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2 **The common house spider, *Parasteatoda tepidariorum*, maintains silk gene expression on sub-**
3 **optimal diet**

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5 **short title: Spiders maintain silk gene expression**

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Abstract

Orb-web weaving spiders and their relatives spin multiple task-specific fiber types. The unique material properties of each silk type result from differences in amino acid sequence and structure of their component proteins, primarily spidroins (spider fibrous proteins). Amino acid content and gene expression measurements of spider silks suggest some spiders change expression patterns of individual protein components in response to environmental cues. We quantified mRNA abundance of three spidroin encoding genes involved in prey capture in the common house spider, *Parasteatoda tepidariorum* (Theridiidae), fed different diets. After 10 days of acclimation to the lab on a diet of mealworms, spiders were split into three groups: (1) individuals were immediately dissected, (2) spiders were fed high-energy crickets, or (3) spiders were fed low-energy flies, for 1 month. All spiders gained mass during the acclimation period and cricket-fed spiders continued to gain mass, while fly fed spiders either maintained or lost mass. Using quantitative PCR, we found no significant differences in the absolute or relative abundance of dragline gene transcripts, major ampullate spidroin 1 (*MaSp1*) and major ampullate spidroin 2 (*MaSp2*), among groups. In contrast, prey-wrapping minor ampullate spidroin (*MiSp*) gene transcripts were significantly less abundant in fly-fed than lab-acclimated spiders. However, when measured relative to *actin*, cricket-fed spiders showed the lowest expression of *MiSp*. Our results suggest that house spiders are able to maintain silk production, even in the face of a low-quality diet.

key words: silk plasticity, major ampullate, minor ampullate, spidroin, gene expression, Theridiidae

Introduction

Spiders synthesize silk in specialized abdominal glands, and most have multiple morphologically and functionally differentiated gland types. Orb-web weaving spiders and their relatives (superfamily Araneoidea) have seven such glands [1]. For instance, silk spun from the major ampullate glands is used both as a dragline silk and structural silk to build the frame and radii of the orb-web. Orb-web weaving spiders then spin an auxiliary spiral using silk made in minor ampullate glands, which keeps the body of the web stabilized until it is replaced by the permanent capture spiral threads [2]. Cobweb weaving araneoid spiders (Theridiidae) also use major ampullate silk as draglines and for the majority of the web [3]. Cobweb weavers do not spin a capture spiral but use minor ampullate silk for prey-wrapping [4].

Spidroins (spider fibrous proteins) are encoded by a gene family found only in spiders [5]. The specialized functions and unique material properties of different spider silk types are the product of composite spidroins [5–7]. For instance, major ampullate glands express major ampullate spidroin 1 (MaSp1) and major ampullate spidroin 2 (MaSp2) [8,9]. Both proteins have a high proportion of β -sheet forming poly-alanine stretches that likely confer strength. However, MaSp2 contains numerous glycine-proline-glycine (GPG) motifs which form β -turn spirals [6,7,10–12]. Across species, those with a higher percentage of the proline-containing MaSp2 relative to the proline-poor MaSp1 have more extensible silk [13]. Therefore, the ratio of MaSp1 to MaSp2 is an important determinant of silk mechanical properties, namely extensibility and strength. Minor ampullate silks, composed of minor ampullate spidroins (MiSp), are typically not as strong but are more extensible than major ampullate silks [14,15]. Although most published MiSp sequences lack proline, the MiSp found in a particular cobweb weaver, the false black widow *Steatoda grossa*, has a high proportion of GPG motifs which is associated with this species having the most extensible minor ampullate silks [16].

Relative spidroin composition and spider silk mechanical properties can respond to environmental conditions (reviewed in [17]). Changes in amino acid content of dragline silks serve as support for changes in spidroin expression levels in response to diet [18–22]. Protein-rich diets lead to

greater fiber strength and extensibility [22]. Under protein deprivation, spider silk is lower in percentage of glutamine, glycine, and proline. Because glutamine and proline are abundant in MaSp2 and extremely limited in MaSp1, it was inferred that MaSp1 is preferentially expressed over MaSp2 under protein constraints [22]. Conflicting results have been found for spidroin expression of the orb-weaver *Nephila pilipes* fed flies versus crickets. Tso et al. [18] found that fly-fed spiders produced major ampullate silk with a higher percentage of alanine and lower percentages of glutamine and proline than cricket fed spiders, suggesting higher MaSp1:MaSp2 ratio in fly-fed spiders. In contrast, Blamires et al. [20] inferred higher MaSp1:MaSp2 in cricket-fed spiders as they found the major ampullate silks of fly-fed spiders were lower in glutamine, glycine, and alanine than cricket-fed spiders. Since the proportion of amino acids cannot be measured independently of each other, and the sequences of MaSp1 and MaSp2 were unknown for the species in these experimental studies, amino acid composition may be a poor indicator of absolute or even relative spidroin expression.

Blamires et al. (2018) [23] tested the effect of protein deprivation on multiple properties of major ampullate silk for five species of araneoid spiders, including amino acid content of major ampullate silk and expression levels of *MaSp1* and *MaSp2* in major ampullate glands. In this study, all species showed a significant difference in *MaSp1* or *MaSp2* expression levels between protein-deprived and protein-fed spiders, but the direction of change varied for each species. Additionally, three of the species showed significant differences in amino acid content as a consequence of protein deprivation. However, only one species' change in amino acid content reflected the change in gene expression levels; *Phonognatha graefei* increased proline in protein-deprived spiders, which is consistent with its observed downregulation of *MaSp1* and upregulation of *MaSp2* under protein-deprivation. In contrast, protein deprived *Argiope keyserlingi* upregulated *MaSp1* but decreased glycine and alanine amino acids. These results highlight the inadequacy of amino acid composition as a proxy for spidroin expression.

Here, we directly tested if expression levels of three spidroin genes change in response to diet. In our experiment, we first acclimated common house spiders, *Parasteatoda tepidariorum* (Theridiidae), to the lab, and then split them into three groups: immediate dissection, cricket-fed for one month, or fly-fed

for one month. Following the feeding trial, the major and minor ampullate glands were separately dissected. We used quantitative polymerase chain reaction (qPCR) to determine mRNA levels from the major ampullate and minor ampullate glands for *MaSp1*, *MaSp2*, and *MiSp*. We hypothesized that spiders fed the high-energy prey crickets would have higher *MaSp1:MaSp2* ratio than fly-fed spiders, because increased *MaSp1* should increase the strength of cobweb fibers [7]. In addition, we predicted higher expression of *MiSp* in cricket-fed than fly-fed spiders, because house spiders would need more *MiSp* to wrap the larger cricket relative to the smaller flies.

Materials and Methods

We collected 61 adult or penultimate female *P. tepidariorum* in June 2014 in Lexington, Virginia, U.S.A. (Rockbridge County) outside of buildings and in the parking garage of Washington and Lee University campus. All individuals were mature by time of dissection. The spiders were split into three groups, with each group having the same average mass (Fig 1), by ranking the spiders by mass, and then assigning individuals to the three groups in the order they were ranked. We individually housed the spiders in cylindrical plastic containers with a paper frame to support the webs (11.5cm across the lid, 9cm across the bottom, and 7.5cm in height). All spiders were fed one mealworm every two days for a total of four mealworms. The day after the fourth mealworm was fed, all webs were destroyed to stimulate silk gene expression.

Fig 1. Weights of spiders. Within the box plots, heavy black lines indicate medians and box hinges are interquartile ranges. Whiskers extend to the largest and smallest values up to 1.5 times the interquartile range. Dots indicate outliers beyond 1.5 times the interquartile range. (A) all spiders start weight: ANOVA results: $F=0.1202$, $p=0.89$. (B) dissected spiders start weight: ANOVA results: $F=0.2948$, $p=0.75$. (C) all spiders end weight: ANOVA results: $F=10.31$, $p=0.00016$. Tukey test results, P (baseline-cricket) = 0.0023; P (baseline-fly) = 0.77; P (cricket-fly) = 0.00024. (D) dissected spiders end weight: ANOVA results: $F=16.85$, $p=0.00015$. Tukey test results, P (baseline-cricket) = 0.001; P (baseline-fly) = 0.33; P (cricket-fly) = 0.00024.

Spiders in the first group were dissected three days after web destruction (hereafter, baseline). We fed the second group of spiders one cricket (~35 mg/cricket) per week and the third group five flies (~7

mg/fly) per week (1-2 times per week) to ensure equal biomass of prey between the two groups (as in [18,20]). At two and four weeks into the feeding trial, we destroyed the webs of the spiders. We then weighed and dissected the spiders two days after the last web destruction. Each spider was subject to CO₂ exposure for two minutes and then dissected under 0.15 M sodium chloride, 0.015 M sodium citrate buffer. Following removal, the major and minor ampullate glands were separately frozen in liquid nitrogen and stored at -80° C.

Total RNA was extracted from individual spider's major ampullate glands, and separately from the minor ampullate glands, using the RNeasy-Micro kit (QIAGEN), which includes DNase treatment. RNA integrity was assessed by denaturing with formamide, electrophoresing on 1% agarose gels, and staining with SYBRTM Gold (Invitrogen). RNA concentration was measured with a NanoDrop 1000 (Thermo Scientific). We synthesized cDNA from 100ng of total RNA with Superscript III® (Invitrogen) primed from an anchored oligo(dT) primer as described in [24].

We amplified the C-terminal encoding regions of *P. tepidariorum* *MaSp1*, *MaSp2*, *MiSp* and *Actin* using primers designed from sequences of cDNA clones (library generated by [16]; Table 1). Major ampullate and minor ampullate cDNAs were used as templates for *MaSp1* and *MaSp2* amplification. Initial experiments indicated no *MiSp* expression in major ampullate glands (Cq values equivalent to no template controls). Thereafter, only minor ampullate cDNA was used for *MiSp* amplification. qPCR amplification was performed using the MylQ5 thermocycler (BioRad) and associated software (Version 2.0). Each 20 microliter reaction volume contained the equivalent of 2.0 ng RNA of the template cDNA and 200 nanomoles of each primer in 1 X SYBR Green Supermix (BioRad). The cycling conditions included one step at 95°C for 3 min, followed by 40 cycles of 15 sec denaturation at 94 °C, 30 sec annealing (Table 1), and 30 sec extension at 72° C. Each reaction concluded with a 65-95°C melt curve analysis of 0.5°C increments every 5 sec to ensure single product amplification. Each biological sample was amplified at least 3 times (3-9 technical replicates, Supplementary File S1).

Table 1. Primers and parameters for quantitative PCR.

Gene	Accession Number	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Annealing Temperature	Efficiency	product length
<i>MaSp1</i>	MH367500	AACCCTGGAGCCTCTGACTG	GCGCCATAGTTGATGTTTCC	60.4 °C	106%	109 bp
<i>MaSp2</i>	MH367501	SGTTAGCTTCTGGAGGACCAGTT	GAAGCACCAGGATTGGATGA	60.4 °C	96%	93 bp
<i>MiSp</i>	KX584022	CTCTGGAGCATTTCAATCCAG	AACCGAGAACAGCTCCTAAAG	60.6 °C	95%	283 bp
<i>Actin</i>	JZ530978	ACGAACGATTCCGTTGTCC	AATACCGCAGGACTCCATACC	60.2	98%	147 bp

We included standard curves with every qPCR reaction. A standard curve was made from serial dilutions of cDNA clones for *MaSp1*, *MaSp2*, *MiSp*, and *Actin* (Table 1). Transcript abundance for each biological sample was calculated by inputting the mean Cq of technical replicates into the best-fit line of regression for the appropriate standard curve (Supplementary File S2, Fig S1). We determined efficiency of each reaction using the equation $\text{Efficiency} = -1 + 10^{(-1/\text{slope of standard curve})}$ (Agilent Genomics). We additionally calculated ratios of transcript abundance to determine expression levels of one gene relative to another gene and to account for any differences in underlying mRNA levels among individuals. We did not calculate relative gene expression levels as ΔCt because primer efficiencies varied among genes (Table 1). Neither did we use $\Delta\Delta\text{Ct}$ because we could not detect all our silk genes in both gland types and did not have another reference tissue. We tested for significant differences among feeding groups using ANOVA followed by post-hoc pairwise testing implemented in R or Python.

Results

At the end of the feeding trial, cricket-fed spiders weighed significantly more than baseline and fly-fed spiders (Fig 1, Supplementary File S3). For all spidroin genes, absolute transcript abundance tended to be lower in cricket and fly-fed spiders relative to baseline for both major and minor ampullate glands. This pattern was only significant ($p < 0.05$) for *MiSp* transcript abundance in minor ampullate glands with fly-fed spiders significantly lower than baseline spiders (Fig 2B; Table 2). *MaSp2* in major ampullate glands approached significance ($p = 0.056$), with fly and cricket-fed spiders lower than baseline

(Fig 2A). Although *Actin* transcript abundance did not significantly differ among feeding groups (Table 2), fly-fed spiders have lower transcript abundance than other feeding groups in minor ampullate glands (Fig 2C) and both fly-fed and cricket-fed had lower transcript abundance than baseline in major ampullate glands (Fig 2C). Thus, when each spidroin gene was measured relative to actin, the pattern of expression changed. This was especially true for *MiSp* in minor ampullate glands. Although not quite significant ($p=0.06$), cricket-fed spiders had the lowest *MiSp* relative to actin (Fig 2D). This same pattern of cricket-fed spiders having lowest spidroin expression relative to actin in minor ampullate glands held true for *MaSp1* and *MaSp2* (Fig 3 D&F). In major ampullate glands, fly-fed spiders had higher average *MaSp1*:actin and *MaSp2*:actin expression than the other two feeding groups (Fig 3 C&E).

Table 2. Results of one-way ANOVA comparing absolute or ratios of transcript abundance among three feeding groups.

Gland type	Response variable	p-value	F-value
Major	Actin	0.22	1.77
	MaSp1	0.20	1.84
	MaSp2	0.056	3.63
	MaSp1:MaSp2	0.064	3.41
	MaSp1:actin	0.18	2.05
	MaSp2:actin	0.20	1.89
Minor	Actin	0.17	2.11
	MaSp1	0.44	0.89
	MaSp2	0.45	0.87
	MaSp1:MaSp2	0.34	1.21
	MaSp1:actin	0.35	1.17
	MaSp2:actin	0.33	1.21
	MiSp	0.046	4.12
	MiSp:MaSp1	0.32	1.27
	MiSp:MaSp2	0.38	1.07
	MiSp:actin	0.06	3.63

Fig 2. Transcript abundance in major and minor ampullate glands. Medians and ranges for box plots as in Figure 1. (A) Total abundance of *MaSp1*, *MaSp2*, and *actin* in major ampullate glands. (B) Total abundance of *MaSp1*, *MaSp2*, *MiSp*, and *Actin* in minor ampullate glands. (C) *Actin* total abundance in major and minor ampullate glands. (D) *MiSp* abundance / *Actin* abundance in minor ampullate glands.

Fig 3. *MaSp1* and *MaSp2* abundance relative to each other and to *Actin*. Medians and ranges for box plots as in Figure 1. (A) *MaSp1/MaSp2* in major ampullate glands. (B) *MaSp1/MaSp2* in minor ampullate glands. (C) *MaSp1/actin* ratio in major ampullate glands. (D) *MaSp1/actin* in minor ampullate glands. (E) *MaSp2/actin* in major ampullate glands (F) *MaSp2/actin* in minor ampullate glands.

The ratio of *MaSp1:MaSp2* is modelled as an important determinant of dragline silk material properties [25]. We found the transcript ratio for *MaSp1:MaSp2* was very similar between major ampullate (mean = 1.60) and minor ampullate (mean = 1.61) glands (Fig 3 A&B). Although some individuals deviated considerably from the mean (Supplementary File S2, Figs S2 and S3), the ratio of *MaSp1:MaSp2* did not significantly differ among feeding groups for major ampullate glands or minor ampullate glands (Table 2), and there was no interaction between gland type and diet (two-way ANOVA: Overall model $F(5, 24) = 1.371, P = 0.2702$).

Discussion

We found little difference in the absolute or relative abundance of dragline gene transcripts, *MaSp1* and *MaSp2* among diet groups. This finding contradicts our expectation that house spiders fed high energy crickets would have higher *MaSp1:MaSp2* expression ratio compared to those fed low-energy flies. Also, we had originally predicted an increased level of *MiSp* expression in cricket-fed spiders compared to the fly-fed spiders. We found the fly-fed group to have the lowest absolute *MiSp* abundance but when measured relative to *actin*, cricket fed spiders had the lowest *MiSp* expression.

Based on similar feeding trials with orb-web weavers, we expected prey type to affect major ampullate spidroin gene expression [18,20,21]. Specifically, we expected the cricket-fed spiders to have increased expression of *MaSp1*, which should increase fiber strength [7]; house spiders fed crickets have stronger major ampullate silk than house spiders fed low-energy pillbugs [26]. However, our results suggest that neither of the major ampullate spidroins significantly respond to prey type. Our results may have differed from those found for orb-web weavers, because orb-web weavers rebuild their web every day, unlike cobweb weavers, which add to their existing web. It is likely that maintaining a cobweb is less

energy consuming than rebuilding an orb-web daily, resulting in no significant change in major ampullate spidroin expression for cobweb weavers eating different prey.

Another functional difference between cobwebs and orb-webs is that cobwebs incorporate major ampullate silk as structural support for the entire three-dimensional webs, whereas orb-webs use major ampullate silk to absorb the impact of flying prey [23]. Thus, it is possible the major ampullate silk of orb-web weavers has greater inherent variability to adjust the web functionality, which may be triggered by changes in nutrient uptake [23,26]. It was found under nutrient deprivation, the major ampullate silk of a cobweb species, *Latrodectus hasselti*, was unaffected in terms of material properties (Blamires et al., 2018). Thus, major ampullate silk of cobweb weavers may not have such variability and are thus less sensitive to changes in nutrient uptake [23]. Our findings could support this hypothesis since we saw minimal changes in major ampullate spidroins across feeding groups, suggesting major ampullate silk of *P. tepidariorum*, was not sensitive to the changes in prey-type. Nonetheless, our results show a fair bit of individual variation in terms of spidroin expression in both major and minor ampullate glands in each feeding group (Supplementary File S2, Figs S2 and S3). It is likely that spidroin expression in house spiders is variable but is not influenced by changes in nutrient uptake, at least as adults.

Although the major ampullate silk of the cobweb weaver *L. hasselti* [23] had minimal changes in material properties in response to nutrient deprivation, the major ampullate silk of house spiders did have changes in material properties in response to prey type. In a study that investigated the silk mechanical properties of *P. tepidariorum* fed either pillbugs or crickets, those fed pillbugs had an average gumfoot thread strength of 1200 MPa and extensibility of about 0.4 ln(mm/mm). The cricket-fed spiders had overall higher strength (1550 MPa) compared to the pillbug-fed spiders as well as modest increases in extensibility (0.43 ln(mm/mm)). Although we did not measure material properties, it is possible the silk strength differed in response to the changes in nutrient intake among the individuals despite an insignificant difference in *MaSp1:MaSp2* ratio among the feeding groups for major and minor ampullate glands.

Comparable to our finding that cricket-fed spiders weighed significantly more than the fly-fed spiders, Boutry and Blackledge [26] found cricket-fed spiders weighed almost twice as much as the pillbug fed spiders. Boutry and Blackledge [26] suggested spiders extract greater biomass from crickets, thus making the difference in material and mechanical properties of silk a response to different levels of starvation. A decrease in expression of *MaSp1* relative to *MaSp2* when starved (fed pillbugs) could be one mechanism through which these house spiders changed the strength of their spider silk. However, a more likely possibility, given our findings of no change in *MaSp1:MaSp2* in house spiders, is that spider body mass plays a direct role in variation of material properties of silk; Boutry and Blackledge [26] found thread diameter and failure load correlated to body mass. Our study aligns with these findings as we found no difference in spidroin expression based on diet, suggesting spider body mass may play a more significant role in modulating silk properties in which spiders may tune their silk in response to their own body mass.

Our spiders in their original environment had access to both low- and high-quality diet, so it is unknown if the spiders were accustomed to a suboptimal diet before capture. In the lab, it is believed the spiders fed crickets received an optimal diet, whereas the fly-fed spiders received a suboptimal diet. An adult cricket has 205 g/kg of protein while a fly has 197 g/kg of protein [27,28]. A cricket has 68 g/kg of fat and 1,402 g/kg of calories, whereas a fly has only 19 g/kg of fat and 918 g/kg of calories. Thus, the group that was fed crickets received greater fat and calories even though the spiders were fed equal biomass of crickets and flies. Thus, the spiders fed flies received less nutrient intake, making the fly diet suboptimal compared to the cricket diet. We found the spiders on this suboptimal diet to maintain dragline gene expression as there were no significant differences in *MaSp1* or *MaSp2* expression compared to the cricket-fed group.

Although we did not observe any effect of diet on dragline genes, we did find evidence for modulated gene expression in the minor ampullate glands. There is no previous literature on *MiSp* expression based on diet for orb-web weavers. We had predicted an increased level of *MiSp* expression in our spiders fed high energy crickets because cobweb weavers use minor ampullate silk for prey wrapping,

whereas orb-web weavers use them for an auxiliary spiral of their web. Crickets, due to their larger size, struggle more in the web and we observed house spiders throwing more silk on crickets than flies. We found slightly higher absolute abundance of *MiSp* in the cricket-fed group, but the fly-fed group was significantly lower than the baseline group in which it is possible even more silk is thrown on meal worms. Interestingly, when normalized against actin transcript abundance, cricket-fed spiders had the lowest *MiSp* abundance which was borderline significant. Our results suggest the fly-fed group potentially upregulate their expression of *MiSp* relative to other transcripts, like *Actin*, in the face of a low-quality diet in order to maintain sufficient prey-wrapping silk.

While most previous studies use amino acid composition to study spidroin levels, our study used qPCR as a direct measure of gene expression levels. A possible limitation of our qPCR design was the choice of the reference gene. Actin was initially chosen as a reference gene because it does not differ much among tissues [29]. However, based on our results, *Actin* may respond to diet as it was lower, albeit not significantly different, in the cricket and fly-fed spiders than the baseline spiders. Therefore, *Actin* might not be the best reference gene to normalize spidroin expression.

In conclusion, the absolute transcript abundance for all spidroins tended to be lower in cricket and fly-fed spiders compared to baseline for both major and minor ampullate glands. However, once compared against *Actin*, it was found cricket-fed spiders had the lowest *MiSp* expression. Additionally, in major ampullate glands, the fly-fed spiders had higher average *MaSp1:Actin* and *MaSp2:Actin* expression than the other two feeding groups. The low weights of fly-fed spiders suggest these spiders were likely starved during the feeding regime. Despite this sub-optimal diet, these fly-fed spiders maintained expression of 3 spidroin genes comparable to the cricket-fed or baseline spiders. Therefore, house spiders appear to maintain silk gene expression, or possibly even increase silk gene expression relative to other genes, even in the face of a low-quality diet.

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References

1. Foelix RF. Biology of Spiders. 3rd ed. New York, New York: Oxford University Press; 2011.
2. Zschokke S. The influence of the auxiliary spiral on the capture spiral in *Araneus diadematus* Clerck (Araneidae). Bull Br Arachnol Soc. 1993;9: 169–173.
3. Blackledge TA, Summers AP, Hayashi CY. Gumfooted lines in black widow cobwebs and the mechanical properties of spider capture silk. Zool Jena Ger. 2005;108: 41–6. doi:10.1016/j.zool.2004.11.001
4. Mattina CL, Reza R, Hu X, Falick AM, Vasanthavada K, McNary S, et al. Spider minor ampullate silk proteins are constituents of prey wrapping silk in the cob weaver *Latrodectus hesperus*. Biochemistry. 2008;47: 4692–4700.
5. Guerette PA, Ginzinger DG, Weber BHF, Gosline JM. Silk properties determined by gland-specific expression of a spider fibroin gene family. Science. 1996;272: 112–115. doi:10.1126/science.272.5258.112
6. Gatesy J, Hayashi C, Motriuk D, Woods J, Lewis R. Extreme diversity, conservation, and convergence of spider silk fibroin sequences. Science. 2001;291: 2603–5. doi:10.1126/science.1057561
7. Hayashi CY, Shipley NH, Lewis RV. Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins. Int J Biol Macromol. 1999;24: 271–275. doi:10.1016/S0141-8130(98)00089-0
8. Xu M, Lewis RV. Structure of a protein superfiber: Spider dragline silk. Proc Natl Acad Sci U S A. 1990;87: 7120–7124.
9. Hinman MB, Lewis RV. Isolation of a clone encoding a second dragline silk fibroin. *Nephila clavipes* dragline silk is a two-protein fiber. J Biol Chem. 1992;267: 19320–19324.
10. Holland GP, Creager MS, Jenkins JE, Lewis RV, Yarger JL. Determining secondary structure in spider dragline silk by carbon-carbon correlation solid-state NMR spectroscopy. J Am Chem Soc. 2008;130: 9871–9877.
11. Holland GP, Jenkins JE, Creager MS, Lewis RV, Yarger JL. Quantifying the fraction of glycine and alanine in β -sheet and helical conformations in spider dragline silk using solid-state NMR. Chem Commun. 2008;43: 5568–5570.

- 311 12. Ayoub NA, Garb JE, Tinghitella RM, Collin MA, Hayashi CY. Blueprint for a high-performance
312 biomaterial: full-length spider dragline silk genes. *PloS One*. 2007;2: e514.
- 313 13. Liu Y, Sponner A, Porter D, Vollrath F. Proline and processing of spider silks. *Biomacromolecules*.
314 2008;9: 116–121.
- 315 14. Colgin MA, Lewis RV. Spider minor ampullate silk proteins contain new repetitive sequences and
316 highly conserved non-silk-like “spacer regions.” *Protein Sci*. 1998;7: 667–672.
- 317 15. Guinea GV, Elices M, Plaza GR, Perea GB, Daza R, Riekel C, et al. Minor ampullate silks from
318 *Nephila* and *Argiope* spiders: tensile properties and microstructural characterization.
319 *Biomacromolecules*. 2012;13: 2087–2098. doi:10.1021/bm3004644
- 320 16. Vienneau-Hathaway JM, Brassfield ER, Lane AK, Collin MA, Correa-Garhwal SM, Clarke TH, et al.
321 Duplication and concerted evolution of MiSp-encoding genes underlie the material properties of
322 minor ampullate silks of cobweb weaving spiders. *BMC Evol Biol*. 2017;17: 78.
323 doi:10.1186/s12862-017-0927-x
- 324 17. Blamires SJ, Blackledge TA, Tso I-M. Physicochemical property variation in spider silk: ecology,
325 evolution, and synthetic production. *Annu Rev Entomol*. 2017;62: 443–460. doi:10.1146/annurev-
326 ento-031616-035615
- 327 18. Tso I-M, Wu H-C, Hwang I-R. Giant wood spider *Nephila pilipes* alters silk protein in response to
328 prey variation. *J Exp Biol*. 2005;208: 1053–1061. doi:10.1242/jeb.01437
- 329 19. Blamires SJ, Wu CL, Tso IM. Variation in protein intake induces variation in spider silk expression.
330 *PLoS ONE*. 2012;7: e31626.
- 331 20. Blamires SJ, Chao I-C, Tso I-M. Prey type, vibrations and handling interactively influence spider silk
332 expression. *J Exp Biol*. 2010;213: 3906–3910. doi:10.1242/jeb.046730
- 333 21. Blamires SJ, Tseng Y-H, Wu C-L, Toft S, Raubenheimer D, Tso I-M. Spider web and silk
334 performance landscapes across nutrient space. *Sci Rep*. 2016;6: 26383. doi:10.1038/srep26383
- 335 22. Blamires SJ, Liao C-P, Chang C-K, Chuang Y-C, Wu C-L, Blackledge TA, et al. Mechanical
336 performance of spider silk is robust to nutrient-mediated changes in protein composition.
337 *Biomacromolecules*. 2015;16: 1218–1225. doi:10.1021/acs.biomac.5b00006
- 338 23. Blamires SJ, Nobbs M, Martens PJ, Tso I-M, Chuang W-T, Chang C-K, et al. Multiscale mechanisms
339 of nutritionally induced property variation in spider silks. *PLOS ONE*. 2018;13: e0192005.
340 doi:10.1371/journal.pone.0192005
- 341 24. Casem ML, Collin MA, Ayoub NA, Hayashi CY. Silk gene transcripts in the developing tubuliform
342 glands of the Western black widow, *Latrodectus hesperus*. *J Arachnol*. 2010;38: 99–103.
343 doi:10.1636/Sh09-20.1
- 344 25. Liu Y, Shao Z, Vollrath F. Elasticity of spider silks. *Biomacromolecules*. 2008;9: 1782–1786.
- 345 26. Boutry C, Blackledge TA. The common house spider alters the material and mechanical properties of
346 cobweb silk in response to different prey. *J Exp Zool Part Ecol Genet Physiol*. 2008;309: 542–552.

27. Finke MD. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biol.* 2002;21: 269–285. doi:10.1002/zoo.10031
28. Finke MD. Complete nutrient content of four species of feeder insects. *Zoo Biol.* 2013;32: 27–36. doi:10.1002/zoo.21012
29. Clarke TH, Garb JE, Haney RA, Chaw RC, Hayashi CY, Ayoub NA. Evolutionary shifts in gene expression decoupled from gene duplication across functionally distinct spider silk glands. *Sci Rep.* 2017;7: 8393. doi:10.1038/s41598-017-07388-1

Supporting Information

Supplementary File S1. Raw Cq values from qPCR. Includes separate sheets for each gene in each gland type including raw Cq for all technical replicates and calculations for estimating absolute abundance.

Supplementary File S2. Three supplementary figures with legends.

Supplementary File S3. The weights of spiders before and after experiment with notes about egg production.

Data accessibility

Raw Cq values are in Supplementary File 1. Spider weights are in Supplementary File 3. Newly sequenced cDNA clones have been deposited in GenBank (MH367500, MH367501).

Ethical statement

The study complied with local legal requirements; no special license was required for this type of research.

A.

B.

C.

D.

Figure 1

Figure 2

A.

MaSp1:MaSp2 in Major

B.

MaSp1:MaSp2 in minor

C.

MaSp1:Actin in major

D.

MaSp1:Actin in minor

E.

MaSp2:Actin in Major

F.

MaSp2:Actin in minor

Figure 3