Capture Silk Scaffold Production in the Cribellar Web Spider

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Research Article

Keywords: Cribellum, scaffold, silk, spider, spigot

DOI: https://doi.org/10.21203/rs.3.rs-478775/v1

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Abstract

Spider capture silk is a kind of natural scaffold material that outperforms almost any synthetic material in its combination of strength and elasticity. Among the various kinds of silk threads, the cribellar thread is the most primitive type of prey-capturing thread found in spider webs. We analyze the functional organization of the sieve-like cribellum spigots and a specialized comb bristles of calamistrum for capture thread production in the titanoecid spider *Nurcia albofasciata*. It's outer surface of the cribellum is covered with thousands of tiny spigots, and this cribellum plate produces the non-sticky threads which composed of thousands of finest nanofibers. Average length of the cribellum spigot in *N. albofasciata* is 10 µm, and each cribellate spigot appeared as singular, long shafts with pagoda-like tiered tips. Each spigot has five distinct segments as a definitive characteristic of this spider. This segmented and flexible structure not only allows it to bend by itself and join together with adjacent spigots, but also enable to draw the silk fibrils from its cribellum with a row of leg bristles of calamistrum to form a cribellar prey capture thread.

Introduction

The araneomorph spiders can be classified into cribellate and ecribellate type along with the presence or absence of a cribellum (Coddington and Levy 1991). The cribellum is a silk spinning organ consisting of one or more plates covered with thousands of tiny spigots (Foelix 2011). These spigots produce extremely fine fibrils which are quickly hackled by the spider’s calamistrum, giving silk with a wooly structure.

It has been reported that the cribellar silk is considered to be a type catching silk with dry-adhesive properties (Opell 1995, 1999). These cribellar fibrils are spun from the spigots of an abdominal spinning plate called the cribellum. Those fibers are very small in diameter, so prey insects easily become entangled in them without any glue substances. The spiders then bite them before they can get away (Opell 2002; Hawthorn and Opell 2003).

While the cribellate web was considered an expensive way to catch prey at high production costs in terms of labor required to comb and deposit cribellar silk (Opell et al. 2000; Opell and Schwend 2009), cribellar silk improves prey capture by allowing the web to retain prey rather than delaying its passage (Opell 2002). The similarity between cribellar silk production and prey wrapping suggests that the cribellum may have evolved from the anterior median spinnerets as a source of dense silk that wraps prey in spider webs (Opell et al. 2011).

Since the number of spigots on the spider cribellum is directly related to the stickiness of its cribellar threads (Opell 2002; Opell and Schwend 2009), the morphology of the cribellar spinning plate and the distribution of the spinning apparatuses are regarded as important characteristics of this cribellate spiders (Park and Moon 2009). Recently, the basic principles of the cribellate spinning process observed in *U. plumipes* demonstrated that the cribellate fibers were organized as a mat surround the paired axial
fibers. Furthermore, the capture threads of *Z. geniculata* showed interconnections between cribellate mat and axial fibers (Joel et al. 2016).

A calamistrum in spider is a row of specialized appendage bristles used to comb out fine bands of silk fibrils (Foelix 2011). This is a kind of comb-like device in certain spiders that separates the silk fibers drawn from the cribellum into many extremely fine fibers, giving it a wool-like structure. The calamistrum and cribellum are used to form the hackled bands of silk that is a characteristic of the webs of the cribellate spiders (Opell et al. 2000; Joel et al. 2016). While the cribellum is an oval spinning field whose spigots produce silk fibrils that form the outer surface of the primitive prey-capture threads found in aerial spider webs (Opell 1999), the calamistrum pulls silk fibrils from the cribellum and helps combine them with supporting strands to form a cribellar prey-capture thread (Opell et al. 2000).

Recent research clearly reveals that the fibers from the cribellate spinning spigots are pass through a rather smooth surface-like region on the calamistrum. Kronenberger and Vollrath (2015) demonstrated that the dry capture threads combines thousands of single nano-scale filaments issuing singly from individual spigots to be electrically charged by specialist combs on the spider's legs. In particular, the contact between the fibers and the calamistrum can be adjusted after finishing thread production without changing the influence of the calamistrum on fiber (Joel et al. 2016). However, it is not resolved how the cribellate nanofibers can effectively assemble a puffy structure within the capture thread using a specialized setae comb of the calamistrum.

The spider *N. albofasciata* is a species that makes cribellate catch silk web with the aid of calamistrum. This spider is one of the most abundant and conspicuous spiders in the temperate zone, but little is known about their spinning system of both cribellum and calamistrum. Thus, this paper describes the functional organization of the cribellum spigots and calamistrum in the titaneocid spider *N. albofasciata* through experiments using the field emission scanning electron microscope (FESEM).

**Materials And Methods**

Adult individuals of the cribellate spiders of the family Titanoecidae (Araneae: Titanoecidae) were collected in a local area near the dumping site of dredging soil at the Busan New Port of Jinhae city, Kyungnam, Korea. All spiders were maintained under ambient conditions with natural lighting in enclosures comprising a wooden cages. They were fed insects and water daily.

Both of female and male specimens were anesthetized with CO₂ and dissected under a dissecting light microscope in a drop of spider Ringer's solution consisting of 160 mM NaCl, 7.5 mM KCl, 4 mM CaCl₂, 1 mM MgCl₂, 4 mM NaHCO₃ and 20 mM glucose, pH 7.4 (Moon and Tillinghast 2020). The specimens for histologic preparation were fixed in alcoholic Bouin's solution, embedded with Paraplast embedding medium (Fisher Scientific Co., Pittsburgh, Pa, USA) and stained with hematoxylin and eosin solution.

For field emission scanning electron microscopy, the whole abdomen were gently removed and pre-fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at
pH 7.4 for 2 hours. Postfixation was performed with 1% osmium tetroxide in the same buffer and washed several times in 0.1 M phosphate buffer for 1 hour. After each fixation step, the samples were rinsed three times with phosphate buffered solution at 15 minute intervals. The samples were then dehydrated through a graded series of ethanol from 50% to absolute ethanol, and then transferred to hexamethyldisilazane (HMDS) for air-dry (Seo et al. 2020).

The samples were then coated with platinum-palladium with a thickness of 20 nm, using a Hitachi E-1030 ion sputter coater (Hitachi Co., Tokyo, Japan). Coated samples were observed with a Hitachi S-4300 (Hitachi Co., Tokyo, Japan) field emission scanning electron microscopy (FESEM) with an accelerating voltage of 5–20 kV.

Results

The specialized spinning plate, called a cribellum is located at the ventral surface on its abdomen. The cribellum in the spider, *N. albofasciata* is placed at the upper region of the spinnerets. Although the term cribellum literally means little sieve and applies to biological structures in the form of tiny perforated plates, this broad plate is set firmly in the abdominal cuticle. There were two defined halves of the spinning field on the cribellum which compose of medially divided plates (Fig. 1A).

Apart from the cribellar spigots, it has been observed that there are three pairs of spinnerets in this spider. Two types of silk spigots, the major ampullate gland and the pyriform gland spigots, were distributed on the anterior lateral spinneret (Fig. 1B, C). On the posterior median spinneret, two types of spigots, the minor ampullate gland and the aciniform gland spigot were observed (Fig. 1D). Individuals of this spider also possessed aciniform gland spigots on the posterior lateral spinnerets (Fig. 1F).

Individuals of *Nurscia albofasciata* possessed bipartite cribellum plate which divided with left and right spinning fields (Fig. 2A, B). The surface of the cribellum is covered with thousands of elongate spigots, all spigots act together to produce cribellate threads made up of thousands of silk fibrils (Fig. 2C, D). The total number of the cribellate spigots vary among individuals or according to their maturity with the variant number from 500 pairs in male to 800 pairs in female spiders (Fig. 2E, F).

This cribellate spider also has a calamistrum comb and this combs the silk that flows from the cribellum, producing a characteristically woolly silk. A specialized comb of bristles is located only on the last pair of legs among 4 pairs of appendages. The calamistrum is found on the upper margin of the metatarsus (Fig. 3A). These bristles are used to simultaneously comb out the mass of cribellate fibrils and their supporting silk lines from cribellum (Fig. 3B). Each bristle of the calamistrum is embedded in a cuticular socket, and is serrated on one side and smooth on the other. The surface of the setae is completely covered with grooves (Fig. 3C). The bristles are long and straight, partly sickle-shaped, strong bristles with pointed longitudinal grooves toward the ends. Each bristles is located in an open articulatory socket, and the gap between each bristles is approximately 5 µm (Fig. 3D).
The cribellate silk in *N. albofasciata* is produced from the spigots of the cribellum, and the cribellate spigots appeared as singular, long shafts with pagoda-like tiered tips. The cribellar silks are produced through these elongated spigots which protruded from the cribellar plates (Fig. 4A-C).

All spigots act together and producing numerous cribellate silk brils at a same time. They are all approximately the same length, and average length of the cribellum spigot is 10 μm. These segmented and flexible structure enable to bent itself and conjoin together with adjacent other spigots (Fig. 4D-F).

All of these spigots are composed of five tubal segments with four thicker regions which will provide the characteristic pearling node of the cribellate threads (Fig. 5A-C). The cribellar silk spinning system consists of tiny silk glands each terminating through exceptionally long and narrow ducts. The cribellar plate is composed of thousands spinning outlets depending on the size of spiders (Fig. 5D-F).

By our fine structural observation using the field emission scanning electron microscopy (FESEM), each cribellar spigot shows segmented flexible structure which enable to bent itself and conjoin together with adjacent spigots (Fig. 6A). The expanded intersegmental spaces finally create pearling of the cribellate thread and provide supporting points to hold silk fibrils during the hackling process by leg combs of the calamistrum (Fig. 6B). Thus, a row of leg bristles of calamistrum draws silk fibrils from its cribellum and helps combine them with supporting strands to form a cribellar prey capture thread (Fig. 6C).

**Discussion**

Spiders can be classified by their shape and number of components of their silk-spinning apparatus, since the apparatus often undergoes adaptative variations, some basic characteristics usually remain unchanged at the familial level (Peters 1987; Shear 1994). Previous researchers have found that the functional specialization of the silk-spinning apparatus involves precise modifications of the spinnerets, anatomical characteristics of the silk glands and the number and morphology of spigots (Peters and Kovoor 1991; Moon and Tillinghast 2004; Foelix 2011; Park and Moon 2014).

Although spiders produce various kinds of silks which are used for the remarkably diverse silk constructs (Denny 1976; Coddington 1986), the main function of the spider silk is prey-catching (Nentwig and Heimer 1987). Therefore, spider webs are classified as either of cribellate or ecribellate groups according to different mechanisms of prey catching system (Foelix 2011). The ecribellate spider relies on a wet glue spinning process that uses liquid silk solution to form aqueous droplets on core filament (Vollrath and Knight 2001; Park and Moon 2014). However, the cribellate spider produces dry capture threads from cribellate spinning organ (Peters 1987; Peters and Kovoor 1991; Bott et al. 2017). The cribellum is a sievelike, transverse plate covered by hundreds or thousands of tiny, elongate spigots, each producing a single fibril of cribellate silk (Nentwig and Heimer 1987; Opell 1995, 1999).
Most spiders produce silk with micrometer scale fibers, but the cribellate spiders spin nanoscopic fibers. Recently, Kronenberger and Vollrath (2015) has shown that the nano-scale fibers spun from the cribellate orb spider, *Uloborus plumipes* are electrically charged for the purpose of their prey capture. According to their hypothesis, the cribellar spigots have an unique morphology with and outer shed uncannily resembling the multilayered ‘weather sheds’ shape of high-voltage insulators designed to prevent flow via leakage (Suwarno 2009; Kronenberger and Vollrath 2015).

Previously, Opell and Schwend (2009) and Opell et al. (2011) also revealed that the cribellum silk captures and holds prey using van der Waals interactions including with the involvement of longer-range electrostatic forces. Our fine structural observation also shown that cribellum structure with long and slendered cribellar spigots and the calmostrium at the hind-leg likely be attributed to electrostatic charging during the spinning of fibers on the nano-scale.

The cribellate silk in *N. albofasciata* is produced from the spigots of an abdominal cribellum with a pair of medially divided plates. It clearly shows that this specialized anatomical characteristic is similar to those cribellate spiders with divided cribellar spinning plates (Opell 2002; Opell et al. 2011; Hjar et al. 2017) On the basis of fine structural analysis using scanning electron microscope, It has been revealed that the surface is covered by hundreds or thousands of tiny, elongate spigots, each producing a single fibril of cribellate silk with the size of true nano-scale. All of these spigots act together to produce a single cribellate thread made up of thousands of the silk fibrils (Eberhard et al. 1993; Park and Moon 2009).

Since the cribellar threads are primitive prey capture threads formed of thousands of fine, looped cribellar fibrils, the number of spigots on a cribellum is related to the stickiness of its cribellar thread (Opell et al. 2011; Hajer et al. 2017). Opell (2002) showed that the linear cribellar thread spun from the divided cribellum of *K. hibernalis* was both wider and stickier than thread from the undivided cribellum of *W. waitakerensis*. Since the divided cribellum of *K. hibernalis* and the undivided cribellum of *W. waitakerensis* had a similar number of spigots and produced cribellar threads with similar stickiness, both a spider’s spinning anatomy and its spinning behavior affect the stickiness of its cribellar threads.

It is therefore likely that a dry web made with a meshwork of these composite wool-like threads is particularly effective at tangling the bristles, claws and spines of insect prey. The fine fibrils of cribellate silk also appear to have dry adhesive with electrostatic properties and will even cling to smooth beetle cuticle (Opell 2002). Previous studies also have shown that the cribellar thread appears to rely on at least two major stickiness mechanisms. The fibrils on its surface can snag on an prey insect's setae, and hold them like the looped side of a Velcro fastener (Autumn et al. 2000; Hawthorn and Opell 2003). Cribellar thread also adheres to nonsnagging surfaces that are fairly smooth even on a microscopic level such as graphite, polished steel and glass by an unknown mechanism (Eberhard 1980, 1988). It holds more tightly to the smooth surface of beetle elytra than to the heavily setose surface of a fly notum (Opell and Schwend 2009).

On the basis of fine structural analysis using scanning electron microscope, It has been revealed that the cribellate spider *N. albofasciata* also possess the calamistrum with a row of toothed bristles on the
metatarsal segment of the last leg. These bristles are used to simultaneously comb out the mass of cribellate fibrils and their supporting silk lines from the cribellum and spinnerets. Therefore silk fibrils are spun by collectively by the comb hairs on the spider’s hind legs and jerked out of their spigots by the rapid hackling.

Recently, Kronenberger and Vollrath (2015) demonstrated that the fiber-forming process of the cribellate orb spider *Uloborus plumipes* is different from the silk-spinning systems of all other known spider. The cribellum glands have long ducts but lack the internal extrusion process draw-down. To be able to flow in the pockets of the spigot, the dope for the cribellate silk must have an exceptionally low viscosity and must be liquid all the way to the spigot since the silk is already a thread when it reaches the spigot (Vollrath and Knight 2001; Davies et al. 2013).

They suggest that the silk solidifies in the milliseconds between each violent hackling pull to be ‘frozen’ into shape during the pulling post-draw. Their electron microscopic examination of the ducts of the cribellate glands revealed this interpretation as we also predict in our separate study using *N. albofasciata* since we also predict the possibilities after observing the specialized pearling chambers of the spigots which filled with silk materials.

The cribellar silk spinning system in *N. albofasciata* is composed of tiny silk glands each terminating through exceptionally long and narrow ducts. In addition, each cribellar spigot shows segmented flexible structure which enable to bent itself and conjoin together with adjacent spigots. In particular, all of the spigots are composed of five tubal segments with four thicker regions which enable to provide the pearling node of the cribellate threads. This expanded intersegmental spaces finally create pearling of the cribellate thread and provide supporting points to hold silk fibrils during the hackling process by leg combs of the calamistrum. Thus, a row of leg bristles of calamistrum draws silk fibrils from its cribellum and helps combine them with supporting strands to form a cribellar prey capture thread.

**Conclusion**

- We investigated the nanoscopic structural features of the sieve-like cribellum spigots for capture thread production in the titanoecid spider *Nurscia albofasciata*.
- Cuticular surface of the cribellum is covered by thousands of tiny spigots, and this cribellum plate produces the non-sticky threads which composed of thousands of finest nanofibers.
- Average length of the cribellum spigot in *N. albofasciata* is 10 µm, and each spigot has five distinct segments as a definitive characteristic of this spider.
- Each cribellate spigot appeared as singular, long shafts with pagoda-like tiered tips.
- Segmented and flexible structure allows it to bend by itself and join together with adjacent spigots to form a cribellar prey capture thread.
- The expanded intersegmental spaces create pearling of the cribellate thread and provide supporting points to hold silk fibrils during the hackling process by leg combs of the calamistrum.
Declarations

Abbreviations

Not applicable

Acknowledgements

This research was supported by the National Research Foundation (NRF) of Korea funded by the Ministry of Education (NRF-2014R1A1A2056398) and the Korea government (MSIT) (NRF-2019R1I1A3A01062105)

Authors' contributions

Myung-Jin MOON (Corresponding Author): contributions to the conception, design of the work as well as contribution to the acquisition, analysis, and interpretation of data. Yan SUN (First Author): contribution to analysis, and interpretation of data. Seung-Min LEE (Second Author), Bon-Jin KU(Third Author): contribution to preparing manuscript and preparing poster presentation. Eun-Ah PARK (fourth Author): contribution to SEM sample preparation and observation.

Funding

This research was supported by the National Research Foundation (NRF) of Korea funded by the Ministry of Education (NRF-2014R1A1A2056398) and the Korea government (MSIT) (NRF-2019R1I1A3A01062105)

Availability of data and materials

Materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes

Competing interests

Not applicable

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**Figures**

**Figure 1**

Scanning electron micrographs of the cribellum and spinnerets in the spider N. albofasciata. A: Cribellum (CB) is located at the upper region of the anterior spinnerets (AS). B, C: On the anterior lateral spinneret, the major ampullate gland (Am) and the pyriform gland (Py) spigots were observed. D: On the posterior median spinneret (MS) the minor ampullate gland and the aciniform gland (Ac) spigots were distributed. E: On the posterior lateral spinnerets (PS), spigots of the aciniform glands are seen. Scale bars indicate 100 µm (A), 20 µm (B,E), 10 µm (D), and 5 µm (C), respectively.

**Figure 2**

Scanning electron micrographs of the cribellum in the spider N. albofasciata. A, B: The cribellar silk spinning system consists of bipartite cribellum (CB) plate which divided with left and right spinning
fields. C, D: A pair of cribellar plate with hundreds to thousands outlets are medially divided to form a symmetrical distribution of spinning apparatuses. E, F: The surface of the cribellum is covered by numerous elongate spigots which producing numerous cribellate silk fibrils. Scale bars indicate 50 µm (A,B) and 20 µm (C-F).

**Figure 3**

Scanning electron micrographs of the calamistrum combs in the spider N. albofasciata. A: On the upper margin of the metatarsus (Mt) segment of the 4th leg, a specialized comb of bristles, called a calamistrum (arrows) is located. B: The bristles (Br) are long, straight, and have a pointed end toward the tip. C: Each bristle of the calamistrum is embedded in a cuticular socket (Sc). D: The surface of the bristle is completely covered with longitudinal grooves (arrowheads). Scale bars indicate 200 µm (A) and 10 µm (B-D).

**Figure 4**

Scanning electron micrographs of the cribellate spigots in N. albofasciata. A-C: The cribellate spigots appeared as singular, long shafts with pagoda-like tiered tips. D-F: All spigots are all approximately the same length (10 µm). These segmented and flexible structure enable to bent itself and conjoin together with adjacent other spigots. Scale bars indicate 5 µm (A,D,E) and 2 µm (B,C,F).
Figure 5

High magnification scanning electron micrographs of the cribellate spigots in N. albofasciata. A-C: Cuticular surface of the cribellum is covered by hundreds or thousands of tiny, elongate spigots, each producing a single fibril of cribellate silk. D-F: All of these spigots are composed of five tubal segments with four thicker regions. Each cribellar spigot shows segmented flexible structure which enable to bent itself and conjoin together with adjacent spigots. Scale bars indicate 1 µm (A) and 0.5 µm (B-F).
Figure 6

A: Scanning electron micrograph of the cribellate spigots in N. albofasciata. The cribellate spigots are composed of five tubal segments with four thicker regions which will provide the characteristic pearling node of the cribellate threads. B: Diagram of the cribellate spigot which composed of five tubal segments with four thicker regions. C: Photo micrograph of the cribellate silk line. Each silk line has a unique outer
morphology resembling the segmented insulators since the silk filaments are spun out from the spigot by the rapid hackling. Scale bar indicates 0.5 µm.