An Investigation of the Stickiness Mechanisms
and the Role of Nodes in Cribellar Spider Threads

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**Abstract**

Sticky prey capture threads are produced by many members of the spider Infraorder Araneomorphae. Cribellar threads are plesiomorphic for this clade, and adhesive threads are apomorphic. The surface of cribellar thread is formed of thousands of fine fibrils. Basal araneomorphs produce cylindrical fibrils, whereas more derived members produce fibrils with nodes. Cribellar fibrils snag and hold rough surfaces, but other forces are required to explain their adherence to smooth surfaces. Threads of *Hypochilus pococki* (Hypochilidae) that are formed of non-noded fibrils held to a smooth acetate surface with the same force under low and high humidities. In contrast, threads of *Hyptiotes cavatus* and *Uloborus glomosus* (Uloboridae) that are formed of noded fibrils held with greater forces to the same surface at intermediate and high humidities. The hygroscopic properties of threads spun by 8 species representing 7 genera and 4 families with noded threads allow them to absorb water, while that of two families, represented by one species each, repels water. Additionally, equations describing van der Waals and hygroscopic forces can predict the observed stickiness of these threads. This supports the hypothesis that van der Waals forces allow non-noded cribellar fibrils to adhere to smooth surfaces, whereas noded fibrils employ van der Waals forces at low humidities and add hygroscopic forces at higher humidities. Thus, there appear to have been two major events in the evolution of spider prey capture thread: the addition of hydrophilic nodes to the fibrils of cribellar threads and the replacement of cribellar fibrils by glycoprotein glue.
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AN INTRODUCTION TO CRIBELLAR THREAD AND ADHESIVE MECHANISMS
**Cribellar thread:**

Capture threads in an aerial web buy time for a spider to respond to prey. An insect that strikes the web is entangled and delayed proportional to the stickiness of the capture threads, allowing the spider to subdue prey that would otherwise escape (Eberhard 1989). The first capture thread was cribellar thread (Fig. 1.1a). This was produced by the ancestral members of the Infraorder Araneomorphae, or “true spiders,” which contains the greater part of araneaid diversity (Coddington and Levi 1991). Cribellar thread is a dry capture thread comprised of a wooly mass of fine fibrils supported by a pair of larger, axial fibers and, in some groups, additional strands. Cribellar fibrils produced by primitive araneomorphs are cylindrical, but those produced by the more derived members of this clade have regularly spaced nodes (Fig. 1.1b) (Eberhard & Pereira 1993). Cribellar thread is produced by the basal members of most major araneomorph clades and the phylogeny of cribellate families mirrors that of the complete araneomorph phylogeny (Coddington & Levi 1991; Griswold et. al. 1999).

A spider produces cribellar thread by drawing fibrils from spigots on the cribellum (Figs. 1.1c,) using the calamistrum, a comb of setae on the metatarsus of the fourth legs (Opell 2001). These fine fibrils are combined with larger, paired supporting strands to produce wooly puffs (Eberhard 1993). The principle determinant of cribellar thread stickiness is the number of fibrils that form a thread, as gauged by the number of spigots on a spider’s cribellum (Opell 1994), although thread configuration can alter stickiness (Opell 1999, 2002).
Mechanisms of Adhesion:

Cribellar thread appears to rely on at least two major stickiness mechanisms. The fibrils on its surface can snag on an insect’s setae, and hold them like the soft, looped side of a Velcro fastener. Cribellar thread also adheres to nonsnagging surfaces such as graphite, polished steel and glass (Eberhardt 1980, Hawthorn, unpublished observation) by an unknown mechanism. It holds more tightly to the smooth surface of beetle elytra than to the heavily setose surface of a fly notum (Opell 1994). This study examines forces that permit cribellar threads to stick to surfaces that are fairly smooth even on a microscopic level (Autumn et al. 2000).

Adhesion is most often achieved by a combination of several mechanisms. These can be grouped as mechanical interlock (such as the snagging described above), adsorption or van der Waals forces, and electrostatic attraction (Allen 1992 a, b, c). Van der Waals forces technically
encompass the hydrogen bonding facilitated by a thin film of water, but as this is described by different equations I treat this separately as hygroscopic attraction. A fourth mechanism, diffusion of polymer chains across the interface of a bond (Allen 1992a), requires the initial presence of a solvent and is unlikely to occur in dry cribellar threads. Peters (1984, 1986) suggested that cribellar threads may derive at least part of their stickiness from electrostatic attraction. However, Opell (1995b) found that cribellar thread sticks with equal force to gold and paraffin surfaces, which have very different dielectrical properties. This observation does not support an electrostatic mechanism in these threads and I do not examine this force further.

Van der Waals forces: Van der Waals (or London dispersion forces, as they are more formally termed) depend only on the presence of nuclei and electrons, and thus can operate between molecules provided they are in sufficiently close proximity (Hobsa and Zahradnik 1988). Van der Waals forces are the main attractive forces among the molecules of most liquids and may cause the molecules of surfaces that are in close proximity to adhere. These relatively weak interactions arise when an instantaneous dipole in one molecule creates a synchronized instantaneous dipole in neighboring molecules, producing a net attractive force between them. Aggregates of molecules can produce enough cumulative force to exert an attractive effect at a distance of 50 nm, making this a potentially major factor in the strength of adhesive bonds (Rigby et al. 1986). There is evidence that this is the major mechanism responsible for the stickiness of gecko toes that have very fine, closely packed arrays of 2 µm diameter setae (Autumn et al. 2000). It is probable that the even finer cribellar fibrils could achieve the small intermolecular gaps required for van der Waals forces to operate.

Hygroscopic mechanism: Opell (1995b) suggested hygroscopic forces as a mechanism for cribellar thread stickiness. Water sticks to surfaces by adhesive forces and to other water molecules by cohesive forces, both of which involve hydrogen bonding. The forces of adhesion are usually stronger than cohesion, so the strength with which a thin film of water holds two surfaces together is determined by surface tension (Stork 1979) and Laplace pressure (Israelachvili 1992). It is not necessary for this film of water to be secreted by an organism, as a sufficiently hydrophilic substance can attract moisture from the atmosphere. For example, viscous capture threads have been shown to absorb moisture from the air to increase their volume (Townley et al. 1991).
Role of Fibril Structure and Composition:

The structure of cribellar fibrils themselves promises to shed some light on the mechanisms of adhesion. The nodes on derived cribellar fibers (Fig. 1b) limit contact to the surface of the nodes rather than to the full surfaces of the fibrils as in primitive, cylindrical fibers. The nodes of these fibrils may contain groups of hydrophilic amino acids that attract and hold atmospheric water and, thereby, enhance the hygroscopic attraction of the fibrils. Since hygroscopic forces are usually stronger than van der Waals forces on a macroscopic scale, molecular changes in the noded regions of fibrils would compensate for reductions in the surface area of contact.

The Cribellar Silk Gene:

Spider silks are coded by a family of genes, of which ten genes encompassing three spider species and two genera have been sequenced. Most spider silks consist of a single exon (Hayashi & Lewis 1998), and all have repetitive, glycine and proline rich regions that contribute to their fibrous and elastic nature (Hayashi & Lewis 1998). The repetitiveness of these sequences means that even a relatively short sequence can yield information about the structure of the entire protein. The most conclusive test of a hydrophilic or hygroscopic stickiness mechanism would be the construction of a molecular model of the cribellar silk protein that contains hydrophilic groups arranged in a manner that explains both the observed stickiness of cribellar thread and the noded configuration of cribellar fibrils.

Research questions and Methods:

In this study I examined the degree to which van der Waals and hygroscopic surface tension forces contribute to the stickiness of cribellar thread, and investigated the putative role of
nodes in maximizing thread stickiness. To this end, I employed five approaches: 1. I expanded a comparison of the stickiness of cribellar thread under different humidities. 2. I examined with the electron microscope cribellar fibrils that had been dusted with nanospheres to determine if there are differences in the molecular structure and adhesive properties of node and internode regions of these fibrils. 3. I compared the hygroscopicity of noded and non noded threads by examining the behavior of a drop of water on the threads under a light microscope. 4. I determined the contact between noded and non-noded fibrils with smooth surfaces, constructed models that predicted stickiness based on van der Waals and hydroscopic forces, and compared these values to measured thread stickiness. 5. I developed a c DNA library and initiated screening for DNA sequences that code for cribellar fibril protein. Approach 2 proved problematic because nanospheres clumped and their liquid carrier did not penetrate the cribellar fibril mat and, consequently, formed an electron-dense surface coat that prevented the examination of underlying fibrils. Therefore, this approach is not described further.

LITERATURE CITED


Chapter 2

EVOLUTION OF ADHESIVE MECHANISMS IN CRIBELLAR SPIDER PREY CAPTURE THREAD: EVIDENCE FOR VAN DER WAALS AND HYGROSCOPIC FORCES *

Abstract

Sticky prey capture threads are produced by many members of the spider infraorder Araneomorphae. Cribellar threads are plesiomorphic for this clade, and viscous threads are apomorphic. The outer surface of cribellar thread is formed of thousands of fine, looped fibrils. Basal araneomorphs produce non-noded cribellar fibrils, whereas more derived members produce noded fibrils. Cribellar fibrils snag and hold rough surfaces, but other forces are required to explain their adherence to smooth surfaces.

Threads of *Hypochilus pococki* (Hypochilidae) formed of non-noded fibrils held to a smooth plastic surface with the same force under low and high humidities. In contrast, threads of *Hyptiotes cavatus* and *Uloborus glomosus* (Uloboridae) formed of noded fibrils held with greater force to the same surface at intermediate and high humidities. This supports the hypothesis that van der Waals forces allow non-noded cribellar fibrils to adhere to smooth surfaces, whereas noded fibrils, owing to the hydrophilic properties of their nodes, add hygroscopic forces at intermediate and high humidities. Thus, there appear to have been two major events in the evolution of adhesive mechanisms in spider prey capture thread: the addition of hydrophilic nodes to the fibrils of cribellar threads and the replacement of cribellar fibrils by viscous material and glycoprotein glue.

KEYWORDS: Araneomorph-character evolution-spider silk-thread stickiness
INTRODUCTION

An aerial web’s sticky prey capture threads retain prey and buy time for a spider to respond. An insect that strikes a web is held and delayed proportional to the stickiness of these threads, allowing a spider to subdue prey that would otherwise escape (Eberhard, 1989). The Infraorder Araneomorphae contains the “true spiders,” that comprise the greater part of araneaid diversity (Coddington & Levi, 1991). Unlike their sister clade, the Mygalomorphae, many araneomorphs produce sticky prey capture threads, the plesiomorphic type being cribellar thread (Fig. 2.1). Cribellar thread is a dry capture thread comprised of a wooly mass of fine fibrils with diameters of 10 – 20 nm (Figs. 2.2, 2.3; Opell, 1994a; Eberhard & Pereira, 1993) supported by a pair of larger, axial fibers and, in some groups, additional strands (Eberhard & Pereira, 1993). A spider produces cribellar thread by drawing fibrils from spigots on the cribellum (Fig. 2.4) using the calamistrum, a comb of setae on the metatarsus of the fourth legs. These fine fibrils are combined with supporting strands to produce wooly puffs (Eberhard & Pereira, 1993). The principle determinant of cribellar thread stickiness is the number of fibrils that form a thread, as gauged by the number of spigots on a spider’s cribellum (Opell 1994a, 1999a).

Cribellar thread is produced by the basal members of most major araneomorph clades and the phylogeny of cribellate families mirrors that of the complete araneomorph phylogeny (Coddington & Levi, 1991; Griswald et. al., 1999). Of the 90 araneomorph families 22 contain cribellate members (Griswald et al., 1999). Cribellar fibrils produced by members of the four most primitive families are non-noded (Fig. 2.2) and those produced by the remaining families have regularly spaced nodes (Fig. 2.3; Eberhard & Pereira, 1993; Opell, 1994a). Although the taxon sample in Eberhardt and Pereira (1993) is limited, the origin of noded fibrils appears to correspond to the origin of the Entelegynae clade (Griswald et al. 1999). This clade is distinguished by features of male and female reproductive anatomy, as well as the appearance of cylindrical silk spigots on the posterior median spinnerets.
Cribellar thread appears to rely on two stickiness mechanisms: mechanical interlock (snagging) and adhesion. The fibrils on its surface can snag on an insect’s setae, and hold them like the soft, looped side of a Velcro fastener. However, cribellar thread also adheres to nonsnagging surfaces by an unknown mechanism. It holds more tightly to the smooth surface of beetle elytra than to the heavily setose surface of a fly notum (Opell 1994b). It also sticks to smooth surfaces such as glass, graphite, and polished steel (Eberhard, 1980; Hawthorn, unpublished observation) that are smooth even on a microscopic level (Autumn et al., 2000).

Adhesion is most often achieved by a combination of several mechanisms. These can be classified as adsorption or van der Waals forces and electrostatic attraction (Allen, 1992a, b, c). Van der Waals forces technically encompass the hydrogen bonding facilitated by a thin film of water, but I will treat this separately as hygroscopic attraction. A third mechanism, diffusion of polymer chains across the interface of a bond (Allen, 1992a), requires the initial presence of a solvent and is unlikely to occur in dry, proteinaceous cribellar threads.

Van der Waals forces are the principle adhesive mechanism by which geckos can climb smooth, vertical surfaces. This is facilitated by very small, closely packed arrays of setae on the adhesive pads of their toes (Autumn et al., 2000). Van der Waals forces are probably one mechanism utilized by cribellar thread, but the small diameter of its fibrils precludes accurate measurements of the force that each fibril exerts. Peters (1984, 1986) suggested that cribellar threads may derive at least part of their stickiness from electrostatic attraction. However, comparisons of the strength with which cribellar threads held to surfaces of different dielectric properties did not support this hypothesis (Opell, 1995a).

Opell (1995a) suggested that hygroscopic forces contribute to cribellar thread stickiness, although this hypothesis has not been tested. Water sticks to surfaces by adhesive forces and to other water molecules by cohesive forces, both of which involve hydrogen bonding. The forces of adhesion are usually stronger than cohesion, so the strength with which a thin film of water holds two surfaces together is determined by surface tension (Stork, 1979) and Laplace pressure (Israelachvili, 1992). It is not necessary for this film of water to be secreted by an organism, as a sufficiently hydrophilic substance can attract moisture from the atmosphere. This has been documented in the viscous adhesive capture threads that, in higher araneomorphs, have replaced
cribellar threads. At typical ambient humidities, hydrophilic compounds in these threads draw water from the air to increase the volume of glue droplets on these threads (Townley et al., 1991).

In this study we examine the mechanisms by which cribellar thread sticks to smooth surfaces. We test two hypotheses: 1. van der Waals forces contribute to thread stickiness and 2. The nodes on fibrils of more advanced cribellate spiders are hydrophilic and increase thread stickiness by implementing hygroscopic forces.

The majority of cribellate species spin thread with noded fibrils (Fig. 2.3), but these nodes are absent in the most ancestral cribellate spiders (Fig. 2.2; Eberhard & Pereira, 1993). As nodes appear to reduce the area of fibril contact to a series of points, this would tend to reduce the stickiness of the cribellar thread with noded fibrils. However, as selection has favored an increase in thread stickiness (Opell, 1997, 1998, 1999a, b) this seems unlikely. As hygroscopic forces are stronger than van der Waals forces (Israelachvili, 1992), we hypothesize that the nodes of these fibrils incorporate hydrophilic amino acid groups that facilitate hygroscopic adhesion by adsorbing water from the atmosphere, thus increasing thread stickiness.

Cribellar threads produced by different spider species are comprised of different numbers of fibrils and have different surface configurations. Therefore interspecific comparisons of stickiness are less useful for testing our hypotheses than are comparisons of the stickiness registered by threads produced by the same spider under different humidity regimes. Our hypotheses predict that threads made of noded fibrils are stickier at high humidities than at very low humidities and that threads made of non-noded fibrils show little or no difference in stickiness under these conditions. Additionally, at near zero humidity noded threads should have a residual (van der Waals) stickiness that is similar to the stickiness of non-noded threads.

This approach also provides information about the operation of electrostatic force. If this force contributes significantly to thread stickiness, then it should diminish because electrostatic charges dissipate more readily at higher humidity. If electrostatic force is weak or nonexistent, then its effect will be overcome by an increase in hygroscopic force at higher humidity.
MATERIALS AND METHODS

We examined the cribellar threads of *Hyptiotes cavatus* (Hentz, 1847) and *Uloborus glomosus* (Walckenaer, 1841) (Family Uloboridae) that are formed of noded fibrils and of *Hypochilus pococki* (Platnick, 1987) (Family Hypochilidae) that are formed of non-noded fibrils. Only threads spun by adult female were used in this study. *Uloborus glomosus* threads were collected on the Virginia Tech campus from July to September, and *H. cavatus* threads were collected from the Virginia Tech campus, the forests of Montgomery and Giles Co. VA (hereafter referred to as the Virginia population), and near the town of Roan Mountain, Craig Co., TN from September to October (hereafter referred to as the Tennessee population). Adult females of both uloborid species were collected and housed individually in large plastic boxes, where they attached their webs to wooden dowel rods glued around the perimeter the boxes. These boxes were kept in an environmental chamber under conditions of 13 h dark - 11 h light, 24°C, and RH that ranged from 80% during the dark phase to 70% during the first and last two hours of the light phase to 60% during the remainder of the light phase. Spiders were misted with water daily and those kept for longer than two days were fed flightless fruit flies every other day. Threads from adult female *H. pococki* webs were collected in September and October from near the town of Roan Mountain, Craig Co., TN and on the eastern slope of Grandfather Mountain, Avery Co., NC. All hypochilid threads were collected in the field, because these spiders could not be induced to spin webs in the lab. Unlike the uloborids that take down and replace their webs nightly, *H. pococki* continues to repair and add cribellar threads to its web. This allowed us to collect newly spun threads from the outer edges of *H. pococki* webs.

We collected threads from webs on microscope slides with parallel raised supports. Double-sided tape atop each support securely anchored threads, ensuring that threads crossing between supports maintained their initial tension when they were cut away from the web. We examined all threads under a dissecting microscope and discarded any that appeared damaged, were intersected by another thread, or were contaminated with dirt or pollen. We also removed
older *Hypochilus* threads, which, under the dissecting microscope, could be distinguished from more recently spun threads by their contracted, collapsed structure.

Stickiness was measured using an instrument that was a modification of the one described by Opell (1989) and was used in previous studies of thread stickiness (Opell, 1993, 1994a, 1994b, 1995a, 1995b, 1999b). This consisted of a stainless steel needle strain gauge mounted in a plexiglass frame, positioned so that the contact plate on the needle’s protruding tip could be brought into contact with the cribellar thread. A motorized screw advanced the thread toward the 2 mm wide contact plate at a constant speed of 10.4 mm per minute, and withdrew it at 10.7 mm per minute. The arbitrary scale over which the needle’s free end passed was calibrated using 5 mg weights, and the force in Newtons required to deflect the needle was calculated by multiplying the scale values by the accelerating force of gravity.

The contact plate was made of aluminum surfaced with acetate from the non-sticky side of Scotch® Magic™ Tape (3M Co., 2002). We chose this surface because scanning electron microscope examination showed it to be fairly smooth even at the scale of tens of nanometers, at which the cribellar fibrils operate. It also has a nonpolar surface that does not attract moisture and allows small water droplets to bead up. Cribellar thread sticks to this surface with a force that is comparable to the force with which it holds fleshfly wings and 320 grit silicon carbide sandpaper, surfaces used in previous studies of capture thread stickiness (Opell, 1993, 1994a, 1994b, 1995a, 1995b, 1999b). Contact plates were prepared by sticking the tape to aluminum strips cut from weighing pans, cutting strips that were approximately 2 mm wide (final widths were measured to the nearest 20 µm under a dissecting microscope), cutting these strips into 4-5 mm lengths, and sealing all edges with silver paint to ensure that none of the tape’s adhesive was exposed.

The stickiness of most threads was measured within 2 weeks after being collected and of all threads within 38 days (Table 1). Each strand was oriented so that it was perpendicular to the long axis of the contact plate. A motorized screw advanced the thread holder towards the contact plate until it pressed against the plate with a force of 19.61 µN, and then immediately reversed the direction of the thread holder. We observed the position of the strain gauge needle along the calibrated scale, and recorded the value at the moment it pulled away from the thread. The
The stickiness of each web is the mean stickiness of three or four threads taken from that web. The force in µN required to pull the plate from the thread was divided by the width of the plate to give the stickiness in µN/mm of thread contact. The number of webs sampled per spider varied from 1 to 3, depending on how readily that individual spun in the lab.

To determine the effect of atmospheric water on the stickiness of cribellar thread, we compared the stickiness of threads from individual webs under conditions of high (near 100% RH), intermediate (between 45 and 50% RH), and low (under 3% RH) relative humidities. To control humidity the entire stickiness measuring apparatus was placed inside a sealed clear plexiglass box. A port in the side admitted the probe of a digital humidity gauge, and another port was connected to a tube through which the chamber could be flushed. The low humidity was achieved by flushing the chamber with pure, dry nitrogen. High and intermediate humidities were achieved by bubbling the nitrogen through distilled water. A small fan in the chamber ensured thorough mixing of the atmosphere in the box. For the high humidity measurements a piece of cloth dampened with distilled water was placed over the fan. We recorded the humidity and temperature at the start and finish of the stickiness measurements of each thread sample and averaged these to determine the mean humidity for each trial.

The conditions under which stickiness was measured are given in Table 2.1. The cribellar threads spun by H. cavatus adult females from Roan Mountain were narrower and less sticky under low humidity than those spun by Virginia specimens (data were normal: Shapiro-Wilke W statistic > 0.1, X width Tennessee 142 µm, SD = 32, N = 6 vs. Virginia 204 µm, SD = 27, N = 8, T-test P = 0.002; X stickiness Tennessee 8.2 µN/mm, SD = 3.6, N = 8 vs. Virginia 27.3 µN/mm, SD = 15.9, N = 8, T-test P = 0.0051). Therefore, the values of these two populations are treated separately. As the stickiness of capture threads spun by different individuals of the same species differ, the stickiness of threads spun by the same individual were compared at different RHs using paired tests. The statistical programs SAS and JMP (SAS Institute, Cary, NC) were used to analyze these data. P-values ≤ 0.05 were considered significant. All data analyzed with parametric statistics were first tested for normality using the Shapiro-Wilke W statistic; values > 0.05 were considered normally distributed.
RESULTS

Noded Thread: The stickiness of threads from Virginia *H. cavatus* populations measured in high RH was greater in all cases than the stickiness of threads from the same webs measured in low RH (Fig. 2.5; X difference 10.4 µN/mm, SD = 3.7, paired T-test: T = 5.399, P = 0.0005, N = 8). The stickiness of *H. cavatus* threads from the Tennessee population showed a similar difference in stickiness. Although the overall stickiness of the threads from the Tennessee population was lower, each thread was stickier when measured at intermediate RH than at low RH (Fig. 2.5; X difference 13.7 µN/mm, SD = 1.6; paired T-test: T = 9.676, P = 0.0001, N = 9). The increase in stickiness from low to intermediate RH (Tennessee population) and the increase from low and high RH (Virginia Population) did not differ (T-test: T = 1.396, P = 0.1845). That is, beyond a RH of 47%, there was no demonstrable increase in thread stickiness.

*Uloborus glomosus* threads showed similar differences in stickiness. Threads from one set of webs were measured at low and intermediate RH and threads from another set were measured at low, intermediate, and high RH. Threads from the first set of webs were stickier at intermediate RH than at low RH (Fig. 2.5; X difference 15.7 µN/mm, SD = 3.8; paired T-test: T = 12.536, P = 0.0001, N = 9). The stickiness of threads from the second set of webs also differed among RHs (Fig. 2.5; ANOVA: F = 24.521, P = 0.0001, N = 7). Pairwise comparisons using the Tukey-Kramer Honest Significant Difference (Sokal & Rohlf, 1969) revealed a difference between low and both intermediate and high RH, but not between intermediate and high RH at the α = 0.05 level experiment wise (X difference between low and intermediate RHs 12.3 µN/mm, SD = 4.8, N = 7; X difference between low and high RHs 18.4 µN/mm, SD = 5.7, N = 7; X difference between intermediate and high RHs 6.1 µN/mm, SD = 5.1, N = 7).

Non-noded Thread: Threads from 6 of the 9 webs of *H. pococki* showed an increase in stickiness when measured at low and high RH (Fig. 2.5). However, there was no significant difference in the stickiness of threads measured under these two conditions (X difference 0.4 µN/mm, SD = 5.3, N = 9); paired T-test: T = 2.100, P = 0.839).
DISCUSSION

This study found that the stickiness of cribellar threads with noded fibrils increased as RH increased, whereas the stickiness of primitive cribellar threads with non-noded fibrils was unaffected. These observations support the hypotheses that both van der Waals and hygroscopic forces contribute to the stickiness of cribellar threads and that the fibril nodes of derived cribellar threads facilitate the hygroscopic mechanism. They also provide no evidence for the operation of electrostatic force. These findings reveal an innovation in spider evolution that was comparable to the replacement of cribellar capture threads by the viscous capture threads spun by modern orb-weaving (Araneoidea) spiders (Opell, 1997, 1998; Opell & Bond, 2001). Just as this latter event is associated with increased species numbers (Bond & Opell, 1998), the replacement of non-noded fibrils by noded fibrils is also associated with increased spider diversity. Families that produce non-noded fibrils are comprised of 12 genera and 142 species, whereas those that spin noded fibrils are comprised of 359 genera and 3,464 species (Eberhard & Pereira, 1993; Platnick 2000). Although these numbers include members of cribellate families that have lost their cribellum, they highlight the disparity between the numbers that spin noded and non-noded fibrils.

Only cribellar threads with noded fibrils show an increase in stickiness with increased humidity, thus the appearance of these nodes was most probably associated with the inclusion of hydrophilic amino acids at these sites on the fibrils. This innovation allowed spiders to spin stickier threads and construct webs that were characterized by a greater stickiness per capture area, a feature that enhances the web's prey capture potential (Eberhard, 1989; Opell, 1999b). As cribellar thread with non-noded fibrils appears to rely only on van der Waals forces for their stickiness, stickiness can only be increased by increasing the number of fibrils that contact a surface. This can be achieved by either increasing the number of fibrils that form a cribellar thread (Opell, 1994a, 1995a, 1999b) or by folding and looping the thread before it is placed in the web (Opell, 2002). Both of these strategies require a spider to invest more material (protein) to increase thread stickiness. Only when noded cribellar fibrils that were capable of
implementing hygroscopic forces appeared was the direct link between silk investment and silk stickiness broken. Even if the amino acids whose incorporation was necessary to implement hygroscopic forces were more metabolically costly (Craig & Weber, 1998) than those they replaced, the benefit appears to be disproportionately great. Under the intermediate RH typical of many habitats noded threads are 30 to 50% stickier than they are under low RH, where we believe that van der Waals forces account for most of the stickiness. As we did not measure thread stickiness at RHs between 2 and 50%, it is possible that noded threads may implement hygroscopic adhesion at values considerably less than 50%.

Evidence for the incorporation of a different suite of amino acids in noded fibrils comes from the configuration of cribellar fibrils. The size of these fibrils (diameter 10 - 20 nm, compared to 1.5 nm for a single collagen fiber; Stryer 1995) suggests that they may represent individual fibrous proteins or a quaternary assembly of protein fibers, and that the nodes are produced by protein folding and aggregation. Hygroscopic proteins would incorporate hydrophilic polar or charged amino acids such as serine, lysine, and aspartate, causing them to fold and assemble to form nodes. These amino acids may also be capable of hydrogen bonding directly with a substrate in the absence of environmental humidity, resulting in a somewhat higher stickiness even in dry environments. However, molecular studies of fibril composition are necessary to confirm this hypothesis. The complex structure of cribellar thread, which incorporates silks from many glands, makes direct amino acid analysis impractical, but the product of the cribellar glands could be determined sequencing its mRNA.

The transition from cribellar capture threads spun by members of the Deinopoidea to viscous capture threads spun by its sister clade, the Araneoidea has been problematic, as transitional states are difficult to envision. Like cribellar threads, viscous threads have a pair of supporting axial fibers (Griswold et al. 1998; Opell & Bond, 2001). However, rather than being sheathed by cribellar fibrils, these fibers are covered by an aqueous solution that contains hydrophilic compounds (Townley et al., 1991) as well as glycoproteins which coalesce to form granules that confer thread stickiness (Tillinghast et al., 1993). It is plausible that the hygroscopic nodes of noded cribellar threads are one step along the path to viscous thread. The secretion of even a small amount of moisture onto threads would further enhance this mechanism and subsequent increases in this material would eliminate the need for relatively expensive
cribellar fibrils, leading to the loss of the cribellum and the production of modern viscous threads.

ACKNOWLEDGEMENTS

This study was supported by grants from the American Arachnological Society, the Virginia Tech Graduate Research and Development Program, and the Virginia Tech Department of Biology.

LITERATURE CITED


Table 2.1. Conditions for stickiness comparisons

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>RH mean and SD</th>
<th>mean age (days)</th>
<th>RH Range</th>
<th>Temperature mean and SD</th>
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<tbody>
<tr>
<td><em>Hypochilus</em></td>
<td>9</td>
<td>0.60% ± 0.48%</td>
<td>13</td>
<td>0.0-1.45</td>
<td>24.5 ± 0.35 C°</td>
</tr>
<tr>
<td><em>pococki</em></td>
<td></td>
<td>99.78 ± 0.36%</td>
<td>14</td>
<td>99.0-100</td>
<td>23.5 ± 0.48 C°</td>
</tr>
<tr>
<td><em>Hyptiotes</em></td>
<td>8</td>
<td>1.89 ± 0.51%</td>
<td>32.3</td>
<td>1.40-2.85</td>
<td>22.3 ± 0.27 C°</td>
</tr>
<tr>
<td><em>cavatus</em> (Virginia)</td>
<td></td>
<td>99.55 ± 1.0%</td>
<td>28.8</td>
<td>97.15-100</td>
<td>21.9 ± 0.24 C°</td>
</tr>
<tr>
<td><em>Hyptiotes</em></td>
<td>8</td>
<td>0.28 ± 0.28%</td>
<td>5.1</td>
<td>0-0.75</td>
<td>25.1 ± 0.38</td>
</tr>
<tr>
<td><em>cavatus</em> (Roan Mt. TN)</td>
<td></td>
<td>46.93 ± 1.45%</td>
<td>8.3</td>
<td>44.5-48.5</td>
<td>25.7 ± 0.61</td>
</tr>
<tr>
<td><em>Uloborus</em></td>
<td>7</td>
<td>1.25 ± 0.58%</td>
<td>12</td>
<td>0.25-1.8</td>
<td>24.8 ± 0.45</td>
</tr>
<tr>
<td><em>glomosus</em></td>
<td></td>
<td>46.6 ± 0.81%</td>
<td>12.5</td>
<td>45.3-47.5</td>
<td>24.7 ± 1.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.98 ± 0.38%</td>
<td>17</td>
<td>99.9-100</td>
<td>25.2 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.1 ± 0.37%</td>
<td>2.3</td>
<td>0.55-1.60</td>
<td>26.4 ± 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.4 ± 2.54%</td>
<td>3.8</td>
<td>45.45-52.9</td>
<td>26.1 ± 0.46</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

**Fig. 2.1.** Scanning electron micrograph of *Hyptiotes cavatus* cribellar thread.

**Fig. 2.2.** Scanning electron micrograph of *Hypochilus pococki* non-noded cribellar fibrils.

**Fig. 2.3.** Scanning electron micrograph of *Hyptiotes cavatus* noded cribellar fibrils.

**Fig. 2.4.** Scanning electron micrograph of *Hyptiotes cavatus* cribellum.

**Fig. 2.5.** Mean stickiness in μN/mm of *Hypochilus pococki* threads in low vs. high RH, *Uloborus glomosus* threads in low vs. intermediate vs. high and low vs. intermediate RH, and *Hyptiotes cavatus* threads in low vs. intermediate RH (Tennessee population) and low vs. high RH (Virginia population).
Figure 2.1: Scanning electron micrograph of *Hyptiotes cavatus* cribellar thread

Figure 2.2: Scanning electron micrograph of *Hypochilus pococki* non-noded cribellar fibrils.
Figure 2.3: Scanning electron micrograph of *Hyptiotes cavatus* noded cribellar fibrils.

Figure 2.4: Scanning electron micrograph of *Hyptiotes cavatus* cribellum.
Figure 2.5: Mean stickiness in µN/mm of *Hypochilus pococki* threads in low vs. high RH, *Uloborus glomosus* threads in low vs. intermediate vs. high and low vs. intermediate RH, and *Hyptiotes cavatus* threads in low vs. intermediate RH (Tennessee population) and low vs. high RH (Virginia population).
Chapter 3

THE EVOLUTION OF HYDROPHILIC NODES IN CRIBELLAR CAPTURE THREAD *

ABSTRACT: Sticky prey capture threads are produced by many members of the infraorder Araneomorphae. Dry fuzzy cribellar capture threads are plesiomorphic for this clade, while viscous adhesive threads are apomorphic. The outer surface of cribellar thread is formed of thousands of fine, looped fibrils spun from the spigots of a spinning plate termed the cribellum. Basal araneomorphs produce smooth, non-noded fibrils, while more derived members produce fibrils with regularly spaced nodes. Cribellar fibrils can snag and hold rough surfaces, but their adherence to smooth surfaces is probably the result of van der Waals and, in some cases, hygroscopic forces. Noded uloborid cribellar threads are significantly less sticky at 2% RH than at 99%RH, but the non-noded threads from Hypochilus pococki, while less sticky than the noded threads, had no loss of stickiness at low RH. This suggests that the nodes of the more derived cribellar threads are hydrophilic, and allow them to adsorb moisture from the environment when it is available and add hygroscopic forces of adhesion. However, these data only include measurements from 3 species representing 2 families. In this study threads from 10 species representing 6 families was tested for hydroscopicity. Threads from families Hypochilidae and Filistatidae, who spin non-noded fibrils repel water, while threads from families Neolanidae, Dictynidae, Desidae, and Uloboridae who spin noded fibrils wet when exposed to water. This supports the hypothesis that the nodes of more derived cribellar thread contribute to their ability to use hygroscopic forces. This may explain why the transition to noded fibrils has never been reversed, and appears to represent the first step in a series of changes that increased the stickiness of prey capture threads.
Introduction

The origin of the Infraorder Araneomorphae, or “true spiders,” was associated with the origin of cribellar thread, the first sticky prey capture thread produced by spiders (Coddington & Levi 1991). Cribellar thread is a dry capture thread comprised of a wooly mass of fine fibrils with diameters of 10 – 20 nm (Fig. 3.1; Opell 1994 a; Eberhard & Pereira 1993) supported by a pair of larger, axial fibers and, in some groups, additional strands (Eberhard & Pereira 1993). Cribellar fibrils produced by basal araneomorphs (Hypochilidae, Austrochilidae, Gradungulidae, and Filistatidae; Coddington and Levi 1991; Griswold et. al. 1999) are smooth (Fig. 3.2), whereas those produced by more derived araneomorphs have regularly spaced nodes (Fig. 3.3; Eberhard & Pereira 1993; Opell 1994a). Families that produce non-noded fibrils are comprised of 12 genera and 142 species, whereas those that spin noded fibrils are comprised of 359 genera and 3,464 species (Eberhard & Pereira, 1993; Platnick 2000). Although these numbers include members of cribellate families that have lost their cribellum, they nonetheless highlight the disparity between the number of taxa that spin noded and non-noded fibrils and suggest that the ability to produce noded cribellar fibrils confers a selective advantage to spiders.

A spider produces cribellar thread by drawing fibrils from spigots on the cribellum using the calamistrum, a comb of setae on the metatarsus of the fourth legs. These fine fibrils are combined with supporting strands to produce wooly puffs (Eberhard & Pereira 1993). The principle determinant of cribellar thread stickiness is the number of fibrils that form a thread, as gauged by the number of spigots on a spider’s cribellum (Opell 1994a, 1999).

Cribellar thread appears to rely on at least two stickiness mechanisms: mechanical interlock (snagging) and adhesion. The fibrils on a thread's surface can snag on an insect’s setae, and hold them like the soft, looped side of a Velcro fastener. However, cribellar thread also adheres to nonsnagging surfaces by an unknown mechanism. It holds more tightly to the smooth surface of beetle elytra than to the heavily setose surface of a fly notum (Opell 1994b). It also
sticks to smooth surfaces such as glass, graphite, and polished steel (Eberhard 1980; Hawthorn, unpublished observation) that are smooth even on a microscopic level (Autumn et al. 2000).

Opell (1995) suggested that hygroscopic forces contribute to cribellar thread stickiness. Water sticks to surfaces by adhesive forces, and to other water molecules by cohesive forces, both of which involve hydrogen bonding. The forces of adhesion are usually stronger than cohesion, so the strength with which a thin film of water holds two surfaces together is determined by surface tension (Stork 1979) and Laplace pressure (Israelachvili 1992). It is not necessary for this film of water to be secreted by an organism, as a sufficiently hydrophilic substance can attract moisture from the atmosphere. For example, at typical ambient humidities, hydrophilic compounds in adhesive capture threads produced by members of the family Araneidae draw water from the air to increase the volume of their viscous droplets (Townley et al., 1991).

When the stickiness of cribellar threads was measured under 2 % RH, using a smooth, acetate surface, the values of threads formed of non-noded and noded fibrils did not differ significantly (Hawthorn & Opell 2002). However, under 99 % RH, threads formed of noded fibrils were 30 to 50% stickier than those formed of non-noded fibrils, a difference that was statistically significant. These results indicate that, when atmospheric moisture is available, noded fibrils implement the hygroscopic mechanism of adhesion, a mechanism unavailable to non-noded fibrils.

If the difference in hydroscopicity between noded and non-noded fibrils is as pronounced as these differences in stickiness indicate, then it should be observable on larger scale. Therefore, we hypothesize that the features of noded thread that appear to allow it to attract atmospheric moisture and implement hygroscopic forces will also make noded thread much more easily wetted by water than non-noded thread. To test this we compare the wettability of cribellar threads produced by two species that spin non-noded fibrils and eight species that spin noded fibrils.
METHODS

Cribellar threads spun by 10 species, representing 9 genera and 6 families (Table 3.1), were collected from webs on raised supports glued to microscope slides. Voucher specimens of these species are deposited in Harvard University's Museum of Comparative Zoology. Double-sided tape atop supports maintained the native tension of the threads. Each thread was then transferred to a glass coverslip surfaced with double-sided tape. A drop of distilled water was placed on each thread using a syringe, and the thread was scored as either wetting or repelling water. Non-wetting thread was identified by the silvery reflective surface of the air retained by its fibrils (Fig. 3.4), whereas thread that wetted did not retain air and was more difficult to see (Fig. 3.5). Threads produced by three individuals of each species were photographed at 12x and 25x magnification under a dissecting microscope, and their hygroscopicity was mapped on a phylogeny (Fig. 3.6).

RESULTS AND DISCUSSION

As hypothesized, all threads comprised of the noded fibrils were wetted by water, whereas all threads comprised of non-noded fibrils repelled water and were not wetted (Fig. 3.6). The non-noded cribellar fibrils of *K. hibernalis* are unique in being ribbon shaped rather than cylindrical (Eberhardt & Pereira 1993). However, like the cylindrical fibrils of *H. pococki*, they did not wet. These results show that the presence of nodes on cribellar fibrils renders them more hydrophilic, an observation consistent with findings that under high atmospheric moisture only noded fibrils implemented the hygroscopic mechanism of adhesion (Hawthorn & Opell in review).

They small diameter (around 20 nm) of non-noded fibrils and of the internode regions of noded fibrils suggests that fibrils are single protein molecules and that the nodes result from protein folding. It is likely that the nodes result either from the addition of hydrophilic amino
acids or from the addition of amino acids in which folding brings into juxtaposition side chains that together form hydrophilic active sites. Nodes appear to increase the volume of noded fibrils and the material cost to producing cribellar threads with noded fibrils. However, the ability of noded fibrils to incorporate the hygroscopic mechanism of adhesion may explain why the transition from non-noded to noded cribellar fibrils has been favored and has never been reversed.

A high evolutionary premium appears to have been placed on the stickiness of spider capture threads. The origin of orb-weaving spiders from ancestors that spun less highly organized webs was accompanied by an increase in the stickiness of their cribellar threads (Opell 1999). Web reduction within the cribellate orb-weaving family Uloboridae was associated with an increase in cribellar thread stickiness (Opell 1994a). The origin of the Araneoidea clade was marked by the replacement of cribellar thread by viscous adhesive thread (Coddington 1986, 1990a, 1990b; Coddington & Levi 1991) and this was associated by an increase in material economy and stickiness of viscous thread (Opell 1997, 1998). The transition from cribellar threads formed of non-noded fibrils to those formed of noded fibrils that implement hygroscopic adhesion appears to represent the first in a series of changes that increased the stickiness of prey capture threads and enhanced the ability of web-building spiders to capture prey.

ACKNOWLEDGEMENTS

This study was supported with funds from an American Arachnology Society Grant, and the Virginia Polytechnic and State University Biology Department


### Table 3.1.—Family, species, population and collection dates of threads examined.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Locality</th>
<th>Date collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypochilidae</td>
<td><em>Hypochilus pococki</em></td>
<td>Avery Co, North Carolina</td>
<td>September, 2001</td>
</tr>
<tr>
<td></td>
<td>(Platnick 1987)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filistatidae</td>
<td><em>Kulkania hibernalis</em></td>
<td>Highlands Co., Florida</td>
<td>April, 1996</td>
</tr>
<tr>
<td></td>
<td>(Hentz 1842)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uloboridae</td>
<td><em>Waitkera waitkerensis</em></td>
<td>Whangarei, New Zealand</td>
<td>February, 1996</td>
</tr>
<tr>
<td></td>
<td>(Chamberlain 1946)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hyptiotes cavatus</em></td>
<td>Montgomery Co., Virginia</td>
<td>September, 2001</td>
</tr>
<tr>
<td></td>
<td>(Hentz 1847)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Octonoba sinensis</em></td>
<td>Montgomery Co., Virginia</td>
<td>May, 1996</td>
</tr>
<tr>
<td></td>
<td>(Simon 1880)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dictynidae</td>
<td><em>Mexitilia trivitatta</em></td>
<td>Cochise Co., Arizona</td>
<td>August, 1996</td>
</tr>
<tr>
<td></td>
<td>(Banks 1901)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neolanidae</td>
<td><em>Neolana pallida</em></td>
<td>Whangarei, New Zealand</td>
<td>February, 1996</td>
</tr>
<tr>
<td></td>
<td>(Forster &amp; Wilton 1973)</td>
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</tr>
<tr>
<td>Desidae</td>
<td><em>Badumna longinqua</em></td>
<td>Whangarei, New Zealand</td>
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</tr>
<tr>
<td></td>
<td>(L. Koch 1867)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><em>Badumna insignis</em></td>
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<td></td>
<td>(L. Koch 1872)</td>
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<td></td>
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<td></td>
<td><em>Matachia livor</em></td>
<td>Whangarei, New Zealand</td>
<td>February, 1996</td>
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<td></td>
<td>(Urquhart 1892)</td>
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FIGURE LEGENDS


Figures 3.4, 3.5. – Light micrographs of cribellar threads immersed in water. 4. *Hypochilus pococki* cribellar thread showing trapped air. 5. *Hyptiotes cavatus* cribellar thread wetted.

Figure 3.6. – Phylogeny of the 10 species studied showing the appearance of noded fibrils and hygroscopic thread.

Figures 3.4, 3.5: Light micrographs of cribellar threads immersed in water. 4. *Hypochilus pococki* cribellar thread showing trapped air. 5. Hyptiotes cavatus cribellar thread wetted.
Figure 3.6: Phylogeny of the 10 species studied showing the appearance of noded fibrils and hygroscopic thread.
Chapter 4

A MATHEMATICAL MODEL FOR VAN DER WAALS AND HYGROSCOPIC ADHESION IN CRIBELLAR SPIDER PREY CAPTURE THREAD
Introduction

Many animals have evolved stickiness mechanisms to aid in locomotion and prey capture. Gecko toes are surfaced with finely branched setae that permit the close conformation to a substrate required to utilize van der Waals forces for adhesion (Autumn et al. 2000). Chrysolina beetles may use van der Waals forces to walk up smooth surfaces, although hygroscopic adhesion produced by a thin layer of adsorbed water has also been hypothesized (Stork 1979). Many spiders also appear to use highly branched setae at the tips of their legs to generate van der Waals forces, allowing them to walk on smooth surfaces (Foelix 1996). The earliest spider prey capture thread may have utilized van der Waals and, later, adsorbed water for hygroscopic adhesion (Hawthorn & Opell 2002).

The large Infraorder Araneomorphae contains the “true spiders.” Its origin was associated with the appearance of aerial prey capture webs designed to intercept flying prey and sticky prey capture thread that. The first sticky prey capture thread was cribellar thread, a dry capture thread comprised of an outer wooly mass of thousands of fine fibrils with diameters of 10-20 nm (Fig 4.1; Opell 1994a; Eberhard & Pereira 1993) supported internally by a pair of larger, axial strands and, in some groups, additional strands (Eberhard & Pereira 1993). Cribellar fibrils produced by basal araneomorphs of the family Hypochilidae are smooth (Fig. 4.2), whereas those of produced by more derived araneomorphs have regularly spaced nodes (Fig. 4.3; Eberhard & Pereira 1993; Opell 1994a; Griswold et al. 1999). Members of the family Filistatidae produce unique, flattened cribellar fibrils that, owing to their phylogenetic position, appear to be derived from noded fibrils (Coddington and Levi 1991; Eberhard & Pereira 1993; Griswold Personal Communication; Griswold et al. 1999).

To spin cribellar fibrils, a spider uses rapid, rhythmic movements of alternating fourth legs to draw the calamistrum, a setal comb on each fourth metatarsus, over the cribellum spinning plate (Eberhard 1989). This pulls protein cribellar fibrils from each of the cribellum’s thousands of spigots and polymerizes the protein. Rhythmic adductions of the median spinnerets (Peters 1984) help combine the fibrils with supporting strands. The completed thread often takes the form of a series of regular puffs (Fig. 4.1). The principle determinant of the stickiness of cribellar thread is the number of fibrils that form a thread, as gauged by the number of spigots on
a spider’s cribellum (Opell 1994a, 1999). Differences in the dimensions of the threads puffs and the manner in which some spiders loop and fold their cribellar threads before they deposit them on non-sticky threads of their webs also affect thread stickiness (Opell 1995a, 2002).

Cribellar thread appears to rely on at least two stickiness mechanisms: mechanical interlock (snagging) and adhesion. The fibrils on a thread’s surface can snag an insect’s setae, and hold them like the soft, looped side of a Velcro fastener. However, cribellar thread also adheres to smooth surfaces such as graphite, polished steel and glass (Eberhard 1980, Hawthorn, unpublished observation). It holds more tightly to the smooth surface of beetle elytra than to the heavily setose surface of a fly notum (Opell 1994b). Although the mechanism by which cribellar thread sticks to smooth surfaces is not well studied, its dual stickiness appears to adapt it to hold a wide range of insect surfaces.

Adhesion is most often achieved by a combination of mechanisms; these can be categorized as mechanical interlock, adsorption or van der Waals forces, and electrostatic attraction (Allen 1992, a, b, c). Van der Waals forces technically encompass the hydrogen bonding facilitated by a thin film of water, but as this is described by different equations than simple adsorption we will treat this separately as hygroscopic adhesion. A fourth mechanism, diffusion of polymer chains across an interface, requires the presence of a solvent and is unlikely to occur in dry cribellar threads.

Mechanical interlock can only account for the adhesion of cribellar threads to rough or setose surfaces. Peters (1984, 1986) suggested that cribellar threads might derive at least part of their stickiness from electrostatic attraction. However, comparisons of the strength with which cribellar threads held to surfaces with different dielectric properties did not support this (Opell 1995b). Therefore, the forces most likely to explain cribellar threads adhesion to smooth surfaces are van der Waals and hygroscopic forces.

Opell (1995b) suggested hygroscopic forces as a possible mechanism for cribellar thread stickiness. Water sticks to surfaces by adhesive forces and to other water molecules by cohesive forces, both of which involve hydrogen bonding. The forces of adhesion are usually stronger than cohesion, so the strength with which a thin film of water holds two surfaces together is determined by surface tension (Stork 1979) and Laplace pressure (Israelachvili 1992). It is not
necessary for this film of water to be secreted by an organism, as a sufficiently hydrophilic substance can attract moisture from the atmosphere. For example, at typical ambient humidities, hydrophilic compounds in the adhesive threads spun by more derived orb-weaving spiders attract atmospheric water to increase the volume of thread (Townley et al. 1991).

Differences in the stickiness of cribellar threads formed of noded fibrils under low and high RH provide support for the operation of a hygroscopic mechanism. These threads were 30 – 50% stickier in 99% than in 2% RH, a difference attributed to the implementation of hygroscopic mechanism under higher humidities (Hawthorn & Opell 2002). In contrast, the cribellar threads of primitive araneomorphs that are formed of non-noded fibrils did not exhibit this difference in stickiness. This suggests that the molecular composition of non-noded fibrils does not permit them to attract atmospheric water and implement the hygroscopic mechanism.

Another force of adhesion that has been documented in an animal system is van der Waals, or London dispersion forces. This force depends only on the presence of nuclei and electrons, and thus can operate between any two molecules provided they are sufficiently close (Hobza & Zahradnik 1988). Van der Waals forces are the main attractive forces among the molecules of most liquids, and may cause surfaces that are in close proximity to adhere. These relatively weak interactions arise when an instantaneous dipole in one molecule creates a synchronized instantaneous dipole in neighboring molecules, producing a net attractive force between them. Aggregates of molecules can produce enough cumulative force to exert an attractive effect at a distance of 50 nm, making this a potentially major factor in the strength of adhesive bonds (Rigby et al. 1986).

There is evidence that Van der Waals forces are the major mechanism that allows gecko toes to stick to smooth surfaces. The tips of gecko toes are surfaced with modified, scales (setae), each of which possesses an array of very fine, closely packed spatulae with diameters of 0.2 - 0.5 µm. This maximizes the area and closeness of contact with surfaces and allows the toes to implement van der Waals forces (Autumn et al. 2000). These investigators documented the sufficiency of Van der Waals forces to explain a gecko's ability to walk on smooth, vertical surfaces by determining the number of spatulae on each seta, approximating the ends of the
spatulae as half spheres, and using a formula that describes the van der Waals forces between a sphere and a plane.

In this paper, we use a similar approach to determine if Van der Waals forces are sufficient to explain the stickiness of cribellar threads formed of noded and non-noded fibrils under low humidities. We then use a formula for predicting hygroscopic forces to determine if the increased stickiness registered under high humidities by cribellar threads formed of noded threads can be attributed to hygroscopic forces. By so doing, we show that cribellar threads relied initially on only van der Waals forces to stick to smooth surfaces. The appearance of nodes on cribellar fibrils added hygroscopic adhesive forces under higher atmospheric humidities.

**Methods**

Collecting and Examining Threads:

This study is based on cribellar threads spun by adult female spiders. Threads of *Hyptiotes cavatus* (Hentz 1847) (Uloboridae) are formed of noded fibrils and were collected near Blacksburg VA in Montgomery and Giles Cos. Threads of *Hypochilus pococki* (Platnick 1987) (Hypochilidae) are formed of non-noded, cylindrical fibrils and were collected near the town of Roan Mountain, Craig Co., TN and on the eastern slope of Grandfather Mountain, Avery Co., NC.

These threads were collecting on microscope slides to which raised supports were glued. To secure threads under their native tension, supports were surfaced with double-sided tape. Supports were separated by 4.8 mm on samplers used to collect threads for stickiness measurements and other samplers used to collect threads for electron microscopy had supports spaced at 2-3 cm intervals. Sectors of threads extending between supports were examined under
a dissecting microscope to ensure that only freshly produced, undamaged threads were included in this study.

We used measurements of fibril and thread features made under the electron and light microscopes to compute the area of contact between a thread and a surface. Threads that were examined under the scanning electron microscope (SEM) were transferred to stubs and sputter coated with 4 nm of gold before being viewed at a magnification of around 100K. Low acceleration of 2.00 kV prevented damage to the fibrils. Images of these fibrils and their included scale bars were saved digitally. Other threads were photographed under dissecting and compound microscopes and these 35 mm slides were scanned to produce digital images. We then used NIH Image© software to measure these images.

Establishing humidity for stickiness measurements:

We measured the stickiness of threads under conditions of high (near 100% RH), intermediate (between 45 and 50% RH), and low (under 3% RH) relative humidities. To control humidity the entire stickiness measuring apparatus was placed inside a sealed clear plexiglass box. A port in the side admitted the probe of a digital humidity gauge, and another port was connected to a tube through which the chamber could be flushed. The low humidity was achieved by flushing the chamber with pure, dry nitrogen. High and intermediate humidities were achieved by bubbling the nitrogen through distilled water. A small fan in the chamber ensured thorough mixing of the atmosphere in the box. For the high humidity measurements a piece of cloth dampened with distilled water was placed over the fan. We recorded the humidity and temperature at the start and finish of the stickiness measurements of each thread sample and averaged these to determine the mean humidity for each trial.
Measuring thread stickiness:

The procedures we follow in developing models to predict thread stickiness are guided in part by the methods used to measure thread stickiness. Therefore, we describe first how stickiness was measured. We use the same procedures and instrument described by Hawthorn & Opell (2002) and Opell (1993, 1994a, 1994b, 1995a, 1995b, 1999). This consisted of a stainless steel needle strain gauge mounted in a plexiglass frame, positioned so that the contact plate on the needle’s protruding tip could be brought into contact with the cribellar thread. A motorized screw advanced the thread, mounted on its collecting slide, toward the 2 mm wide contact plate at a constant speed of 10.4 mm per minute, and withdrew it at 10.7 mm per minute. The plate was pressed against a thread sample until a force of 19.61 µN was achieved, at which point the motor was reversed. The needle passed over a scale calibrated in mg necessary to deflect the needle. The position of the needle on this scale at the instant the plate pulled free of the thread was recorded and this value multiplying by the accelerating force of gravity to yield the force in Newtons required to pull the plate from a thread.

As in our previous study (Hawthorn & Opell 2002), we use contact plates made of aluminum surfaced with acetate from the non-sticky side of Scotch® Magic™ Tape (3M Co., 2002). We chose this surface because scanning electron microscope examination showed it to be fairly smooth even at the scale of tens of nanometers, at which the cribellar fibrils operate. It also has a nonpolar surface that does not attract moisture and allows small water droplets to bead up. Cribellar thread sticks to this surface with a force that is comparable to the force with which it holds fleshfly wings and 320 grit silicon carbide sandpaper; surfaces used in previous studies of capture thread stickiness (Opell, 1993, 1994a, 1994b, 1995a, 1995b, 1999). Contact plates were prepared by sticking the tape to aluminum strips cut from weighing pans, cutting strips that were approximately 2 mm wide (final widths were measured to the nearest 20 µm under a dissecting microscope), cutting these strips into 4-5 mm lengths, and sealing all edges with silver paint to ensure that none of the tape adhesive was exposed.

In previous studies the force required to pull the contact plate from a thread was divided by the width of the contact plate to yield stickiness, reported as µN/mm thread contact. This method provides a consistent and sensitive measure of thread stickiness upon which the
conclusions of previous comparisons depended. However, the model of stickiness that we
develop here requires a more detailed understanding of how a thread releases from the contact
plate. This in turn causes us to treat the force registered by the strain gauge differently.

    We believe that as a contact plate is pulled away from a thread force is concentrated
along the two edges of the plate (Fig. 4.4). When the force pulling on each edge overcomes the
adhesive force(s) of the thread along these edges, then the thread peels from the edges of the
plate toward its center. Thus, we believe that our instrument measures the threshold force
necessary to initiate this peeling action. We model the adhesive force to be overcome as that
exerted by the narrow band of cribellar fibrils in contact with each edge of the measuring
instrument’s contact plate. As this plate is oriented parallel to the cribellar thread, we also
consider that the force the instrument exerts is divided evenly between these two bands of
cribellar fibrils. Consequently, the predicted force of each band of thread should equal half of the
force registered by the instrument.

Determining the cribellar fibril contact:

    To predict thread stickiness we determined the number of contact points (cribellar fibril
nodes for *H. cavatus* and designated fibril contact points for *H. pococki*) within the bands of
contact at the margins of contact plates and multiplied this total by the adhesive forces computed
for a single contact point.

    The first step in this process was to determine the density of cribellar fibril contact with a
smooth surface. To do this for *H. cavatus* we counted the number of nodes on the surface (in
sharp focus and not lying behind another fibril) in SEM micrographs and divided this number by
the total area of the micrograph to determine contact points per thread area. Fibril nodes have
diameters about 1.5 times those of internode regions. Therefore, we assumed that only these
nodes contact smooth surfaces. From these SEM micrographs, we also determined mean node
diameter (from measurements of 10 randomly selected nodes per thread) and internode distance
(from 10 randomly selected internode spaces per thread).
The threads of *H. pococki* are formed of non-noded fibrils. Therefore, we assumed that their fibrils on their surfaces contact a smooth surface along their entire lengths (unless overlain by another fibril). We measured the length of these surface fibrils in printed micrographs using a digital map measurer. To conservatively model the stickiness of these fibrils in a manner consistent with that of noded fibrils, we converted this contact length to a series of closely spaced contact points separated by a distance equal to fibril diameter (calculated from the mean diameter of 10 fibrils per thread). We then divided the number of these points by the micrograph area represented to determine contact points per thread area.

The second step in determining the number of contact points within the bands of contact was to determine the footprint area of a thread when pressed against a smooth surface. To do this we placed a microscope cover slip atop the threads on a sampler and photographed of these threads at 40x under a dissecting microscope. The threads of *H. cavatus* are formed of regularly spaced puffs. We measured the maximum contact width of three puffs per thread and used the mean puff contact width as the width of the band of thread whose stickiness must be overcome before a thread can peel from a contact plate.

We measured the contact footprint area of *H. pococki* in a manner similar to that of *H. cavatus*. However, as these threads do not have puffs and are irregular in outline, we divided the total measured contact area of a thread by the length of the thread to obtain footprint area in $\mu m^2$ per $\mu m$ length of thread.

The final step in determining the number of contact points within the bands of contact was to multiply node or contact point density by the areas of the bands of contact. For *H. cavatus*, we set the length of each band of contact to the maximum contact puff width and the width of the band to a distance equal to the mean internode spacing plus the mean node diameter. For *H. pococki* we set the length of each band to the mean width of the cribellar thread and the width equal to the mean width of a cribellar fibril. In both cases, we reasoned that the forces of adhesion to be overcome were generated by a single band of fibril nodes (or, in the case of *H. pococki*, assigned fibril contact points) along the outer edges of the zone where a thread pressed against a contact plate.
Modeling the van der Waals and Hygroscopic forces of a single fibril node:

We modeled the van der Waals force of a single fibril node of *H. cavatus* or one of the arbitrarily designated contact points of *H. pococki* as the force generated by the contact between a sphere and a plane, as described by the equation

\[ F = \frac{AR}{6D^2} \]

where A is the Hamaker constant, taken to be $10^{-19}$, R is the radius of the sphere, and D is 0.3nm, the distance between the sphere and the substrate where van der Waals forces become significant (Autumn 2000; Israelachvili 1992).

The hygroscopic adhesive force created by a thin film of adsorbed water between a sphere and a plane is described by the equation

\[ F = \frac{AR}{6D^2} \lambda_L \cos \theta \]

where R is the radius of the sphere, $\lambda_L$ is the surface energy of water (76 mJm$^{-2}$), and $\theta$ is the angle of contact between the water and the substrate (Israelachvili 1992), which was estimated to be 60º by observing a drop of water on the acetate surface used to measure thread stickiness in Hawthorn & Opell (2002).

**Results**

Appendix 1 gives the calculations used to predict the stickiness of cribellar threads formed of noded and non-noded fibrils under conditions of high and low RH. The model predicts that under perfectly dry conditions where only van der Waals forces operate *H. cavatus* thread, which is formed of noded fibrils, will begin peeling away from the contact point when a
force of 18.3 µN is applied. Hydroscopic forces acting alone would require 48.9 µN to initiate peeling. If these forces are considered to be cumulative, as they probably are under humid conditions, the model predicts that a force of 66.9 µN would be required to begin the peel. These predictions are within the range of the observed mean values (Fig.4.5). Under dry conditions stickiness ranged from 19.4 to 86.8 µN (X = 53.5 µN, SD 31.1, N = 8). Under humid conditions, stickiness ranged from 47.7 to 116.6 µN (X = 73.8 µN, SD = 27.7, N = 8).

The threads of H. pococki are formed of non-noded fibrils and did not exhibit increased stickiness under high humidity (Hawthorn & Opell 2002). Therefore we modeled this stickiness only under dry conditions where van der Waals forces are postulated to operate. This model predicts that a force of 11 µN should be required to begin peeling. This is consistent with measured stickiness values that range from 5.5 – 23.8 µN (X = 12.9 µN, SD 5.5, N = 9).

**Discussion**

These simple mathematical models yield predictions that are well within the range of observed values for the stickiness of noded and non-noded cribellar threads under high and low RH. For non-noded H. pococki threads, the predicted value of 11 µN is very near the observed mean release force of 12.9 µN. The predicted value of H. cavatus threads under the assumption of van der Waals forces alone, while still within two standard deviations of the mean, is lower than the observed stickiness of the thread under dry conditions. This may be due to the crudeness of the model. It may also suggest that this thread either retains some residual moisture even in a 2% RH environment, or that it is capable of hydrogen bonding directly with a substrate. To understand what is happening at the molecular level to produce cribellar thread stickiness it will be necessary to know the sequence of amino acid that forms cribellar fibrils, the folding and aggregation patterns of the fibril protein, and the functional groups present on the surface of this folded protein.
The support that this study provides for two mechanisms of cribellar thread adhesion points to an important event in the evolution of spider capture thread. Ancestral, non-noded threads relied only on van der Waals forces to stick to smooth surfaces. These threads could increase their stickiness only by increasing the amount of fibrils that formed a thread, a change that required the expenditure of more protein. The addition of hydroscopic adhesion not only increased thread stickiness, but also uncoupled thread stickiness from a direct reliance on the amount of protein invested in a thread. The evolution of nodes therefore allowed spiders to catch more prey with less material (protein) investment. In many ways, this is comparable to the subsequent evolution of adhesive thread which replaced cribellar thread in the webs of modern orb-weaving spiders and their descendents (Bond & Opell 1998; Coddington & Levi 1991). These threads achieve an even greater stickiness relative to their material investment than do cribellar threads formed of noded fibrils (Opell 1997, 1998).
Appendix 4.1: Calculations

For *H. cavatus* noded thread, the calculations are as follows:

intenode length × puff width = area of loaded edge
85.5 nm × 209.9 μm = 17.9 μm²

edge area × node density = nodes per edge
17.9 μm² × 170 nodes/μm = 3043 nodes per edge

van der Waals force per node:

\[ F_v = \frac{10^{-19}(17.5 \times 10^{-9})}{6(3 \times 10^{-10})^2} = 3 \times 10^{-9} N = 3 \times 10^{-3} \mu N / node \]

van der Waals force to begin peel:

3 × 10⁻³ μN per node × 3043 nodes × 2 edges = 18.3 μN

Hygroscopic force per node:

\[ F_H = 4\pi R \lambda_c \cos \theta = 4\pi(17.5 \times 10^{-9} M) \times 76 mJM^{-2} \times 0.5 = 8.36 \times 10^{-6} mN = 8.36 \times 10^{-3} \mu N / node \]

Hygroscopic force to begin peel:

8.36 × 10⁻³ μN per node × 3043 nodes × 2 edges = 48.6 μN
For H pococki non noded thread the calculations are:

\[
\text{Fibril density} \quad \text{Fibril width} = \frac{\text{contact points}}{\mu m^2} = \frac{11797 \text{nm}/\mu m^2}{29 \text{ nm}} = 406 \text{ points} / \mu m^2
\]

\[
\text{Edge area} = \text{fibril width} \times \text{thread area per } \mu m = 0.03 \mu m \times 168 \mu m^2/\mu m = 5.04 \mu m^2
\]

\[
\text{Contact points / edge} = 5.04 \mu m^2 \times 406 \text{ points} / \mu m^2 = 2046 \text{ points}
\]

van der Wals force per contact point:

\[
F_v = \frac{10^{-19}(14.5 \times 10^{-9})}{6(3 \times 10^{-10})^2} = 2.7 \times 10^{-9} N = 2.7 \times 10^{-3} \mu N / \text{node}
\]

van der Waals force to begin peel:

\[
2.7 \times 10^{-3} \mu N / \text{node} \times 2046 \text{ points} \times 2 \text{ edges} = 11 \mu N
\]

**Literature Cited**


FIGURE LEGENDS


Figure 4.4 – Diagram of thread peeling from contact plate

Figure 4.5 – Measured and calculated stickiness of cribellar threads.

Figure 4.4: Diagram of thread peeling from contact plate.
Figure 4.5: Measured and calculated stickiness of cribellar threads. Bars represent one standard deviation.
CONSTRUCTION OF A *KUKULKANIA HIBERNALIS* CRIBELLAR SILK GLAND cDNA LIBRARY FOR CHARACTERIZATION OF THE CRIBELLAR SILK PROTEIN
INTRODUCTION

Capture Threads: Most aerial spider webs are comprised of non-sticky supporting threads and sticky prey capture threads. Sticky threads hold insects securely, and therefore buy time for a spider to subdue prey (Eberhard 1989). Cribellate spider silk is a primitive, dry capture thread that is spun by basal members of the infraorder Araneomorphae, or true spiders (Coddington & Levi 1991). It consists of a wooly mass of thousands of 18 nm diameter fibrils that surround a two or more supporting axial fibers. These fibrils are spun from spigots on an abdominal spinning plate termed the cribellum, and the axial fibers are produced by spigots on the spider’s spinnerets.

Cribellate fibrils spun by members of the primitive family Hypochilidae are cylindrical, but those spun by all but one of the remaining 21 cribellate families have regularly spaced nodes along their length (Eberhard & Pereira 1993). Members of the family Filistatidae, to which Kukulkania hibernalis (Hentz 1842), the subject of this study, belongs produce flat, ribbon shaped fibrils without nodes. The phylogenetic position of Filistatidae indicates that the fibrils are derived from noded fibrils (Griswold et al., 1999). Cribellar thread sticks to rough or setose surfaces when its fibrils snag these irregularities (Opell 1994). However, it also adheres to smooth surfaces such as glass, polished steel, and graphite with an unknown mechanism (Eberhard 1980.; Hawthorn, unpublished observation).

Adhesion is most often the result of multiple mechanisms, which can be grouped as electrostatic, adsorption or van der Waals, mechanical interlock, and diffusion (Allen 1992a). Cribellar threads stick with the same force to surfaces with very different dielectric properties, an observation which does not support an electrostatic mechanism (Opell 1995a). Mechanical interlock relies on microscopic projections on which the fibrils can catch and snag, and so can
not explain the adhesion of cribellar thread to smooth surfaces (Allen 1992b). Diffusion is the migration of polymer chains across an interface and requires the presence of a solvent, making it unlikely in dry, proteinacious cribellar thread (Allen 1992a). The most likely mechanism is adsorption (also called van der Waals or London dispersion forces), which technically encompass hygroscopic adhesion from the hydrogen bonding facilitated by a thin film of water. Van der Waals forces are attractive forces between any two molecules in close enough proximity, produced by an instantaneous dipole interaction (Allen 1992c). I treat hygroscopic adhesion separately from simple adsorption, since hygroscopic adhesion is stronger due to the polar nature of water, and the surface chemistry that promotes the adsorption of water for hygroscopic adhesion is more specific (Israelachvili 1992).

Cribellar thread formed of noded thread is stickier in a humid environment than a dry environment (Hawthorn & Opell 2002), providing support for the ability of these threads to attract atmospheric water and implement hygroscopic forces of adhesion (Israelachvili 1992; Allen 1992a; Stork 1979). Mathematical models also support this hypothesis (Chapter 4). I hypothesize that fibril nodes contain hydrophilic amino acids responsible for the thread’s ability to attract water.

Silk Genes: Spiders produce an array of silks. Although all are formed of protein (or protein overlain with an aqueous solution in the case of viscous capture threads), each one has unique chemical and physical properties, is secreted by a different type of gland, and is drawn from different spinnerets at the tip of the abdomen (Foelix 1996). These silks are coded by a family of genes, of which ten genes encompassing three spider species and two genera have been sequenced. Most spider silks consist of a single exon (Hayashi & Lewis 1998), and all have repetitive, glycine and proline rich regions that contribute to their fibrous and elastic nature (Hayashi & Lewis 1998). The repetitiveness of these sequences means that even a relatively short sequence can yield information about the structure of the entire protein.

A molecular model of the cribellar fibril protein would provide the most thorough explanation for the stickiness mechanism of cribellar silk. Fibril protein can best be characterized by sequencing the mRNA, produced by the cells of the cribellar silk glands. Results described in chapters 2 and 3 predict that noded fibrils would contain hydrophilic amino acids absent from
non-noded, cylindrical fibrils. The tertiary structure of noded fibril protein should also help explain the presence of nodes and characterize the active sites at these nodes.

I chose to characterize the cribellar fibril protein of *K. hibernalis* because this is a robust spider with a large number of cribellar glands and easily collected in fairly large numbers within it’s range. Because filistatids have lost fibril nodes, DNA sequences from this species may provide access to species that produce noded and non-noded fibrils. The cribellar silk amino acid sequence from *K. hibernalis*, which lack nodes, should be similar to that of noded fibrils, but lack the hydrophilic node forming amino acids.

**METHODS**

I used the Micro poly(A) pure kit from Ambion® to purify the mRNA from the cribellar silk glands of 50 adult female *Kukulkania hibernalis* that were collected near Gainesville, Alachua Co., Florida. Dissecting these spiders yielded 12 mg of tissue, which was stored in RNA later (Ambion) at 4° before being ground in liquid nitrogen. The purification kit used a microcentrifuge filter column with oligo (dT) cellulose. This bound mRNA, and allowed ribosomal and transfer RNA to be washed off the column. The purified mRNA was then eluted, precipitated overnight with ethanol and ammonium acetate, and stored in DEPC water/ EDTA at -80 C° for two months. One µl of the purified mRNA was loaded onto a formaldehyde agarose gel with molecular weight markers, and a faint band was visible at 1.5 Kb. The exact yield of RNA was difficult to determine, but the total was probably not more than 50 ng.

The cDNA library was constructed in the lambda Zap phagemid vector using the ZAP-cDNA® Synthesis and Gigapack® III Gold Cloning Kit from Stratagene (© 2000). This created a unidirectional ³²P labeled cDNA with 5’ Xho 1 ends and 3’ Eco R1 ends. This was ligated into the pBluescript® phagemid which contains a polylinker flanked by T3 and T7 regions within the β-galactosidase gene. Autoradiography of 1µl of both the first strand and second strand reactions
on an 0.8% agarose gel was not successful. This was attributed to the small amount of mRNA used. No bands could be visualized, but radiation was detected in the tube after the cDNA was precipitated.

The cDNA was purified using the Qiaquick® PCR purification kit from Qiagen, instead of size fractioning with the drip column as described in the Stratagene kit. This was done to minimize loss of cDNA while removing digested or unligated linkers and free nucleotides. The purified cDNA was precipitated with ethanol and sodium acetate and resuspended in 2.5 µl of water. This allowed all of the cDNA to be used in the subsequent vector ligation reaction.

The cDNA was ligated into the Uni-Zap XR vector, and packaged using the Gigapack® III Gold kit. The resulting library was immediately amplified in XL1-Blue F’ E. coli host cells, and then plated in the same host with IPTG and X-gal for blue-white screening. In order to get a reasonable density of plaques it was necessary to use 1µl of amplified phage to 200µl of host cells, indicating a low titer in the amplified library.

Eight clear plaques were selected for sequencing. These were cored from the plate and the pBluescript plasmid was excised using the ExAssist helper phage and the SOLR E. coli host strain. Of these, five produced successfully transformed colonies from which plasmids were purified for sequencing. Cycle sequencing reactions were performed using Applied Biosystems BigDye (version 2.0) Terminator chemistry. Reactions were purified using the Millipore Multiscreen plates and then dried and resuspended as per manufacturer’s protocols for loading on the automated sequencer. Automated DNA sequencing was performed in the Core Laboratory Facility of the Virginia Bioinformatics Institute using standard methods on an ABI 377 automated DNA Sequencer or an ABI 3100 capillary sequencer.
RESULTS AND DISCUSSION

Of 5 sequencing reactions, four were successful. The sequences obtained from these were from base pairs 652 through 1250 of the pBluescript phagemid (Fig. 5.1). None contained a cDNA insert. Since these were derived from white plaques, which imply a disruption in the lacZ gene I had expected a cDNA insert. However, it is possible to have white plaques in the absence of any insert. Even a point mutation in either the lacZ or _-galactosidase genes can disrupt the marker and produce white colonies. Normally these false positives are far outnumbered by insert-containing vectors, but cases where the insert titer is very low relative to the vector titer the false positive background may dominate. I believe that this is what happened in my investigation.

According to the Zap-cDNA synthesis kit protocol, white plaques should outnumber blue ones by 100 or more to 1. When this library was plated for blue/white selection, the white and blue plaques appeared in approximately equal numbers. This was not unexpected, since the protocol was optimized for 5 µg of mRNA, and the library construction began with no more than 50 ng. These false positives would, in a successful library, be outnumbered by insert containing positives by approximately 100 to 1. This does not rule out the possibility that there may be filistatid cDNA lurking somewhere in the library, but it is in a lower concentration than the false positives, making it very difficult to extract. Figures 5.2 and 5.3 provide a flow diagram of the procedure indicating the point at which the vector ligated to itself, rather than to a cDNA insert.
Figure 5.1: “Insert” sequences.
Figure 5.2: Steps from cribellar gland tissue to cDNA

- Dissect *K. hibernalis* cribellar silk glands, stored in RNUlater (Ambion)
  - Extract mRNA using poly(A) pure kit (Ambion)
  - 50 ng mRNA
  - Make into cDNA using ZAP-cDNA synthesis kit (Stratagene); the first strand synthesis is done with poly (T) primers containing a *Xho I* restriction site and methylated dCTP to protect internal restriction sites, the second with nick-translation of the RNA.
  - 5'-cDNA-3'
  - Blunt cDNA ends, and ligate Eco R I adapters to cDNA ends
  - 5'-EcoR I - cDNA - Xho I - Eco R I - 3'
  - Digest with *Xho I* to produce unidirectional cDNA
  - 5'-Eco R I - cDNA - Xho I - 3'
Figure 5.3: Steps from unidirectional cDNA to sequences

5'-Eco R I-Xho I-3'

- Purify cDNA from unligated ends and free nucleotides using Qiaquick® PCR purification kit (Qagen®). (Incomplete purification at this stage may have left free nucleotides that facilitated self-ligation of the vector in later steps)

Ligation into λ-ZAP® phagemid vector (Stratagene)

- At this stage, the paucity of cDNA in the reaction allowed the formation of a larger than usual percentage of empty vector ligations, some of which had disruption of the β-galactosidase or lac-Z genes

Amplification of phage in F′XL1-Blue cells (Stratagene®)

- Amplified library screened with blue-white selection, which detects disruption of the lac-Z gene. cDNA inserts within the lac-Z gene, so white colonies (which are unable to break down IPTG to form a blue precipitate) usually contain a cDNA insert

Library

6 individual white plaques

- pBluescript® phagemid (Stratagene) isolated by infecting F′XL1 Blue cells with phage from plaques and ExAssist® (Stratagene) helper phage. The resulting lysogeny produces phagemids that are then introduced into the SOLR® strain (Stratagene), where they replicate as plasmids.

pBluescript Plasmids

- Purified using the Qiaquick® plasmid miniprep kit (Qagen)

Sequenced using T7 primers

- Sequences - BLAST search reveals match with pBluescript multiple cloning site and no cDNA insert for 4 plaques (2 did not sequence)
LITERATURE CITED


Anyah Hawthorn attended Antioch University for two years, and graduated from Radford University with a B.S. in biology in May, 2000. She lives with partner Sonya Perkins, their children Peregrine, Jamie and Tonya, and pets of many species (but no tarantulas).

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PUBLICATIONS:

ABSTRACTS:

PAPERS:


GRANTS:
2001 Virginia Polytechnic Institute and State University, Graduate Research and Development Grant. Title: An Investigation of Hygroscopic and van der Waals Forces in the Stickiness Mechanism of Cribellar Thread. Amount: $300, plus $300 matching funds.


2002 Virginia Polytechnic Institute and State University, Graduate Research and Development Grant. Title:. A Molecular Model of the Stickiness Mechanism of Spider Cribellar Silk. Amount: $300, plus $300 matching funds.

PRESENTATIONS OF ORIGINAL RESEARCH

2002 26th Annual Meeting of the American Arachnological Society. Riverside, CA. Title: How does Cribellar Silk stick to smooth surfaces, evidence foe van der Waals and hygroscopic forces.

2001 25th Annual Meeting of the American Arachnological Society. Lexington, KY. Title: How does Cribellar Silk stick to smooth surfaces?

2000 Radford University Undergraduate Forum. Title: Kin Recognition in the Zebrafish (Danio danio)

2000 Undergraduate Honors Convention, Hampton College. Title: A Behavioral and Ecological Study of the Funnel Web Spider.

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