The Journal of Experimental Biology 208, 1053-1061
Published by The Company of Biologists 2005
doi:10.1242/jeb.01437

Giant wood spider *Nephila pilipes* alters silk protein in response to prey variation

I-Min Tso[1,2,*], Hsuan-Chen Wu[1] and In-Ru Hwang[1]

[1]*Department of Life Sciences, Tunghai University, Taichung 407, Taiwan* and [2]*Center for Tropical Ecology and Biodiversity, Tunghai University, Taichung 407, Taiwan*

*Author for correspondence (e-mail: spider@mail.thu.edu.tw)

Accepted 13 December 2004

Summary

Recent studies have demonstrated that orb-weaving spiders may alter web structures, foraging localities or silk output in response to prey variations. In this study we conducted field surveys and food manipulations to examine whether orb-weaving spiders may also adjust the protein of silk to prey variations. A comparison of dragline silks collected from nine giant wood spider *Nephila pilipes* populations in Taiwan showed a spatial variation. The percentage of all amino acids (except alanine and glycine) exhibited significant differences among populations. A survey of prey composition also revealed a significant spatial variation among *N. pilipes* populations. To determine whether prey variation was responsible for silk protein variation, we fed *N. pilipes* with different types of prey (dipteran *vs* orthopteran) then compared the percentage of five major dragline amino acids and secondary structures. The results showed that dragline of *N. pilipes* fed with orthopteran prey contained significantly higher proline and glutamine but lower alanine. Congruent with this result were those from FTIR spectroscopy, which showed that dragline of *N. pilipes* fed with crickets exhibited significantly higher percentage of proline- and glutamine-containing β turns, and lower percentage of alanine-containing β sheet structures. Since the results of feeding manipulations showed that diet significantly affected the compositions of dragline silks, the observed spatial variation seemed to reflect the different types of prey these spiders had consumed. Results of this study thus indicated that orb-weaving spiders can alter dragline protein in response to prey variations.

Key words: spider silk, dragline, major ampullate gland, *Nephila.*

Introduction

Orb-weaving spiders construct webs to catch prey and this trait is easy to quantify, therefore they have been a popular model to study how foragers respond to various foraging conditions (Eberhard, 1990; Craig, 1992; Heiling and Herberstein, 1999, 2000). In addition to changes in foraging site (Enders, 1973, 1977; Rypstra, 1981; Smallwood, 1993; Chmiel et al., 2000), web structures (Higgins and Buskirk, 1992; Pasquet et al., 1994; Sandoval, 1994; Sherman, 1994; Blackledge, 1998; Heiling and Herberstein, 2000; Tso, 1999; Watanabe, 1999; Venner et al., 2000) and silk output (Higgins and Buskirk, 1992; Sherman, 1994; Tso, 1999), more and more studies have demonstrated that orb-weaving spiders may also alter various properties of the silk. Madsen et al. (1999) suggested that malnutrition would decrease the amount of available amino acids of spiders, which might affect silk chemistry and mechanical properties. Higgins et al. (2001) reported that the low molecular weight (LMW) organic compounds of adhesive spirals of *Nephila clavipes* changed significantly when spiders were moved from field to laboratory. Higgins et al. (2001) suggested that diet variations might have induced the production of different LMW organic compounds by aggregate glands. Craig et al. (1996) also demonstrated that *Nephila clavipes* could adjust silk pigment production according to the intensity and spectral composition of its light environment. In a field survey and manipulative study, Craig et al. (2000) showed that *Argiope* spiders may alter the properties of their dragline silk according to different prey intakes. In their study, dragline silks obtained from *A. argentata* of 13 Caribbean islands exhibited an inverse relationship between percentages of glycine and serine. Moreover, when the diet of *A. keyserlingi* was changed from fly to cricket, the composition of serine changed significantly. Results of Craig et al. (2000) suggested that in addition to changes of web structure and silk quantity, orb-weaving spiders might also change silk protein in response to prey variation.

Recent research has revealed that dragline silks are composed of the products of at least two genes: major ampullate spidroin 1 (*MaSp1*; Xu and Lewis, 1990) and major ampullate spidroin 2 (*MaSp2*; Hinman and Lewis, 1992). The MaSp1 protein exhibits poly (GA), poly (A) and poly (GGX) motifs (G, glycine; A, alanine; X, any amino acid; Xu and Lewis, 1990). Among these motifs, poly (GA) and poly (A) are major components of the β-sheet crystal structure (Gosline

et al., 1999) and they are regarded as being responsible for the tensile strength of the silk (Winkler and Kaplan, 2000). The GGX repeat regions might form helix structures that function as linkages between crystalline and non-crystaline portions of the molecule, or function to help align protein molecules in the silk (Hayashi et al., 1999). By contrast, the MaSp2 protein exhibits GPGXX and GPGQQ motifs (P, proline; Q, glutamine; Hayashi et al., 1999). These motifs formed β-turn spirals of the dragline silk (Hayashi et al., 1999) and, thus, are responsible for the extensibility of the silk. The relative composition of amino acids and secondary structures determines the strength and extensibility of the silk, which in turn will greatly affect the type and efficiency of the prey trapped by the web (Olive, 1980; Craig, 1987, 1992). In this study we investigated whether spiders will physiologically adjust the protein of silk when they encounter changes in prey type.

Recently, orb-weaving spiders of the genus *Nephila* have been extensively used in silk-related studies ranging from spinning process (Vollrath and Knight, 2001), physical properties (Vollrath, 1999, 2000), molecular biology (Winkler and Kaplan, 2000) to genetic engineering (Fahnestock et al., 2000). An understanding of whether or not *Nephila* spiders alter silk protein in response to prey variation will provide important insights to future silk-related studies. In the present study, we first conducted field surveys to see whether a spatial variation in silk amino acid percentages and prey compositions existed in wild populations. Secondly, we conducted feeding manipulations by giving spiders different types of prey (dipterans *vs* orthopterans) to see if these treatments would cause changes in amino acid percentages and secondary structures of the silk.

Materials and methods

Survey of dragline composition of wild populations

We conducted an island-wide survey to see if populations in Taiwan exhibited spatial variation in percentages of silk amino acid composition. Only dragline silk produced by major ampullate glands was examined because this type of silk was the focus of most relevant studies and therefore relevant information was available (Vollrath, 1999, 2000; Winkler and Kaplan, 2000). We collected draglines from spider populations in the following locations: Taipei (N=10), Baushan (N=7), Fouyenshan (N=12), Dakeng (N=5), Taiwu (N=9), Fusan (N=9), Fuyuan (N=5), Ziben (N=5) and Lanyu (N=5) (Fig. 1). All sites were located in Taiwan except Lanyu, which is a tropical island 92 km off the southeastern coast of Taiwan. Draglines were reeled the same day the spiders were collected and silk samples were kept at room temperature during the field survey. After the field survey they were kept in –20°C freezer before further analysis on amino acid percentages. In this study we used forced silking to collect dragline silks from *Nephila pilipes* Fabricius 1793. Spiders were first placed on a platform with the ventral side facing upward and the legs and abdomen were fixed with non-sticky tapes and insect pins. Threads of major ampullate silks were pulled from the spinneret and were taped on a rotor powered by a motor. The platform was placed under a dissecting microscope to make sure that no experimental error or contamination occurred during forced silking. Dragline silks were drawn at a speed of 1–2 m min^{-1}, a speed similar to that of natural silking during web building (Knight et al., 2000). The silks obtained were first weighted, then submerged in hexafluoro-isopropanol (HFIP, 500 μl for each milligram of silk), and then the silk solution was carefully examined to make sure that no suspended particles existed and that the protein was completely in solution. 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 Waters PICO.TAG Amino Acid Analysis System to obtain percentages of various amino acids. In the standard amino acid hydrolysis procedure, glutamines will be converted into glutamic acids and thus the percentages of these two amino acids are difficult to determine. However, according to Xu and Lewis (1990) and Hinman and Lewis (1992), the ratios of glutamine to glutamic acid in the proteins MaSp1 and MaSp2 were 66:2 and 74:2, respectively. Therefore, it should be valid to assume that the percentage of glutamic acids could well represent that of glutamine. Because the percentages of various dragline amino acids were not independent variables, we used a multivariate analysis of variance (MANOVA) test using SYSTAT 5.2.1 (Wilkinson et al., 1992) to compare the dragline amino acid percentages of *N. pilipes* collected from various populations.

Survey of prey composition of wild populations

This part of our study aimed to understand whether or not *N. pilipes* exhibited spatial variation in the composition of prey consumed. We recorded the foraging histories of *N. pilipes* populations in Lanyu in 1999, in Taipei and Fouyenshan in 2000, and in Fushan in 2001. All these censuses lasted about 10 days and were conducted in August of each year, at which time *N. pilipes* were the most abundant. During the censuses, the web sites of about 20 female spiders were marked and monitored for trapped prey hourly between 8:00 h to 18:00 h. The number and taxonomic orders of the trapped prey were recorded. A pair-wise comparison of ratios of different orders of insects caught by *N. pilipes* in these localities was performed using χ^2 tests of homogeneity to see whether prey composition of this spider in Taiwan exhibits spatial heterogeneity.

Effect of diet on dragline amino acid compositions

In this part of our study, we used female *N. pilipes* (body length 15–20 mm) to examine the effect of manipulating diet on silk amino acid percentages. Spiders were collected from secondary forests in central Taiwan and were reared in large cages made of wooden frames and screens (40×40×30 cm). We kept *N. pilipes* in large cages to facilitate their normal web building and recycling behaviors. The caged spiders were

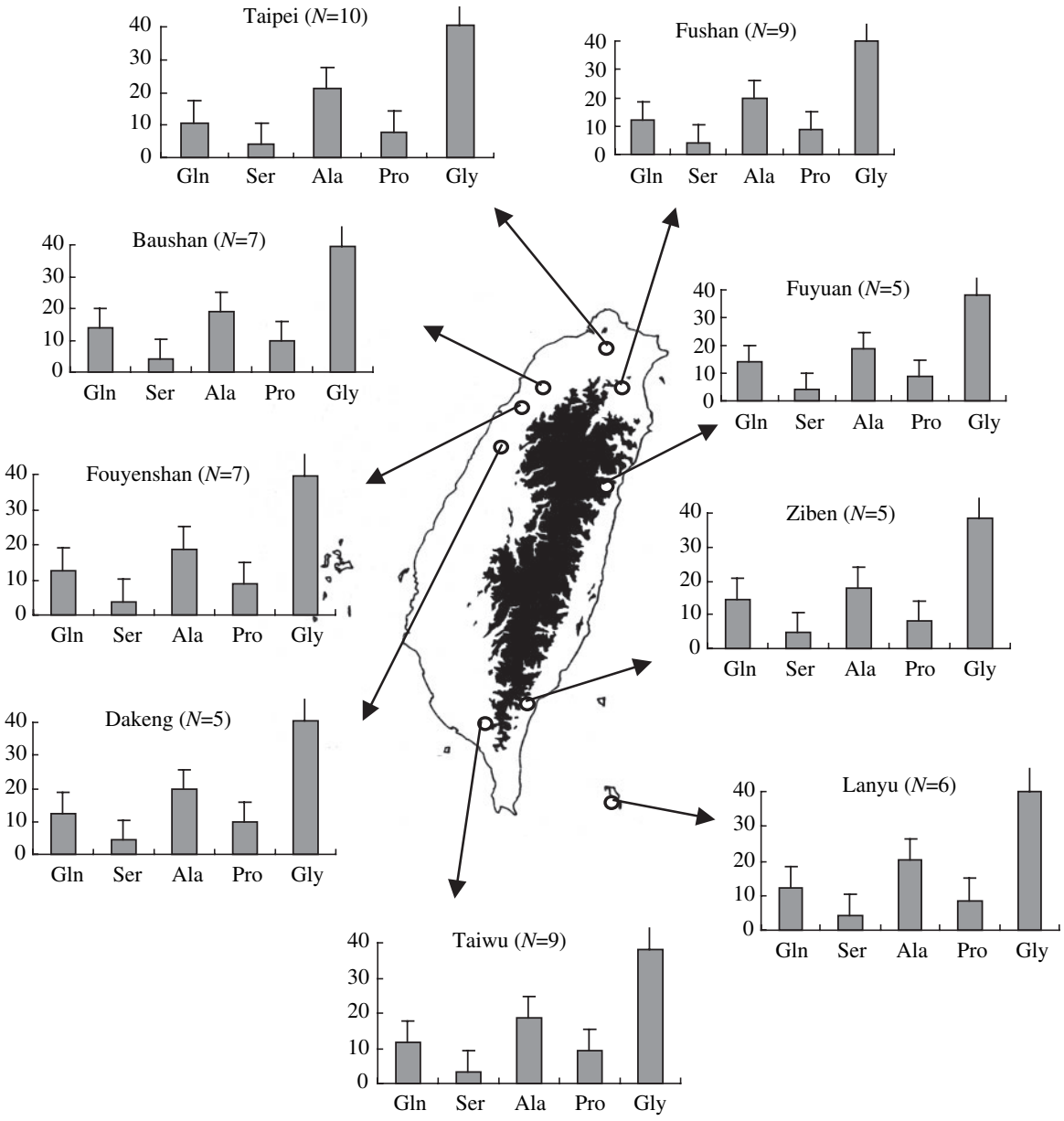

Fig. 1. Percentages of five major dragline amino acids of *Nephila pilipes* collected in nine localities in Taiwan.

placed in an outdoor screenhouse (5×5×3 m) thus the spiders were kept in physical conditions similar to those of their normal habitats. Craig et al. (2000) demonstrated that *Argiope keyserlingi* significantly altered the amount of serine in dragline when the diet was switched from fly (Diptera) to crickets (Orthoptera). Therefore, we randomly assigned 20 caged spiders into two groups (N=10 each) and fed them with either crickets or flies of equal biomass. Before the food manipulation all the spiders were first fed with mealworms till they had constructed three orbs. Before these spiders received food manipulation their draglines were force-reeled. The dragline amino acid percentages of spiders designated to be fed with either cricket or fly were compared to make sure that these spiders exhibited similar dragline composition before the food treatment. Spiders in the first group were fed with one cricket each day and in the second group spiders were fed with equal weights of flies (the weight of one cricket was about that of three flies). After the caged spiders had constructed seven more webs, dragline silks were force-reeled, and amino acid percentages were analyzed and statistically compared.

Effect of diet on dragline secondary structures

Fourier Transform Infrared (FTIR) spectroscopy is a powerful tool in studying the folding conformation of peptide chains and secondary structures of proteins (Goeden-Wood et al., 2003; Sethuraman et al., 2003) and this method had been used in spider silk researches (Wilson et al., 2000; Chen et al., 2002). In this study, the FTIR spectra of draglines produced by *N. pilipes* fed with different prey types were analyzed to compare the percentages of various secondary structures. To

Table 1. *Results of MANOVA tests comparing the percentages of major amino acids of draglines collected from nine* Nephila pilipes *populations in Taiwan*

	Glutamine	Serine	Proline	Glycine	Alanine
F	2.37	3.97	1.83	4.07	1.82
P	0.03	0.00	0.09	0.00	0.09

obtain dragline silk FTIR spectra, an ATR-Perkin Elmer Spectrum GX FTIR spectroscopy equipped with MCT detector was used. The resolution of MCT detector was 4 cm^{-1}, and the operation condition of scan number was 128. We used the program Peakfit 4.11 to facilitate the separation of peaks and assignment of the amide I bands (1600–1700 cm^{-1}). The proportion of secondary structures was calculated by integrating the area of each peak and then normalizing to the total area under the spectral curve. A MANOVA test was used to compare the percentages of various secondary structures in draglines of *N. pilipes* fed with different prey.

Results

Dragline amino acid compositions of wild populations

Among the amino acids of dragline, we examined the percentages of alanine, glycine, glutamine, proline and serine because of their higher quantity in the silk (Work and Young, 1987; Craig et al., 1999). The means and standard errors of percentages of major dragline amino acids in nine Taiwanese *N. pilipes* populations are given in Fig. 1. Results of MANOVA tests showed that significant differences were found in most of the major dragline amino acids, except proline and alanine (Table 1). The percentages of glutamine ranged from 10.7 to 14.7%, which represented a 37% difference between populations exhibiting the lowest and highest values. The percentages of serine ranged from 3.4 to 4.6%, which accounted to a 34% variation between populations. Finally, the percentages of glycine ranged from 38.3 to 40.6%, which constituted a 6% difference between populations. Although the percentages of proline and alanine did not differ significantly among populations, their statistical values were marginally significant (P=0.09 for both of them). The percentages of these two amino acids ranged from 7.6 to 9.5%, and 17.9 to 21.0%, respectively, which represented a difference of 25% and 17% between populations.

Prey composition of wild populations

Nephila pilipes populations in four localities in Taiwan varied considerably in composition of prey ingested. In all four localities, insects of the order Diptera, Hymenoptera and Coleoptera were the major prey (Fig. 2). Compared with other insect orders, Orthoptera was less represented in the diet. Although insects of these four orders were the major prey in all four populations, results of pairwise χ^2 tests of homogeneity showed that the relative proportion of different insect orders differed significantly among these populations (Table 2).

Effect of diet on dragline amino acid compositions

After *N. pilipes* fed with mealworms built three webs, dragline collected from 20 individuals designated to fed with different prey type did not show significant variations in amino acid percentages (F=0.002 for glutamine, 0.431 for serine, 3.111 for glycine, 0.785 for alanine, 0.176 for proline, all of them P>0.05). This result indicated that before the spiders received different type of prey, they exhibited similar amino acid composition in their dragline. At the end of food manipulation, complete data were available from eight spiders fed with flies and six spiders fed with crickets. Results of the MANOVA test showed that the food manipulation used in this study generated a significant variation in dragline amino acid percentages. The means and standard errors of percentages of the main dragline amino acids of spiders of the two groups are given in Table 3. With the exception of glycine, the percentages of the major amino acids differed significantly between spiders fed with different prey. Compared with *N. pilipes* fed with flies, those fed with cricket exhibited 40% higher glutamine and proline but 20% lower alanine. Among the silk samples collected from spiders subjected to feeding treatments, the abundance relationships between various major amino acids differed (Fig. 3). A significantly positive relationship was found between percentages of glutamine and proline (r^2=0.94, P<0.001; Fig. 3A). Conversely, significantly negative relationships were found

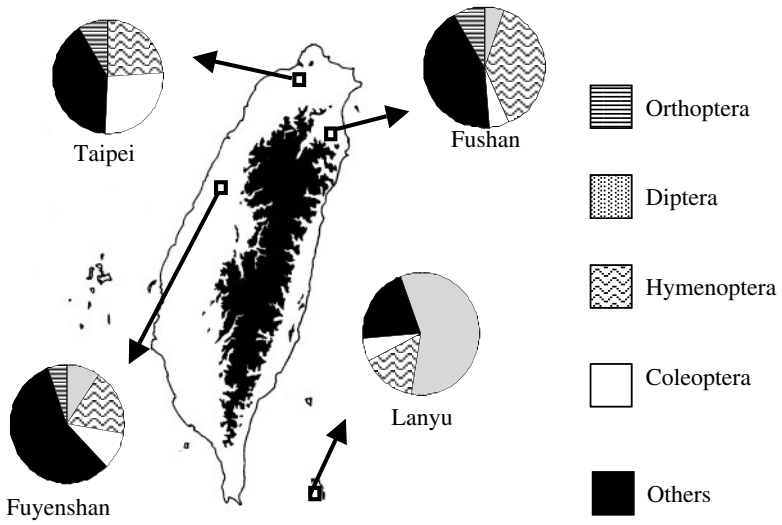

Fig. 2. Weight percentages of different taxonomic orders of prey intercepted by *Nephila pilipes* in four localities in Taiwan.

Table 2. *Results of pairwise χ^2 tests of homogeneity comparing prey composition of four* N. pilipes *populations in Taiwan*

	Taipei	Fushan	Fuyenshan
Fushan	23.086***	–	–
Fuyenshan	20.482***	11.418*	–
Lanyu	88.707***	69.94***	55.928***

*P<0.05, **P<0.01, ***P<0.005.

between percentages of glycine and serine (r^2=0.54, P<0.001; Fig. 3B) and between those of alanine and proline (r^2=0.88, P<0.001; Fig. 3C).

Effect of diet on dragline secondary structures

According to peak separation analyses conducted *via* Peakfit 4.11, the FTIR spectra of *N. pilipes* draglines were separated into five major peaks. We used the results of previous studies to assign these peaks to various secondary structures. The peak at 1612.5±0.6 cm^{-1} was assigned as protein side chains that contained amide groups or aromatic rings generating vibration at this region (Tatulian et al., 1998; Iconomidou et al., 2000). Chen et al. (2002) suggested that some helical conformations, such as α-helix and 3_{10}-helix, had absorption bands similar to those of random coil (unordered structure) and were sometimes not well resolved. Therefore, they assigned the absorption peaks around 1647 cm^{-1} to random coil and/or helical conformation. We followed the suggestion of Chen et al. (2002) and assigned the peak of 1646.8±0.4 cm^{-1} as random coil/helix. The peaks at 1663.5±0.2 cm^{-1} and 1682.1±0.3 cm^{-1} were both assigned as β-turn structures according to Huang et al. (2003) and the percentages were combined in the MANOVA analysis. The peak at 1628.6±0.4 cm^{-1} was assigned as β-sheet according to Goeden-Wood et al. (2003) and Sethuraman et al. (2004). Results of the MANOVA test comparing percentages of secondary structures also showed a significant effect by food manipulation. The means and standard errors of percentages of secondary structures were given in Table 4. Draglines from spiders fed with crickets contained a significantly higher percentage of β-turn and marginally significantly (P=0.06) lower percentage of β-sheet structures (Table 3).

Discussion

Nephila spiders have been known to alter web structures (Higgins and Buskirk, 1992), LMW substances of the sticky spiral (Higgins et al., 2001) and pigments on dragline and spiral silks (Craig et al., 1996) to various foraging conditions. In this study, results from field surveys and diet manipulations suggest that *N. pilipes* can alter dragline proteins in response to prey variation. Results of the field surveys showed that the dragline silk composition of *Nephila pilipes* populations in Taiwan exhibited a significant spatial variation. Among various populations, the dragline amino acid percentages could differ as much as 37% in glutamine, 34% in serine, 6% in glycine, 25% in proline and 17% in alanine. Congruent with such spatial variation in silk protein was the spatial variation in prey composition of four *N. pilipes* populations in Taiwan. Although we did not have foraging histories of the nine populations, results of field surveys demonstrated that relative compositions of flying insects (dipterans and hymenopterans) and orthopterans consumed by various *N. pilipes* populations varied greatly. Therefore, the spatial heterogeneity of different prey types might be responsible for the observed spatial variation in dragline proteins of *N. pilipes*. Moreover, results from food manipulations further indicated that the observed result was very likely to be induced by diet variations. Results from food manipulation showed that it took *N. pilipes* only seven foraging bouts to generate significant changes in silk protein. Such a response rate is congruent with those of web structures, LMW substances and silk pigments of other *Nephia* species to various foraging conditions (Higgins and Buskirk, 1992; Craig et al., 1996; Higgins et al., 2001).

Results of food manipulation showed that *N. pilipes* altered dragline silk amino acid when they encounter a stable temporal variation of prey type. The dragline silks produced by *N. pilipes* fed with crickets had significantly higher percentages of glutamine and proline but lower alanine. Conversely, the dragline produced by *N. pilipes* fed with flies exhibited the opposite pattern. All these amino acids are major components of various motifs of the silk protein. According to series of studies conducted on a South American *Nephila* species, *N. clavipes*, dragline silk is composed of the products of at least two genes, *MaSp1* and *MaSp2* (Xu and Lewis, 1990; Hinman and Lewis, 1992), and each exhibits different amino acid compositions and motifs. The MaSp1 protein contains higher proportion of β-sheet crystals comprising poly (GA) and poly (A) motifs, both of which are regarded as responsible for the tensile strength of the dragline. Conversely, MaSp2 is unique in containing β-turn structures composed of poly (GPGXX) or poly (GPGQQ) motifs, which are related to the extensibility of dragline. While alanine is the major component of MaSp1,

Table 3. *Mean (±S.E.M.) percentages of major amino acids of dragline silks produced by* Nephila pilipes *fed with different prey and results of MANOVA tests*

	Glutamine	Serine	Proline	Glycine	Alanine
Cricket	11.9±0.4	3.7±0.1	9.3±0.4	38.7±0.1	18.5±0.4
Fly	8.6±0.8	3.4±0.1	6.8±0.7	39.8±0.5	21.9±0.9
F	10.77	8.94	8.24	3.59	8.88
P	0.00	0.01	0.01	0.08	0.01

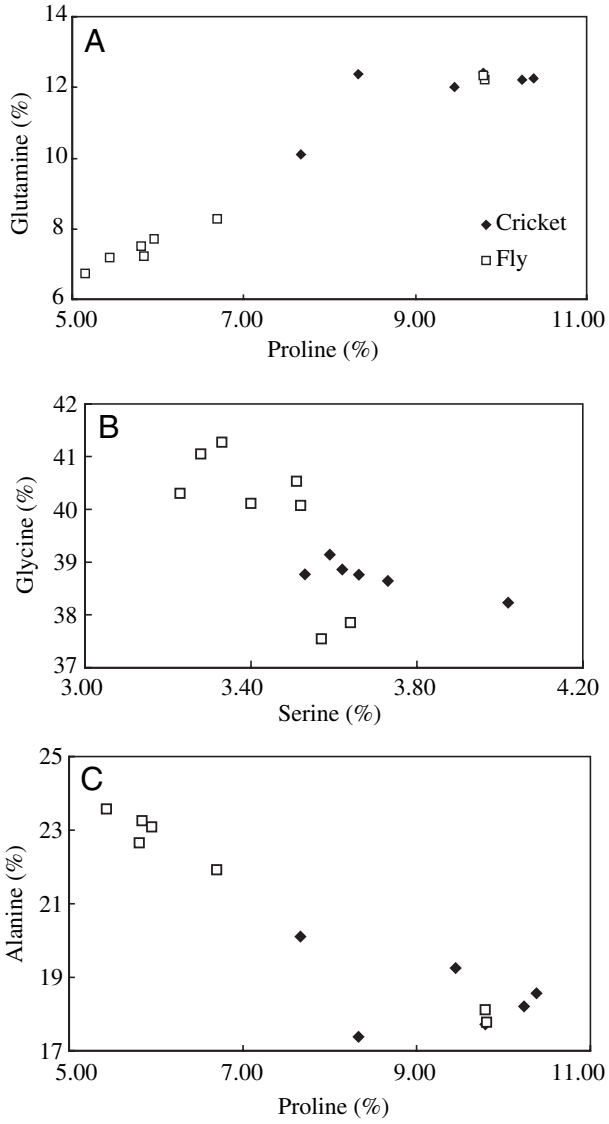

Fig. 3. The relationship of five major amino acids in dragline silk of *Nephila pilipes* fed with different prey. (A) glutamine *vs* proline. (B) glycine *vs* serine. (C) alanine *vs* proline.

proline and glutamine are relatively unique to MaSp2 proteins (Hinman and Lewis, 1992). Currently, the full length DNA sequences of *MaSp1* and *MaSp2* are not available. However, based upon the published partial sequences (~2 kb) the percentages of alanine, proline and glutamine in MaSp1 are 28.0, 0.0 and 10.2 (Xu and Lewis, 1990; Hayashi and Lewis, 1998). The corresponding percentages in MaSp2 are 22.3, 15.5 and 12.8 respectively (Hinman and Lewis, 1992; Hayashi and Lewis, 1998). Although these data are estimated from dragline of *N. clavipes*, results of a recent study conducted by Tai et al. (2004) showed that the *MaSp1* sequences of *N. pilipes* were very similar to those of *N. clavipes*. Therefore, the percentages of major amino acids in dragline of *N. pilipes* should be similar to those of *N. clavipes*. In addition to significant amino acid percentage differences between two *N. pilipes* groups, the abundance relationships between various major amino acids also suggested that our observed results were generated by differential expressions of *MaSp1* and *MaSp2* gene products. A significantly positive relationship was found between percentages of glutamine and proline, and significantly negative relationships were found between glycine and serine and between those of alanine and proline. Moreover, in all three amino acid abundance relationships in Fig. 3, individual *N. pilipes* were separated into two distinct groups according the prey type they ingested. Such results provided supports for our hypothesized model of differential expression of two dragline silk genes. In addition, two *N. pilipes* fed with flies adopted the same proportion of amino acids as those fed with crickets (Fig. 3). The reason for such a phenomenon might be that these two individuals, for some reason, were not responsive to the flies they had ingested and therefore exhibited the same gene expression and thus amino acid abundance patterns with those fed with crickets.

Significantly higher proline and glutamine percentages in dragline of *N. pilipes* fed with crickets suggested that in these silks there were relatively higher proportion of MaSp2, and consequently more β-turn structures and higher extensibility. Conversely, a significantly higher proportion of alanine in dragline of *N. pilipes* fed with flies indicated a relatively higher proportion of MaSp1 protein, more β-sheet crystals and, thus, a higher tensile strength. Results from FTIR spectroscopy were congruent with these predictions. Draglines from spiders fed with crickets contained a higher percentage of β-turn and a lower percentage of β-sheet. Previous studies have demonstrated that silk properties are vital for successful prey catching (Craig, 1987, 1992). Silks suitable for catching large orthopterans and flying insects differ in physical properties, and *Nephila* spiders may have evolved genetic plasticity of the silk protein to cope better with both prey types. As flying insects are intercepted by the web, they tend to pull perpendicularly away from the web. Therefore, webs with stronger radial and spiral silks will better retain flying insects (Olive, 1980). Conversely, intercepted large orthopterans tend to rip down along the panel of the web, accumulating more and more of the sticky spiral. Orbs with higher numbers of spirals, a larger catching area and more elastic silk will better retain this type of prey (Olive, 1980). Results of this and other studies (Robinson and Robinson, 1970; Nyffeler and Breene, 1991; Craig et al., 2001) indicated that the prey of orb-weaving spiders varies both spatially and temporally, therefore, they may have evolved the ability to adjust silk protein according to the presence of different prey type. We suggest that the relative abundance of different prey types in the diet can induce orb-weaving spiders to adjust relative strength and extensibility of silk by altering silk protein to enhance the catching success.

In this study we did not perform HPLC analysis by ourselves but three lines of evidence suggested that the analyses were accurate. First, the amino acid composition of dragline produced by *N. pilipes* in this study was very similar to those of other *Nephila* spiders. Second, we sent in several major ampullate silk samples collected from *Cyrtophora moluccensis*

Table 4. *Mean (±S.E.M.) percentages of various secondary structures of dragline silks produced by* Nephila pilipes *fed with different prey estimated by FTIR spectroscopy and results of MANOVA tests*

	Side chain (1612.5±0.6 cm^{-1})	β-sheet (1628.6±0.4 cm^{-1})	Random coil/helix (1646.8±0.4 cm^{-1})	β-turn (1663.5±0.2 cm^{-1}) (1682.1±0.3 cm^{-1})
Cricket	15.3±0.8	32.2±0.4	25.9±0.5	26.6±0.5
Fly	17.9±1.2	33.5±0.4	25.0±0.5	23.7±0.9
F	3.14	4.47	1.40	8.44
P	0.11	0.06	0.26	0.02

for analysis. The amino acid percentages of these samples were quite different from that of *Nephila* spiders but were very similar to those reported in Craig et al. (1999). Third, we simultaneously sent in samples obtained from the same silking process but diluted in different quantity of HFIP (1× *vs* 5×). The amino acid percentages of these two samples were almost identical and the differences ranged only from 0.01 to 0.5%. All evidence indicated that our data reflected the actual percentages of amino acids in the samples.

One potential mechanism of the observed spatial variation in *N. pilipes* dragline protein might be the accumulation of mutations among isolated populations. It is possible that the unusually long and repetitive nature of dragline genes may constantly generate variations in both DNA and protein levels in different *N. pilipes* populations. Dragline silks are encoded by *MaSp1* and *MaSp2*, both of which are unusually long and repetitive (Winkler and Kaplan, 2000). The length of mRNA in major ampullate glands was reported to range from 4.4–15.5 kb (Hayashi, 2002). The whole sequence can be divided into repetitive and non-repetitive regions (Beckwitt and Arcidiacono, 1994). The repetitive regions are composed of glycine- or alanine-rich units and a comparison of repetitive units from different portions of both genes showed that much variation existed (Gatesy et al., 2001). The unusually long and repetitive nature of dragline genes may greatly enhance the occurrence of point mutations, biased base composition, replication error and unequal crossing-over (Beckwitt et al., 1998; Hayashi, 2002). If *N. pilipes* populations are isolated, it is likely that the aforementioned genetic events generating rapid evolution of dragline genes would result in local divergences in dragline proteins. However, a recent study by Lee et al. (2004) on population genetic structure of *N. pilipes* in Taiwan and neighboring islands suggested that the populations were not isolated. Instead, owing to the excellent aerial dispersal by the ballooning ability of *N. pilipes* similar to those of other orb weavers (Dean and Sterling, 1985; Decae, 1987; Greenstone et al., 1987), there were strong gene flows even between populations across high mountain ranges (4000 m) and oceans. Therefore, the level of gene flow should be rather high and local population divergence is unlikely to occur. The observed variation in dragline amino acid composition is probably generated by differential expression of *MaSp1*/*MaSp2* gene products in response to local prey composition variations.

Orb-weaving spiders have long been known to be able to adjust dragline properties behaviorally in response to different foraging conditions and results of this present study demonstrated that they can also adjust silk proteins. Several studies have already demonstrated that spiders can fine-tune mechanical properties of webs without changing silk proteins. Vollrath and Köller (1996) reported that when the body weight of orb-weavers was artificially increased, the spiders initially increased the diameter of the radial threads and subsequently doubled or tripled the number of radial threads when building the web. In addition, reeling speed was also shown to be a significant factor on physical properties of dragline silk. As the reeling speed increased the arrangement of microstructures become more oriented and so the strength of the silk increased (Madsen et al., 1999; Riekel and Müller, 1999; Knight et al., 2000). Madsen et al. (1999) also reported that food quantity significantly affects the property of dragline by decreasing the breaking elongation. Since Craig et al. (2000) reported that hungry and satiated *Argiope keyserlingi* did not differ in dragline amino acid composition, the findings of Madsen et al. (1999) might be generated physiologically or behaviorally. Vollrath (1999) suggested that spider silks involved in prey catching did not seem to be selected to maximize a narrow set of physical properties but were products of compromises of different selective forces. Therefore, given the complicated foraging conditions faced by orb-weaving spiders, the ability to adjust silk properties in response to different conditions will be quite adaptive. Concluding from the results of this and previous studies, we suggest that in face of prey variation orb-weaving spiders adjust the mechanical properties of the webs by manipulating silk diameter, number of radial threads and also the protein of the silk.

Concluding from the results of this and previous studies, *Nephila* spiders seem to exhibit plasticity on a behavioral and a molecular level to varying foraging conditions. However, how such plasticity is achieved remains unclear. It is possible that differential expression of different silk genes is induced by different chemical composition of dipteran and orthopteran prey. According to Ramos-Elorduy et al. (1997), these two orders of insects differed significantly in percentages of major amino acids. Conversely, the different intensity of mechanical stimuli exhibited by smaller dipterans and heavier orthopterans might also be potential factors. Based on the results of the present study, future investigations on how *Nephila* spiders achieve differential expressions of various dragline genes in response to prey variation will be quite

valuable to the genetic studies of spider silks. In addition, further studies on how a variation in diet affects silk physical properties and the efficiency of prey capture will provide a new insights into the understanding of foraging ecology using orb-weaving spiders.

We greatly thank S. C. Wu, W. H. Lin, I. C. Chou, K. C. Chen, Y. H. Chang, J. C. Yang, H. Y. Lee and C. K. Yang for all sorts of assistances. This study was supported by the National Science Council, Taiwan grants (NSC 91-2311-B-029-006, NSC 92-2311-B-029-002) to I-M.T.

References

Beckwitt, R. and Arcidiacono, S. (1994). Sequence conservation in the C-terminal region of spider silk proteins (spidroin) from *Nephila clavipes* (Tetragnathidae) and *Araneus bicentenarius* (Araneidae). *J. Biol. Chem.* **269**, 6661-6663.

Beckwitt, R., Arcidiacono, S. and Stote, R. (1998). Evolution of repetitive proteins: spider silks from *Nephila clavipes* (Tetragnathidae) and *Araneus bicentenarius* (Araneidae). *Insect Biochem. Mol. Biol.* **28**, 121-130.

Blackledge, T. A. (1998). Stabilimentum variation and foraging success in *Argiope aurantia* and *Argiope trifasciata* (Araneae: Araneidae). *J. Zool.* **246**, 21-27.

Chen, X., Knight, D. P., Shao, Z. and Vollrath, F. (2002). Conformation transition in silk protein films monitored by time-resolved Fourier transform infrared spectroscopy: effect of potassium ions on *Nephila* spidroin films. *Biochemistry* **41**, 14944-14950.

Chmiel, K., Herberstein, M. E. and Elgar, M. A. (2000). Web damage and feeding experience influence web site tenacity in the orb-web spider *Argiope keyserlingi* Karsch. *Anim. Behav.* **60**, 821-826.

Craig, C. L. (1987). The ecological and evolutionary interdependence between web archetecture and web silk spun by orb web weaving spiders. *Biol. J. Linn. Soc.* **30**, 135-162.

Craig, C. L. (1992). Aerial web-weaving spiders: linking molecular and organismal process in evolution. *Trends Ecol. Evol.* **7**, 270-273.

Craig, C. L., Hsu, M., Kaplan, D. and Pierce, N. E. (1999). A comparison of the composition of silk proteins produced by spiders and insects. *Int. J. Biol. Macromol.* **24**, 109-118.

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

Craig, C. L., Weber, R. S. and Bernard, G. D. (1996). Evolution of predator–prey systems: spider foraging plasticity response to the visual ecology of prey. *Am. Nat.* **147**, 205-229.

Craig, C. L., Wolf, S. G., Davis, L. D., Hauber, M. E. and Maas, J. L. (2001). Signal polymorphism in the web-decorating spider *Argiope argentata* is correlated with reduced survivorship and the presence of stingless bees, its primary prey. *Evolution* **55**, 986-993.

Dean, D. A. and Sterling, W. L. (1985). Size and phenology of ballooning spiders in Texas. *J. Arachnol.* **13**, 111-120.

Decae, A. E. (1987). Dispersal: ballooning and other mechanisms. In *Ecophysiology of Spider* (ed. W. Nentwig), pp. 348-356. Berlin: Springer-Verlag.

Eberhard, W. G. (1990). Function and phylogeny of spider webs. *Annu. Rev. Ecol. Syst.* **21**, 341-372.

Enders, F. (1973). Selection of habitats by the spider *Argiope aurantia* Lucas (Araneidae). *Am. Midl. Nat.* **90**, 47-55.

Enders, F. (1977). Web-site selection by orb-web spiders, particularly *Argiope aurantia* Lucas. *Anim. Behav.* **25**, 694-712.

Fahnestock, S. R., Yao, Z. and Bedzyk, L. A. (2000). Microbial production of spider silk proteins. *Rev. Mol. Biotechnol.* **74**, 105-119.

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

Greenstone, M. H., Morgan, C. E., Hultsch, A. L., Farrow, R. A. and Dowse, J. E. (1987). Ballooning spiders in Missouri, USA and New South Wales, Australia: family landmass distribution. *J. Arachnol.* **15**, 163-170.

Goeden-Wood, N. L., Keasling, J. D. and Muller, S. J. (2003). Self-assembly of a designed protein polymer into sheet fibrils and responsive gels. *Macromolecules* **36**, 2932-2938.

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.

Hayashi, C. Y. (2002). Evolution of spider silk proteins: insight from phylogenetic analyses. *Molecular Systematics and Evolution: Theory and Practice.* Basel, Switzerland: Birkhäuser Verlag.

Hayashi, C. Y. and Lewis, R. V. (1998). Evidence from flagelliform silk cDNA for the structural basis of elasticity and modular nature of spider silks. *J. Mol. Biol.* **275**, 773-784.

Hayashi, C. Y., Shiply, 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.

Heiling, A. M. and Herberstein, M. E. (1999). The role of experience in web-building spiders (Araneidae). *Anim. Cognition* **2**, 171-177.

Heiling, A. M. and Herberstein, M. A. (2000). Interpretations of orb-web variability: a review of past and current ideas. *Ekológia (Bratislava)* **19**, 97-106.

Higgins, L. E. and Buskirk, R. E. (1992). A trap-building predator exhibits different tactics for different aspects of foraging behaviour. *Anim. Behav.* **44**, 485-499.

Higgins, L. E., Townley, M. A., Tillinghast, E. K. and Rankin, M. A. (2001). Variation in the chemical composition of orb webs built by the spider *Nephila clavipes* (Araneae, Tetragnathidae). *J. Arachnol.* **29**, 82-94.

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

Huang, J., Valluzzi, R., Bini, E., Vernaglia, B. and Kaplan, D. L. (2003). Cloning, expression, and assembly of sericin-like protein. *J. Biol. Chem.* **278**, 46117-46123.

Iconomidou, V. A., Chryssikos, D. G., Gionis, V., Pavlidis, M. A., Paipetis, A. and Hamodrakas, S. J. (2000). Secondary structures of chorion proteins of the teleostean Fish *Dentex dentex* by ATR FT-IR and FT-Raman spectroscopy. *J. Struct. Biol.* **132**, 112-122.

Knight, D. P., Knight, M. M. and Vollrath, F. (2000). Beta transition and stress-induced phase separation in the spinning of spider dragline silk. *Int. J. Biol. Macromol.* **27**, 205-210.

Lee, J. W., Jiang, L., Su, Y. T. and Tso, I. M. (2004). Is Central Mountain Range a barrier to giant wood spider *Nephila pilipes*? A population genetic approach. *Zool. Stud.* **43**, 112-122.

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.

Nyffeler, M. and Breene, R. G. (1991). Impact of predation upon honey bees (Hymenoptera, Apidae), by orb-weaving spiders (Araneae, Araneidae and Tetragnathidae) in grassland ecosystems. *J. Applied Ecol.* **111**, 179-189.

Olive, C. W. (1980). Foraging specialization in orb-weaving spiders. *Ecology* **61**, 1133-1144.

Pasquet, A., Ridwan, A. and Leborgne, R. (1994). Presence of potential prey affects web building in an orb-weaving spider *Zygiella x-notata*. *Anim. Behav.* **47**, 477-480.

Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L. and de Guevara, O. L. (1997). Nutritional value of editable insects from the state of Oaxaca, Mexico. *J. Food Composition Anal.* **10**, 142-157.

Riekel, C. and Müller, M. (1999). *In-situ* x-ray diffraction during forced silking of spider silk. *Macromolecules* **32**, 4464-4466.

Robinson, M. H. and Robinson, B. (1970). Prey caught by a sample population of the spider *Argiope argentata* (Araneae: Araneidae) in Panama: a year's census data. *Zool. J. Linn. Soc.* **49**, 345-358.

Rypstra, A. L. (1981). The effect of kleptoparasitism on prey composition and web relocation in a Peruvian population of the spider *Nephila clavipes*. *Oikos* **37**, 179-182.

Sandoval, C. P. (1994). Plasticity in web design in the spider *Parawixia bistriata*: a response to variable prey type. *Funct. Ecol.* **8**, 701-707.

Sethuraman, A., Vedantham, G., Imoto, T., Przybycien, T. and Belfort, G. (2004). Protein unfolding at interfaces: slow dynamics of alpha-helix to beta-sheet transition. *Proteins* **56**, 669-678.

Sherman, P. M. (1994). The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Anim. Behav.* **48**, 19-34.

Smallwood, P. D. (1993). Web-site tenure in the long-jawed spider: is it risk-sensitive foraging, or conspecific interactions? *Ecology* **74**, 1826-1835.

Tai, P. L., Hwang, G. Y. and Tso, I. M. (2004). Inter-specific sequence

conservation and intra-individual sequence variation in a spider silk gene. *Int. J. Biol. Macromol.* **34**, 295-301.

Tatulian, S. A., Cortes, D. M. and Perozo, E. (1998). Structural dynamics of the *Streptomyces lividans* K+ channel (SKC1): secondary structure characterization from FTIR spectroscopy. *FEBS Lett.* **423**, 205-212.

Tso, I. M. (1999). Behavioral response of *Argiope trifasciata* to recent foraging gain: a manipulative study. *Am. Midl. Nat*. **141**, 238-246.

Venner, S., Pasquet, A. and Leborgne, R. (2000). Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. *Anim. Behav.* **59**, 603-611.

Vollrath, F. (1999). Biology of spider silk. *Int. J. Biol. Macromol.* **24,** 81-88.

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

Vollrath, F. and Knight, D. P. (2001). Liquid crystalline spinning of spider silk. *Nature* **410**, 541-548.

Vollrath, F. and Köller, T. (1996). Mechanics of silk produced by loaded spiders. *Proc. Roy. Soc. Lond. B* **263**, 387-391.

Watanabe, T. (1999). The influence of energetic state on the form of stabilimentum built by *Octonoba sybotides* (Araneae: Uloboridae). *Ethology* **105**, 719-725.

Wilkinson, L., Hill, M. and Vang, E. (1992). *SYSTAT: Statistics. Version 5.2.* Evanston, Illinois: SYSTAT Inc.

Wilson, D., Valluzzi, R. and Kaplan, D. (2000). Conformational transitions in model silk peptides. *Biophys. J.* **78**, 2690-2701.

Winkler, S. and Kaplan, D. L. (2000). Molecular biology of spider silk. *Rev. Mol. Biotechnol.* **74**, 85-93.

Work, R. W. and Young, C. T. (1987). The amino acid composition of major and minor ampullate silks of certain orb-web-building spiders (Araneae, Araneidae). *J. Arachnol.* **15**, 65-80.

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