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The color of dragline silk produced in captivity by the spider *Nephila clavipes*

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Abstract

Fifty-six *N. clavipes* spiders from the same region of Florida were kept in captivity under the same conditions and fed a similar diet of crickets. Their major ampulate glands were forcibly silked. Dates, silking times, and the colors of the dragline silk produced were recorded. The colors ranged from all white through various combinations of white and yellow upon different silkings to all yellow. If a spider had been producing white silk for at least 4 h, the color being produced could suddenly change to yellow 38% of the time. These observations indicate that factors beyond diet and environment influence the color of silk produced in captivity by forcible silking. They also indicate that the spiders store both pigmented and unpigmented silks and that some aspect of forcible silking precludes the spiders' choosing the color. The yellow and white silks exhibit similar exterior surface morphologies as well as similar tensile properties.

Keywords: Color; Dragline silk; *N. clavipes*

1. Introduction

Silks are increasingly considered for use as materials of technology as well as for fabrics [1–3]. Spiders produce a rich variety of silks for different purposes including foraging under different environmental conditions such as light [4–7]. Some silks have a variety of optical properties extending from the visible to the UV wavelengths [8]. One example is *Nephila clavipes* which was described in 1767 [9] and later in one of Hahn's Monographs [10]. It is known as a golden orb weaver because of the bright yellow color of its dragline silk which has been woven into fabric [11–13] and studied for its properties [14–19]. The silk for such studies is often gathered by forcible silking. In this note we report our discoveries (1) that forcible silking of *N. clavipes* can produce dragline silk of a different color that is not necessarily representative of a particular foraging strategy and (2) that *N. clavipes* can store both pigmented and unpigmented dragline silk. Some aspect of forcible silking appears to preclude the spiders' choosing the color.

2. Experimental

2.1. Materials

The 56 mature female spiders (no males) were collected from the same region by Mark Stowe of the University of Florida, Gainsville, FL. They were kept in the same laboratory in individual plastic boxes approximately 18×18 in. square and 6 in. deep. Twine was strung just inside the circumference of the boxes so that the spiders could make small webs. One side of the boxes was exposed to northern daylight and the other to 'cool white' fluorescent light during the day with the boxes in darkness at night. The laboratory temperature was 24.5–25 °C with 36–42% relative humidity. A small damp towel was kept in each box. The boxes, towels, and webs were misted once a day. The spiders were fed approximately one cricket per day. The

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crickets were fed apples, popcorn and water. The spiders lived for four to five months in captivity.

2.2. Silking

For collection of the dragline silk, the spider was placed in a container and briefly anesthetized with carbon dioxide. It was then placed on a soft stage and held in an inverted position by a soft restraint. The restraint kept the spiders' legs away from the fibers and spinnerets. The spinnerets were illuminated with an MKH Fiber Optics Light (Nikon) which allowed continuous observation using an SMZ-10 Reflected Light Microscope (Nikon) with a 10 × eyepiece and a 1 to 4 × objective. The apparatus was similar to one described relatively recently [20] and not too different mechanically from one used before 1807 [21].

The two major ampulate dragline fibers from the anterior spinnerets were isolated, drawn and attached to a motor driven rotating mandrel lined with Teflon® sheets. The silking rate was 1.1 cm/s which is similar to the natural spinning rate for web building [22,23]. The entire apparatus was placed in a small chamber made with PVC tubing and polyethylene sheets to prevent dust, etc. from settling on the fibers. The environment in the chamber was similar to that in the boxes. After silking, the spiders were returned to their boxes and fed. The silk was wrapped in Teflon® sheet, placed in reclosable Ziploc® bags and stored under vacuum in a desiccator in the dark. The average time between silkings of a spider was 12.4 ± 6.4 days and average duration of a silking was 2.9 ± 2.0 h (the variations are standard deviations.). Most of the silking was carried out between late May and late October.

2.3. Atomic force microscopy (AFM)

An optical lever type AFM, TopoMetrix 2010, was used in the repulsive contact mode at ambient conditions. The fiber samples were mounted on the sample holder disk with the thermoplastic adhesive *Tempfix* ®. Images were obtained with a constant force of approximately 10^{-10}–10^{-9} N. Cantilevers with a silicon nitride tip of approximately 50 nm radius were used. The scanning frequency was 1 Hz with 300 data points being taken on each of the scan lines. A total of 90 AFM images were taken to obtain representative images for fibers of each color.

2.4. Tensile measurements

Single fibers were glued onto the inner edges of cardboard frames in a manner similar to that reported previously [24]. They were tested in extension using an ARES rheometer (Rheometric Scientific, USA). The frames were clamped at the inner edges with the bottom clamp being attached to an electronic analytical balance (Sartorius Handy H110) and the top to the rheometer. The gauge length was 12.7 mm. The sample was elongated at a constant rate of 0.025 mm/s after the two sides of the cardboard frame were cut. The balance was connected to a computer and the reading was recorded automatically. The force on the fiber was determined from the decrease of the initial weight measurement. The average fiber diameters were determined microscopically for each sample before testing. The testing temperature was 24.5–25 °C and the relative humidity was 36–42%.

3. Results and discussion

The individual spiders produced a considerable variation in dragline silk color. Not all the spiders produced yellow silk upon arrival. Some produced only white silk during their time in captivity and some produced only yellow silk. Others produced yellow at first silking and all white on subsequent silkings. The remainders produced different colors on different silking occasions. Table 1 summarizes these variations.

Another observation was that sometimes when the spiders were silked for at least 4 h and were producing white dragline silk, the color of the fiber being produced could spontaneously change to yellow and remain that color for the remainder of the silking. Note that no change occurred during a 4 h silking, which was producing yellow silk. The change occurred from white to yellow only. For the 26 silkings, which were producing white silk and for which the silking duration had been at least 4 h, 10 underwent the change to yellow and 16 continued as white. Of all the silkings, approximately 7% were at least 4 hs long and exhibited the change. It is interesting to note that a change of color has been reported for *Cyrtophora molluccensis*. It produced white silk for the egg sac and then switched to green silk for the final covering [25].

It also was observed that the yellow silks produced by the spiders varied from pale yellow to bright yellow to vibrant mustard yellow. When these silks were dissolved in a solvent such as LiBr solution, the silk solution exhibited the yellow color (A similar effect has been observed for *Nephila madagascariensis.*) [26]. Further, a film cast from the solution retained the color. However, dialysis of the solution removed the color and fibers regenerated from the dialyzed

Table 1
Number of spiders producing dragline silks of various colors during captivity

Color	Number
White only	16
White at first silking and then all yellow on subsequent silkings	0
White at first and then mixed[a] on subsequent silkings	16
Yellow at first and then mixed[a] on subsequent silkings	8
Yellow at first silking and then all white on subsequent silkings	7
Yellow only	9

[a] Mixed means producing different colors on different silking occasions.

solution did not exhibit the color of the original fibers. That is, they were white. These observations are consistent with the visible coloring materials being xanthurenic acid (low amounts)—weakly yellow and fluorescent, a hydroxylated benzoquinone or napthaquinone—yellow and another quinone with similar properties but unstable [27].

As noted above, 16 of the spiders produced only white silk and 9 produced only yellow while the others produced silks with some variation in the color. The spiders were collected from the same region, kept in captivity under the same conditions and fed a similar diet of crickets, but produced different color fibers. Therefore, these observations indicate that the differing colors are not necessarily representative of a particular foraging strategy. Some factors other than diet and environment appear to have influenced the colors produced in captivity by forcible silking. It is not clear what these factors might be. The sudden change from white to yellow silk in some (but not all) of the spiders silked for at least 4 h give a similar indication. The data also indicate that the spiders retain both pigmented and unpigmented silks. Further, the data indicate that some aspect of forcible silking might preclude the spiders from choosing when one color of silk is produced relative to another. Depending on the lighting conditions, the coloring materials can be incorporated into the body of the fibers [7]. Thus, it is possible that these silks of different color might have somewhat different physical properties. This possibility is reinforced by the fact that the composition of major ampulate silk is not uniform even when the silk is taken from a single spider during a single forcible silking [28]. Some variation of the mechanical properties of dragline silks has been observed [18,24,29]. Therefore, both the surface morphology and the tensile properties of the fibers were examined.

Typical AFM images of the exterior surfaces of the yellow silk fibers are shown in Fig. 1(a) and (b). They are similar to those of the white silk in Fig. 1(c) and (d). Most fibers such as those in Fig. 1(a) and (c) exhibit surface features similar to those reported previously for yellow silk of *N. clavipes* [30] and unstretched scaffolding fibers of *Latrodectus hesperus* [31]. A smaller number of fibers such as those in Fig. 1(b) and (d) also exhibited a 'transverse'

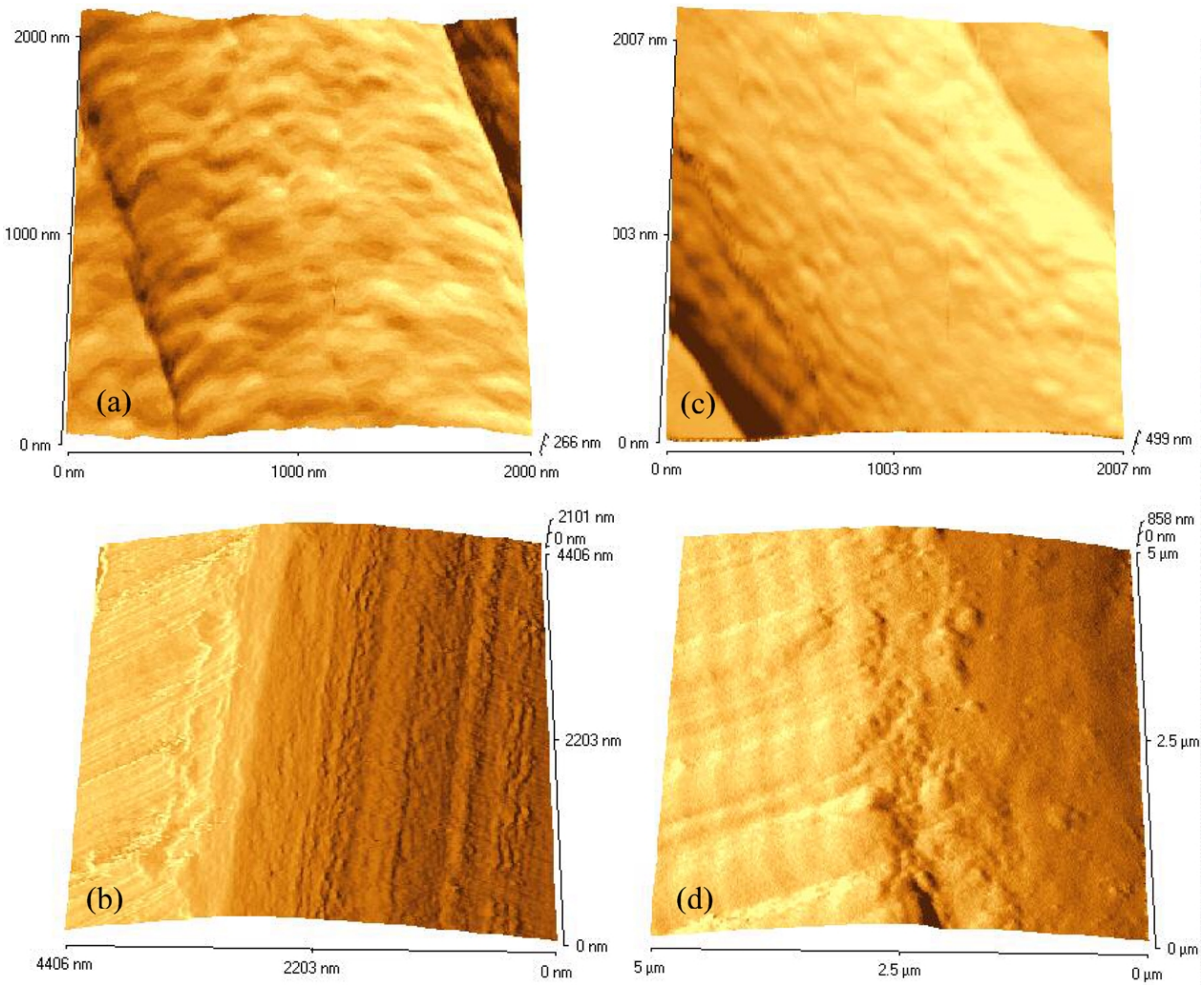

Fig. 1. AFM images of the exterior surface of yellow (a), (b) and white (c), (d) dragline silk of *N. clavipes*.

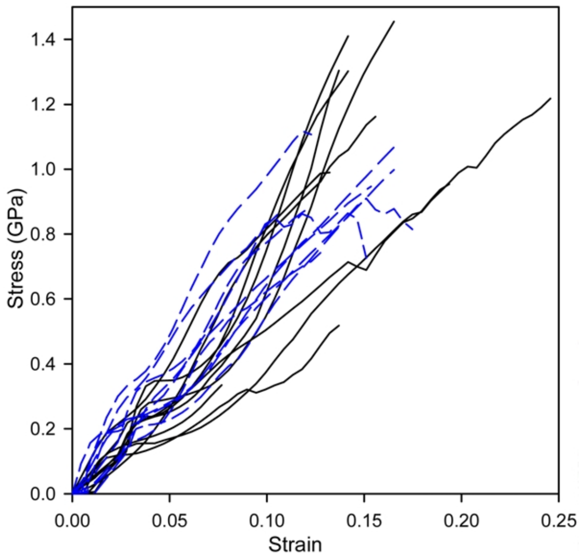

Fig. 2. Tensile engineering stress–strain curves for white (10 continuous lines) and yellow (9 broken lines) dragline silks.

morphology similar to that observed in the interior surface of the yellow silk of *N. clavipes* exposed by abrasion [30]. Fig. 2 shows tensile stress–strain curves for single fibers of the white and yellow silks. Table 2 presents a summary of the data. The results for the two types of fiber are not too different and overlap by about one standard deviation. Thus, it appears that the silks of different colors have similar tensile properties.

4. Conclusions

Factors other than diet and environment appear to influence the color of the dragline silk produced in captivity by forcible silking. The spiders retain both pigmented and unpigmented silks. Some aspect of forcible silking appears to preclude the spiders from choosing the color. It is not clear what these factors might be. The yellow and white silks exhibit similar surface morphologies as well as tensile properties.

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Table 2
Summary of tensile measurements on the white and yellow dragline silks. The results are averages and the uncertainties are standard deviations

Color	White	Yellow
Modulus (GPa)	7 ± 2	9 ± 3
Yield stress (GPa)	0.22 ± 0.07	0.14 ± 0.02
Strength (Gpa)	1.1 ± 0.3	0.9 ± 0.1
Failure strain (%)	15 ± 4	14 ± 2

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