RESEARCH ARTICLE

The mechanical properties of the non-sticky spiral in *Nephila* orb webs (Araneae, Nephilidae)

Thomas Hesselberg* and Fritz Vollrath

Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK

*Author for correspondence (thomas.hesselberg@zoo.ox.ac.uk)

SUMMARY

Detailed information on web geometry and the material properties of the various silks used enables the function of the web's different structures to be elucidated. In this study we investigated the non-sticky spiral in *Nephila edulis* webs, which in this species is not removed during web building. This permanent non-sticky spiral shows several modifications compared with others, e.g. temporary non-sticky spirals – it is zigzag shaped and wrapped around the radial thread at the elongated junctions. The material properties of the silk used in the non-sticky spiral and other scaffolding structures (i.e. radii, frame and anchor threads) were comparable. However, the fibre diameters differed, with the non-sticky spiral threads being significantly smaller. We used the measured data in a finite element (FE) model of the non-sticky spiral in a segment of the web. The FE analysis suggested that the observed zigzag index resulted from the application of very high pre-stresses to the outer turns of the non-sticky spiral. However, final pre-stress levels in the non-sticky spiral after reorganisation were down to 300 MPa or 1.5–2 times the stress in the radii, which is probably closer to the stress applied by the spider during web building.

Key words: orb web, *Nephila*, silk material properties, finite element analysis, pre-stresses, non-sticky spiral.

INTRODUCTION

Spider silk is an extraordinary biomaterial with dragline silk displaying a superb combination of tensile strength and extensibility (Agnarsson et al., 2010). Of specific interest, however, is not a silk's material properties in the abstract but the functional inter-relationships of the various silks deployed in the spider's web, which determine the co-evolution of material and structure (Vollrath, 1992). The highly ordered two-dimensional orb web consists of radii that radiate outward from the hub towards the frame. The frame is attached using pyriform silk to the surrounding vegetation with anchor threads. The scaffolding threads themselves consist of a variable number of silk fibres of different diameters from the spider's major and minor ampullate glands (Foelix, 1996). These scaffolding threads support the sticky spiral, which spirals inward from the frame towards the hub and usually consists of hygrophilic, gluey aggregate silk surrounding a pair of flagelliform core fibres (Peters, 1955). During web construction most orb spiders build a transient non-sticky spiral (also referred to as an auxiliary or temporary spiral) that strengthens the radii and frame structure while the sticky spiral is being built (Zschokke and Vollrath, 1995). As the spider builds the sticky spiral it simultaneously removes the non-sticky spiral, which has traditionally been thought to consist of a pair of minor ampullate silk fibres (Andersen, 1970). However, experimental evidence of this claim is currently lacking, but could be tested by experiments that include chromatography-mass spectroscopy comparisons of non-sticky spiral silk with reeled minor and major ampullate silk or freezing of the spider while it is spinning the non-sticky spiral to locate the spigot of origin.

Research on silk has traditionally been done with two separate aims in mind. Engineers and biomaterial scientists have predominantly focused on the chemical and mechanical properties of silk that has been forcibly reeled directly from the major ampullate glands of the spiders in an attempt to reproduce its unique properties in artificial silk (Eisoldt et al., 2011; Vollrath et al., 2011), while biologists have focused on the web itself as a means to study optimal foraging, predation and behavioural flexibility in trap-building animals (Scharf et al., 2011). However, an integrative approach is necessary to understand how silk properties, web structure and spider behaviour have co-evolved (Harmer et al., 2011; Vollrath and Selden, 2007; Lin, 1997). Examples of such studies include those on silk taken directly from the web that show how the silk is fine-tuned to the size and structure of the web and the resident spider as well as to the web's main function of dissipating energy from impacting prey (Kelly et al., 2011; Sensenig et al., 2010; Sensenig et al., 2011; Lin et al., 1995).

Orb spiders in the genus *Nephila* are particularly suitable for the study of function of *in situ* silks, as *Nephila* major ampullate silk is one of the most studied types of silk (Gosline et al., 1999; Vollrath et al., 2001) and *Nephila* species build webs that, in adult females, can be exceptionally large and asymmetrical, while still being very densely meshed (Hesselberg, 2010; Robinson and Mirick, 1971). Importantly, all *Nephila* webs retain the non-sticky spiral, which in most other spider families is typically removed by the spider when it lays down the sticky capture spiral (Kuntner and Agnarsson, 2009; Kuntner et al., 2008; Robinson and Mirick, 1971). Yet, like the temporary non-sticky spiral in other orb webs, the permanent non-sticky spiral in *Nephila* webs is fully homologous in origin, i.e. it is an arithmetic (logarithmic) spiral with an increasing spacing of the spiral turns with distance from the hub, in contrast to the geometric (constant spiral turn spacing) spiral found in the typical sticky capture spiral (Vollrath and Mohren, 1985). However, the permanent non-sticky spiral in *Nephila* differs from other non-sticky

spirals in that its inter-radial segments do not meet at a single point (Eberhard, 1988; Zschokke, 1993). Instead, the *Nephila* non-sticky spiral and radius junctions are elongated along the radius, which, presumably as a result of high pre-stresses, results in a zigzag pattern of the non-sticky spiral (Kullmann and Stern, 1973) (this study). The non-sticky spiral, though, is continuous in each spiral turn and scanning electron microscopy (SEM) studies show how the non-sticky spiral wraps around the radius at the junctions (Kullmann and Stern, 1973) (Fig. 2B this study).

It is not totally clear why *Nephila* spiders retain the non-sticky spiral in their webs. Landolfa and Barth studied the importance of the non-sticky spiral in *Nephila clavipes* webs in relation to vibratory signals similar to those being generated by struggling prey and found that the presence of the non-sticky spiral degrades the directional transmission of the signals from the prey (Landolfa and Barth, 1996). To offset this negative impact on the fitness of the spider, the authors hypothesised that the non-sticky spiral must have a positive function by strengthening the web (Landolfa and Barth, 1996). However, this hypothesis has not yet been investigated experimentally. One way to unravel the functional significance of the different structures that make up the orb web is to simulate the behaviour of the web using numerical engineering methods such as finite element (FE) analysis (Harmer et al., 2011). Although the use of FE analysis in biology is still in its relative infancy, an important set of studies has demonstrated the role of aerodynamic damping in dissipating prey impact energy (Lin, 1997; Lin et al., 1995), the importance of silk strength and toughness during prey impacts (Ko and Jovicic, 2004), the web's damage tolerance (Alam and Jenkins, 2005; Alam et al., 2007) and the importance of the non-linear properties of the silk for the robustness of the web (Cranford et al., 2012). To improve the accuracy of FE models it is important not only to use realistic web geometry and experimentally obtained silk material properties but also to incorporate the pre-tensile forces of the different silk structures (Lin, 1997). However, apart from the seminal study by Wirth and Barth on the forces in the webs of four species of orb spiders (Wirth and Barth, 1992), there is little information available on pre-tensile forces in completed webs, probably because they are difficult to measure accurately.

In the present study we investigated the shape and geometry of the non-sticky spiral in *N. edulis* webs in detail while comparing its mechanical properties with those of the other structural silk types in the web. Finally, we used the silk properties measured as well as the detailed shape of the zigzag pattern of the non-sticky spiral in a FE analysis to estimate the level of pre-stress that the spider applied to the non-sticky spiral during its construction.

MATERIALS AND METHODS
Spiders
Adult *N. edulis* (Labillardière 1799; family Nephilidae) females were taken from our greenhouse colony and placed individually in $40 \times 40 \times 10$ cm Perspex frames (see Zschokke and Herberstein, 2005). The spiders were allowed at least 1 week to acclimatize to laboratory conditions (20–23°C, 40–60% relative humidity RH and a 16 h:8 h light–dark cycle) during which they were inspected and sprayed with water every second day. Spiders that had built a web were fed a housefly and their webs were collapsed into a single thread for the spider to re-ingest. Only complete webs from spiders that had built at least one previous web in the frames were included in the analysis.

Structure and geometry of the non-sticky spiral
Spiders were gently removed from the webs and webs were then placed in a back-lit black box and photographed. The resulting digital web images were analysed with ImageJ (v. 1.42, National Institutes of Health 2009) to extract the following parameters (see Fig. 1). (1) Web area, the area enclosed by the outermost sticky spiral. (2) The number of radii in the web. (3) The number of sticky spiral turns in the web. (4) The number of non-sticky spiral turns in the web. (5) The ratio between the number of sticky and non-sticky spiral turns. (6) The mesh size or the distance between successive sticky spiral turns, measured along a radius in the eastern, southern and western region of the web as the length from the innermost turn to the outermost turn divided by the number of turns minus one.

For each web, we selected the region spanned by the eight southern-most radii and measured the following parameters from the seven enclosed segments of each of the non-sticky spiral turns. Segments where the non-sticky spiral was missing or radically changed direction were ignored. (1) The height–width ratio of the non-sticky spiral zigzag pattern (h/w). (2) The zigzag index of the non-sticky spiral, measured as the sum of the two angles the non-sticky spiral makes with its junction at the radius divided by 180 deg $[(\theta_1 + \theta_2)/180]$.

SEM images were obtained by gluing selected silk threads onto superconducting tape on a SEM stub. To ensure that threads remained in their natural configuration, the ends were additionally fixed onto the tape with superglue (Loctite Ultra Gel). The stub was held up to the web with a retort stand and the threads were carefully cut free from the web with a pair of iridectomy scissors once the glue had dried. The sample was then coated with a thin layer of gold using a sputter coater (Quorum Technologies SC 7620, East Grinstead, UK) and examined under a scanning electron microscope
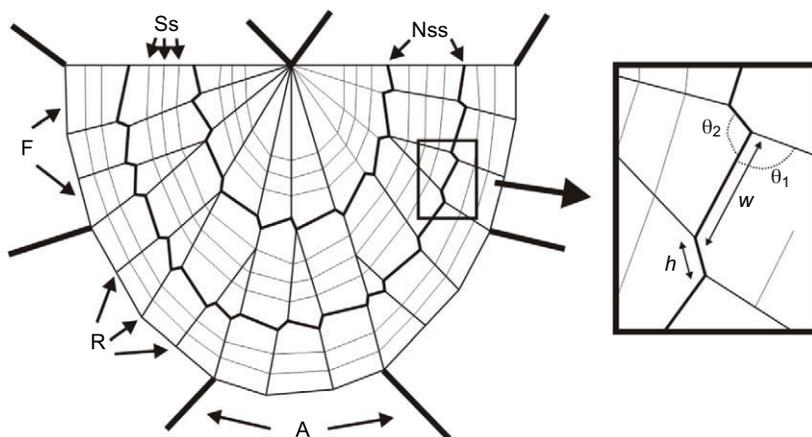

Fig. 1. Schematic drawing of a simplified *Nephila* orb web showing anchor threads (A), frame threads (F), radii (R), sticky spiral threads (Ss) and non-sticky spiral threads (Nss). The inset shows the height (length of non-sticky spiral and radius junction, *h*) and width (*w*) of the non-sticky spiral zigzag pattern as well as the angle between the non-sticky spiral and the radius (θ_1) and the angle between the non-sticky spiral and the junction (θ_2).
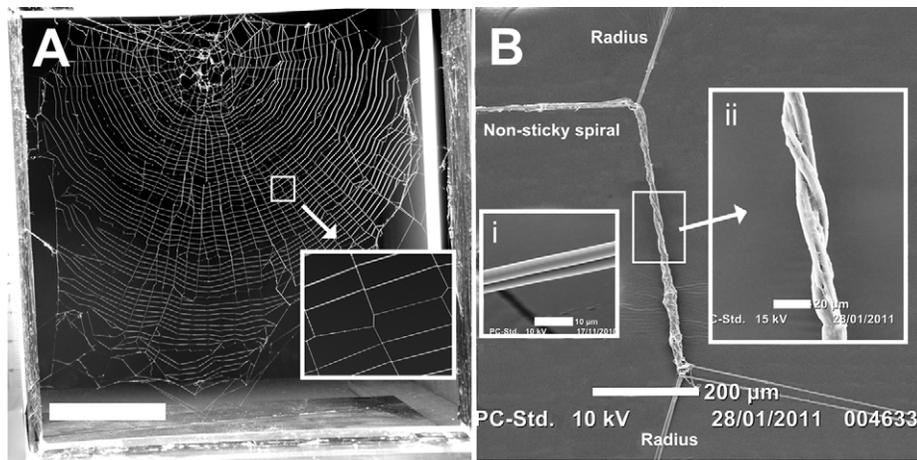
Fig. 2. The structure of the non-sticky spiral. (A) A photo of a *Nephila edulis* web that shows the presence of the non-sticky spiral in the gaps of the sticky spiral. The inset shows a close-up of the non-sticky spiral, revealing its characteristic zigzag pattern. Scale bar, 10 cm. (B) A scanning electron microscopy (SEM) image of the junction between the radius (running vertically) and the non-sticky spiral (running horizontally). Scale bar, 200 μm. The background in the main image was cleaned up with the clone tool in Corel PHOTO-PAINT (v. X4, Corel Corporation 2008). Inset i shows a close-up of the non-sticky spiral from further to the left of the image. Scale bar, 10 μm. Inset ii is a close-up of the junction, which shows how the non-sticky spiral wraps around the radius. Scale bar, 20 μm.

(Jeol Neoscope JCM-5000, Welwyn Garden City, UK) at 10 or 15 kV.

Comparison of the material properties of the non-sticky spiral silk with other silk types in the *Nephila* web

Silk samples were taken from the outer region of 13 normal-looking adult webs (i.e. clearly structured orb webs looking similar to the one in Fig. 2A). Individual silk threads were sampled by gluing them with superglue (Loctite Ultra Gel) to cardboard frames. Once the glue had dried, the threads were carefully cut free from the web with a pair of iridectomy scissors so that in-web tensions were maintained in the sample. Five samples were taken from each of the webs (one 10 mm thread from each of the following structures: the non-sticky spiral, the sticky spiral, the radius, the frame and the anchor threads), although it did not always prove possible to get good samples of each of the structures because of damage to the web from cutting out previous samples. The samples were transferred to the experimental laboratory (20–25°C, 40–70% RH) where the number and diameter of individual fibres in the threads were measured under a polarising microscope, a method that is as precise as SEM for measuring silk fibre diameters and has the further advantage that it does not damage the fibres (Blackledge et al., 2005). The samples were tested in a tensile tester (Instron 5542, Norwood, MA, USA) with a speed of 5 mm min^{-1}. Initially, we attempted to get material properties from the sticky silk samples, but the sensitivity of our load cell was not good enough to obtain reliable data, so only the structural silk data were analysed further. Engineering stress–strain curves were generated from the raw data by inserting the number and cross-sectional area of the individual silk fibres into an Excel macro, made by Tom Gheysens from our group, from which we found the stiffness (Young's modulus), tensile strength and extensibility of the silk threads.

FE analysis of pre-stresses in the non-sticky spiral

We used a digitised cut-out section comprising seven non-sticky sectors from one of the outer spiral turns from a real *Nephila* web to ensure that the numeric simulation was as similar as possible to the physical measurements from real webs. We constructed two models by re-drawing the length of the junction between the radius and the non-sticky spiral to match a low (0.1) and a high (0.2) zigzag width–height ratio based on the result of the geometry data from the real webs (Fig. 3). The cut-out sections were modelled with the FE software ABAQUS (v. 6.8-4, 2008, Dassault Systèmes S.A., Vélizy-Villacoublay, France) as 3D deformable wires and meshed with quadratic hybrid truss elements (T3D3H), generating a total of 3025 elements in each model. A non-linear general static step (allowing non-linear geometry, i.e. Nlgeom was turned on) was generated to calculate the deformations caused by the pre-stresses. All thread ends were fixed with the encastre boundary condition.

The material properties of the three silk types were incorporated into ABAQUS using smoothed [the average method with span 10 using the smooth() function in Matlab] representative (engineering) stress–strain data from a real spider (Table 1, Fig. 4) as uniaxial test data in a hyperelastic material model fitted with a second order polynomial strain energy potential. For the sticky spiral, we used a typical stress–strain curve (stiffness: 0.8 MPa, tensile strength: 98 Mpa, extensibility: 800%) from an adult female *N. clavipes* kindly supplied to us by Ken Savage from the University of British Columbia. The material properties of structural silk do not appear to differ much between *N. clavipes* and *N. edulis*, so a large
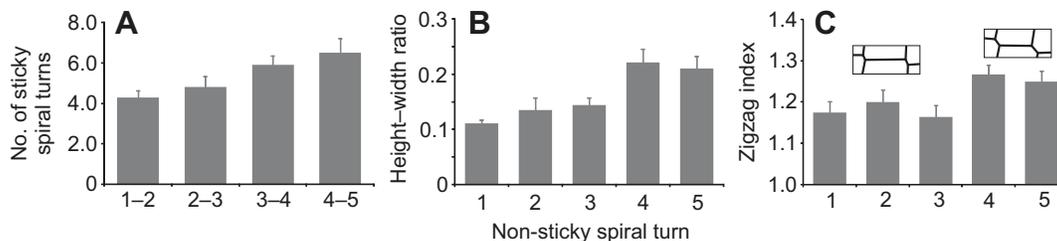

Fig. 3. The geometry of the non-sticky spiral in *N. edulis* webs. (A) The number of sticky spirals between successive turns of the non-sticky spiral (*N*=10 webs). (B) The ratio of the height of the zigzag pattern to its width along non-sticky spiral turns (turn 1: *N*=59, turn 2: *N*=63, turn 3: *N*=62, turn 4: *N*=71, turn 5: *N*=72 sectors from 11 webs). (C) The zigzag index (see Materials and methods) along non-sticky spiral turns (turn 1: *N*=59, turn 2: *N*=63, turn 3: *N*=62, turn 4: *N*=71, turn 5: *N*=72 sectors from 11 webs). Non-sticky spiral turn 1 is the first turn from the hub that is fully within the sticky spiral. The error bars represent s.e.m. The insets in C show a digitised inner (left) and outer (right) turn segment.

Table 1. Morphology of silk threads used in the finite element model

	Radial silk	Sticky spiral silk	Non-sticky spiral silk
Spider mass (mg)	595	595	595
Fibre diameter (μm)	3.2	2.3	2.2
No. of fibres in thread	2	2	2
Total cross-sectional area (mm^2)	1.6×10^{-5}	8.4×10^{-6}	7.6×10^{-6}
Force (N)	8.0×10^{-4}	2.0×10^{-5}	3.8×10^{-4} to 1.5×10^{-2}
Pre-stress (MPa)	50	2	50–2000

difference in the sticky silk between the two species is unlikely (Gosline et al., 1999; Madsen et al., 1999).

The material properties were assigned to the geometry by splitting the web into three ABAQUS sets (radii, sticky and non-sticky spiral). The pre-stress level in the radial threads was found by first estimating a pretensile force of 800 μN using the published ratio of radial force to spider mass for *N. clavipes* ($1.35 \mu N mg^{-1}$) (Wirth and Barth, 1992) and then dividing this by the total cross-sectional area of the two fibres in the thread, which gives a pre-stress value of 50 MPa (Table 1). The pretensile force in the sticky spiral of *Araneus diadematus* webs is about 10 times smaller than that in the radii (Wirth and Barth, 1992) and the stiffness of *A. diadematus* sticky silk is about 4 times higher than that *N. clavipes* sticky silk (Gosline et al., 1999) (K. Savage, personal communication), which suggests that the tensile force in the *Nephila* sticky spiral is 40 times lower than that in the radial threads, which gives a pretensile force of 20 μN and a pre-stress value of approximately 2 MPa (Table 1). To find the non-sticky spiral pre-stress value that gives the best fit to the measured zigzag index, we tested a range of pre-stress levels from 1 to 40 times the radial pre-stress value (Table 1). The values of the applied model pre-stresses were entered into ABAQUS as a predefined initial condition (*PRESTRESS) using the keyboard editor.

It is important here to emphasise that the pre-stress values we input as initial conditions to ABAQUS (hereafter referred to as applied model pre-stress) are a measure of the energy required to deform the original model (Fig. 5A) into the deformed model with the zigzag non-sticky spiral (Fig. 5B). This allows our FE model to generate zigzag patterns similar to the ones observed in real *Nephila* webs. However, as discussed in more detail in the Discussion, the real spider adds pre-stresses to the non-sticky spiral segment by segment as it lays it down and our applied model pre-stresses are therefore not similar to the pre-stresses applied by the spider during construction.

These are instead more likely to be similar to, or slightly higher than, the final pre-stresses calculated by our FE model.

Statistics

The height–width ratio and the zigzag index of the first five (i.e. inner) turns of the non-sticky spiral were analysed with a repeated measures (RM)-ANOVA with spiral turn as a within-subject factor. Assumptions of equal variance and sphericity where met in both tests. Webs with fewer than five turns of the non-sticky spiral were excluded from the analysis. The material properties were compared across all structural silk types with a RM-ANOVA. To ensure normal distribution and equal variance the fibre diameter and tensile strength data were log transformed and as the assumption of sphericity was not met for stiffness, the Greenhouse–Geisser correction was applied. We furthermore compared the properties of non-sticky spiral and radial silk with a paired Student's *t*-test from all 13 webs.

A significance level of 0.05 was used for all tests. Tests were carried out in PASW (v. 18. SPSS Inc. 2009).

RESULTS
Structure and geometry of the non-sticky spiral

The turns of the non-sticky spiral were clearly visible as distinct gaps between the sticky spiral turns in all 15 *N. edulis* webs recorded (Fig. 2A). The number of non-sticky spiral turns ranged between 4 and 8, averaging 4.5 (Table 2). There was a mean of 6.0 sticky spiral turns between each non-sticky spiral turn (Table 2), but outer non-sticky spiral turns had more sticky spiral turns between them than inner turns (Fig. 3A, RM-ANOVA: $F_{3,27}=11.4$, $P<0.001$). The non-sticky spiral was recognisable by its zigzag shape, where the length of the junction between the radius and the non-sticky spiral made up the height of the zigzag pattern, while the inter-radial length made up the width (Fig. 1, Fig. 2A). The junction consisted of the non-sticky
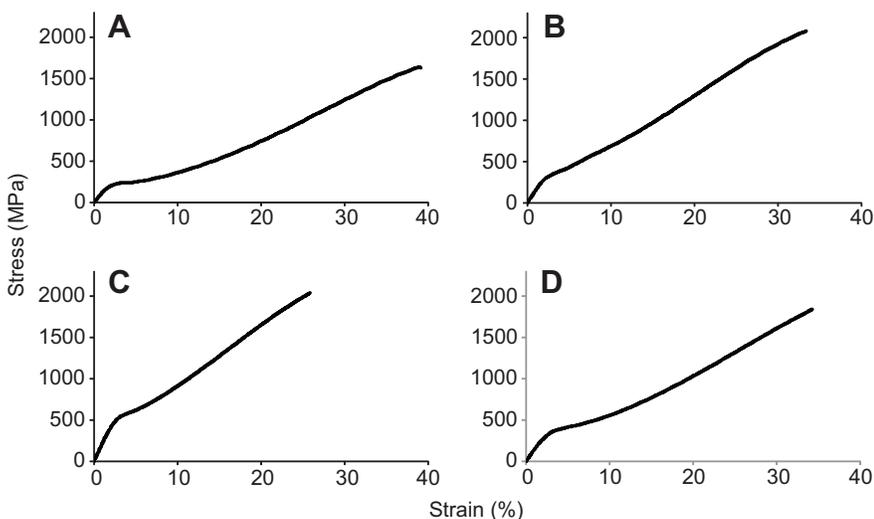

Fig. 4. Stress–strain curves of the different structural silk types from a *N. edulis* web (spider weighing 595 mg). The collected data have been smoothed using the moving average with *N*=30 for A–D and *N*=50 for E. (A) Non-sticky spiral silk. (B) Radial silk. (C) Frame silk. (D) Anchor silk.
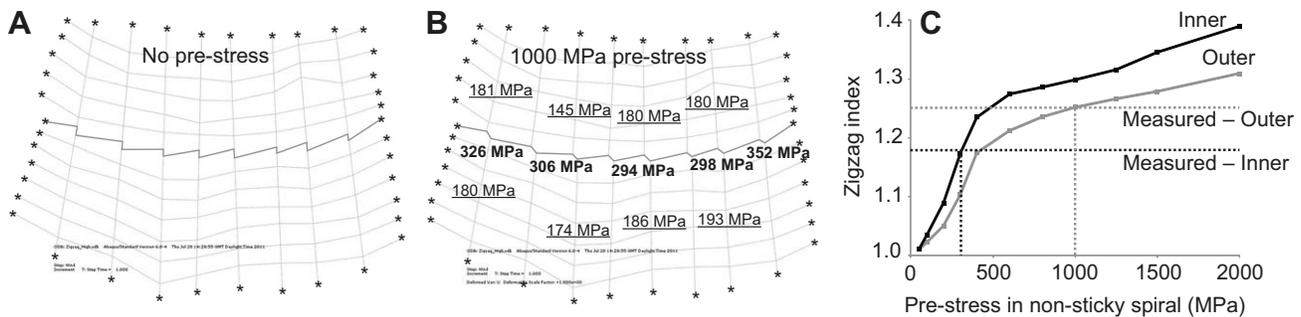
Fig. 5. Results of finite element analysis of the zigzag pattern of the non-sticky spiral in *N. edulis* webs. (A) The section from a real *N. edulis* web that was used in the simulation, before any pre-stresses were added. The non-sticky spiral (thick grey lines) had a height–width ratio of 0.2 (outer turn). Asterisks indicate where silk threads were fixed. (B) The same section after a 1000 MPa pre-stress was applied to the non-sticky spiral (50 MPa applied to radial threads and 2 MPa to the sticky spiral). The underscored values give the maximum principal stresses at selected points on the radii and the bold values give the maximum principal stresses along the length of the non-sticky spiral. (C) The relationship between zigzag index and pre-stress in the non-sticky spiral. The black curve represents the inner non-sticky spiral turns (height–width ratio of 0.1), while the grey curve represents the outer turns (height–width ratio of 0.2). The black horizontal line shows the measured zigzag index of the inner non-sticky spiral turns (~1.18, Fig. 3C), while the grey shows the outer non-sticky spiral turns (~1.26, Fig. 3C).

spiral fibres wrapping around the radius, which, together with smaller gluey silk threads, probably ensures that there is no slip between the non-sticky spiral and the radius (Fig. 2B) (Kullmann and Stern, 1973). The shape of the zigzag pattern changed from the innermost to the outermost turn of the non-sticky spiral with a height–width ratio of around 0.1 in the former and 0.2 in the latter (Fig. 3B, RM-ANOVA: $F_{4,40}$=9.6, P<0.001). Similarly, the outer non-sticky spiral turns showed a more pronounced zigzag pattern with a zigzag index of about 1.26, compared with the inner turns with a zigzag index of about 1.18 (Fig. 3C, RM-ANOVA: $F_{4,40}$=5.9, P=0.001).

Comparisons of the material properties of the non-sticky spiral silk with other silk types in the *Nephila* web

The stress–strain curves obtained from the uni-axial tensile tests were highly non-linear with a high initial stiffness (Young's modulus) followed by a lower post-modulus (Fig. 4). No differences in material properties between the silk types were observed, although the fibre diameter was significantly different, with the lowest diameter found in the non-sticky spiral and the highest in the anchor threads (Table 3). In addition, we compared pair-wise the material properties of the non-sticky spiral and the radial threads, as we wished to determine whether the non-sticky spiral was made of a different silk (i.e. minor ampullate silk) from the other structural silks and as only the full sample size was available for these two types. However, similar to the full comparison, this revealed that the fibre diameter of the non-sticky spiral was significantly smaller than the diameter of radial fibres (paired *t*-test: *t*=–5.2, d.f.=12, *P*<0.001), while no significant differences were found for stiffness (paired *t*-test: *t*=–2.0, d.f.=12, *P*=0.07), tensile strength (paired *t*-test: *t*=–2.2, d.f.=12, *P*=0.06) or extensibility (paired *t*-test: *t*=1.0, d.f.=12, *P*=0.34).

FE analysis of pre-stresses

We used the FE software ABAQUS to estimate the pre-stress in the non-sticky spiral based on the observed zigzag index (Fig. 3C) and given the pre-stress values in radial and sticky spiral threads from the literature (Wirth and Barth, 1992) (Table 1). As the height–width ratio differed between non-sticky spiral turns near the hub and turns near the frame, we used two different geometry models for the FE analysis; one simulating the inner turns with a height–width ratio of 0.1 and one simulating the outer turns with a height–width ratio of 0.2.

From our FE analysis, we found that the applied pre-stresses in the non-sticky spiral varied between 300 MPa in the inner turns and 1000 MPa in the outer turns (Fig. 5C). To estimate the sensitivity of the estimated range to the pre-stress values from the literature, we repeated the simulations both with half pre-stress values in the radial (25 MPa) and sticky spiral threads (1 MPa) and with twice as high pre-stress values (radial threads: 100 MPa, sticky spiral threads: 4 MPa). This gave estimated ranges of 300–900 MPa for half and 400–1250 MPa for twice the normal pre-stresses in radial and sticky spiral threads (data not shown).

Higher pre-stresses in the non-sticky spiral resulted in a contraction of the width of the non-sticky spiral (Fig. 5B), causing the height–width ratio to increase with an increase in pre-stress up to 0.13 for the inner and up to 0.23 for the outer non-sticky spiral turns at a pre-stress level of 2000 MPa. However, both of these values were within the height–width ratios observed in real webs (Fig. 3B), so should not significantly influence the estimated ranges of pre-stress reported above.

In the previous paragraphs, we focused on the pre-stresses in the non-sticky spiral that were applied to the non-deformed geometry (Fig. 5A). However, after reorganisation into the zigzag geometry (Fig. 5B), the pre-stresses in the model changed as the high stress applied to the non-sticky spiral distributed itself into the radii. The final stress in the model for the outer non-sticky spiral turns (with an applied model pre-stress of 1000 MPa) was in the range 294–326 MPa (excluding the stress near the two fixed end points) for the non-sticky spiral and 145–193 MPa for the radii (Fig. 5B). For the inner non-sticky spiral turns (with an applied model pre-stress of 300 MPa) the stresses were 78–85 MPa for the non-sticky spiral and 71–85 MPa for the radii (data not shown).

Table 2. Web and non-sticky spiral geometry of *Nephila edulis*

	Mean ± s.e.m. (*N*)
Mass (mg)	677±89 (15)
Web area (cm²)	634±30 (15)
No. of radii	51.9±2.5 (15)
No. of sticky spiral turns	26.3±0.7 (15)
No. of non-sticky spiral turns	4.5±0.2 (15)
Sticky/non-sticky spiral ratio	6.0±0.2 (15)
Mesh size (cm)	0.49±0.01 (15)

Table 3. Mechanical properties of different structural silks in *Nephila edulis* webs

	Non-sticky spiral	Radius	Frame	Anchor	Statistics*	P	Sticky spiral
Sample size	13	13	11	9			7
Spider mass (mg)	882±99	882±99	820±80	803±91			931±159
No. of fibres	2	2	2–8	2–8			2
Fibre diameter (μm)	2.6±0.1	3.2±0.2	3.8±0.3	4.0±0.3	$F_{3,24}$=14.1	<0.001	2.8±0.3
Stiffness (MPa)	12,900±1300	14,800±900	17,300±1700	16,000±2000	$F_{2.0,15.8}$=1.3	0.291	–
Tensile strength (MPa)	1600±120	1770±110	1560±180	2030±340	$F_{3,24}$=0.7	0.580	–
Extensibility (%)	37.9±2.6	35.1±2.5	32.5±4.5	33.4±2.5	$F_{3,24}$=0.6	0.648	–

Data are means ± s.e.m.
*Repeated measures ANOVA.

DISCUSSION

The non-sticky spiral in webs of the golden orb spider *Nephila* spp. differs from non-sticky spirals in webs of other orb spider families in three major ways (Kullmann and Stern, 1973). Firstly, the non-sticky spiral is retained in the finished orb webs, where it separates groups of *ca*. 6 spiral turns of the sticky spiral (Fig. 2A, Table 2). Secondly, the non-sticky spiral has a clear zigzag shape (Fig. 2A). Thirdly, the junction of the non-sticky spiral and the radius is elongated and appears not to allow slip, as the non-sticky spiral wraps around the radius in the elongated junction (Fig. 2B). However, while the non-sticky spiral in other orb spiders is thought to consist of different silk (minor ampullate) with different mechanical properties compared with the other scaffolding structures (major ampullate) in the web (Andersen, 1970), no such differences were found for the *Nephila* non-sticky spiral in this study (Table 3, Fig. 4). The non-sticky spiral did not differ in stiffness, tensile strength or extensibility compared with radial, frame and anchor threads, although it did have a significantly smaller fibre diameter (Table 3, Fig. 4). We estimated from a FE model that in order to achieve the observed zigzag pattern, an applied model pre-stress level up to 20 times higher than those found in the radial threads would have to be applied (Fig. 5), although the final pre-stresses in the model were similar for the non-sticky spiral and radii in the innermost non-sticky spiral turns and 1.5–2 times higher in the outer non-sticky spirals than in the radii. In the following sections, we discuss the geometry, material properties and pre-stress of the *Nephila* non-sticky spiral in greater detail.

Structure and geometry of the non-sticky spiral

The permanent non-sticky spiral turns were clearly visible in *N. edulis* orb webs in the gaps of the sticky spiral (Fig. 2A). The presence of the non-sticky spiral in the completed web is a unique basal trait in the family Nephilidae (Kuntner and Agnarsson, 2009; Kuntner et al., 2008). In all other orb spider families, except anapids where the non-sticky spiral is not constructed at all (Eberhard, 1987), the non-sticky spiral is removed during sticky spiral construction, where it serves as a scaffold and a guideline for the placement of the sticky spiral turns (Peters, 1937; Zschokke, 1993; Eberhard and Hesselberg, 2012). In laboratory webs, *N. edulis* built non-sticky spirals with 4–5 turns and an average of 6 sticky spiral turns per non-sticky spiral turn (Table 2), although this varied with distance from the hub so that outer non-sticky spiral turns had significantly more sticky spiral turns between them than inner turns (Fig. 3A). Thus, the non-sticky spiral in *N. edulis* can be classified as an arithmetic spiral similar to the non-sticky spiral in other orb spiders (Vollrath and Mohren, 1985). However, whereas the junction between the radius and the non-sticky spiral in other orb spiders consists of a single point (Eberhard, 1988; Peters, 1937), the junction in *N. edulis* is elongated, with the non-sticky spiral wrapping around the radius (Fig. 2B), probably to avoid the slippage and sliding seen in other types of junctions (Eberhard, 1976). How this wrapping is achieved by the spider is not currently known. It is possible that the spinnerets generate it by rubbing the two silk threads together, but detailed high-speed photography of a building spider is needed to answer this question. The elongated junction together with the high pre-stresses in the non-sticky spiral give rise to a characteristic zigzag pattern that becomes more pronounced with each spiral turn away from the hub (Figs 2, 3, 5). The geometry of the non-sticky spiral webs of the Australian *N. edulis* reported here is comparable to the structure and geometry of the closely related neotropical *N. clavipes* (Kullmann and Stern, 1973). Similarly, zigzag patterns in the non-sticky spiral can be seen in the genus *Nephilengys*, but not in *Herennia* and *Clitaetra* (T.H., personal observation; M. Kuntner, personal communication), which suggests that this trait is only found in the nephilid species that build large and dense aerial webs.

Comparisons of the material properties of the non-sticky spiral silk with other silk types in the *Nephila* web

The function of the sticky spiral is to retain prey long enough for the spider to reach it, which it achieves by using gluey substances and being very elastic, making it difficult for prey to push against it, whereas the function of the radii, frame and anchor threads, and presumably the permanent non-sticky spiral, is to ensure that the web does not fail during prey impact and during wind exposure (Agnarsson and Blackledge, 2009; Denny, 1976). The material properties of the silk reflect these different functions in that the scaffolding silk has high stiffness and tensile strength but a modest extensibility, whereas capture spiral silk has very high extensibility but relatively low stiffness and tensile strength (Blackledge and Hayashi, 2006; Denny, 1976).

The difference between sticky and scaffolding silk arises because the silk is drawn from different silk glands. The scaffolding silk consists of a varying number of major ampullate fibres, whereas sticky silk consists of a pair of flagelliform core fibres enclosed within gluey aggregate silk (Foelix, 1996). Non-sticky silk is thought to arise from a different gland, the minor ampullate gland, from the other scaffolding silks (Andersen, 1970), although experimental evidence to support this claim is currently lacking. Minor ampullate silk has, as the name implies, smaller diameter fibres, a slightly higher stiffness and extensibility, and a lower tensile strength than major ampullate silk (Blackledge and Hayashi, 2006). However, except for a smaller diameter, our study found no differences in mechanical properties between silk from the non-sticky spiral and silk from the other scaffolding structures (Fig. 4, Table 3). Our results can be interpreted in four different ways. (i) The non-sticky spiral is made of minor ampullate silk which, in *N. edulis*, differs only slightly in terms of mechanical properties from major ampullate silk. The difference in fibre diameter between non-sticky spiral and the other scaffolding silks could indicate that the

former consists of minor ampullate silk (Table 3). However, spiders are known to be able to use drawing speed to regulate the diameter of the fibres they extrude from their glands (Vollrath et al., 2001) and in our study there is a smaller difference in the diameter of the fibres between the non-sticky spiral and the radii than between the radii and the anchor threads (Table 3). In one study on reeled minor and major ampullate silk in *N. clavipes*, the elasticity was not found to differ, but major differences were found in tensile strength with up to four times higher strength in major ampullate silk (Stauffer et al., 1994). However, the methodology of this study has later been heavily criticised and indeed their estimate of the tensile strength of major ampullate silk is more than twice as high as reported in any other studies (Blackledge and Hayashi, 2006). (ii) All scaffolding threads arise from the same gland and consist of either minor or major ampullate silk. Orb spider dragline and frame threads are known to consist of major ampullate silk (Blackledge and Hayashi, 2006; Foelix, 1996; Gosline et al., 1999), so it is highly unlikely that all scaffolding *N. edulis* silk is minor ampullate silk. However, if all silk is major ampullate silk, it would mean that the spider is able to vary the diameter of major ampullate silk from 2.6 μm in the non-sticky spiral to 4.0 μm in the anchor threads (Table 3), which is higher than the maximum variation found in *N. edulis* silk reeled at speeds varying from 0.1 to 100 mm s^{-1} (Vollrath et al., 2001). (iii) Scaffolding threads consist of a combination of minor and major ampullate silk fibres. There are some indications that dragline, bridgeline and scaffolding threads occasionally can consist of both minor and major ampullate silk (Peters, 1990; Peters, 1993). In addition, a study on the mechanical properties of reeled *N. edulis* major ampullate silk (although with spiders of half the mass of our study) found an extensibility of around 40%, a stiffness of 7000 MPa and a tensile strength of 1000 MPa (Madsen et al., 1999), where we found significantly higher stiffness and tensile strength (Table 3), which could perhaps suggest a combination of different silks as minor ampullate silk in *Nephila* may have a higher stiffness and tensile strength (Stauffer et al., 1994; Vollrath and Porter, 2006). However, we did not observe any non-sticky spiral or radial threads that consisted of more than two fibres or had fibres that differed widely in diameter, although this could have occurred in four- and eight-stranded frame and anchor threads where it was not always possible to view all fibres or compare their diameters directly. (iv) Both the non-sticky spiral and the radial threads are made of minor ampullate silk. We found no difference in stiffness, extensibility and strength between the non-sticky spiral and the radial threads, but perhaps this is because both consist of minor ampullate silk. The three-dimensional *Cyrtophora* (family Araneidae) webs have no sticky spiral, but have instead an interwoven sheet of radii and spiral threads in a zigzag pattern very similar to the one found in the *Nephila* non-sticky spiral, including even helically shaped junctions (Peters, 1993) (Fig. 2). Some of the radial and spiral threads in *Cyrtophora* webs were found to originate from the minor ampullate gland (Peters, 1993). Something similar could be occurring in *Nephila* webs, although *Cyrtophora* is not a close relative. Even though we found no significant differences in mechanical properties between different structures, the non-sticky spiral and radial threads had lower diameter fibres, slightly lower stiffness and slightly higher extensibility than the frame and anchor threads, which could perhaps indicate material differences (Table 3). It is also possible that primary radii (originating at the hub) consist of major ampullate silk and the secondary radii (originating outside the hub) consist of minor ampullate silk, especially as secondary radii are added during the construction of the non-sticky spiral in *Nephila* spiders (Zschokke and Vollrath, 1995). In the present study

we did not differentiate between primary and secondary radii, so we cannot rule out the possibility that we tested both minor and major ampullate radii. However, considering that all tested radial threads, without exception, had a larger diameter than the non-sticky spiral threads, this is perhaps less likely.

All of our reported silk mechanical properties showed relatively large variations between different webs and spiders. This is not surprising given that silk properties are known to vary between individual spiders as well as with ambient humidity and temperature (Madsen et al., 1999; Vehoff et al., 2007; Vollrath et al., 2001). However, this variation should not have an impact on the conclusions of the intra-web comparisons made here, as all silk types from each web were drawn at roughly the same time during web building in the laboratory and tested within an hour of each other, thus minimising any impact of natural fluctuations in humidity and temperature in the laboratory.

FE analysis of pre-stresses

The use of FE analysis clearly demonstrates that, as expected, the zigzag pattern in the *Nephila* non-sticky spiral arises from high pre-stresses in the non-sticky spiral, which pulls the intersection points with the radius together (Fig. 5). By comparison with the height–width ratios and the zigzag indices in real webs (Fig. 3), we estimated that applied model pre-stress values in the non-sticky spiral during construction ranged from 300 MPa in the inner turns of the non-sticky spiral to 1000 MPa in the outer turns (Fig. 5C). In the FE model used in this study, we chose an average constant pre-stress for the radial and sticky spiral threads, where in the real webs the pre-stresss in the radii vary substantially with position and distance from the hub (Wirth and Barth, 1992). However, based on our sensitivity analysis, where pre-stresses were halved and doubled without significantly affecting the required pre-stresses in the non-sticky spiral, this is unlikely to have any major influence on the results presented here. Thus, in our FE model, we found that the model pre-stress applied to the non-sticky spiral is up to 70% of its breaking stress (Table 3). However, it is important to realise that these are initial stresses applied to the non-deformed geometry. The spider does not first complete the entire web and then apply the pre-stress as we, out of necessity, have done in our FE model. Instead, the spider probably applies stress to the individual non-sticky spiral segments by tugging on the spiral thread after gluing it to the radial thread, which probably requires the application of stresses significantly below 1000 MPa and only slightly higher than the observed pre-stresses in the final web. The result of the FE analysis suggests that the stresses distribute in the finished web in such a way that the final pre-stress in the non-sticky spirals is only about 1.5–2 times higher than the pre-stress in the radii in the outer turns (~300 *versus* ~180 MPa) (Fig. 5B) and equal to the pre-stress of the radii in the inner non-sticky turns (~80 *versus* ~78 MPa), which is comparable to values measured in incomplete webs of *A. diadematus* (Wirth and Barth, 1992). As always with computer models, the reliability of the output depends on the accuracy of the input. At the highest pre-stresses, the actual pre-stress values observed along the radii in the FE model output varied between 143 and 193 MPa and were thus significantly higher than those observed in real *N. clavipes* webs (Wirth and Barth, 1992), probably because of the limited size of the cut-out section and the fixed boundary conditions at the periphery of the cut-out section (Fig. 5).

CONCLUSIONS

Our FE analysis provides estimates of the pre-stress levels found in *Nephila* webs; the accuracy of these estimates needs to be

validated by direct measurements. In addition, our combination of geometry and FE model results suggest that the zigzag index could be an easy way to estimate pre-stresses in the non-sticky spiral. Thus the zigzag index could prove useful to indicate whether pre-stresses change with geometry and silk properties in *Nephila* webs built by spiders experiencing windy conditions (Liao et al., 2009; Vollrath et al., 1997) or by spiders capturing large prey (Blamires et al., 2011; Tso et al., 2007; Nentwig, 1985).

Finally, we may speculate as to why the non-sticky spiral is retained in *Nephila* webs, when it reduces the effective capture area and the efficiency of the radii to transmit vibrations from impacting prey to the spider in the hub (Landolfa and Barth, 1996). Landolfa and Barth hypothesised that the non-sticky spiral has a mechanical role in stabilising the web (Landolfa and Barth, 1996). This, in combination with the fact that, within the family Nephilidae, the zigzag pattern is only found in the *Nephila* and *Nephilengys* genera, which both build large and dense aerial webs, suggests that the non-sticky spiral strengthens and increases the stiffness of the structural parts of the web, possibly to reduce the risk that adjacent sticky spiral turns cling together. Windy conditions would pose a particular problem in these dense and exposed webs. Results from our laboratory from wind-tunnel experiments and FE analysis confirm the 'mechanical role' hypothesis and demonstrate that both the zigzag shape and the pre-stress values play a large role in reducing the deformation of webs during wind loading (T.H. and F.V., in preparation).

REFERENCES

Agnarsson, I. and Blackledge, T. A. (2009). Can a spider web be too sticky? Tensile mechanics constrains the evolution of capture spiral stickiness in orb-weaving spiders. *J. Zool. (Lond.)* **278**, 134-140.

Agnarsson, I., Kuntner, M. and Blackledge, T. A. (2010). Bioprospecting finds the toughest biological material: extraordinary silk from a giant riverine orb spider. *PLoS ONE* **5**, e11234.

Alam, M. S. and Jenkins, C. H. (2005). Damage tolerance in naturally compliant structures. *Int. J. Damage Mech.* **14**, 365-384.

Alam, M. S., Wahab, M. A. and Jenkins, C. H. (2007). Mechanics in naturally compliant structures. *Mech. Mater.* **39**, 145-160.

Andersen, S. O. (1970). Amino acid composition of spider silk. *Comp. Biochem. Physiol.* **35**, 705-711.

Blackledge, T. A. and Hayashi, C. Y. (2006). Silken toolkits: biomechanics of silk fibers spun by the orb web spider *Argiope argentata* (Fabricius 1775). *J. Exp. Biol.* **209**, 2452-2461.

Blackledge, T. A., Cardullo, R. A. and Hayashi, C. Y. (2005). Polarized light microscopy, variability in spider silk diameters, and the mechanical characterization of spider silk. *Invertebr. Biol.* **124**, 165-173.

Blamires, S. J., Chao, Y.-C., Liao, C.-P. and Tso, I.-M. (2011). Multiple prey cues induce foraging flexibility in a trap-building predator. *Anim. Behav.* **81**, 955-961.

Cranford, S. W., Tarakanova, A., Pugno, N. M. and Buehler, M. J. (2012). Nonlinear material behaviour of spider silk yields robust webs. *Nature* **482**, 72-76.

Denny, M. (1976). The physical properties of spider's silk and their role in the design of orb-webs. *J. Exp. Biol.* **65**, 483-506.

Eberhard, W. G. (1976). Physical properties of sticky silk spirals and their connections: sliding connections in orb webs. *J. Nat. Hist.* **10**, 481-488.

Eberhard, W. G. (1987). Web-building behavior of anapid, symphytognathid and mysmenid spiders (Araneae). *J. Arachnol.* **14**, 339-356.

Eberhard, W. G. (1988). Memory of distances and directions moved as cues during temporary spiral construction in the spider *Leucauge mariana* (Araneae: Araneidae). *J. Insect Behav.* **1**, 51-66.

Eberhard, W. G. and Hesselberg, T. (2012). Cues that spiders (Araneae: Araneidae, Tetragnathidae) use to build orbs: lapses in attention to one set of cues due to dissonance with others? *Ethology* **118**, 610-620.

Eisoldt, L., Smith, A. and Scheibel, T. (2011). Decoding the secrets of spider silk. *Mater. Today* **14**, 80-86.

Foelix, R. F. (1996). *Biology of Spiders*, 2nd edn. New York: Oxford University Press.

Gosline, J. M., Guerette, P. A., Ortlepp, C. S. and Savage, K. N. (1999). The mechanical design of spider silks: from fibroin sequence to mechanical function. *J. Exp. Biol.* **202**, 3295-3303.

Harmer, A. M. T., Blackledge, T. A., Madin, J. S. and Herberstein, M. E. (2011). High-performance spider webs: integrating biomechanics, ecology and behaviour. *J. R. Soc. Interface* **8**, 457-471.

Hesselberg, T. (2010). Ontogenetic changes in web design in two orb-web spiders. *Ethology* **116**, 535-545.

Kelly, S. P., Sensenig, A., Lorentz, K. A. and Blackledge, T. A. (2011). Damping capacity is evolutionarily conserved in the radial silk of orb-weaving spiders. *Zoology* **114**, 233-238.

Ko, F. K. and Jovicic, J. (2004). Modeling of mechanical properties and structural design of spider web. *Biomacromolecules* **5**, 780-785.

Kullmann, E. and Stern, H. (1973). *Leben am Seidenen Faden – Die rätselsvolle Welt der Spinnen.* München, Germany: C. Bertelsmann Verlag.

Kuntner, M. and Agnarsson, I. (2009). Phylogeny accurately predicts behaviour in Indian Ocean *Clitaetra* spiders (Araneae: Nephilidae). *Invertebr. Syst.* **23**, 193-204.

Kuntner, M., Coddington, J. A. and Hormiga, G. (2008). Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. *Cladistics* **24**, 147-217.

Landolfa, M. A. and Barth, F. G. (1996). Vibrations in the orb web of the spider *Nephila clavipes*: cues for discrimination and orientation. *J. Comp. Physiol.* **179**, 493-508.

Liao, C.-P., Chi, K.-J. and Tso, I.-M. (2009). The effects of wind on trap structural and material properties of a sit-and-wait predator. *Behav. Ecol.* **20**, 1194-1203.

Lin, L. H. (1997). Biomechanics of spiders' silks and orb-webs. PhD Thesis, University of Oxford, Oxford, UK.

Lin, L. H., Edmonds, D. T. and Vollrath, F. (1995). Structural engineering of an orb-spider's web. *Nature* **373**, 146-148.

Madsen, B., Shao, Z. Z. and Vollrath, F. (1999). Variability in the mechanical properties of spider silks on three levels: interspecific, intraspecific and intraindividual. *Int. J. Biol. Macromol.* **24**, 301-306.

Nentwig, W. (1985). Prey analysis of four species of tropical orb-weaving spiders (Araneae: Araneidae) and a comparison with araneids of the temperate zone. *Oecologia* **66**, 580-594.

Peters, H. M. (1937). Studien am Netz der Kreuzspinne (*Aranea diademata*): II. Über die Herstellung des Rahmens, der Radialfäden und der Hilfsspirale. *Z. Morphol. Ökol. Tiere* **33**, 128-150.

Peters, H. M. (1955). Über den Spinnapparat von *Nephila madagascariensis. Z. Naturforsch. B* **10b**, 395-404.

Peters, H. M. (1990). On the structure and glandular origin of bridging lines used by spiders for moving to distant places. *Acta Zool. Fenn.* **190**, 309-314.

Peters, H. M. (1993). Functional organization of the spinning apparatus of *Cyrtophora citricola* with regard to the evolution of the web (Araneae, Araneidae). *Zoomorphology* **113**, 153-163.

Robinson, M. H. and Mirick, H. (1971). The predatory behaviour of the golden-web spider *Nephila clavipes* (Araneae: Araneidae). *Psyche (Stuttg.)* **78**, 123-139.

Scharf, I., Lubin, Y. and Ovadia, O. (2011). Foraging decisions and behavioural flexibility in trap-building predators: a review. *Biol. Rev. Camb. Philos. Soc.* **86**, 626-639.

Sensenig, A., Agnarsson, I. and Blackledge, T. A. (2010). Behavioural and biomaterial coevolution in spider orb webs. *J. Evol. Biol.* **23**, 1839-1856.

Sensenig, A. T., Agnarsson, I. and Blackledge, T. A. (2011). Adult spiders use tougher silk: ontogenetic changes in web architecture and silk biomechanics in the orb-weaver spider. *J. Zool. (Lond.)* **285**, 28-38.

Stauffer, S. L., Coguil, S. L. and Lewis, R. W. (1994). Comparison of physical properties of three silks from *Nephila clavipes* and *Araneus gemmoides. J. Arachnol.* **22**, 5-11.

Tso, I.-M., Chiang, S.-Y. and Blackledge, T. A. (2007). Does the giant wood spider *Nephila pilipes* respond to prey variation by altering web or silk properties? *Ethology* **113**, 324-333.

Vehoff, T., Glisović, A., Schollmeyer, H., Zippelius, A. and Salditt, T. (2007). Mechanical properties of spider dragline silk: humidity, hysteresis, and relaxation. *Biophys. J.* **93**, 4425-4432.

Vollrath, F. (1992). Analysis and interpretation of orb spider exploration and web-building behavior. *Adv. Stud. Behav.* **21**, 147-199.

Vollrath, F. and Mohren, W. (1985). Spiral geometry in the garden spider's orb web. *Naturwissenschaften* **72**, 666-667.

Vollrath, F. and Porter, D. (2006). Spider silk as archetypal protein elastomer. *Soft Matter* **2**, 377-385.

Vollrath, F. and Selden, P. (2007). The role of behavior in the evolution of spiders, silks and webs. *Annu. Rev. Ecol. Evol. Syst.* **38**, 819-846.

Vollrath, F., Downes, M. and Krackow, S. (1997). Design variability in web geometry of an orb-weaving spider. *Physiol. Behav.* **62**, 735-743.

Vollrath, F., Madsen, B. and Shao, Z. Z. (2001). The effect of spinning conditions on the mechanics of a spider's dragline silk. *Proc. Biol. Sci.* **268**, 2339-2346.

Vollrath, F., Porter, D. and Holland, C. (2011). There are many more lessons still to be learned from spider silks. *Soft Matter* **7**, 9595-9600.

Wirth, E. and Barth, F. G. (1992). Forces in the spider orb web. *J. Comp. Physiol. A* **171**, 359-371.

Zschokke, S. (1993). The influence of the auxiliary spiral on the capture spiral in *Araneus diadematus* Clerk (Araneidae). *Bull. Br. Arachnol. Soc.* **9**, 169-173.

Zschokke, S. and Herberstein, M. E. (2005). Laboratory methods for maintaining and studying web-building spiders. *J. Arachnol.* **33**, 205-213.

Zschokke, S. and Vollrath, F. (1995). Web construction patterns in a range of orb-weaving spiders (Araneae). *Eur. J. Entomol.* **92**, 523-541.