Persistent Stickiness of Viscous Capture Threads Produced by Araneoid Orb-Weaving Spiders

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ABSTRACT The most commonly encountered spider orb-webs rely on sticky, viscous capture threads to retain prey. These threads are composed of supporting fibers covered by a complex aqueous solution that forms a series of droplets, each with a glycoprotein granule that confers adhesion. This adhesive system normally functions for less than a day before being replaced. Despite their ephemeral nature, we found that the stickiness of viscous threads persists for much longer. When measured over the course of 7 days, small decreases in the adhesion of Larinioides cornutus threads were not statistically significant. Threads of Araneus marmoreus, Argiope trifasciata, and Metepeira labyrinthica were aged for 8–10 months and remeasured under environmental conditions similar to those under which initial measurements were made. When returned to humidity similar to that under which measurements were initially made, neither the droplet volumes nor the stickiness of aged threads differed significantly from those of newly spun threads. These observations indicate that when viscous threads are protected from contamination, the compounds responsible for their hydrophilic and adhesive properties do not degrade easily. J. Exp. Zool. 309A:11–16, 2008. © 2007 Wiley-Liss, Inc.

How to cite this article: Opell BD, Schwend HS. 2008. Persistent stickiness of viscous capture threads produced by araneoid orb-weaving spiders. J. Exp. Zool. 309A:11–16.

Orb-weaving spiders of the Araneoidea clade appeared in the Cretaceous (Selden, ’89; Péanlver et al., 2006) and have since spun viscous capture threads. These composite threads are spun from the spigots of two adjacent silk glands (Foelix, ’96): flagelliform glands produce a pair of supporting axial fibers and aggregate glands coat these fibers with a complex aqueous solution (Gosline et al., ’84; Peters, ’86, ’95; Vollrath et al., ’90). Soon after threads are spun, this solution forms regularly spaced droplets, whose size and spacing are probably determined by the amount of material deposited, the axial fibers’ diameters, and the solution’s viscosity. A glycoprotein granule coalesces inside each droplet. This granule confers thread stickiness and may also affect droplet size (Vollrath and Tillinghast, ’91). Hydrophilic compounds in the viscous fluid maintain droplet size by attracting atmospheric moisture and preventing droplets from drying and cause the droplet volume to change with ambient humidity (Vollrath et al., ’90; Townley et al., ’91).

Most araneoid orb-weaving species take down and replace their webs daily (Carico, ’86), using recycled material to construct a new web (Breed et al., ’64; Peakall, ’71; Townley and Tillinghast, ’88). Arachnura melanura removes and replaces the capture spiral daily, but reuses the frame and radial components for several days (Robinson and Lubin, ’79). A few spiders, such as members of the genera Nephila and Herennia, repair damaged web sectors and replace their webs less frequently (Robinson and Robinson, ’73; Robinson and Lubin, ’79; Higgins, ’87). The ephemeral nature of most orb-webs has lead to the general impression

Grant sponsor: National Science Foundation; Grant number: IOB-0445137.
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Received 20 July 2007; Revised 9 September 2007; Accepted 30 September 2007

Published online 26 October 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.426
that viscous threads quickly lose their stickiness (Eberhard, '80). In many situations, dust, pollen, and rain probably do rapidly diminish the stickiness of these threads. Indeed, there seems to be little adaptive value for these threads to remain sticky for more than a few days. This study determined if the stickiness of viscous threads produced by orb-weaving species that replace their threads daily degrades when these threads are protected from typical environmental contaminants. We did so by measuring the stickiness of viscous threads produced by one species daily for 7 days after web construction. We measured the threads of three other species on the day of web construction, and again 8–10 months later after their threads were returned to temperature and humidity conditions similar to those under which their stickiness was initially measured.

MATERIALS AND METHODS

Collecting methods

We collected threads from webs constructed by adult females of four species in the family Araneidae from Montgomery County, Virginia: Larinioides cornutus (Clerck, 1757), Araneus marmoreus Clerck, 1757, Argiope trifasciata (Forsskål, 1775), and Metepeira labyrinthica (Hentz, 1847). Large web sectors were first collected on one or two 18-cm diameter aluminum rings, on whose upper rims a double-sided tape was applied to hold threads securely. From these rings we collected threads on samplers that allowed us to photograph threads and measure their stickiness. We made these samplers by gluing raised 4.8-mm wide brass supports to microscope slides at 4.8-mm intervals. The double-sided tape secured threads to these supports under their native tensions. Before photographing threads and measuring their stickiness, we inspected threads under a dissecting microscope and removed damaged sectors.

Aging threads

We collected enough threads sectors from the webs constructed by four L. cornutus females to measure the stickiness of three sectors per day for 7 days. These threads were kept at laboratory humidity (47–59%) in covered microscope slide boxes until measurements were completed. After finding that the stickiness of these threads did not decrease appreciably with age, we measured threads taken from the webs of A. marmoreus, A. trifasciata, and M. labyrinthica. These threads were photographed and their stickiness was measured on the day of web construction. The samplers on which threads were collected were then stored in microscope slide boxes and kept in a dessicator, where humidity ranged from 18 to 29%, depending on the freshness of the desiccant. To confirm that droplet volume had diminished during storage, we photographed threads 3–4 months after they were collected when laboratory humidity had dropped to 25–30% during the winter and then returned these threads to the dessicator. When laboratory humidity again returned to initial levels of around 50%, we allowed the threads to equilibrate to ambient laboratory humidity for 2 days and again photographed them and measured their stickiness.

Measuring thread stickiness

We measured thread stickiness with an instrument composed of an interchangeable contact plate attached to the lever arm of a jeweled escapement, which transferred force to a load cell machined to increase its sensitivity (Opell and Hendricks, 2007). A linear actuator pressed the thread against a contact plate at a speed of 0.06 mm/sec until a force of 25 μN was generated, at which time the direction of travel was immediately reversed. As the strand was withdrawn, its adherence to the plate exerted force on the plate and the maximum force achieved before the strand pulled free of the plate was recorded as the strand’s stickiness.

The stickiness of three thread sectors from an individual’s web was measured and the mean value was used as that individual’s stickiness. Contact plates were covered with Scotch Magic tape (St. Paul, MN), which provided a smooth acetate surface (3M Co., 2002) that maximized thread contact. The acetate surface was replaced shortly before measurements were taken, allowing us to measure each thread with a fresh, unused region of the plate. Contact plates used to measure L. cornutus threads were 1,613 μm wide and those used to measure the stickiness of the other three species were 2,133 μm wide. Immediately before measuring the stickiness of a thread sector, we recorded temperature (T), relative humidity (RH), and barometric pressure (BP). These values are reported in Tables 1 and 2. For L. cornutus, we computed the successive changes in stickiness on

J. Exp. Zool. DOI 10.1002/jez
days 2–7 by subtracting the stickiness on that day from the stickiness recorded on day 1.

**Measuring thread droplet features**

Using techniques described more fully by Opell and Hendricks (2007), we photographed the threads of each species, measured these digital images with Image J (Bethesda, MD; Image J, 2006), and computed the droplet volume (DV) using the following formula:

\[ DV = (2 \pi \text{droplet width}^2 \times \text{droplet length})/15. \]

This formula was derived from the equation of a parabola rotated around its x-axis because this shape resembled most closely the profile of viscous droplets.

Although some viscous threads have smaller secondary droplets between their primary droplets (Opell and Hendricks, 2007), we measured only primary droplets. The secondary droplets of *A. marmoreus*, *A. trifasciata*, and *M. labyrinthea* comprised 4.2, 10.8, and 4.9%, respectively, of these threads’ total viscous volumes (Opell and Hendricks, unpublished observations). Thus, primary droplet volumes accounted for most of the threads’ viscous volume. Additionally, volumetric changes of secondary droplets resulting from changes in ambient humidity probably mirror those of primary droplets.

**Statistical analysis**

We used the SAS statistical package (S.A.S. Institute Inc., Cary, NC) to test the normality of data and compare stickiness and droplet volume values. Data were considered normally distributed if \( P > 0.05 \) for a Shapiro-Wilk W-statistic test. We examined normally distributed data with one-way analyses of variance (ANOVA), \( t \)-tests (T), and regression. Data that were not normally distributed were compared with Wilcoxon two-sample tests (W2). \( P \)-values of 0.05 or less were considered significant.

**RESULTS**

For threads of *L. cornutus*, which were aged over the course of days, the regression models showed that the T, RH, and BP were not related to stickiness, either collectively (\( P = 0.38 \)) or individually (\( P = 0.15, 0.14, \) and 0.12, respectively). ANOVA showed no differences in the stickiness measured over the course of 7 days (Table 1): days 1–7 (\( P = 0.37 \)), days 1, 3, 5, and 7 (\( P = 0.78 \)), days 1, 4, 7 (\( P = 0.79 \)), and days 1, 2, 6, 7 (\( P = 0.25 \)). There was no difference between the stickiness registered on days 1 and 7 (\( T \) \( P = 0.55 \)) or between the pooled stickiness values of days 1 and 2 and the pooled values of days 6 and 7 (\( T \) \( P = 0.07 \)). Likewise, progressive changes in thread stickiness from days 1 though 7 (Table 1) did not differ (ANOVA \( P = 0.51 \)) or show a continuous change when regressed against day (\( P = 0.10 \)).

The droplet volumes of thread produced by *A. marmoreus*, *A. trifasciata*, and *M. labyrinthea* changed with ambient humidity. Under low laboratory humidity during the winter, the droplet volumes of 3-month-old threads were 499.18 ± 115.83 (SEM), 76.19 ± 19.20, and 17.73 ± 8.39 × 10^2 µm³. These volumes are 73, 90, and 72%, respectively, of the initial droplet volumes. After equilibrating to the higher laboratory humidity under which final stickiness measurements were made, thread volumes were 74, 144, and 127%, respectively, of the initial volumes (Table 2).

For threads that were aged for 8–10 months, the T, RH, and BP under which new and aged threads were measured (Table 2) did not differ for *A. marmoreus* (W2 \( P = 0.07, 0.21, \) and 0.20, respectively) and *M. labyrinthea* (W2 \( P = 0.42, T \) \( P = 0.88, \) and \( T \) \( P = 0.22, \) respectively). The T and BP under which *A. trifasciata* threads were measured did not differ (\( T \) \( P = 0.28 \) and W2

| Table 1. Stickiness of threads produced by four Larinioides cornutus females and the conditions under which they were measured over the course of 7 days (mean ± 1 SEM) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Day 1           | Day 2           | Day 3           | Day 4           | Day 5           | Day 6           | Day 7           |
| Temperature (°C) | 23.8 ± 0.3      | 24.0 ± 0        | 23.8 ± 0.3      | 23.8 ± 3        | 22.8 ± 0.3      | 22.0 ± 0        | 24.0 ± 0        |
| % RH            | 59 ± 0          | 53 ± 0          | 55 ± 0          | 49 ± 1          | 47 ± 0          | 48 ± 0          | 54 ± 1          |
| (mm Hg)         | 1018 ± 0        | 1017 ± 0        | 1014 ± 0        | 1009 ± 0        | 1011 ± 0        | 1016 ± 0        | 1016 ± 0        |
| Stickiness (µN) | 378.1 ± 79.9    | 389.7 ± 50.6    | 376.5 ± 20.9    | 357.4 ± 38.3    | 327.1 ± 44.2    | 247.0 ± 32.9    | 325.2 ± 30.7    |
| Change in stickiness (µN) | +11.7 ± 33.2 | −1.6 ± 61.4 | −20.6 ± 46.0 | −53.2 ± 43.1 | −131.1 ± 77.8 | +52.9 ± 55.0 |

RH, relative humidity.

J. Exp. Zool. DOI 10.1002/jez
TABLE 2. Stickiness of newly collected and aged threads produced by three species and the conditions under which they were measured (mean ± 1 SEM)

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Temperatures °C (°C)</th>
<th>% RH (mm Hg)</th>
<th>Drop length (µm)</th>
<th>Drop volume ($\times 10^2 \mu m^3$)</th>
<th>Stickiness (µN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>New</td>
<td>Aged</td>
<td>New</td>
<td>Aged</td>
<td>New</td>
</tr>
<tr>
<td>New Aged</td>
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<td></td>
</tr>
</tbody>
</table>

Araneus marmoreus (N = 5)

New: 284 ± 5 | Aged: 270 ± 3

Argiope trifasciata (N = 7)

New: 22.3 ± 0.3 | Aged: 22.0 ± 0.0

Metepeira labyrinthina (N = 5)

New: 23.3 ± 0.3 | Aged: 23.0 ± 0.0

RH, relative humidity.

$P = 0.37$, respectively), although RH did differ (T $P = 0.04$). The droplet lengths of new and aged threads of A. marmoreus, A. trifasciata, and M. labyrinthina did not differ (T $P = 0.23$, 0.37, and 0.34, respectively). The droplet widths of new and aged threads of A. marmoreus and M. labyrinthina did not differ (T $P = 0.56$ and 0.65), although those of A. trifasciata differed (T $P = 0.01$). However, the droplet volumes of new and aged threads of A. marmoreus and A. trifasciata did not differ (Table 2; T $P = 0.83$ and 0.14, respectively), nor did those of new and aged threads of M. labyrinthina (W2 $P = 0.53$). Likewise, aging did not alter the stickiness of A. marmoreus, A. trifasciata, and M. labyrinthina threads (Table 2; T $P = 0.92$, 0.89, and 0.43, respectively).

To provide a preliminary assessment of the effect of droplet volume on the stickiness of A. marmoreus, A. trifasciata, and M. labyrinthina threads, we regressed the droplet volumes of the combined new and aged threads of each species against the stickiness of its threads. For A. marmoreus and A. trifasciata, whose threads had the greatest droplet volumes and the greatest variance in droplet volumes (Table 2), the droplet volume was directly related to thread stickiness (N = 10, $P = 0.037$, $R^2 = 0.44$ and N = 14, $P = 0.041$, $R^2 = 0.30$, respectively). However, for M. labyrinthina, this relationship was not significant (N = 10, $P = 0.134$, $R^2 = 0.26$).

DISCUSSION

Our observations show that when viscous threads are stored under protective conditions, they retain substantial amounts of adhesion for long periods of time. Moreover, the droplets of these threads continue to respond volumetrically to changes in ambient humidity. These observations indicate that the compounds responsible for both droplet adhesion and droplet hygroscopicity do not degrade easily. Our storage conditions may be more protective of glycoproteins and other droplet compounds than those found in nature, although they were less stringent than those routinely used to lyophilize proteins. The persistent stickiness of viscous threads has practical implications for those who study these threads. If these threads are properly stored and measured under controlled conditions, their features remain substantially unchanged for long periods, allowing more time to complete studies of their properties.

Although not significant, the stickiness of L. cornutus threads seemed to decrease (Table 1). Even if the variance of our data prevented us from statistically documenting small decreases in thread adhesion over time, our results show that the adhesive system of viscous threads is very robust. For spiders that replace their webs daily, such a system may seem overdesigned. However, there may be no selective advantage to producing capture threads that degrade easily unless the durability of capture threads interferes with the digestive recycling of the silk’s components. In fact, the practice of thread ingestion and component recycling, which seems to have evolved along with orb-web architecture (Opell, ’98), may have reduced constrains on the chemical composition of thread, as much of the cost of thread production could be recovered when the thread was reused. Thus, the persistent stickiness of viscous threads may simply be a by-product of selection for an effective adhesive system.

The stickiness of newly produced A. marmoreus, A. trifasciata, and M. labyrinthina threads recorded
in this study was similar to the values of 62, 240, and 110 μN, respectively, reported by Opell and Hendricks (2007). The intraspecific tendency for droplet volume to be directly related to thread stickiness observed in our study is consistent with the interspecific relationship between these features noted by Opell (2002). However, a closer examination of the threads of *A. marmoreus*, *A. trifasciata*, and *M. labyrinthina* raises questions about the generality of this relationship.

With a droplet volume of 727 μm$^3$, *A. marmoreus* threads register only 75.5 μN, whereas the threads of *M. labyrinthina* have a droplet volume of 22 μm$^3$ and register a stickiness of 119.5 μN and the threads of *A. trifasciata* have a droplet volume of only 102 μm$^3$ and register a stickiness of 231.5 μN (Table 2). This suggests that droplet volume is not the sole determinant of thread stickiness. However, a fuller analysis of these differences requires information about the number of droplets per millimeter length of *A. marmoreus*, *A. trifasciata*, and *M. labyrinthina* threads (4.3, 6.7, and 38.0, respectively; Opell and Hendricks, 2007) and the manner in which adhesion is recruited from multiple droplets in a thread span. Although viscous thread spans of increasing lengths register increasing stickiness, the efficiency with which adhesion increases diminishes as the number of contacting droplets increases, indicating that droplets interior to the edges of thread contact contribute successively less adhesion (Opell and Hendricks, 2007). Modeling this pattern as a reduction by half for each successive interior pair of droplets (Fig. 1), Opell and Hendricks (2007) estimated that, regardless of the droplet size and distribution, thread stickiness does not increase beyond a span of approximately 12 droplets.

Thus, only in the case of *A. marmoreus* do all the droplets that contact a 2,133 μm plate (9.17 droplets) contribute to a thread’s stickiness. In the case of *A. trifasciata* and *M. labyrinthina* threads, contributing spans comprising 12 droplets are 1,791 and 316 μm long, respectively, less than the full plate width. If only one-half of the stickiness of the pair of droplets immediately interior to the edge droplets of a contributing thread span is recruited and only one-quarter of the stickiness of the droplet pair interior to these is recruited, then only one-half and one-quarter of these droplets’ volumes, respectively, contribute to the thread’s stickiness. When this pattern is extended to the threads of *A. marmoreus*, a total of $\frac{3}{13}\frac{16}{16} (=3.8125)$ droplets worth of viscous volume contributes to the stickiness registered by a contact plate (Fig. 1). In the case of *A. trifasciata* and *M. labyrinthina*, a total of $\frac{30}{32} (=3.9375)$ droplets worth of viscous volume contributes to a thread’s stickiness. Dividing the mean stickiness registered by fresh threads of *A. marmoreus*, *A. trifasciata*, and *M. labyrinthina* by their total contributing volumes (2772, 402, and $87 \times 10^2 $ μm$^3$, respectively) yields efficiency values of 0.027, 0.576, and 1.374 μN/×10$^2$ μm$^3$, respectively.

Although our sample size is very small and these values are only approximations, they indicate that droplet composition differs among species and strongly influences the adhesion generated by a given volume of viscous material. Moreover, as the stickiness per droplet volume of these three species is inversely related to the droplet size, a droplet’s surface to volume ratio may also enhance thread stickiness. Thus, the direct relationship between the droplet volume per millimeter thread length and thread stickiness noted by Opell (2002) may be an oversimplification of a more complicated set of relations. We are conducting more extensive studies to determine how factors such as the water content of viscous droplets and the size of their glycoprotein granules may impact thread stickiness.

![Fig. 1. Contribution of droplets to thread stickiness as modeled after Opell and Hendricks (2007). Edge droplets contribute the greatest adhesion and the successive pairs of inner droplets half as much adhesion.](image-url)
ACKNOWLEDGMENT

Mary Lee Hendricks and Charles Hannum helped with the field and laboratory work.

REFERENCES


