

SHORT COMMUNICATION

The mechanism behind plasticity of web-building behavior in an orb spider facing spatial constraints

Thomas Hesselberg¹: Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panamá. E-mail: thomas.hesselberg@zoo.ox.ac.uk

Abstract. Orb spiders demonstrate an impressive ability to adapt their web-building behavior to a wide range of environmental and physiological factors. However, the mechanisms behind this plasticity remain poorly understood. Behavioral plasticity can be categorized as either developmental, where new neural pathways arise from learning, or activational, which rely on more costly pre-existing neural pathways. Here I argue that orb spiders and their webs in general and their response to spatial constraints in particular make an ideal model system in which to explore these two mechanisms further. I show that the spider *Eustala illicita* (O. Pickard-Cambridge 1889) immediately modifies its first orb web after being placed in spatially confined experimental frames without showing subsequent improvements in design of the second web. Thus, these data are in accord with the hypothesis that this spider relies on activational behavioral plasticity, which might be linked to its preferred habitat in the wild.

Keywords: Behavioral flexibility, learning, experience, web geometry, *Eustala illicita*

The ability of an animal to rapidly adapt its behavior to changes in its environment, so-called behavioral plasticity or behavioral flexibility, has been described from a wide range of vertebrate and invertebrate taxa. Phenotypic plasticity in general, and behavioral plasticity in particular, has previously been recognised as arising either from an innate pre-programmed pathway or from internal physiological or external environmental changes including developmental changes and learning (West-Eberhard 2003; Mery & Burns 2010). Most studies focus on the interaction between environmental change and the evolution of learning. Initially it was assumed that learning was always favored in variable environments, but more detailed experimental and theoretical studies show that learning is only favored when the environment changes relatively little within an individual lifetime and shows predictable changes between generations (so-called coarse-grained environmental variation). Innate behavior is favored when the environment changes randomly and unpredictably within generations (so-called fine-grained environmental variation) (Stephens 1991; Dunlap & Stephens 2009).

The above and similar studies have significantly increased our understanding of the evolution of learning, but the relationship between behavioral plasticity and learning remains poorly defined. However, this relationship has recently been the subject of a review by Snell-Rood (2013), in which she defined two different kinds of behavioral plasticity based on separate costs and benefits. Developmental behavioral plasticity is the slower process that requires a physical re-organisation of the underlying neural pathways caused by, for example, learning, which is hypothesised to be favoured in environments that show coarse-grained variation. Activational behavioral plasticity, which is an immediate response that relies on pre-configured neural pathways, is favoured in environments that show fine-grained variation. Both require significant initial investment in costly neural tissue, but developmental behavioral plasticity allows animals to prune and optimize the neural network over time, while activational behavioral plasticity relies on a constant amount of neural tissue (Snell-Rood 2013). However, the two mechanisms do not necessarily operate completely separately. What may look like activational behavioral plasticity in the adult animal may have arisen through interactions between the genes and the environment including learning processes in the juvenile animal. Thus activational

behavioral plasticity that does not involve any learning in the present may be the result of neural pathways that were fixed through developmental behavioral plasticity in the past. More experimental data is required to investigate the prevalence and interaction of these two types of behavioral plasticity.

Here I propose that orb spiders and their webs constitute an ideal model system in which to study behavioral plasticity. Orb spiders show an impressive ability to modify their webs to a range of environmental and physiological factors including temperature (Vollrath et al. 1997), wind (Vollrath et al. 1997; Liao et al. 2009), prey size and type (Nakata 2007; Blamires et al. 2011), silk availability (Eberhard 1988; Vollrath et al. 1997), leg loss (Pasquet et al. 2011) and spatial constraints (Ades 1986; Vollrath et al. 1997; Harmer & Herberstein 2009). However, the majority of these studies tested either only the first web or allowed the spiders a week or more to acclimatize to experimental conditions before testing them, and so do not allow us to unravel whether spiders immediately adapt their webs to the new condition (i.e., activational behavioral plasticity) or improve their webs gradually as they gain more experience with the condition (i.e., developmental behavioral plasticity). Given that inexperienced spiders build perfectly normal webs (Reed et al. 1970) and that spiders do not improve webs with age or size (Eberhard 2007; Hesselberg 2010), a reliance on developmental behavioral plasticity is perhaps less likely. However, orb spiders readily learn to avoid dangerous and distasteful prey (Hénaut et al. 2014); gradually alter their sticky spiral mesh size, web size and web asymmetry based on recent prey capture experiences (Heiling & Herberstein 1999; Venner et al. 2000); improve the size, planarity and verticality in subsequent webs built at the same site (Zschokke & Vollrath 2000; Nakata & Ushimaru 2004); and also seem to gradually improve their locomotory and web-building skills under weightless conditions in space (Witt et al. 1977).

Here I propose that spiders' adaptation to building webs in spatially constrained spaces is particularly useful for studying behavioral plasticity as it is ecologically relevant and has been studied in a number of different species (Ades 1986; Vollrath et al. 1997; Krink & Vollrath 2000; Harmer & Herberstein 2009; Barrantes & Eberhard 2012; Hesselberg 2013). I re-analysed previously collected data on behavioral flexibility in *Eustala illicita* (O. Pickard-Cambridge 1889), which successfully built webs in size-limited experimental frames (Hesselberg 2013). Late instar female spiders were collected in a dry tropical rain forest in Panama City, Panama (9°N, 80°W) and

¹Current Address: Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, United Kingdom

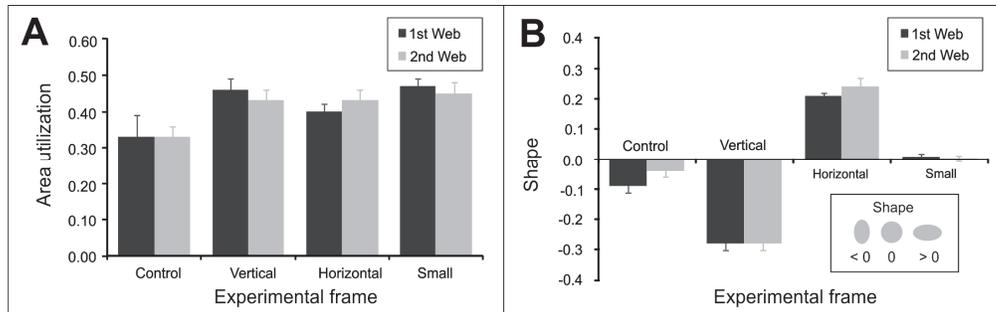


Figure 1.—Differences in area utilization (A) and shape (B) between first (dark grey bars) and second (light grey bars) webs of *Eustala illicita* built in experimental frames (Control ($N = 5$): 30×30 cm; Vertical ($N = 8$): 15×30 cm; Horizontal ($N = 6$): 30×15 cm; Small ($N = 10$): 15×15 cm). The error bars indicate the standard error of the mean. The inset on figure B gives an interpretation of the numerical shape values with a value of 0 indicating a perfect circle. Shape was calculated using the following equation $(d_h - d_v)/(d_h + d_v)$, where d_h and d_v is the horizontal and vertical diameter of the web.

were given a week to acclimatize to building webs in standard frames ($30 \times 30 \times 5$ cm) in the laboratory during which they were watered and fed fruit flies regularly after which their webs were cut into a single strand with a soldering iron (see Hesselberg 2013 for a more detailed description of methods). Spiders that built normal-looking webs nearly daily were included in the experiment, which consisted of transferring spiders to experimental frames (control frames: $30 \times 30 \times 5$ cm, vertical frames: $15 \times 30 \times 5$ cm, horizontal frames: $30 \times 15 \times 5$ cm and small frames: $15 \times 15 \times 5$ cm), where they were kept for three days with any webs being photographed and subsequently destroyed with a soldering iron as described above. Spiders were given water but not fed throughout the three-day experimental period. In the present study I used the spiders that built multiple webs in the three day period to compare the first web built on day 1 with the second web built on day 2 or day 3. As only about half the spiders built three webs, I decided to compare only web 1 and 2. A range of web parameters were measured from digital photographs using ImageJ (v1.41, National Institute of Health, USA) and were analysed with IBM SPSS v. 20 (IBM Corporation 2011) using a significance level of 5%. The tests performed were either a repeated measures ANOVA with web number as the within-subject factor and experimental frame as between-subject factor or a paired t-test.

The main parameters of interest were the area utilization (i.e. the proportion of the available area in the frame taken up by the capture spiral) and the shape of the web (Vollrath et al. 1997; Krink & Vollrath 2000; Hesselberg 2013). As shown in Fig. 1A, this study found only minor and non-significant differences in area utilization between first and second webs across all four experimental treatments (repeated measures ANOVA: $F_{(1,25)} = 0.12$, $P = 0.915$) but, as expected, spatially constrained spiders utilized a significantly higher proportion of the available area than the control spiders (repeated measures ANOVA: $F_{(3,25)} = 5.56$, $P = 0.005$). Similarly, there were no differences in shape between the first and the second web across the different experimental frames (repeated measures ANOVA: $F_{(1,25)} = 2.17$, $P = 0.153$) but, as expected, there were significant differences in shape between webs in the different treatments (repeated measures ANOVA: $F_{(3,25)} = 173.39$, $P < 0.001$), with control and small frames resulting in almost round webs while the vertical frames had vertically elongated webs and the horizontal frames had horizontally elongated webs (Fig. 1B). The repeated measures ANOVA found no significant interactions between web number and frame shape for either area utilization or shape (test results not shown). The similarity of first and second webs across all the experimental frames was further supported by the lack of differences between first and second webs in a range of web parameters for all four treatments (Table 1), except that mesh height in the horizontal frame was slightly larger in the second web.

In conclusion, the data presented here suggest that *E. illicita* immediately adjusts its first orb web to match the experimentally

constrained space with no improvements in shape or area utilization in the second webs built under the same conditions. Although the present lack of statistical differences could be attributed to the relatively small sample size, none of the measured parameters show any consistent trends towards better adapted, larger or denser second webs. *Eustala illicita* therefore appears to rely on activational behavioral plasticity to adapt its web to spatial constraints, which the spider probably frequently encounters in its natural habitat. It is almost exclusively found in relative high densities within the branches of the ant acacia *Acacia collinsii*, which might give rise to competition for available space (Hesselberg & Triana 2010; Styrsky 2014). As the individual spiders grow larger, they are therefore likely to be subject to fine-grained environmental variation as they move around on the acacia in search of suitable web-building sites. Since the spiders used in this study were caught in the wild, however, it is possible that the present behavior is the result of earlier developmental behavioral plasticity that has resulted in fixed neural pathways for dealing with spatial constraints. In this regard the present behavior can be viewed as an example of context-dependent behavior in that spiders utilize earlier learning to adapt their web-building behavior when facing similar constraints. Such context-dependent learning has previously been found in spiders (Skow & Jakob 2006), although the two very different contexts in this study in terms of learning in the complex natural environment and using this learning in the much simpler artificial environment in the laboratory renders this less likely. Finally, there is also the possibility that no learning or plasticity takes place and that the ability to adapt their webs to spatial constraints is a passive emergent property of the spider's web-building behavioral rules. This, however, is unlikely for the following reasons: the spiders in this experiment and in others (Vollrath et al. 1997) readily adapt their webs to many different types of spatial constraints; orb spiders in general match the size and shape of their webs to their available silk resources (Eberhard 1988) and therefore probably gather information during their exploratory behavior relevant to the size and shape of their future webs (Vollrath 1992); and other species of orb spiders, likely using similar behavioral rules, are unable to adapt their webs to limited space (Hesselberg 2013). Given the discussion above and because the present study only investigates learning over a short period of time for only one situation, that of web-building behavior in spatial constraints, this study provides a relative weak test for the role of learning in behavioral plasticity of web-building behavior generally. However, the activational behavioral plasticity hypothesis is further supported by the strong either-or response in web-building frequency between spiders that match their webs to available space (Vollrath et al. 1997) and those that do not (Hesselberg 2013) as well as the immediate response in web parameters observed in *Cyclosa octotuberculata* (Karsch 1879) to feeding and prey detection experiences (Nakata 2007, 2012). To determine whether orb spiders

Table 1.—A comparison between first and second webs of *Eustala illicita* facing spatial constraints. Measures are given as mean ± standard error.

	First web	Second web	Paired <i>t</i> -test	<i>P</i> -value
CONTROL FRAME				
Number of webs	5	5		
Number of radii	28.8 ± 3.3	31.0 ± 2.2	-1.77	0.151
Number of spirals	32.5 ± 4.7	33.1 ± 4.4	-0.58	0.591
Mesh height (cm)	0.25 ± 0.03	0.24 ± 0.04	0.37	0.733
Vertical asymmetry ¹	-0.51 ± 0.04	-0.54 ± 0.02	0.82	0.458
VERTICAL FRAME				
Number of webs	8	8		
Number of radii	31.4 ± 1.6	33.1 ± 1.5	-1.07	0.320
Number of spirals	31.9 ± 1.7	29.2 ± 1.8	1.52	0.173
Mesh height (cm)	0.20 ± 0.01	0.20 ± 0.01	-0.73	0.487
Vertical asymmetry ¹	-0.44 ± 0.03	-0.40 ± 0.06	-0.70	0.506
HORIZONTAL FRAME				
Number of webs	6	6		
Number of radii	33.5 ± 1.4	35.7 ± 1.7	-1.23	0.273
Number of spirals	33.9 ± 2.4	31.7 ± 1.4	1.49	0.193
Mesh height (cm)	0.18 ± 0.01	0.19 ± 0.01	-3.10	0.027*
Vertical asymmetry ¹	-0.45 ± 0.02	-0.47 ± 0.04	<i>Z</i> = -0.67	0.500
SMALL FRAME				
Number of webs	10	10		
Number of radii	29.2 ± 1.4	29.4 ± 1.3	-0.12	0.907
Number of spirals	25.4 ± 1.6	24.7 ± 1.3	0.49	0.639
Mesh height (cm)	0.17 ± 0.01	0.18 ± 0.01	-1.44	0.184
Vertical asymmetry ¹	-0.42 ± 0.05	-0.37 ± 0.07	-0.85	0.419

¹ Vertical asymmetry was calculated using the following equation: $(r_u - r_l)/(r_u + r_l)$, where r_u and r_l are the upper (above hub) and lower (below hub) web radii. The Wilcoxon Signed Rank test (*Z*) was used where data could not be normalized.

generally rely exclusively on activational behavioral plasticity, or on a combination of the two behavioral plasticity mechanisms, to adapt their behavior to changes in the environment requires further comparative studies in a range of situations including naturally occurring ones such as leg loss and experimental ones such as changes in the magnitude or direction of gravity.

ACKNOWLEDGMENTS

The collection of the original data upon which this study was based was funded by a Smithsonian Institution Postdoctoral Fellowship. The author would like to thank William Eberhard for his useful comments on an earlier version of this paper as well as the very valuable comments from two anonymous reviewers.

LITERATURE CITED

Ades, C. 1986. A construção de teia geométrica como programa comportamental. *Ciência e Cultura* 38:760–775.
 Barrantes, G. & W.G. Eberhard. 2012. Extreme behavioral adjustments by an orb-web spider to restricted space. *Ethology* 118:438–449.
 Blamires, S.J., Y.-C. Chao, C.-P. Liao & I.M. Tso. 2011. Multiple prey cues induce foraging flexibility in a trap-building predator. *Animal Behaviour* 81:955–961.
 Dunlap, A.S. & D.W. Stephens. 2009. Components of change in the evolution of learning and unlearned preference. *Proceedings of the Royal Society of London. Series B* 276:3201–3208.
 Eberhard, W.G. 1988. Behavioral flexibility in orb web construction: effects of supplies in different silk glands and spider size and weight. *Journal of Arachnology* 16:295–302.
 Eberhard, W.G. 2007. Miniaturized orb-weaving spiders: behavioural precision is not limited by small size. *Proceedings of the Royal Society of London. Series B* 274:2203–2209.

Harmer, A.M.T. & M.E. Herberstein. 2009. Taking it to extremes: what drives extreme web elongation in Australian ladder web spiders (Araneidae: *Telaprocera maudae*)? *Animal Behaviour* 78:499–504.
 Heiling, A.M. & M.E. Herberstein. 1999. The role of experience in web-building spiders (Araneidae). *Animal Cognition* 2:171–177.
 Hénaut, Y., S. Machkour-M'Rabat & J.-P. Lachaud. 2014. The role of risk-avoidance strategies during spider-ant interactions. *Animal Cognition* 17:185–195.
 Hesselberg, T. 2010. Ontogenetic changes in web design in two orb-web spiders. *Ethology* 116:535–545.
 Hesselberg, T. 2013. Web-building flexibility differs in two spatially constrained orb spiders. *Journal of Insect Behavior* 26:283–303.
 Hesselberg, T. & E. Triana. 2010. The web of the acacia orb-spider *Eustala illicita* (Araneae: Araneidae) with notes on its natural history. *Journal of Arachnology* 38:21–26.
 Krink, T. & F. Vollrath. 2000. Optimal area use in orb webs of the spider *Araneus diadematus*. *Naturwissenschaften* 87:90–93.
 Liao, C.-P., K.-J. Chi & I.-M. Tso. 2009. The effects of wind on trap structural and material properties of a sit-and-wait predator. *Behavioral Ecology* 20:1194–1203.
 Mery, F. & J.G. Burns. 2010. Behavioral plasticity: an interaction between evolution and experience. *Evolutionary Ecology* 24: 571–583.
 Nakata, K. 2007. Prey detection without successful capture affects spider's orb-web building behaviour. *Naturwissenschaften* 94: 853–857.
 Nakata, K. 2012. Plasticity in an extended phenotype and reversed up-down asymmetry of spider orb webs. *Animal Behaviour* 83:821–826.
 Nakata, K. & A. Ushimaru. 2004. Differences in web construction behavior at newly occupied web sites between two *Cyclosa* species. *Ethology* 110:397–411.

- Pasquet, A., M. Anotaux & R. Leborgne. 2011. Loss of legs: is it or not a handicap for an orb-weaving spider? *Naturwissenschaften* 98:557–564.
- Reed, C.F., P.N. Witt, M.B. Scarboro & D.B. Peakall. 1970. Experience and the orb web. *Developmental Psychobiology* 3:251–265.
- Skow, C.D. & E.M. Jakob. 2006. Jumping spiders attend to context during learned avoidance of aposematic prey. *Behavioral Ecology* 17:34–40.
- Snell-Rood, E.C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. *Animal Behaviour* 85:1004–1011.
- Stephens, D.W. 1991. Change, regularity, and value in the evolution of animal learning. *Behavioral Ecology* 2:77–89.
- Styrsky, J.D. 2014. An orb-weaver spider exploits an ant–acacia mutualism for enemy-free space. *Ecology and Evolution* 4:276–283.
- Venner, S., A. Pasquet & R. Leborgne. 2000. Web-building behavior in the orb-weaving spider *Zygiella x-notata*: influence of experience. *Animal Behaviour* 59:603–611.
- Vollrath, F. 1992. Analysis and interpretation of orb spider exploration and web-building behavior. *Advances in the Study of Behavior* 21:147–199.
- Vollrath, F., M. Downes & S. Krackow. 1997. Design variability in web geometry of an orb-weaving spider. *Physiology & Behavior* 62:735–743.
- West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press, New York.
- Witt, P.N., M.B. Scarboro, R. Daniels, D.B. Peakall & R.L. Gause. 1977. Spider web-building in outer space: evaluation of records from the skylab experiment. *Journal of Arachnology* 4:115–124.
- Zschokke, S. & F. Vollrath. 2000. Planarity and size of orb-webs built by *Araneus diadematus* (Araneae: Araneidae) under natural and experimental conditions. *Ekologia* 19:307–318.

Manuscript received 10 January 2014, revised 7 July 2014.