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Testing the prey attraction function of stabilimenta in orb-webs of *Argiope luzona* and *Argiope aemula* (Araneae: Araneidae)

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Abstract. Y-maze choice experiment were used to examine the prey-attraction hypothesis for the function of stabilimentum on the orb-webs of *Argiope aemula* and *A. luzona*. Fruit flies (*Drosophila* spp.), common prey species for spiders were used to determine whether webs with stabilimenta attract more flies in Y-maze choice experiment. The result showed that under well-lit condition (bright light), webs with stabilimenta significantly attracted more prey compared to those without stabilimenta in *A. aemula* and *A. luzona*. However, regarding the prey capture rate in bright light, only *A. aemula* showed an increased in the number of prey captured in decorated webs. Under dim condition, the results still showed a significant difference in the number of approaching prey, but not in the number of prey captured by the webs. In both species, the number of prey approached and intercepted in the decorated webs were higher in well-lit condition than in dim condition, suggesting that fruit flies are attracted to webs with stabilimenta in both species studied. Our data is congruent with the prey-attraction hypothesis as function of stabilimentum.
Keywords: *Argiope*, stabilimentum, prey attraction hyothesis, Y-maze.

Rezumat. Experimente bazate pe folosirea labirintului Y au fost folosite pentru a examina ipoteza atragerii prăzii şi funcţia stabiliment-ului de pe pânzele speciilor de păianjeni *Argiope aemula* şi *A. luzana*. Musculiţele de oţet (*Drosophila* spp.), prada comună pentru speciile de păianjen au fost folosite în experimentele cu labirinte Y pentru a determina dacă pânzele cu stabiliment atrag mai multe musculiţe. Experimentul a arătat că la cele 2 specii (*A. aemula* şi *A. luzona*) în condiţii bune de iluminat (condiţii normale) pânzele cu stabiliment tind să atragă mai multă pradă decât cele fără stabiliment. Totuşi, doar la specia *A. aemula* s-a înregistrat o creştere în rata capturii efective. La lumină slabă s-a observat că un număr mai mare de musculiţe se apropiau de pânzele cu stabiliment, dar rata de capturare a prăzii nu s-a modificat semnificativ. La ambele specii numărul de musculiţe care s-au apropiat sau au fost interceptate de pânze a fost mai mare in condiţii de iluminat normal comparativ cu lumina obscura. Acest lucru sugerează că prada este atrasă de pânze cu stabiliment în cazul ambelor specii studiate. Datele noastre sunt in concordanţă cu ipoteza în care stabiliment-ul are rol de atragere a prăzii.
Cuvinte cheie: *Argiope*, stabiliment, ipoteza atragerii prăzii, labirint Y.

Introduction. Spiders in genus *Argiope* spin orb-webs and have the tendency to build a silk decoration called stabilimentum in center most part of the web. Juveniles were noted to spin discoid stabilimenta while adults spin cruciate forms (Abrenica-Adamat et al 2009; Abrenica-Adamat et al 2013; Adamat et al 2011). Stabilimenta are very conspicuous white zigzag silk constructs that have caught the attention of many arachnologist for over a century (Tso 1996). Many functions have been proposed for the stabilimenta, however, evidence is lacking for most. Among the proposed function of these constructs, the prey-attraction hypothesis as function of stabilimentum is the most supported (Craig & Bernard 1990; Craig 1994; Hauber 1998; Tso 1996; Tso 1998a; Tso 1998b).

It has been suggested that the ability to see ultraviolet (UV) light is crucial in insect vision, since insects use UV light to locate open space for flying or escape routes, and silk stabilimentum reflects UV light (Goldsmith 1961; Hu & Stark 1977; Watanabe 1999). Insects that has been suggested to have UV photoreceptors that are very

sensitive in the ultraviolet range includes fruit flies (Watanabe 1999; Hu & Stark 1977; Li et al 2004), which are common insect-prey of orb-web spiders. UV-reflectance as a key luring feature of prey-attraction has been supported by several studies. Like for instance, in the presence of UV light, webs of juveniles containing discoid stabilimenta captured more *Drosophila* than non-decorated webs in Y-choice experiments (Li et al 2004). In *Octonoba sybotides* webs, *Drosophila* was significantly attracted to decorated webs in UV-positive light (Watanabe 1999). In favor of the prey attraction function, Gálvez (2009) found out that the decorated webs of *A. savignyi* intercepted more stingless bees (*Tetragonisca angustula)* than non-decorated webs. Artificial webs with stabilimenta from *A. aurantia* intercepted more flying insects than the control group of artificial webs containing non-decorative silk from the same area of the web (Tso 1998a). Thus, this study primarily aims to reexamine the prey-attraction hypothesis as the function of the stabilimenta using the orb-webs of *A. luzona* and *A. aemula.* Specifically this study aims to test whether webs with stabilimenta attract more prey compared to webs without stabilimenta, and whether decorated webs attract more prey in well-lit conditions than in dimly-lit condition. In this paper, decorated webs are webs with stabilimentum while non-decorated webs are webs without stabilimentum.

Materials and Methods

Enclosure. A total of 20 enclosures were constructed to house the spiders individually, a frame of 20x20x12cm was put together using 1x1in of precut wood. The four 12x20cm sides were covered with mosquito net to provide ventilation for the spider while the one 20x20cm side was covered with a clear plastic to provide easy viewing of the spider and the other 20x20cm side was strip of 20x20 cardboard that can be easily removed to be able to open and close the enclosure.

Prey Choice Experiment. The theory that the stabilimentum attracts prey was tested with the use of a free-choice apparatus, Y-maze (Figure 1). The constructed Y-maze was derived from the study of Walter (2008) and Li et al (2004), however modifications were made. Such alterations include the dimensions of the maze and the species used in the experiment. The maze was covered with plastic cover on the top and sides while plywood was used in the base of the apparatus. The three ends of the maze were kept open for easy attachment of enclosures.

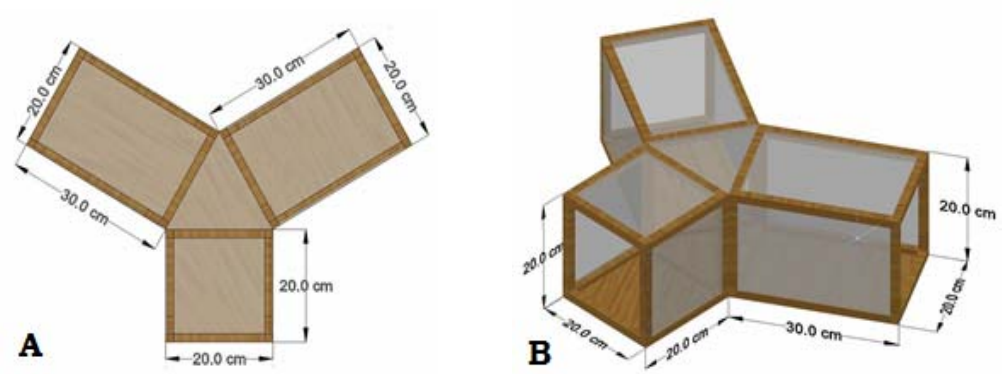

Figure 1. Graphic design of the Y-maze apparatus designed through AutoCAD 2014: top view (A) and isometric view of the Y-maze apparatus graphic design (B).

For testing the prey attracting property of the stabilimenta, an enclosure with built web was attached to each long end of the apparatus: a web with stabilimentum on one and a web without stabilimentum on the other. Included are two webs of almost similar web area, diameter, and mesh distance (distance between sticky spirals). On the short leg of the apparatus is where an approximate of 20-30 fruit flies, *Drosophila* spp., were set free to serve as the prey. The number of fruit flies approaching and the number of those which got stuck in each of the spiders' webs per minute was recorded individually. This method was done in two conditions—well-lit/bright light and dim. Natural/bright light was

used in the well-lit condition (light intensity: 1835.64±410.274 lux) while in dimly-lit condition (light intensity: 15.3636±3.23335 lux), an indoor set-up was used wherein no artificial lights were turned on and the only illumination came from the tinted windows. The intensity of light illumination in the two light conditions were measured using EasyView™ Light Meter 01588-07. In *A. aemula*, the experiment was done with 54 webs in well-lit conditions and 45 webs in dim conditions, totaling to 99 webs. Two webs were used per trial giving a total of 198 webs. In *A. luzona*, a total of 60 trials was done using 120 webs.

In has been proposed that UV-reflecting stabilimentum, together with the UV-reflecting body surfaces of *Argiope* spiders act as a visual display that attracts prey (Elgar et al 1996). To eliminate the posible effect of the UV-reflecting body surface of the spider, the spiders were removed prior to the experiment.

Statistical Analysis. One-way ANOVA Test was used to determine if there were any significant differences among the prey approached and prey captured in the web in the prey choice experiment. All statistical calculations were done with the aid of Paleontological Statistics Software version 2.17c (Hammer et al 2001).

Results and Discussion

Prey Choice Experiment in *A. aemula*. Table 1 shows the number of approaching prey and the number of captured prey in well-lit (bright light) and dimly-lit condition in decorated and non-decorated webs of *A. aemula*. Under well-lit condition, the number of prey approached and intercepted significantly differed between decorated and non-decorated webs. A higher number of prey approached and were captured was observed in webs with stabilimenta. In dim condition, the number of prey approached is significantly higher in decorated webs than in non-decorated webs suggesting that even at a reduced light intensity (15.3636±3.23335 lux), the stabilimenta in orb-webs of *A. aemula* are able to attract prey. However, the number of prey captured by the two kinds of webs showed no significant difference.

Table 1

Summary and One-way ANOVA result for prey choice experiment in well-lit and dim conditions of *A. aemula*

	With stabilimenta	No stabilimenta	*Q*	*P*
Well-lit condition, N=54				
No. of prey captured	3.48±2.74	1.69±1.61	5.88	0.0002*
No. of prey approached	16.83±4.95	10.13± 3.10	11.94	0.0001*
Dim condition, N=45				
No. of prey captured	1.36 ±1.43	0.91 ±0.97	2.435	0.089
No. of prey approached	13 ±2.98	11.4 ±2.21	4.081	0.005*

*Relationship is significant at $p \leq 0.05$

Table 2 shows the One-way ANOVA results for prey choice experiment in webs in bright light versus in dim light of *A. aemula*. In this experiment, only the decorated webs were used in the analysis. A significant difference was observed in the number of prey approached and captured in the decorated webs between dim and well-lit conditions. The number of prey approached and intercepted in decorated webs in bright light is greater than that in dim light. The fact that there is a difference in prey captured/approached between the two light conditions further confirms the prey attraction abilities of stabilimentum. The results may indicate that ultraviolet light plays a role in the efficiency of stabilimentum functioning. The stabilimentum of *A. aemula* might reflect the UV light to attract UV-attracted insects such as fruit flies. And because of its capability to attract insects, it has also increased the number of prey captured by the web.

Table 2

One-way ANOVA results for prey choice experiment in decorated webs in bright light versus in dim light in *A. aemula*

	Well-lit condition (N=54)	Dim condition (N=45)	*Q*	*P*
No. of prey captured	3.48 ± 2.74	1.36± 1.43	6.64	0.0001*
No. of prey approached	16.84 ± 4.95	13.00 ± 2.98	6.44	0.0001*

*Relationship is significant at $p \leq 0.05$.

Prey Choice Experiment in *A. luzona*. Table 3 shows the number of approaching prey and the number of captured prey in well-lit and dim conditions between decorated and non-decorated webs of *A. luzona*. The result showed that in bright light, the web with stabilimenta attracted more prey than the web without one, but the number of prey captured did not differ between decorated and non-decorated webs. These results implies that the presence of stabilimenta increases the attractiveness of the webs but does not necessarily increase the prey capture rate of the webs. This might be because these spiders spun orb-webs with mesh distances unable to effectively intercept small-sized prey such as fruit flies. In dim conditions, the number of prey approached and captured did not differ between decorated and non-decorated webs. These results may suggest that the prey-attracting capabilities of the stabilimenta is dependent on the availability and intensity of light. It could be that the UV reflectance of the silk stabilimenta at light intensity of 15.3636±3.23335 lux was insufficient to attract insects.

Table 4 shows the One-way ANOVA results for prey choice experiment in webs of *A. luzona* in bright light versus in dim light. Only decorated webs are used in the analysis. The result showed that the number of prey approached and intercepted significantly differs between webs in bright light and in dim light. The decorated webs attracted and captured more prey in well-lit condition than in dimmer condition which further confirms the UV-reflecting property of stabilimenta.

Table 3

Summary and One-way ANOVA result for prey choice experiment in well-lit and dim conditions of *A. luzona*

	With stabilimenta	No stabilimenta	*Q*	*P*
Well-lit condition, N=45				
No. of prey captured	1.01±1.11	0.57±0.77	2.47	0.085
No. of prey approached	12.6±3.23	10.43±2.31	4.21	0.004*
Dim condition, N=45				
No. of prey captured	0.43±0.63	0.27±0.45	0.24	1.675
No. of Prey approached	6.73±2.10	6.4±1.69	0.50	0.957

*Relationship is significant at $p \leq 0.05$

The Y-maze experiment showed that under well-lit condition, webs with stabilimenta tend to attract more prey compared to those without stabilimenta in both species [*A. aemula* (decorated: 16.83±4.95 *vs.* non-decorated: 10.13±3.10; p=0.0001), and *A. luzona* (decorated: 12.6±3.23 *vs.* non-decorated: 10.43±2.31; p=0.004)]. However, the presence of stabilimentum could not necessarily affect the prey capture rate of the web even in bright light (light intensity: 1835.64±410.274 lux).

Table 4

One-way ANOVA results for prey choice experiment in decorated webs in bright light versus in dim light in *A. luzona*

	Well-lit condition N=45	Dim condition N=45	*Q*	*P*
No. of prey captured	1±1.11	0.43±0.63	3.4	0.018*
No. of prey approached	12.6±3.23	6.73±2.10	11.7	0.0001*

*Relationship is significant at $p \leq 0.05$.

In particular, no significant difference in the number of captured prey between decorated and non-decorated webs of *A. luzona* in well-lit condition, an increased prey capture was only observed among decorated webs of *A. aemula* (decorated: 13±2.98 *vs.* non-decorated: 11.4±2.21; p=0.005). This might be because *A. luzona* spiders spun orb-webs with large mesh distances which were unable to efficiently intercept small-sized prey such as fruit flies given a very short time. It should be noted that these spiders were previously fed with medium-sized mealworm (*Tenebrio molitor* larvae; body length: 1.4-1.8 cm). It has been suggested that spiders fed with large prey were more likely to spin webs with large web meshes (Adamat et al 2011), and web with large meshes tended to capture large prey (unpublished result).

The importance of light in prey-attracting efficiency of silk stabilimenta is supported in the present study. In dimmer habitat (dim conditions, illumination: 15.3636±3.23335 lux), specifically, among A. *luzona* spiders, the number of prey attracted and captured did not differ between decorated and non-decorated webs. Likewise, among *A. aemula* spiders, the number of prey approached in decorated webs is significantly higher in habitat illuminated by brighter light than in dimmer habitat.

Although there was a significant difference in the number of approaching prey between decorated and non-decorated webs in both well-lighted and dim condition, however, decorated webs in well-lit condition yielded more approaching prey than in the dim condition in both species [*A. luzona* (well-lit=12.60±3.23 *vs*. dim=6.73±2.10; p=0.0001), and *A. aemula* (well-lit=16.84±4.95 *vs*. dim=13.00±2.98; p=0.0001)]. This suggests that light availability contributes to the functioning of stabilimentum and that its presence yields more number of approaching prey. This supports the statement that stabilimenta reflect UV rays from the sun to draw UV attracted insect prey near to the web. It also indicates that *Drosophila* spp. are more attracted to decorated webs in the presence of a brighter light than to that in dimmer light.

Foraging efficiency of any predator is dependent on its ability to scout prey. Both *A. aemula* and *A. luzona* are stationary orb-web spiders, in which foraging success is influence by any tendency of webs either to lure or repel prey. The web of *Argiope* spiders reflects very little UV light and to increase its foraging performance, *Argiope* decorate their webs with bright, UV reflecting silk constructs called stabilimenta that attract prey (Craig & Bernard 1990). The evolutionary significance of these decorations is poorly understood, but the silk decorations of many species reflect UV light, suggesting that they may function to attract insects (Elgar et al 1996).

Insect prey of many taxa are attracted to orb-webs with stabilimenta and stabilimenta excised from webs and this attraction is presumed by most arachnologists to be visual, as only diurnal orb-web spiders include stabilimenta in their webs (Herberstein 2000). A fundamental assumption of the prey-attraction hypothesis for stabilimenta is that these constructs reflect UV light. Li et al (2004) have listed several proposed mechanisms as to how insects are attracted by these UV-reflecting silk constructs, but neither have been explicitly tested. The UV-reflecting stabilimenta may increase the spider's foraging success by mimicking a gap between vegetation, or an open space for flying or escape routes (Goldsmith 1961; Craig & Bernard 1990). Silk stabilimenta attract prey in search of high light intensity or by imitating flowers with UV-reflecting properties

which visually guide insects to find food (nectar or pollen reward) or mating sites (Frolich 1976; Craig & Bernard 1990), and this has been proposed as one potential mechanism for the evolution of visually similar prey-attracting mechanism of spider body surfaces and their webs (Crowe 2009; Craig & Bernard 1990).

Conclusions. The fact that more prey approached the decorated webs in both light conditions, and more prey approached and captured in decorated webs in well lighted condition supports with the prey-attracting function of stabilimenta. In relation with the prey-attracting function of stabilementum, a study investigating the UV reflectance of silk stabilimenta in orb-webs of *A. luzona* and *A. aemula* and if these spiders may adjust the quantity of silk decoration to their webs, adding more silk decoration when the web is located in dim light rather than bright light should be considered in a future study.

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