© 2017. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2017) 220, 2243-2249 doi:10.1242/jeb.157594

RESEARCH ARTICLE

Nanofibre production in spiders without electric charge

Anna-Christin Joel[1,*] and Werner Baumgartner[2]

ABSTRACT

Technical nanofibre production is linked to high voltage, because nanofibres are typically produced by electrospinning. In contrast, spiders have evolved a way to produce nanofibres without high voltage. These spiders are called cribellate spiders and produce nanofibres within their capture thread production. It is suggested that their nanofibres become frictionally charged when brushed over a continuous area on the calamistrum, a comb-like structure at the metatarsus of the fourth leg. Although there are indications that electrostatic charges are involved in the formation of the thread structure, final proof is missing. We proposed three requirements to validate this hypothesis: (1) the removal of any charge during or after thread production has an influence on the structure of the thread; (2) the characteristic structure of the thread can be regenerated by charging; and (3) the thread is attracted to or repelled from differently charged objects. None of these three requirements were proven true. Furthermore, mathematical calculations reveal that even at low charges, the calculated structural assembly of the thread does not match the observed reality. Electrostatic forces are therefore not involved in the production of cribellate capture threads.

KEY WORDS: Cribellar, Processing, Uloboridae, Filistatidae, Silk, Protein

INTRODUCTION

We all have experienced the effects of rubbing a plastic balloon against our hair: the single hairs are repelled from each other, but attracted towards the balloon. This is induced by Coulomb forces, also called electrostatic forces, which are caused by frictional charging in this example (triboelectrification). Even without friction, electrons drift from one material to the other whenever two surfaces are in contact, and after separation, the materials become oppositely charged. Most materials are easily discharged again by protons and electrons occurring in the air. The final charging of an object hence depends not only on the physical and chemical nature of the given material but also on the conductivity of the medium surrounding the object (Chang et al., 1995).

Many biological systems use the attraction between oppositely charged objects: this charging facilitates, for example, the landing of pollen on the stigma during pollination by wind (Bowker and Crenshaw, 2007) and also attracts the gluey capture threads of spiders to positively charged flying insects (Ortega-Jimenez and Dudley, 2013). Another type of capture thread, the cribellate capture thread, is even suggested to employ electrostatic forces not for the attraction of prey but for the formation of its own structure, using the repulsion between two identically charged objects (Opell, 1995a; Joel et al., 2015; Kronenberger and Vollrath, 2015). Prey is captured here by a combination of hygroscopic forces, van der Waals' forces and an entanglement of the prey in a wool-like mat of nanofibres (cribellate fibres) surrounding two larger stabilizing axial fibres (Opell, 1994; Hawthorn and Opell, 2003).

The cribellate fibres shape the outer structure of the capture thread with two characteristic regions: the puff with a larger diameter than the intermediate zones, clearly separating two puffs with a constriction (Fig. 1) (Peters, 1984; Opell, 1989; Joel et al., 2015). It is commonly assumed that the adhesive cribellate fibres are kept separate within the puff by being charged uniformly during their extraction, leading to a repulsion of the fibres (Peters, 1984; Sahni et al., 2011; Kronenberger and Vollrath, 2015). During the production of the thread, the nanofibres are extracted from the cribellum, a spinning plate with up to 40,000 spigots anterior to the spinnerets (Bertkau, 1882; Foelix and Jung, 1978). To assemble all these fibres to one functional thread, the spiders deploy a highly sophisticated movement of the spinnerets as well as the fourth pair of legs, finally wrapping the sheet of nanofibres around the axial fibres (Joel et al., 2015, 2016). On the metatarsus of the fourth leg, cribellate spiders bear a comb-like row of specialized setae, the calamistrum. It is assumed that the charge is transferred to the cribellate fibres by triboelectrification when they are brushed over a continuous area composed of the specialized setae of the calamistrum (Fig. 1B) (Peters, 1984; Kronenberger and Vollrath, 2015; Joel et al., 2016).

This kind of nanofibre production involving only low electric charge has attracted the interest of researchers, as technical nanofibres are typically produced by electrospinning, involving high tension (Teo and Ramakrishna, 2006; Kronenberger and Vollrath, 2015). From the biological perspective, evidence for a charging of cribellate fibres by the action of the calamistrum is missing so far. However, there are indications that electrostatic charges are involved in the formation of the puffy structure: high humidity can increase the conductivity of air and hence remove charge. Indeed, the thread loses its characteristic structure after being exposed to the fine mist of a nebulizer (Zheng et al., 2010; Elettro et al., 2015). Furthermore, spiders without calamistra produce capture threads without puffs (Joel et al., 2015). This could indicate missing electrostatic charges to keep the fibres separate, though the use of a nebulizer, i.e. fine droplets of water, could also destroy the structure of the thread as a result of capillary forces or a reaction with the spider silk. The aim of this study was hence to resolve the involvement of Coulomb forces in the nanofibre production of cribellate spiders.

There are several ways to determine whether cribellate nanofibres are indeed electrostatically charged and whether such charging helps formation of a puffy structure: (1) one can try to regenerate the puffy structure of threads by charging them; (2) one can remove any charge by (i) making the surrounding conductive or (ii) making the fibres conductive; and (3) one can determine whether they are attracted to

[1]RWTH Aachen University, Institute of Biology II, Worringerweg 3, 52074 Aachen, Germany. [2]JKU Linz, Institute of Biomedical Mechatronics, Altenberger Straße 69, 4040 Linz, Austria.

*Author for correspondence (joel@bio2.rwth-aachen.de)

A.-C.J., 0000-0002-7122-3047

Received 2 February 2017; Accepted 3 April 2017

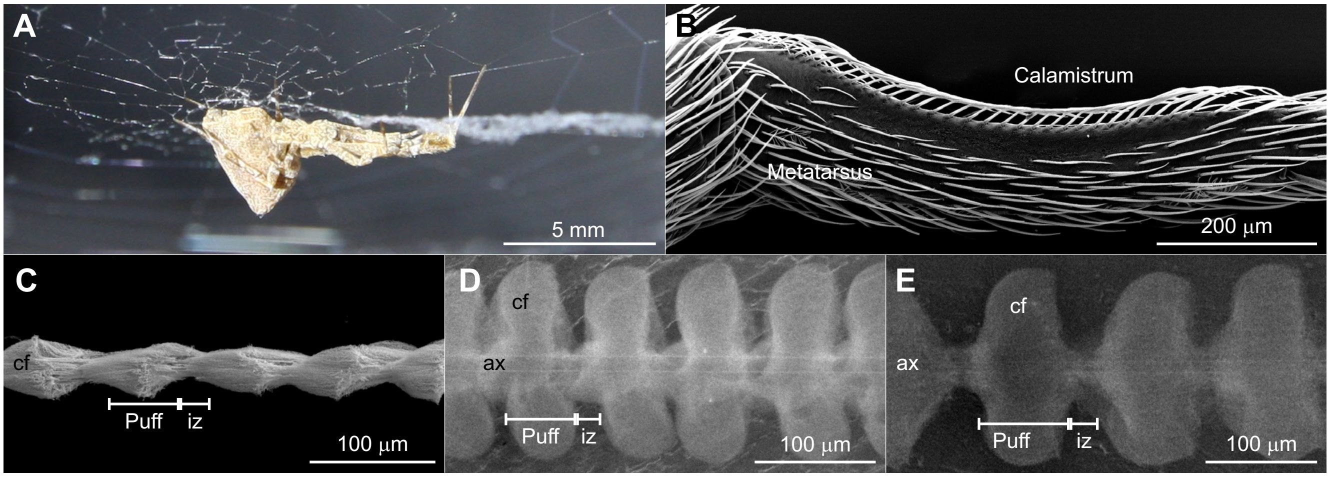

Fig. 1. The cribellate spider *Uloborus plumipes*. (A) An adult female in its resting position in the hub of its web. (B) Enlarged view of the calamistrum on the metatarsus of the fourth leg of the spider. Scanning electron microscope (SEM) image, sample coated with gold. (C–E) Capture threads of *U. plumipes* after different treatments (C, coated with gold; D, untreated; E, coated with carbon). SEM images. ax, axial fibre; cf, cribellate fibres; iz, intermediate zone.

or repelled from differently charged objects. On the basis of these three requirements, we aimed to determine whether electrostatic forces are deployed during the formation of the cribellate thread. All experiments were performed with *Uloborus plumipes* (Uloboridae) whose capture thread formation has been best described to date (Fig. 1A). To evaluate the general validity of the findings, threads of another Uloboridae, *Zosis geniculata*, as well as the distantly related Amaurobiidae *Amaurobius ferox*, Desidae *Badumna longinqua* and the Filistatidae *Kukulcania hibernalis* were examined (Bond et al., 2014; Fernández et al., 2014; Garrison et al., 2016). Capture threads of these species differ greatly in shape from those of Uloboridae (Fig. 2). Comparable results would indicate a conserved cribellate fibre production system in all cribellate spiders, whereas differences would hint at a diverging mechanism involved in the formation of cribellate capture threads.

MATERIALS AND METHODS

Ethics

The species used in the experiments (*Amaurobius ferox*, *Kukulcania hibernalis*, *Uloborus plumipes* and *Zosis geniculata*) are not endangered or protected species and special permits were not required. All applicable international, national and institutional guidelines for the care and use of animals were followed.

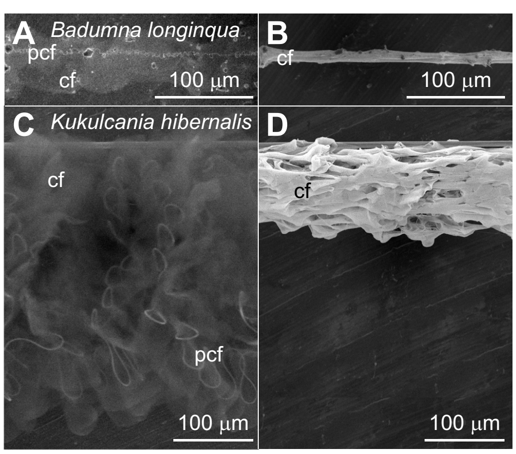

Fig. 2. Shape changes of capture threads after gold coating. Same region of capture thread of *Badumna longinqua* (A,B) and *Kukulcania hibernalis* (C,D) before (A,C) and after (B,D) coating it with gold. SEM images. ax, axial fibre; cf, cribellate fibres; iz, intermediate zone; pcf, paracribellate fibres.

Study animals

Adult female *Uloborus plumipes* Lucas 1846 and *Zosis geniculata* (Olivier 1789) were raised in larger terraria shared by several spiders of the same species as a lab colony under room temperature (~21°C), room humidity (~30%) and northern European diurnal rhythm. Each spider was able to build a web of its own. Once a week, spiders were fed with *Drosophila melanogaster* or juvenile *Acheta domestica*. Water was provided once or twice per month by sprinkling the web. Threads of such sprinkled webs were not used for further research.

Kukulcania hibernalis (Hentz 1842) were raised separately in 1 litre containers covered with gauze under elevated room temperature (~26°C), normal room humidity and northern European diurnal rhythm. Once a week, spiders were fed with juvenile *A. domestica* or bean weevils. Water was provided once or twice per month by applying droplets near the burrow.

Thread samples of *Amaurobius ferox* were collected near the Institute of High Frequency Technology in Aachen (Germany). Thread samples of *Badumna longinqua* were provided by the Queensland Museum (Brisbane, Australia). Samples were stored in the dark and protected against dust in a box at room temperature and room humidity. Because of their origin, threads of *B. longinqua* were stored for a longer period of time (about a year) until use, whereas *A. ferox* threads were stored for no more than 2 weeks after collection. These samples were used only for observation in the scanning electron microscope, where we can exclude distorted thread behaviour due to the age of the threads.

Thread preparation

Thread samples were taken from the web by picking them up gently with two strips of conductive foil on a sample holder. Special care was taken not to stretch the sample with this procedure. The samples could be observed without any further preparation (native), or after coating with carbon or a 10 nm gold layer (Hummer, Technics Inc., Alexandria, VA, USA) before examination in a scanning electron microscope (SEM 525 M, Philips AG, Amsterdam, The Netherlands).

Artificially charging capture threads
Threads of *U. plumipes* without the typical puffy structure were taken as samples for these experiments. This lack of puffs can be achieved by either exposing normal threads to fine mist (see 'Reaction to high humidity', below) or taking the capture threads of *U. plumipes* from which calamistra were previously removed according to Joel et al. (2015). Samples were transferred into the SEM without any previous treatment. Each sample was exposed directly to the electron beam (15 kV and a spot size of 30 nm) for 5–15 min to observe thread structure and potential changes in the structure. Extensive exposure to an electron beam might damage nanofibres, but no indications of such damage were observed.

Reaction to high humidity
Thread samples of *U. plumipes* were picked up with two arms of a metal filament and thread shape, i.e. puff width, was initially controlled with light microscopy. Afterwards the thread was placed in a climate chamber with a constant temperature of 28°C. Humidity was raised to 80% either by letting tap water evaporate or by controlling humidity with the help of a nebulizer (Super Fog Nano, Lucky Reptile, Import Export Peter Hoch GmbH, Waldkirch, Germany). Because of the size of the climate chamber (volume about 10 l), the impact of the nebulizer is omnipresent. After an hour, the samples were taken from the climate chamber and again measured under a light microscope. To observe whether thread shape changes after drying the thread again, samples were stored in a desiccator for a week, regularly monitoring the shape of the threads.

To observe the influence of high humidity on the production of the threads, *U. plumipes* were taken from the terrarium and placed overnight in a climate chamber at room temperature but 95% humidity, kept high by the evaporation of tap water. The next morning, samples of freshly spun capture threads were taken as described above and examined with the SEM.

Ionization of the surrounding air
Control threads of *U. plumipes* and *K. hibernalis* were taken from three webs of each species and their configuration characterized with a video microscope (VW 9000C, Keyence Corporation, Osaka, Japan) whilst ionizing the surrounding air with the help of Milty Zerostat 3 (Armour Home Electronics Ltd, Bishop's Stortford, UK). This anti-static device prevents the formation of static charges and is used, for example, to protect sensitive electronic devices during handling.

To observe the influence of ionizing the air during the production of threads, the diurnal rhythm of one *U. plumipes* was shifted 12 h by turning white light on from 06:00 h to 07:00 h. For observation, the spider was illuminated from 07:00 h to 18:00 h using red LED (Paulmann Licht GmbH, Springe, Germany). During production of the web, the air surrounding the spider was ionized. After the spider had finished building the web, thread samples were taken and measured. These experiments were performed under room humidity.

Statistical analysis
If the puffs are established by a repulsion of the nanofibres, the removal of any charge should lead to the collapse of this structure, producing a uniformly structured thread with a constant diameter, resembling the intermediate zone. Based on this premise, we performed a Power Analysis (G*Power version 3.1.9.2) to calculate how large our sample has to be to get reliable data with a power above 0.95. Because of the large difference between the diameter of the puff and the diameter of the intermediate zone as well as the low standard deviation of the data, achieved by only taking threads of adult spiders, two samples per experiment would be enough to determine any significant differences (assumed if $P<0.05$) using a two-tailed *t*-test with a power of 0.999. Nevertheless, if not indicated otherwise, we calculated the mean between three spiders, with four puffs per spider measured. p_{puff} refers to the comparison between the measured diameter of the sample and the typical diameter of a native puff, whereas p_{iz} refers to the comparison between the measured diameter of the sample and the typical diameter of the intermediate zone of a native thread.

Controlling thread charge
Whole webs (about 230 m^2) of *U. plumipes* and *K. hibernalis* were taken as a sample. A positively charged glass rod or a negatively charged piece of foamed plastic was brought near to single capture threads without touching them (the distance was always >0.5 cm). Because of the elasticity of these threads, any deformation could be easily detected with the naked eye (>1 cm). Please note that Movie 1 was recorded with a single thread and not a whole web and hence deflection is not as pronounced.

RESULTS
Charging capture threads missing the puffy structure
When feeding *U. plumipes* and *Z. geniculata* with live *D. melanogaster*, we observed that cribellate capture threads attract flying fruit flies, copying the effect described for gluey capture threads (Movie 1). Because flying insects charge themselves positively, the potential charge leading to a repulsion between the cribellate nanofibres should be negative (Ortega-Jimenez and Dudley, 2013). Hence, if only electrostatic forces are keeping the cribellate fibres apart, the exposure of threads without a puffy structure to the electron beam of a SEM should lead to recovery of the structural features.

Threads of spiders with previously removed calamistra or threads after exposure to fine mist both lack the puffy structure. Taking these threads as samples and charging them negatively with an electron beam, however, did not lead to a recovery of the puffy structure ($n\geq3$). The puffy structure of cribellate capture threads of *U. plumipes* cannot be retroactively generated by negatively charging the fibres after thread production.

Removal of charge
If the protruding structure of cribellate threads was only maintained by a repulsion of the nanofibres, the removal of charge should lead to a collapse of the puffy structure, producing threads of uniform structure with a constant diameter of the intermediate zone (in case of *U. plumipes*: 65±10 µm, $n=6$). To test whether cribellate fibres are indeed repelled by one another, the fibres were made conductive by coating the complete thread with either gold or carbon. Both coatings are typically used to make biological samples electrically conductive for electron microscopy. Threads changed shape after being coated with gold: untreated thread of *U. plumipes* had a puffy structure resembling beads on a string with a puff diameter of 168 µm (Fig. 1D, Table 1) but gold coating led to a significant collapse and a more tooth-shaped structure of the puffs (Fig. 1C, Table 1). In contrast, threads coated with carbon had a structure resembling that of the native state (Fig. 1E, Table 1). This collapsing of the thread after gold coating, but not after carbon coating, was reproducible for the Uloboridae *Z. geniculata* as well as for the Amaurobiidae *A. ferox*, the Desidae *B. longinqua* and the Filistatidae *K. hibernalis*

Journal of Experimental Biology

Table 1. The structural changes of cribellate capture threads after different treatments to remove electrostatic charge

Treatment	Diameter of the puff (μm)	p_{puff}	p_{iz}
None	168±13 (*n*=8)	–	**0.00**
Gold coating	50±9 (*n*=3)	**0.00**	**0.04**
Carbon coating	164±36 (*n*=3)	0.75	**0.00**
Nebulizer (80% RH)	49±22 (*n*=3)	**0.00**	0.15
Evaporation (80% RH)	175±2 (*n*=4)	0.22	**0.00**
Ionizer	179±18 (*n*=3)	0.28	**0.00**
Web production at 95% RH	178±21 (*n*=4)	0.30	**0.00**
Web production in ionized air	156±35 (*n*=3)	0.37	**0.00**

To evaluate the influence of electrostatic charges on the puffy shape of capture threads of *U. plumipes*, the diameter of the puff after different treatments was measured and compared with the diameter of the puff and the intermediate zone of a native thread. Significant differences (two-tailed *t*-test; α=0.05) between the diameter of the puff of a treated thread and the diameter of the puff of a native thread (p_{puff}) or of the intermediate zone of a native thread (p_{iz}) are marked in bold. RH, relative humidity.

(Fig. 2). Hence, making the cribellate fibres conductive alone does not lead to a change of thread shape.

This experiment does not exclude the possibility that electrostatic forces are deployed to form the structure in the first place, i.e. during extraction of the fibres. Therefore, the surroundings of the spider have to be made conductive during thread production. Raising the humidity to 80% with the help of a nebulizer led to the collapse of the puffs, which was not reversible by drying the threads (*U. plumipes*; Table 1). However, we found that the cribellate threads lost their puffy structure only when raising the humidity with the help of a nebulizer, not when letting water simply evaporate, despite reaching the same humidity (*U. plumipes*; Fig. 3, Table 1). Furthermore, the threads did not change their structure when ionizing the surrounding air [tested for *K. hibernalis* (*n*=3) and *U. plumipes*; Table 1].

Using this knowledge, we placed *U. plumipes* in a climate chamber with a humidity of 95% or ionized the air during thread production and hence made the air conductive. *Uloborus plumipes* was indeed able to build the same structured capture threads at room humidity (∼30%) and in a climate chamber with elevated humidity (Table 1). The same was true for threads produced by a spider in ionized air (Table 1, Fig. 4). Hence, reducing the spider's ability to electrostatically charge fibres in the first place has no impact on cribellate thread production.

Calculating the forces needed to keep two fibres separate, we found these to be rather small (0.01–0.3 elementary charges per µm; for details concerning the calculation, see the Appendix). Assuming the nanofibres are indeed charged with just 0.01–0.3 elementary charges per µm, removal of this low charge is not simple. Our further mathematical calculations revealed, however, that nanofibres with such a low charge do accumulate in a denser outer layer as observed in reality (Figs S1 and S2); however, single fibres are also uniformly distributed within the cross-section of the capture thread. But this does not fit the observed structural assembly of the capture thread, building a hollow structure around the two axial fibres (Joel et al., 2015, 2016). Any employment of Coulomb forces to generate the structure of the cribellate thread is therefore very unlikely.

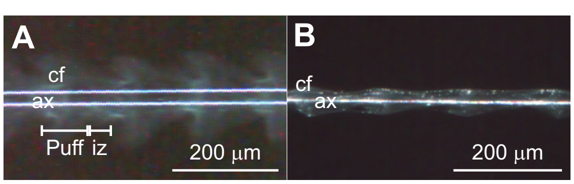

Fig. 3. Thread structure does not collapse without contact with liquid water. Structural changes of capture threads of *U. plumipes* after different treatments to raise the humidity to 80% in a climate chamber. (A) Raising the humidity by evaporation does not lead to any shape changes. (B) Exposure to the fine water droplets of a nebulizer leads to the collapse of the capture thread structure. Light microscopic pictures. ax, axial fibres; cf, cribellate fibres; iz, intermediate zone.

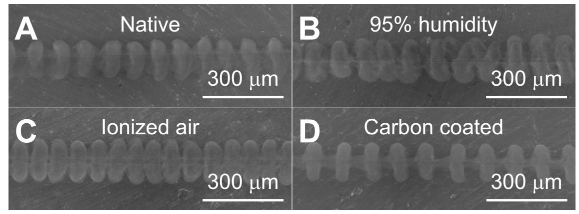

Fig. 4. No electrostatic charge involved in capture thread formation of cribellate spiders. (A–C) The capture thread structure is not changed, despite being produced by spiders kept under different conditions. Raising the humidity or ionizing the surrounding air should inhibit charging of the nanofibres during thread production. All samples were uncoated. (D) The capture thread was produced under normal conditions, but any possible charge was removed afterwards by coating the sample with carbon. The thread structure is nevertheless not impaired. SEM images.

Attraction to charged objects

Finally, to survey whether cribellate capture threads are charged at all, we approached webs of *U. plumipes* and *K. hibernalis* with a negatively charged piece of plastic or a positively charged glass rod. However, capture threads were attracted to both and the deflection could be seen with the naked eye (Movie 1). This demonstrates that the thread as a whole structure is not charged. In addition, the cribellate thread behaves like a dipole.

DISCUSSION

Although it is commonly assumed that cribellate fibres are electrostatically charged, our data do not support this hypothesis. Reviewing three previously proposed requirements to identify the impact of electrostatic forces on the capture thread structure, we could neither (1) regenerate the puffy structure of threads lacking puffs by charging them, nor (2) detect any structural change of the capture thread by making either the thread or its surrounding conductive (Fig. 4). Hence, the puff neither can be retroactively generated nor is preserved by electrostatic forces. Instead, when investigating our third condition, we found that the cribellate capture thread indeed acts as a dipole and is attracted to both positively and negatively charged objects. As we were not able to detect any differences between the threads of distantly related species, we suggest no cribellate spider deploys Coulomb forces to establish the structure of its capture thread.

Opell (1993) suggested that no electrostatic forces are involved in the formation of the puffy structure, because he likewise observed no collapsing of puffs when raising humidity only by evaporation. This exclusion of electrostatic forces in the formation of capture thread by cribellate spiders fits the described occurrence of cribellate spiders not only in deserts (like *Uloborus diversus*) but also in humid areas with 60–90% relative humidity (like *U. plumipes*, *Octonoba sinensis*, *Stegodyphus pacificus* or *Waitkera waitakerensis*) (Eberhard, 1971, 1972; Kullmann et al., 1971; Kumhof et al., 1992; Opell and Bond, 2000). In habitats with high humidity, fibres would more easily be discharged again. The same would be true for the spider, which should be oppositely charged

Journal of Experimental Biology (2017) 220, 2243-2249 doi:10.1242/jeb.157594

after capture thread production. In contrast, in arid areas, not only the fibres but also the spiders are, and stay, more easily charged. Without any special behaviour to discharge themselves again, the spiders would accumulate electric charge during thread production and afterwards attract their own capture threads. Because neither a compensatory behaviour nor an attraction between spiders and their capture threads was observed, we conclude that cribellate spiders cannot employ permanent larger charge through triboelectrification during capture thread production. Although we calculated that extremely low charges are sufficient to repel two nanofibres, the simulation of their arrangement within the thread only as a result of repulsion does not fit the characterized structure of the cribellate capture thread (Joel et al., 2015, 2016). Hence, the employment of any electrostatic charge to establish the thread's structure can be excluded. Being charged could actually counteract the purpose of the capture threads as insects can sense electric fields (Clarke et al., 2013; Greggers et al., 2013; Sutton et al., 2016). Hence, a charged capture thread would be detected and avoided by potential prey. The observed dipole behaviour of cribellate capture threads instead benefits the capturing of prey, because, like ecribellate capture threads, cribellate capture threads attract approaching flying charged prey (Ortega-Jimenez and Dudley, 2013).

Two experimental setups nevertheless led to the collapse of the puffs: coating them with gold and contact with fine mist. Both results are more difficult to explain when excluding the involvement of electrostatic forces in structure formation. Collapse of puffs after coating threads with gold not only is visible for the four species studied here but can also be found in the literature on other Uloboridae and Deinopidae (Opell, 1989, 1990; Peters, 1992). Data comparing native threads and gold-coated ones of other spiders are missing so far, but we presume a similar effect would be observed there. Although this treatment should remove any charge, other effects must have led to the collapse of the puffs, as puffs and intermediate zones are still discriminable in the gold-coated samples. In contrast, following exposure of the threads to fine mist, the puffs were no longer discriminable from the intermediate zones. If both methods removed only electrostatic charge, threads should look alike following treatment. Because coating the thread with carbon is a thermal process, any distortion of the thread due to heat formation during the gold-coating process can be excluded. We suggest that coating with gold leads to artefacts by covering the cribellate fibres with too much material, leading to compression of the puff. The conserved structure after coating the thread with the typically thinner carbon layer supports this hypothesis.

The exposure to fine mist must have another impact on the fibres. High humidity alone indeed improves the adhesion force, whereas exposure to fine mist (droplets of liquid water) annihilates the adhesion force (Hawthorn and Opell, 2003; Opell, 2013; Elettro et al., 2015). Hence, liquid water has to influence the spider silk directly. Water has the effect of resetting the protein conformation of other silks into its unprocessed state (Blamires et al., 2012). Because the structure of capture threads after contact with water is very similar to that of threads produced by spiders without calamistrum, the calamistrum might modify the protein conformation instead of charging the fibres. This could lead to an autonomous curling, finally forming the puff. Though we tried to eliminate any electrostatic charge during capture thread production, it is possible that local Coulomb forces caused a change in protein folding as a result of the polar amino acids, which are more abundant in cribellate silk than in ecribellate silk (Perutz, 1978; Liao et al., 2011). A modification at the protein level would also explain the irreversibility of the loss of puffs. Further studies have to specify how the calamistrum processes the cribellate fibres to form the puffy structure and whether this crimping is indeed a modification at the protein level.

The finding that no electrostatic forces are involved in the nanofibre production of cribellate spiders annuls the possibility of transferring a corresponding biological model to a technical application involving only low electric charge. Nevertheless, the capture thread production system of cribellate spiders remains of interest for biomimetic approaches: the nanofibre production of cribellate spiders can be used as an inspiration to produce nanofibres without any electric charge at all, and additionally to reduce the typical diameter of technical nanofibres from 100–1000 nm to 10–30 nm (Friedrich and Langer, 1969; Opell, 1995b; Nayak et al., 2012).

APPENDIX

Calculation of the cribellate nanofibre arrangement with an approximated charge

To estimate the surface charge density that is necessary to deflect the nanofibres in the way observed, a simple electromechanical estimation was performed. The deflection of a fibre can be closely approximated according to linear elastic beam theory. If a distributed load (force per length) of $k(x)$ is applied to a fibre oriented in the x-direction, the deflection w in the z-direction behaves according to the differential equation:

$$\frac{\partial^4 w}{\partial x^4} = \frac{k(x)}{Y \cdot J_y}, \tag{A1}$$

where Y is the elastic modulus (Young's modulus; we chose Y instead of the usual E to avoid any mix-up with the electric field $\vec{E}$) and J_y is the area moment of inertia in the y-direction, which in our case of a circular cross-section is:

$$J_y = J = \int_A z^2 \, \mathrm{d}A = \frac{r^4 \cdot \pi}{4}. \tag{A2}$$

To calculate the load $k(x)$, one needs to initially calculate the electric field according to the first Maxwell equation (Gauss equation):

$$\oiint_{\partial V} \varepsilon_0 \, \vec{E} + \vec{P} \, \mathrm{d}V = \iiint_V \rho \, \mathrm{d}V, \tag{A3}$$

where $\vec{E}$ is the electric field, ε_0 is the permittivity of a vacuum, $\vec{P}$ is the polarization of matter and ρ stands for the charge density in space. In our case, we have a symmetrical cylinder set-up with uniform charge density along the fibre. Thus, the electric field is radially from the fibre with a field strength:

$$\|\vec{E}\| = \frac{q}{2 \cdot w(x) \cdot \pi}, \tag{A4}$$

where q is the charge per unit length of the fibre. The load $k(x)$ is now q times the field strength, leading to:

$$\frac{\partial^4 w}{\partial x^4} = \frac{2 \cdot q^2}{Y \cdot r^4 \cdot w(w) \cdot \pi^2}. \tag{A5}$$

For a single puff of length L, this differential equation has to be solved using the boundary conditions $w(0)$=$w(L)$=w_0 and $w'(0)$=$w'(L)$=0. Here, w_0 is the initial distance of the nanofibres. Unfortunately, this differential equation cannot be solved analytically. However, using an implicit difference scheme, a numerical solution can be found. The results for a puff of length

L=50 µm, a fibre diameter of 25 nm, an initial distance w_0 of 500 nm and a Young's modulus of Y=8×10^9 N m^{-2} and with a different charge per unit length ranging from q=1×10^{-15} to q=5×10^{-14} C m^{-1} are shown in Fig. S1. It must be emphasized that our calculation only considers two charged nanofibres. The other fibres are of course also charged and are repelled by one another; however, the order of magnitude of charge needed to form a puff can be roughly estimated.

The minimal distance of two fibres in a puff is about 1 µm; thus, a charge in our range shown in Fig. S1 is more than sufficient for the required deformation. This means that about 0.01–0.3 elementary charges would be necessary per µm length of the fibre to yield deflections as observed. This ridiculously small charge can be explained by the low diameter of the nanofibres leading to very low moment of inertia and thereby to low bending resistance. Thus, rather than modelling the fibres as beams they can be better modelled as ropes which can only transfer longitudinal stress and no bending momentum. Under these conditions it is possible to estimate how the fibres would be arranged in space if they are uniformly charged and their length within a puff is limited. Thus, a number of fibres N arrange themselves in a limited radius r so that force equilibrium is reached. The outer fibres at radius r are held back as a result of the limited length and the inner fibres need to be in equilibrium with reference to the Coulomb forces. The electric field acting on a single fibre can be obtained using Maxwell's first law (Gauss' law) for non-polarized media:

$$\oint\!\!\oint_{\partial\Omega} \vec{E}\cdot \mathrm{d}\vec{s} = \frac{1}{\varepsilon_0}\cdot \iiint_{\Omega} \rho\, \mathrm{d}V, \tag{A6}$$

where ρ is the charge density and ε_0 is the permittivity of a vacuum, meaning that the electric flux leaving a volume is proportional to the charge inside. This equation is used for all other fibres, and the field of the fibre under consideration is obtained by superposition of all other fields. Now the force acting on the fibre considered is calculated according to:

$$\vec{F} = q\vec{E}, \tag{A7}$$

where q is the charge of our fibre. Calculating the forces for all fibres at a given geometry, one can vary the geometry numerically to yield a distribution of the fibres which fulfils equilibrium conditions. A typical result for the radial distribution of N=500 fibres which face a length restriction so that they cannot exceed a radius of 1 from the centre-axis is shown in Fig. S2A.

Clearly, a lot of fibres form a rim at radius 1 but other fibres are distributed uniformly within the cross-section. The fibre density for 2000 fibres according to the radius is shown in Fig. S2B. Evidently, there is a high density close to the outermost radius of 1 in our case and uniform distribution inside this circle. This would be expected if in fact electrostatic repulsion led to the arrangement of charged fibres in space, given the length limitation. This contradicts the observation that in the centre of the puff hardly any fibres can be observed (Fig. S3; Joel et al., 2015).

Acknowledgements
We want to thank Robert Raven, Barbara Baehr and Robert Whyte from Queensland Museum for the supply of capture threads of *B. longinqua*. Many thanks to Moritz to Baben and Jan-Ole Achenbach from the Institute for Material Chemistry at RWTH Aachen University for coating the threads with carbon. Furthermore, we want to thank Peter Bräunig and Björn Kampa for supporting this study with lab equipment.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.-C.J., W.B.; Methodology: A.-C.J., W.B.; Validation: A.-C.J., W.B.; Formal analysis: A.-C.J., W.B.; Investigation: A.-C.J.; Writing - original draft: A.-C.J.; Writing - review & editing: A.-C.J., W.B.; Visualization: A.-C.J., W.B.; Supervision: W.B.

Funding
This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/10.1242/jeb.157594.supplemental

References
Bertkau, P. (1882). Über das cribellum und calamistrum: ein beitrag zur histiologie, biologie und systematik der spinnen. *Archiv für Naturgeschichte* **48**, 316-362.
Blamires, S. J., Wu, C.-L., Blackledge, T. A. and Tso, I.-M. (2012). Post-secretion processing influences spider silk performance. *J. R. Soc. Interface* **9**, 2479-2487.
Bond, J. E., Garrison, N. L., Hamilton, C. A., Godwin, R. L., Hedin, M. and Agnarsson, I. (2014). Phylogenomics resolves a spider backbone phylogeny and rejects a prevailing paradigm for orb web evolution. *Curr. Biol.* **24**, 1765-1771.
Bowker, G. E. and Crenshaw, H. C. (2007). Electrostatic forces in wind-pollination - Part 2: Simulations of pollen capture. *Atmos. Environ.* **41**, 1596-1603.
Chang, J.-S., Kelly, A. J. and Crowley, J. M. (1995). *Handbook of Electrostatic Processes*. New York: Marcel Dekker, Inc.
Clarke, D., Whitney, H., Sutton, G. and Robert, D. (2013). Detection and learning of floral electric fields by bumblebees. *Science* **340**, 66-69.
Eberhard, W. G. (1971). Ecology of the web of Uloborus diversus (Araneae: Uloboridae). *Oecologia* **6**, 328-342.
Eberhard, W. G. (1972). Web of Uloborus diversus (Araneae: Uloboridae). *J. Zool.* **166**, 417-465.
Elettro, H., Neukirch, S., Antkowiak, A. and Vollrath, F. (2015). Adhesion of dry and wet electrostatic capture silk of uloborid spider. *Naturwissenschaften* **102**, 41.
Fernández, R., Hormiga, G. and Giribet, G. (2014). Phylogenomic analysis of spiders reveals nonmonophyly of orb weavers. *Curr. Biol.* **24**, 1772-1777.
Foelix, R. F. and Jung, H. (1978). Some anatomical aspects of Hypochilus thorelli with special reference to the calamistrum and cribellum. In *Symposia of the Zoological Society of London*, pp. 417-422. New York: Academic Press.
Friedrich, V. and Langer, R. M. (1969). Fine structure of cribellate spider silk. *Am. Zool.* **9**, 91-96.
Garrison, N. L., Rodriguez, J., Agnarsson, I., Coddington, J. A., Griswold, C. E., Hamilton, C. A., Hedin, M., Kocot, K. M., Ledford, J. M. and Bond, J. E. (2016). Spider phylogenomics: untangling the Spider Tree of Life. *PeerJ* **4**, e1719.
Greggers, U., Koch, G., Schmidt, V., Durr, A., Floriou-Servou, A., Piepenbrock, D., Gopfert, M. C. and Menzel, R. (2013). Reception and learning of electric fields in bees. *Proc. R. Soc. B Biol. Sci.* **280**, 8.
Hawthorn, A. C. and Opell, B. D. (2003). van der Waals and hygroscopic forces of adhesion generated by spider capture threads. *J. Exp. Biol.* **206**, 3905-3911.
Joel, A.-C., Kappel, P., Adamova, H., Baumgartner, W. and Scholz, I. (2015). Cribellate thread production in spiders: Complex processing of nano-fibres into a functional capture thread. *Arthropod. Struct. Dev.* **44**, 568-573.
Joel, A.-C., Scholz, I., Orth, L., Kappel, P. and Baumgartner, W. (2016). Morphological adaptation of the calamistrum to the cribellate spinning process in Deinopoidae (Uloboridae, Deinopidae). *R. Soc. Open Sci.* **3**.
Kronenberger, K. and Vollrath, F. (2015). Spiders spinning electrically charged nano-fibres. *Biol. Lett.* **11**, 20140813.
Kullmann, E., Nawabi, S. and Zimmermann, W. (1971). Neue ergebnisse zur brutbiologie cribellater spinnen aus afghanistan und der serengeti (Araneae, Eresidae). *Z. Koeln. Zool.* **14**, 87-108.
Kumhof, E., Sengonca, C., Montagne, E., Deut Gesell, A. and Angewandte, E. (1992). Laboratory studies on the development and feeding-activity of Uloborus plumipes Lucas (Araneae, Uloboridae). *Mitt. Dtsch. Ges. Allg. Angew. Entomol.* **8**, 204-208.
Liao, X., Yin, G., Huang, Z., Yao, Y., Gu, J. and Han, D. (2011). Supercontraction on cribellate spider spiral silk with wet-rebuilt micro-structure. *Mater. Sci. Eng. C* **31**, 128-133.
Nayak, R., Padhye, R., Kyratzis, I. L., Truong, Y. B. and Arnold, L. (2012). Recent advances in nanofibre fabrication techniques. *Text. Res. J.* **82**, 129-147.
Opell, B. D. (1989). Functional associations between the cribellum spinning plate and capture threads of Miagrammopes animotus (Araneida, Uloboridae). *Zoomorphology* **108**, 263-267.
Opell, B. D. (1990). Material investment and prey capture potential of reduced spider webs. *Behav. Ecol. Sociobiol.* **26**, 375-381.
Opell, B. D. (1993). What forces are responsible for the stickiness of spider cribellar threads? *J. Exp. Zool.* **265**, 469-476.

Journal of Experimental Biology (2017) 220, 2243-2249 doi:10.1242/jeb.157594

Opell, B. D. (1994). The ability of spider cribellar prey capture thread to hold insects with different surface-features. *Funct. Ecol.* **8**, 145-150.
Opell, B. D. (1995a). Do static electric forces contribute to the stickiness of a spider's cribellar prey capture threads? *J. Exp. Zool.* **273**, 186-189.
Opell, B. D. (1995b). Ontogenetic changes in cribellum spigot number and cribellar prey capture thread stickiness in the spider family Uloboridae. *J. Morphol.* **224**, 47-56.
Opell, B. D. (2013). Cribellar thread. In *Spider Ecophysiology* (ed. W. Nentwig), pp. 303-315. Berlin: Springer-Verlag.
Opell, B. D. and Bond, J. E. (2000). Capture thread extensibility of orb-weaving spiders: testing punctuated and associative explanations of character evolution. *Biol. J. Linnean Soc.* **70**, 107-120.
Ortega-Jimenez, V. M. and Dudley, R. (2013). Spiderweb deformation induced by electrostatically charged insects. *Sci. Rep.* **3**, 4.
Perutz, M. (1978). Electrostatic effects in proteins. *Science* **201**, 1187-1191.
Peters, H. M. (1984). The spinning apparatus of Uloboridae in relation to the structure and construction of capture threads (Arachnida, Araneida). *Zoomorphology* **104**, 96-104.
Peters, H. M. (1992). On the spinning apparatus and the structure of the capture threads of Deinopis subrufus (Araneae, Deinopidae). *Zoomorphology* **112**, 27-37.
Sahni, V., Blackledge, T. A. and Dhinojwala, A. (2011). A review on spider silk adhesion. *J. Adhesion* **87**, 595-614.
Sutton, G. P., Clarke, D., Morley, E. L. and Robert, D. (2016). Mechanosensory hairs in bumblebees (Bombus terrestris) detect weak electric fields. *Proc. Natl. Acad. Sci. USA* **113**, 7261-7265.
Teo, W. E. and Ramakrishna, S. (2006). A review on electrospinning design and nanofibre assemblies. *Nanotechnology* **17**, R89.
Zheng, Y., Bai, H., Huang, Z., Tian, X., Nie, F.-Q., Zhao, Y., Zhai, J. and Jiang, L. (2010). Directional water collection on wetted spider silk. *Nature* **463**, 640-643.

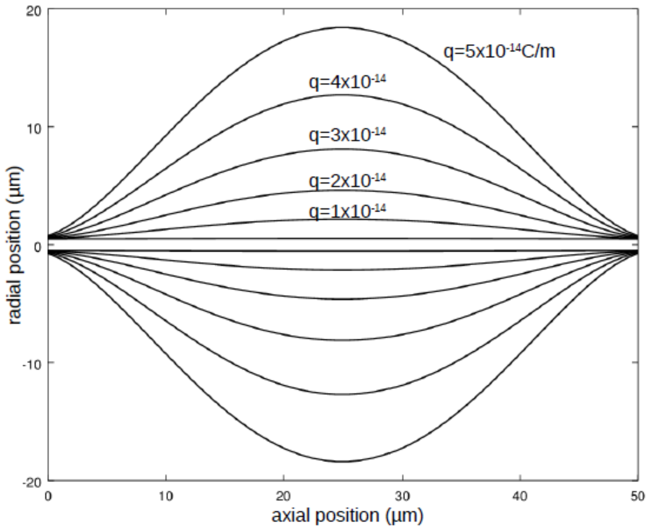

Fig. S1. Calculation of the deformation of two repelled nanofibres by given charge.

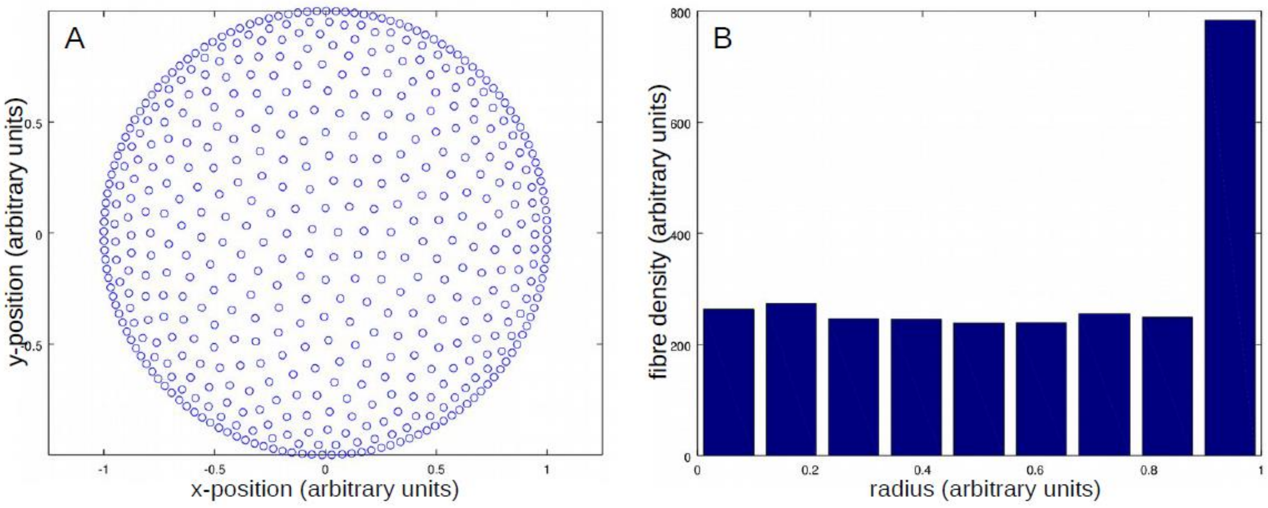

Fig. S2. Calculation of the distribution of the nanofibres within the thread. A) Calculated assuming 500 nanofibres within one thread. B) Calculated assuming 2000 nanofibres within one thread.

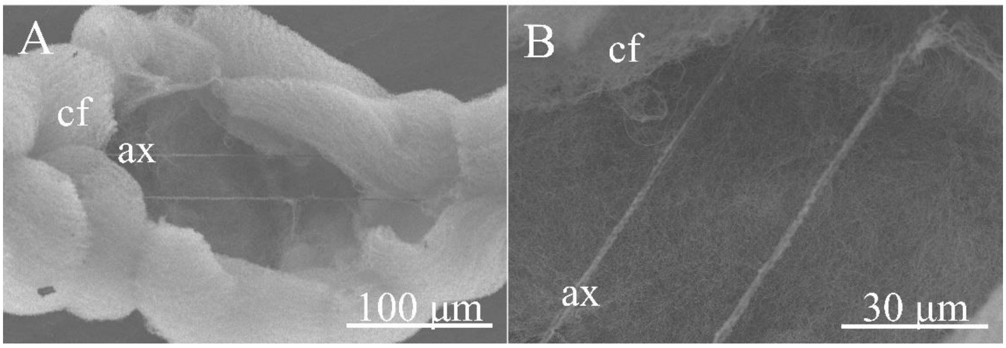

Fig. S3. Scanning electron microscopy pictures of opened thread showing the hollow inner structure of the cribellate capture thread. Cribellate nanofibres (cf) are not uniformly distributed within the capture thread, but build a sheet of nanofibres encasing the axial fibres (ax).

Movie 1. Attraction of cribellate capture threads to differently charged objects.

About 1 cm long pieces of cribellate capture threads were taken from the web of *U. plumipes*. When approaching this sample with either (1) a negatively charged piece of foamed plastic, (2) a positively charged glass rod or (3) with a fruit fly (*D. melanogaster*), charged by flapping its wings, the capture threads behave like a dipole and are attracted to all objects.

Please note that the severity of deformation due to the attraction depends not only on the charge quantity, but also on the length of the piece of thread, because longer pieces are more easily deformable.