

Adhesive efficiency of spider prey capture threads

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Abstract

Cribellar capture threads are comprised of thousands of fine silk fibrils that are produced by the spigots of a spider's cribellum spinning plate and are supported by larger interior axial fibers. This study examined factors that constrain the stickiness of cribellar threads spun by members of the orb-weaving family Uloboridae in the Deinopoidea clade and compared the material efficiency of these threads with that of viscous capture threads produced by members of their sister clade, the Araneoidea. An independent contrast analysis confirmed the direct relationship between cribellar spigot number and cribellar thread stickiness. A model based on this relationship showed that cribellar thread stickiness is achieved at a rapidly decreasing material efficiency, as measured in terms of stickiness per spigot. Another limitation of cribellar thread was documented when the threads of two uloborid species were measured with contact plates of four widths. Unlike that of viscous threads, the stickiness of cribellar threads did not increase as plate width increased, indicating that only narrow bands along the edges of thread contact contributed to their stickiness. As thread volume increased, the gross material efficiency of cribellar threads decreased much more rapidly than that of viscous threads. However, cribellar threads achieved their stickiness at a much greater gross material efficiency than did viscous threads, making it more challenging to explain the transition from deinopoid to araneoid orb-webs.

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Introduction

Prey capture threads are crucial for the operation of aerial spider webs, as they retain insects, giving spiders more time to locate, run to, and subdue their prey. Investigations using web analogs show that increased stickiness increases insect retention time (Chacón and Eberhard, 1980). Therefore, it is not surprising that

changes in the adhesive mechanisms and stickiness of capture threads have accompanied the evolution of aerial webs (Opell, 1999a, b; Opell and Hendricks, 2007). Cribellar thread (Fig. 1A) was the first type of capture thread spun by spiders. These dry threads are comprised of an outer sheath of fine, looped protein fibrils that surrounds one or more pairs of larger, supporting fibrils (Eberhard and Pereira, 1993; Peters, 1984, 1986, 1992; Opell, 1994a, b). Fibrils are drawn from the spigots (Fig. 1B) of an oval or medially divided spinning plate, termed the cribellum (Fig. 1C), using the calamistrum, a setal comb on the metatarsus of each of a spider's fourth

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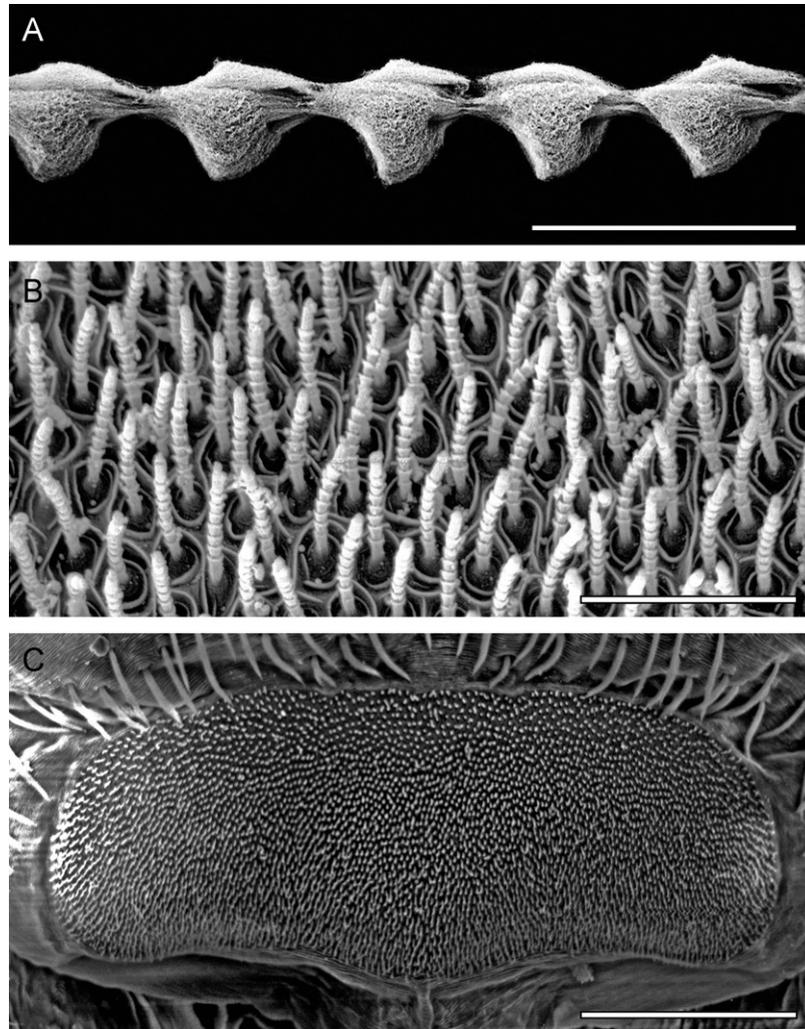


Fig. 1. Cribellar thread (A), cribellum spigots (B), and cribellum (C) of an adult female *Waitkera waitakerensis*. Scale bars represent 200, 10, and 100 μm , respectively.

leg (Eberhard, 1988; Opell, 2001). Rhythmic adductions of the median spinnerets press the fibril sheath around the supporting strands to produce a thread that has a complex, but often regular surface configuration (Peters, 1986).

Cribellar thread is spun by the most basal members of the large infra-order Araneomorpha, a clade that contains over 93% of the 39,490 living spider species (Platnick, 2008). However, members of only 22 of the 95 families that comprise this clade continue to produce cribellar threads (Griswold et al., 1999), the remainder having lost the ability to produce cribellar threads when they adopted alternative capture tactics and when araneoid orb-weavers replaced cribellar thread with viscous capture thread (Coddington, 1986, 1989; Coddington and Levi, 1991). The 11 species that comprise the Hypochilidae, the most basal araneomorph family, produce cylindrical cribellar fibrils and the 109 species of the family Filistatidae (Platnick, 2008) produce unique ribbon-like cribellar fibrils (Eberhard and Pereira, 1993).

However, the remaining cribellate spiders spin fibrils that have regularly spaced nodes along their lengths (Eberhard and Pereira, 1993; Opell, 1994a; Hawthorn and Opell, 2002, 2003). These include about 3739 species in 20 families (Platnick, 2008), although some families and even some genera contain both cribellate and ecribellate members.

Cribellar threads employ three adhesive mechanisms to stick to a range of surfaces. They adhere to rough surfaces when mechanical interlock causes fibrils to snag on setae surface irregularities in a VelcroTM-like fashion (Opell, 1994c). Smooth surfaces are held by either van der Waals forces or capillary action, depending on the nature of the fibrils and on the ambient humidity (Hawthorn and Opell, 2002, 2003). Primitive cribellar threads with cylindrical fibrils implement van der Waals forces, but show no increase in adhesion when ambient relative humidity increases from 2% to 99%. In contrast, threads formed of noded fibrils generate van der Waals forces under low humidity, but register much

greater adhesion at 45% and 95% relative humidity. At 45% humidity, cribellar threads of *Uloborus glomus* achieved 95% of the stickiness that they registered at 99% humidity, although thread performance at humidities between 45% and 2% was not evaluated. Hawthorn and Opell (2002, 2003) interpreted these results as support for the ability of noded cribellar fibrils to implement capillary forces at humidity in excess of 45%. However, capillary forces may also operate at low humidity and the observed increases in stickiness may result from increases in the thickness of the water film that covers fibrils at higher humidity.

Changes in a cribellar thread's surface configuration can alter its stickiness slightly (Opell, 1995), but the stickiness of a cribellar thread strand is determined principally by the number of fibrils from which it is formed and, therefore, is directly related to the number of spigots on the spider's cribellum (Opell, 1994a, 1995, 1999a). A few spiders increase the stickiness of their threads by folding completed strands (Opell, 2002a), although this is achieved at still greater material and behavioral cost per mm of modified thread. As the production of cribellar thread is both materially and behaviorally costly (Lubin, 1986; Opell, 1998), increases in thread stickiness relative to spider size appear to occur only when changes in web architecture result in shorter lengths of cribellar thread being included in a web. Cribellate orb-weavers of the family Uloboridae provide a striking example of this. These spiders restrict cribellar threads to regular spirals and spin threads that, relative to spider mass, are comprised of many more fibrils than those constructed by cribellate spiders that construct other web forms (Opell, 1999a). Other examples are seen within the uloborid genera *Hyptiotes* and *Miagrammopes*, which construct more reduced triangle- and simple webs, respectively, formed of shorter lengths of stickier cribellar threads that are comprised of greater numbers of cribellar fibrils (Opell, 1994a, b, 1999a). Members of the genus *Matachia* of the family Desidae construct ray webs that might be viewed as orb-web analogs (Opell, 1999a). Of the non-orb-web cribellar spiders studied, their cribellar threads are, relative to spider mass, the stickiest and comprised of the greatest number of cribellar fibrils (Opell, 1999a).

A major change in capture thread occurred during the Cretaceous (Selden, 1989; Peñalver et al., 2006) when viscous threads replaced cribellar threads in orb-webs constructed by members of the Araneoidea, the sister clade of Deinopoidea, to which cribellate orb-weavers belong (Coddington, 1986, 1989; Coddington and Levi, 1991; Garb et al., 2006). These composite threads are spun from the spigots of two adjacent silk glands (Foelix, 1996): flagelliform glands produce a pair of supporting axial fibers and aggregate glands coat these fibers with a viscous, aqueous solution that quickly forms into droplets (Peters, 1986; Vollrath et al., 1990;

Vollrath, 1992; Peters, 1995). The glycoprotein granules that coalesce inside each droplet contribute to thread adhesion (Vollrath and Tillinghast, 1991; Tillinghast et al., 1993) and the hydrophilic compounds in the surrounding fluid attract atmospheric moisture to prevent droplets from drying (Vollrath et al., 1990; Townley et al., 1991).

Orb-weaving araneoid species outnumber deinopoid orb-weavers by 13–1 and, when cob- and sheet-web araneoid species are included, the Araneoidea comprise 27% of all living spider species (Platnick, 2008). This has led to the hypothesis that viscous capture threads are superior to cribellar capture threads and have contributed to the success of the Araneoidea (Bond and Opell, 1998). Indeed, viscous threads tend to reflect less UV light than cribellar threads and, therefore, are less visible (Craig and Bernard, 1990; Craig et al., 1994; Zschokke, 2002), a characteristic that enhances prey interception. Data on the volumes of cribellar fibrils and the number of cribellar spigots (Opell, 1994a, 1996) have been used to show that viscous threads achieve greater stickiness per volume of material investment (Opell, 1997, 1998). However, a transition from horizontal to vertical web orientation that is also associated with the origin of araneoid orb-weavers (Bond and Opell, 1998) and favors prey interception (Chacón and Eberhard, 1980; Eberhard, 1989) may also have contributed to the success of araneoid orb-weavers.

Although monophyly of the orbiculariae is firmly supported (Griswold et al., 2005), several unsettling issues are associated with araneoid origin. It is difficult to envision a capture thread intermediate between dry, fuzzy cribellar threads and wet, viscous threads. This may suggest that araneoid ancestors spun axial fibers devoid of a cribellar fibril sheath before evolving the aggregate glands that cover these fibrils with viscous material. However, such a loss of thread adhesion seems contrary to the increased number of cribellar fibrils and enhanced capture thread stickiness associated with both the origin of orb-web architecture in the Deinopoidea and the architectural changes in uloborid webs described above. Moreover, araneoid orb-webs have a greater stickiness per cm² of web capture area than deinopoid orb-webs (Opell, 1999b), providing additional evidence that capture thread stickiness is favored evolutionarily. Second instar uloborids lack a cribellum and orb-weaving species construct silken sheets formed of numerous, closely spaced radii, but do not deposit spiral threads on these radii (Szlep, 1961). Consequently, they provide no indication of the role that uncoated axial fibers could play in araneoid evolution.

Our study does not resolve the dilemma that surrounds the transition from cribellar to viscous capture threads. However, it does address three hypotheses related to the adhesive efficiency of prey capture threads, an issue that is central to the origin

and diversification of the Orbiculariae. Like viscous thread, cribellar thread generates adhesion when it presses against a surface. Unlike viscous threads, whose droplets flatten and spread upon contact, cribellar thread appears to flatten less completely, permitting only the outer fibrils on the thread's lower contacting surface to generate adhesion (Hawthorn and Opell, 2003). Increases in the stickiness of cribellar threads require the addition of many fibrils that increase the thread's volume and, thus, the breadth of its contact area with a surface. However, only fibrils on the surface of this contact area appear to contribute directly to thread adhesion. Therefore, the first hypothesis that we tested was that increased cribellar thread stickiness is achieved at a rapidly decreasing material efficiency, as measured in terms of stickiness per cribellar spigot, which we equate with stickiness per cribellar fibril.

The same surface-to-volume constraint hypothesized to limit the adhesion of cribellar thread may also limit the adhesion of viscous thread, as suggested by Opell (2002b, Fig. 5). Therefore, the second hypothesis that we tested was that the material efficiency of cribellar thread decreases more rapidly than that of viscous capture thread, whose stickiness has been shown to be directly related to the volume of its droplets (Opell, 2002b). To test this hypothesis, we compared the stickiness achieved by the total cribellar fibril volumes of threads spun by nine deinopoid species with the stickiness achieved by the total wet and desiccated volumes of viscous threads spun by five araneoid species and then examined the effects that increasing material investment had on the stickiness of each type of capture thread.

Preliminary data indicate that much of a cribellar thread's adhesion is generated by narrow bands along the two edges of its contact with a surface (Hawthorn and Opell, 2003). Thus, not only do the fibrils on a thread's non-contacting surface fail to contribute to its stickiness, but it appears that the contacting fibrils along most of the thread's length also fail to contribute to the thread's expressed stickiness. Therefore, the third hypothesis that we tested was that the material efficiency with which cribellar thread generates adhesion is further limited by its inability to recruit adhesion from thread regions that lie between the edges of a thread's contact with a surface.

We tested the first two hypothesized limitations to cribellar thread stickiness using values reported in the literature. We confirmed the relationship between cribellar thread stickiness and cribellum spinning spigot number using independent contrast phylogenetic methods. We then computed and compared the adhesion achieved per volume of material in each mm length of cribellar and viscous thread and used independent contrast analyses and standard regressions to examine how rapidly the stickiness of each type of thread increases, as the volume of its material increases.

Finally, we compared the stickiness that two uloborid species' cribellar threads register on contact plates of four widths to determine if increasing lengths of thread contact are accompanied by increases in stickiness, as they are in viscous threads (Opell and Hendricks, 2007). Together, these investigations provide a clearer picture of factors that affect the adhesion of cribellar and viscous capture threads and may have influenced the evolution of orb-weaving spiders.

Materials and methods

Cost of achieving stickiness – cribellar thread

To constrain differences in the spinning behavior and the configuration of cribellar thread puffs, we restricted our study to nine species of the family Uloboridae and the out-group, *Neolana pallida* Forster and Wilton from New Zealand. This latter species is a member of the family Neolanidae, the most closely related family to Uloboridae for which data on linear cribellar threads (those that are not looped prior to deposition, Opell, 2002a) are available. Uloborid representatives include five orb-web species: *Waitkera waitakerensis* (Chamberlain) from New Zealand, *Siratoba referens* (Muma and Gertsch) from Arizona, *Uloborus glomus* (Walckenaer) from Virginia, *Philoponella arizonica* (Gertsch) from Arizona, and the introduced Asian species *Octonoba sinensis* (Simon) collected in Virginia; two triangle-web species: *Hyptiotes cavatus* (Hentz) from Virginia and *Hyptiotes gertschi* (Chamberlin and Ivie) from Washington; and two simple-web species: *Miagrammopes animotus* Chickering from Puerto Rico and an undescribed *Miagrammopes* species from Costa Rica.

Using the ancestor reconstruction method described by Huey and Bennett (1986); Opell (1994a, 1999a) demonstrated a direct relationship between the number of cribellum spigots and the stickiness of cribellar thread. We reexamined this relationship using independent contrast analysis (Garland et al., 1999; Garland and Ives, 2000), the currently preferred method for documenting functional relationships between features in a phylogenetic context. The stickiness values used in this analysis were measured with 2000 μm wide contact plates covered with 320 grit 3M waterproof silicon carbide sandpaper using methods described by Opell (1994a, 1999a) and are reported as the force in μN required to pull a 1 mm length of thread from the contact plate. These values as well as those of viscous threads taken from the literature (Opell, 2002b) might also be considered as tenacity (pull-off force normalized for thread contact). We also present previously unpublished measurements of the maximum puff widths of the same cribellar threads included in these earlier studies

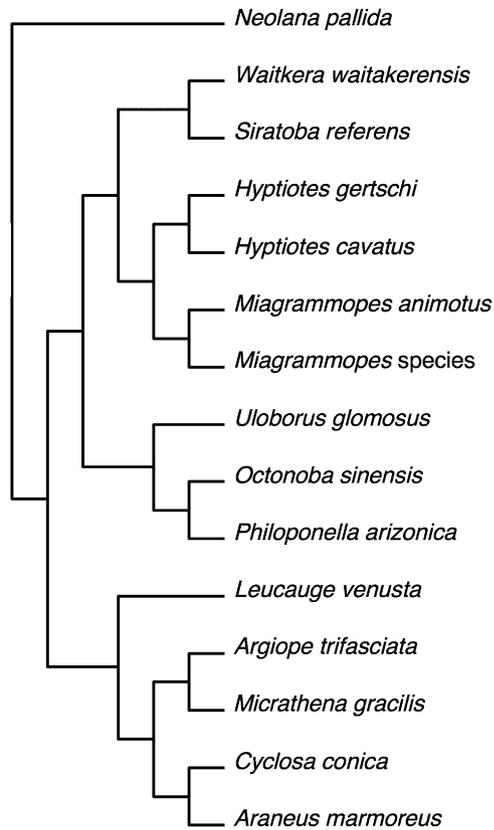


Fig. 2. Relationship of the species included in this study after Coddington (1990); Scharff and Coddington (1997), and Griswold et al. (1999).

(Opell, 1994a, 1999a). These measurements were made at the time that these threads were collected by examining suspended threads under a compound microscope. From these width measurements, we computed the maximum cross-sectional areas of cribellar thread puffs using the formula for the area of a circle. These cross-sectional areas are probably related to the flattened widths of threads that have contacted a surface, but we have no specific information on this relationship.

The pruned phylogeny of cribellate species that we used (Fig. 2) is based on Coddington (1990) and Griswold et al. (1999). We performed this analysis using the “Y contrasts vs. X contrasts (positivized)” option of the PDAP module (Midford et al., 2005) run under the Mesquite 1.06 program (Maddison and Maddison, 2005). All branch lengths were set to 1. After the independent contrast analysis confirmed the direct relationship between cribellum spigot number and thread stickiness, we performed a standard regression analysis to establish the actual relationship between spigot number and thread stickiness and used this regression model to compute the stickiness per spigot number for threads produced by cribellae of increasing spigot numbers.

We used the values of cribellar fibril features (Table 1, Fig. 3) given by Opell (1994a) to compute cribellar fibril volume. These measurements of cribellar fibrils were taken from uncoated threads observed under the transmission electron microscope and include values for all species in the present study except *H. gertschi*, *P. arizonica*, and *N. pallida*. For *H. gertschi*, we used the values of *H. cavatus*. For *P. arizonica*, we used the mean values of *U. glomus* and *O. sinensis*, the two most closely related species. For *N. pallida*, we used the mean values of the nine uloborid species. These values may be conservative, as Hawthorn and Opell (2003) report a 35.3 nm node diameter and a 85.5 internode spacing from sputter-coated threads of *H. cavatus* examined under the scanning electron microscope (SEM). We computed CFV, the volume of protein in a mm length of cribellar fibril, as follows:

$$\text{CFV} = (\text{NVOL} + \text{INVOL}) \times \text{NIUPMM}$$

where NVOL = node volume, computed as the volume of a sphere, INVOL = internode volume computed as the volume of a cylinder, and NIUPMM = the number of node–internode units per mm length of thread. Cribellar fibrils assume a highly coiled configuration (e.g., Opell, 1999a, Figs. 1–3; Hawthorn and Opell, 2003, Fig. 1B and C). Therefore, we doubled the CFV to better estimate an effective fibril volume (EFV), expressed as $\mu\text{m}^3/\text{mm}$. We then multiplied each species’ EFV by the number of spigots on its cribellum to determine the total fibril volume per mm length of its cribellar thread. Dividing the stickiness per mm length of a species’ thread by the total fibril volume per mm of thread yields an index of the thread’s gross adhesive efficiency.

Cost of achieving stickiness – viscous thread

The stickiness of viscous threads and the features of droplets (Table 2) were taken from Opell (2002b), and based on the threads of one member of the family Tetragnathidae: *Leucauge venusta* (Walckenaer) and four members of the family Araneidae: *Argiope trifasciata* (Forskål), *Micrathena gracilis* (Walckenaer), *Cyclosa conica* (Pallas), and *Araneus marmoreus* Clerck, all of which were collected from Virginia. The volume per mm length of viscous thread (VPMM) was computed as follows:

$$\text{VPMM} = \text{VPD} * \text{DPMM}$$

where VPD = volume per droplet and DPMM = droplets per mm thread length. We computed VPD using the following formula provided by Opell and Schwend (2007):

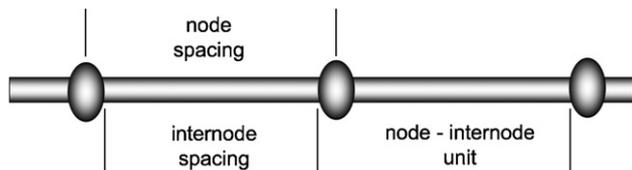
$$\text{VDP} = (2\pi \text{ droplet width}^2 \times \text{droplet length})/15$$

The percent water in these droplets is taken from a survey of araneoid species. These values are derived

Table 1. Cribellum and cribellar fibril features from Opell (1994a, 1999a) and the material efficiency with which threads achieve their stickiness

	Stickiness ($\mu\text{N}/\text{mm}$)	Cribellar puff width (μm)	Cribellar puff C. S. area (μm^2)	Spigot number	Fibril node diameter (nm)	Fibril internode diameter (nm)	Node spacing (nm)	Effective fibril volume ($\mu\text{m}^3/\text{mm}$)	Total fibril volume ($\mu\text{m}^3/\text{mm}$)	Efficiency ($\mu\text{N}/\mu\text{m}^3 \times 10^3$)
<i>Neolana pallida</i>	5.52	134 \pm 4 <i>N</i> = 17	14,103	1835	(18.1)	(9.9)	(61.1)	0.211	387.95	14.23
<i>Waitkera waitakerensis</i>	15.45	154 \pm 2 <i>N</i> = 33	18,627	3894	17.4	10.1	51.4	0.213	830.67	18.60
<i>Siratoba referens</i>	11.46	115 \pm 3 <i>N</i> = 26	10,387	1800	17.5	8.7	63.9	0.174	313.49	36.56
<i>Hyptiotes gertschi</i>	29.84	182 \pm 3 <i>N</i> = 32	26,016	7724	(19.4)	(10.3)	(63.1)	0.237	1830.59	16.30
<i>Hyptiotes cavatus</i>	26.18	180 \pm 2 <i>N</i> = 56	25,447	7276	19.4	10.3	63.1	0.237	1721.38	15.21
<i>Miagrammopes animotus</i>	32.11	167 \pm 2 <i>N</i> = 103	21,904	8990	16.2	8.6	42.3	0.177	1590.65	20.19
<i>Miagrammopes species</i>	24.41	164 \pm 3 <i>N</i> = 24	21,124	7254	18.4	9.5	52.7	0.216	1567.25	15.58
<i>Uloborus glomus</i>	15.38	174 \pm 4 <i>N</i> = 32	23,779	4717	18.4	10.4	54.5	0.232	1095.45	14.04
<i>Octonoba sinensis</i>	17.02	195 \pm 3 <i>N</i> = 36	29,865	4465	18.8	10.7	87.7	0.221	985.12	17.28
<i>Philoponella arizonica</i>	14.97	149 \pm 4 <i>N</i> = 27	17,437	5110	(18.6)	(10.6)	(71.2)	0.225	1149.91	13.02

Cribellar puff widths (mean \pm standard deviation) and the cribellar puff cross-sectional areas derived from them are newly reported measurements from these data sets. Values in parentheses denote inferred features, as described in the study's *Materials and methods*.

**Fig. 3.** Features used to compute the volumes of cribellar fibrils.

from measurements of threads that were photographed under a compound microscope on the day of their production. Other capture threads were collected from the same web at the same time and stored in a desiccator, until they were photographed under the high vacuum of an SEM 8–10 months later. The number of individuals whose wet and dry threads were measured and the mean number of droplets measured from each individual's web (in brackets) was for *L. venusta* 10 (6.0), 5 (13.8); *A. trifaciata* 11(6.0), 3 (11.3); *M. gracilis* 5 (6.0), 4 (10.5); *C. turbinata* 9 (5.7), 4 (8.0); and *A. marmoreus* 10 (5.6), 4 (4.0), respectively. Values for *C. conica* are based on those of *C. turbinata*, a species of similar adult female body size. We determined the percent water content of viscous threads by dividing the mean droplet volume based on SEM images by the mean droplet volume based on light microscope images and multi-

plying the result by 100. This permitted us to compute an adhesive efficiency for both wet and dry viscous thread volumes. To confirm the relationship between thread stickiness and wet and dry viscous thread volumes, we performed independent contrast analyses using the methods described above and the phylogeny (Fig. 2) of Scharff and Coddington (1997).

Contact width and stickiness

Adult female *U. glomus* were collected from shrubbery on the Virginia Tech Campus, near Blacksburg, Montgomery County, Virginia. Adult female *O. sinensis* were collected from a barn at Virginia Tech's Kentland Farm, located in Montgomery County 22 km WSW of Blacksburg. Each spider was placed in a 24 cm \times 37 cm plastic box with wooden dowel rods glued to the sides to provide attachment points for webs. On the day that webs were constructed, sectors were collected on 18 cm diameter aluminum rings, on whose upper rims double-sided tape was applied to securely hold threads. From these rings, threads were subsampled on microscope slides to which 4.8 mm wide brass supports were glued at 4.8 mm intervals. Double-sided tape also secured threads to these supports under

Table 2. Viscous thread features from Opell (2002b) and the material efficiency with which threads achieve their stickiness

	Stickiness ($\mu\text{N}/\text{mm}$)	Droplet length (μm)	Droplet width (μm)	Droplets per (mm)	Thread volume ($\mu\text{m}^3/\text{mm}$)	Percent water Mean \pm SE	Dry volume ($\mu\text{m}^3/\text{mm}$)	Wet efficiency ($\mu\text{N}/$ $\mu\text{m}^3 \times 10^3$)	Dry efficiency ($\mu\text{N}/$ $\mu\text{m}^3 \times 10^3$)
<i>Leucauge venusta</i>	19.3	12.5	10.1	41.4	22,112.70	69.74 \pm 8.00	6691.30	0.87	2.88
<i>Argiope trifaciata</i>	28.2	43.0	29.3	9.4	145,351.71	41.37 \pm 15.01	85,219.71	0.19	0.33
<i>Micrathena gracilis</i>	26.4	24.9	19.8	20.2	82,598.03	43.46 \pm 9.21	46,700.93	0.32	0.57
<i>Cyclosa conica</i>	9.6	11.8	9.6	34.0	15,487.88	57.44 \pm 17.85	6,591.64	0.62	1.46
<i>Araneus marmoreus</i>	32.0	59.6	46.9	6.9	378,904.40	48.08 \pm 11.46	196,727.16	0.08	0.16

their native tensions. Thread samples were inspected under a dissecting microscope to ensure that at least 12 undamaged thread sectors were obtained from each spider's web.

The stickiness of three thread sectors from an individual's web was measured with each of the four contact plates having widths of 963, 1230, 1613, and 2133 μm . These plates were covered with Scotch[®] Magic Tape 810, which provided a smooth acetate surface (3M Co., St. Paul, Minnesota) that maximized the thread contact. This acetate has a surface energy of 38.2 mN/m. The acetate was replaced frequently, ensuring that each thread was measured with an unused region of the surface. The mean value registered by a spider's threads on each plate was recorded as that of the individual's stickiness profile. The stickiness of a spider's threads was measured with contact plates of all four widths before the thread samples from another individual were measured. Immediately before taking each set of stickiness measurements, we measured temperature (T) and relative humidity (RH). Threads of ten *U. glomosus* were measured at a mean T of 24.3 $^{\circ}\text{C} \pm 1$ standard error of the mean (sem) of 0.2% and 49% RH ± 0.7 . Threads of nine *O. sinensis* were measured at 23.7 $^{\circ}\text{C} \pm 0.2$ and 51% RH ± 0.6 .

We measured stickiness with an instrument (illustrated and described in detail by Opell and Hendricks, 2007) that incorporated interchangeable contact plates attached to the lever arm of a jeweled escapement, which transferred force to a load cell that was machined to increase its sensitivity. A linear actuator pressed the thread against a contact plate at a speed of 0.06 mm/s, 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. Unlike the stickiness values of cribellar and viscous threads taken from previously

published studies, we report these stickiness values as the μN generated by each plate width and not as stickiness normalized for length of thread contact.

We used the S.A.S package (S.A.S. Institute Inc., Cary, North Carolina) to perform the statistical tests reported in this study.

Results

Material cost of thread stickiness

Tables 1 and 2 summarize the features of cribellar and viscous capture threads included in this study. When computing the total fibril volumes invested in cribellar threads we discovered that, due to improper conversions, the fibril volumes reported by Opell (1996) were 1000 times greater than they should have been. For example, Opell (1996) reported that threads of *W. waitakerensis* had an uncoiled cribellar fibril volume of 107 $\mu\text{m}^3/\text{mm}$, whereas we found that they had a volume of 0.1065 $\mu\text{m}^3/\text{mm}$ (doubled in Table 2 to account for fibril looping, where fibril volume is reported as EFV). Consequently, the material efficiency with which cribellar threads achieve their stickiness was not only much greater than previously thought (Opell, 1997, 1998), but was also much greater than that of the viscous threads, even when compared to the stickiness per dry volume of viscous threads.

Independent contrast analyses showed no relationship between cribellar thread stickiness and either cribellar puff width or cribellar puff cross-sectional area ($P = 0.1538$, $R^2 = 0.24$ and $P = 0.1677$, $R^2 = 0.22$, respectively). However, an independent contrast analysis did show that thread stickiness was directly related to the total number of cribellar spigots ($P = 0.0002$, $R^2 = 0.69$). A standard regression also showed that thread stickiness was directly related to the cribellar

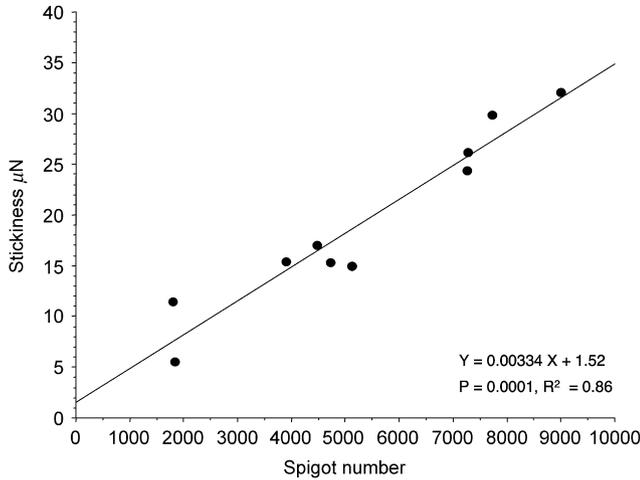


Fig. 4. Relationship between cribellum spigot number and cribellar thread stickiness.

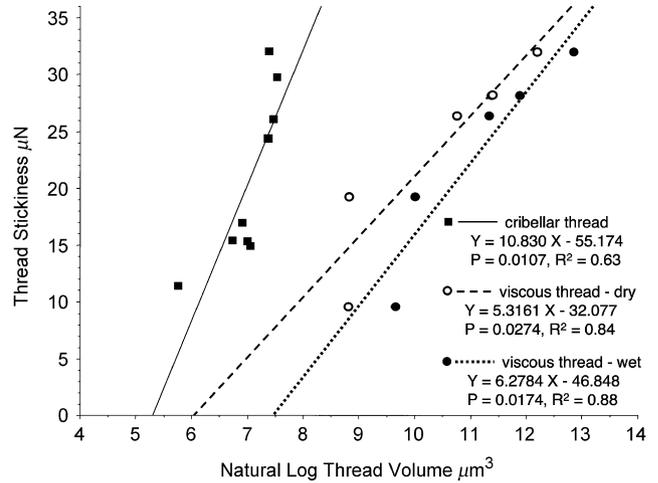


Fig. 6. Comparison of the stickiness achieved per volume of cribellar thread and viscous thread material. For viscous threads values are provided for fresh threads and for desiccated threads.

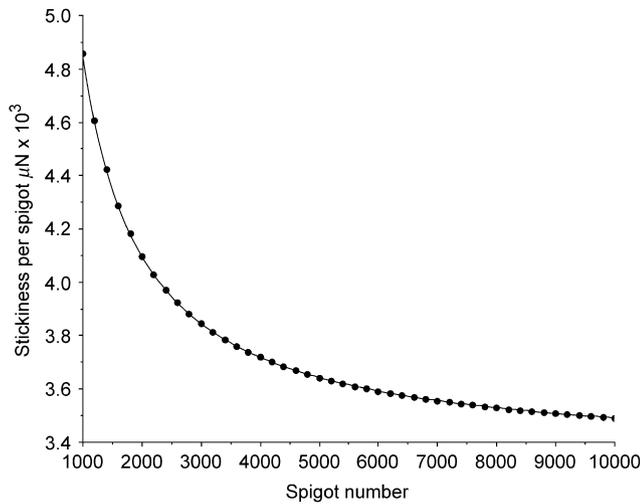


Fig. 5. Relationship between cribellum spigot number and the stickiness per cribellum spigot as derived from the regression formula presented in Fig. 6.

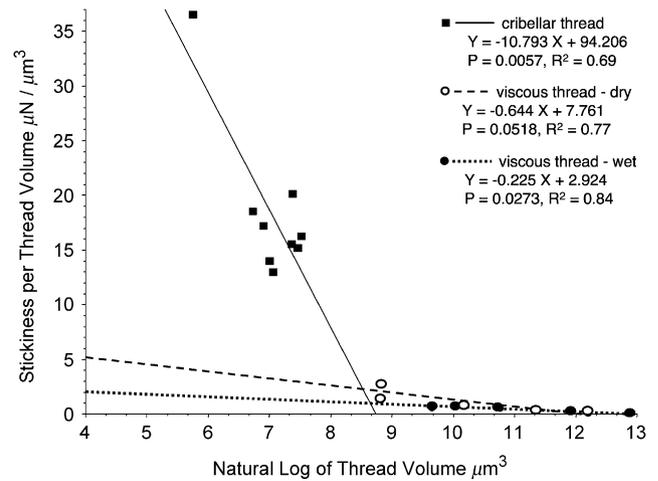


Fig. 7. Comparison of the material efficiency with which cribellar threads and viscous threads achieve their stickiness. For viscous threads, values are provided for fresh threads and for desiccated threads.

spigot number (Fig. 4) and a model based on this regression documented a rapid decline in the mean per spigot adhesion as spigot number increased (Fig. 5).

Independent contrast analyses also showed that the total volume of cribellar fibrils and the total wet and dry volumes of viscous threads were directly related to the stickiness of these threads ($P = 0.0068$, $R^2 = 0.62$; $P = 0.0323$, $R^2 = 0.83$; and $P = 0.0292$, $R^2 = 0.84$, respectively). Standard regressions of cribellar thread volumes and efficiencies included only the nine uloborid species to provide a more appropriate comparison with araneoid viscous threads. These regressions also showed a direct relationship between thread stickiness and the volumes of cribellar thread fibrils and both wet and desiccated viscous thread volumes and showed that cribellar threads achieved a much greater stickiness per thread volume than either wet or dry viscous threads

(Fig. 6). However, as thread volume increased the material efficiency of cribellar threads decreased much more rapidly than that of viscous threads (Fig. 7).

Contact width and stickiness

All stickiness values were normally distributed (Shapiro–Wilk W -statistic test $P > 0.34$) except those of *O. sinensis* threads measured with 2133 μm wide contact plates ($P = 0.033$). Threads of both *U. glomosus* and *O. sinensis* registered the same stickiness when measured with contact plates of four widths (Fig. 8; ANOVA $P = 0.15$ and Kruskal–Wallis test chi square $P = 0.71$,

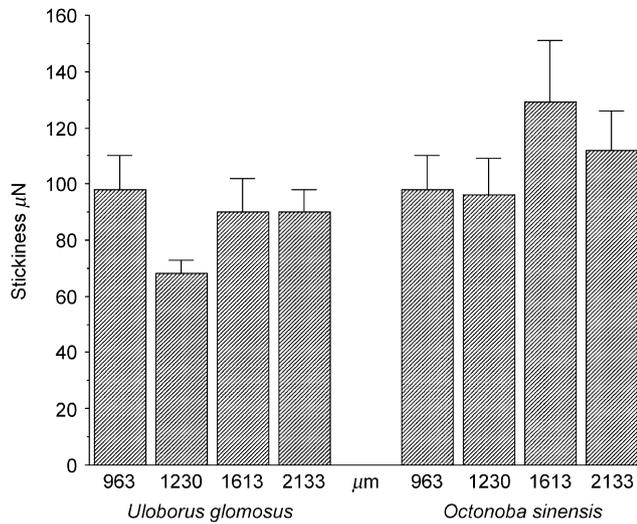


Fig. 8. Comparisons of the stickiness registered by cribellar threads of *Uloborus glomus* ($N = 10$) and *Octonoba sinensis* ($N = 9$) on contact plates of four widths. Error bars represent ± 1 standard error.

respectively). Moreover, for both *U. glomus* and *O. sinensis* there was no difference in the stickiness registered by 963 and 2133 µm plates (t -test $P = 0.56$ and Wilcoxon 2-Sample Test with normal approximation $P = 0.66$, respectively).

Discussion

The results of this study support the hypothesized limitations to the stickiness of cribellar capture threads: their adhesion is achieved at a rapidly decreasing material efficiency, as measured both in terms of stickiness per cribellar spigot and stickiness per μm^3 of cribellar fibril protein. Moreover, in contrast to the viscous capture thread, the stickiness of cribellar threads does not increase as greater lengths of thread contact surfaces. These limitations to the material efficiency of increasing cribellar thread stickiness may have constricted the size of cribellate orb-weavers. The largest uloborid species have a female body mass of 13.5 mg (Opell, 1999a), whereas some araneoid species attain adult female masses of more than 500 mg (e.g., Opell and Hendricks, 2007). However, the correction of a previous systematic computational error in determining cribellar fibril volumes presents a drastically improved picture of the material economy with which cribellar thread achieves its stickiness and shows it to be superior to the viscous thread in this regard.

These estimates of the material investment in both cribellar and viscous threads are based on gross thread volumes. However, just as not all fibrils of a cribellar

thread contribute to its stickiness, not all contacting droplets of viscous threads contribute fully to the stickiness of the viscous threads. Each successive pair of viscous droplets interior to the outermost droplets at the two contacting edges of a thread contributes less adhesion, successively, and beyond a span of roughly 12 droplets no further adhesion appears to be added (Opell and Hendricks, 2007; Opell and Schwend, 2008).

The rapid decline in material efficiency of cribellar threads formed of increasing numbers of fibrils appears to be related to the thread's intricate composition. Although the coiled and looped configurations of these fibrils allow them to press tightly against a contacting object, this configuration also prevents fibrils beneath the surface and those on the thread's upper surface from contributing to the thread's adhesion. This probably explains why neither cribellar thread width nor puff cross-sectional area is directly related to the thread stickiness. Hawthorn and Opell (2003) used measurements of the areas of flattened cribellar threads along with those of the density of surface cribellar fibrils to infer the lengths of fibrils that contacted a surface. Such estimates of contacting cribellar fibril lengths might also explain the stickiness of threads included in this study.

The inability of cribellar threads to recruit adhesion from greater lengths of contact may result from the limited elasticity of their axial fibers, which are stiffer than those of viscous capture threads (Blackledge and Hayashi, 2006). Consequently, cribellar threads probably establish smaller angles with a contacting surface as force is generated at each end of a loaded thread. This configuration would produce greater force vectors that are perpendicular to the contacting surface and smaller force vectors that are parallel to this surface (Opell and Hendricks, 2007). As it is the latter force vectors that recruit adhesion from inner regions of the thread contact, the stickiness that a cribellar thread expresses appears to come principally from outer bands of the thread contact.

Our study confirmed that differences in surface texture affect the stickiness expressed by cribellar threads (Opell, 1994c). In contrast to the absolute stickiness values registered by *U. glomus* and *O. sinensis* on 2000 µm sandpaper plates ($31.0 \mu\text{N} \pm 1$ sem of 2.2, $N = 30$ and $34 \mu\text{N} \pm 2.4$, $N = 35$, respectively, as reported by Opell, 1999a), those registered on 2133 µm acetate plates were much greater ($89.5 \mu\text{N} \pm 8$, $N = 10$ and $115.1 \mu\text{N} \pm 14$, $N = 9$, respectively). As increasing lengths of cribellar thread contact do not result in a significant increase in stickiness, the 6.65% greater widths of the acetate plates cannot explain these increases in stickiness (2.89- and 3.37-fold, respectively). Instead, the smooth acetate surface appears to maximize the extent of fibril contact with the plate's surface and perhaps also to allow fibrils to more fully generate

capillary adhesive forces (Hawthorn and Opell, 2003), rather than a combination of capillary force and mechanical interlock. The sandpaper used on contact plates had a surface energy of 65.9 mN/m and the acetate one of 38.2 mN/m. Increasing the roughness of a surface can increase its surface energy (Pizzi and Mittal, 2003). Therefore, the greater surface energy of sandpaper is probably due to its more highly textured surface. Because surface energy is computed from the contact angle of a liquid droplet placed on a surface, the high surface energy of sandpaper may not reflect the functional surface energy encountered by the very thin cribellar fibrils when they contact the silicon carbide particles and the glue used to bind them to the sandpaper.

Araneoid orb-webs and their viscous threads may be superior to deinopoid orb-webs and cribellar threads in many ways and these differences may be key innovations that contributed to the much greater diversity of the Araneoidea, as suggested by Bond and Opell (1998). However, three challenges lie before any attempt to explain the transition from cribellar to viscous capture threads in the Orbicularia: (1) it is difficult to understand how cribellar thread stickiness could have degraded as a step leading to the origin of viscous threads when an increase in the spider-size-specific stickiness of cribellar thread accompanied the origin of orb-webs in the family Uloboridae (Opell, 1999a) and subsequent architectural changes in uloborid webs (Opell, 1994b), (2) it is difficult to visualize a functional form of capture thread intermediate between cribellar and viscous threads, and (3) it is difficult to imagine how an orb-web could continue to function with spirals formed of axial fibers devoid of their adhesive component until aggregate glands evolved to cover these fibrils with viscous material. Our discovery that the gross material economy of cribellar threads exceeds that of the viscous threads makes it even more difficult to explain how a capture thread's cribellar fibril sheath could have been gradually reduced and replaced by the viscous material, as such a transition would appear to reduce the material economy of prey capture. The greater material economy with which cribellar threads achieve their stickiness may be offset by higher behavioral and metabolic costs of production, as suggested by Lubin (1986) observations, or by a lower efficiency of protein recycling, although these issues have not been thoroughly studied.

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