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

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

Jeremy Miller¹,², Jannelle Vienneau-Hathaway¹, Enkhbileg Dendev¹, Merrina Lan¹, Nadia A. Ayoub¹, *

¹ Washington and Lee University, Department of Biology, Lexington, VA 24450
² University of Kentucky College of Medicine

*Author for correspondence: ayoubn@wlu.edu*
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.](image)

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 SYBR™ 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 MyIQ5 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).
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 Efficiency = \(-1+10^{(-1/slope \text{ of \ standard \ curve})}\) (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 \(\Delta C_t\) because primer efficiencies varied among genes (Table 1). Neither did we use \(\Delta \Delta C_t\) 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 pill-bug 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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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.
Figure 1

(A) Start Weight for all spiders
(B) Start Weight for all dissected spiders
(C) End Weight for all spiders
(D) End Weight for all dissected spiders

Baseline n=20, Cricket-fed n=18, Fly-fed n=19
Baseline n=6, Cricket-fed n=6, Fly-fed n=6
Figure 2
Figure 3