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Prey type, vibrations and handling interactively influence spider silk expression

S. J. Blamires[1], I.-C. Chao[2] and I.-M. Tso[1,3,*]

[1]Department of Life Sciences, Tunghai University, Taichung 407, Taiwan, [2]Affiliated High School of National Taiwan Normal University, Taipei 105, Taiwan and [3]Center for Tropical Ecology and Biodiversity, Tunghai University, Taichung 407, Taiwan

*Author for correspondence (spider@thu.edu.tw)

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SUMMARY

The chemical and mechanical properties of spider major ampullate (MA) silks vary in response to different prey, mostly *via* differential expression of two genes – *MaSp1* and *MaSp2* – although the spinning process exerts additional influence over the mechanical properties of silk. The prey cues that initiate differential gene expression are unknown. Prey nutrients, vibratory stimuli and handling have been suggested to be influential. We performed experiments to decouple the vibratory stimuli and handling associated with high and low kinetic energy prey (crickets *vs* flies) from their prey nutrients to test the relative influence of each as inducers of silk protein expression in the orb web spider *Nephila pilipes*. We found that the MA silks from spiders feeding on live crickets had greater percentages of glutamine, serine, alanine and glycine than those from spiders feeding on live flies. Proline composition of the silks was unaffected by feeding treatment. Increases in alanine and glycine in the MA silks of the live-cricket-feeding spiders indicate a probable increase in *MaSp1* gene expression. The amino acid compositions of *N. pilipes* feeding on crickets with fly stimuli and *N. pilipes* feeding on flies with cricket stimuli did not differ from each other or from pre-treatment responses, so these feeding treatments did not induce differential *MaSp* expression. Our results indicate that cricket vibratory stimuli and handling interact with nutrients to induce *N. pilipes* to adjust their gene expression to produce webs with mechanical properties appropriate for the retention of this prey. This shows that spiders can genetically alter their silk chemical compositions and, presumably, mechanical properties upon exposure to different prey types. The lack of any change in proline composition with feeding treatment in *N. pilipes* suggests that the *MaSp* model determined for *Nephila clavipes* is not universally applicable to all *Nephila*.

Key words: major ampullate silk, *MaSp*, *Nephila pilipes*, prey nutrients, prey vibratory stimuli, prey handling.

INTRODUCTION

There is immense industrial interest in developing synthetic materials that mimic the mechanical performance of proteinaceous biopolymers. The high mechanical strength, biodegradability, biocompatibility and ease of manipulation of spider dragline, or major ampullate (MA), silk make it attractive as a possible biomimetic (Gosline et al., 1999; Vollrath, 2000; Du et al., 2006; Kluge et al., 2008). Despite such an interest, the mechanisms regulating spider silk function are not well understood (Craig, 2003). Research aimed at emulating silk synthesis has focused on understanding the relationships between genetics, amino acid sequences and the physical properties of silk (Gosline et al., 1999; Hayashi et al., 1999; Vollrath, 2000; Hayashi et al., 2004). Nonetheless, a generally poor understanding of the influence of spider ecology and physiology on silk production impedes our discernment of how genetic traits translate into silk properties (Craig, 2003). It has been shown that the mechanical and chemical properties of spider silks are responsive to variations in the quality and quantity of prey consumed (Craig et al., 2000; Tso et al., 2005; Tso et al., 2007; Guehrs et al., 2008). Thus, the mechanisms by which prey affect silk production, biochemistry and mechanics are of interest to ecologists, physiologists and bioengineers.

The web designs and silk properties of orb web spiders (Araneidae) are plastic in their responses to different diets (Craig et al., 2000; Tso et al., 2005; Blackledge and Eliason, 2007; Tso et al., 2007; Blamires, 2010); hence, they make excellent models for investigating the processes of prey-induced silk properties. Indeed, research has been done to investigate the role of prey variation on the chemical and physical properties of silk and has found that the amino acid constituency of spider MA silk is influenced by the type of food the spider has consumed. For example, the amino acid composition of MA silk from *Nephila pilipes* and *Argiope* spp. differs between spiders feeding on crickets and those feeding on flies (Craig et al., 2000; Tso et al., 2005; Tso et al., 2007). The elicited changes in silk amino acids, nonetheless, are generally incongruent with the amino acid composition of the different prey (Craig et al., 2000; Zax et al., 2004; Guehrs et al., 2008). Therefore, the amino acid composition of the silk is unlikely to be a result of direct assimilation of the ingested materials into silk. Rather, the silk composition is a product of gene expression (Hayashi et al., 1999; Craig, 2003). Amino acid compositional changes, additionally, do not always coincide with changes in silk mechanical properties (Madsen et al., 1999; Zax et al., 2004; Tso et al., 2007; Liao et al., 2009). Alterations in lumen hydration, pH, ions or temperature alone during spinning can exert influences over the mechanical properties of MA silk (Madsen et al., 1999; Vollrath, 2000; Dicko et al., 2004).

Genetic control of MA silk composition is a product of the expression of two fibroin-encoding genes within the MA glands: major ampullate spidroin 1 (*MaSp1*) and major ampullate spidroin 2 (*MaSp2*) (Xu and Lewis, 1990; Gatesy et al., 2001). MaSp1 and MaSp2 fibroins are both characterized by amino acid motifs containing alanine (A) and glycine (G). Poly-A and GGX repeat units are present in both fibroins (Gatesy et al., 2001; Sponner et al., 2005). MaSp2, however, differs from MaSp1 in that it has a glycine-rich GPG region (Xu and Lewis, 1990; Gatesy et al., 2001; Ayoub and Hayashi, 2008). The poly-A repeat units of both fibroins

result in proteins folded into β-sheet crystalline structures (Xu and Lewis, 1990). These β-sheet structures contribute to the high ultimate strength of the silk (Craig, 2003; Lefevre et al., 2007; Brookes et al., 2008). The GPG units in MaSp2 result in the protein exhibiting β-spiral arrangements (Ayoub and Hayashi, 2008), which are responsible for the extensibility of the fibers (Hayashi et al., 1999; Brookes et al., 2008). Any changes in the ratios of the fibroins cause alterations in the mechanical properties of MA silk (Sponner et al., 2005; Brookes et al., 2008; Guehrs et al., 2008).

MaSp1 has more abundant poly-A and GGX motifs, so alterations in the relative ratios of MaSp1 and MaSp2 may be detected *via* alanine and glycine concentrations, higher concentrations of either amino acid representing greater *MaSp1* expression. Many authors, nonetheless, advocate proline as an identifier amino acid as it is present only in the GPG region of MaSp2 (Hinman and Lewis, 1992; Savage and Gosline, 2008a; Guehrs et al., 2008). A model was developed to differentiate spiders in the genus *Nephila* from other Araneidae based on the relative contributions of MaSp1 and MaSp2 in their native MA silks. *Nephila* were described as having low (1–4%) proline and silks comprising principally *MaSp1* products whereas most other spiders have high (14–16%) proline and silks that comprise principally *MaSp2* products (Savage and Gosline, 2008a; Savage and Gosline, 2008b). Nonetheless, at least one *Nephila* species, the giant wood spider, *Nephila pilipes*, has MA silk proline values of 7–10% (Tso et al., 2005; Tso et al., 2007), contradicting the model's generalizations. Additionally, Craig et al. found that the glycine composition of *Argiope argentata* MA silks differ (by 30–60%) geographically without major compositional changes in proline, which, incidentally, were found to be ~3–5% (Craig et al., 2000). The influence of genes over amino acid composition thus appears to be species-specific and the model appears to be inadequate for describing the genetic inputs across all araneid species.

Although spider diet can induce changes in silk amino acid composition *via* differential *MaSp1* and *MaSp2* expression (Craig et al., 2000; Tso et al., 2005; Tso et al., 2007; Guehrs et al., 2008), it is not known which prey properties induce changes in silk gene expression. The web and silk properties of orb web spiders feeding on prey of different protein content may vary (Zax et al., 2004; Blamires et al., 2009; Mayntz et al., 2009), suggesting that prey nutrients, especially protein, are important in inducing changes in silk gene expression. The precise role of nutrients in web and silk properties are, nonetheless, uncertain, as other influences – such as prey size, allelochemicals, vibratory stimuli and prey handling – confound the influence of prey nutrients (Nentwig, 1987). Decoupling the multiply acting variables is thus needed to gain a clearer understanding of how different prey types induce alterations in spider silk properties.

Here we experimentally manipulated the food (crickets or flies) and vibratory stimuli/prey handling (handling as used here includes prey encounter, subduing and killing) experienced by *N. pilipes* in order to decouple the vibrations and handling from nutritional factors affecting MA silk properties. Individual *N. pilipes* were fed one of the following diets: (1) live flies, with fly vibratory stimulation and handling characteristics; (2) live crickets, with cricket vibratory stimulation and handling characteristics; (3) dead flies, with cricket vibratory stimulation and inert fly handling characteristics; or (4) dead crickets, with fly vibratory stimulation and inert cricket handling characteristics. We chose diets of crickets or flies because the amino acid composition of their MA silk differs depending on which of these prey they feed on (Tso et al., 2005; Tso et al., 2007). Our study differed from those of Craig et al. (Craig et al., 2000) and Tso et al. (Tso et al., 2005; Tso et al., 2007) in that we decoupled the vibratory stimuli and prey handling from prey consumption. We thus effectively determined whether changes in silk amino acid composition are mediated by prey nutrients or by vibratory stimuli/prey handling. We did not measure the mechanical properties of the MA silk because we could not control or measure the spinning process, so we could not be sure to what extent induced amino acid sequence changes correspond to any changes in mechanical properties. We separately compared the amino acid composition of *N. pilipes* MA silk when: (1) prey nutrients and vibratory stimuli/prey handling were coupled and (2) prey nutrients and vibratory stimuli/prey handling were decoupled, to assess whether changes found in the amino acid composition of the MA silk persist upon decoupling, allowing us to identify which prey factor induces differential silk expression. If the changes in amino acid composition do not persist when prey nutrients and vibratory stimuli/prey handling are decoupled, it indicates that these factors interactively induce the spiders to alter *MaSp* expression. We did not rely on the composition of any single amino acid to indicate altered gene expression because, without the silk genome of *N. pilipes* being known, we cannot be sure which amino acid is the most reliable indicator in *N. pilipes*. Rather, we examined five key amino acid indicators: glutamine, serine, proline, glycine and alanine.

MATERIALS AND METHODS

Pre-treatment feeding and silk collection

We collected 60 female giant wood spiders, *Nephila pilipes* Fabricius 1793, 15–20 mm body length, from secondary forests in northern Taiwan. We collected only penultimate instar females to ensure that reproductive condition did not alter amino acid reserves or amino acid allocation into silk in any of the spiders. Any spider that molted during our experiments was removed and replaced. As *N. pilipes* can build webs exceeding 2 m in diameter, we housed the spiders in large rooms. The spiders were fed one mealworm daily for 3 days to allow them to fully express their web-building potential. Prior to experimentation, a sample (~10 m) of MA silk was mechanically spooled to ensure that all spiders within comparative groups produced silk of similar amino acid composition.

Spooling was accomplished by anaesthetizing spiders with CO_2 and placing them on a foam platform, ventral side up. The legs and abdomen were fixed in position with non-sticky tape and insect pins. We waited 30 min to ensure that the effect of the anaesthesia on silk properties was minimal (Madsen and Vollrath, 2000; Du et al., 2006). Threads of MA silks were manually pulled from the spinneret and taped onto a rotor powered by an electronic motor. The other two threads of minor ampullate silks were also pulled and taped onto the platform to prevent them from contaminating the MA silks. The spider on the platform was placed under a dissecting microscope in order to view the spinnerets and ensure that silk was being drawn from one MA spigot without intervention by any other spinnerets. The spooling process was terminated whenever we suspected contamination by other spinnerets. Silks were drawn at a speed of 1–2 m min^{-1}, a speed similar to that of natural silking during web building. Silk samples were kept at –20°C in a freezer before analysis of amino acid percentages.

Prey manipulation and spider feeding

After the pre-treatment, 53 *N. pilipes* (seven spiders failed to complete pre-treatment feeding) were assigned to one of four feeding treatment groups, designated CC, FF, CD or FD. Spiders in the CC group (N=13) were given one live cricket (high kinetic energy prey; ~300 mg body mass) at a time, to interact with and to feed on. Spiders in the FF group (N=14) were given five live house flies (low kinetic energy prey; ~60 mg body mass each) at a time to interact with and

to feed on. For spiders in the CD group (N=13), live flies were initially placed on the web but were removed as the spiders approached and replaced with a freshly killed dead cricket. For spiders in the FD group (N=13), one live cricket was initially placed on the web but was removed when the spider approached and replaced by an equal biomass of freshly killed dead flies. Because the amino acid composition of *N. pilipes* silk can be influenced by foraging history, which is correlated with location (Tso et al., 2005), the CC and FF treatment groups and the CD and FD treatment groups used spiders from similar locations to control for the effect of location. Apart from this initial criterion, the allocation of spiders to treatment groups was random.

Crickets were manually thrown onto the webs of spiders in the CC and FD groups in order to hit the sticky capture spirals with enough velocity to become entangled. Flies were introduced to spiders in the FF and CD groups by placing vials close to the web and allowing one fly at a time out of the vial as the previous fly was intercepted by the web. Prey nutrients and vibratory signal/prey handling were coupled for the CC and FF groups, but decoupled for the CD and FD groups. Only when the spiders built new webs, usually every other day, were they given the feeding treatments. All feeding treatments lasted until the spiders had built seven webs. Separate comparisons were performed between the CC and FF groups and between the CD and FD groups to determine the relative influence of prey nutrients and vibratory stimuli/prey handling. The carapace width of all *N. pilipes* designated to one of the four treatment groups was measured with digital callipers (accuracy to 0.1 mm). Spider carapace width was used as an indication of spider body size because body size has been shown to influence silk properties in penultimate instar females (Tso et al., 2007).

Chemical and statistical analyses of MA silk amino acids

MA silks were drawn using a mechanical spool from each spider. The silk samples were weighed (to the nearest 0.01 mg on an electronic balance), submerged in hexafluoro-isopropanol (HFIP; 500 μl mg^{-1} of silk), carefully examined to ensure there was no suspended particles, dried and hydrolyzed (115°C) in 6 mol l^{-1} HCl for 24 h. Silk samples were analyzed by reverse-phase HPLC and the analyses were performed in the Instrument Center, Department of Chemistry, National Tsing Hua University, Taiwan. The silk solution samples were first dried and then hydrolyzed at 115°C in 6 mol l^{-1} HCl for 24 h. The resulting product was transferred to a Waters Pico-Tag Amino Acid Analysis Column (Milford, MA, USA) to obtain the relative percentages of the five amino acids.

Table 1. Mean (±s.e.m.) carapace width of *Nephila pilipes* subjected to different treatment groups

Comparisons	Carapace width (mm)	t	d.f.	P
CC *vs* FF	7.51±0.25 *vs* 7.37±0.16	0.432	16	0.671
CD *vs* FD	7.24±0.27 *vs* 6.91±0.21	0.973	17	0.344

Results of t-tests are shown.
CC, spiders fed live crickets; FF, spiders fed live flies; CD, spiders fed dead crickets but receiving fly vibration stimuli/prey handling; FD, spiders fed dead flies but receiving cricket vibratory stimuli/prey handling.

Two Student's t-tests were used to compare the carapace width of spiders used in various treatment groups (one for spiders in the CC and FF groups and the other for those in the CD and FD groups) to ensure that spiders used in this study were similar in size. We used an ANOVA, with Bonferroni corrections to P-values to accommodate multiple testing (Rice, 1989), in order to compare the amino acid composition of pre- and post-treatment MA silks. We compared silks collected from the CC treatment group with those of the FF treatment group, and silks from the CD treatment group with those of the FD treatment group. Prior to analyses we ensured that the data were normally distributed and variances were homogeneous using Kolmogorov–Smirnov and Levene's tests, respectively.

RESULTS

For spiders that completed the treatments, carapace width did not differ significantly between the CC (N=10) and FF (N=9) treatment groups or between the CD (N=9) and FD (N=10) treatment groups (Table 1). Upon pre-treatment, the percentages of glutamine, serine, proline, glycine and alanine in MA silk did not differ significantly between spiders in the CC and FF groups (Table 2A), or between spiders in the CD and FD groups (Table 2B). There were differences in the amino acid composition of MA silk between CC–FF and CD–FD treatment group pairs (Table 2A *vs* Table 2B), most likely a result of the spiders in these treatment pairs originating from different locations. These differences, nonetheless, did not influence the outcomes of our experiments because our design did not entail post-treatment cross-comparisons.

Post-treatment glutamine, serine, alanine and glycine compositions of MA silk from *N. pilipes* fed live crickets (CC treatment group) differed from those fed live flies (FF treatment group) (Table 3A). The higher glycine and alanine in MA silks from spiders in the CC treatment group is consistent with an increased *MaSp1* expression. There was no difference in any of the amino

Table 2. Mean (±s.e.m.) percentages of major amino acids in major ampullate silks collected prior to the experiments from *Nephila pilipes* designated to different treatment groups

Treatment	Glutamine	Serine	Proline	Glycine	Alanine
A. CC–FF					
CC group	11.67±0.38	4.51±0.12	9.24±0.27	36.40±1.26	17.67±0.42
FF group	12.14±0.12	4.59±0.12	8.71±0.23	37.25±1.39	17.70±0.27
$F_{1, 25}$	1.001	0.260	2.237	0.204	0.003
P	0.327	0.621	0.147	0.655	0.954
B. CD–FD					
CD group	11.92±0.44	4.15±0.08	8.12±0.33	41.12±0.79	20.10±0.46
FD group	11.92±0.50	4.03±0.07	8.14±0.31	41.89±0.69	19.78±0.44
$F_{1, 24}$	<0.001	1.112	0.001	0.546	0.257
P	0.999	0.302	0.974	0.467	0.617

Results of ANOVAs are shown.
A and B are separate analyses of the CC–FF and CD–FD treatment group pairs, respectively.
CC, spiders fed live crickets; FF, spiders fed live flies; CD, spiders fed dead crickets but receiving fly vibration stimuli/prey handling; FD, spiders fed dead flies but receiving cricket vibratory stimuli/prey handling.

Table 3. Mean (±s.e.m.) percentages of major amino acids in draglines collected from *Nephila pilipes* in different treatment groups

Treatment	Glutamine	Serine	Proline	Glycine	Alanine
A. CC–FF					
CC group	12.68±0.13	4.29±0.06	8.92±0.28	40.24±0.25	18.22±0.21
FF group	12.14±0.12	4.62±0.05	8.96±0.17	35.00±0.65	17.06±0.26
$F_{1,17}$	9.491	15.859	0.015	61.502	11.789
P	0.007*	0.001*	0.903	<0.001*	0.003*
B. CD–FD					
CD group	13.40±0.23	3.70±0.13	9.30±0.44	44.03±1.38	19.79±0.43
FD group	12.36±0.50	3.85±0.28	8.91±0.48	42.66±2.36	19.94±1.05
$F_{1,17}$	3.357	0.219	0.350	0.236	0.015
P	0.084	0.646	0.562	0.663	0.905

Results of ANOVAs are shown.

A and B are separate analyses of the CC–FF and CD–FD treatment group pairs, respectively.

*, significance after Bonferroni correction.

CC, spiders fed live crickets; FF, spiders fed live flies; CD, spiders fed dead crickets but receiving fly vibration stimuli/prey handling; FD, spiders fed dead flies but receiving cricket vibratory stimuli/prey handling.

acids measured between spiders in the CD and FD treatment groups (Table 3B). The percentages of glycine and alanine in silks from the post-treatment CC and FF groups were less variable (i.e. lower s.e.m. values; Table 3A) compared with those of pre-treatment CC and FF silk (Table 2A *vs* Table 3A) and with those of post-treatment CD and FD silk (Table 3A *vs* Table 3B); however, these variations did not influence our results.

DISCUSSION

We found that, when feeding on live crickets, *N. pilipes* altered the amino acid composition of their MA silks, producing silks of higher glutamine, alanine and glycine concentrations, whereas serine concentration was reduced. No such alterations occurred under our other feeding treatments. We predict that greater expression in *MaSp1* genes is responsible for this response, as alanine and glycine are more abundantly encoded by *MaSp1* than *MaSp2* genes (Craig, 2003; Hayashi et al., 2004; Sponner et al., 2005). Because alanine and glycine repeats form crystals that correspond to β-sheet structures, and β-sheet structures are associated with stronger silks (Hayashi et al., 1999), prey-induced amino acid compositional changes can potentially alter silk strength. Nonetheless, results from a previous study (Tso et al., 2007) suggest that amino acid compositions do not always lead to altered silk strength, and mechanisms such as spinning conditions (Madsen et al., 1999; Vollrath, 2000) are also responsible for altering the mechanical properties of silk.

According to the model developed for *Nephila clavipes*, and assumed to hold for all species in the genus (Savage and Gosline, 2008a; Savage and Gosline, 2008b), proline concentration should decrease if *MaSp1* is upregulated. Nonetheless, we did not find any change in proline concentration. Our proline concentrations are consistent with those published for *N. pilipes* (Tso et al., 2005; Tso et al., 2007) and similar results have been obtained by at least two independent studies in our laboratory (S.J.B., unpublished data; Y. H. Tseng, unpublished data). Hence, our values obtained for proline are reliable. Thus, it seems that the models developed for *N. clavipes* and *Araneus diadematus* to explain silk gene expression patterns in all nephiliids and all araneids (Savage and Gosline, 2008a; Savage and Gosline, 2008b) do not hold for many species, including *N. pilipes* (Tso et al., 2005; Tso et al., 2007) (this study), *Nephila senegelensis* (Liu et al., 2008), *A. argentata* (Craig et al., 2000) and some species of *Cyclosa* (Liao et al., 2009).

Previous studies showing prey-induced changes in the chemical and/or mechanical properties of spider MA silk have explained these changes as a mechanism by which spiders modify their web properties to suit commonly encountered prey (Craig et al., 2000; Tso et al., 2005; Tso et al., 2007; Guehrs et al., 2008; Boutry and Blackledge, 2008; Boutry and Blackledge, 2009). However, prior to this study, the prey properties responsible for inducing such changes had not been investigated. By decoupling the vibratory signal and handling from the nutrients consumed we showed that both of these factors are required to induce *N. pilipes* to alter its MA silk properties. The lower s.e.m. values in glycine and alanine composition in the post-treatment MA silks from spiders in the CC and FF treatments may indicate that when vibratory stimuli/prey handling and nutrients of a specific prey are combined, the induced response is less variable. Perhaps exposure to crickets, which are larger and thus strike the web with greater kinetic energy, have handling costs that necessitate the spiders to produce stronger radial threads. Because amino acid compositions differed in our experiments, such a change in thread properties appears to occur in *N. pilipes via* differential gene expression, although the spinning process may exert influences over a shorter time frame.

In the presence of prey that are difficult to subdue, it is profitable for spiders to modify their web architecture and silk properties (Boutry and Blackledge, 2008; Blamires, 2010). The first cue detected by spiders in the presence of prey is the vibratory signals generated by the prey as it struggles in the web, followed by further assessments of the prey if it does not struggle free (Frohlich and Buskirk, 1982; Landolfa and Barth, 1996). The frequency of the vibrations may signal the size, handling time required and energetic profitability of prey (Landolfa and Barth, 1996). These factors and the responses they induce are thus strongly linked. Various vibratory stimuli activate complex behaviors in spiders *via* neuronal triggers (Barth, 1985). *MaSp* expression may thus also be under the influence of neuronal triggers that may be induced by vibrations or prey handling. Alternatively, vibrations may act as a cue to adjust the silk spinning processes, leading to changes in silk mechanical properties. We found that vibrations and prey handling altered the amino acid composition of *N. pilipes* MA silks when feeding on live crickets or live flies. It thus appears that differential *MaSp* expression is working, at least partially, to alter the properties of *N. pilipes* MA silk. As vibratory stimuli are directly related to the mechanical properties of the radial threads (Barth, 1985), there may be a positive feedback cycle between prey type, vibrations and silk properties. Prey handling may interact with, or correlate with, the vibratory-stimuli–silk feedback cycle. Future experiments should thus aim to decouple vibrations from prey handling to fully understand the roles of each type of stimulus on silk expression.

Nutrients in prey are often used and expressed by spiders in silk; however, there is often disagreement between prey nutrient composition and the nutrient investment of the resultant silk (Craig, 2003; Zax et al., 2004). For example, depriving *N. clavipes* of alanine and glycine does not affect the amino acid composition of their MA silk (Zax et al., 2004). The amino acids ingested are thus not utilized directly to produce particular types of silk. According to current models, the relative proportions of glycine and alanine are a product of the different *MaSp1*- and *MaSp2*-encoded fibroins (Sponner et al., 2005; Sponner et al., 2007; Brookes et al., 2008). Guehrs et al. found that in starved *N. clavipes* the dragline silk became stiffer and less elastic and they attributed these changes to *MaSp2* fibroin down-regulation indicated by changes in alanine, glycine and proline (Guehrs et al., 2008). Such a result indicates that orb web spiders can adjust the relative expression of *MaSp* genes according to their nutritional state. Nonetheless, experiments of *N. clavipes* fed specific nutrients in the absence of vibratory stimuli/prey handling have shown that the nutrients induce a minimal response in silk amino acid composition or mechanical properties (Zax et al., 2004); thus, prey nutrition only partially explains the variation in silk amino acid composition.

We demonstrated that spider silk protein expression is plastic and our findings suggest that the plastic response is mediated by interactions between prey vibrations, prey handling and prey nutrients. As the MA silks of *N. pilipes* feeding on live crickets contained more glutamine, more crystal-forming alanine and glycine, and less serine, *MaSp1* appears to be upregulated. Nonetheless, no changes in the composition of proline were detected among any feeding treatment, and proline is often considered a key indicator of the genetic outputs of *MaSp1* and *MaSp2* (Hayashi et al., 1999; Liu et al., 2008; Savage and Gosline, 2008a). Perhaps a different MaSp1 ortholog, such as that found in *Latrodectrus* (Motriuk-Smith et al., 2005), is downregulated. Such an ortholog, however, has not been demonstrated in any species of *Nephila*. It appears, however, that the relative role and expression of silk genes may substantially differ from species to species. Clearly, more studies, and gene sequences for a wider range of spider species, are needed before any generalizations can be made. Likewise, more research is needed to understand how spiders regulate MaSp protein synthesis at a molecular level to determine how changes in foraging conditions lead to compositional changes in transcription factors or compositional variation in key amino acids in the silk-producing epithelial cells. Post-transcriptional editing may be a mechanism used to adjust amino acid compositional input into silk proteins (Sponner et al., 2005), but further studies are needed to understand precisely how spiders genetically or physiologically exert this control. Studies using methods such as antibody labeling (Sponner et al., 2005; Gruers et al., 2008) would be useful to identify how gene expression influences protein molecular arrangement in the solid silk and how this then influences silk mechanics.

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REFERENCES

Ayoub, N. A. and Hayashi, C. Y. (2008). Multiple recombining loci encode MaSp1, the primary constituent of dragline silk, in widow spiders (*Latrodectus*: Theridiidae). *Mol. Biol. Evol.* **25**, 277-286.

Barth, F. G. (1985). Neuroethology of the spider vibration sense. In *Neurobiology of Arachnids* (ed. F. G. Barth), pp. 203-229. Berlin: Springer-Verlag.

Blackledge, T. A. and Eliason, C. M. (2007). Functionally independent components of prey capture are architecturally constrained in spider orb webs. *Biol. Lett.* **3**, 456-458.

Blamires, S. J. (2010). Plasticity of an extended phenotype: spider web architectural response to varying prey parameters. *J. Exp. Biol.* **213**, 3207-3212.

Blamires, S. J., Hochuli, D. F. and Thompson, M. B. (2009). Prey protein influences growth and decoration building in the orb spider *Argiope keyserlingi*. *Ecol. Entomol.* **34**, 545-550.

Boutry, C. and Blackledge, T. A. (2008). The common house spider alters the material and mechanical properties of cobweb silk in response to different prey. *J. Exp. Zool.* **309A**, 542-552.

Boutry, C. and Blackledge, T. A. (2009). Biomechanical variation of silk links spinning plasticity to spider web function. *Zoology* **112**, 451-460.

Brookes, A. E., Nelson, S. R., Jones, J. A., Koenig, C., Hinman, M., Stricker, S. and Lewis, R. V. (2008). Distinct contributions of model MaSp1 and MaSp2 like peptides to the mechanical properties of synthetic major ampullate silk fibers as revealed in silico. *Nanotechnol. Sci. Appl.* **1**, 9-16.

Craig, C. L. (2003). *Spiderwebs and Silk: Tracing Evolution from Molecules to Genes to Phenotypes*. Oxford: Oxford University Press.

Craig, C. L., Riekel, C., Herberstein, M. E., Weber, R. S., Kaplan, D. L. and Pierce, N. E. (2000). Evidence for diet effects on the composition of silk proteins produced by spiders. *Mol. Biol. Evol.* **17**, 1904-1913.

Dicko, C., Vollrath, F. and Kennedy, J. M. (2004). Spider silk protein refolding is controlled by changing pH. *Biomacromolecules* **5**, 704-710.

Du, N., Liu, X. Y., Narayanan, J., Li, L., Lim, M. L. M. and Li, D. (2006). Design of superior spider silk: from nanostructure to mechanical properties. *Biophys. J.* **91**, 4528-4535.

Frohlich, C. and Buskirk, R. E. (1982). Transmission and attenuation of vibration in orb spider webs. *J. Theor. Biol.* **95**, 13-36.

Gatesy, J., Hayashi, C. Y., Motriuk, D., Woods, J. and Lewis, R. V. (2001). Extreme diversity, conservation, and convergence of spider silk fibroin sequences. *Science* **291**, 2603-2605.

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.

Guehrs, K. H., Schlott, B., Grosse, F. and Wiesshart, K. (2008). Environmental conditions impinge on dragline silk protein composition. *Insect Mol. Biol.* **17**, 553-564.

Hayashi, C. Y., Shipley, N. H. and Lewis, R. V. (1999). Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins. *Int. J. Biol. Macromol.* **24**, 271-275.

Hayashi, C. Y., Blackledge, T. A. and Lewis, R. V. (2004). Molecular mechanics and characterization of aciniform silk: uniformity of iterated sequence modules in a novel member of the spider silk fibroin gene family. *Mol. Biol. Evol.* **21**, 1950-1959.

Hinman, M. B. and Lewis, R. V. (1992). Isolation of a clone encoding a second dragline silk fibroin. *J. Biol. Chem.* **267**, 19320-19324.

Kluge, J. A., Rabotyagova, O., Leisk, G. G. and Kaplan, D. L. (2008). Spider silks and their applications *Trends Biotechnol.* **26**, 244-251.

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. A* **179**, 493-508.

Lefevre, T., Rousseau, M. E. and Pezolet, M. (2007). Protein secondary structure and orientation in silk as revealed by Raman spectromicroscopy. *Biophys. J.* **92**, 2885-2895.

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.

Liu, Y., Sponner, A., Porter, D. and Vollrath, F. (2008). Proline and processing of spider silks. *Biomacromolecules* **9**, 116-121.

Madsen, B. and Vollrath, F. (2000). Mechanics and morphology of silk reeled from anaesthetized spiders. *Naturwissenschaften* **87**, 148-153.

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.

Mayntz, D., Toft, S. and Vollrath, F. (2009). Nutrient balance affects foraging behaviour of a trap-building predator. *Biol. Lett.* **5**, 735-738.

Motriuk-Smith, D., Smith, A., Hayashi, C. Y. and Lewis, R. V. (2005). Analysis of the conserved N-terminal domains in major ampullate silk proteins. *Biomacromolecules* **6**, 3152-3159.

Nentwig, W. (1987). The prey of spiders. In *Ecophysiology of Spiders* (ed. W. Nentwig), pp. 249-263. Berlin: Springer-Verlag.

Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution* **43**, 223-225.

Savage, K. N. and Gosline, J. M. (2008a). The role of proline in the elastic mechanism of hydrated spider silks. *J. Exp. Biol.* **211**, 1948-1957.

Savage, K. N. and Gosline, J. M. (2008b). The effect of proline on the network structure of major ampullate silks as inferred from their mechanical and optical properties. *J. Exp. Biol.* **211**, 1937-1947.

Sponner, A., Schlott, B., Vollrath, F., Unger, E., Grosse, F. and Wiesshart, K. (2005). Characterization of the protein components of *Nephila clavipes* dragline silk. *Biochemistry* **44**, 4727-4736.

Sponner, A., Vater, W., Monajembashi, S., Unger, E., Grosse, F. and Wiesshart, K. (2007). Composition and hierachical organization of a spider silk. *PLoS ONE* **10,** e998.

Tso, I. M., Wu, H. C. and Hwang, I. R. (2005). Giant wood spider *Nephila pilipes* alters silk protein in response to prey variation. *J. Exp. Biol.* **208**, 1053-1061.

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.

Vollrath, F. (2000). Strength and structure of spider's silks. *Rev. Mol. Biotechnol.* **74**, 67-83.

Xu, M. and Lewis, R. V. (1990). Structure of a protein superfiber: spider dragline silk. *Proc. Nat. Acad. Sci. USA* **87**, 7120-7124.

Zax, D. B., Armanios, D. E., Horak, S., Malowniak, C. and Yang, Z. (2004). Variation of mechanical properties with amino acid content in the silk of *Nephila clavipes*. *Biomacromolecules* **5**, 732-738.