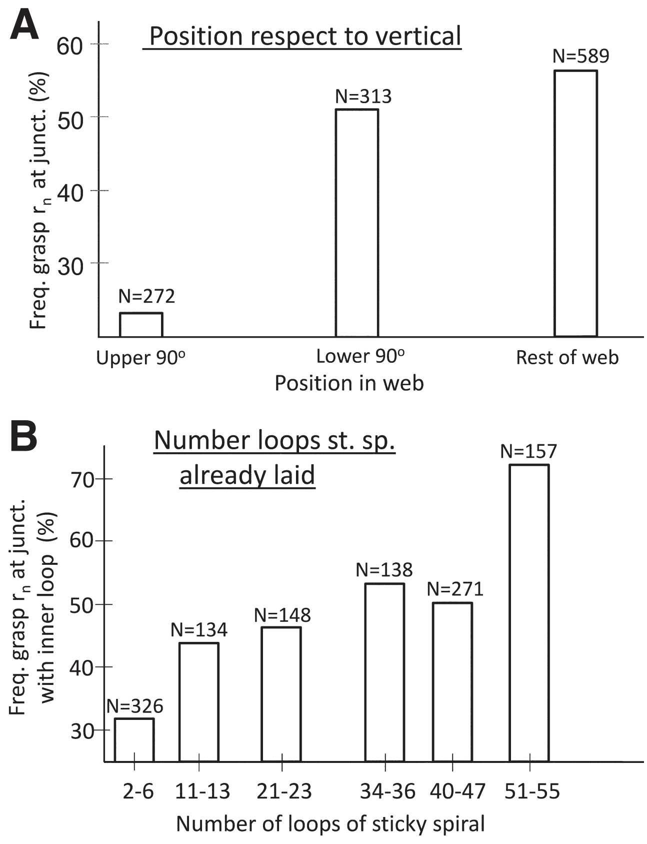

Fig. 3: **A** The frequency with which the tarsus oIV of a *M. duodecimspinosa* grasped r_n at the junction with the inner loop (position a in Fig. 1) was lower when the spider was above the hub as compared to when she was below it or elsewhere in the web at approximately the same distance from the hub (data from the construction of the same early sticky spiral loops (χ^2 = 110, dl = 2, p < 0.0001); **B** When the spider was at greater distances from the hub during the construction of a given loop (and thus above as well as below the hub), she also grasped the radius at the junction less often (R = 0.91, F = 19.4, dl = 1,4, p = 0.012).

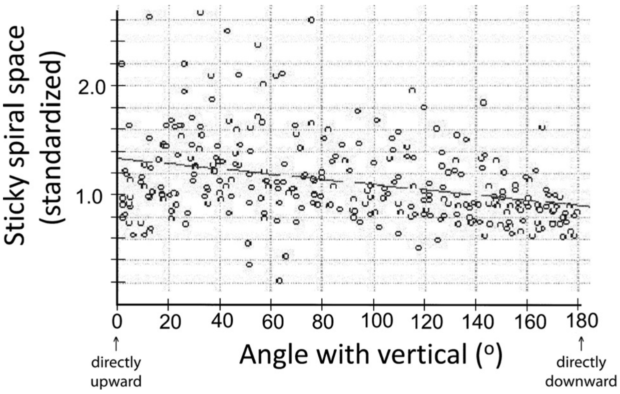

Fig. 4: The standardized sticky spiral space showed a significant negative correlation with the angle of the radius with vertical (0° = directly above the hub, 180° = directly below the hub) (R = 0.33, $F_{1,291}$ = 35.5, $p < 0.0010$) (12 webs).

Fig. 5: A finished orb of *M. duodecimspinosa* whose construction was video-taped, illustrating the larger spaces between loops of sticky spiral farther from the hub, and in the upper as opposed to the lower portion of the web.

by leg oIV, and differences in the sites grasped were correlated with differences in sticky spiral spacing. The sticky spiral spacing in photos of finished orbs was in accord with these patterns: the sticky spiral spaces in areas of the orb where leg oIV grasped the junction less frequently (above the hub, near the edge) were larger (Figs. 4, 5).

Discussion

The conclusion here, that the behaviour of leg oIV functions to locate the inner loop of sticky spiral in order to guide sticky spiral placement, is in accord with previous deductions that *M. duodecimspinosa* uses the site of the inner loop of sticky spiral as a point of reference to guide its decision where to attach the sticky spiral (Eberhard & Hesselberg in press). The pushing movement of oIV that brings the dorsal surface of its tarsus into contact with the sticky spiral differs from the tapping movements of leg oI in other araneid spiders, but closely resembles the convergent oIV inner loop localization movements of *Nephila* and other nephilids during sticky spiral construction (Eberhard 1982; Kuntner *et al*. 2008). Presumably, the relatively small distances in these two groups between radial and sticky spiral lines with respect to the spider's body size is responsible for this convergence. Experimental modifications of orbs during construction also support the inner loop localization function hypothesis for these movements in *M. duodecimspinosa* as well as in other orb weavers (Hingston 1920; Peters 1954; Eberhard & Hesselberg in press).

An orb web is a geometrically regular structure, and many different variables are correlated with each other. Take, for example, the fact that some radial lines are under more tension than others, and that there is probably a within-orb pattern to these differences (Wirth & Barth 1992). The arguments made here are based on the supposition that stimuli guiding the spider are sensed by direct contact (and lack of contact) with lines in the web, and ignore the possibility that, instead, the spider responds to possibly correlated differences in tensions or vibrations of the radii. This reasoning is justified by the fact that experimental reduction in radius tension has no perceptible effect on sticky spiral spacing in this species (Eberhard & Hesselberg in press).

Comparisons of the positions of legs indicated that sticky spiral spacing in *M. duodecimspinosa* was partially determined by the site at which tarsus oIV grasped the radius just prior to attachment of the sticky spiral. When the tarsus oIV grasped a site farther from the inner loop of sticky spiral, the attachment tended to be farther from the inner loop (Table 1). Thus, in the causal chain of events, stimuli related to the site of the inner loop affected the site at which the spider grasped the radius, which may have, in turn, affected the site at which the sticky spiral was attached to the radius. It should be noted, however, that the correlation between changes in the site grasped by leg oIV and changes in the sticky spiral spacing was far from perfect (Table 1). Whether this variation was due to the effects of other variables,or to errors by the spider (or both), is not clear.

Figure 6 provides a perspective for the discoveries documented here with respect to the different decisions that may influence sticky spiral spacing and the variables known to affect spacing. The general lesson is that much remains to be learned about mechanisms of implementation. Some other variables, in addition to the spider's site in the web, may also influence sticky spiral spacing via the mechanism of determining the site at which leg oIV grasps the radius (3 in Fig. 6). Perhaps others affect the site at which the spider touches her spinnerets to the radius with respect to the positions of tarsi oIII and oIV (4 in Fig. 6), or the site originally grasped by oIII (1 in Fig. 6). Further studies that combine video analyses with experimental manipulations of different factors (for example body weight, silk supply) could test these ideas.

Failure to reach the inner loop with inner loop localization tapping behaviour was mentioned as a cause for changes in sticky spiral spacing in *A. diadematus* ('These gaps occurred when the spider failed to reach a previously constructed outer sticky thread'—Krink & Vollrath 1999, p. 230), but no quantitative data were given. The present study is the first quantitative demonstration that larger spaces are associated with failures to contact the inner loop. The apparent chain of decisions in *M. duodecimspinosa*

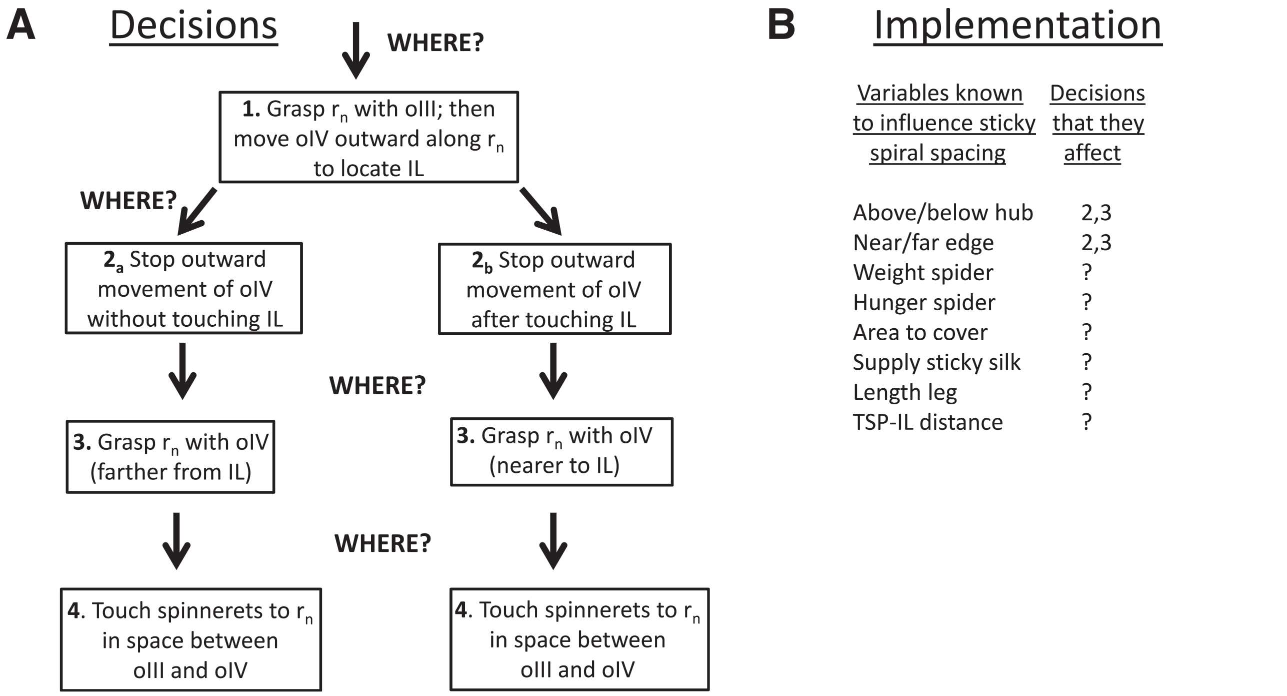

Fig. 6: **A** The order of behavioural decisions and spider movements that can influence sticky spiral spacing; **B** Current knowledge of the roles that these decisions play in implementing effects of different variables on behaviour that results in changes in sticky spiral spacing.

concerning where to grasp each radius and where to attach the sticky line must occur very rapidly, given the rapid rate of sticky spiral construction in this species.

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