Behavioural analysis of web building anomalies in the orb-weaving spider *Zygiella x-notata* (Araneae, Araneidae)

Camille Toscani, Raymond Leborgne & Alain Pasquet

Abstract: Among animal constructions, spider's orb webs represent regular geometrical architecture models. Their construction is the result of successive, simple and reproducible behavioural patterns, often considered as stereotyped. It has recently been shown that spider's building behaviours vary, which can alter web regularity. The final capture spiral results from the laying of successive threads between two radii, here termed 'spiral units'. We defined a theoretical normal web, as a web in which each turn of the final spiral should be parallel to the preceding one. Weaving of the spiral units sometimes leads to anomalies in the orb web. Anomalies were identified and analysed in the orb-weaving spider *Zygiella x-notata* (Clerck, 1757). From video recordings of web construction, we noted the displacements of the legs and of the abdomen of the spider. We compared the frequency of displacements, and their duration, between the construction of spiral units that produce a normal turn and ones that produce an anomalous turn. The position of the legs on the web's threads was also analysed. Results showed that anomalies were not the consequences of a modification in activity but more likely the result of the position on the radii of the fourth leg. These results suggest that spiders use local information to build the final capture spiral.

Key words: behavioural variability, building behaviour

Many animal species belonging to different taxa (mammals, birds, reptiles, arthropods) can build more or less complex constructions. The success of these building behaviours is linked to morphological capacities and to coordination of movements (HANSELL 2005, 2007). These constructions are the result of a succession of repeated behaviours, which involve cognitive complexity (HANSELL & RUXTON 2008). A wide diversity of constructions can be observed at the inter-specific level, but at the intra-specific level, variations in building behaviours can lead to differences in construction. By comparison with a normal structure defined by the observer, some variations can appear as anomalies of construction. For example, some wasp nests do not possess a pedicel, which results from confusion in the program of construction (KARSAI & THERAULAZ 1995).

In spiders, the orb-web is the result of successive, simple and reproducible behavioural patterns organised in time (VOLLRATH 1992). The architecture of the web contains radii and a final – also called the capture or sticky – spiral. This spiral consists of a succession of segments of silk line attached to two successive radii. We name these linear segments 'spiral units'. They are the elementary parts of the final capture spiral. We defined a regular final spiral by the continuity of the spiral unit arrangement around the hub and by the parallelism of each turn of the spiral with the preceding one; i.e. the spiral unit is expected to be parallel with the preceding unit in the same sector, as defined by two successive radii. Despite the apparent regularity of the orb-web, capture spirals vary in form, size and density (estimated by the distance between two spiral turns). Different factors can affect this regularity, such as gravity (VOLLRATH & MOHREN 1985), loss of legs (VOLLRATH 1987) and experimental application of neurotoxins or other substances (WITT & REED 1965, HESSELBERG & VOLLRATH 2004).

Given that the orb-web is a direct reflection of successive behaviours (ZSCHOKKE & VOLLRATH 1995), it is a good model for studying variability in the sequence of building behaviour. Previous studies have shown that the spider uses the first pair of legs (L1) to determine spacing relative to the turn of the auxiliary spiral (VOLLRATH 1987) and that leg position is decisive in the control of interspiral distance (KRINK & VOLLRATH 1999). Construction of the final spiral is accomplished by producing a silk line, step by step between two successive radii. At each step, the spider executes the same behaviour in order to deposit the thread line between the site on the

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Abbreviations	Explanation
NSU	Normal spiral unit
NP	Non-parallel spiral unit. In a sector between two radii, two adjacent spiral units meet at a point on a radius.
T2	Two adjacent spiral units stuck together.
R2	Second radius of the spiral unit.
SU	Spiral unit
L1	The first pair of legs.
L4P	The fourth leg oriented to the web's periphery, i.e., the direction opposite to the hub.

radius where the thread was just attached and a new site on the next radius. We hypothesize that leg displacement during final spiral building is dictated by local configuration (THERAULAZ et al. 1998) – i.e. immediate stimuli at the time of building, such as the position of an already laid preceding spiral unit – rather than knowledge of the global web structure. Anomalies, defined as alterations or discontinuities within a regular final spiral, have recently been identified in *Zygiella x-notata* (Clerck, 1757) (Araneae, Araneidae) (Pasquet unpubl.). We used this species to understand the mechanisms of building anomalies through the behaviours of spiral unit construction. The understanding of anomalous building behaviour will help to provide a better comprehension of orb-web building behaviour and of how simple organisms can build large, complex structures.

Material and methods

Zygiella x-notata is an orb-weaving spider abundant in the west Palearctic region. Adult females were collected is the north-east of France (Nancy, 48°41'N, 6°17'E, 272 m a.s.l.) in 2008 and 2010. Spiders were maintained in the laboratory in plastic boxes (10×7×2 cm), where they were fed with flies (*Lucilia caesar*) and supplemented with water once per week.

To allow observations of web-building behaviour, spiders were placed into wooden frames (50×50×10 cm) closed by two panes of glass that were suitable for *Zygiella* to build webs with the same characteristics as webs built in their natural habitat. After a maximum of 96h, or after construction was complete, spiders were returned to their boxes. Video recordings of capture spiral building (n=17) (Fig. 1) were made (camera Sony HDR-CX550) and analysed using the software "The Observer XT-10.0".

Three types of construction were observed and compared. The first type (NSU) was the pattern found in all webs (control). Here, the spider built a spiral unit parallel to the previous one in the same sector (Fig. 2). The second pattern (NP) was when the unit built was not parallel to the previous one in the same sector and the two units met at the same point on the next radius of the sector. The third pattern (T2) was when the unit stuck to the previous one in the same sector. In the 17 webs recorded, we took at random 15 T2 anomalies and 15 NP anomalies. For each anomaly, we associated a normal spiral unit (NSU, n=30) constructed just before an NP or T2 anomaly. We never sampled the same type of anomaly twice in the same web.

By analysing video recordings (24 images/s) we noted the building time of the spiral units, and we counted all the displacements of each of the eight legs for the construction of a spiral unit (i.e. the number of leg displacements). We noted the number and the duration of displacements of L4P – the fourth leg oriented to the web's periphery, i.e. the direction opposite to the hub – between the moment when it stopped extending the thread from the spinneret and the moment when it was placed on R2 (the second radius, where the current spiral unit was fixed) (Fig. 2). These legs were observed because they are known to play an important role in the final capture spiral construction (EBERHARD 1988). Finally we noted the individual positioning of the abdomen and of L4P on R2 (position R2/0, R2/1 or R2/2), where the current spiral unit was fixed (see Fig. 2). If the current spiral unit was attached at R2/2, a normal spiral unit was formed. If it was fixed at R2/0 or R2/1, an anomalous spiral unit appeared. The position in R2/0 or R2/1 was not linked to a particular anomaly.

We compared the construction of an anomalous spiral unit and the associated control one by conducting paired t-tests or Wilcoxon nonparametric tests (in case of non-normality of the data). Four parameters were taken into account: building time of the spiral unit, number of all leg displacements, number and mean duration of L4P displacements (n=15 for each type of anomaly). Normality of the data was tested by Shapiro-Wilk tests. Position of the abdomen and L4P during spiral unit building was compared between normal and anomalous spiral units using a McNemar test (abdomen: n=30, (the two anomalies were combined) L4P, n=15 for each anomaly). The means were given with standard deviation (mean ±

standard deviation), and the medians with lower and upper quartiles, and p<0.05 was considered as significant. The statistical software R 2.15.0 and StatXact3 were used (R 2012, STATXACT3 1995).

Results

Analysis of leg displacement

When normal and anomalous final spiral unit construction were compared, no difference in mean building time between a normal spiral unit (NSU) and an anomalous one (NP or T2) was found (paired t-test, mean NP=5.93 ± 0.37s, mean NSU=6.01 ± 0.43s, n=15, t=0.28, p=0.78; Wilcoxon test, median T2=6.24s (5.38s, 7.28s), median NSU=6.08s (5.36s, 7.82s), n=15, W=-5, p=0.88).

The mean number of leg displacements did not differ when the spider constructed a normal spiral unit or an anomalous spiral unit (Wilcoxon test: median NP=75 (60, 95), median NSU=75 (59, 89), n=15, W=-14, p=0.71; median T2=72 (68, 87), median NSU=80 (71, 88), n=15, W=11, p=0.78).

The mean duration of L4P displacements when the spider built a normal spiral unit was not significantly different than when an anomalous one was built (Wilcoxon test: median NP=0.36s (0.28s, 0.42s), median NSU=0.24s (0.16s, 0.30s), n=15, W=-37, p=0.16, median T2=0.20s (0.12s, 0.36s), median NSU=0.28s (0.22s, 0.44s), n=15, W=24, p=0.47).

The mean number of L4P displacements also did not differ significantly when the spider built a normal or an anomalous spiral unit (Wilcoxon test: median NP=3 (2, 3), median NSU=2 (2, 3), n=15, W=-16, p=0.49, median T2=2 (2, 4), median NSU=4 (2, 5), n=15, W=10, p=0.75).

Position of the abdomen
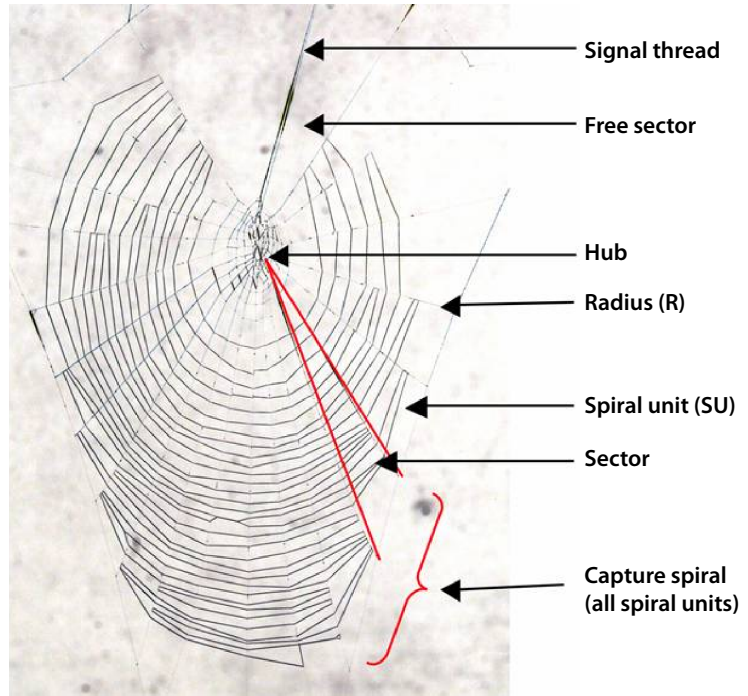
During the attachment of the spiral

Fig. 1: Web of *Zygiella x-notata*. The hub, the radii and the capture spiral of the web are represented; a part of the frame is not visible. The spiral unit is represented by the thread between two radii and as a segment of the final capture spiral.

unit, the position of the abdomen on R2 was not significantly different between an anomalous spiral unit and a normal one (McNemar test, n=30, p=1). In both cases, the abdomen was predominantly positioned in the same location as the L4P (84.4% of cases). In 15.6% of cases, it was located above the L4P on R2.
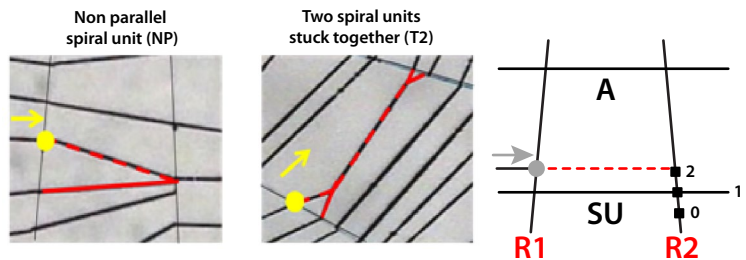

Fig. 2: The two pictures on the left represented the two anomalies that were analysed (NP and T2). The scheme on the right represents the normal situation, with the red dotted line, which symbolizes the spiral unit (SU) recorded and analysed from radius 1 to radius 2. 0, 1 and 2 were the possible positions of the attachment of the line on R2; if the attachment was made at position 2, we obtained a normal spiral unit, if it was in 0 or 1 we obtained an anomalous spiral unit. The grey or yellow arrow represents the direction of spider movement and the grey or yellow point represents the starting point of spiral thread unit construction on radius R1.

Position of the L4P

The position of the L4P was significantly different when the spider deposited a NP spiral unit than when it built a normal one (McNemar test, n=15, Q_{obs}=10.1, p=0.001). The L4P positions were in R2/2 in 80% of cases when the spider built a normal spiral unit, whereas this position was never selected when building a NP spiral unit. In this case, the L4P positions were on R2/1 or R2/0 in 100% of the cases. Positioning of the L4P was also different between T2 spiral unit and a normal one (McNemar test, n=15, Q_{obs}=11.1, p<0.001). Indeed, in 93.3% of normal spiral unit construction, the L4P was positioned on R2/2, whereas this position was selected in only 6.7% of cases for T2 spiral units. Therefore, in a normal spiral unit construction the position of the L4P was different than during the construction of an anomalous one (Fig. 3).
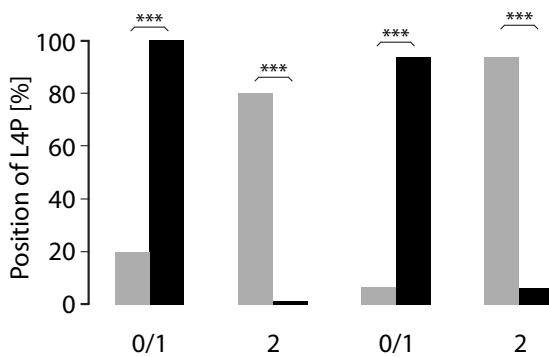

Fig. 3: Comparisons of the positions of L4P (0/1 or 2, see text) between an anomalous spiral unit (black bars) and a normal parallel one (grey bars). On the left, the case of NP anomalies and on the right the case of T2. ***: McNemar test p<0.001.

Discussion

Parallelism between turns of the final capture spiral in orb webs can fail to occur in some sectors of the webs. We studied here the effects of weaving behaviour on these anomalies. To do this, we observed the behaviour of the spider *Zygiella x-notata* during building spiral units (segments of thread attached to two successive radii). We defined normal spiral units as a sector with two consecutive parallel spiral units and anomalous spiral units – i.e. a sector with two consecutive non-parallel spiral units – and we compared different parameters measured during spiral unit construction. No difference in activity (leg displacements and abdomen positioning) was found between the construction of a normal spiral unit and an anomalous one for the four parameters measured: building time, number of leg displacements, mean duration and number of L4P displacements. Therefore, activity does not seem to result in anomalous construction of spiral units.

We analysed the position of L4P on the second radius (R2) at the end of the construction of the spiral units, and we observed a difference in its position between anomalous and normal spiral units. The position of L4P was almost always in R2/0 or R2/1 for the two anomalies investigated in this study (NP and T2), whereas the L4P positions in R2/2 produce normal units. The position of the abdomen, however, did not differ between an anomalous and a normal spiral unit. In conclusion, it is a modification in the position of L4P on the radius, which leads to the building of an anomalous spiral unit. Nevertheless, placement of L4P on the radius remains one of the last steps of the building sequence, and any modification of its position is most likely caused by a change in the position of L1 (first pair of leg) because L4 took the place of L1 on the next radius. Thus we concur with previous studies which hypothesized that L1 is largely implicated in the establishment of the final capture spiral (VOLLRATH 1987). This shows that spiders use information to decide where to attach the spiral of the previous unit on the radius.

It is known that the local configuration of the environment may influence the building behaviour of an animal (HANSELL 2000, THERAULAZ et al. 1998). During the completion of complex structures, animals may use the initial parts of the construction as markers for subsequent stages of construction, as in the building of nests by termites or bees (GRASSÉ 1959, DOWNING & JEANNE 1990). Thus, a construction that is the result of a repeated response to local stimulus is subject to variation at each building step (HANSELL 2005). This is the case in orb-web building; the spider takes into account previous elements of the construction for the building of further elements, and errors can occur in this process. Such errors via architecture modification could lead to modifications in the performance of the web, as studied by CRANFORD et al. (2012).

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