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The route to synthetic silks

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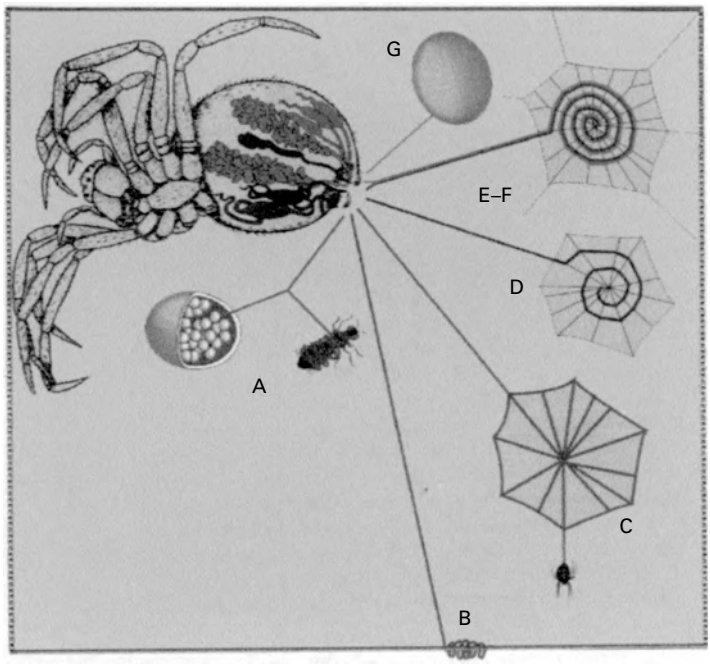
8.1 Introduction

Natural silks are extremely fine, tough, strong and extensible. Many spiders produce, from a widely diverse 'battery' of glands, a wide variety of silks (Fig. 8.1). Silkworms only have one type of gland. A single silk filament can be anything up to 1200 metres (as in silkworm silk reeled from a high quality cocoon) or up to 500 metres (as in spider dragline silk reeled directly from the immobilised animal). Industrialists have long dreamt of artificially producing silk fibres with the properties of spider dragline silk. To this end attempts have begun to express spider silk genes in organisms that are more easily and cheaply cultured than spiders ranging from micro-organisms to potatoes and even mammals [1–3]. While such a biotechnological approach in itself poses a range of problems, both with the transfer of genes as well as the expression and extraction of the relevant proteins, it emerges that the exceptional properties of a silk do not depend solely on the unique nature of the silk precursor-feedstock. There is now strong evidence that a spider's (as well as a silkworm's) spinning mechanism may be no less important in determining a filament's material properties than the feedstock polymer (e.g. [4–6]).

8.2 Silk structures

Spider silk, like insect silk, was long thought to be a composite consisting of protein crystals embedded in a protein matrix with the crystals giving the silk biopolymer its strength and the matrix its elasticity [7–9]. This strength would largely be determined by the length, the diameter and the composition of the crystals while the elasticity is thought to derive from the entropic qualities of the matrix molecules [10]. However, it now appears that this simple model is far from correct for most silks. Indeed, the draglines of both the golden silk spider *Nephila* (Fig. 8.2) and of the garden spider *Araneus* are among the toughest silks so far investigated. Both are not simple

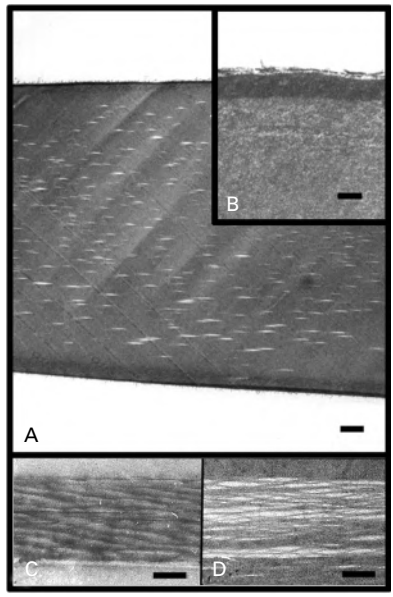


8.1 The female cross spider *Araneus* (like most other advanced orb weaving spiders) spins 7 different silks from as many different glands: soft silk, to enshroud prey and cradle the eggs in the cocoon or to balloon (A); tough silk for the egg sac 'shell' (G); cement silk to affix the web to support (B); strong but relatively stiff silk for the scaffolding of the web (C); additional scaffolding silk also used for the temporary support spiral (D); and, finally, very elastic sticky capture silk consisting of a core thread and a watery coat that forms the web's droplets and glue (E–F).

homogeneous filaments (as was long thought) but resemble microscopic climbing ropes with nano-engineered strands of micro fibrils interspersed with filled inclusion channels and covered by several layers of coating [11].

Another interesting silk composite, the sticky capture silks of *Nephila* and *Araneus*, are complex, albeit microscopic, mechanical windlass systems that make good use of the physics of biological micro-engineering (Fig. 8.3). In the 'windlass' silk (which operates in the wet state) the elasticity is given by a combination of surface tension of the aqueous coat and recoil of the plasticised silk fibre [12–14] while adhesion is bestowed by a separate glycoprotein complex [12, 15].

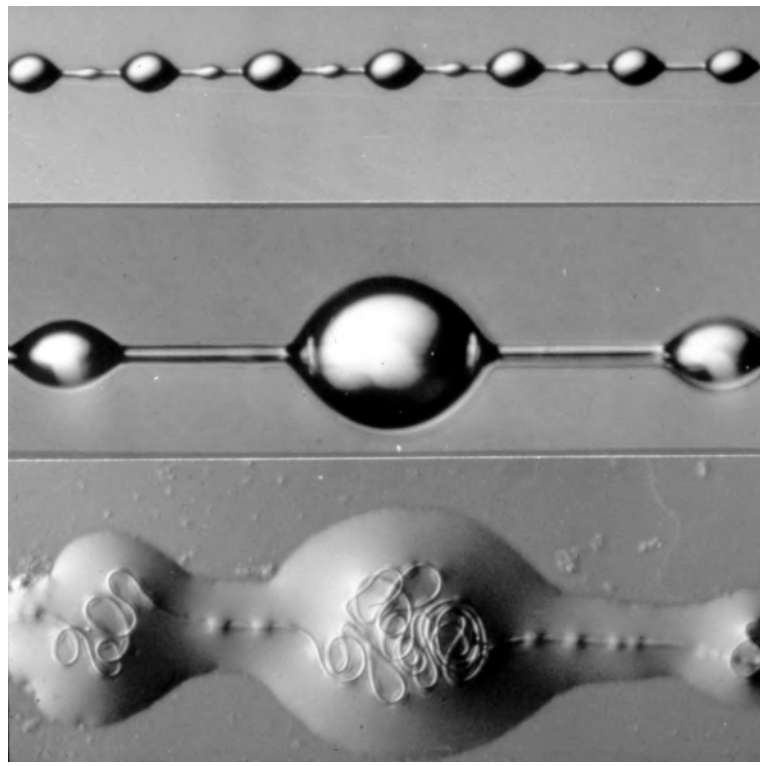
The mechanical behaviour of radial and capture silks differs greatly. For example, the wet and soft sticky spiral of the *Araneus diadematus* garden spider absorbs energy by large extendibility (*circa* 500%) of the wetted thread which develops substantial force only after 100–200% extension with



8.2 Electron micrographs of *Nephila* dragline silk: (A) longitudinal section of a fully urea-swollen fibre – note the numerous electron-light canaliculi and the electron-dark outer coat (bar 1μm); (B) magnification of the coat showing the various levels (bar 100 nm); (C) cryo-section of an untreated fibre (bar 1 μm); (D) enzyme K treated fibre (bar 1 μm). (A/B modified from ref. 85; C/D: we thank W. Hu for help with the preparation.)

the thread breaking suddenly at around 400–500% extension [12, 16]. Nevertheless, the engineering strength of these *Araneus* capture threads is 1338 + 80 MN m^{-2} with a breaking energy of 163 J cm^{-3} (N = 6) and thus is comparable to that of the radial threads at 1153 + 144 MN m^{-2} with a breaking energy of 194 J cm^{-3}. Finally, a third basic type of web silk employs very fine filaments of only nanometres in diameter which is combed into hackled bands of many threads onto supporting axial threads which are often sprung. In this kind of silk, which operates in the dry state the hackled bands provide some elasticity as well as tremendous adhesion presumably by electrostatic forces [16]. The mechanical behaviour of these threads resembles more that of the wet capture silks than that of the dry radial threads.

The functional and developmental details of the two so very different elastic recoil mechanisms of the two types of capture silk micro-machines are interesting and deserve deeper studies. However, at present we do not even understand the interaction of form and function in the much more 'typical' spider silk fibre such as a dragline filament. Recent studies indicate that the toughness of spider dragline silk may depend on the complex hierarchical structure of the fibre [11] which in turn depends on a complex



8.3 A capture thread of *Araneus diadematus* under increasing magnification. The windlass mechanism is seen in the lower picture where the core fibres after a large extension–contraction cycle have been reeled into a droplet (for details see ref. 12).

spinning process [17, 18], as well as on tuned dopants in the polymer feedstock [19]. Several factors may contribute to the toughness of a spider silk fibre: (a) the design of the protein monomers and their conformations and interactions; (b) the hierarchy of protofibrils and fibrils within the filament which provide several levels of energy dissipation; (c) numerous narrow, highly elongated channels in the silk that serve as crack deflectors or fluid filled shock absorbers; (d) a multi-layered coat to the filament surrounding a central core to prevent surface crazing.

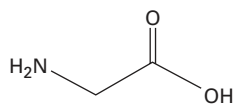
8.3 Development of fibre: the feedstock

8.3.1 Biochemistry

Neither spider nor silkworm silks are a simple single protein bio-polymer. Indeed, the typical spider major ampullate dragline silk contains many different

organic and inorganic components like neurotransmitter proteins, glycoproteins, lipids, sugars, phosphates, calcium, potassium or sulphur [15, 20–28]. Notwithstanding the plethora of associated compounds in many silks, the main constituents of typical silks are a class of proteins called fibroins for insects and spidroins for spiders. These proteins are considered to be principally responsible for the mechanical properties of the silk fibres although the non-fibrous protein compounds (e.g. those mentioned above or the sericins of the *Bombyx* silks) also affect mechanical properties [28, 29]. However, strength, elasticity and toughness are not the only tasks that a spider's silk must fulfil in nature. Like silkworm cocoon silks, spider silks also have to withstand microbial attack as well as other physiological/environmental challenges, therefore, some components of the spun thread may not be required for the mechanical properties; in fact, a proteinase inhibitory function could be demonstrated for smaller peptides found in some insect silks [30]. Additionally, some components found in spun silk may have their function in the formation of the spinning dope or the spinning process, but may not play any substantial role in the fibre.

Quantitative amino acid analyses showed already nearly 100 years ago that silks like those of *Bombyx mori* and *Nephila madagascariensis* are very rich in glycine (8.1) and alanine (8.2) [31]. A predominance of amino acids with short side chains can be stated for almost all silks [32]. The chemical composition of spider silk, however, can vary between subsequent webs of the same animal in dependence of its diet and living conditions [20, 33]. Very different values have been reported for the molecular weights of spider spidroins derived from solved silk and the liquid spinning dope of the major ampullate glands, ranging between 30 to 740 kDa [34]. Various technical difficulties implied by the peculiar amino acid compositions and the nature of the proteins may interfere with different methods. Values derived from SDS-PAGE are reported to be 323.6 kDa [35], 195 and 220 kDa [36] for *Nephila clavipes* spidroins; the latter values were obtained under reducing and non-reducing conditions indicating covalent bonds via disulfide bridges. Disulfide bridges are also found in *Bombyx mori* where heavy and light chain fibroins are coupled [37].



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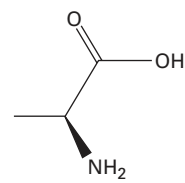

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Recent investigations of the gel-like protein content gathered and stored in the sac-like glands of the major ampullate glands revealed that the majority

of the protein fraction is of high molecular weight (>200 kDa) but migrates as multiple bands in SDS-PAGE. Partly this observation is explained by the presence of the two different major ampullate spidroins Masp1 and Masp2 (see Section 8.3.2). This is evident from both antibody staining [38, 39] and isoelectric focusing [39], but the results also show that multiple sizes exist from one protein species. Aside from splicing especially degradational processes are discussed as an explanation; and the question was put forward if and how this heterogeneity impinges on the spinning process and quality of the fibres.

8.3.2 Molecular biology

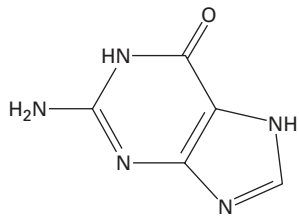
The first sequence data for spidroins were obtained from c-DNA clones of *Nephila clavipes* major ampullate glands that form the dragline [40]. Two different spidroins, Masp1 and Masp2, were identified and approximately 2000 base pairs from the 3′ end of their reading frames were sequenced. Different values of the transcript sizes were published and the actual size remains somewhat doubtful (Chinali, personal communication). The highest values, however, indicate that only approximately 20–25% of the sequences might be known yet (Table 8.1).

Table 8.1 Published transcript sizes of various spidroins (only the highest values are given)

Gland	Species	Gene	Transcript size ($\times 10^3$ bases)	Ref.
Major ampullate	*N. clavipes*	MASp1 (NCF-1)	12.0	[47]
Major ampullate	*N. clavipes*	MASp2 (NCF-2)	11.5	[47]
Major ampullate	*A. diadematus*	ADF-3	9.0	[46]
Major ampullate	*A. diadematus*	ADF-4	7.5	[46]
Minor ampullate	*N. clavipes*	MiSp1	9.5	[45]
Minor ampullate	*N. clavipes*	MiSp2	7.5	[45]
Minor ampullate	*A. diadematus*	ADF-1	9.5	[46]
Flagelliform	*N. clavipes*	Flag	15.5	[43]
Cylindrical	*A. diadematus*	ADF-2	14	[46]

The sequences reveal that the genes are highly repetitive in their main parts [40]. Due to the codons of the most prominent amino acids alanine and glycine they are very rich in guanine (8.3) and cytosine (8.4) although these nucleotides are avoided in the third position. The latter is reflected by clusters of tRNA genes with the respective anticodons [41].

The repetitive parts of the protein sequences are made up of several peptide motifs that are iterated multiple times to form repetitive structural modules. These motifs are used in various combinations in the different



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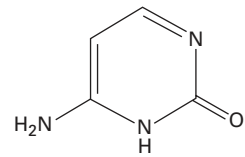

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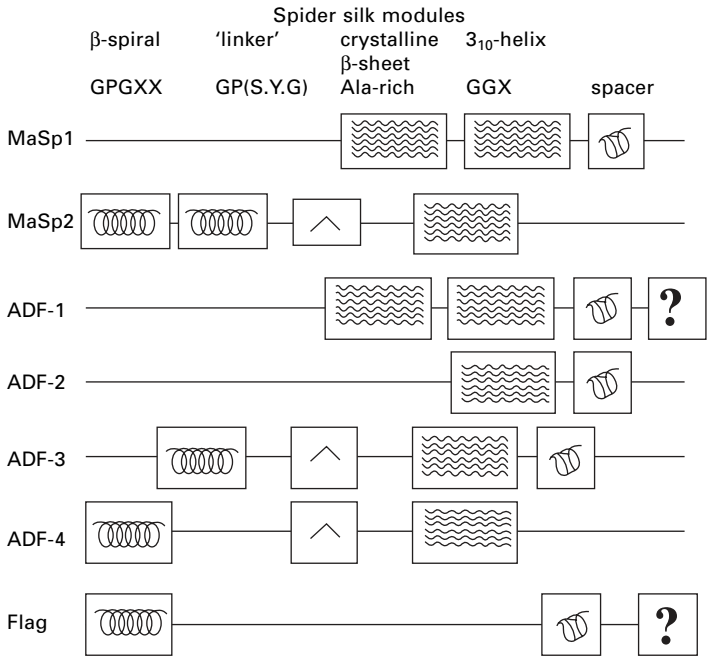
spidroins and are believed to confer the observed mechano-physical properties to the respective silk [42, 43] (see Fig. 8.4).



8.4 Distribution of structural motifs within different spidroins according to Hayashi *et al.*[47] X indicates a residue that may vary.

These repetitive sections are framed by non-repetitive N- and C-termini [40, 44–51]. The N-terminal sequences contain putative signal sequences and might play a role in the secretion of the spidroins into the glandular lumen. However, although they are potentially very important for our understanding of silk production/storage, to date N-terminal sequences are known only from Flag spidroins and one minor ampullate spidroin [45, 50–52].

The C-termini of the spidroins derived from ampullate glands are highly

conserved, not only between different spidroins but also between different spider species [44] and are considered to be of high functional importance [38]. Various functions have been proposed, for example, that they might play a role in the solubility of spidroins in the highly concentrated spinning dope. In addition (or alternatively) the C-termini might represent proto-peptides with signalling capabilities, which are cleaved off after secretion. Studies of *Bombyx mori* fibroins have demonstrated emulsion formation and micellar structures from aqueous solutions of reconstituted silkworm silk fibroin [53]. The hydrophilic N- and C-terminal peptide sequences of *B. mori* might be required for this micellar-like organisation that subsequently leads to the highly concentrated gel-like state of the spinning dope. It may be speculated that spidroin C-termini, although substantially shorter, might fulfil a similar function [53].

New experimental evidence [54] indicates that the C-termini of *Nephila clavipes* major ampullate spidroins, which are present in the high molecular weight fractions of both the proteins derived from the secretions of the glands and the spun thread, are involved in the formation of disulfide bridges. However, it is rather unlikely that such covalent cross-linking has a strong impact on the material properties as the C-termini of the minor ampullate spidroins that form a very stiff fibre do not contain cysteines [50].

8.3.3 Molecular structure

Most structural investigations of silks on the molecular level use X-ray diffraction and crystallography. In this way, so far, over 100 different silks have been characterised and grouped according to their structures [32]. Most of these structures contain β-pleated sheets orientated either parallel or antiparallel to the fibre axis, but one can also find silks with cross β-pleated sheets, β- and/or 3(1)-helices or α-helical conformations. The α-helical silks have a relatively low content of glycine and are high in acidic residues.

The dragline silks of spiders belong to the first group with β-pleated sheets. The investigation of major ampullate silks (MAS) of *Nephila madagascariensis* and *Nephila clavipes* revealed amorphous regions in which crystalline domains of antiparallel β-pleated sheets are interspersed [55]. The crystallinity was estimated to be 30–50% in Nephila clavipes MAS [56]. For the diameters of the crystallites a size in the range of 70–100 nm [28] and later 70–500 nm [57, 58] have been published. Calcium detected by EELS (Electron Energy Loss Spectroscopy) was found exclusively in the crystallites [56]. Hence it could be important either for the conformation or the generation of the crystalline structure.

The β-pleated sheets are formed by the poly-alanine stretches. Solid state NMR revealed that about 78% of the alanine in *Nephila clavipes* MAS are included in the crystalline fraction. It consists of two types of alanine-rich

regions. About 40% make up a dense and highly orientated fraction. The second fraction, of lower density and less well orientated, is suspected to contain the GAG motifs on the flanks of the highly orientated poly-alanine stretches [59]. As was evident from REDOR (rotational-echo double-resonance) NMR data the sequence motif LGXQG (X = G, S, N) which is present in front of the poly-alanine stretches possibly is a β-turn of type I that could reverse the chain direction [60].

From the size of the crystallites as well as the measured intersheet spacing it was concluded that the crystalline domains must also include glycine rich sequence parts [56–58, 61]. The inclusion of GGX motifs, however, is still an open issue [62].

The molecular and functional organisation of spider silk has been compared to that of rubber [8]. It was proposed that the crystals formed by the β-pleated sheets cross-link the fibroins into a polymer network and thus provides toughness and stiffness. The amorphous regions, made of randomly oriented chains, would account for the elastic properties. The glycine rich fraction that is thought to make up the amorphous matrix, however, seems to be much more orientated as NMR experiments with *Nephila madagascariensis* MAS indicate; it adopts a 3(1)-helical conformation that could contribute substantially to the mechanical properties by the high forces of hydrogen bonds [62–65].

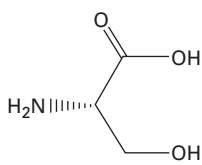
The poly-alanine stretches in spidroin 2 are somewhat longer than those of spidroin 1 and therefore would be more suitable for the formation of β-sheets. However, the proline residues in the glycine rich neighbourhood are rather detrimental to a formation of β-pleated sheets and would disrupt the integrity of the conformation. It was proposed that spidroin 2 should therefore be exclusively found in the amorphous matrix while solely motifs found in spidroin 1 are responsible for the formation of non-periodic lattices (NPL) in the crystalline fraction [57, 58]. Yet, attempts to investigate the conformation of spidroin 2 with NMR failed as no labelled proline could be found [66]. Thus the role of spidroin 2 in the molecular structure remains speculative.

8.3.4 Insect silks

The silks of insects like the silkworm *Bombyx mori* have been mentioned several times in this chapter; in fact, far more is known about the biochemistry, molecular biology and genetics of the silkworm than of any spider. There are many similarities between insect silks and spider silks, e.g. the predominance of amino acids with short side chains or the occurrence of some sequence motifs, which can help to understand the general principles involved in the generation of their material properties. Yet, there are also major differences that have to be taken into account. Most insect silks are used as cocoons or protective webs in larval stages and originate in labial glands [32]. In contrast,

in almost all spider species the silk glands are situated in the so-called opisthosoma, the abdominal body segment of these animals. Apart from the different phylogenetic as well as ontogenetic origin of the glands, there are far more different gland types in spiders and they are usually not restricted to a certain developmental stage. The main difference lies in the usage of the silks and the subsequent adaptations of the material. Threads used for protective shelters for instance do not require elasticity whereas the capture threads of spider webs would just not function without it.

Bombyx mori silk fibres show a different morphology to spider fibres. The silkworm fibres (as we know them so well from commercial fabrics) actually do not represent the natural fibre but its core only, having been separated from its outer shell by a process called 'degumming'. The coating layer is produced in the middle parts of the silk duct and added on top of the passing liquid core material so that, upon extrusion, a two-layered fibre is created. The shell represents an extensive layer of glycoproteins that are named sericins due to their high content in the amino acid serine (8.5). A high variability is observed in sericins in which splicing is involved [67–70]. The core of a silkworm silk consists of three different proteins: a protein called P25, the heavy chain (H-fibroin) and the light chain fibroins, which are exclusively produced in the posterior (PSG) section of silk glands. H-fibroin is a highly repetitive protein with a high molecular weight of 391.5 kDa, whereas P25 and L-fibroin with app. 25–30 and 25 kDa, respectively, are rather small. Such small proteins were not identified in spider silks. The heavy and light chains are linked by disulfide bonds and their assembly is essential for the efficient intracellular transport and secretion of fibroin as is the participation of P25 [37, 71, 72]. Analogues of these genes have also been found in other insect silks.



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Similar to spider silk, the fibre is composed of microcrystalline arrays alternating with amorphous regions. The crystalline arrays are composed of anti-parallel beta sheets that run parallel to the fibre axis. This conformation results from long $(\text{-Gly-Ser-Gly-Ala-Gly-Ala-})_n$ stretches in the sequence of the H-fibroin which are interrupted by regions containing bulkier residues [73]. The GSGAGA motif is also found in the sequences of minor ampullate spidroins [50, 51]. Interestingly, the fibroin of some insects, for example,

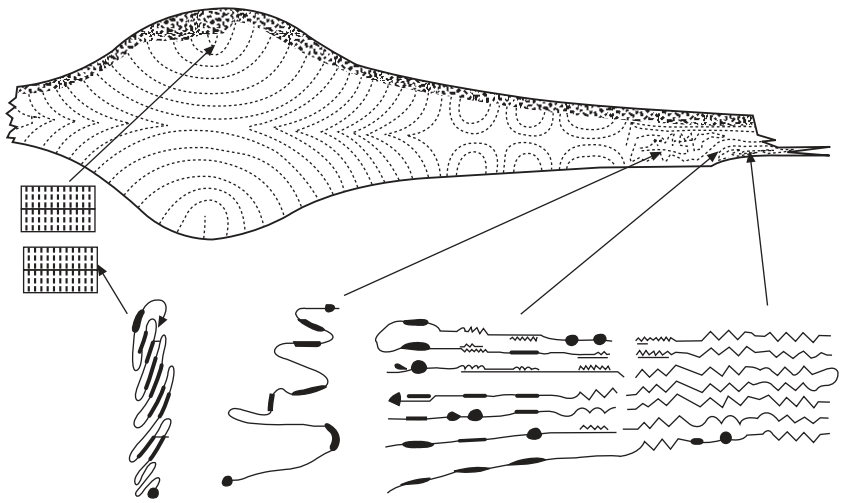
Antheraea, which produces a tougher silk than *Bombyx* also contains poly-alanine of the type $(A)_n$ [74].

8.4 Development of fibre: spinning

Clearly, in the dragline silks as in the capture silks, the different components and their interactions heavily depend on processing conditions during natural silk spinning. Silk is converted from the liquid feedstock in the gland into a solid thread inside a tapering tubular duct, which exits at the spigot [75]. The core and coat composite structure of the dragline thread is formed by the co-drawing of at least two feedstocks through a single die and followed by further coating in the duct [11].

The spider modifies the mechanical properties of its silk not only on a medium-term basis (e.g. in response to starvation [76]) but also in rapid response to immediate static requirements of the web, which are complex structures with highly specific design characteristics relying heavily on silk properties [77, 78]. To be able to alter silk properties so rapidly, the animal cannot do it by changing the molecular structure because the silk feedstock dope is prepared well in advance of spinning. Instead the spider must use its ability to control the refolding of these molecules during the extrusion process, which would mean controlled modifications as the fibre is formed. Hence we must assume that a proper spider silk fibre is not self-assembling but instead forms during an assembly process that is highly controlled. Nevertheless, outside the animal, raw silk pre-cursor (as well as recombinantly produced silk peptides) can self-assemble to some degree into some sort of filament simply by drying out or if shared [34, 79, 80]. The details of this assistance are clearly important but as yet only poorly understood (Fig. 8.5).

Nevertheless, we assume that control is asserted all along the spinning production process. Here the feedstock can be chemically modified for example by subtle pH alterations [81] or the rate by which salts and water is pumped in and out of the duct [5]. Moreover, the spider can influence the rate (i.e. the spinning speed) by which the dope moves through the duct (e.g. by walking or running [82]). This rate of flow through affects the mechanisms of flow elongation and flow shear [6, 83] with direct consequences on silk mechanics [11] via the degree of β-sheeting [5] and molecular order in general [84]. Furthermore, the coating of the fibres is important [85] and the action of the ratchet clamp has some effect, possibly as internal post-extrusion draw-down [11]. Finally, the post-processing external draw-down settles the silk's mechanical behaviour [86–88] by locking the molecules into position. All of these are parameters and variables in a centrally controlled production system with considerable scope for feedback [76].



8.5 Generalised spider silk gland based on typical major gland producing structural threads. The liquid crystalline dope solution produced in different zones of the gland is drawn through a tapering S-shaped duct that converts it into an elastomeric thread with a composite core-coat structure. A funnel (A) links gland and duct while reducing turbulence and mixing. The duct (B) removes water and adds auxiliary compounds facilitating shear stressing and has an internal draw-down. The 'valve' (C) is not a shaping device but a clamp for gripping and a ratchet to retrieve a broken thread. The spigot (D) strips off the last of the lumen solvents and surfactants. Magnifications (height), funnel 350 μm, draw-down 40 μm, valve 300 μm, spigot 190 μm. Both gland and duct have a complex histology. The so-called A-zone of the gland secretes the spidroin forming the core of the thread and the compounds filling the canaliculi while the B-zone secretes the mantle. The secretory granules of the A-zone contain finely granular/filamentous material while those of the B-zone contain polydomain hexagonal columnar liquid crystalline material. The thick cuticle of the short funnel reduces movement while the long and flexible spinning duct has a thin cuticle acting as an advanced hollow fibre dialysis membrane. The epithelium of the S-shaped duct increases in height from the first to the second and third limbs of the duct indicating increasing specialisation for pumping water and ions on the outflowing dope. Just before the draw-down taper, single flask-shaped gland cells contribute surfactants and extra coating. The final section of duct past the clamp is highly specialised for pumping with its tall cells full of mitochondria and covered with apical microvilli with a highly folded plasma membrane. Finally, the lips of the spigot seal the duct to the outside world (for further details see ref. 75).

8.5 Performance characteristics

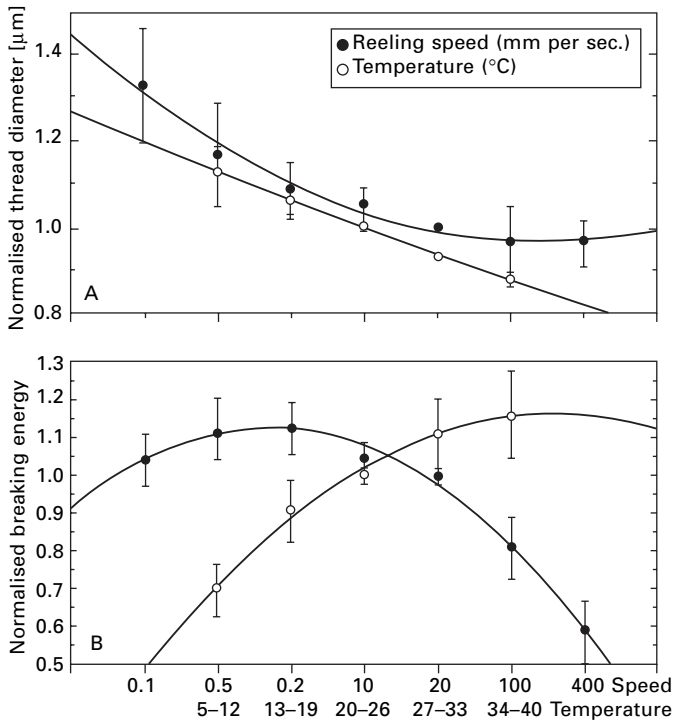
The dry and very tough radius threads of orb web-building spiders such as *Nephila spp.* or *Araneus diadematus* show good extendibility (up to and

even above 40%) as well as high tensile strength (*circa* 1.2 GPa) and large hysteresis (*circa* 50%). Together all performance characteristics indicate that in the web these fibres function as both shock absorbers and structural elements [16]. For example, radial/dragline thread drawn from the major ampullate glands of mature females *Nephila edulis* (average weight 527 ± 103 mg, mean ± s.d.) at control spinning conditions (a drawing speed of 20 mm s^{-1} and a temperature of 25°C) have an average silk diameter of 3.35 ± 0.63 μm with a normalised average breaking strain of 0.39 ± 0.08%, a breaking stress of 1.15 ± 0.20 GPa, an initial modulus of 7.87 ± 1.85 GPa, a yield stress of 0.153 ± 0.058 GPa and a breaking energy of 165 ± 28 kJ kg^{-1} [89]. Like most fibres, silk has a moderate positive Poisson ratio with a thinning ratio of *circa* 5% for each 10% of strain in a linear fashion until the maximum extensibility of 40% when the fibre typically breaks [89].

The mechanical properties of a fibre are greatly affected by the conditions of manufacture. For example, in *Nephila* dragline silk produced under highly controlled conditions, not only diameter but also most mechanical properties were affected significantly by both speed and temperature of spinning [89] (Fig. 8.6 and Fig. 8.7). Micro-X-ray diffraction shows that spinning speed and temperature both affect the molecular structure of a silk filament [87, 90], which in turn is responsible for the observed mechanical properties. In addition to these environmentally induced variablities, the mechanical properties of dragline silks show also large variability between individual spiders as well as differences between species [76] (Fig. 8.8).

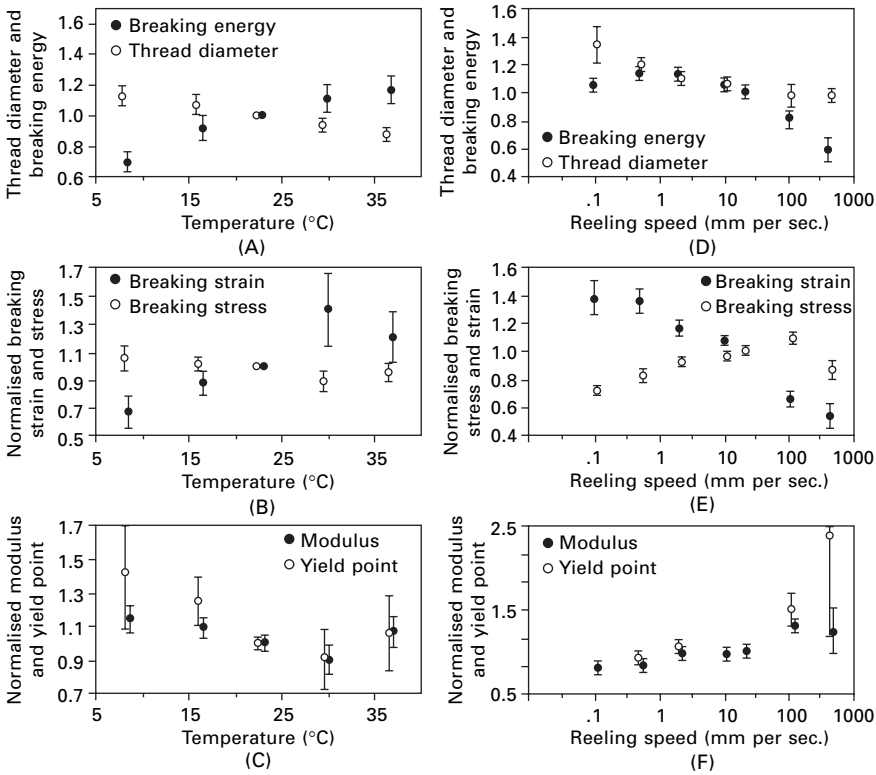
It can be strongly argued that silk is tuned for the average conditions that the different spiders would encounter in nature. Thus, as each silk of a particular species is optimised, in some cases the ability for a rapid (and temporary) adaptation to the environment could offer a great selective advantage [20]. Orb webs have been designed by evolution to take out-of-plane load in maximum deflection [78]. Their performance is greatly enhanced by incorporating into one web the mechanical properties of different types of silk [7]. Environmental conditions affect both the architecture of the web [77] and the mechanical properties of the silken threads [89]. Consequently the spider is under considerable and sustained selection pressure to modify web engineering including silk mechanics [4, 20] and silk genetics [42].

As shown earlier, orb weavers like the garden spider *Araneus* or the golden silk spider *Nephila* coat the capture threads with an aqueous solution that forms sticky droplets. Coat and droplets are crucial for the function of these capture threads as their elasticity derives largely from the high water content of the coat. However, water is important not only for these threads, but for many other types of thread as well, and the role of water as well as other solvents for understanding and manipulating the mechanical properties of spider silk cannot be understated (Fig. 8.9). This can be of special interest if we aim to produce bio-engineered silks with specific properties.



8.6 The effect of drawing speed and abdominal temperature at spinning on normalised silk diameter (A) and normalised energy (B) required to break a thread. For non-normalised data under control conditions see Table 8.1. Reeling speeds (•) are denoted in mm s^{-1}; body temperatures (○) are given in °C. Control temperature was 25°C when reeling speed was varied, and control speed was 20 mm s^{-1} when body temperature was varied; 95% confidence intervals are given for each data point (after ref. 89). At 20 mm s^{-1} and 25°C females with an average weight of 527 + 103 mg (mean + s.d., N = 4) had an average silk diameter of 3.35 + 0.63 µm with a normalised average breaking strain of 0.39 + 0.08, a breaking stress of 1.15 + 0.20 GPa, an initial modulus of 7.87 + 1.85 GPa, a yield stress of 0.153 + 0.058 GPa and a breaking energy of 165 + 28 kJ kg^{-1} (for details see ref. 89).

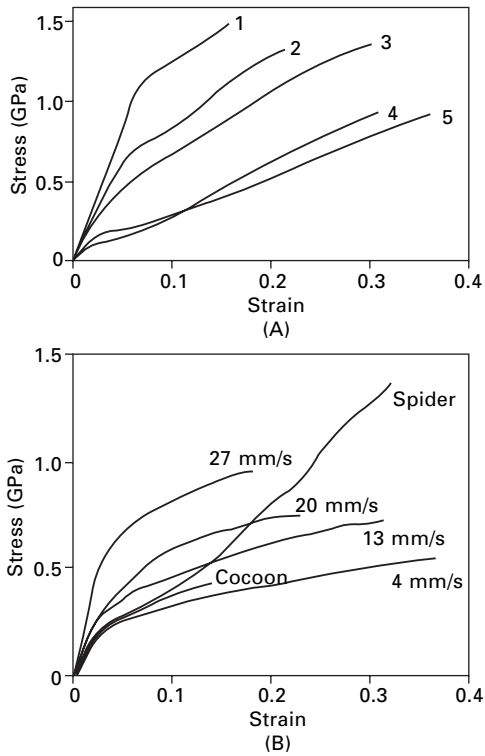
Many spider silks contract in water [91] and other small-molecular solvents [92]. Indeed some silks such as, for example, the typical *Nephila* and *Araneus* dragline threads, can super-contract up to 50%. The degree of super-contraction seems to be a function of the crystallinity of the material and can be used to study both the gross morphology of silks [93] and their molecular structure [92, 94]. The glass transition temperature of spider silk of about –75°C suggests that at room temperature the molecular chains are held in place by intermolecular hydrogen bonds. As these bonds are gradually destroyed by



8.7 The effect of abdominal temperature (A, B, C) and drawing speed (D, E, F) on normalised silk parameters. For non-normalised data under control conditions see Fig. 8.6 Shown here are (A, D) thread diameter and breaking energy, (B, E) stress and strain at breaking as well as (C, D) initial Young's modulus and point of yielding. Each point represents the average taken from several spiders, with 3 measurements for each animal; the vertical bars are 95% confidence intervals (for details see ref. 89).

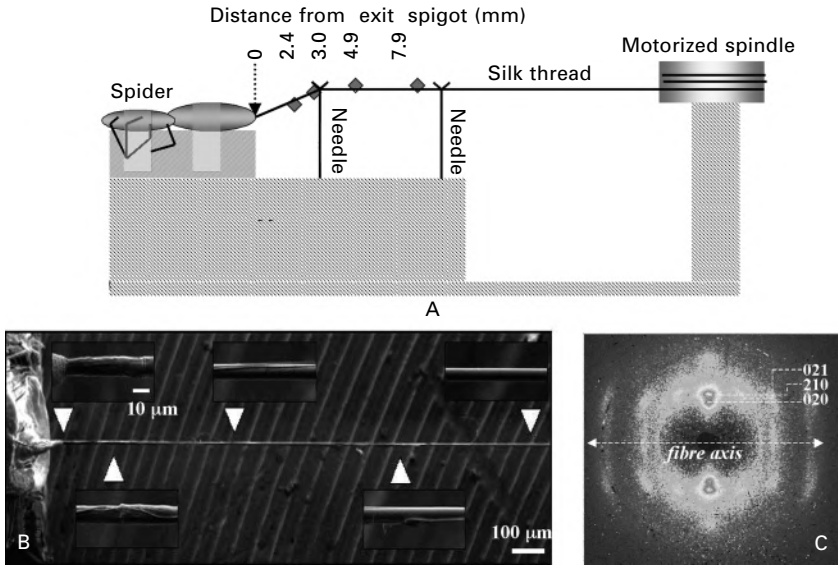
the actions of the solvents, the molecular chains begin to 'disorient', i.e. lose order. Thus, in essence, the contraction of spider silk in water results from the disorientation of the molecular chains. The more hydrogen bonds are destroyed, the larger the shrinkage until, finally and in a strong solvent, the silk is totally dissolved. It has been shown that, in water, silk with high birefringence shrinks less than silk with low birefringence [95] and we may assume that birefringence is positively correlated with degree of hydrogen bonding. Thus, birefringence would also be positively correlated with molecular orientation and higher density, i.e. more so-called 'β-sheet crystal areas'; one might hypothesise that such very dense areas keep water molecules out with the result of fewer broken hydrogen bonds and lesser shrinkage.

Major ampullate dragline silk from *Nephila* spiders has high initial modulus



8.8 (A) The mechanical properties of different spider dragline silks. Stress–strain characteristics of silk reeled from spiders belonging to widely diverging taxa: (1) *Euprosthenops sp.* (Pisauridae), (2) *Cyrtophora citricola* (Araneidae), (3) *Steatoda bipunctata/ Latrodectus* (Theridiidae), (4) *Araneus diadematus* (Araneidae) and (5) *Nephila edulis* (Tetragnathidae). The large inter-specific differences in drag and structural major ampullate threads which might correlate to web type: *Euprosthenops, Leatrodectus* and *Cyrtophora* build 3-D space knock-down webs that catch by breaking threads and that have a long active life (several months) whereas *Araneus* and *Nephila* build 2-D orb webs that catch by net-action and that have a short service life (a few days at most). Note the differences in strength, extendibility and yielding, all, of course, affecting toughness (for details see ref. 76). (B) Influence of reeling speed on the mechanical properties demonstrated with *Bombyx mori* cocoon silk. For comparison, the properties of spider dragline (spider) and cocoon silk (cocoon) are given.

(~14GPa), good tensile strength (~1.5Gpa) and high extensibility (~40%) even below 0°C. However, unlike most synthetic filaments, this silk also shows remarkable toughness at temperatures below –60°C as well as tough fracture behaviour even in liquid nitrogen [96]. Even more unusual, the elongation to break decreases with the increasing temperature and reaches a



8.9 (A) Experimental set-up for *in situ* X-ray diffraction during forced silking. The spider is fixed by soft tape and mylar bandages to a metal support. The path of the thread from the spinnerets to the motorised reel is schematically indicated. Distance indications (to the spinneret exit) correspond to points where X-ray diffraction data were recorded. (B) Optical image of draw-down of *N. edulis* spider silk at a drawing speed of 20 mm s^{-1}. (C) Diffraction pattern obtained at 23.5 ± .5°C. Miller's indices are indicated for selected reflections (for details see ref. 87).

minimum around +70°C. Strength and toughness of this silk begins to decrease around +100°C while the failure temperature lies around 370°C [96]. Thus, this benchmark spider silk retains its exceptional mechanical properties over a temperature range from at least –66°C and probably down to liquid nitrogen temperatures and up to about 100°C while breaking up just below 400°C. Clearly, spider silk can teach even the modern synthetic chemist a trick or two.

A quantitative model for silk can be developed [84] by looking at silk from the perspective of a user. The spider uses silk threads to manage mechanical energy (without the silk actually breaking) for different tasks: to store elastic energy in supporting its own weight or for the structural framework of a web, and to absorb kinetic energy to capture flying insects. The mechanisms at a molecular level that dictate energy storage and dissipation in a polymer can be identified and analytical relations can be derived for the full range of possible mechanical properties in silk. Such relations can then be expressed in terms of a small number of energy-based parameters with a direct fundamental link to chemical composition and morphological order [84].

Ideally, thus the key design principles in natural polymers fibres can be elucidated and compared to man-made polymers. Such comparison would offer the potential for the design of greatly improved synthetic biopolymers.

8.6 Applications

During Napoleonic times, in Madagascar and France, silk from the giant *Nephila madagascariensis* golden silk spider was collected and used to spin gloves, for example. This industry was not successful, not least due to the cannibalistic nature of the spiders. But ever since (and probably before, see the Greek story of Arachne) spider silks were seen as an exemplary fibre for many applications that required strength and toughness. Recently, spider fibres are often associated with bullet-proof vests, perhaps in analogy to the spider's insect prey which can be seen as flying protein-bullets. However, the way in which both web and silk deal with the kinetic energy of the insect projectile is not readily transferable to a flak-jacket [7], certainly not if one does not want the bullet to dent the fabric by more than a few inches. After all, the toughness of spider silk depends on its extensibility. Hence artificial spider silks are best envisioned for requirements where stretch is required and where water is not likely to be a problem.

But in addition to toughness spider silks seem to be bio-compatible [6], as well as being decorated for function [75], making them excellent biomedical materials. Accordingly, it seems that today much effort is spent looking for ways to develop spider silks for this application.

8.7 Future trends

Spider silks are bio-polymers with a wide range of interesting mechanical properties [97]. If these silks could be manufactured in quantity and quality and with comparably cheap and environmentally friendly production methods [6] then they could indeed become interesting alternative fibres to low-tech materials such as nylon or cotton (which are cheap, but environmentally costly) or hi-tech materials such as Kevlar or Twaron (which are expensive and environmentally costly). The worldwide production of synthetic fibres exceeds several million tons p.a. and requires an equal amount of fossil carbohydrates; the consumption of energy is not even considered [75]. Although decay of deposited synthetics is slow, in the end the degradation of these fibres will add to the overall balance of the greenhouse gas carbon dioxide. Recombinant spider silk on the other hand can be generated from sustainable resources and could be recycled since they are made of proteins and therefore are fully degradable [98]. Thus, even the replacement of low-tech fibres would be beneficial by lowering carbon dioxide output and saving valuable resources. However, the key to low-tech applications lies in the capabilities

to find cheap and efficient production methods to deliver the huge amount of material that is demanded.

Initially, artificial dragline-type spider silk will probably find use in medicine [99] partly because of the traditionally high return on investment in this field, partly because here spider silks have already a long tradition as *ad hoc* emergency plaster. However, there would also be a potential future in other markets; it is likely that techno-silks in addition to replacing some now traditional man-made fibres might find a use in novel applications. Magnetic silk-fibre composites, for example, can be made by binding colloidal magnetite (Fe_3O_4) nanoparticles to threads of dragline spider silk [100]. Such mineralised fibres retain their high strength and elasticity but can be oriented by an external magnetic field. Finally, artificial silks could find profitable employment in lightweight composites where their toughness and good thermal stability might be rather desirable.

In order to find an efficient way to produce spider silk proteins, a number of researchers and companies have attempted to express the relevant genes in a range of organisms that are relatively easily and cheaply cultured. This included transgenic plants (e.g. potato tubers) and mammals (e.g. goat's milk), which could provide a substantial harvests in agricultural production systems [1–3]. These more 'advanced' and not necessarily more productive systems were used in addition to the more typical fermentation systems where spider silk genes were expressed in microorganisms such as *E. coli* and *Pichia pastoris* [38, 101–103]. Other host systems like MAC-T or BHK mammal cell lines were also used, but due to the high costs these were more of scientific interest [1]. Commercially more interesting could be the use of specialist spider or insect cell lines [104]. Products of sizes up to 150 kDa were successfully expressed in these systems and the applied gene cassette models are capable to extend the product size at wish. However, it remains unclear whether the natural protein size is a requirement for the fibre quality and, aside from molecular size, whether the heterogeneity observed in the sequence repeats might also be important.

It is, of course, very much hoped that one or several of these production systems will be able to supply (some time in the not-too-distant future) sufficient amounts of raw materials to allow spinning silk-like fibres on a commercial scale. However, we must not forget the parallel development of appropriate 'spinning' extruders. Once a good and reliable expression system is up and running then we can test and optimise both the artificial spinning dopes and the spinning methods. Only by tuning both to act in synergy will we be able to manufacture fibres to match the spider's threads and their millions of years of co-evolution of feedstocks and extrusion systems.

8.8 Acknowledgements

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