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 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 plu-
mipes (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 move-
ment 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 anaesthetised in
chloroform vapour. Opisthosoma were fixed in alcoholic
Bouin’s at room temperature for 24 h. Samples were then
embedded in 5% agarose, vibratomed in 200 µm sections,
stained for 3 min with Langeron’s carmine, dehydrated in a
graded methanol series (50, 70, 95, 100%) for 30 min each,
cleared and mounted in 100% benzyl benzoate (BAAB) in
Bouin’s at room temperature for 24 h. Samples were 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 stand 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 acqui-
sition 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
cribellar 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 cribellum 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 single gland and a long duct (figure 1g), which are,
respectively, ancestral and highly derived [15].

The spherically shaped cribellar glands are situated in the
posterior third of the spider’s opisthosoma and form a com-
pact 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 unu-
sually shaped spigot (figure 1g,h; 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 514.8 ± 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 spi-
gots 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 cribellum 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 hackling
(figure 1l), which suggests that the silk somehow forms/solidsify in the milliseconds between each violent hackling
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 draw-
down cum post-draw is not performed by 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...
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 chambering 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 hackling 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 electrosprinning.

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

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Author’s contributions. F.V. posed the problem, K.K. designed and carried out the observations and drafted the paper. K.K. and F.V. wrote the paper.

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