Ethology

RESEARCH PAPER

Cues that Spiders (Araneae: Araneidae, Tetragnathidae) Use to Build Orbs: Lapses in Attention to One Set of Cues Because of Dissonance with Others? 1

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Abstract

Even for small animals such as spiders, behavioral decisions are sometimes influenced by multiple cues. Orb webs constitute exquisitely precise records of the stimuli the spider experienced and the decisions that it made while building its web. In addition, because spiders appear to sense their webs largely by touch, direct behavioral observations can determine which stimuli they probably sense. Previous studies have shown that when an orb-weaving spider decides how far apart to space successive sticky lines during orb construction, it responds to at least five different kinds of stimuli, all of which apparently use a cue from the web, the location of the previous, inner loop of sticky spiral (IL location), as a point of reference. Here we show that two additional cues from the web, which are related to the position of the temporary spiral (TS), also influence sticky spiral spacing. A combination of direct observations of spider movements, analyses of complete and partially complete webs, and responses to experimental modifications of the web of two species in different families, *Micrathena duodecimspinosa* (Araneidae) and *Leucauge mariana* (Tetragnathidae), indicate that both the TS-IL distance itself and the short-term memory of the change in TS-IL distance compared with that on other recently encountered radii correlate with sticky spiral spacing. When the TS-IL distance was large, the spiders apparently ceased to attend to other cues. Thus, even the relatively stereotyped behavior of orb construction includes variation that stems from attention-like mental processes.

Introduction

Animals receive floods of information from the environment through their sense organs, much more than their brains can process at a given moment (Dukas 2004). It is thus reasonable to expect that animals may bias which subsets are processed and acted upon at any given moment. If an animal can bias input and processing of information appropriately ('pay attention'), it can respond more consistently and more efficiently to the particular subsets of stimuli that are most relevant to its current behavioral context (Shettleworth 2010). We argue here that attention plays an important role in spider orb web construction.

Attention is a well-established phenomenon in vertebrates, and there are indications that insects and spiders also present 'attention-like' phenomena (Shettleworth 2010). Study of attention, and of possible behavioral errors that are associated with changes in attention, has important implications. They may help explain present-day variations in behavior and may influence the directions in which evolution can most readily proceed. Inevitably, the behavioral variants present in a population will determine how natural selection can act and thus future evolution (Eberhard 1990; West-Eberhard 2003).

Orb Construction and Attention to Multiple Cues

In this study, we explore attention-like phenomena during orb web construction. Orb construction is a well-known example of an apparently difficult behavior that is executed by animals that possess only limited neural capabilities (Witt et al. 1968; Foelix 1996; Quesada et al. 2011). It offers an unusual opportunity to study attention-like phenomena for several reasons. Stimuli from the web can be easily modified. Some operations are repeated over and over. And both the stimuli and the spider's responses to them can be relatively easily quantified in fine detail, using the web itself as an exquisitely precise record of both the stimuli available to the spider and some aspects of the spider's responses to them (Witt et al. 1968; Vollrath 1992; Zschokke & Vollrath 1995). We will focus on one stage of orb construction, sticky spiral construction, in which all of these advantages are especially manifest.

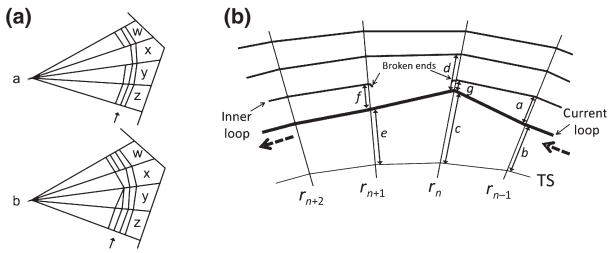
During sticky spiral construction, a spider makes literally thousands of measurements and behavioral decisions in the space of the approximately 20–30 min it takes to lay the sticky spiral. Starting at the edge of the web, she circles inward, attaching the sticky line she is producing to each radius that she encounters at a high rate. A mature female *Araneus diadematus* near the edge of her orb makes one attachment every 2.7 ± 0.7 s, and one every 1.3 ± 0.3 s near the center (reanalysis of data from Hesselberg & Vollrath 2004). Some other species, such as *Micrathena duodecimspinosa*, *Leucauge mariana*, *Scoloderus* sp, *Mangora* sp., and *Acacesia haemata*, work even more rapidly (Eberhard 1975; W. Eberhard unpubl. data).

At least five different behavioral functions are performed in the few seconds during which the spider moves from one attachment of the sticky spiral to the next: locate radial and sticky spiral lines with the anterior legs; find and seize the radius with legs oIII and oIV at appropriate positions to make the attachment (see Table 1 for definitions of terms); locate the inner loop of sticky spiral; align the radius with the abdomen very precisely, so that the radius runs through the center of the cluster of spinnerets; and execute a brief burst of spinneret movements that attaches the spiral to the radius (Hingston 1920; Mayer 1952; Eberhard 1972, 2010) (in the moderately sized species *M. duodecimspinosa*, these spinneret movements last only about 0.03 s/attachment) (W. Eberhard unpubl. data). In a non-horizontal orb, quite different leg movements are needed when the spider moves along radii and temporary spiral lines above, as opposed to below, the hub (Hingston 1920; Mayer 1952; Eberhard 1982). In sum, sticky spiral construction involves repeated, rapid decisions, and rapid and variable yet very precise behavior patterns. Lapses in attention could result in imprecision in placement of the sticky line.

Table 1: Definition of terms used throughout the text

Beyond a radius	The far side of the radius as the spider approaches it
Inner loop of SS	The innermost loop of the sticky spiral already present in the web (Fig. 1b)
Inward	Direction toward the hub
iI, iII, iIII, iIV	Legs on the side closest to the hub; iI is the front leg, iIV the hind leg, etc.
Outward	Direction away from the hub
oI, oII, oIII, oIV	Legs on the side furthest away from the hub; oI is the front leg, oIV the hind leg, etc.
r_{n-1}, r_n, r_{n+1}	r_n is the next radius to which the spider will attach the sticky spiral, while r_{n-1} is the radius preceding this radius and r_{n+1} is the radius following it (in the direction the spider is moving) (Fig. 1b)
SS	The sticky spiral
Sticky spiral space	The distance between adjacent loops of sticky spiral
TS	The temporary spiral
TS-IL distance	The distance between the outermost intact loop of temporary spiral and in the inner loop of sticky spiral

Sticky spiral construction is also complex at a higher level of analysis. Up to six different stimuli influence the spaces between successive loops of sticky spiral lines. These depend on a stimulus that must be perceived anew each time the spider encounters the next radius – the point where the innermost loop of sticky spiral crosses that radius. Hingston (1920) found that when he removed a segment of the innermost loop of sticky spiral in *Neoscona nautica* (= *Araneus nauticus*; Platnick 2011), the next loop of sticky spiral attached to that radius was displaced sharply outward (Fig. 1). Similar experiments resulted in outward displacements in *Nephila clavipes* and *Zygiella x-notata* (= *Zilla litterata*; Platnick 2011) (Peters 1939, 1954). In effect, Hingston's experiment produced an outward displacement of the site at which the spider encountered the inner loop of sticky spiral when she searched for it with her leg oI. Hingston noted that the new attachment was at the point where the previous loop had been attached (on top of the broken end of the inner loop) and concluded that the spider uses the site of the inner loop of sticky spiral that is already in place as a reference point to guide subsequent sticky spiral placement.



(c)

t tests	p
$d>a$	<0.001
$d>f$	<0.001
$g<a$	0.001
$g<f$	0.0006

Regressions

d/a on $(c+d)/(b+a)$: $R = 0.502$, $F_{1,28} = 9.43$, $p = 0.005$

$d-a$ on $(c+d)-(b+a)$: $R = 0.40$, $F_{1,28} = 5.3$, $p = 0.029$

Fig. 1: (a) The original figure of Hingston (1920) (redrawn) depicting the experiment in which the segment of the inner loop in sector X was broken (upper) and the outward displacement of the next sticky spiral attachment to the radius between sectors X and Y (lower). The arrows indicate the direction in which the spider was moving. (b) The Hingston experiments in webs of *Leucauge mariana* (sticky lines are thicker, and the current loop of sticky spiral is thickest). The segment of the inner sticky spiral loop between radii r_n and r_{n+1} was broken experimentally, while laying the sticky spiral from right to left (thick arrows). *a*, *d*, *f*, and *g* indicate distances from the current loop to the inner loop of the sticky spiral, while *b*, *c*, and *e* indicate differences between the current loop and the temporary spiral (TS). (c) A summary of the results of the Hingston experiments in 34 webs of *L. mariana*. As the spider moved outward on r_n, she failed to encounter this missing segment with her leg iI, so the TS-IL distance she encountered on r_n ($c + d$) was thus greater than the TS-IL distance she had encountered on r_{n-1} ($b + a$). The new loop of sticky spiral was displaced outward on r_n ($c > b$; $g < a$). In contrast to the results reported by Hingston, however, *g* was generally >0, and *d* was greater than either *a* or *f* (32 of 34 cases).

Later experiments suggested that four stimuli apparently combine with the basic guiding inner loop (IL) reference point stimulus, setting the distance from the IL reference point at which attachments will be made (e.g., setting the value(s) of *a* and *f* in Fig. 1). These cues include the length of the spider's legs (Vollrath 1987), the amount of material in its silk glands (Eberhard 1988b), the direction of gravity (Vollrath 1986, 1988), and the distance from the hub (LeGuelte 1966; Vollrath 1992). A fifth cue that signals the availability of a special type of prey for which the spider needs a special orb design (the time of the day), may also influence sticky spiral construction in one species (Sandoval 1994). An additional decision that is generally made at each attachment site is whether to turn back and begin to circle in the opposite direction (Eberhard 1969; Zschokke 1993); we will not discuss turn back decisions in this article.

A second stimulus that may be perceived anew with each encounter with a new radius is the distance of the inner loop from the temporary spiral (TS-IL distance). This stimulus was assumed by Krink & Vollrath (1999) to provide another cue guiding sticky spiral placement in a simulation study. No convincing empirical evidence was given, however, to support this possibility. These authors stated that the spider itself 'always kept the legs that were gripping the supporting auxiliary spiral bent in a certain posture. This adjusted posture apparently resulted in the spider keeping a fixed distance between its body and auxiliary thread …' and they assigned this behavior to their robot. Recordings of the path of the body of *A. diadematus* during sticky spiral construction show, however, pronounced variations in the spider's distance from the temporary spiral, even within a single orb (Zschokke & Vollrath 1995). It was true that virtual robots using this stimulus generated orbs that were similar in some respects to those produced by spiders. The ability to produce a spider-like design in this or other simulation studies (Eberhard 1969; Gotts & Vollrath 1991) does not, however, constitute evidence that the spider actually uses the cues employed in the simulation. The general shape and coiling direction of the temporary spiral of *A. diadematus* was found to correlate with the shape and coiling direction of the sticky spiral (Zschokke 1993), but no precise measurements of TS-IL distances or sticky spiral spacing were given. In sum, use of the TS-IL distance remains a likely but untested hypothesis.

If the TS-IL distance is indeed perceived and used each time the spiders encounters a radius, then it is possible that stimuli from the IL location and the TS-IL distance are sometimes in conflict regarding the point where the sticky line should be attached to the radius. In such situations, it is thus possible to check for consistent patterns in the relative attention paid by the spider to these two cues. We show here that the IL location cues tend to be ignored when conflict with the TS-IL distance is especially large (i.e., attention is focused on the TS-IL cue). This study was performed using the tetragnathid *Leucauge mariana*, which builds an approximately horizontal orb, and the araneid *Micrathena duodecimspinosa*, which builds a strongly slanted web (usually 60–80° with horizontal – W. Eberhard unpubl. data). We use a combination of direct observations of spider movements, analyses of complete and partially complete webs, and responses to experimental modifications of the web from two distantly related orb spiders to show that both the TS-IL distance and the memory of recently encountered TS-IL distances appear to influence sticky spiral spacing. By experimental manipulation of webs, we also show that spiders pay attention to different combinations of stimuli under different circumstances.

Methods

Only adult females and their webs were observed. Webs were photographed after being coated with corn starch. Webs of free-ranging *M. duodecimspinosa* were photographed in the field near San Antonio de Escazú, San José Province, Costa Rica (el. 1300 m). The orbs of *L. mariana* were built in stiff wire hoops (Eberhard 1987) hung in an outdoor screen cage. Voucher specimens of *M. duodecimspinosa* and *L. mariana* have been deposited in the Museum of Comparative Zoology, Harvard University, and the Museo de Zoología of the Escuela de Biología, Universidad de 4 Costa Rica (Fig. 1).

The following set of observations and experiments were performed (see Table 1 for a definition of terms used throughout this article):

Behavior During Sticky Spiral Construction

Close-ups of the construction behavior of five mature female *M. duodecimspinosa* and two *L. mariana* were videotaped with ambient light in the field in webs built after dawn with a hand-held SONY DCR-TRV50 5 digital camera equipped with +6 close-up lenses to determine the movement of individual legs during sticky spiral construction.

Sites of Attachments in Web

Eighteen *L. mariana* and 16 *M. duodecimspinosa* were disrupted during web building after they had laid down the first two to three sticky spiral loops and photographed. Measurements of the sticky spiral space (distance between loop 1 and 2), the TS-IL distance, change in TS-IL distance (from fist to second loop of the sticky spiral), and the angle of the radius with vertical in the plane of the spider's orb (as a measure of the importance of gravity) were extracted from each web from digital photographs using ImageJ soft- 6 ware (National Institute of Health, USA). Data from different webs were standardized before being combined for analysis by dividing all values from a given web by the median for that web, to reduce possible variation because of 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. However, to avoid pseudo-replications, the significance of the performed single and multiple linear regressions were calculated using a linear mixed model (restricted maximum likelihood estimation criteria) with web as a random effect and degrees of freedom fixed as the number of spiders minus one (note that each spider contributed with one web). Sample sizes differed for different species and different variables and are given in the text and the figures.

Experimental Modifications of Webs – Inner Loop of Sticky Spiral

Sticky spiral lines of 30 *L. mariana* webs were experimentally severed during construction (e.g., to perform 'Hingston experiments' – Fig. 1) in captivity using the tip of a hot soldering iron, thus producing a minimum of vibration.

Experimental Modifications of Webs – Outer Loop of Temporary Spiral

Similar experiments as above, but where the outer loop of the temporary spiral was cut, were performed on 29 *L. mariana* webs. Lines in an additional five webs of *M. duodecimspinosa* webs in the field were cut with a small, sharp pair of scissors. We attempted to avoid disturbance by cutting lines while the spider was on the opposite side of the web and minimizing the vibrations produced by cutting.

Experimental Modifications of Webs – Radii

Three adjacent radii were cut near the hub in 35 *L. mariana* webs just before sticky spiral constriction to determine whether the resultant lack of tension influenced the spaces between sticky spiral loops or the TS-IL distance.

Statistical analyses were performed using the program STATISTICA (Statsoft, USA), and the linear mixed models were performed with SAS (v. 9.2, SAS Institute Inc., 2008) (Table 1). 7, 8

Results

Behavior During Sticky Spiral Construction

Contact with the inner loop of sticky spiral

Video recordings showed that both *M. duodecimspinosa* and *L. mariana* usually appeared to touch the inner loop with one leg (oIV and iI respectively) just before the spider attached the sticky spiral, as in nearly all other orb spiders that have been observed (Eberhard 1982; Kuntner et al. 2008). Video recordings of five *M. duodecimspinosa* building the sticky spiral showed that leg oIV generally contacted the inner loop, but that leg oI did not tap it as in most other araneids (Eberhard 1982). Direct observations of >20 *L. mariana* and video recordings of two others showed that they used leg iI, as is typical in other tetragnathids. In both species, the leg appeared to contact the inner loop just beyond the r_n (between r_n and r_{n+1} in Fig. 1b). These are the 'inner loop localization' movements that are thought to function to guide the placement of the sticky spiral for orb spiders (Hingston 1920; Peters 1939; Mayer 1952; Eberhard 1982; Vollrath 1987; Kuntner et al. 2008). This hypothesis of function was supported in both species by the fact that as soon as the spider touched the inner loop she immediately terminated inner loop localization movements and turned to attach the sticky spiral to the radius. The interpretations below are based on this attribution of function.

Sites of Attachments in Webs

Spaces between the first two loops of sticky spiral

Using photographs of webs in which sticky spiral construction was interrupted after only two or three loops of sticky spiral had been laid, and before the spider had broken any segments of the temporary spiral (Fig. 2), it was possible to determine the direction in which the spider moved as she laid the first and second loops of sticky spiral, as well as the distances between temporary spiral, sticky spiral, and frame lines that she encountered. In the second loop of sticky spiral of 18 *L. mariana* webs and 16 *M. duodecimspinosa* webs, there was a positive relation between the TS-IL distance along r_n and the sticky spiral space (Fig. 3).

A previous study of *L. mariana* showed that the recent memory of previous distances between lines influences temporary spiral construction; spiders adjusted temporary spiral attachment sites to make the current space more similar to the space on the previous radius, in effect buffering responses to changes in stimuli on successive radii (Eberhard 1988a). When we checked the 16 *M. duodecimspinosa* webs for memory effects in sticky spiral construction that might buffer variations in the TS-IL distances in a similar way, we found a similar pattern. Regressions of sticky spiral space on both TS-IL distance and the

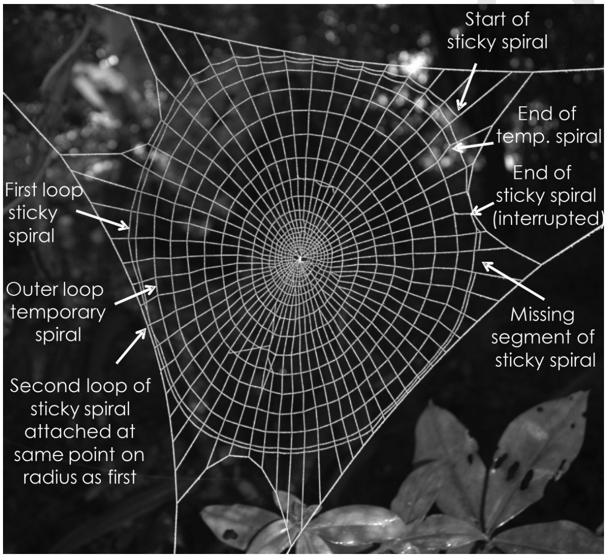


Fig. 2: A *Micrathena duodecimspinosa* web in which construction was interrupted when two loops of sticky spiral had been completed and before any temporary spiral lines had been broken. The positive relation between the spaces between the first and second loops of sticky spiral and the distance from the outer loop of temporary spiral is clear. In extreme cases in which the outer loop of the temporary spiral was especially close to the frame, the second loop was attached at the same point on the radius as the first loop. The spider built the sticky spiral in a counterclockwise direction (after laying a clockwise temporary spiral).

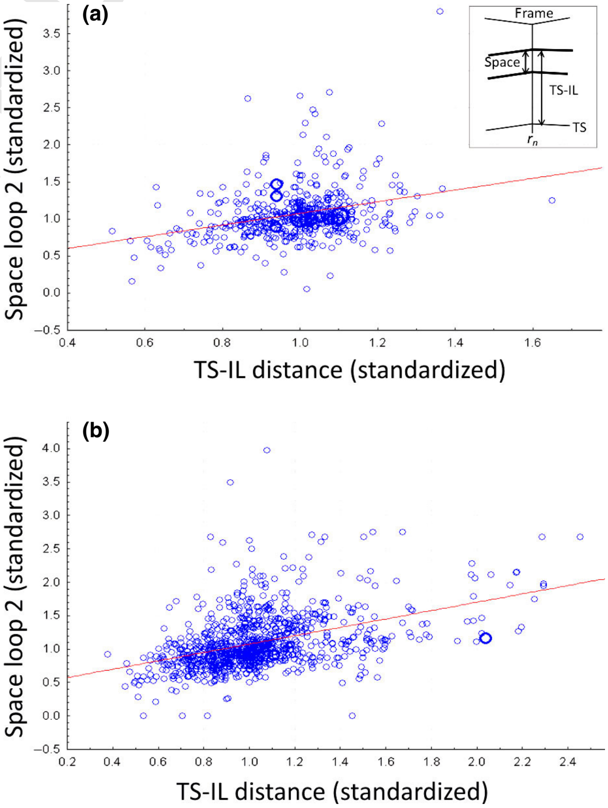


Fig. 3: The positive correlation between the normalized sticky spiral space (distance between first and second loops) and the standardized TS-IL distance (see inset). (a) *Leucauge mariana* (data from 18 webs; regression: $y = 0.29 + 0.79x$, $R = 0.31$, linear mixed model using web identity as a random variable: $F_{1,17} = 55.6$, $p < 0.001$). (b) *Micrathena duodecimspinosa* (data from 16 webs; regression: $y = 0.52 + 0.56x$, $R = 0.41$, linear mixed model using web identity as a random variable: $F_{1,15} = 168.1$, $p < 0.001$).

change in TS-IL distance (comparing r_{n-1} with r_n) were both significant ($R = 0.31$, linear mixed model using web identity as a random variable: $F = 168.1$, df = 1, 15, $p < 0.001$ and $R = 0.26$, linear mixed model using web identity as a random variable: $F = 45.2$, df = 1, 15, $p < 0.001$ respectively). A multiple regression of sticky spiral space on TS-IL distance and the change in TS-IL ($R = 0.35$) gave significant values for both independent variables (linear mixed models using web identity as a random variable: $F = 39.3$, df = 1, 15, $p < 0.001$ and $F = 21.7$, df = 1, 15, $p < 0.001$ respectively). A similar multiple regression with data from 18 *L. mariana* webs also gave significant positive relationships. The correlation coefficients for TS-IL and change in TS-IL (from r_{n-2} to r_n) were 0.31 and 0.21, respectively, and in a multiple regression ($R = 0.29$), they both had significant values (linear mixed models using web identity as a random variable: $F = 6.7$, df = 1, 17, $p = 0.019$ and $F = 6.4$, df = 1, 17, $p = 0.022$, respectively).

The distance between the first and second loops of sticky spiral was larger above the hub and smaller below the hub in 11 *M. duodecimspinosa* webs, but this effect disappeared when analyses also included TS-IL distance and recent change in TS-IL distance as variables. A multiple regression ($R = 0.50$) was significant for TS-IL distance (linear mixed model using web identity as a random variable: $F = 48.3$, df = 1, 10, $p < 0.001$) and change in TS-IL distance (linear mixed model using web identity as a random variable: $F = 23.4$, df = 1, 10, $p < 0.001$), but not for gravity (linear mixed models using web identity as a random variable: $F = 0.6$, df = 1, 10, $p = 0.476$). Thus, it appears that the correlation of the distance between sticky spiral loops with gravity in *M. duodecimspinosa* webs is only a secondary effect of the relations with TS-IL values.

Experimental Modifications of Webs

Break a segment of the inner loop of sticky spiral

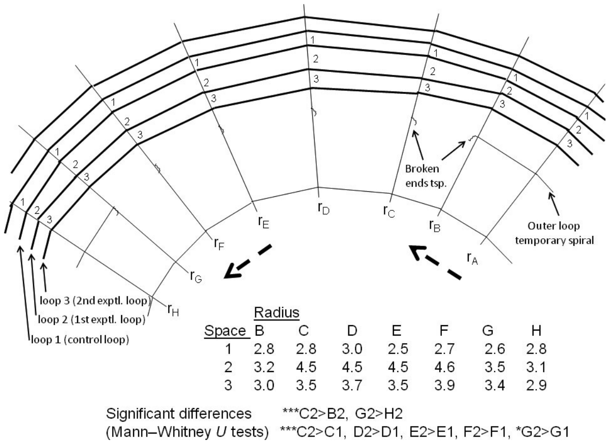
Qualitative responses (judged by eye) to 'Hingston Experiments' (Fig. 1) performed in the field with *L. mariana* varied. In only 16% of 135 experiments in 10 webs of as many females did the spider produce a complete or nearly complete outward displacement of the new loop to the broken end of the sticky spiral, as described by Hingston (Fig. 1a); 33% resulted in no perceptible outward deflection, and 51% resulted in intermediate deflections (as in Fig. 1b). The responses were somewhat stronger in 212 additional experiments in the same webs in which two loops of sticky spiral were broken rather than one (respectively, 35%, 26%, and 38%) ($p < 0.001$ with chi-square test comparing experiments with one and two broken loops). Field experiments with a single broken loop with another araneid, *Argiope argentata*, gave similar, often incomplete responses (33.5%, 27.7%, and 38.8% respectively; N = 180 in four webs) (W. Eberhard unpubl. data).

Similar, inconsistent responses were obtained when similar one-loop experiments were performed with 30 *L. mariana* females in captivity. These webs were photographed while the temporary spiral segments used by the spider were still intact (thus permitting precise measurements of thread positions). The mean displacement was substantially less drastic and showed more variation than that described by Hingston (Fig. 1a). On average, the outward displacement resulted in a reduction of about 30% in the space (in terms of the labels in Fig. 1b, the x of $g/a = 0.71 \pm 0.50$, range 0.0–1.92, N = 30; g was significantly less than a (Mann–Whitney U-test; $Z = 3.20$, $p = 0.0014$). Similarly, the distance d from the new sticky spiral loop to the remaining intact sticky line between r_n and r_{n+1} was substantially greater than the 'control' distance a on the previous radius, rather than being equal to a as reported by Hingston (the x for d/a was 1.99 ± 0.76, range 1.00–3.47, N = 30; d was significantly greater than a, U-test; $Z = 5.83$, $p = 0.001$). There was a significant positive relation between the increase in the TS-IL distance, comparing the distance on r_n ($c + d$) with that on r_{n-1} ($a + b$), and the increase in the sticky spiral space comparing that on r_n (d) with that on r_{n-1} (a) (Fig. 1).

In sum, spiders clearly responded to the experiment (which, in effect, produced an outward displacement of site where the spider encountered the inner loop) by moving the attachment site for the sticky spiral outward, as described by Hingston. But the displacement was generally less dramatic than that described by Hingston, and at least part of this difference may have been due to the influence of a second cue, the TS-IL distance.

Break segments of the outer loop of temporary spiral

When several segments of the outer loop of temporary spiral were cut experimentally in 29 *L. mariana* webs, the sticky spiral spaces in the experimental sector increased sharply over previous values on the same radii (2 vs. 1 on radii C-F in Fig. 4) and over those on immediately adjacent control radii (two on radius C vs. radius B in Fig. 4). Greater TS-IL distances were associated with larger spaces between sticky spiral loops (Fig. 5).



	Radius						
Space	B	C	D	E	F	G	H
1	2.8	2.8	3.0	2.5	2.7	2.6	2.8
2	3.2	4.5	4.5	4.5	4.6	3.5	3.1
3	3.0	3.5	3.7	3.5	3.9	3.4	2.9

Significant differences ***C2>B2, G2>H2
(Mann–Whitney *U* tests) ***C2>C1, D2>D1, E2>E1, F2>F1, *G2>G1

Fig. 4: Schematic representation of the results of experimental elimination of five segments of the outer loop of temporary spiral in 29 horizontal webs of *Leucauge mariana* after loop 1 was laid and before loop 2. The spider moved counterclockwise (heavy arrows). The distances between loops 1 and 3 in the drawing only approximate the median values in the table.

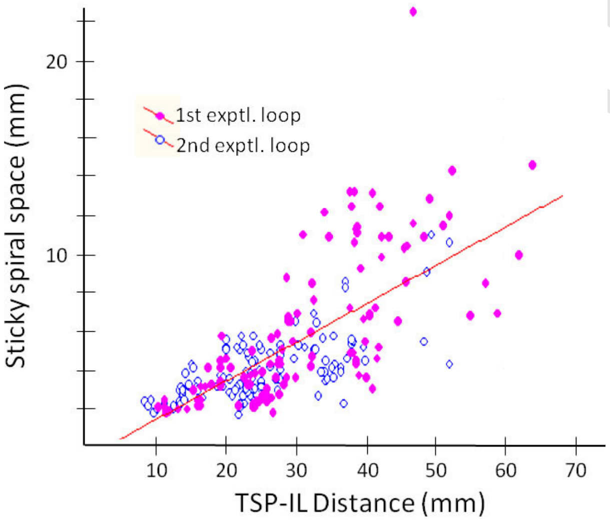


Fig. 5: Relation between the sticky spiral space and the TS-IL distance for the first and second experimental loops in 29 *Leucauge mariana* webs on radii B–H, when five segments of the outer loop of temporary spiral were experimentally removed (Fig. 4). When the TS-IL distance was lower than about 30 mm, the spaces of the first and second experimental loops showed the same relation; but at higher TS-IL values, the spider increased sticky spiral spaces more sharply during the first loop than during the second.

In contrast, the second loop of sticky spiral across the experimental sector was often displaced inward much less than the first and had a more complex relation with the TS-IL distance.

At lower TS-IL distances during construction of the second experimental loop, the relation between the

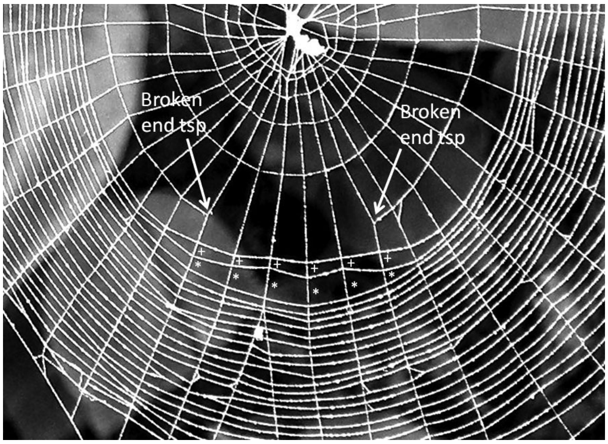


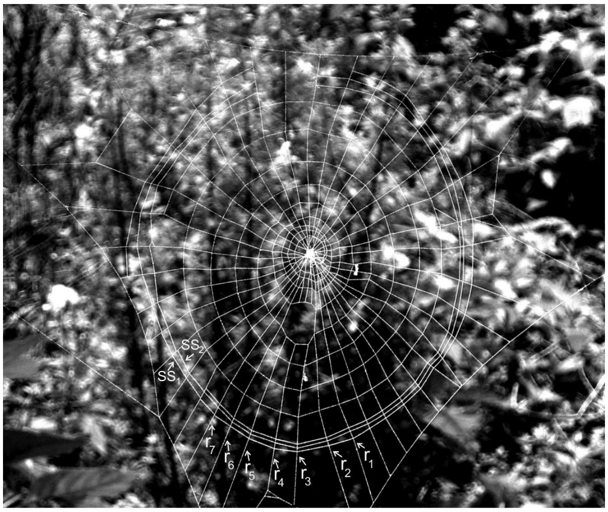
Fig. 6: The response of a *Micrathena duodecimspinosa* to experimental removal of five segments of the outer loop of temporary spiral (solid arrows) during sticky spiral construction; the photograph was taken after the spider had laid two more loops of sticky spiral following temporary spiral removal. The first time she moved across the modified area of the web (from right to left), the spider sharply increased the sticky spiral spaces compared with the previous attachments to these same radii ('*' in the figure), as expected if a greater distance from the outer loop of temporary spiral has a positive effect on sticky spiral spacing. On her next pass across this sector, however, the spaces ('+' in the figure) were only slightly larger than normal, despite the still relatively large TS-IL distances, suggesting that memory may have a buffering effect on this response (see text).

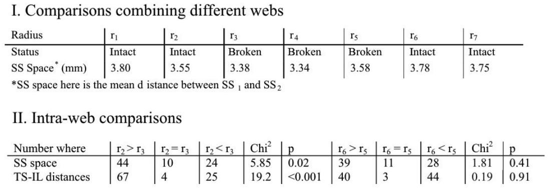
sticky spiral space and the TS-IL distance was the same as that in the first loop. At higher TS-IL distances, however, the sticky spiral spaces in the second experimental loop were smaller than in the first. In other words, the spider responded more dramatically while building the first loop to especially large TS-IL distances than while building the second loop. Five similar experiments were performed with *M. duodecimspinosa*, and the first subsequent loop of sticky spiral was sometimes displaced inward, followed by reduced displacement of the second (Fig. 6), but these responses were not consistent.

Direct observations and video recordings of both species showed that spiders often (though not always) stopped short of making contact with the inner loop of sticky spiral when laying the first loop in the experimental sector.

Break radii during sticky spiral construction

Interpretations of the observations described above depend on whether the spider senses the positions of lines by touching them directly (as we have assumed), or whether she can sense them at a distance (e.g., by vibrations or differences in tension). To check the feasibility of at least some of the mechanisms by which long distance sensing might occur, we broke





I. Comparisons combining different webs

Radius	r_1	r_2	r_3	r_4	r_5	r_6	r_7
Status	Intact	Intact	Broken	Broken	Broken	Intact	Intact
SS Space* (mm)	3.80	3.55	3.38	3.34	3.58	3.78	3.75

*SS space here is the mean d istance between SS_1 and SS_2

II. Intra-web comparisons

Number where	$r_2 > r_3$	$r_2 = r_3$	$r_2 < r_3$	Chi^2	p	$r_6 > r_5$	$r_6 = r_5$	$r_6 < r_5$	Chi^2	p
SS space	44	10	24	5.85	0.02	39	11	28	1.81	0.41
TS-IL distances	67	4	25	19.2	<0.001	40	3	44	0.19	0.91

Fig. 7: (upper) Lack of effect on sticky spiral spacing of experimentally breaking three adjacent radii after the temporary spiral was complete but before the sticky spiral had been initiated in a web of *Micrathena duodecimspinosa*. (below) The second and third loops of sticky spiral in 35 webs of *Leucauge mariana* in which three radii were broken at the end of temporary spiral construction (sticky spiral construction was interrupted before any temporary spiral lines had been broken, allowing determination of TS-IL distances). The expected values in the chi-square tests were calculated on the basis of the number of sticky spiral spaces that showed changes.

adjacent radii near the hub just before or during sticky spiral construction (Fig. 7), thus sharply reducing the tensions on these radii in the outer portion of the web where the spider was working. There was little, if any, change in either species in the site of the first loop of sticky spiral, or the spaces between loops of sticky spiral that were laid subsequently on these broken radii (Fig. 7). This implies that variables altered by cutting the radius, such as tensions, frequencies of vibrations, and transmission of vibrations, have little, if any, influence on decisions regarding the distance between sticky spiral loops.

Discussion

Cues Guiding Sticky Spiral Placement

The reference point: site of the inner loop of sticky spiral

Two types of data indicate that the spiders used the site of the inner loop of sticky spiral as a point of reference to guide their decision where to attach the sticky spiral. Leg oIV of *M. duodecimspinosa* and leg iI of *L. mariana* were moved in exploratory patterns just preceding contact with the sticky spiral, and immediately after contact with the inner loop, these movements ceased and attachment behavior occurred (for further details see W. Eberhard in prep.). In addition, *L. mariana* responded to outward displacement of the IL of sticky spiral by displacing the attachment of the sticky spiral outward ('Hingston' experiments Fig. 1). Along with the similar observations of exploratory behavior in many other orb weavers (Mayer 1952; Eberhard 1982; Kuntner et al. 2008), and similar experimental responses by the araneids *A. argentata* (W. Eberhard, unpubl. data), *N. nautica* (Hingston 1920), *Z. x-notata,* and *A. diadematus* and the nephilid *N. clavipes* (Peters 1939, 1954), these results support previous conclusions that tapping movements of the legs of many different orb weavers function to sense the 'reference site' where the inner loop crosses the radius. This conclusion has important implications regarding possible changes in attention (below).

Hingston reported that the new sticky spiral loop was displaced all the way to the broken loop ($g = 0$ in Fig. 1b) and concluded that the IL reference point stimulus was the only cue from the web that guides sticky spiral placement. The responses by *L. mariana* in similar experiments were variable, however, suggesting that other stimuli from the web also guide sticky spiral placement (below). Hingston experiments also resulted in variable responses in *A. argentata* (above), *A. diadematus*, *Z. x-notata*, and *N. clavipes* (Peters 1939, 1954). It is likely, in fact, that similar variation also occurred in Hingston's observations of *N. nautica*, despite his sometimes categorical descriptions. He mentioned variation '… the experiment seldom succeeds when the spider is working at the inner and smaller turns …' (p.104, Hingston 1920) and even suggested that the differences in spider responses might result from use of the distance from the temporary spiral (as we have shown to be the case in this study). He was working in a different time, in which variation was more difficult to deal with quantitatively, and in which typological thinking was more common.

Additional cues: TS-IL distances and recent memories

In both *L. mariana* and *M. duodecimspinosa*, responses to experimental modifications of the temporary spiral, as well as the positive relations in intact webs between the TS-IL distance and with sticky spiral spacing, and also similar positive relations in *L. mariana* webs that were modified for Hingston experiments, all suggest that the spiders use distances from the outer loop of temporary spiral to guide sticky spiral attachment. Sticky spiral spaces were larger when TS-IL distances were larger (Fig. 3). Recent memories of these distances also apparently influence sticky spiral placement, because recent changes in TS-IL distance also correlated with sticky spiral spaces. These are the first empirical demonstrations that the TS-IL distance influences sticky spiral spacing.

In their simulation study of sticky spiral placement behavior in *A. diadematus*, Krink & Vollrath (1999) assumed that the spider uses the TS-IL distance by keeping its body a fixed distance from the temporary spiral, but the empirical justification for this assumption in this species was not convincing (see above). Our video recordings also showed that both *M. duodecimspinosa* and *L. mariana* failed to maintain constant distances from the temporary spiral. In sum, we believe the assumption in the Krink & Vollrath (1999) simulation study that the TS-IL distance is an important cue was correct, but for different reasons. In addition, it also seems likely that the site of the TS affects sticky spiral placement in spiders in families such as Theridiosomatidae in which the spider never contacts the inner loop during sticky spiral construction (Eberhard 1982).

A second, longer-term memory of the changes in TS-IL distances could be responsible for the sharper reaction (in terms of increased sticky spiral spacing) when the first experimental loop was compared with the second experimental loop after temporary spiral lines were experimentally broken (Fig. 5). Further observations are needed to test this possibility.

In sum, seven and perhaps eight different stimuli can influence each of the up to 1000 or more sticky spiral attachment decisions a spider makes in building a given orb. We have not reviewed the published literature on the numbers of types of stimuli that influence given decisions in invertebrate animals, but this number seems surprisingly large, especially in light of the rapidity, the high number of repetitions, and the extended period of time (20–30 min) during which spiders make these decisions. It may be that orb-weaving spiders are unusual in being able to concentrate in such a sustained manner. In any case, the spiders reliably changed the relative attention they gave to different stimuli under different conditions (below).

Importance of the lack of effect of tension changes

An orb web is a geometrically regular structure, so many different variables are correlated with each other. For example, some radial lines are under more tension than others, and there are within-orb patterns to these differences (Denny 1976; Wirth & Barth 1992). We have argued that web variables that the spider senses by direct contact (and lack of contact) (e.g., legs touching lines) provide the cues that guide sticky spiral placement, and have not discussed the possibility that instead the spider senses lines at a distance by responding to tensions or vibrations of the radii. We feel justified in ignoring such possibilities, because experimental reduction in radius tension had no perceptible effect on sticky spiral spacing (Fig. 7).

Interspecific Uniformity

We have jumped erratically from one species to another in discussing the cues guiding sticky spiral placement, assuming that many different species use the same cues. This assumption originates in the apparent monophyletic origin of orb web construction in araneoid spiders (Scharff & Coddington 1997; Kuntner et al. 2008; Blackledge et al. 2009). In addition, empirical behavioral evidence also supports this assumption, as species in different families showed similar trends whenever similar types of evidence were available. In both the araneid *M. duodecimspinosa* and the tetragnathid *L. mariana*, the TS-IL distance and memories of changes in this distance both apparently affect sticky spiral spacing. Similar responses to Hingston experiments occur in araneids, a tetragnathid and a nephilid (Hingston 1920; Peters 1954; this study). An araneid and a tetragnathid also made similar use of cues associated with differences in the amounts of silk in their silk glands (Eberhard 1988b), and the uloborid *Zosis geniculata* and the tetragnathid *L. argyra* made several similar adjustments in orb design when obliged to build in very small spaces (T. Barrantes & W. Eberhard submitted, W. Eberhard & T. Barrantes in prep.).

Combining Cues

During normal sticky spiral construction, both the reference point IL cue and the TS-IL distance cue are correlated simultaneously with sticky spiral spacing (e.g., Fig. 3). When we experimentally removed temporary spiral threads during sticky spiral construction, the TS-IL distance was very large, and its positive correlation with sticky spiral spacing thus directed the spider to use an attachment site far from that indicated by the cues which depend on the IL reference point cue. Faced with this conflict, the spider apparently ignored the IL reference point cue. In fact, she often did not even sense it, stopping short and attaching the sticky line before she contacted the IL. There are other species in which use of one cue 'over-rules' that of another in a similar manner when the two are in conflict (Shettleworth 2010). In some species, the animal gives precedence to more reliable cues (Vander Wall 1982). In the species of the present study, the order in which stimuli become available to the spider may be important. The stimulus that overrides, based on the TS location, is sensed prior to the stimulus that is overridden (IL location). After exploring outward along the radius a certain distance without encountering the inner loop, the spider may simply give up the search and attach the sticky line. This would imply that the spider has a previous expectation of how far she will need to move to encounter the inner loop. The data presented here are compatible with such an expectation hypothesis, but do not prove it.

Changes in attention to different cues sometimes resulted in abrupt changes in sticky spiral spacing (e.g., Fig. 6). Perhaps changes in attention to different, dissonant cues can help explain the association of 'errors', in the form of over-sized space, with nearby temporary spiral (W. Eberhard & T. Hesselberg, in prep.). Owing to the gradual process of removing the temporary spiral during sticky spiral construction, spiders normally probably experience occasional abrupt increases in the TS-IL distance.

General Implications

The ability to selectively bias the attention paid to different stimuli under different conditions is probably advantageous in many contexts for many animals (Shettleworth 2010), and it is thus not surprising that orb weavers should also show this ability. Variation in the degree of attention paid to different cues could be an important source of variation in the degree of correlation between particular cues and behavioral responses in orb web construction, as well as in the behavior of many other species.

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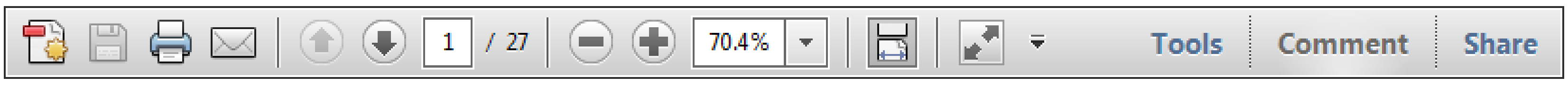

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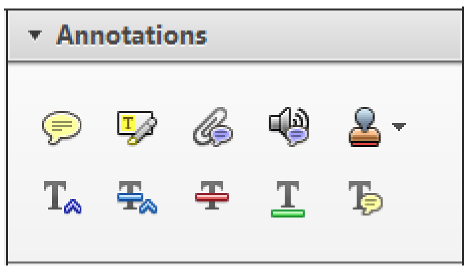

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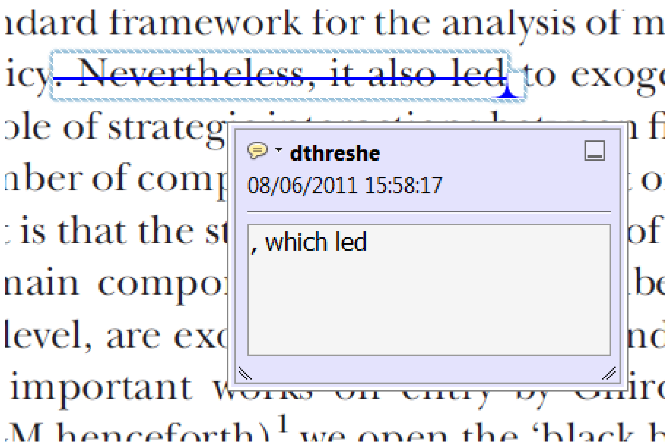

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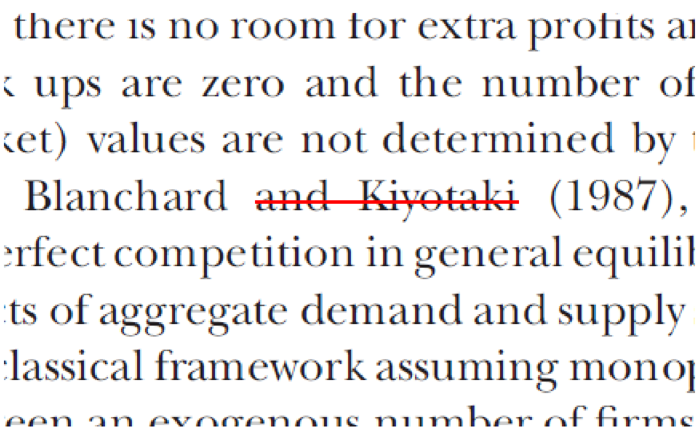

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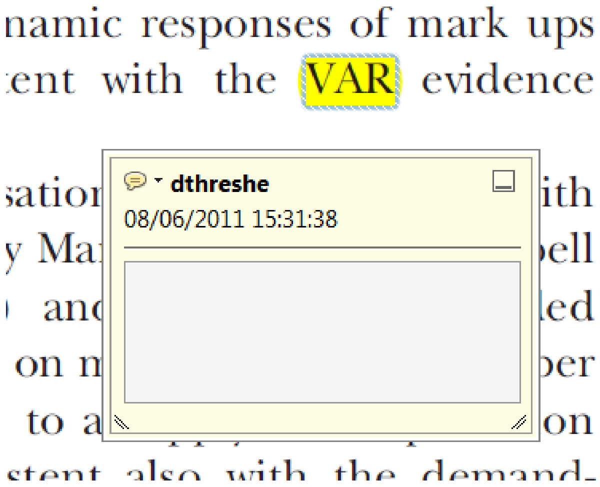

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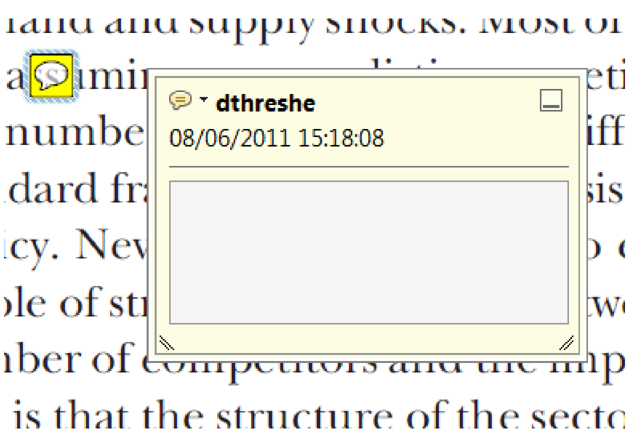

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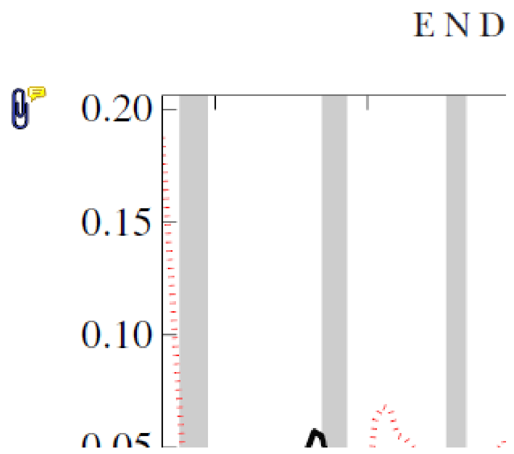

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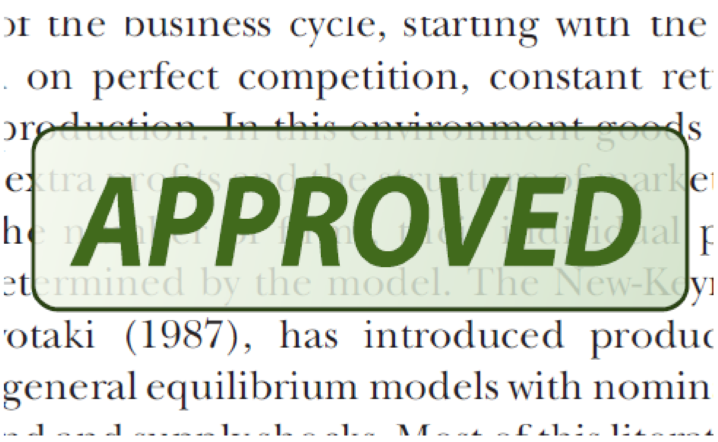

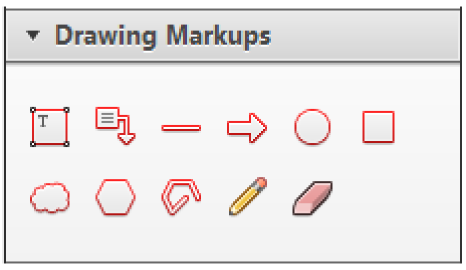

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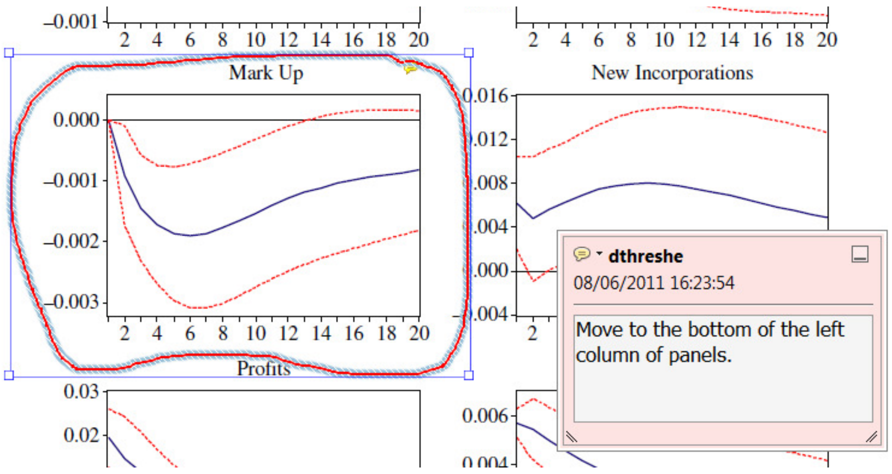

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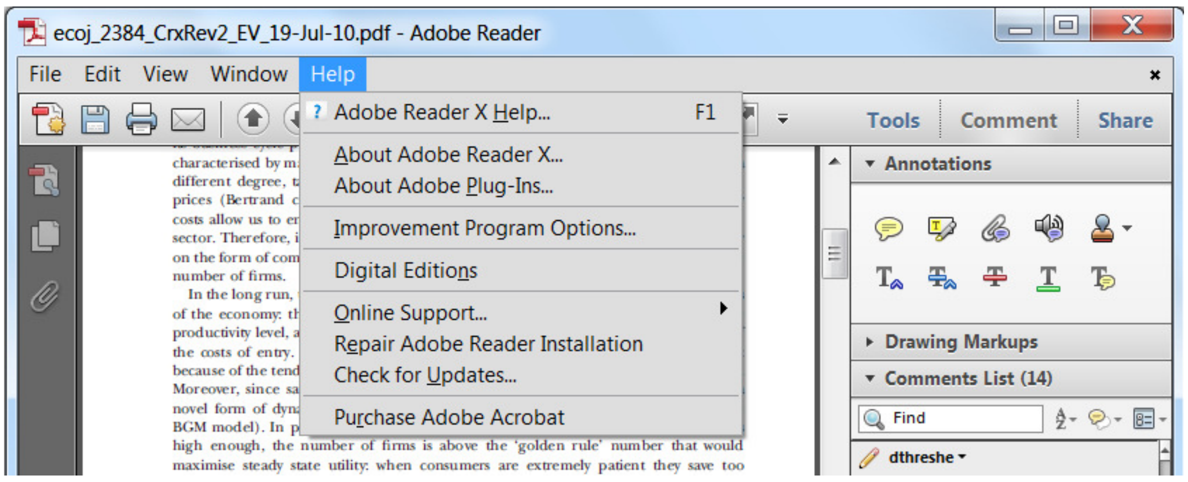

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