

Does the Giant Wood Spider *Nephila pilipes* Respond to Prey Variation by Altering Web or Silk Properties?

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Abstract

Recent studies demonstrated that orb-weaving spiders may alter web architectures, the amount of silk in webs, or the protein composition of silks in response to variation in amount or type of prey. In this study, we conducted food manipulations to examine three mechanisms by which orb-weaving spiders may adjust the performance of webs to variation in prey by altering the architectures of webs, making structural changes to the diameters of silk threads, and manipulating the material properties or amino acid composition of silk fibers. We fed *Nephila pilipes* two different types of prey, crickets or flies, and then compared orb structure and the chemical and physical properties of major ampullate (MA) silk between groups. Prey type did not affect orb structures in *N. pilipes*, except for mesh size. However, MA silk diameter and the stiffness of orbs constructed by spiders fed crickets were significantly greater than for the fly group. MA fibers forcibly silked from *N. pilipes* fed crickets was significantly thicker, but less stiff, than silk from spiders fed flies. Spiders in the cricket treatment also produced MA silk with slightly, but statistically significantly, more serine than silk from spiders in the fly treatment. Percentages of other major amino acids (proline, glycine, and glutamine) did not differ between treatments. This study demonstrated that orb-weaving spiders can simultaneously alter some structural and material properties of MA silk, as well as the physical characteristics of webs, in response to different types of prey.

Introduction

Historically, orb webs constructed by spiders in the Araneoidea have been used in a variety of studies of foraging ecology because the spinning behaviors used to construct webs are easy to quantify (Eberhard 1990; Craig 1992; Pasquet et al. 1994; Sandoval 1994; Blackledge 1998; Heiling & Herberstein 1999; Watanabe 2000; Venner et al. 2000). Orb webs function during prey capture primarily to resist and absorb the kinetic energy of flying insects and to retain prey long enough to be captured by spiders (Denny 1976; Craig 1987; Eberhard 1990). There are

a variety of different behavioral and physiological mechanisms by which spiders could potentially influence how webs capture different prey. These mechanisms include changing the architectures of webs as well as altering structural or material properties of the silk fibers used to spin those webs.

An orb web consists of a spiral of elastic sticky silk suspended upon a framework of stiff, dry radii (Fig. 1). The radii are spun from major ampullate (MA) silk and function to support the web, transmit vibrations from prey to the spider, and to absorb kinetic energy during prey capture (Denny 1976; Masters 1984; Eberhard 1986). In contrast, sticky silk

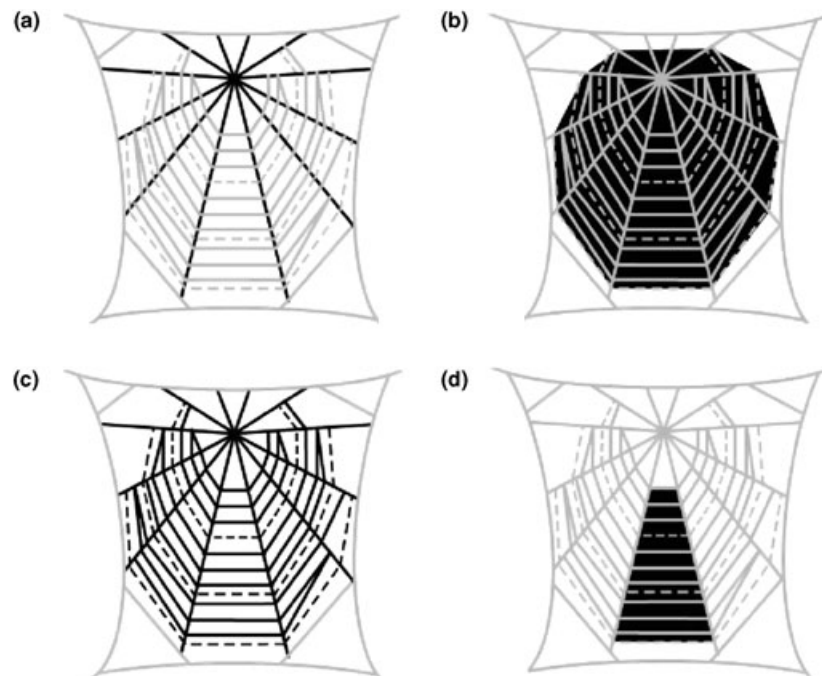


Fig. 1: Architectural features of the orb web of *Nephila pilipes*. Aspects that were studied are indicated in black. (a) The total number of supporting radii were counted. (b) Capture area was estimated as the total area of the surface delimited by the sticky capture spiral. (c) The total silk length in the web was estimated as the length of the sticky spiral, auxiliary spiral (dashed line) and the lengths of all radii. (d) Mesh size was estimated as the average space between adjacent rows of the capture and auxiliary spirals along the vertical axis of the web

functions primarily to intercept and adhere to prey (Eberhard 1990; Opell 1997). Spiders show species-specific differences in web architectures both in the length and spacing of sticky spirals and in the number of supporting radii (Eberhard 1986). Craig (1987) proposed that web architectures of different species of spiders varied functionally along a continuum from high energy-absorbing webs, characterized by tightly packed sticky spirals and many radii, to low energy-absorbing webs that have open sticky spirals suspended on fewer radii. While early studies focused primarily on interspecific variation in web shape, more recent studies have reported that individual spiders can control silk output in response to changes in foraging conditions, altering the shapes of webs (Higgins & Buskirk 1992; Sherman 1994; Blackledge 1998; Tso 1999).

The structural and material properties of the silk used to spin webs largely determine how effectively prey are stopped and retained by webs (Denny 1976; Gosline et al. 1986; Craig 1987) and there is growing evidence that spiders can actively control many aspects of silk performance (Pan et al. 2004; Guinea et al. 2005). For example, orb-weaving spiders under different prey or light environments can adjust the mechanical properties of dragline silk (Madsen et al. 1999), the low molecular weight compounds in sticky silk (Higgins et al. 2001), or even the pigments coating silk (Craig et al. 1996). Furthermore, spiders

have the capability to actively regulate the thickness of threads spun under different conditions (Madsen et al. 1999; Garrido et al. 2002). Thus, in addition to changing the architectures of webs, spiders could potentially affect the function of webs by adjusting the qualitative attributes of the silk used to spin those webs.

The mechanical performance of MA spider silk is impressive, exhibiting a capacity to absorb kinetic energy without breaking which exceeds that of most natural and man-made fibers (Vollrath 1992; Gosline et al. 1999; Blackledge et al. 2005a). MA silk has therefore been studied from a variety of perspectives including material science (Gosline et al. 1999; Vollrath 2000), silk-spinning physiology (Vollrath & Knight 2001; Ortlepp & Gosline 2004), and biotechnology (Fahnestock et al. 2000; Winkler & Kaplan 2000). However, few studies have examined the physical performance of MA silk in the context of spider foraging ecology and the function of orb webs (e.g. Craig 1987; Swanson et al. 2006).

Dragline silks primarily consist of the products of two genes: major ampullate spidroin 1 (*MaSp1*) and major ampullate spidroin 2 (*MaSp2*) (Xu & Lewis 1990; Hinman & Lewis 1992; Gatesy et al. 2001). *MaSp1* is composed largely of amino acid motifs that fold proteins into β -sheet crystalline structures (Xu & Lewis 1990), which likely contribute to the high ultimate strength and Young's modulus of the silk

(Winkler & Kaplan 2000). *MaSp2*, on the other hand, exhibits amino acid motifs that form β -spirals, which are hypothesized to increase the extensibility of the fibers (Hayashi et al. 1999). Therefore, changes in the ratios of these two proteins may alter the physical performance of MA silk. Several studies demonstrated that orb-weaving spiders can adjust the relative quantity of *MaSp1* and *MaSp2* products to different types of prey (Craig et al. 2000; Tso et al. 2005). However, because these studies did not measure the physical properties of the MA silk, it is not clear whether variation in prey types can result in changes in the physical performance of silk fibers and webs.

In this study, we investigated the ability of spiders to alter web performance at three different levels. We first tested the performance of webs spun by spiders receiving two different types of prey. We then tested three hypotheses about how spiders would alter web performance in response to variation in prey capture by: (1) altering the architectures of webs, (2) making structural changes to the diameters of silk threads, and (3) manipulating the material properties or amino acid composition of silk fibers. We provide evidence that *Nephila pilipes* generated orb webs that exhibited different physical properties in response to two different types of prey, but did so primarily through structural changes in silk threads, by adjusting the diameters of MA silk threads.

Materials and Methods

Manipulating the Prey Intake of *N. pilipes*

Female *N. pilipes* of body lengths 15–20 mm were collected from secondary forests in central Taiwan and then reared in large cages consisting of wooden frames covered by mesh screens (40 × 40 × 30 cm). The cages were placed in an outdoor screenhouse (5 × 5 × 3 m) with physical conditions similar to the spiders' natural habitat. We randomly assigned 20 caged spiders to two groups (n = 10 each), one of which was fed crickets while the other was fed an equal biomass of flies. Prior to this food manipulation, all spiders had been fed equal quantities of mealworms till they had constructed three orbs. After the pre-manipulation treatment, spiders in the first group were fed one cricket (body weight about 300 mg) each day while spiders in the second group were fed equal weights of flies (five flies of body weight about 60 mg each) until the spiders had constructed seven new webs (within a range of 2–3 wk).

Web Architecture

The mesh size and the numbers of radii in webs affect how webs can absorb the kinetic energy of different types of prey. In particular, webs with higher ratios of radii to rows of sticky spirals are hypothesized to function better at capturing high energy prey (Craig 1987). We compared the architectural characteristics of orb webs (Fig. 1) constructed by *N. pilipes* that were fed crickets vs. flies to test the prediction that spiders fed crickets would build webs with more radii and smaller mesh sizes. The orbs constructed by *N. pilipes* differ from those of most araneid and tetragnathid spiders in their extreme vertical asymmetry, where the lower halves of the webs are considerably larger than the upper halves (Zschokke 2002; Kuntner 2006). *Nephila* webs are also unusual in retaining auxiliary spirals in finished orbs and in exhibiting subsidiary radii (Zschokke 1999). In this study, we measured the total number of radii in the webs and, along each of the four cardinal directions of the orb: hub radius, orb radius and number of sticky spirals. Although webs built by *N. pilipes* exhibited subsidiary (split) radii, the number of these threads was very low in the webs examined (two to three out of more than 60 radii). Therefore, we counted the number of radii from the edge of the orb and used the total number of all radii in the analyses. Similarly, we did not differentiate auxiliary and regular sticky spiral silks and used their pooled number in the analyses because either of these threads could contribute to the stopping of flying insects. Because the orbs constructed by *N. pilipes* were not symmetrical, we used the formula provided by Herberstein & Tso (2000) to calculate catching area by:

$$\left[\frac{1}{2} \pi r_{\text{au}}^2 - \frac{1}{2} \pi (Hr_{\text{u}})^2 \right] + \left[\frac{1}{2} \pi r_{\text{al}}^2 - \frac{1}{2} \pi (Hr_{\text{l}})^2 \right],$$

where

$$r_{\text{au}} = \frac{r_{\text{u}} + (d_{\text{h}}/2)}{2}$$

and

$$r_{\text{al}} = \frac{r_{\text{l}} + (d_{\text{h}}/2)}{2}$$

Mesh size was estimated by a formula modified from Herberstein & Tso (2000):

$$\frac{r_{\text{u}} - Hr_{\text{u}} + r_{\text{l}} - Hr_{\text{l}}}{S_{\text{u}} + S_{\text{l}} - 2},$$

where r_{u} is the radius of the upper orb half, r_{l} is the radius of the lower orb half, d_{h} is the horizontal orb

diameter, H_{ru} is the radius of the upper hub half, H_{rl} is the radius of the lower hub half, S_u is the number of spirals in the upper half of the orb web and S_l is the number of spirals in the lower half of the orb web. As the orb of *Nephila* is quite asymmetric vertically, r_u and S_u tend to be very small so that this formula down weights the mesh size of the upper orb half.

Total silk length was estimated by:

$$\pi (\bar{x}_{Rweb} + \bar{x}_{Rhub}) \bar{x}_{spiral} + (\bar{x}_{Rweb} + \bar{x}_{Rhub}) \bar{x}_{radii},$$

where \bar{x}_{Rweb} is the mean orb radius, \bar{x}_{Rhub} is the mean hub radius, \bar{x}_{spiral} is the mean number of spirals (both capture and auxiliary spirals; Fig. 1d) and \bar{x}_{radii} is the mean number of radii. For each orb characteristic, we used an ANCOVA test, with carapace width as the covariable, to test for significant differences between spiders fed different prey.

Measuring the Performance of the Orb Web

After the architectures of orbs had been measured we collected the orbs from the cages to measure the structural stiffness of the webs. We defined structural stiffness as the ability of the web to resist deflection when force is applied perpendicular to its surface. While changes in web stiffness could clearly result from architectural differences between webs such as Craig (1987) described for high vs. low energy-absorbing webs, we reasoned that this was only one of the three non-mutually exclusive factors that could affect web stiffness: (1) spiders could alter the attachments of different threads to one another, (2) spiders could alter the structures of silk threads by spinning thicker or thinner fibers, (3) spiders could alter the material properties or amino acid composition of the silk itself.

We first carefully mounted the orb onto a wooden ring (30 cm in diameter) and oriented it horizontally. We then measured the force needed to lift a single radial thread to a fixed height of 3 cm, using a hook attached to a microbalance (accuracy 0.1 g). We attached the hook to a radial thread 10 cm from the hub, used the moveable arm of a dissecting microscope to raise the balance 3 cm (accuracy 1 mm), and then measured the force generated. Previous studies demonstrated that the maximum extensibility of *Nephila* MA silk was at least 20% (Vollrath 2000; Swanson et al. 2006). Therefore, the distance we extended the radius was well within the performance limits of dragline silk and did not deform other radii in the web.

We only tested radii from the lower orb half because *N. pilipes* constructs an asymmetric orb

where the radii in the upper half of orb were usually very short (Tso & Severinghaus 1998), and thus were not suitable for measuring stiffness. A total of eight radii were measured from the lower half of each orb and we computed the mean for each web to use in subsequent analyses. We used separate ANCOVA tests to compare the stiffness of the webs obtained from *N. pilipes* fed different types of prey, incorporating number of radii, silk diameter, mesh size and carapace width as separate covariates.

Fiber Structure – Diameter of Dragline Silk from the Orb

To test the hypothesis that spiders would alter the diameters of silk threads in response to different prey, we collected three radii from the left, middle and right cardinals of the lower orb half, after determining the stiffness of the orb web, and then measured the diameter of the MA silk using SEM. Wirth & Barth (1992) demonstrated that the diameter of radii collected from different regions within orb webs did not differ significantly. Therefore, in this study, we used the mean of those three samples as a representation of the whole orb. Fibers were coated with gold for 3 min under 7 mA electrical current and were then measured using a Hitachi S-2300 Scanning Electronic Microscope (Tokyo, Japan) under 15 kV voltage. Because there is a positive relationship between silk diameter and body size for *N. pilipes* (Tso & Severinghaus 1998), we performed an ANCOVA test to compare feeding treatments using carapace width of the spider as the covariant.

Material Properties of Dragline Silk

We also tested the hypotheses that differences in prey capture would alter the material properties of the MA silk spun by *N. pilipes* and the amino acid composition of the silk, as spiders manipulate the ratios of the MaSp1 and MaSp2 proteins in fibers. We used forcibly silked MA silk from *N. pilipes* to gather large enough pure samples of MA silk. Spiders were first restrained ventral side upward on a platform using non-sticky tape and insect pins. We then used a rotor powered by a motor to pull threads of MA silk directly from the MA spigot at a fixed speed of 320 cm per minute.

Throughout this process, the spider was observed under a dissecting microscope to make sure that only fibers extruded from the MA spigots were collected. The dragline of *N. pilipes* was typically composed of two threads from the MA spigots and two

additional fibers from minor ampullate spigots (Folix 1996). Therefore, to obtain pure MA silk, we affixed all but a single fiber of MA to the platform, and then collected only the single remaining silk thread from the MA gland. Initially, five samples of silk were collected across slide holders to be used in mechanical analysis. We then spooled both threads of MA gland silk for amino acid composition analyses and stored them in a $-20\text{ }^{\circ}\text{C}$ freezer until further analysis. Finally, we collected another five samples of a single MA silk thread from the spider. This allowed us to characterize the mechanical performance of samples of silk just prior to and just after the sample that was analyzed for amino acid composition to verify that the physical properties were homogenous across the sample.

Material Properties – Estimating Amino Acid Composition

Silk samples for amino acid analysis were first weighed and then submerged in hexafluoro-isopropanol ($500\text{ }\mu\text{l}$ for each mg of silk). Silk solutions were then dried and subsequently hydrolyzed at $115\text{ }^{\circ}\text{C}$ in 6N HCl for 24 h. The resulting product was transferred to a Waters PICO.TAG Amino Acid Analysis System (Boston, MA, USA) to obtain percentages of various amino acids. Because the amino acid percentages were not independent of one another, we used a multivariate analysis of variance (MANOVA) to compare the dragline amino acid percentages of *N. pilipes* collected from different treatment groups. We were primarily interested in whether or not samples varied in the ratios of amino acids such as proline and to a lesser extent serine, which will vary inversely with the ratio of MaSp1 to MaSp2 proteins within silk based upon their different cDNA sequences.

Material Properties – Characterization

Samples of silk for mechanical analysis were drawn from the same fibers that were used in the amino acid analysis, but were affixed onto 30-mm slide mounts. They were then stored for 2–3 wk before being airmailed to the USA from Taiwan. Silk samples were then transferred onto cardboard mounts and secured across 10-mm gaps using cyanoacrylate glue, as previously described (Blackledge et al. 2005a). The diameter of each fiber was measured using polarized light microscopy (Blackledge et al. 2005b). Finally, we used a Nano Bionix tensile tester (MTS Systems Corp., Oak Ridge, TN, USA) to gener-

ate load-extension data for fibers, pulling fibers at an extension rate of $1\%/sec$ (see Blackledge et al. 2005a for more details). We used the raw load-extension data to calculate true stress (σ_t), where load is normalized to the instantaneous cross-sectional area of fibers, as:

$$\sigma_t = \frac{F}{A},$$

where F is the force applied to the specimen and A is the actual cross-sectional area of the specimen calculated from the original cross-sectional area under an assumption of constant volume (Vollrath et al. 2001). True strain (ϵ_t) was calculated as:

$$\epsilon_t = \log_e \frac{L}{L_0},$$

where L is the instantaneous length of the fiber at each extension value and L_0 is the original gage length of the fiber.

We used true stress and true strain measurements to calculate six variables of interest (Fig. 2). Young's modulus measured the stiffness, or the ability of fibers to resist deformation, and was calculated as the slope of the linear region of the stress–strain curve prior to the yield point. The yield strain and yield stress measured the point at which the mechanical behavior of fibers changed from purely elastic. Extensibility was the true strain at the point of failure of the fiber. Ultimate strength was the true stress at the point of failure of the fiber. Toughness (i.e.

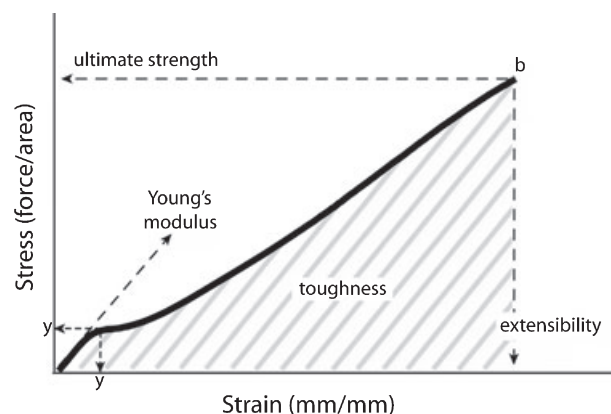


Fig. 2: Typical stress–strain curve for viscoelastic polymers, such as spider dragline silk. Young's modulus, or stiffness, is the initial elastic slope of the stress–strain curve. Fiber yield (y) occurs just after this linear elastic region and is indicated by a decrease in the slope of the stress–strain curve. Ultimate strength and extensibility are the stress and strain respectively when the fiber breaks (b). Toughness measures the energy absorbed by the fiber and is calculated from the area under the stress–strain curve

work of extension or work to fracture) provided a measure of the energy necessary to rupture a fiber of a given volume and was calculated as the area under the true stress–true strain curve. Toughness measures the energy absorbed by a given volume of fiber and is therefore unaffected by use of engineering vs. true stress–strain values.

To analyze these data we first performed paired *t*-tests to determine whether the silk diameter or mechanical properties differed between silk obtained before and after the amino acid samples. The results showed that there was no significant difference between pre- and post-amino acid analysis samples so we pooled all 10 samples and used the mean for each individual spider in the subsequent analyses. Because silk diameter, stress, strain and Young's modulus were not necessarily independent of one another, we first performed a MANOVA analysis to see whether silk mechanics differed between spiders that were fed crickets vs. flies. We then conducted post-hoc comparisons for individual properties using Tukey's HSD tests.

Results

Web Architecture and Web Performance

Data on web architecture were obtained for 18 of the 20 spiders that completed the feeding treatment (nine in each feeding treatment). Body size of spiders fed crickets did not differ significantly from those fed flies. Capture areas of webs did not differ significantly between spiders in the two prey treatments (Table 1). However, webs constructed by spiders fed flies had significantly smaller mesh sizes and tended to consist of more silk and fewer radii, although the latter two variables did not reach statistical significance. The diameters of radii spun by spiders fed crickets were significantly larger than those spun by spiders fed flies (Table 1). The stiffness of

Table 1: Mean (\pm SE) of the properties of webs collected from *Nephila pilipes* fed crickets vs. flies ($n = 9$ each). Results of ANCOVA tests are shown

Variables examined	Cricket	Fly	F	p
Carapace width (mm)	7.19 \pm 0.21	7.32 \pm 0.32	2.014	0.178
Capture area (cm ²)	1263 \pm 128	1293 \pm 104	0.081	0.778
Mesh size (cm)	0.57 \pm 0.04	0.44 \pm 0.03	6.189	0.025
Silk length (cm)	1575 \pm 191	1807 \pm 192	0.356	0.558
Number of radii	67.7 \pm 3.1	61.3 \pm 3.60	2.371	0.143
Silk diameter (μ m)	3.96 \pm 0.22	3.43 \pm 0.32	7.954	0.013
Web stiffness (g)	1.525 \pm 0.164	0.83 \pm 0.10	12.684	0.003

the webs (force needed to lift up a radius 3 cm) constructed by spiders fed crickets was 80% greater than that of webs constructed by spiders fed flies (Table 1). Although the number of radii did not individually differ between treatments, results of an ANCOVA showed that this variable was a significant covariate with feeding treatment in determining web stiffness (Table 2).

Amino Acid Composition

Amino acid composition analyses were performed on silk from eight spiders fed crickets and seven spiders fed flies. A MANOVA ($F_{5,9} = 9.515$, $p = 0.002$) test showed that feeding treatment was associated with a slight, but statistically significant, increase in serine content when *N. pilipes* were fed crickets (Table 3). While congruent with the findings of a similar study of the amino acid compositions of *N. pilipes* MA silk by Tso et al. (2005), the difference was only 0.2%. Furthermore, our study did not find significant variation in proline, as predicted if spiders were manipulating the ratios of MaSp1 and MaSp2 proteins in their silk.

Structural and Material Properties of Dragline Silk

The physical properties of forcibly drawn MA silk varied between spiders that were fed crickets vs. flies (MANOVA $F_{2,7} = 4.7$, $p < 0.01$). In particular, like silk collected directly from webs, the diameter of MA silk drawn from spiders fed crickets was significantly larger than that of spiders fed flies (Table 4). In addition, silk drawn from spiders fed flies exhibited significantly higher Young's modulus and stress at fiber yield than MA silk from spiders that were fed crickets, although variation between individual spiders was high. However, the other measures of physical performance – strain at fiber yield, ultimate strength, extensibility, and toughness – did not differ between the two groups of spiders.

Table 2: Result of ANCOVA tests comparing the stiffness of webs collected from *Nephila pilipes* fed crickets vs. flies ($n = 9$ each)

Source	Sum of squares	df	Mean square	F	p
Treatment	0.096	1	0.096	5.404	0.038
Carapace width	0.027	1	0.027	1.535	0.239
Mesh size	0.008	1	0.008	0.441	0.519
Number of radii	0.220	1	0.220	12.406	0.004
Silk diameter	0.020	1	0.020	1.146	0.181
Error	0.213	12	0.018		

Treatment	Glutamine	Serine	Proline	Glycine	Alanine
Cricket	13.4 ± 0.2	4.3 ± 0.0	10.0 ± 0.2	38.1 ± 0.2	18.1 ± 0.2
Fly	13.2 ± 0.2	4.1 ± 0.0	10.3 ± 0.2	37.6 ± 0.2	18.6 ± 0.2
F	2.014	24.632	1.768	2.832	4.283
p	0.178	0.01	0.203	0.115	0.057

Table 4: Mean (\pm SE) of the physical properties of dragline silk collected from *Nephila pilipes* fed different prey (MANOVA test, $F_{7,12} = 4.7$, $p < 0.01$). Results of post-hoc comparison using Tukey's HSD tests are shown

	Cricket	Fly	p
Silk diameter (μ m)	6.1 ± 1.1	4.6 ± 1.4	0.017
Young's modulus (GPa)	12.2 ± 2.5	14.7 ± 1.6	0.018
Stress at yield (MPa)	260 ± 52	310 ± 30	0.017
Strain at yield	0.027 ± 0.002	0.027 ± 0.005	ns
Ultimate strength (MPa)	1431 ± 100	1524 ± 187	ns
Extensibility (%)	0.26 ± 0.03	0.25 ± 0.05	ns
Toughness (MPa)	183 ± 32	190 ± 30	ns

Discussion

Our study demonstrated that orb-weaving spiders can alter the physical performance of webs in response to variation in prey, by adjusting both how silk is spun and how threads are assembled into webs. *Nephila pilipes* fed crickets produced webs with greater structural stiffness than did spiders fed flies. Spiders seemed to achieve this change primarily by altering the diameters of silk threads. Except for mesh size, most properties of webs did not differ statistically between treatments. One possible explanation is that we kept *N. pilipes* in cages that were too small to allow the spiders to fully express variation in size of orbs or length of silk in webs (see also Blackledge 1998). In contrast, properties that were not inherently constrained by the cage size, such as silk diameter, exhibited significant differences between the two feeding treatments. Despite the constraint of cage size, the number of radii in webs was a significant predictor of the overall stiffness of webs. The tendency for *N. pilipes* fed crickets to spin webs with higher numbers of radii may have acted in concert with the significantly thicker diameters of their silk threads to explain why their webs exhibited greater stiffness than when spiders were fed flies.

Previous studies showed that spiders would alter dragline silk amino acid composition when they encountered a relatively long-term change in type of insect prey (Craig et al. 2000; Tso et al. 2005). This variation in amino acid composition is predicted to

have a strong effect on the physical performance of silk, and hence webs, because of how different amino acid motifs affect the secondary and tertiary structures of proteins (Hayashi et al. 1999; Winkler & Kaplan 2000). However, in our study, we found little effect of diet on the amino acid composition of the dragline silks produced by *N. pilipes* (Table 3). Together with the results of Craig et al. (2000) and Tso et al. (2005), these results suggest that orb web spiders seem to be flexible in how it adjusts silk proteins in response to the prey environment, sometimes exhibiting a strong response and other times spinning silk that is remarkably similar in amino acid composition even in different prey environments, such as we found in this study. This may occur in part because spiders have alternative mechanisms by which they can influence the mechanical performance of their webs, such as the structural changes in the diameters of threads that we document here.

Vollrath & Köller (1996) reported that when the body weights of orb-weavers were artificially increased, the spiders initially responded by increasing the diameters of radii in orb webs and then increasing the number of radii in new webs, but they did not measure the effects of these changes on web performance. In our study, when *N. pilipes* encountered persistent presence of large cricket prey, they spun fibers with 30% greater cross-sectional area than those spun by spiders that were fed an equal biomass of smaller fly prey. Spiders in the cricket treatment also exhibited a slight trend toward including more radii in webs, which was associated with increased web stiffness. Moreover, we found that this effect of prey types was still expressed when silk was 'artificially' drawn from spiders. The diameter of MA silk is controlled by valves in the MA gland (Ortlepp & Gosline 2004). These valves are under neural control, allowing spiders to actively manipulate the diameters of fibers spun under different conditions (Garrido et al. 2002). This suggests that spiders can freely control the diameter of their MA silks when constructing orb webs. Because the breaking force of a fiber is directly proportional to its cross-sectional area, even small changes in fiber diameter would greatly alter the physical perform-

Table 3: Mean (\pm SE) of percentages of major amino acids in draglines collected from *Nephila pilipes* fed crickets ($n = 8$) or flies ($n = 7$). Results of MANOVA tests ($F_{5,9} = 9.515$, $p = 0.002$) are shown

ance of webs (Fig. 3). Therefore, the observed variations in MA silk diameter in orbs can be interpreted as spiders actively adjust fiber thickness according to past foraging experiences.

While very convenient to obtain, the study of forcibly silked threads has sometimes been criticized because they can have different mechanical properties compared with silk obtained from natural draglines or webs (e.g. Madsen & Vollrath 2000; Ortlepp & Gosline 2004). Forcibly silked MA threads were consistently thicker than MA silk from the radii of webs, however we found that regardless of whether MA fibers were produced voluntarily or were drawn passively, fibers from spiders fed crickets always exhibited significantly larger diameters than those from spiders fed flies. This result suggests that differences in ingestion of different prey can have long-lasting effects on the MA gland and spigot that

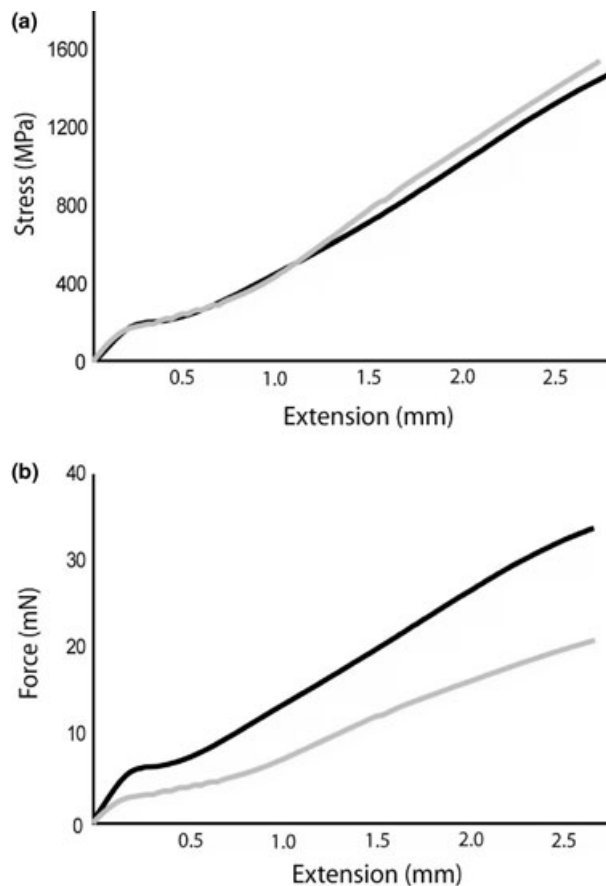


Fig. 3: Tensile tests for single exemplar fibers from spiders fed flies (gray line) and crickets (black line). (a) Stress-extension characteristics are nearly identical for major ampullate silk from the two treatment groups. (b) However, the larger diameter of silk spun by spiders in the cricket treatment results in their fibers breaking at approx. 65% greater force than fibers spun by spiders in the fly treatment

makes it persistently produce finer or thicker silk under many different conditions. The proximate mechanisms involved in the control of MA silk diameter when spiders experience different prey might include differences in amino acid composition of dipteran vs. orthopteran prey, the vibratory signals generated by these different prey, or changes in spider weight or gut distention as a result of feeding.

While the changes in MA silk diameter and stiffness have clear implications for physically stopping prey that are intercepted by orb webs, these changes may also influence how spiders detect the vibrations of different insects. The prey that we used in this experiment differed significantly in body size and thus in the nature of the vibratory signals that they would produce when entangled. Crickets are large and generate high amplitude, but low frequency, vibratory signals when entangled. In contrast, entangled flies produce lower amplitude, but higher frequency, vibrations in the range of 500–1000 Hz (Frohlich & Buskirk 1982; Landolfi & Barth 1996). High frequency vibrations generated by flying insects tend to attenuate more quickly on silks with lower tension (Masters 1984). For instance, Watanabe (2000) demonstrated that when the silk tension of orbs spun by *Octonoba sybotides* was increased, spiders were more sensitive to low-amplitude vibratory signals generated by smaller prey. In our study, in the persistent presence of flies, *N. pilipes* decreased silk diameter, increased fiber stiffness, and marginally increased the alanine content of MA silk (Tables 3 and 4). These changes are consistent with the production of a fine silk fiber with high crystalline structure and Young's modulus, which might be better at transmitting the higher frequency and lower amplitude vibrations generated by flies. This suggests that these spiders may be altering the properties of their silk in ways that would alter transmission of information about prey in webs. We suggest that in the persistent presence of large prey such as crickets, *N. pilipes* produced thicker MA silk and thus stronger and stiffer orbs to cope with the mechanical challenges presented by large prey. On the other hand, in the persistent presence of smaller flying insects that may be harder to detect in webs, *N. pilipes* produced finer MA silk that would better detect the vibrations of entangled prey. Thus, spiders may sometimes face trade-offs between the ability of their webs to effectively stop high energy prey vs. signaling the presence of prey in the web. In summary, spiders likely have many different mechanisms to adjust the physical performance of webs, including changes in web architecture, fiber structure, or silk

composition. One of the challenges for future research will be to determine if each of these mechanisms can act independently of one another and how they interact with other aspects of web function, such as signal transmission.

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Literature Cited

- Blackledge, T. A. 1998: Stabilimentum variation and foraging success in *Argiope aurantia* and *Argiope trifasciata* (Araneae: Araneidae). *J. Zool.* **246**, 21–27.
- Blackledge, T. A., Swindeman, J. E. & Hayashi, C. Y. 2005a: Quasistatic and continuous dynamic characterization of the mechanical properties of silk from the cobweb of the black widow spider *Latrodectus hesperus*. *J. Exp. Biol.* **208**, 1937–1949.
- Blackledge, T. A., Cardullo, R. A. & Hayashi, C. Y. 2005b: Polarized light microscopy, variability in spider silk diameters, and the mechanical characterization of spider silk. *Invertebr. Biol.* **124**, 165–173.
- Craig, C. L. 1987: The ecological and evolutionary interdependence between web architecture and web silk spun by orb web weaving spiders. *Biol. J. Linn. Soc.* **30**, 135–162.
- Craig, C. L. 1992: Aerial web-weaving spiders: linking molecular and organismal process in evolution. *Trends Ecol. Evol.* **7**, 270–273.
- Craig, C. L., Weber, R. S. & Bernard, G. D. 1996: Evolution of predator-prey systems: spider foraging plasticity response to the visual ecology of prey. *Am. Nat.* **147**, 205–229.
- Craig, C. L., Riekel, C., Herberstein, M. E., Weber, R. S., Kaplan, D. & Pierce, N. E. 2000: Evidence for diet effects on the composition of silk proteins produced by spiders. *Mol. Biol. Evol.* **17**, 1904–1913.
- Denny, M. 1976: Physical properties of spider silks and their role in design of orb-webs. *J. Exp. Biol.* **65**, 483–506.
- Eberhard, W. G. 1986: Effects of orb-web geometry on prey interception and retention. In: *Spiders, Webs, Behavior and Evolution* (Shear, W. A., ed.). Stanford Univ. Press, Stanford, CA, pp. 70–100.
- Eberhard, W. G. 1990: Function and phylogeny of spider webs. *Ann. Rev. Ecol. Syst.* **21**, 341–372.
- Fahnestock, S. R., Yao, Z. & Bedzyk, L. A. 2000: Microbial production of spider silk proteins. *Rev. Mol. Biotechnol.* **74**, 105–119.
- Foelix, R. F. 1996: *Biology of Spiders*. Oxford Univ. Press, New York.
- Frohlich, C. & Buskirk, R. E. 1982: Transmission and attenuation of vibration in orb spider webs. *J. Theor. Biol.* **95**, 13–36.
- Garrido, M. A., Elices, M., Viney, C. & Pérez-Rigueiro, J. 2002: Active control of spider silk strength: comparison of drag line spun on vertical and horizontal surfaces. *Polymer* **43**, 1537–1540.
- Gatesy, J., Hayashi, C., Motriuk, D., Woods, J. & Lewis, R. V. 2001: Extreme diversity, conservation, and convergence of spider silk fibroin sequences. *Science* **291**, 2603–2605.
- Gosline, J. M., Demont, M. E. & Denny, M. W. 1986: The structure and properties of spider silk. *Endeavour* **10**, 37–43.
- Gosline, J. M., Guerette, P. A., Ortlepp, C. S. & Savage, K. N. 1999: The mechanical design of spider silks: from fibroin sequence to mechanical function. *J. Exp. Biol.* **202**, 3295–3303.
- Guinea, G. V., Elices, M., Pérez-Rigueiro, J. & Plaza, G. R. 2005: Stretching of supercontracted fibers: a link between spinning and the variability of spider silk. *J. Exp. Biol.* **208**, 25–30.
- Hayashi, C. Y., Shipley, N. H. & Lewis, R. V. 1999: Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins. *Int. J. Biol. Macromol.* **24**, 271–275.
- Heiling, A. M. & Herberstein, M. E. 1999: The role of experience in web-building spiders (Araneidae). *Anim. Cogn.* **2**, 171–177.
- Herberstein, M. E. & Tso, I. M. 2000: Evaluation of formulae to estimate the capture area and mesh height of orb webs (Araneoidea, Araneae). *J. Arachnol.* **28**, 180–184.
- Higgins, L. E. & Buskirk, R. E. 1992: A trap-building predator exhibits different tactics for different aspects of foraging behaviour. *Anim. Behav.* **44**, 485–499.
- Higgins, L. E., Townley, M. A., Tillinghast, E. K. & Rankin, M. A. 2001: Variation in the chemical composition of orb webs built by the spider *Nephila clavipes* (Araneae, Tetragnathidae). *J. Arachnol.* **29**, 82–94.
- Hinman, M. B. & Lewis, R. V. 1992: Isolation of a clone encoding a second dragline silk fibroin. *J. Biol. Chem.* **267**, 19320–19324.
- Kuntner, M. 2006: Phylogenetic systematics of the Gondwanan nephilid spider lineage Clitaetrinae (Araneae, Nephilidae). *Zool. Scr.* **35**, 19–62.
- Landolfi, M. A. & 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.

- Madsen, B. & Vollrath, F. 2000: Mechanics and morphology of silk drawn from anesthetized spiders. *Naturwissenschaften* **87**, 148—153.
- Madsen, B., Shao, Z. Z. & 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.
- Masters, W. M. 1984: Vibration in orb webs of *Nuctenea sclopeteria* (Araneidae). I. Transmission through the web. *Behav. Ecol. Sociobiol.* **15**, 207—215.
- Opell, B. D. 1997: The material cost and stickiness of capture threads and the evolution of orb-weaving spiders. *Biol. J. Linn. Soc.* **62**, 443—458.
- Ortlepp, C. S. & Gosline, J. M. 2004: Consequences of forced silking. *Biomacromolecules* **5**, 727—731.
- Pan, Z. J., Li, C. P. & Xu, Q. 2004: Active control on molecular conformations and tensile properties of spider silk. *J. Appl. Polym. Sci.* **92**, 901—905.
- Pasquet, A., Ridwan, A. & Leborgne, R. 1994: Presence of potential prey affects web-building in an orb-weaving spider *Zygiella x-notata*. *Anim. Behav.* **47**, 477—480.
- Sandoval, C. P. 1994: Plasticity in web design in the spider *Parawixia bistriata*: a response to variable prey type. *Funct. Ecol.* **8**, 701—707.
- Sherman, P. M. 1994: The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Anim. Behav.* **48**, 19—34.
- Swanson, B. O., Blackledge, T. A., Beltrán, J. & Hayashi, C. Y. 2006: Variation in the material properties of spider dragline silk across species. *Appl. Phys. A-Mater.* **82**, 213—218.
- Tso, I. M. 1999: Behavioral response of *Argiope trifasciata* to recent foraging gain: a manipulative study. *Am. Midl. Nat.* **141**, 238—246.
- Tso, I. M. & Severinghaus, L. L. 1998: Silk stealing by *Argyrodes lanyuensis* (Araneae: Theridiidae): a unique form of kleptoparasitism. *Anim. Behav.* **56**, 219—225.
- Tso, I. M., Wu, H. C. & Hwang, I. R. 2005: Giant wood spider *Nephila pilipes* alters silk protein in response to prey variation. *J. Exp. Biol.* **208**, 1053—1061.
- Venner, S., Pasquet, A. & Leborgne, R. 2000: Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. *Anim. Behav.* **59**, 603—611.
- Vollrath, F. 1992: Spider webs and silks. *Sci. Am.* **266**, 70—76.
- Vollrath, F. 2000: Strength and structure of spider's silks. *Rev. Mol. Biotechnol.* **74**, 67—83.
- Vollrath, F. & Knight, D. P. 2001: Liquid crystalline spinning of spider silk. *Nature* **410**, 541—548.
- Vollrath, F. & Köller, T. 1996: Mechanics of silk produced by loaded spiders. *Proc. R. Soc. Lond. B* **263**, 387—391.
- Vollrath, F., Madsen, B. & Shao, Z. Z. 2001: The effect of spinning conditions on the mechanics of a spider's dragline silk. *Proc. R. Soc. Lond. B* **268**, 2339—2346.
- Watanabe, T. 2000: Web tuning of an orb-web spider, *Octonoba sybotides*, regulates prey-catching behaviour. *Proc. R. Soc. Lond. B* **267**, 565—569.
- Winkler, S. & Kaplan, D. V. 2000: Molecular biology of spider silk. *Rev. Mol. Biotechnol.* **74**, 85—93.
- Wirth, E. & Barth, F. G. 1992: Forces in the spider orb web. *J. Comp. Physiol. A* **171**, 359—371.
- Xu, M. & Lewis, R. V. 1990: Structure of a protein super-fiber: spider dragline silk. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7120—7124.
- Zschokke, S. 1999: Nomenclature of the orb-web. *J. Arachnol.* **27**, 542—546.
- Zschokke, S. 2002: Form and function of the orb-web. In: *European Arachnology 2000* (Toft, S. & Scharff, N., eds). Aarhus Univ. Press, Aarhus, pp. 99—106.