Most spider threads are on the micrometre and sub-micrometre scale. Yet, there are some spiders that spin true nano-scale fibres such as the cribellate orb spider, *Uloborus plumipes*. Here, we analyse the highly specialized capture silk-spinning system of this spider and compare it with the silk extrusion systems of the more standard spider dragline threads. The cribellar silk extrusion system consists of tiny, morphologically basic glands each terminating through exceptionally long and narrow ducts in uniquely shaped silk outlets. Depending on spider size, hundreds to thousands of these outlet spigots cover the cribellum, a phylogenetically ancient spinning plate. We present details on the unique functional design of the cribellate gland–duct–spigot system and discuss design requirements for its specialist fibrils. The spinning of fibres on the nano-scale seems to have been facilitated by the evolution of a highly specialist way of direct spinning, which differs from the aqua-melt silk extrusion set-up more typical for other spiders.

1. Introduction

Spider webs have two very different mechanisms of holding onto entrapped prey: electrically charged nano-thin filaments or dollops of sticky glue [1,2]. The two adherents differ greatly in their evolutionary age as well as in their costs of production—with the glue being significantly younger and also cheaper to make for the spider [3]. The wet glue spinning process relies on a self-assembling mechanical system that cunningly uses the Rayleigh Plateau instability of the highly water-diluted, liquid silk solution to form aqueous droplets on core filaments, which then gather the core filaments into a micro-windlass system apparently powered by surface tension [4,5]. The dry capture thread, on the other hand, combines thousands of single nano-scale filaments issuing singly from individual spigots to be quickly hackled and apparently electrically charged by specialist combs on the spider’s legs into fluffed-up ropes [6].

Somehow this cribellate micrometre-scale extrusion system manages to spin nano-scale filaments, i.e. not only a new spider silk-spinning technology, but one that might also provide important hints for the commercial development of novel polymer extrusion technologies. After all, thread diameter is one key determinant of filament strength [7]. In contrast to spider silk-spinning, synthetic polymer fibres are produced by hot-melt extrusion, whereby a solution is pumped or drawn through cylindrical dies and spun/drawn by a take-up device to produce filaments with typical diameters of 10 μm and above [8]. To achieve diameters in the nanometre range, electrospinning is today’s most efficient and versatile polymer processing technique [9]. This leads to the question: How does a spider like *Uloborus* spin its nano-filaments? Has this spider evolved a novel way of spinning or has it somehow refined the typical spider aqua-melt extrusion-spinning setup [8,10]?

A number of studies have examined the spinning apparatus of uloborids without, so far, elucidating details of the production process of these cribellate nano-filaments (e.g. [11–14]). By employing a combination of new sample preparation and novel imaging approaches we obtained detailed information on this
silk production system allowing us to comment on (i) the design of the gland production system and (ii) its possible function.

2. Material and methods

(a) Specimen collection. Adult female specimens of Uloborus plumipes (Lucas, 1846) were collected in pesticide-free garden centres in Hampshire, UK.

(b) Photography. Photographs of Uloborus were taken with a Canon 5D Mark III; a Canon EF 100 mm f/2.8 macro USM lens (manual focus and live view mode).

(c) Video. Uloborus was recorded ‘hacking’ cribellate silk with a Canon 5D Mark III in full HD (1920 × 1080) using LED-lamps (Dedolight Ledzilla). Still frames of a video were selected to measure the distance of a single hacking movement from and back to the cribellum. The frequency of hacking movements was assessed using the software iMovie (v. 9.09). Frame rate was reduced to 5 s per frame; 10 frames were analysed.

(d) Confocal scanning microscopy. Spiders were anaesthetized in chloroform vapour. Opisthosoma were fixed in alcoholic Bouin’s solution at room temperature for 24 h. Samples were then embedded in 5% agarose, vibratomed in 200 μm sections, stained for 3 min with Langor’s carmine, dehydrated in a graded methanol series (50, 70, 95, 100%) for 30 min each, and then cleared and mounted in 100% benzyl benzoate (BAAB) in Ibidi coverslip-bottom 8-well μ-slides (Thistle Scientific, Glasgow). Autofluorescence of carmine-stained samples was imaged on a Leica TCS SP5 laser scanning confocal microscope on a Leica DMi6000 microscope using 10× HCX PL FLUOTAR 10.0 × 0.30 dry or 20× HC PL APO immersion (used with oil) objectives and Leica LAS-AF confocal acquisition software (v. 2.2.1) (Biomedical Imaging Unit, University of Southampton).

(e) Transmission electron microscopy. Opisthosoma of adult female specimens were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer at room temperature for 24 h. The cribellum was isolated, post-fixed in OsO₄ and stained en bloc in uranyl acetate. Samples were then dehydrated in a graded ethanol series, embedded in the low viscosity medium resin Spurr. Gold interference-coloured sections were collected on uncoated copper grids, contrasted with lead citrate and examined with an FEI Tecnai 12 transmission electron microscope.

(f) Scanning electron microscopy. Samples were fixed in alcoholic Bouin’s at room temperature for 24 h, dehydrated in a graded ethanol series, mounted on stubs with double-sided sticky tape, critical point dried and sputter coated with gold. Samples were viewed with an FEI Quanta 200 scanning electron microscope.

(g) Morphological measurements. Mean length, mean internal diameter and mean thickness of the cuticle of a typical cribellate duct were measured in Photoshop CS2.

3. Results

The full complement of silk glands in the spider Uloborus is one of the most complex known in spiders and consists of eight different silk systems (figure 1b). Here, we focus on the unique cribellar glands that yield the ultra-fine ‘catching wool’ of the cribellate prey capture thread—glands that are also among the smallest of silk glands described from any spider (figure 1b,g,h). Externally, these glands terminate in micrometre-scale spigots, which densely cover the cribellum plate located in front of three pairs of spinnerets (figure 1c–f).

The spigots have a unique outer morphology and geometry, strangely resembling voltaic insulators (figure 1f). Internally, this system combines the typically unconnected features of a simple gland and a long duct (figure 1g), which are, respectively, ancestral and highly derived [15].

The spherically shaped cribellare glands are situated in the posterior third of the spider’s opisthosoma and form a compact mass containing thousands of tiny, single glandular units (figure 1b,h). Each gland leads via a strikingly long, straight and small-diameter cuticular duct to a tiny and unusually shaped spigot (figure 1g,j; see also the electronic supplementary material). Many hundreds (to thousands in some species) of these spigots share the cribellum spinning plate (figure 1d,e). Typical length of a duct in a mature female Uloborus is 514.88 ± 224.70 μm, with typical inner gland diameter of 48.61 ± 9.07 μm; typical diameter at the middle of a duct is 0.19 ± 0.03 μm inside and 0.11 ± 0.03 μm outside, with inner diameter of 0.13 ± 0.02 μm at the entry into the spigot and 0.05 ± 0.01 μm at its exit. Silk filaments are extrusion spun by being individually or collectively hooked by the hairs on the specialist combs on the spider’s hind legs (figure 1a) and jerked out of their spigots by the rapid hacking (8 Hz, i.e. 8 ± 0.52 pulls per second, see the electronic supplementary material).

4. Discussion

During their history of 400+ years, spider silks have evolved an interesting and presumably highly adapted combination of molecular composition and processing conditions [15]. We found that the cribellate glands studied have long ducts but lack the internal draw-down so characteristic for other long ducts, where the silk is already a thread when it reaches the spigot [10,16]. Indeed, the cribellate silk is so liquid at this stage that it even fills the pockets of this unusually shaped spigot, indicating a fibre-forming process that is different from that of all other known spider silk-spinning systems. To be able to flow in this kind of extruder, the dope must have an exceptionally low viscosity and must be liquid all the way to the spigot rather than form filaments early in the duct (see the electronic supplementary material). The filling of the ‘pearling’ chambers of the spigot with silk confirms this interpretation (figure 1k). Importantly, some of this ‘pearling’ seems to remain in place on the finished thread after hacking (figure 1i), which suggests that the silk somehow forms/solidifies in the milliseconds between each violent hacking pull to be ‘frozen’ into shape during the pulling post-draw.

If this interpretation were correct, then the hacking action would determine the morphology as well as the mechanical properties of the fibre, providing post-draw stretching far exceeding any post-draw experienced by typical silk threads. In commercial polymer spinning, post-draw can lead to extensions of ca 500% and increases of modulus of ca 500%, with the increased mechanical properties deriving from higher molecular ordering and lower disorder ‘void’ fractions [17]. Importantly, in the spider the combing action draws the solidified post-draw thread onto the earthed roller system of industrial spinning but by the very rapid combing of chitin hairs, which in all likelihood (being chitin [18]) are non-conducting [19]. This then would lead to electrostatic friction charging of the silk as well as some filament crimping due to uneven post-draw loading.
The concept of charges carried on this type of silk is not new. Opell and his collaborators [1,2] have long maintained that cribellum silk captures and holds prey using van der Waals forces, while strongly implying that longer-range electrostatic forces are also involved. The formation and maintenance of the silk ‘puffs’ (figure 1b, SEM) provides further, and independent, evidence for post-draw charging as the forces keeping these nano-dimensioned filaments apart can most

*Figure 1. Nano-silk production in *Uloborus plumipes* (adult, ♀). (a) *Uloborus* in lateral view; arrow indicates the position of the setal comb (calamistrum) on the metatarsus of the fourth leg; arrowhead indicates the position of the spinning apparatus. (b) Illustrated is the complete silk gland system of *Uloborus*. Coloured in are the glands producing the silks making up the prey capture thread. Cribellate silk is produced by the cribellar glands (pink) and laid down upon two axial fibres produced by the pseudoflagelliform glands (green) and paracribellar glands (blue). The scanning electron micrograph (SEM) demonstrates the cribellate multi-fibre composite produced by *Uloborus*. (c) SEM of part of the spinning apparatus in frontal view with the cribellum (cr) in the centre. Anterior spinnerets (as); posterior spinnerets (ps); median spinnerets (ms) are in inactive position. (d) SEM of the cribellum spinning field featuring high density of specialized cribellate spigots. (e) Cribellate spigots at higher magnification. Each spigot is about 6 μm long. (f) Same as in (e) but at higher magnification. Note the unique geometry of these silk outlets. (g) Illustration of the cribellate silk thread production system showing details of glands (g), glandular lumen (l), excretory ducts (d) and spigots (s). (g1) Illustration of a longitudinal section of a cribellate spigot. (h) Confocal micrograph of a section through the opisthosoma showing mass of cribellar glands (g), musculature (m), and ducts (d) connecting glands with spigots covering the cribellum (cr). (i) Confocal micrograph showing details of numerous excretory ducts (d), each feeding into a single spigot on the surface of the cribellum. Note the presence of giant nuclei (n). (j) Same as in (i) but at higher magnification; nuclei (n). (k) Transmission electron micrograph (TEM) of a longitudinal section of a cribellate spigot. (l) TEM illustrating the ‘pearling’ of the cribellate thread.
likely be attributed to electrostatic charging [20]. Finally, the hypothesis of active electrostatic charging during spinning might be supported by the unique morphology of the cribellum spigots, with an outer shape uncannily resembling the multi-layered ‘weather sheds’ shape of high-voltage insulators designed to prevent current flow via leakage [21]. Could it be that the cribellum spigot ‘sheds’ have evolved for a comparable purpose?

Alternatively, the unique geometry of annular constrictions in the outer surface of a large part of the cribellate spigot may impart considerable overall pliability, while local stability may be due to the longitudinal ridges in the cuticle, stiffening the crucial constricted region of the die (figure 1c/f). This kind of combination of compliance and rigidity may be necessary to prevent spigot damage when the rigid comb hairs hackle abruptly over the cribellum plate in 8 Hz sequences of quick and forceful pulls. Indeed, the unique choreography of the duct in its final stretch may have evolved to provide reservoirs of still-liquid silk (perhaps inside a somewhat solidified coating) that could serve to distribute the forces generated by the hacking force separated by periods of zero-flow and thus prevent local concentrations and breakage. After all, individual filaments are only a few tens of nanometres in diameter and hence only able to withstand sub-nano-Newton pulling forces. A buffer of liquid or highly hydrated silk would greatly reduce the shear forces inside the length of the spigot and duct, which is so thin (average diameter 50 nm at the very tip) that even non-Newtonian flow would presumably have problems coping [22].

However we may seek to explain the function of the cribellate spinning system we studied, it is obvious that Uloborus is able to spin nano-scale filaments of great length and it may be assumed that the animal also somehow manages to electrostatically charge them. The spinning system observed has key features not found in other spiders studied so far and clearly presents a challenge that needs to be tackled in detailed follow-up studies. Given our growing understanding of the interaction between fibre dimensions and mechanical properties, the growing importance should be obvious for thin-fibre technology seeking commercial production of nano-scale filaments by highly controllable extrusion electro-charge spinning, rather than the somewhat uncontrollable traditional electrospinning.

Data accessibility. Data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.nl3ts

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