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Diversity and properties of key spider silks and webs

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Abstract - Spider silks are natural materials whose diversity surpasses other known silks and man-made polymers. This review covers exciting aspects of the diversity of spider silks and focuses on orb web silks. We argue that to understand silks as biological materials, their functionality must always be considered. The evolution of major ampullate silk provides a good example, as do the properties and functions of the six other silks produced and used by araneid orbweavers. The adhesive snares of the orb webs provide an excellent example of the evolution of two very different forms of silk to capture and hold insect prey, both the wet-glue ecribellate spiders, like the araneids, and the dry-hackled silk of the much older cribellate spiders, like the uloborids. The review concludes by examining how silks may be actively tuned by the spider by adjusting spinning and post-processing conditions. Throughout we argue that this diversity makes spider silks excellent models for polymer research and applications.

Keywords: Orbweaver, spider silk, dragline, ecribellate, capture spiral.

1. Introduction

Silks are diverse materials produced by a wide range of arthropods. Characterized as hierarchical spun biopolymers, they are natural proteins defined by their processing from a gel-like dope to a dry fibre (Vollrath and Knight, 2001). Thus silks are not grown, unlike other biopolymer proteins such as collagen and keratin, and are used exclusively outside the body (Porter and Vollrath, 2009).

Silks have evolved many times independently, including 23 times within the insects, once within the spiders and at least once within the crustaceans (Craig, 1997; Kronenberger *et al*., 2012; Sutherland *et al*., 2009). Whereas insects will produce one type of silk at one particular life stage, spiders produce multiple types of silk throughout their life. These silks have evolved similar protein sequences and processing conditions, but they differ in the origin of the silk-storing gland(s) (Hayashi, 2001; Holland *et al*., 2006; Sutherland *et al.,* 2009). This diversity of structure and properties reveals both remarkable adaptations as well as considerable constraints on the protein composition and processing of silk, indicating that there are a limited number of ways to make a silk fibre. Importantly, the diversity seen in silks concerning both natural functions and material properties originates from a combination of spinning feedstock diversity and complex spinning and processing of these silk precursors. Thus a constrained number of protein motifs can lead to many different materials.

Given the diversity in silk properties and natural applications, silks are a superb resource to inspire novel biopolymers (Gatesy *et al.,* 2001). Nature has evolved these materials over hundreds of millions of years of trial and error and this is now providing intellectual stimuli for industrial materials research and development (Kluge *et al*., 2008). Our review covers recent insights into the diversity of selected spider silks, concentrating on orbweavers. We assert that our bio-centric approach is critical for a solid understanding of the links between molecular polymer structure, material properties and natural functionality, including the many evolutionary constraints that nature imposes on its materials.

2. Material innovation: The evolution of ampullate silk

All 40,000 + species of spider produce silk from spinnerets located on their abdomen (Coddington and Levi, 1991; Vollrath and Selden, 2007). The 2000 or so more ancestral spiders make up only a small proportion of this remarkable diversity, while the more derived spiders, the Araneomorphae, form the rest (Coddington and Levi, 1991). The radiation of the Araneomorphae coincided with the evolution of ampullate silk, around 150 million years ago, which can be considered to be a key 'evolutionary innovation' (Brunetta and Craig, 2010; Hayashi *et al*., 2005). Across the Araneomorphae, ampullate silks show consistent high toughness and high strength (this Section), with variability controlled by processing conditions (see Section 5) (Boutry *et al*., 2011).

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The low diversity of ancestral-type spiders (mostly mygalomorphs) can perhaps be explained in part by the fact that they have a rather limited armoury of silk types with, apparently, rather poor mechanical properties (Swanson *et al.,* 2006). This may restrict their habitats to the ground where their silks are used primarily as sensors that alert the spider of an insect by propagating vibrations rather than catching the prey as adhesive snares (Costa and Perez-Miles, 1998; Naftilan, 1999; Singer *et al.,* 2000).

The mechanical property differences between basal/ancestral and more derived spiders can be explained by their silk gene sequences, which lead to different protein secondary structures (Table 1). Major ampullate, or dragline silks, have similar gene sequences across the Araneomorphae, including glycine-alanine (GA) and repeats of alanine (polyA), with some novel repeats (Table 1) (Gatesy *et al.*, 2001; Tian *et al.*, 2004). Dragline silks are made up of two proteins: MaSp1 and MaSp2 (Hinman and Lewis, 1992). In contrast, the basal silks contain some unique repetitive sequences in their silk genes involving amino acids such as serine and threonine (Garb *et al.*, 2007; Gatesy *et al.,* 2001). However, all spider silk genes share polyA repeats, suggesting that these sequences have been maintained over 240 million years (Gatesy *et al.,* 2001).

Table 1. Amino acid sequence and proposed secondary structure for a range of spider silks. Amino acids: A = alanine, S = serine, G = glycine, X = varied/unknown, V = valine, Q = glutamine, P = proline and a lower case n means it is repeated a number of times.

Silk type (gene name)	Repetitive sequence	Proposed secondary structure	Reference(s)
Major ampullate (MaSp1)	A_n, GA_n, GGX	Orientated beta sheet	Gatesy *et al.,* 2001; Xu and Lewis 1990
Major ampullate (MaSp2)	$GPGX_n$	Beta spiral	Hinman and Lewis, 1992
Non-orbweaver major ampullate silk	A_n, GA_n, GV_n, GS_n	Orientated beta sheet	Gatesy *et al.*, 2001; Tian *et al.*, 2004
Basal spider fibroins	A_n, S_n, GX, AQ_n	Varied/unknown	Garb *et al.*, 2007; Gatesy *et al.*, 2001
Minor ampullate (MiSp1 and 2)	GGX, GA_n + serine rich spacers	Orientated beta sheet	Colgin and Lewis, 1998
Flagelliform	GPGGX (GGX + spacer)	Beta spiral, no preferential orientation	Hayashi and Lewis, 2001
Cylindriform/Tubuliform: (TuSp1)	Long repetitive sequence	Orientated beta sheet	Garb and Hayashi, 2005; Rousseau *et al.*, 2009
Aciniform (AcSp1)	Long repetitive sequence	Less orientated beta sheet, alpha helix	Hayashi *et al.,* 2004; Rousseau *et al.*, 2009

Although the emergence of ampullate silk corresponds with a huge increase in spider diversity, it cannot alone explain the success of the Araneomorphae. One group of the Araneomorphae that has received a large amount of research interest are the orbweavers. These spiders have evolved a veritable ‘toolbox’ of materials, with different properties for different uses, as outlined in the next section.

3. Materials diversity: Orbweaver spider silks

Orbweavers produce up to seven different types of silk, with different protein primary sequences (Table 1). Spiders use gland-specific expression of these silks, and the silk fibres leave the body through different spigots on the spinnerets (Guerette *et al.,* 1996). The seven different types of silks made by orbweavers show an impressive range of mechanical performance (Fig. 1) (Blackledge and Hayashi, 2006; Vollrath and Porter, 2006).

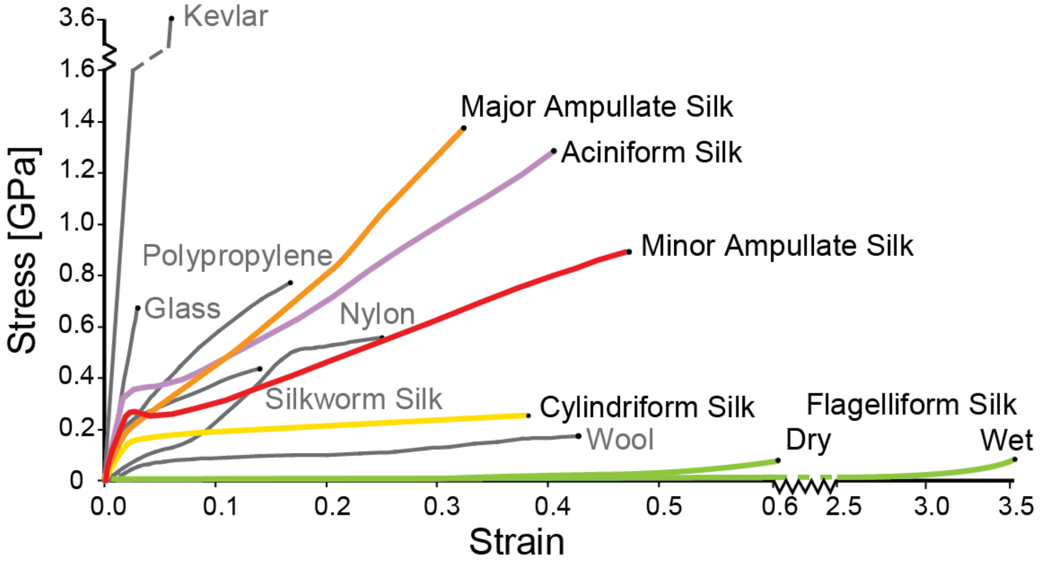


Figure 1. Stress-strain curves comparing orbweaver spider silk mechanical properties: major ampullate (orange), aciniform silk (purple), minor ampullate silk (red), cylindriform silk (yellow) and wet and dry flagelliform silk (green, where the former contains an aggregate silk coating). Reference curves for silkworm silk (blue) and synthetic and other biopolymers are also given in grey. Data taken from (Blackledge and Hayashi, 2006; Van Nimmen *et al.,* 2005; Vollrath and Edmonds, 1989; Wynne, 1997).

Major ampullate silk has impressive mechanical performance and particularly superb toughness (Fig. 1) (Gosline *et al.,* 1999; Porter and Vollrath, 2009). Shaped by over 150 million years of evolution, the mechanical properties of dragline silk are suited to absorb the energy of a falling spider or prey kinetic energy in the web (Gatesy *et al*., 2001; Ortlepp and Gosline, 2008; Sensenig *et al*., 2012). A particularly tough dragline silk is made by the large orb-weaving Darwin's bark spider, which also makes an exceptionally large web (Agnarsson *et al*., 2010). As well as mechanical and structural roles, spider dragline silks have vital functions to the spider as transmitters of sensory information (see Section 5) (Landolfa and Barth, 1996; Masters and Markl, 1981). In the orb web, the major ampullate radial threads are well positioned for their sensory roles, as they transmit vibrations from the capture area to the spider in the web hub and spiders will orientate towards the vibrating radii within milliseconds (Klarner and Barth, 1982; Masters and Markl, 1981).

As a companion to the major ampullate silks, minor ampullate is another tough silk that is used as an auxiliary spiral in the orb web and contains proteins that are expressed from two genes (MiSp) (Colgin and Lewis, 1998; Gatesy *et al.,* 2001). Aciniform silks (gene AcSp) form the wrapping silk used to enshroud prey, as well as some of the inner lining of the egg sac (Hayashi *et al*., 2004). These silks are relatively poorly understood because they are both very thin, the glands are small, and it is difficult to force-reel them under controlled conditions (Blackledge and Hayashi, 2006). However, they seem to show impressive toughness (Blackledge and Hayashi, 2006). Cylindriform, or tubuliform silk (gene TuSp) is used in the outer coating of egg sacs. It is less well studied than the major and minor silks but seems to show intermediate mechanical performance (Van Nimmen *et al.,* 2005). Piriform silks form adhesive disks to enable the spider to stick its silks to the substrate in the spider's environment (Perry *et al.,* 2010). Capture spiral silk is formed of a core flagelliform silk, with a coating of hygroscopic aggregate silk glue (Vollrath and Edmonds, 1989). Capture spiral silks show impressive extensibility and poor strength, and are closely reviewed in Section 4 (Blackledge and Hayashi, 2006; Gosline *et al.,* 1999; Swanson *et al*., 2007).

The diverse properties of orbweaver spider silks are explained by their differences in material structure. The tougher silks (ampullates, aciniform) show orientated beta sheet structures, the less tough cylindriform silks show less oriented beta sheet and alpha helix structures, whereas flagelliform silks have no preferential orientation and are thought to contain beta spiral structures (Table 1) (Liivak *et al.,* 1997; Rousseau *et al*., 2009; Simmons *et al*., 1996). These structures, in turn, are explained by amino acid composition and sequence, where higher glycine and alanine content leads to more crystalline structures, whereas the presence of proline leads to more spiral structures (Hayashi *et al.,* 1999). Major ampullate silk combines both types of structure at the nanometer scale, which is thought to lead to dragline silk's exceptional toughness (Porter and Vollrath, 2007). In addition to the highly ordered and totally disordered regions, major ampullate silk also has sections of intermediate order (Guan *et al.,* 2011); Nuclear Magnetic Resonance experiments have shown that *Nephila* dragline glycine-rich segments form 3(1)-helical structures (Beek *et al.,* 1999; Kummerlen *et al.,* 1996).

Clearly, the macro-morphologies and micro/nano-structures are key to explaining the properties of spider silks (as with other silks). An important and often overlooked contributor to strength is simply the thin diameters and flawlessness of most spider silks (draglines are typically 1-3 μm in diameter). Due to the failure mechanism of thin fibres, a small size allows for higher fibre strength (Porter *et al.,* 2013). This has implications for the attempt to create dragline silk-like properties from genetic engineering of silkworms to include spider silk genes, as silkworm spinning ducts are larger (Teule *et al*., 2012; Vollrath *et al*., 2011), which prevents the production of small diameter filaments. Major ampullate silk ducts have evolved to optimise the processing of these silks into thin fibres, even as the spider grows (Davies *et al.,* 2013). Thinness, additionally, has advantages in terms of material conservation, saving energy for the spider, and reducing visibility to aerial prey (Craig, 1986; Foelix, 2010).

In terms of architectural macro-structure, *Nephila* major ampullate silk fibres show a range of structural elements in the transmission electron microscope (Frische *et al*., 1998). A thin outer layer surrounds a column of apparently homogeneous material that contains elongated cavities oriented parallel to the silk fibre axis that is the result of the spinning process and rheology (Vollrath and Knight, 2001). The cavities appear similar to 'elongate vacuolar droplets' observed in the silk of some moth larva (Akai *et al.,* 1993). These structures resemble small filled rifts (called canaliculi) being extremely fine and elongated in line with the fibre axis. Stressing a fibre until fracture can lead to cracks forming between canaliculi (Shao *et al.,* 1999), and it can be argued that by diverting 'crack energy', the canaliculi may contribute further to the exceptional tensile strength and toughness of the threads that have them.

4. Adhesive solutions: Orb web spirals

Spider orb webs are aerial filters adapted to the task of capturing high velocity insect 'missiles'. The spider's orb web is a fine example of animal engineering, which combines the spider's toolkit of silks (Section 3) as building materials (Lin *et al*., 1995). A typical orb web consists of a major ampullate frame supporting a wheel of major ampullate spokes radiating from a hub; these radii in turn support the sticky spiral that entangles the insect (Fig. 2). Strong radii and a soft spiral collaborate to absorb the insect's impact, hold it aloft and act as a vibration transmission surface (Lin *et al.,* 1995; Masters and Markl, 1981).

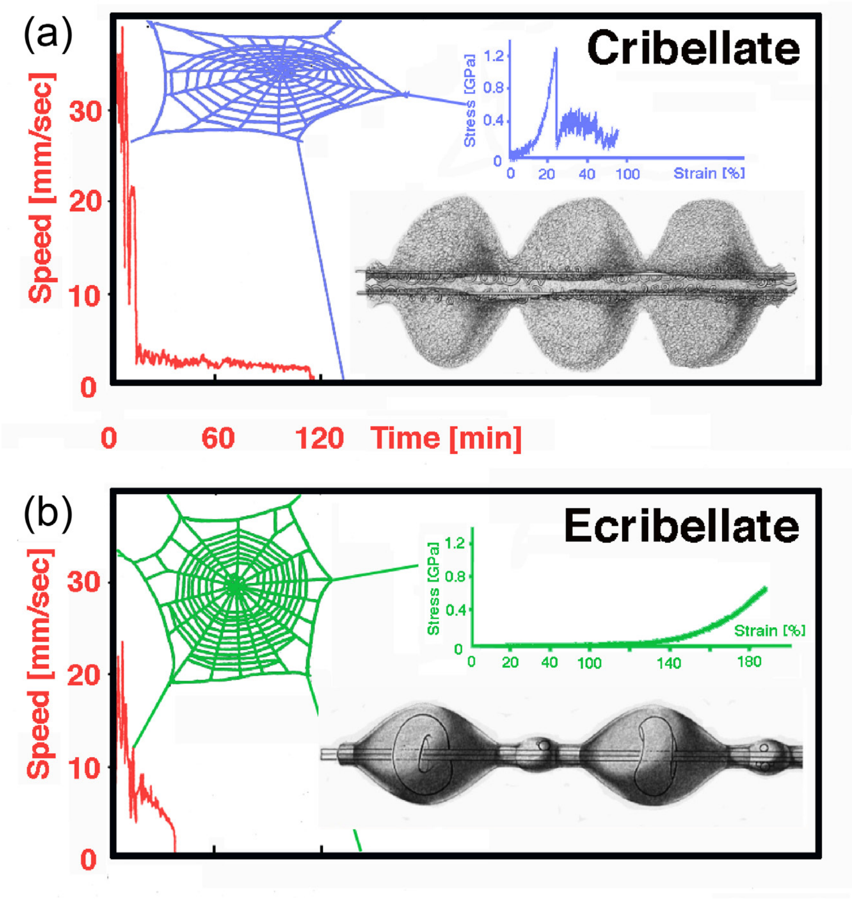


Figure 2. Comparison of cribellate and ecribellate webs and silks. The red curves show the time spent on web construction by (a) the cribellate *Uloborus walkenarius* and (b) the ecribellate *Araneus diadematus* (modified from Vollrath, 2006 CB). The blue and green insets show the orb web shape and stress–strain characteristics of the uloborid hackled dry-adhesion (blue) capture thread and the araneid wet-glue (green) capture thread (for data see Köhler T, Vollrath F. (1995)). Each of the two capture spiral insets shows ca. 150 μm in length.

Orbweavers have evolved two very different types of spiral thread, which used to be important for taxonomic classification (Foelix, 2010). Cribellate spiders produce very fine silk filaments of nanometer diameter, which are combed in hackled bands of thousands of threads onto supporting central threads (Fig. 2a). These hackled bands provide elasticity by spring action and adhesion by electrostatic forces (Opell, 1993; Opell, 1994c; Opell, 1995). Ecribellate spiders (with the garden cross spider *Araneus diadematus* being the archetypal example) coat the axial threads with an aqueous solution that forms glue-sticky droplets with the spring action coming from microscopic windlasses inside the droplets (Fig. 2b). In both cases the interaction of core and coat are crucial for the function of the capture threads.

The ancestral cribellate spiders solved the problem of highly extensible and sticky capture threads over 150 million years ago (Bond *et al.,* 2014; Fernandez *et al*., 2014). The spiral core fibres of cribellate orbweavers such *Uloborus* or *Deinopis* carry crimped spiralling threads that are further surrounded by puffs of exceedingly fine silk with filament diameters of a few tens of nanometers (Opell, 1994a; Opell, 1994b). The cribellum, an ancient spinning plate on the abdomen near the anterior spinnerets, is covered by hundreds or thousands of very fine spigots, each connected with a tiny gland of its own (Kronenberger and Vollrath, 2015). The fibres of the puffs are brushed out from the cribellum by the calamistrum, a highly specialised comb on the metatarsus of each fourth leg (Eberhard, 1988; Opell, 1982). The hackled band of *Uloborus walckenaerius* has shorter overall extensibility (125 %) than the *Araneus* capture silk (where extensibilities are in many hundred %, see Fig. 1) and it seems to absorb energy by friction of the fine hackled fibres, many of which need to break in succession before a thread fails (Köhler and Vollrath, 1995). The stickiness of this hackled capture silk seems to depend on electrostatic van der Waals forces possible from the close contact between the superfine thread and the prey's surface (Eberhard, 1980; Opell, 1993; Opell, 1995; Vollrath, 1994). Important for this action are the puffed nano-filaments, which function only when dry.

The ecribellate spiders have 'lost' the cribellum (as the name implies) and instead have evolved the use of a specialist, liquid glue coating for the core fibres of the capture threads, which function only when wet. Examples of ecribellate orbweavers are the temperate garden *Araneus* spiders or the tropical forest *Nephila* golden orbweaver spiders. The effectiveness of their webs rely on the coating of the naturally dry capture threads with a thin layer of water and glue (Edmonds and Vollrath, 1992) and the windlass mechanism that allows the threads to absorb the high kinetic energy of the prey without breaking (Vollrath and Edmonds, 1989). This intriguing mini-mechanism relies on water as plasticiser of the core fibres, and water surface tension powering the windlasses. To facilitate this double action, these spiders have evolved an aqueous coating with hygroscopic compounds that act to maintain the aqueous coating and attract the required water from the atmosphere (Edmonds and Vollrath, 1992; Tillinghast *et al.,* 1991; Vollrath and Tillinghast, 1991). The high water content of the coat (80 %) plasticises these threads and renders them highly extensible (see Figure 1 for a comparison of wet and dry threads). The coat also renders the threads elastic via the surface tension that reels the axial threads into the droplets when the thread is slackened, and lets them out when stretched.

The glue-component of this composite micro-machine comes from adhesive glycoproteins (Tillinghast and Sinohara, 1984), which self-assemble into microscopic glycoprotein toruses straddling the capture threads beneath the droplets in the aqueous coat (Vollrath, 1994; Vollrath and Tillinghast, 1991). Excellent stickiness is as important as the web's overall mechanical properties, and the fine threads of the capture spiral must stick firmly to hold the prey and ensnare it even when it struggles to get free. Chemical analysis of the coating reveals high concentrations of five water-soluble organic compounds and low concentrations of inorganic salts, as well as at least one glycoprotein. In *Araneus diadematus* webs, this aqueous coating is a strong 5M solution of compounds that are unrelated to the usual silk amino acids, even though they are produced by homologous silk glands. Some of these compounds are close relatives of neurotransmitters being either derivatives or hydrolysates i.e. GABAmide, N-acetyltaurine, choline, betaine and isethionic acid (Vollrath *et al.,* 1990). Other compounds, in lower concentrations are either ionic salts like potassium nitrate (7 %) and potassium dihydrogenphosphate (3 %) or free amino acids such as lysine (2 %), serine (2 %) and cysteic acid (2 %) (Fischer and Brander, 1960; Schildknecht, 1972). In addition, the web contains traces of KH_2PO_4 and KNO_3 as well as glycine and highly saturated fatty acids (Vollrath *et al.,* 1990). Taurin is a protein-stabilising compound while Betaine and GABA are osmoprotectives and osmolytes in a wide range of organisms. We may assume that the organic additives act osmotically and may be necessary to maintain the capture silk's aqueous coat without denaturing the various silk proteins. Moreover, they may act as fungicides or bacteriocides and may even affect the nervous system of the prey (Vollrath *et al.,* 1990).

Importantly for the energy household of the ecribellate orbweavers, the sticky droplets are self-assembling. This is in stark contrast to the hackled threads of the cribellate spiders, which have to be combed out at considerable cost to the spider, meaning that a web takes longer to build (Fig. 2) (Vollrath, 2006). With the ecribellate orbweaver capture threads, the mixture of the compounds listed earlier is applied from two pairs of glands (called aggregate glands) onto the core thread produced from a pair of flagelliform glands and issuing from spigots that are bracketed by the aggregate spigots (Peters, 1987). We note that like all other silks, here every gland is bilaterally symmetric; after all, they have evolved from abdominal appendages (Shultz, 1987), which had to be bilateral. However this may be, the core fibres merge under their thin and viscous coating, which begins to swell as its compounds attract atmospheric water. This swelling leads to surface (Rayleigh) instability, which in turn leads to separation into drops that continue to swell further (Edmonds and Vollrath, 1992). The rate of swelling and the subsequent droplet size is dominated by a dynamic equilibrium between the water in the glue coating and water vapour in the air, and droplets begin to form within seconds after the sticky silk has been spun onto the web.

Clearly, water and hydration is rather important to orbweaver capture silks with their mechanical performance changing under different environmental humidities. This is also true, albeit to a lesser degree, for most other silks. Due to the details of the spinning process and the ensuing polymeric structure of spider silks, their properties can be changed not only passively by the environment (e.g. humidity and temperature), but also actively by the conditions during spinning (e.g. reeling speed) and the post-processing after spinning (e.g. stretching and tension). The interaction of these mechanisms allows the spider to tune its materials to changing situations and environments, as outlined in the next section.

5. Tuning your material: Spinning & processing

Tuning the physical properties of a material is a very practical way to adapt a material to particular functions. Biologically speaking, this means harnessing the material's variability to match ever-changing biotic and abiotic conditions. The insights gained from biological systems sit very comfortably with the present trend of 'smart' or adaptable engineering materials that can change their structure or properties in response to stimuli (Kopecek and Yang, 2007). This makes spiders interesting models as they can actively adapt materials in two broad ways: (i) altering spinning conditions as the fibres are forming and (ii) apply processing conditions post-spinning. The latter will be explored with an example: tuning spider dragline silks for sensory versus mechanical functions.

5.1 Spinning conditions

Variations in spider silk structure and beta-sheet content are greatly affected by the conditions during spinning, i.e. the conditions of 'manufacture'. For example, studying the stress-stain characteristics of *Nephila* dragline silk produced under highly controlled conditions reveals that diameter as well as all mechanical properties is affected significantly by the spinning conditions (Vollrath *et al*., 2001). This trend is similar to that seen in the forced reeling of paralysed silkworms (Mortimer *et al.,* 2013). For example, speed of spinning affects silk properties, where increasing speed decreases breaking elongation, and increases breaking stress and Young's modulus up to the natural spinning speed (Madsen *et al.,* 1999; Vollrath *et al*., 2001). On-line X-ray scattering experiments on single threads as they are being spun further elucidates this correlation (Riekel *et al*., 1999). For example, normal (ca 2 cm s^{-1}) and fast (ca 80 cm s^{-1}) reeling speeds show that the rate of silk production has an influence on silk properties, as does the temperature of the spider (Vollrath *et al.,* 2001). Unnaturally slow speeds of reeling (ca 0.2 cm s^{-1} to 0.9 cm s^{-1}) show silks to be remarkably similar with a semicrystalline morphology (Riekel *et al*. 1999), with nano-scale patterns suggestive of small crystalline blocks in a matrix containing both oriented and unoriented amorphous material (Grubb and Jelinski, 1997; Simmons *et al*.,1996).

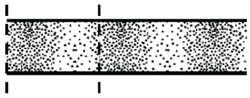
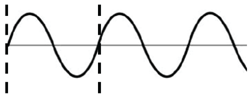
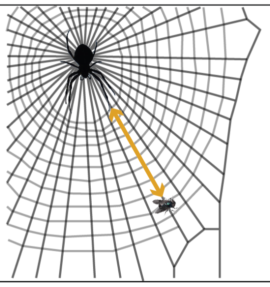
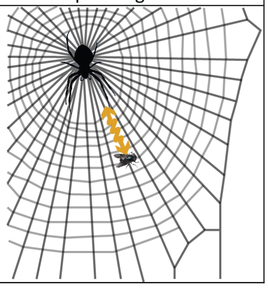
The spinning pathway that controls much of these properties is a highly complex silk production system, for example the major ampullate gland and duct of *Nephila* (Vollrath and Knight, 2001). The gland consists of a sac or ampulla with an apical 'tail' that produces some part of the silk feedstock and a funnel that leads to the duct. The duct is folded back on itself in an elongated 'S' to give three limbs (first, second and third) that progressively narrow to form a hyperbolic die (Davies *et al.,* 2013). Throughout, the flow of droplet inclusions within the silk feedstock confirms that elongational flow at a slow shear rate assists in defining axial molecular orientations (before the final draw-down). Congo red-staining experiments suggest that the combination of wall shear and elongational flow, together with subtle changes in the pH, induce the beta-sheet transitions in the proteins as they travel through the duct (Vollrath and Knight, 2001).

5.2 Post-spinning processing: Supercontraction

In addition to the internal extrusion spinning process, the conditions immediately following this - such as post-draw - further allow the animal to tune properties. Moreover, environmental conditions (such as ambient humidity) further affect the degree to which the spun fibre self-modifies or adapts. This is especially important in the example of supercontraction, when spider silks contract when submersed in water, for example the dragline threads of all *Nephila* species. Importantly, the process (and degree) of supercontraction is correlated to the ratio of the amino acid proline in the silk molecules (Liu *et al.,* 2008) and moreover is a two-phase process (Guan *et al.,* 2011). Different species contain different proportions of proline, which directly alters the extent to which their dragline silks can supercontract (Liu *et al*., 2008). The link between supercontraction and protein structure has been quantifiably modelled to explain the range of mechanical profiles possible for a given proline content (Liu *et al.,* 2008).

The role of supercontraction in tuning silk properties can be illustrated with the example of spider dragline silk, which balances mechanical and sensory roles of transmitting vibrations. Silks can transmit both transverse and longitudinal waves, depending on the direction of the vibration displacement (Frohlich and Buskirk, 1982; Mortimer *et al.,* 2014). Combining experimental techniques of laser vibrometry and ballistic impact with classical vibrating string theory demonstrates that longitudinal waves are more consistent in their signalling properties compared to transverse waves (Table 2) (Mortimer *et al*., 2014). In contrast to transverse waves, longitudinal waves have little to no change in their propagation speed with fibre tension or number and diameter of fibres (Mortimer *et al*., 2014). Transverse waves are sensitive to all these features, so are likely to be used for assessing the condition of the web when a spider plucks its radial threads (Szlep, 1964).

Table 2. Transverse and longitudinal wave sonic properties, including hypothesised use of each wave type in spider silks in the web. Reproduced under the CC-BY license from Mortimer, B., Gordon, S. D., Holland, C., Siviour, C. R., Vollrath, F. and Windmill, J. F. C. (2014), The Speed of Sound in Silk: Linking Material Performance to Biological Function. Adv. Mater. doi: 10.1002/adma.201401027.

Wave type	**Longitudinal** 	**Transverse** 
Wavespeed variation between materials	Governed by storage modulus	Does not vary between polymers as densities and response to tension is similar
Wavespeed sensitivity	Storage modulus, (slight effect of tension), (slight effect of frequency)*	Tension, (slight effect of frequency)
Resonance sensitivity	Distribution of structures in the material	Fibre number, fibre diameter, distribution of structures in the material
Hypothesised use in web	Vibration propagation of all kinds, where any resonance will be above 1 kHz	Web condition signals, resonance at short silk lengths from silk plucking
		

Compared to other materials, spider dragline silk is special in the way that it can control and tune its longitudinal waves. Longitudinal waves are governed by the material's storage modulus, which also influences mechanical stiffness. This could lead to a property trade-off, as storage modulus values could limit the optimality of mechanical or signalling function. However, spiders can tune the storage modulus day-to-day to adjust the balance of mechanical and vibrational properties. The tuning can be through actively adjusting the modulus through modifying tension in the threads or changing reeling speed during spinning (Vollrath *et al*., 2001). Additionally, spiders can passively reduce the storage modulus of their dragline silks through supercontraction (Guan *et al*., 2011; Liu *et al.,* 2008). Importantly, the silks can be stretched following supercontraction to alter mechanical properties predictably, allowing spiders to access the full range of moduli from 3-30 GPa – the largest range of moduli available to a single material (Boutry and Blackledge, 2010). These multifunctional and tuneable properties make spider dragline silk a unique material and an excellent model for adding signalling functionality to high-performance fibres.

6. Conclusions

As materials, spider silks have a much larger diversity than both silkworm silks and man-made polymers. They vary hugely in molecular composition and mechanical properties with the 'option' of tuning properties by the extrusion spin process to environmental requirement. This is possible because the protein dope and spinning apparatus to control spinning and processing, including post-processing such as through silk draw-down stretching, have co-evolved with the spider's spinning behaviour (Boutry *et al.,* 2011; Liu *et al.,* 2005; Vollrath *et al*., 2001). This allows rapid adaptation to a wide range of conditions (Vollrath, 1999). Which of course is crucial to optimal performance of the orb web, which incorporates many different types of silk (Lin *et al.,* 1995) with properties of specific silks tuned to specific conditions, for example, balancing mechanical stiffness with fast vibration propagation in windy conditions (Mortimer *et al.,* 2014).

Protein primary sequence, spinning, processing and environmental conditions combine to modify fibre hydration, hydrogen bonding and ratios of order to disorder within the spider fibre's molecular structures (Guan *et al.,* 2012; Guan *et al*., 2013; Guan *et al.,* 2011). These parameters can be quantified to model the range of mechanical properties of the whole range of spider and insect silks (Porter and Vollrath, 2009). This makes all sorts of silks very valuable models in the search to better understand the wider (and generic) aspects of bio-polymer structure and properties. This, in turn, can be used to develop or inspire future polymeric materials for specific applications. Furthermore, as silks are biodegradable, natural, high performance materials that are made at room temperatures and pressures (Bunning *et al.,* 1994; Cao and Wang, 2009; Holland *et al.,* 2012; Vollrath *et al.,* 2011), there is a great potential benefit for applying silk or silk-like materials to applications in engineering and medicine. The diversity in mechanical performance and structures of silks could therefore provide a broad library of materials that can suit a variety of applications. Harnessing the diversity and variability of silk will be a future challenge with huge potential outcomes for green, smart and adaptive materials.

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