The adhesive delivery system of viscous capture threads spun by orb-weaving spiders

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INTRODUCTION

Capture thread is the sticky, spiral component of a spider’s orb-web that is supported by the web’s non-sticky radial lines. By lengthening prey retention time, these sticky threads give a spider more time to locate, run to and subdue prey before they escape from the web (Chacón and Eberhard, 1980), a capability that is particularly important for the capture of large, profitable prey (Blackledge and Eliason, 2007). Two sub-clades of orb-weaving spiders comprise the Araneoidae clade, the Deinopoidea and the Araneoidae (Coddington and Levi, 1991; Griswold et al., 1998; Griswold et al., 2005). Like their non-orb-weaving ancestors, the Deinopoidea produce dry, cribellar capture threads formed of thousands of fine protein fibrils that are supported by a pair of axial lines (Eberhard and Pereira, 1993; Opell, 1994; Opell, 1999; Peters, 1984; Peters, 1986; Peters, 1992). In contrast, the orb-weaving members of the more diverse Araneoidae produce viscous capture threads composed of regularly spaced aqueous droplets supported by a pair of axial fibrils (Peters, 1986; Tollinghast et al., 1993; Vollrath, 1992; Vollrath et al., 1990; Vollrath and Tollinghast, 1991). At the center of each droplet is a glycoprotein granule that is thought to confer thread stickiness (Tollinghast et al., 1993; Vollrath and Tollinghast, 1991). The fluid that covers these granules and surrounds the axial fibers contains hydrophilic compounds, which attract atmospheric moisture, maintaining the droplet volume (Townley et al., 1991; Vollrath et al., 1990).

Each of a spider’s paired median spinnerets bears a single flagelliform gland spigot and two aggregate gland spigots. The flagelliform glands produce axial fibers and the aggregate glands produce viscous aqueous material. Aggregate gland material initially forms a continuous cylinder around the fibers, but quickly condenses into a series of regularly spaced droplets. In some species these droplets have a pattern of larger primary droplets with smaller secondary droplets between them (Fig. 1).

Just as a thread’s axial fibers and viscous droplets are physically linked, so too are they functionally linked (Agnarsson and Blackledge, 2009). Thread adhesion is generated when droplets contact a surface and, as the thread is pulled from this surface, the adhesion of multiple droplets is recruited by the axial fibers in what has been termed a suspension bridge mechanism (Opell and Hendricks, 2007). This is demonstrated by the observation that when the stickiness of viscous threads is measured with contact plates of increasing width, thread stickiness increases, something that is not observed when this procedure is used to measure the stickiness of cribellar threads (Opell and Schwend, 2008a). This mechanism operates perfectly, with each successive pair of droplets interior to the edge of thread contact contributing progressively less adhesion until a limiting number of droplets contacts a surface, after which no additional thread stickiness is achieved (Opell and Hendricks, 2007). The contribution of axial fiber extensibility can be documented by stretching threads to reduce their extensibility and then measuring their stickiness with contact plates whose widths are increased in proportion to thread elongation, thereby maintaining the number of droplets that contribute to a thread’s stickiness (Opell et al., 2008). This procedure shows that the per droplet stickiness of stretched threads is less than that of threads at their native tensions and indicates that axial fiber extensibility accounts for roughly one-third of a viscous thread’s stickiness.

In this study we confirm the hypotheses supported by these earlier studies and examine other features that affect the stickiness of viscous threads by studying threads produced by 16 araneoid species.
from three families. We include viscous threads with a wide range of droplet sizes and spacing (Fig. 1; Table 1). We measure the stickiness of each species’ threads with contact plates of four widths and determine the size, shape, spacing and water content of their droplets. We also characterize the residual extensibility of their axial fibers. The six-variable model that we develop accounts for the contributions of the thread’s larger primary droplets and of its smaller secondary droplets and explains 88% of the variance observed in these 64 mean stickiness values. Dividing these species into three groups, whose models weigh the contributions of variables differently, explains over 96% of the variance and shows that there are different pathways to achieving thread adhesion.

**MATERIALS AND METHODS**

**Species studied and thread collection**

We collected threads from newly spun orb-webs constructed by adult females of 16 Araneoidea species: one species from the family Theridiosomatidae: *Theridiosoma gemmosum* (L. Koch); three species from the family Tetragnathidae: *Meta ovalis* (Gertsch), *Leucauge venusta* (Walckenaer) and *Tetragnatha elongata* Walckenaer; and 12 from the family Araneidae: *Micrathena gracilis* (Walckenaer), *Micrathena sagittata* (Walckenaer), *Argiope aurantia* Lucas, *Argiope trifasciata* (Forskål), *Metapeira labyrinthina* (Hentz), *Larinioides cornutus* (Clerck), *Cyclosa turbinata* (Walckenaer), *Verrucosa arenata* (Walckenaer), *Araneus pegnia* (Walckenaer), *Araneus bicentenarius* (McCook), *Araneus marmoreus* Clerck and *Mangora maculata* (Keyserling). Threads of *Meta ovalis* and *Araneus bicentenarius* were collected from forests at the base of Grandfather Mountain, Avery County, NC, USA. Threads of the other species were collected from sites within 10 km of Blacksburg, Montgomery County, VA, USA.

We collected threads on samplers made by gluing 4.8 mm wide brass bars at 4.8 mm intervals to microscope slides. Double-sided Scotch® tape (Tape 665; 3M, St Paul, MN, USA) on the bars held thread strands securely at their native tension. To ensure that physical and performance characteristics of an individual spider’s threads were not affected by changes in environmental conditions, we photographed its threads and measured stickiness under the same laboratory temperature and per cent relative humidity (RH). Threads of all species except *Meta ovalis* and *Araneus bicentenarius* were photographed and tested for stickiness on the same day they were collected. Threads of these two species were processed 2–4 days after being collected. During this time these threads were kept either on microscope slide samplers or on the 15 cm diameter rings used to collect orb-web sectors. Threads were secured to the 5 mm wide rings and 5 mm wide central supporting bar of these rings by double-sided tape. Samplers were kept in closed microscope slide boxes and rings in tightly sealing plastic food storage boxes. These storage conditions combined with the high summer humidity probably maintained threads in an environment in which RH was above 50% while they were being transported to the laboratory, where they were then stored at 49% RH before being photographed and measured. This short delay in measuring threads that are protected from dust and other damage appears to have little effect on their droplet volumes and thread stickiness (Opell and Schwend, 2008b). However, as another study has shown that the axial fibers of viscous threads became stiffer and lost extensibility and strength when

![Fig. 1. Viscous threads of some of the species that were studied.](image)

| Species                  | N  | Spider mass (mg) | Primary droplets | Secondary droplets | Temperature (°C) | Relative humidity |
|--------------------------|----|------------------|------------------|-------------------|------------------|------------------|
| *Argiope aurantia*       | 5  | 841.9±138.7      | 3.5±0.4          | 62.7±3.2          | 20.9±2.3         | 23.7±0.1         |
| *Araneus marmoreus*      | 10 | 498.5±74.2       | 3.7±0.3          | 67.1±4.5          | 20.9±2.3         | 23.7±0.1         |
| *Argiope trifasciata*    | 11 | 510.8±82.0       | 6.1±0.6          | 41.5±2.5          | 22.5±2.0         | 23.7±0.1         |
| *Araneus bicentenarius*  | 6  | 407.0±67.6       | 6.2±1.7          | 50.5±5.1          | 21.5±3.4         | 23.7±0.1         |
| *Larinioides cornutus*   | 4  | 265.9±27.2       | 6.2±1.4          | 41.6±6.4          | 33.1±8.4         | 33.2±0.2         |
| *Verrucosa arenata*      | 5  | 74.3±12.2        | 7.4±0.8          | 27.9±1.0          | 9.7±1.4          | 33.2±0.2         |
| *Araneus pegnia*         | 9  | 65.7±7.1 (8)     | 8.6±0.7          | 38.6±3.6          | 16.2±5.7 (3)     | 33.2±0.2         |
| *Micrathena gracilis*    | 5  | 73.4±9.5         | 9.9±1.9          | 30.2±2.2          | 14.3±1.5 (4)     | 33.2±0.2         |
| *Theridiosoma gemmosum*  | 5  | 2.8±0.2 (4)      | 11.0±1.4         | 14.4±3.4          | 9.2±2.9          | 24.0±1.5         |
| *Micrathena sagittata*   | 5  | 46.8±5.5         | 11.4±2.0         | 25.9±1.4          | 10.7±1.8         | 24.0±1.5         |
| *Tetragnatha elongata*   | 4  | 71.0±17.2 (3)    | 13.0±1.4         | 28.2±4.8          | 10.3±0.6 (3)     | 26.0±0.0         |
| *Metapeira labyrinthina* | 8  | 10.4±1.0         | 17.5±3.5         | 23.5±1.9          | 9.3±1.3          | 23.5±0.2         |
| *Meta ovalis*            | 8  | 19.3±2.2 (7)     | 20.6±2.4         | 14.9±0.9          | 5.2±0.6          | 23.5±0.2         |
| *Leucauge venusta*       | 10 | 22.0±3.1         | 29.9±2.1         | 13.8±1.2          | 5.6±0.5 (7)      | 22.6±0.2         |
| *Cyclosa turbinata*      | 9  | 7.2±0.8 (8)      | 33.4±6.9         | 13.2±1.0          | 7.0±0.3          | 22.6±0.2         |
| *Mangora maculata*       | 6  | 11.2±1.0         | 67.6±9.0         | 4.4±0.3           | 3.0±0.2 (4)      | 23.6±0.2         |

Means ± 1 s.e. Numbers in parentheses give the sample size if less than that listed in the second column.
measured 20 days after they were spun (Agnarsson et al., 2008), it is not possible to completely rule out aging affects on thread stickiness.

**Measuring droplet features**

We made digital images of three suspended viscous threads from each spider’s web at ×50 (for species with large droplets), ×125 (for species with smaller droplets) or ×250 magnification (for the species with tiny droplets) and of two threads at the next higher magnification. To simulate an insect adhering to the droplets, we then placed a glass cover slip across the brass supports on the microscope slide sampler to flatten thread droplets and made images of two threads at the higher magnification. We used ImageJ (ImageJ, 2006; http://www.uhnresearch.ca/facilities/wcif/imagej/) to measure these images.

The threads of many species feature large droplets with one or more smaller droplets between each pair of larger droplets (Fig. 1). We refer to larger droplets as primary droplets and smaller droplets as secondary droplets. To obtain the number of primary droplets per millimeter (DPMM) we measured the length of a thread span from the left edge of one primary droplet to the left edge of another primary droplet farther along the thread. We measured two or three thread spans per individual. To maximize the number of droplets included in the span we measured some Araneus marmoreus, Argope aurantia and Argiope trifasciata threads under a dissecting microscope. The droplet distributions of other threads were determined from images made at low magnification under a compound microscope. For each individual spider we calculated DPMM as the total number of primary droplets included in the measured spans divided by the total span length and then computed the mean value for each species. The mean number of primary droplets included in a thread span ranged from 14 in Argiope aurantia to 27 in Micrathena gracilis to 54 in Mangora maculata. As thread stickiness was measured with contact plates of four widths, we computed the number of primary droplets that contacted each plate (PDPP) by multiplying DPMM by plate width (PWIDTH) expressed in mm.

We measured the length (dimension parallel to the thread’s axial fibers) and width of three primary droplets (PL and PW, respectively) on two higher-magnification images of each spider’s threads and the length and width of the secondary droplets, if any, included in these spans (SL, SW, respectively). We divided the total number of secondary droplets in a span by the total number of primary droplets to obtain the ratio of an individual’s secondary to primary droplets (SPRATIO) and then computed mean values for individuals and species.

The outline of the lower half of a droplet is close to the shape of a parabola (Opell and Hendricks, 2007). Therefore, we computed the volume of each individual’s primary and secondary droplets (PV and SV, respectively) as the volume of a parabola rotated around the x-axis using the following formulas:

\[
PV = \frac{2\pi PW^2 PL}{15}, \quad (1)
\]

\[
SV = \frac{2\pi SW^2 SL}{15}. \quad (2)
\]

Fig. 2 shows the relationship between DPMM and primary droplet volume. We also calculated an index that we term SHAPE from the mean PL and PW of each species as P in equation of a parabola defined by the outline of the lower half of a droplet: \( Y = X^2/(2P) \), where \( X = PL/2 \) and \( Y = PW/2 \). Thus, \( P = PL^2/(4PW) \). The larger the value of \( P \), the more elongated the droplet along the axial fibers; the smaller the value of \( P \), the more spherical the droplet.

We computed the total number of secondary droplets per contact plate (SDPP) as the product of PDPP and SPRATIO, and the total volume of secondary droplets per contact plate (SVPP) as the product of SDPP and SV.

We calculated the proportion of water in primary droplets because their larger size permitted us to measure their dimensions more accurately than those of secondary droplets and because they comprise the greatest amount of the thread’s viscous material. At the time we collected thread samples for use in the measurements described above, we also collected thread samples from each individual’s web on microscope slides with more widely spaced supports, also covered with double-sided tape on their upper surfaces. These we placed in microscope slide boxes and stored in a desiccating cabinet. Approximately 1 year later, we sputter-coated these threads with 50nm of gold palladium and photographed them under the high vacuum of a scanning electron microscope (Fig.3). We measured the droplets as above and computed the desiccated primary droplet dry volume (PDV) of each individual’s threads using the formulas given above. We then calculated the volume of water in each individual’s fresh primary droplets (PVW) as its PV–PDV and calculated the proportion of water in the primary droplets (PH2O) as PV/PV.

In species whose threads had closely spaced primary and secondary droplets, we could not clearly delineate the boundaries of the primary and secondary droplets after these threads were flattened on cover slips. Therefore, we measured the total flattened
droplet area of two threads from each individual’s web and the lengths of these thread spans. We then divided the thread’s surface area by its span length to obtain the surface area per millimeter (APMM). Multiplying APMM by PWIDTH in mm yields the surface area of primary and secondary droplets that contact a plate (APP). Flattening droplets also makes it possible to observe a thread’s glycoprotein granules. We characterized granule shape, measured their dimensions and, from these data, determined their flattened surface areas and estimated their volumes.

**Determining effective droplet number**

Droplets at the edges of thread contact make the greatest contribution to thread stickiness and those at the center the least (Opell and Hendricks, 2007). This decreasing adhesive contribution of successive interior droplets should result in a droplet span beyond which no additional thread stickiness is registered. To account for this pattern of declining adhesion we used the same common denominator to compute an effective droplet number (EDN) for each species’ threads on each of the four contact plate widths. EDN describes the total droplet equivalents that contribute to a thread’s stickiness.

For this purpose we previously (Opell and Hendricks, 2007) used a common denominator of 2.0 to compute an approximate EDN. However, in this study we empirically determined which common denominator produced the 64 EDN values (one value for threads of each of the 16 species on each of the contact plates of four widths) used to model stickiness per contact plate. We did this by first computing 10 sets of 64 EDN values, one for each denominator from 1.1 to 2.0. After finding that the $R^2$ of the six-variable regression model described in the Results was greatest for EDN values computed with a common denominator of 1.4, we then computed sets of 64 EDN values for denominators 1.40 to 1.45. A comparison of the fit of these denominator values shows that a denominator of 1.43 produces the six-variable regression model with the greatest $R^2$ (Fig. 5).

Therefore, we computed EDN such that each successively interior droplet contributed $1/1.43 = 0.6993$ the adhesion of the next outermost droplet (Fig. 5).

**Measuring residual extensibility of threads**

We define the residual extensibility (RE) of a thread as the ratio of its length at rupture to its native, in-web length. This index is the same as breaking extension and describes the amount of extensibility remaining in a viscous thread after a spider manipulates it during web construction. We measured RE by capturing viscous capture threads on 5 mm wide bars that were attached to the jaws of a digital caliper opened to a distance of 3 mm. Double-sided carbon tape (used for mounting specimens to be examined with a scanning electron microscope) secured threads to bars. Threads were then gently pressed into the tape with a smooth surface. To hold these threads even more securely, we applied Kores® mimeograph correction fluid (Ink Technology, Tenafly, NJ, USA) along the length of thread spans that contacted the tape. This red fluid is a fast-drying paint whose principal solvent appears to be ether. It did not wick onto suspended threads but immediately adhered to the double-sided tape and, when dry, formed a thin seal on the tape’s surface. We then slowly separated the jaws of the caliper at a speed of approximately $232 \mu\text{m s}^{-1}$ until each of the strands broke. We computed the mean RE of at least 10 threads for most individuals and 4–11 individuals per species. Although we believe that the combined use of tape and mimeograph fluid held the threads securely, it is possible that the viscous material allowed some axial fiber slippage. However, RE exhibited an interspecific range of 3.53 to 9.06, indicating that this index is useful in characterizing the extensibility of threads at their native web tensions.

**Thread extensibility and contact plate width effects**

Differences in both the RE of a thread and the width of the contact plate used to measure thread stickiness have the potential to introduce artifacts into measurements of thread stickiness. As force increases, threads that are more extensible permit a contact plate to move further from the supports to which the thread is anchored before generating force and, in so doing, form a greater angle with the contact plate (Fig. 6, C vs B). Likewise, as the width of a contact plate increases and the lengths of suspended threads on either side of the contact plate decrease, these threads form a greater angle with the contact plate (Fig. 6, B vs A). As the angle between a thread and a contact plate increases, more force is directed perpendicular rather than parallel to the contact plate, causing the outer-most thread droplet to be pulled from the plate more easily and reducing the efficiency with which the adhesion of inner droplets is recruited. Consequently, greater RE and greater PWIDTH both have the potential to introduce negative artifacts into measured stickiness. We tested these hypothesized artifacts by determining whether RE, PWIDTH, or an interaction between RE and PWIDTH was related to stickiness.
SHAPE: twice the focal length of the parabola defined by the outline of the lower half of a primary droplet. As SHAPE is computed from droplet length and width, these variables were not included in the model.

SVPP: secondary droplet volume per contact plate.

PH2O: the proportion of water in primary droplets.

APP: the surface area of flattened primary and secondary droplets contacting a plate.

RE: residual extensibility (equivalent to breaking extension).

The failure of glycoprotein granules to contribute to the model

Those familiar with viscous threads may find the absence of glycoprotein granule features from this list of model variables surprising. These granules form within thread droplets soon after they coalesce and are thought to be largely responsible for the thread’s adhesion (e.g. Vollrath and Tillinghast, 1991; Tillinghast et al., 1993). However, each of our attempts to find a relationship between any granule feature and thread stickiness either failed or showed a small, negative contribution of granule size to thread stickiness. Therefore, as the granule data set and the images required to document it are extensive, we present these data in a separate study.

RESULTS

Tables 1 and 2 describe the features of the 16 species’ threads. Spider mass exhibits an inverse, allometric relationship to the number of droplets per millimeter (DPMM = 38.85–5.5834 ln mass; $P$ = 0.0169, $R^2$ = 0.34) and a direct, isometric relationship to primary droplet volume ($PV = 78.3823$ mass $+ 1916.44$; $P$ = 0.0001, $R^2$ = 0.68). This is reflected in an allometric decline in both primary droplet volume and the volume of primary droplets per millimeter thread length as the number of droplets per millimeter increases (Fig. 2).

Using EDN it is possible to estimate the number of droplets in a thread span beyond which little or no additional stickiness accrues, a value that we (Opell and Hendricks, 2007) have termed ‘maximum efficiency span’, as the number of contacting droplets beyond which EDN shows no appreciable increase. Fig. 7 shows this value to be in the range of 20 droplets.

Correlations among the model variables are shown in Table 3. For each plate width RE was negatively, but insignificantly, correlated with SPP (963 μm: $-0.34$, $P$ = 0.20; 1230μm: $-0.29$, $P$ = 0.27; 1613 μm: $-0.24$, $P$ = 0.38; and 2133 μm: $-0.26$, $P$ = 0.33). When all plate widths were included RE was negatively and significantly related to SPP ($P$ = 0.0141), although neither PWIDTH nor the interaction between RE and PWIDTH was significant ($P$ = 0.1958 and 0.1221, respectively). Consequently, there is evidence for an RE negative artifact, but no support for a PWIDTH artifact.

Figs 8–10 show the mean stickiness values that threads of the 16 study species registered on contact plates of 963, 1230, 1613 and 2133 μm width. When included in a regression model, each of the six variables described above had $P$ = 0.0001 except RE, which had $P$ = 0.0019. Together these variables constitute the following regression model that describes thread stickiness per plate (SPP) and has $P$ = 0.0001 and $R^2$ = 0.88:

$$SPP = (62.2537EDN) + (15.052SHAPE) + (0.002SVPP) – (213.6091PH2O) – (11.452APP) – 202.3569 . (3)$$

A maximum $R^2$ improvement analysis added these values in the following order as it increased the model’s fitness: PH2O ($R^2$ = 0.42),
Table 2. Computed features of viscous threads

| Species                  | N | Primary droplet volume (μm³) | Secondary droplet volume (μm³) | Secondary to primary droplet ratio | Primary droplet shape (μm) | Proportion of water | Flattened droplet area (μm²; mm⁻²) | Residual extensibility |
|--------------------------|---|------------------------------|-------------------------------|-----------------------------------|---------------------------|----------------------|------------------------------------|------------------------|
| Argiope aurantia         | 5 | 59297±11404                  | 1073±364                      | 1.14±0.19                         | 24.45                     | 0.49±0.03 (3)         | 63363±11385                     | 6.33±0.49              |
| Araneus marmoreus        | 10| 79730±13708                  | 3284±725 (7)                  | 0.89±0.15                         | 22.44                     | 0.48±0.11 (4)         | 128561±15379                     | 6.53±0.62              |
| Araneus trifasciata      | 11| 12005±2150                   | 1314±263                      | 1.41±0.29                         | 17.15                     | 0.41±0.15 (3)         | 30834±2747                      | 6.41±0.68              |
| Araneus bicaenarius      | 6 | 43234±1694                   | 2353±107                      | 0.79±0.12                         | 15.33                     | 0.63±0.12 (3)         | 63117±8216                      | 9.06±1.06              |
| Larinioides cornutus     | 4 | 20264±9409                   | 12021±6994                    | 0.58±0.14                         | 14.13                     | 0.29±0.08 (4)         | 34513±1935                      | 3.97±0.50              |
| Verrocusa arenata        | 5 | 6055±694                     | 243±162 (4)                   | 0.33±0.18                         | 8.65                      | 0.39±0.06 (3)         | 27084±5456                      | 3.53±0.33              |
| Araneus pegnia           | 9 | 15124±3566                   | 1340±1176 (3)                 | 0.08±0.04                         | 13.15                     | 0.66±0.05 (5)         | 120726±5554 (7)                 | 6.74±0.82              |
| Microthella gracilis     | 5 | 7510±1510                    | 453±129 (4)                   | 0.65±0.20                         | 9.71                      | 0.43±0.09 (4)         | 100813±38605                    | 8.69±0.60              |
| Theridiosoma gemmosum    | 5 | 1249±746                     | 387±284                       | 0.81±0.39                         | 4.49                      | 0.53±0.15 (2)         | 8743±2323                       | 3.82±0.34              |
| Microthella sagittata    | 5 | 4622±766                     | 286±135                       | 0.72±0.13                         | 8.37                      | 0.32±0.11 (4)         | 131080±9793                     | 5.39±0.18              |
| Tetragonatha elongata    | 4 | 5846±2332                    | 161±113 (3)                   | 0.60±0.25                         | 9.93                      | 0.03±0.02 (3)         | 26665±8293                      | 5.13±0.98              |
| Metepeira labyrinthina   | 8 | 3014±632                     | 203±124                       | 1.05±0.21                         | 8.42                      | 0.68±0.07 (4)         | 31117±3946                      | 6.89±0.64              |
| Meta ovalis              | 8 | 896±146                      | 37±16                         | 0.39±0.09                         | 4.68                      | 0.57±0.08 (5)         | 12989±2071 (7)                  | 4.53±0.27              |
| Leucage venusta          | 10| 698±186                      | 39±7 (7)                      | 0.35±0.11                         | 4.74                      | 0.70±0.08 (5)         | 15106±2314 (8)                  | 4.98±0.19              |
| Cyclosa turbinata        | 9 | 608±149                      | 74±10                         | 0.85±0.07                         | 4.43                      | 0.57±0.18 (4)         | 19679±4114 (8)                  | 5.63±0.38              |
| Mangora maculata         | 6 | 40±7                         | 13±3 (4)                      | 0.29±0.14                         | 1.09                      | 0.68±0.06 (5)         | 390±222                         | 4.93±0.28              |

Means ± 1 s.e. Numbers in parentheses give the sample size if less than that listed in the second column.

Fig. 7. Relationship between the number of primary droplets that contact a plate and effective droplet number (EDN), showing that there is little increase in EDN, and, therefore, thread stickiness, beyond a value of 20 droplets.

SVPP ($R^2=0.64$), APP ($R^2=0.68$), SHAPE ($R^2=0.75$), EDN ($R^2=0.85$), RE ($R^2=0.88$).

Although the modeled and measured stickiness correspond well overall (Figs 8–10) the values of Araneus marmoreus, Araneus pegnia, Microthella gracilis, Theridiosoma gemmosum, Microthella sagittata and Cyclosa turbinata differed conspicuously from their modeled values. When modeling the proportional contribution of the model’s six variables according to the above composite model (Fig. 11, composite model), we identified three groups of species in which a particular variable made an unusually high or low contribution to modeled stickiness. The regression model of each of these three groups had $P=0.0001$ and a higher $R^2$ value than the composite 16 species model. Consequently, their modeled stickiness values were consistently more similar to measured stickiness than were their values under the composite model (Figs 8–10).

Group 1 included Argiope aurantia, Araneus bicaenarius, Argiope trifasciata, Larinioides cornutus, Leucage venusta, Metepeira labyrinthina, Tetragonatha elongata and Verrocusa arenata, which in the composite model were characterized by large APP and SHAPE contributions. In this model all variables

Table 3. Correlations among the variables used to model thread stickiness

| SHAPE | SVPP | PH2O | EDN | APP | RE |
|-------|------|------|-----|-----|----|
| SHAPE | Composite | 0.34* | -0.22 | 0.48** | 0.41** |
|       | Group 1   | 0.22  | -0.10 | -    | 0.38* |
|       | Group 2   | 0.79** | 0.34  | 0.11 | -    |
|       | Group 3   | 0.46  | -0.94** | 0.69* | -1.20 |
| SVPP  | Composite | -0.29* | 0.07  | -0.13 | -    |
|       | Group 1   | -0.23 | -     | -0.23 | -    |
|       | Group 2   | -0.14 | 0.38  | -    | -    |
|       | Group 3   | -0.66 | 0.38  | -0.32 | -    |
| PH2O  | Composite | -0.10 | 0.24  | -    | 0.48* |
|       | Group 1   | -     | -     | -    | -    |
|       | Group 2   | -0.04 | -     | -    | -    |
|       | Group 3   | -0.54* | 0.42  | -    | -    |
| APP   | Composite | 0.47** | -     | -    | -    |
|       | Group 1   | -     | -     | -    | -    |
|       | Group 2   | -     | -     | -    | -    |
|       | Group 3   | 0.38  | -     | -    | -    |

SHAPE, twice the focal length of the parabola defined by the outline of the lower half of a primary droplet; EDN, effective droplet number; SVPP, total volume of secondary droplets per contact plate; PH2O, proportion of water in the primary droplets; APP, surface area of primary and secondary droplets that contact a plate; RE, residual extensibility.

*0.0007<P<0.05. **P=0.0007.
This model retained EDN, SHAPE, SVPP and APP (composite model were characterized by a small APP contribution. This model excluded PH2O and RE (P=0.62) and had $R^2=0.96$. The contribution of SVPP changed from positive to negative and the contribution of APP changed from negative to positive (Fig. 11).

A maximum $R^2$ improvement analysis added these values in the following order as it increased the model’s fitness: APP ($R^2=0.96$), SHAPE ($R^2=0.75$), SHAPE ($R^2=0.88$), EDN ($R^2=0.96$).

Group 3 included Cyclosa turbinata, Mangora maculata, Meta ovalis and Theridiosoma gemmosum, which in the composite model were characterized by a small SHAPE contribution. This model retained all variables ($P<0.0021$) except PH2O ($P=0.2511$) and had...
$R^2=0.97$. As in the group 2 model the contribution of SVPP changed from positive to negative and the contribution of APP changed from negative to positive (Fig. 11).

\[
\text{SPP} = (34.4850 \text{EDN}) + (8.1900 \text{SHAPE}) - (0.0121 \text{SVPP}) + (698.8896 \text{PH2O}) - (31.0785 \text{RE}) + (0.0013 \text{APP}) - 46.0925. \quad (6)
\]

A maximum $R^2$ improvement analysis added these values in the following order as it increased the model’s fitness: SHAPE ($R^2=0.25$), SVPP ($R^2=0.72$), RE ($R^2=0.77$), APP ($R^2=0.89$), EDN ($R^2=0.92$), SHAPE ($R^2=0.97$).

**DISCUSSION**

These results confirm the observation that capture threads register increased stickiness when measured with contact plates of increasing width (Figs 8–10) (Opell and Hendricks, 2007). However, with the exception of *Theridiosoma gemmosum*, this progression is restricted to threads of the 13 species having 20 or fewer DPMM. This assessment corresponds with an empirical examination of the rate of EDN increase with increasing DPMM, which also suggests that beyond a thread span composed of 20 droplets, little additional stickiness accrues (Fig. 7). This value is greater than the ‘maximum efficiency span’ of 12 droplets we estimated previously (Opell and Hendricks, 2007) because in the current study we used an empirically determined common denominator of 1.43 rather than an estimated value of 2.0 to compute EDN. Stickiness does not increase in proportion to plate width because of the declining increase in EDN as droplet number increases (Fig. 5).

In all models EDN makes the greatest positive contribution to stickiness, followed by SHAPE (Fig. 11), indicating that, for most species, the total effective amount of viscous material that contacts a surface is the greatest determinant of thread stickiness. For droplets of a given volume, SHAPE increases as droplets elongate along axial fibers. Consequently, other features being equal, droplets that are more viscous should have larger SHAPE values. SVPP makes either the smallest positive or the smallest negative contribution to stickiness. Although it is difficult to understand why this variable would reduce stickiness, its small contribution is easily explained by the small size of secondary droplets.

A positive correlation between APP and PH2O might be expected, as droplets with a higher proportion of water should be less viscous and, therefore, flatten to a larger area. However, the only correlation between APP and PH2O is a negative one seen in group 3 species (Table 2), suggesting that droplet viscosity is not dependent on droplet water content. PH2O makes either no contribution or a large negative contribution to stickiness, indicating that droplets with a higher percentage of water have a lower concentration of chemicals that contribute to adhesion. In the comprehensive model, APP makes a small negative contribution to stickiness, although it either fails to contribute to the group models or makes a small positive contribution to stickiness.

RE has either no effect or a negative effect on stickiness, supporting the hypothesis that more elastic threads form greater edge angles of contact with plates, reducing their ability to recruit adhesion from interior droplets and making it easier for them to be pulled from a contact plate. The negative impact of RE is most strongly expressed in group 3, where the small number of species results in a contrast between the low measured RE of *Theridiosoma gemmosum* and the higher values of the other three species. We found no evidence that PWIDTH imposed an artifact on the measurement of thread stickiness.

The issue of measurement artifact bears on our recent study of axial fiber extensibility and viscous thread stickiness (Opell et al., 2008). This study showed that thread extensibility contributes to thread stickiness by facilitating the recruitment of adhesion from multiple viscous droplets. By stretching threads to reduce their extensibility and then measuring the stickiness of these threads with contact plates whose widths increased proportionately to thread elongation, the study maintained the number of droplets contributing to stickiness as the thread’s extensibility decreased. The current study indicates that thread elongation also reduced the edge angles of contact between threads and contact plates (Fig. 6, B vs C), thereby slightly reducing their stickiness. Consequently, in the 2008 study it was only possible to document the positive contribution of thread extensibility to thread stickiness because this contribution overcame the negative artifact documented in the current study. Our inability to detect a measurement artifact of PWIDTH also meant we failed to call into question the conclusions of the previous study.

We attempted to measure and characterize all of the features of capture threads that our instrumentation would permit. However, other features such as the chemical composition of the viscous material and the Young’s modulus of the axial fiber may affect thread stickiness. Our effort to standardize the humidity under which threads were photographed and their stickiness measured probably resulted in the threads of some species being measured under a humidity different from that of their typical habitats. We do not know whether the concentration or strength of hydrophilic compounds in viscous droplets differs among species or, if they do, whether droplets from dry or moist habitats are more hydrophilic. When we divide the species we studied into putative high humidity (HH) and low humidity (LH) habitat groups (HH: *Araneus bicentenarius*, *Leucauge venusta*, *Mangora maculata*, *Meta ovalis*, *Micrathena gracilis*, *Micrathena sagittata*, *Tetragnatha elongata*, *Theridiosoma gemmosum* and *Verrucosa arenaeta*; LH: *Argiope aurantia*, *Araneus marmoreus*, *Araneus pegnia*, *Argiope trifasciata*, *Cyclosa turbinata*, *Larinioides cornutus* and *Metepeira laevigata*); there was no difference in either the humidity under which threads were photographed (mean ± s.e., HH 50.7±1.2%, LH 47.5±3.3%; t-test P=0.39) or the proportion of water in their droplets (mean ± s.e., HH 0.48±0.07, LH 0.51±0.05; t-test P=0.73). Consequently, differences between environmental and laboratory humidity do not appear to have imposed a systematic bias on our results.

Our results show that there is no single scaling factor that explains the range of features observed in viscous threads and that similar adhesion can be achieved by threads with quite different combinations of features, even among species in the same model group. This is seen clearly when features of the two stickiest threads, those produced by *Larinioides cornutus* and *Tetragnatha elongata*, both in group 1, are compared (Figs 8 and 10). Threads of *Tetragnatha elongata* have primary and secondary droplet volumes that are only 30% and 1%, respectively, those of *Larinioides cornutus* (Table 2). However, two other variables compensate for this difference in droplet size. With twice the droplets per millimeter, *Tetragnatha elongata* has a greater effective droplet number (5.934 for a 963–μm wide contact plate, compared with 4.125 for *Larinioides cornutus*) and, with only 3% water content, little stickiness is lost to this variable. The more pronounced increase in stickiness that *Larinioides cornutus* threads register on contact plates of increasing width is explained by their smaller number of droplets per millimeter and an associated larger increase in EDN. With only 6.21 droplets per millimeter, the EDN of *Larinioides cornutus* threads show a 46% increase from the narrowest to the widest plate; whereas with 13.0 droplets per millimeter, *Tetragnatha elongata* threads show only an 11% increase.

However, droplet number alone is not a good predictor of stickiness. *Araneus bicentenarius*, also in group 1, has the same
number of droplets per millimeter and the same EDN as Larinioides cornutus, but its threads exhibit only one-quarter the stickiness (Fig. 8), even though its primary and secondary droplets have twice the volume of those of Larinioides cornutus (Table 2). The low stickiness of Araneus bicentenarius threads can be attributed to two factors: (1) a residual extensibility that is more than twice that of Larinioides cornutus and (2) primary droplets with twice the water content of Larinioides cornutus.

Within the context of droplet sizes, which tend to be directly related to spider size, and droplet spacing, which tends to be inversely related to spider size, two hypotheses may account for the combinations and range of features seen in the threads of the 16 species that we studied. Thread features may be selected to optimize thread performance in the context of a particular habitat, web architecture or prey type. Alternatively, thread features may be shaped largely by other factors, such as a species’ phylogenetic history or the metabolic cost or efficiency of producing thread components. A test of these hypotheses could come from comparison of pairs of similarly sized congeners that occupy similar and dissimilar habitats.

Although our data are not sufficient for a comprehensive test, the three qualifying species pairs that they do contain provide tentative support for a habitat effect on thread features. Members of the Argiope aurantia–Argiope trifasciata (group 1 model) and of the Micrathena gracilis–Micrathena sagittata (group 2 model) species pairs are found in the same habitats, sometimes with webs less than a meter apart. The Argiope species pair inhabits exposed weedy areas and the Micrathena species pair inhabits moist forests. The members of each group have similar stickiness values and, when body size-related differences in droplet size and spacing are taken into account, each member has similar thread features. In contrast, members of the third species pair live in different habitats and are characterized by different group models. Araneus marmoreus (group 2) has a holarctic distribution and is a common inhabitant of trees and shrubs of the forest edge (Levi, 1971), whereas in our region Araneus bicentenarius (group 1) appears restricted to moist, high elevation forests of the Appalachian Mountains (Levi, 1971). Although the threads of these two species have similar stickiness values (Fig. 8), their values are described by different models, which assign different weights to their thread features (Fig. 11), indicating that different selective factors have shaped their thread features. A potential problem with this preliminary analysis is that, in the absence of phylogenetic studies, it assumes a similar degree of relatedness of the members of each species pair.

Our findings on the positive contributions to stickiness by both effective droplet number and the size and shape of primary viscous droplets, and the negative artifact of axial fiber residual extensibility appear to apply to most viscous threads. The role of other features, such as the small positive contribution to stickiness made by secondary droplets and the negative contributions of both the proportion of water in viscous droplets and the flattened area of threads may apply to many but not all threads. A fuller understanding of interspecific differences in the chemical composition of viscous droplets, in the performance characteristics of individual droplets, and in the mechanical properties of the thread’s axial fibers may be necessary for a better understanding of the adhesive delivery system of viscous threads.

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**LIST OF ABBREVIATIONS**

| Acronym | Description |
|----------|-------------|
| APP | surface area of flattened droplets on a contact plate of given width |
| DPM | number of primary droplets per millimeter thread length |
| EDN | the total droplet equivalents that contribute to a thread’s stickiness on a contact plate |
| PDPP | primary droplets per contact plate |
| PDV | desiccated volume of a primary droplet |
| PDV | length of a primary droplet |
| PHO | proportion of water in a primary droplet |
| PV | volume of a primary droplet |
| PW | width of a primary droplet |
| PWIDTH | contact plate width |
| PWV | volume of water in a primary droplet |
| RE | residual extensibility of a thread |
| SDPP | secondary droplets per contact plate |
| SHAPE | twice the focal length of a parabola defined by the lower half of a primary droplet |
| SL | length of a secondary droplet |
| SPP | stickiness per plate |
| SPRATIO | ratio of the number of secondary to primary droplets |
| SV | volume of a secondary droplet |
| SVPP | secondary droplet volume per contact plate |
| SW | width of a secondary droplet |

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