

Review article

Strength and structure of spiders' silks

Fritz Vollrath^{a,b,*}

^aDepartment of Zoology, South Parks Road, Oxford OX1 3PS, UK

^bDepartment of Zoology, University B135, 8000 Aarhus C, Denmark

Accepted 17 April 2000

Abstract

Spider silks are composite materials with often complex microstructures. They are spun from liquid crystalline dope using a complicated spinning mechanism which gives the animal considerable control. The material properties of finished silk are modified by the effects of water and other solvents, and spiders make use of this to produce fibres with specific qualities. The surprising sophistication of spider silks and spinning technologies makes it imperative for us to understand both material and manufacturing in nature before embarking on the commercialization of biotechnologically modified silk dope. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Spider; Silk; Variability; Platicization; Water; Solvent; Spinning technology

1. Introduction

Before we can biotechnologically mimic or copy silk (or any complex protein structure) we have to understand it. By 'understanding' I mean not only knowing the sequence of protein motifs but also knowing their function. Here, it seems to me, is the gap in our present knowledge. Silk proteins have been patented by several groups — even expressed in goat milk (by Nexia Biotech Ltd, Canada) but no one, to my knowledge, has succeeded in successfully spinning those proteins into

anything resembling the natural fibre neither in its microstructure (which is rather complex) nor in its mechanical properties (which are outstanding by any measure). This discrepancy between sequencing and spinning is due mainly to the fact that it is rather easy with modern technology to identify, manipulate and express protein motifs (Case and Thornton, 1999); it is 'easy' because many people do it for many organisms and purposes and thus are constantly developing new and better techniques. Spinning these proteins into fibres is an altogether different proposition because it involves (actually it relies on) classical production engineers taking a totally new view towards machinery in order to manipulate protein

*Tel. +44-1865-271-234; fax: +44-1865-310-447.

E-mail address: fritz.vollrath@zoo.ox.ac.uk (F. Vollrath).

feedstock which will not perform nor be spun in the present day commercial spinnerets. Thus, silk biotechnology (in the wider sense of the word, i.e. creating a product from a 'gene' sequence) constitutes an unusual challenge. In order to appreciate the extent of this challenge, we must acknowledge the complexity of the material. Since spider silk has better mechanical properties than commercial insect silk, and since spider silk has evolved independently from those silks, we could do worse than use spiders to begin our investigation into the function and form of silk. Since silks as biological materials have evolved over hundreds of millions of years (Shultz, 1987) and have adapted to very particular selection pressures, examining the natural function of a particular silk might reveal interesting features about its structure which is, after all, what we are trying to copy biotechnologically.

Micro-morphological studies on spider dragline silk already show that it differs significantly from the silks of moths (Kaplan et al., 1994; Vollrath et al., 1996; Sirichaisit et al., 1999; Craig et al., 1999). This is not really surprising because the two silks have different biological histories, and detailed analyses of both using the most modern of techniques are beginning to show why, where and how they differ (Lewis, 1999). In the following I shall concentrate on an overview of a few selected spider silks, with very specific features. Most spiders have a whole battery of silks they can employ, each for a particular task (Fig. 1), and I am sure that in due course we will discover many interesting traits in this diversity, but at the moment most research concentrates on only a few silks, trying to understand them mostly on either gene sequence or mechanical terms. In particular I am referring here to the ubiquitous

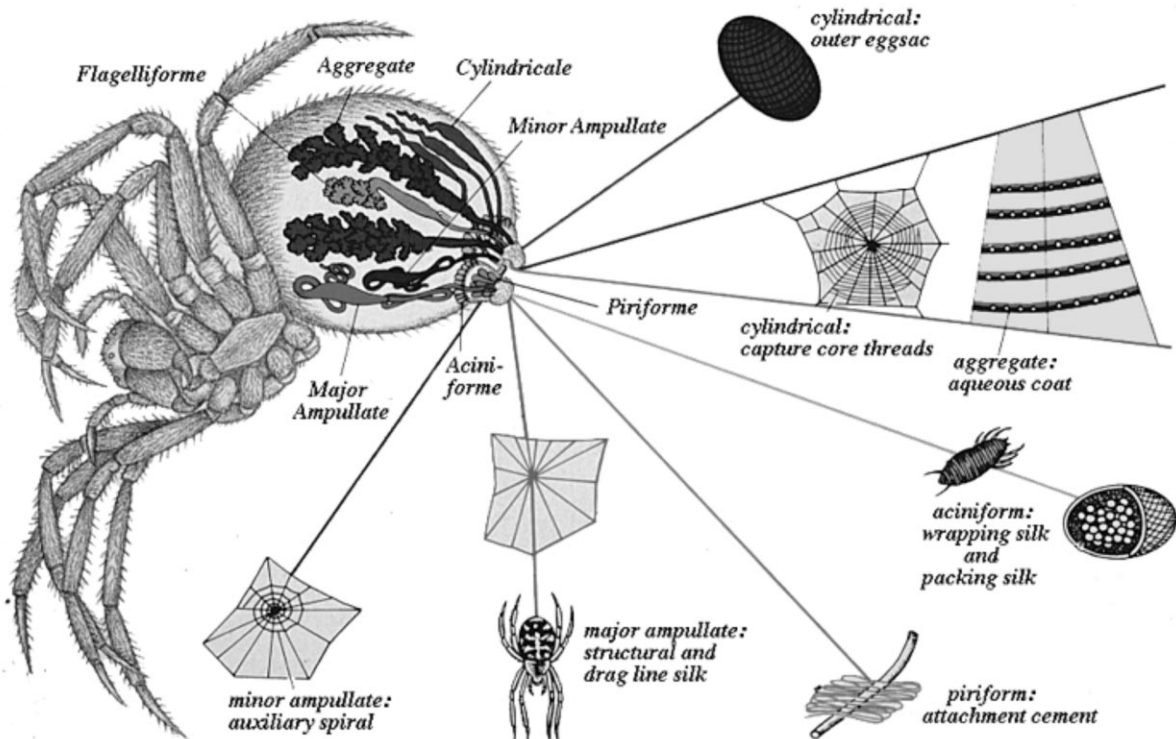


Fig. 1. The silk glands and threads of *Araneus diadematus*. The glands are called by their Latin names, which are again referred when associated with the type of silk they produce (reprinted from Wynne, 1992).

workhorse of spider silk research, the golden silk spider *Nephila* (Kaplan et al., 1994).

Until recently, consensus had it that both spider and insect silks are simple composite materials of protein crystal-inclusions embedded cleanly in protein rubber-matrices (e.g. Gosline et al., 1984, 1986; Dong et al., 1991; Vollrath, 1992; Hinman et al., 1994) but that is now changing and both spider (Vollrath et al., 1996) and silkworm silk (Miller et al., 1999) are thought to contain longish fibrils and other microscopic structures. Personally I am not surprised that an orb spider's main silk turns out to be a material with a rather complex structure; after all, in particular the capture silks of orb spiders are intricate silk 'machines' using either a microwindlass system with glycoprotein glue (e.g. Vollrath and Edmonds, 1989; Vollrath et al., 1990; Vollrath and Tillinghast, 1991) or a hackled band of hundreds of microfibrils (e.g. Peters, 1984; Vollrath, 1994; Opell, 1997). Obviously, spiders can go to great trouble to make silks for specific occasions but the main silks of a spider are web, dragline and capture threads which — we must assume — are specialized to deal with the annihilation of kinetic energy.

Spiders use their complement of different silk types for many different tasks, unlike insects which typically employ silk only for the two tasks of cocoon cover and protective webbing. Accordingly, spider silks have evolved a wide range of different mechanical properties. Information on these silk properties is still quite limited and is restricted mainly to data on a few web and dragline silks (e.g. Denny, 1976, 1980; Griffiths and Salanitri, 1980; Vollrath and Edmonds, 1989; Kaplan et al., 1994). However, already the few silks studied in detail give a fine indication of the high degree of variability (Madsen and Vollrath, 1999; Madsen et al., 1999) and illustrate well the amazing degree of nature's tinkering through clever modifications often subverting phylogenetic design constraints (in the silk's case particularly plasticization by water, see below).

Studies have shown that spider silks are highly variable in both amino acid composition and mechanical properties (Andersen, 1970; Work and Young, 1987; Kaplan, 1990; Lombardi and Ka-

plan, 1990). This can extend to a degree of having variability specifically build into the genome (Vollrath, 1999a,b) as recently demonstrated for capture silk core fibres (Hayashi and Lewis, 2000). It is clear that both external and internal conditions of the spider affect silk production and thus ultimately the mechanical properties of the finished thread. An argument can be made that silk is optimized for a wide range of conditions and that rapid temporal adaptation to the environment is an advantage (Vollrath, 1999a,b). After all, the performance of an orb web — which is designed by evolution to take out-of-plane load in maximum deflection — is greatly enhanced by incorporating into one web the mechanical properties of different types of silk (Lin et al., 1995) as well as different properties of the same silk type tailored to the specific conditions (Madsen et al., 1999). Some spiders, like the garden cross spider *Araneus diadematus* have solved the problem of absorbing the high kinetic energy of the insect's impact rather elegantly. This includes the evolutionary invention of a windlass system powered by the surface tension of water (Vollrath and Edmonds, 1989) as well as using aerodynamic damping by ambient air (Lin et al., 1995).

2. Function and form of three important spider silks

Some spider webs are protective housing, others are trap/signal-lines to monitor walking prey (Foelix, 1996). The most interesting 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 with a combination of high and low tensile silks as building materials (Witt et al., 1968). A typical orb web consists of a frame of silk supporting a wheel of silken spokes radiating from a hub; these radii in turn support the sticky spiral that entangles the insect. Soft spiral and tough radii work together to absorb the insect's impact and hold it aloft. Orb web builders have invented two very different ways of doing this, and thus fall into two groups classified by the type of capture spiral stickiness (Foelix, 1996). Cribellate spiders use

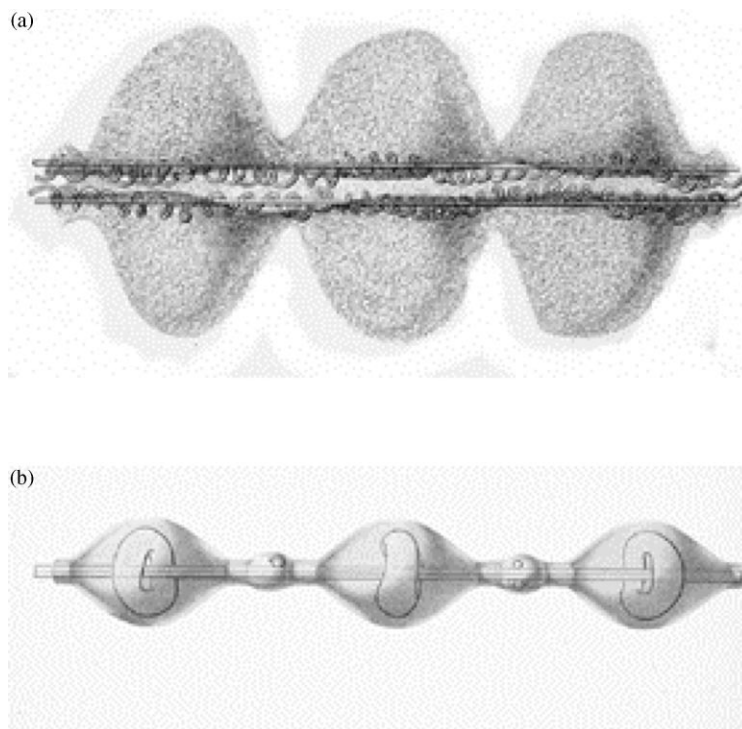


Fig. 2. The two forms of sticky capture silk. Cribellate silk (top) is dry and has strong axial core threads, the crimped spring threads and the fine hackled capture threads. Ecribellate silk (bottom) is wet and has plasticized and thus soft core threads, is surrounded by an aqueous coat and has sticky glycoprotein nodules. Reprinted with permission from F. Vollrath, 'General Properties of Spider Silk', pp. 17–28 in ACS Books Title: Silk Polymers: Materials Science and Biotechnology (eds.: D. Kaplan, W. Adams, C. Viney, B. Farmer), ACS Symposium Series No. S44, Washington (1994). Copyright (1994) American Chemical Society.

very fine silk of nanometer diameter which is combed in hackled bands of many threads onto supporting axial threads (Fig. 2a). These hackled bands provide elasticity as well as adhesion presumably by electrostatic forces. Ecribellate spiders (such the garden cross spider *Araneus diadematus* or the golden silk spider *Nephila clavipes*) lack cribellum silk. Instead they coat the axial threads with an aqueous solution that forms sticky droplets (Fig. 2b). Coat and droplets are crucial for the function of these capture threads.

In the well known orb webs of our garden spider *Araneus diadematus*, architecture and material have co-evolved to form a highly efficient structure assembled from only a few components. Essential for the effectiveness of such a web are the rather different mechanical properties for the two main silks used for construction: stiffness in the radials and elasticity in the circumferential

capture spiral. The difference is in kind not in degree, and it is due to a simple trick (illuminating nature's way of creative tinkering) whereby an inherent functional weakness is turned to advantage. The silk we know and appreciate in everyday life is stiff and strong, qualities which disappear when the material is waterlogged and becomes plasticized (Work, 1985; Vollrath and Edmonds, 1989; Bonthron et al., 1992). In her web, the cross spider coats the naturally dry capture threads with a thin layer of water and glue to do just that (Edmonds and Vollrath, 1992).

The dry and very tough radius threads of *Araneus diadematus* show good extensibility (approx. 40%), high tensile strength (approx. 1200 MPa) and large hysteresis (approx. 50%) which indicates that they function as shock absorbers as well as structural elements (Köhler and Vollrath, 1995) (Fig. 3a, Fig. 4). The upper half of this kind

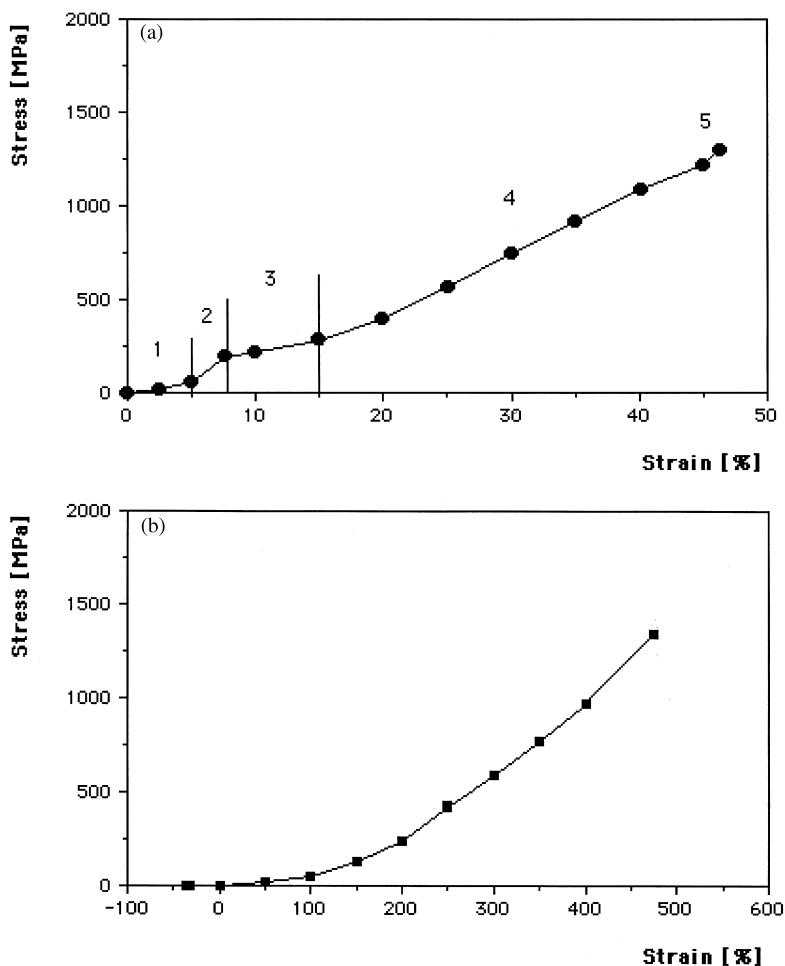


Fig. 3. Typical stress strain curves of *Araneus diadematus* web silks. (a) The dry silk of the major ampullate glands which make the radii (web spokes) as well as the safety dragline; (b) the wet silk of the flagelliform and aggregate glands which together form the capture spiral. The curve for dragline silk can be subdivided (Köhler and Vollrath, 1995) into five parts denoted by significant changes in the Young's modulus (i.e. different modes of energy absorption by the molecular or the supramolecular structure); the last part (5), for example, is thought to mark the formation of energy absorbing cracks between the canaliculi (Shao et al., 1999b).

of orb web typically has fewer radii than the lower half because the spider can run down faster than up making the lower half more valuable for prey capture where speed matters (Rhisiart and Vollrath, 1994). Threads have similar pre-tension although pre-tensions in the upper half of the web show greater variation (Wirth and Barth, 1992; Köhler and Vollrath, 1995).

The two major threads used in a spider web have very different mechanical properties (Fig. 3a,b). The wet and soft sticky spiral of the same *Araneus diadematus* absorbs energy by large ex-

tendibility (approx. 500%) of the plasticized thread which develops substantial force only after 100–200% extension with the thread breaking suddenly at approximately 400–500% extension (Vollrath and Edmonds, 1989; Köhler and Vollrath, 1995) (Fig. 3b). The engineering strength of these fibres of $1338 \pm 80 \text{ MN/m}^2$ with a breaking energy of 163 J/cm^3 ($N = 6$) is comparable with that of the radial threads of $1153 \pm 144 \text{ MN/m}^2$ with a breaking energy of 194 J/cm^3 (both $N = 6$, Köhler and Vollrath, unpublished data) (Fig. 3a). However, since the core fibres of the capture

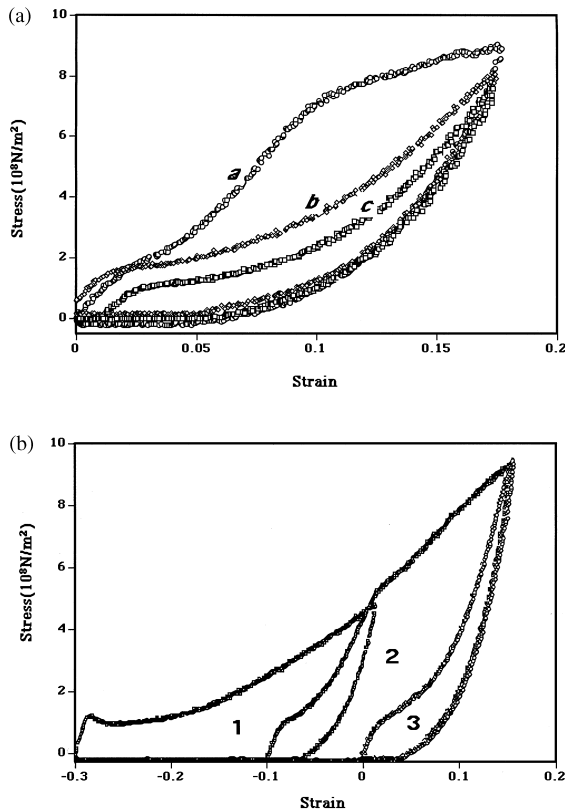


Fig. 4. The mechanical properties of *Nephila* silk is affected by plasticization in water (Shao and Vollrath, 1999). (a) Multiple loading–unloading cycles of *Nephila edulis* silk: (a) for normal (virgin) silk; (b) for pre-stretched silk recovered to its initial length by soaking in water for 2 min and drying in air for 20 min; (c) for pre-stretched silk recovered only in air for 20 min. (b) Loading–unloading cycles of 30% supercontracted silk submerged in water; between each of the three cycles the thread was allowed to record for 10 min.

threads are much thinner, the force required to snap them is obviously much lower. *Araneus* like other orb weavers of ecribellate families employs the windlass mechanism which allows the threads to absorb the high kinetic energy of the prey without breaking (Vollrath and Edmonds, 1989; Edmonds and Vollrath, 1992). This intriguing mini-mechanism relies on water both as plasticizer of the core fibres and to provide surface tension for the windlasses; these spiders have evolved an aqueous coating, supplied and maintained by hygroscopic compounds (Tillinghast et

al., 1991; Vollrath and Tillinghast, 1991) that attract the necessary water from the atmosphere (Edmonds and Vollrath, 1992). Warburton had shown in 1890 that the ecribellate sticky spiral thread is covered with a viscid coat which turned out to be 80% water (Tillinghast and Townley, 1987) containing adhesive glycoproteins (Tillinghast and Sinohara, 1984). More recently Vollrath and Edmonds (1989) have shown how these aqueous droplets, which hang on the spiral thread like pearls on a string, act like tiny windlasses, collecting the loose core fibres inside (Vollrath and Edmonds, 1989). For this reason the threads do not sag even after strong plastic deformation. Moreover, it is largely the water in the coating that is responsible for the high plasticity of these threads (Work, 1977, 1984; Gosline et al., 1984; Bontrone et al., 1992). Plasticizing with water softens the core silks and the aqueous coating provides additional elasticity with its surface tension, called upon during rapid strain cycles (Vollrath and Edmonds, 1989; Edmonds and Vollrath, 1992). The stickiness of this windlass capture silk depends on the glycoproteins which stick like syrup due to their sugars (Vollrath and Tillinghast, 1991). Adhesiveness is as important as the silk's 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. In the ecribellate orb weavers, the capture glue consists of microscopic glycoprotein toruses (Fig. 2b) straddling the capture threads beneath the droplets in the aqueous coat (Vollrath and Tillinghast, 1991; Vollrath, 1994). The toruses make up between 5 and 10% of the aqueous coat which accords with the proportion of glycoprotein in the aqueous web wash (Tillinghast, 1981). Reaction with specific lectins indicates that the toruses in the webs of *Araneus* and *Argiope* contain *N*-acetylgalactosamine (Vollrath and Tillinghast, 1991). A study of the adhesion of the *Araneus* capture threads indicates that the toruses alone are responsible for a web's stickiness (Vollrath and Tillinghast, 1991). Separation of the glycoproteins from the initially uniform viscous coat coincides with the separation of the coat into droplets. The speed in which the droplets form

depends on the ambient humidity: in very dry conditions (9% RH) droplet and nodule formation could require several hours, in more natural conditions (45% RH) it requires only minutes. Webs built under conditions of unusually low (10%) or high (90%) humidity have atypical glycoprotein nodules (Vollrath, 1994), even if the webs had subsequently been exposed to several hours of a more natural ambient humidity (e.g. 45% RH) suggesting that water conditions during formation are crucial for the glycoprotein disassociation.

I shall return to discuss the mechanism of the role of water in cribellate capture and structural threads in some more detail, after a brief introduction of a very different way of being sticky, because to my mind it highlights a number of important constraints of spider silk. These evolutionary design constraints of natural silks are likely to be important for the approaches towards the biotechnological design of artificial silks.

Cribellate spiders have solved the problem of highly extensible and sticky capture threads using a method altogether different from that of the ecribellate spiders such as *Araneus* or *Nephila* (Lehmensick and Kullmann, 1956; Friedrich and Langer, 1969; Peters, 1984; Lubin, 1986; Eberhard and Pereira, 1993). The spiral core fibres of cribellate spiders such as *Uloborus* or *Deinopis* (Fig. 2) are not covered by an aqueous coat but instead carry crimped spiralling threads that are further surrounded by puffs of exceedingly fine silk (fibre diameter approx. 20 nm diameter) (Peters, 1987; Opell, 1994; Vollrath, 1994). The cribellum, a plate in front of the anterior spinnerets, is covered by numerous, very fine spigots, each connected to a tiny gland of its own. The fibres of the puffs are brushed out from the cribellum by the calamistrum, a highly specialized comb on the metatarsus of each fourth leg (Opell, 1982; Peters, 1984; Eberhard, 1988). The hackled band of *Uloborus walckenaerius* has shorter over-

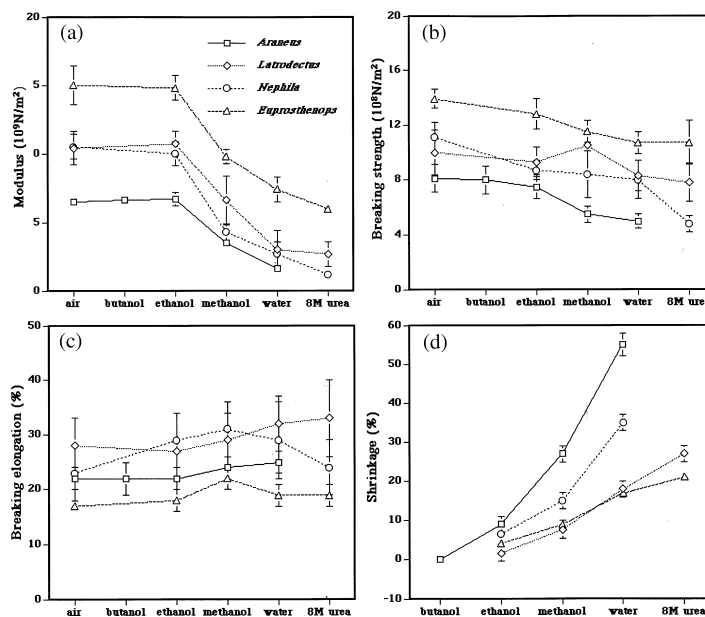


Fig. 5. The effects of solvents with different polarities on the mechanical properties of four different species of dragline silks (Shao and Vollrath, 1999). The silks were taken from four species of spider belonging to four rather unrelated families. Threads were reeled under controlled conditions and submerged in the solvent before being air dried and measured in our custom tensile tester. Shown are for each of the silks and conditions the mechanical properties: (a) Young's modulus E ; (b) breaking strength; (c) breaking elongation and (d) shrinkage. Shrinkage denotes the contraction of the native silk in the solvent. Note that the different silks have on the whole already very different properties in the native state (i.e. in air).

all extendibility (125%) than the *Araneus* capture silk and it seems to absorb energy by friction of the fine hackled fibres, many of which needs 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 by the close contact between the superfine thread and the prey's surface (Eberhard, 1980; Opell, 1993, 1995; Vollrath, 1994).

3. The effect of water on silk

As pointed out above, ecribellate orb weavers like the garden spider *Araneus* or the golden silk spider *Nephila* coat their capture threads with an aqueous solution that forms sticky droplets (Fig. 2b). Coat and droplets are crucial for the function of these capture threads. The high water content of the coat (80%) plasticizes the core threads and renders them elastic and the droplets rely on surface tension to keep the threads tight by reeling in the axial threads when slackened and letting them out when stretched. Chemical analysis of the coat shows high concentrations of five water-soluble organic compounds and low concentrations of inorganic salts as well as at least one glycoprotein. Water is important not only for these fibres, but for many other types of thread as well, and it is becoming clear that the role of water and other solvents for understanding and manipulating the mechanical properties of spider silk cannot be overstated (Fig. 4a, Fig. 5) especially if we aim to produce bioengineered silks with specific properties.

Although there are some basic — and probably important — differences in the molecular make-up of the core threads of these wet capture threads when compared to dry radial silks (Guerette et al., 1996; Hayashi and Lewis, 2000), their extraordinary elasticity depends largely on the water film in and between the droplets on its silk fibres (Vollrath and Edmonds, 1989; Bonthron et al., 1992; Edmonds and Vollrath, 1992). This aqueous solution is a strong 5 M solution of compounds that are unrelated to the usual silk amino acids. These substances are close relatives of neurotran-

smitters being either derivatives or hydrolysates, i.e. GABamide, *N*-acetyltaurine, choline, betaine and isethionic acid (Vollrath et al., 1990). The web is coated in lower concentrations with cysteic acid (2%), lysine (2%), serine (2%), potassium nitrate (7%) and potassium dihydrogenphosphate (3%) (Fischer and Brander, 1960; Schildknecht et al., 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). Betaine and GABA are osmoprotectives and osmolytes in a wide range of organisms whereas taurin is a protein-stabilizing compound. Such organic compounds which act osmotically while allowing for normal protein function are necessary to maintain the capture silk's aqueous coat while keeping the silk proteins in favourable conformations. They allow the formation and stabilization of the droplets crucial for the function of the capture spiral. Furthermore, they may act as fungicides or bacteriocides and may even affect the nervous system of the prey (Vollrath et al., 1990).

The sticky droplets originate when the mixture of these compounds is applied to the core thread; each core thread is paid out from its own spigot in a triad with two spigots adding the coating compounds (Foelix, 1996). The two core threads (doubled because they originate from bilateral legs) and their viscous coats merge and begin to attract water, swells, becomes unstable and separates into drops that continue to swell further (Edmonds and Vollrath, 1992). Droplet formation follows as a consequence of the surface tension of thin films and the distinct droplets are connected by a fine layer of liquid (Edmonds and Vollrath, 1992). The rate of swelling and the subsequent droplet size is dominated by a dynamic equilibrium between water in the glue and water vapour in the air. Along with the droplets, the glycoprotein toruses form within minutes after the sticky silk has been laid down. *Araneus*, like all ecribellate orb weavers, confines sticky silk to the capture spiral. Distantly related ecribellate spiders like the black widow *Latrodectus mactans* or the social *Anelosimus eximius* (Vollrath and Rohde-Arndt, 1983) use glue to immobilize by throwing it over the struggling prey before administering a

bite. Clearly, purposely wetted silks are important for a range of spiders and modes of action. However, practical when used judiciously, water can be a serious problem for spider silks, and studying the role of water — or other hydrogen bond breakers, will eventually lead to some important insights into this fascinating material which is as tough as Kevlar yet produced by the spider under ambient temperatures, only slightly raised pressures and water as production solvent.

Many, although not all, silks contract when submersed in water; the dragline threads of *Nephila* or *Araneus* are good examples (Work, 1981). Urea is a stronger breaker of hydrogen bonds than water and accordingly has an even stronger effect (Fig. 5). Sections of *Nephila* dragline silk submersed in urea undergo a brief period of violent rotations before super-contracting (Vollrath et al., 1996), and *Araneus* threads dissolve in 8 M urea (Shao and Vollrath, 1999). Silk treated in this way not only contracts to a fraction of its length but also swells to many times its diameter. Moreover, it shows structures that are not visible in the native state and there is strong evidence that these structures are already present in the native state albeit hidden (Vollrath et al., 1996). Silk ends — in the native state in air — observed under an X-ray microscope at 10000 × magnification showed a tube, not a rod (F. Vollrath, unpublished observations). *Nephila* silk shows surface and marginally subsurface structures indicative of fine fibrils (Li et al., 1994; Mahoney et al., 1994; Thiel et al., 1994a,b; Vollrath et al., 1996). Electron microscopy suggests an obliquely textured thread (Thiel et al., 1994a,b) or even a fibril wall surrounding a fibrilless core (Mahoney et al., 1994). Atomic force microscopy (AFM) of untreated fibre surfaces shows microfibrils (possibly covered by a thin coat and apparently in places ‘interwoven’) oblique to the thread axis (Mahoney et al., 1994). AFM analysis of silk cross-sections suggests a trinity of coat, tube and core (Li et al., 1994). A skin and core structure was already observed by Work (1984) in *Araneus diadematus* for silk still in the glandular ducts as, in one case, was an apparently helical structure in this unextruded silk. Finally, the coiling and swelling behaviour that we found to accompany

supercontraction would be explained by fibril bands wound helically into a tube with a ‘skin’ enclosing a less dense and more hygroscopic core ‘body’. A microfibril skin would impart the advantages of a multiple stranded thread, which always has greater tensile strength than a single stranded fibre of the same material diameter simply since it is better at resisting the propagation of cracks (Chou, 1992).

Major ampullate silk fibres from the orb weaving spider *Nephila madagascariensis* under transmission electron microscopy show further structural elements (Frische et al., 1997). There is a thin envelope (maybe lipids) surrounding an outer layer and a column of apparently homogeneous material which contain elongated cavities oriented parallel to the silk fibre axis. The cavities appear similar to ‘elongate vacuolar droplets’ observed in the silk of some moth larva (Akai et al., 1993). The overall skin-core structure is the result of a rheological pattern originating in the two secreting regions recognized in *Nephila* silk glands; the cavities indicate material inhomogeneities (Knight and Vollrath, 1999a,b). These structures resemble small, filled rifts (called canaliculi) being extremely fine and elongated in line with the fibre axis. Stressing a fibre until fracture may lead to cracks forming between canaliculi (Shao et al., 1999b), 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.

Whatever the details of their fine structure, a wide range of solvents (water and urea are the most prominent) consistently modify the material properties of many dragline silks (Shao and Vollrath, 1999; Shao et al., 1999c) (Fig. 4a,b; Fig. 5). Raman spectra of single silk fibres allow the study of silk ultrastructure immediately before and after solvent action (Shao et al., 1999a). Solvents with different polarities affect different regions of the silk’s composite microstructure by modifying the conformation of the different molecular chains. A comparison shows clear native as well as solvent induced differences in a wide range of material properties in the major ampullate dragline silks in four spiders representing differ-

ent families *Araneus diadematus* (Araneid orb-weavers), *Nephila edulis* (Tetragnathid orb-weavers), *Latrodectus mactans* (Theridiid tangle web-weavers) and *Euprosthenoops* sp. (Pisaurid nursery web-weavers). Raman spectra allow the assignment of conformational sensitive regions. Compared to the silk of the silkworm *Bombyx mori* all spider silks have less β -sheet and more helix material (Shao et al., 1999a; Sirichaisit et al., 1999). Polarized spectra suggest that the molecular chains of the spider silk are aligned parallel to the axis of the fibre. The differences in the mechanical properties between native and supercontracted silks can be attributed to variations in β -sheet content. The mechanism of contraction of spider silks in solvents correlates well with conformational changes in the supermolecular structure.

Raman spectroscopy is an important tool for the study of the deformation of polymeric fibres and with modern techniques even fine single spider threads can be studied (Young et al., 1991). In *Nephila edulis* silk bands at 1095 and 1230/cm are the most prominent in the spectrum and can be used to follow molecular deformation during stretching (Sirichaisit et al., 1999). Although at present we cannot be sure, it is most likely that the 1085 band can be assigned to a disordered configuration of the $\nu(\text{CC})$ skeletal band (by analogy to this band in polypeptide chains; Yeh and Young, 1998). The band located at 1230/cm is thought to correspond to a band at *Bombyx mori* silk denoting a disordered configuration (Edwards and Farwell, 1995) and to a band in synthetic polypeptides denoting a beta-configuration (Xue, 1994). In spider silk both bands shift with a very good positive and linear correlation when the silk is stressed and strained (Sirichaisit et al., 1999). This corresponds with *Bombyx* silk where the correlation is not only weaker but also non-linear (Sirichaisit et al., 1999). The linear dependence during stretching can be interpreted as the silk's microstructure being subjected to an overall and uniform stress during deformation (Sirichaisit et al., 1999). Thus, behaviour under tension can reveal interesting information on structure (Grubb and Jelinski, 1997; Valluzzi et al., 1999).

Mechanical properties are affected by the action of chemicals and also by the conditions of manufacture. That both affect a material is not surprising but they can be used to good effect when studying specific aspects of it. For example, when analysing the stress–strain characteristics of *Nephila* dragline silk produced under highly controlled conditions, we found that both diameter and all mechanical properties were affected significantly (Madsen and Vollrath, 2000) (Fig. 6). The degree of spider control over its silk production system can be shown elegantly by on-line X-ray diffraction patterns of single threads as they are being spun (Riekel et al., 1999). In this way, using state-of-the-art micro-X-ray diffraction, molecular structure can be recorded for a wide and natural range of silking speeds and/or spider body temperatures. Normal (approx. 2 cm/s) and fast (approx. 80 cm/s) reeling speeds show that the rate of silk production has an influence on silk properties, as does the temperature of the spider (Riekel et al., in preparation). A sample from a silk specimen stored for approximately 1 month shows the same features as the fresh fibre with an additional meridional reflection. Unnaturally slow speeds of reeling (approx. 0.2–0.9 cm/s) show silks to be remarkably similar with a semicrystalline morphology which is characterized by Bragg reflections, an oriented amorphous halo and a diffuse halo (Riekel et al., 1999). These patterns agree with the model of a small crystalline block in a matrix containing both oriented and unoriented amorphous material (Simmons et al., 1996; Grubb and Jelinski, 1997) with the crystalline fraction showing the pattern of the b-poly(L-alanine) structure (Riekel et al., 1999).

The secondary structures of the alanine-rich and glycine-rich segments of spider dragline silk can be further differentiated using nuclear magnetic resonance (NMR) experiments (McNamee et al., 1994; Simmons et al., 1994, 1996; Hijirida et al., 1996). The best analysis of NMR data so far — using proton-driven ^{13}C 2D — suggests a structural model for spider dragline where the poly-Ala segments adopt a highly ordered β -sheet structure and the glycine-rich segments form

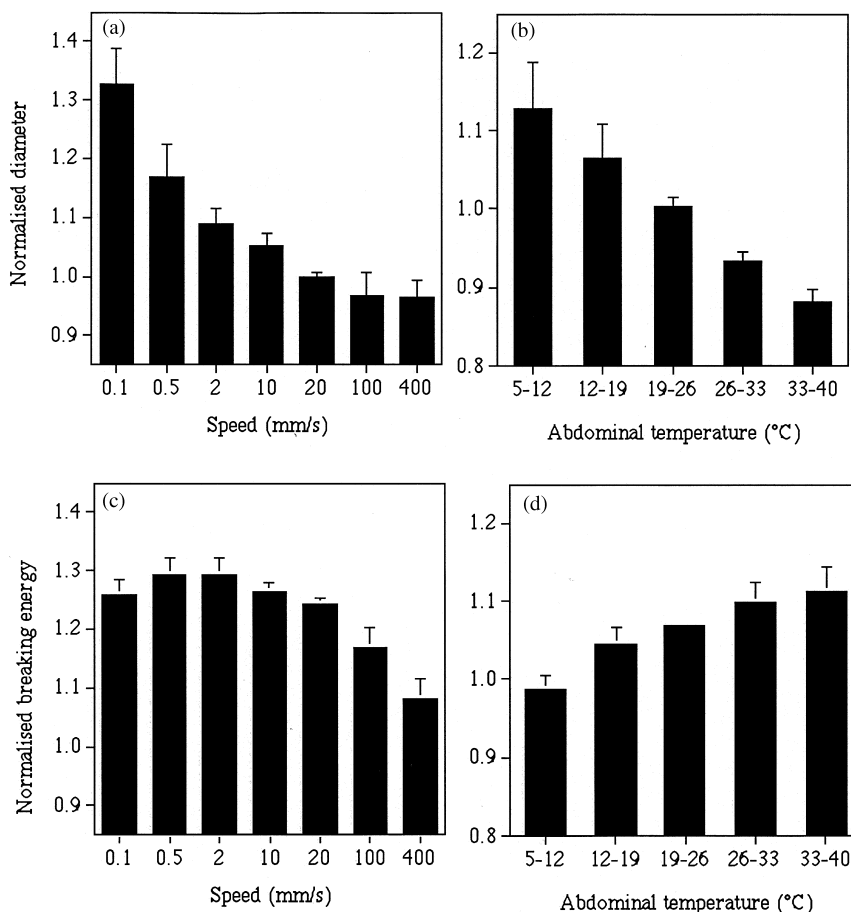


Fig. 6. The effect of production conditions on the diameter and mechanical properties of *Nephila* dragline (Vollrath and Madsen, submitted). The live and fully awake spider was affixed firmly to a platform that could be cooled or heated by a liquid pumped through it from a control unit; and the silk was reeled out using a little motor with variable speeds. Since spiders showed individual differences in diameters and properties, the measured silk data were normalized for each spider under the control conditions. The temperature was 25°C for the reeling experiments; and reeling speed was 20 mm/s for the temperature experiments. For each condition, the column height shows the average taken from several spiders (N), with $n = 4$ measurements per spider (temperature: $N = 7, 8, 10, 5, 5$; speed: $N = 4, 4, 5, 8, 11, 10, 5$). The vertical bars are 95% C.I.

3(1)-helical structures (Kümmerlen et al., 1996; Beek et al., 1999).

Studies like these that aim to integrate structural and mechanical data are important because a full understanding of the macroscopic properties of spider silk demands a deep knowledge of the secondary structures of the different domains (Hayashi et al., 1999) in the protein as well as of the higher order structures, i.e. the domain sizes of the β -sheet structures and the packing of the helices. Finally, the final explanation requires also knowledge of higher order structures and the

microscopic fine structures discussed earlier. In short, mapping protein and peptide sequences of specific silks (e.g. Kaplan and Lombardi, 1990; Hinman and Lewis, 1992; Mello et al., 1994; Hayashi and Lewis, 2000) and modelling their interactions in the thread (e.g. Termonia, 1994) are a first step but eventually we have to test specific hypotheses of their interaction, and here we should be concerned particularly with the mechanical properties of the thread. Since thread properties can be highly variable, even for the same thread of the same spider (Madsen et al.,

1999), unravelling the cause of this diversity could open an interesting and important window into the folding of those proteins and microstructures that are responsible for silk strength.

The stress–strain characteristics of dragline silks show large inter- and intraspecific differences between spiders in different families (Madsen et al., 1999). Moreover, there can be a large daily variability in silk from individuals of the same species; and spider condition can (and frequently does) affect silk properties, starvation for example can lead to decreased breaking elongation (Madsen et al., 1999). Speed of silk production also effects silk properties such that with increasing speed: (i) breaking elongation decreases; (ii) breaking stress increases; and (iii) Young's modulus increases (Madsen et al., 1999). Finally, the spider's body temperature during silk production plays also an important role. Since spiders are ectotherms, this means that an individual can to some extent modify silk parameters by adjusting the time of building and the speed of running. However, the spider also seems to be able to modify silk parameters by direct nervous control (Madsen and Vollrath, 2000) as well as indirectly by its diet (Madsen et al., 1999; Vollrath, 1999a,b).

4. Spinning

The mechanisms by which the proteins fold and assemble in the spider's silk press are unknown. However, evidence is accumulating on the form and function of the silk pathway in the major ampullate dragline silk of the golden silk spider *Nephila edulis*. Silk is converted from the liquid feedstock in the gland into a solid thread via a tapering tubular duct which exits at the spigot (Wilson, 1969). 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 (Vollrath and Knight, 1999). The cuticle that lines the gland's duct has the structure of an advanced hollow fibre dialysis membrane and is thought to facilitate a rapid removal of water and change in ionic composition involved in the spinning process (Vollrath et al., 1998). It seems that

the thread is formed by the elongational flow of the highly oriented liquid crystalline feedstock through a hyperbolic extrusion die (Knight and Vollrath, 1999a,b). Bi-refringence patterns and silk droplet inclusions show that elongational flow assists in defining molecular orientations throughout the duct and that a combination of solvent (i.e. water) extrusion and subsequent acidification helps the process of alignment and folding. Thus the spider uses a liquid crystalline spinning process which — in terms of human engineering — is highly advanced and it is clear that details of this process are crucial in determining the mechanical qualities of the silk (Vollrath and Knight, in preparation).

The spinning pathway of the major ampullate silk in *Nephila edulis* is a technologically highly advanced silk production system (Vollrath et al., 1998; Knight and Vollrath, 1999a,b; Vollrath and Knight, 1999; Knight and Vollrath, in preparation). The gland consists of a sac or ampulla with an apical 'tail' which produces some part of the silk feedstock and a funnel that leads to the duct. The secretory portion of the gland ampulla is divided into two transverse zones, A and B which produce different silk proteins. These secretions are then passed through a 'funnel' into a long duct. The duct is folded back on itself in an elongated 'S' to give three limbs (first, second and third) which progressively narrow to form a hyperbolic die. The duct terminates in a structure often termed the 'valve' (Wilson, 1962a,b, 1969) because it was thought to squeeze the silk and provide shear stress. 'Ratchet' might be a better term for this structure because it does not act like a valve but rather as a mechanism to advance thread especially silk broken inside the duct (Vollrath et al., 1998). Finally, before exiting the spider the thread is stripped of its bathing and coating liquid by a tight cuticular lip at the spigot (Vollrath et al., 1998).

The dragline thread is drawn from a hyperbolic die using a previously oriented lyotropic liquid crystalline feedstock (Vollrath et al., 1998; Knight and Vollrath, 1999a; Knight and Vollrath, in preparation) which is produced and stored in at least two different cell types. Of these, one, the coat produces outer proteins that presumably

form the coat, is stored tightly packed already in liquid crystalline form in hexagonal columns (Knight and Vollrath, 1999b). Studies using polarizing microscopy have shown that in the early part of the production pathway (i.e. within the ampulla of the gland) the feedstock flows in a curved pattern that suggests elongational flow orientation through a divergent/convergent die (Vollrath and Knight, 1999). Later in the pathway (i.e. in the S-shaped duct) one can see a remarkable double helical arrangement suggesting a flow elongated cholesteric liquid crystalline phase. 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 thread is drawn (Vollrath and Knight, 1999). Congo red-staining experiments suggest that the combination of wall shear and elongational flow induces the β -sheet transitions in the proteins as they travel through the duct (Knight et al., 2000).

Dragline silk is produced by the draw down of a pre-oriented liquid crystalline feedstock. Pre-orientation reduces the viscosity of the feedstock and thus the energy required to produce an axial alignment of the molecules within the draw-down taper. The cuticle that lines the gland's duct has the structure of an advanced hollow fibre dialysis membrane which facilitates the rapid removal of water. This part of the duct is thus also specialized for ion transport and the management of the pH inside the lumen (Vollrath et al., 1998). The chemical treatment within the secretory pathway combined with the elongational flow of liquid crystalline feedstock assists the optimal folding of long chain silk proteins (Cappello and McGrath, 1994; Valluzzi et al., 1999). In the spider, as in engineering, liquid crystallinity and flow elongation must be taken to contribute to the extreme toughness and strength of the remarkable properties of spider silk (Knight and Vollrath, 1999a, in preparation).

5. Outlook

I have demonstrated that spider silks are semicrystalline biopolymers with extraordinary

mechanical properties which have evolved into a wide range of forms and functions. Because of the potential of high strength and superb toughness it is likely that dragline-like techno-silks will sooner or later find applications in impact- and tear-proof textiles or other structural fabrics where strong, flexible materials are desirable (Heslot, 1998). If we can follow the spider's lead and can manufacture these silks with comparably cheap and environmentally friendly production methods then these silks might quickly replace some present-day low-tech materials such as nylon or cotton (which are cheap but environmentally costly) or even hi-tech materials such as Kevlar or Twaron (which are expensive and environmentally-costly). Techno-silks might benefit from the fact that environmental concerns are growing and that the market is already primed and waiting for artificial spider's silks.

I predict that artificial dragline silk will first appear in medicine: 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 plasters (Newman and Newman, 1995). In future, silk threads, pads or films presumably will have other functions such as artificial skin, biodegradable suture material or even carriers for delayed release drugs. The silk's droplet inclusions, for example, could be filled with compounds that in addition to imparting strength would be released to advance healing as the thread dissolves. In a controlled study to study tolerance we have already implanted spider silks and silk derivatives subcutaneously in pigs (Barth et al., in preparation). As natural silks we used major ampullate dragline silk reeled from the golden silk spider *Nephila clavipes* and web silk of the tarantula *Brachypelma* sp. Within a 14-day period all implantation sites healed well with only a few inflammatory reactions showing lympho-plasmacellular infiltrations, phagocytosis and granulation tissue. Experiments such as these bode well for the future of techno-silks in medicine.

However, as outlined above, there is also a potential future in markets other than pharmaceuticals. I am confident that, in addition to replacing some now traditional man-made fibres,

techno-silks might find a use in novel applications. It is likely that in the foreseeable future we will be able to design silk proteins from scratch and thus make fibres to order assuming we fully understand the form–function relationship. First experiments on natural dragline silk that has been modified suggest that its desirable mechanical properties can indeed be maintained while at the same time adding totally new properties. Magnetic silk-fibre composites, for example, can be made by binding colloidal magnetite (Fe_3O_4) nanoparticles to threads of dragline spider silk (Maynes et al., 1998). Such mineralized fibres retain their high strength and elasticity but can be oriented by an external magnetic field. Elemental mapping of sectioned fibres showed a dense, coherent surface coating of the iron oxide which is partially retained after washing and sonication. Functionalized silk-fibre composites can be obtained by similar procedures using conductive (metallic Au) or semiconductive (CdS) nanocolloidal precursors (Maynes et al., 1998). This study (like others, e.g. Sun et al., 1997a,b) show that organic/inorganic silk-fibre composites can be produced to order. Such composites might have a future in microelectronics and fibre optics or as ‘smart’ structural fabrics with anti-static properties. Electrostatic properties may also lead to a first market for the more complex mini-machine silks of the capture thread type, be they of the droplet or woolly kind, and they might find employment in active filters which, after all, is an area where orb webs already excel (Samu et al., 1992; Vollrath and Edmonds, unpublished).

Finally, research into silk and spinning might teach us more than just how to make threads. The details of the spinning process are crucial in determining the mechanical qualities of the silk. In silk, the mechanics of protein folding can be studied using experimental perturbation during the spinning phase, and the final end product can easily and quickly be tested for its design properties. Thus, artificial even more than natural silk has the potential to become not only a valuable commercial resource but also a benchmark material to study the principles of folding in fibrous proteins.

Acknowledgements

The research in my group has previously been funded by the British Science and Engineering Research Council, the Volkswagenfoundation, the US-Army and the Swiss National Fonds; and it is presently funded by the Danish Statens Naturvidenskabelige Fonds, the European Synchrotron Research Facilities, the British Biology and Biotechnology as well as its Engineering and Physical Science Research Councils and, finally, the European Science Foundation through its generous Silk Network. Obviously, this is the work of a great many collaborators whom I thank gratefully. Since this review is supposed to be a review of research in which I was personally involved; I regret that I have had to omit and leave unacknowledged many contributions more important than mine to the field.

References

- Akai, H., Nagashima, T., Aoyagi, S., 1993. Ultrastructure of posterior silk gland-cells and liquid silk in Indian tasar silkworm, *antheraea-myliitta drury* (lepidoptera, saturniidae). *Int. J. Insect Morphol. Embryol.* 22, 497–506.
- Andersen, S.O., 1970. Amino acid composition of spider silks. *Comp. Biochem. Physiol.* 35, 705–711.
- Barth, F., Basedow, A., List, H., Vollrath, F. Local tolerance to spider silks after subcutaneous implantation in pigs. (in preparation).
- Beek, J.V., Kümmerlen, D., Vollrath, F., Meier, B.H., 1999. Solid state NMR on supercontracted spider dragline silk. *Int. J. Biol. Macromol.* 24, 173–178.
- Bonthrone, K.M., Vollrath, F., Hunter, B.K., Sanders, J.K.M., 1992. The elasticity of spider’s webs is due to water-induced mobility at a molecular level. *Proc. R. Soc. Lond.* 248, 141–144.
- Cappello, J., McGrath, K.P., 1994. Spinning of protein polymer fibers. In: Kaplan, D., Adams, W.W. et al. (Eds.), *Silk Polymers. Materials Science and Biotechnology*. American Chemical Society, Washington, pp. 311–327.
- Case, S.T., Thornton, J.R., 1999. High molecular mass complexes of aquatic silk proteins. *Int. J. Biol. Macromol.* 24, 89–101.
- Chou, T.W., 1992. *Microstructural Design of Fiber Composites*. Cambridge: Cambridge University Press.
- Craig, C.L., Hsu, M., Kaplan, D., Pierce, N.E., 1999. A comparison of the composition of silk proteins produced by spiders and insects. *Int. J. Biol. Macromol.* 24, 109–118.
- Denny, M., 1976. The physical properties of spider’s silk and their role in the design of orb-webs. *J. Exp. Biol.* 65, 483–506.

- Denny, M.W., 1980. Silks — their properties and functions. In: Vincent, J.F.V., Currey, J.D. (Eds.), *The Mechanical Properties of Biological Materials: Society for Experimental Biology Symposium*, 34. Cambridge University Press, Cambridge, pp. 245–271.
- Dong, Z., Lewis, R.V., Middaugh, C.R., 1991. Molecular mechanism of spider silk elasticity. *Arch. Biochem. Biophys.* 284, 53–57.
- Eberhard, W., Pereira, F., 1993. Ultrastructure of cribellate silk of nine species in eight families and possible taxonomic implications (Araneae: Amaurobiidae, Deinopidae, Desidae, Dictynidae, Filistatidae, Hypochilidae, Stiphidiidae, Tengellidae). *J. Arachnol.* 21, 161–174.
- Eberhard, W.G., 1980. Persistent stickiness of cribellum silk. *J. Arachnol.* 8, 283.
- Eberhard, W.G., 1988. Combing and sticky silk attachment behaviour by cribellate spiders and its taxonomic implications. *Bull. Br. Arachnol. Soc.* 7, 247–251.
- Edmonds, D., Vollrath, F., 1992. The contribution of atmospheric water vapour to the formation and efficiency of a spider's web. *Proc. R. Soc. Lond.* 248, 145–148.
- Edwards, H.G.M., Farwell, D.W., 1995. *J. Raman Spectrosc.* 26, 901–909.
- Fischer, F., Brander, J., 1960. Eine Analyse der Gespinste der Kreuzspinne. *Hoppe-Seyler's Z. Physiol. Chem.* 320, 92–102.
- Foelix, R., 1996. *Biology of Spiders*. Oxford University Press, Oxford.
- Friedrich, V.L., Langer, R.M., 1969. Fine structure of cribellate spider silk. *Am. Zoologist* 9, 91–96.
- Frische, S., Maunsbach, A., Vollrath, F., 1997. Microstructure of two spider silks. *J. Microsc.* 189, 64–70.
- Gosline, J., Denny, M., DeMont, M., 1984. Spider silk as rubber. *Nature (Lond.)* 309, 551–552.
- Gosline, J.M., DeMont, M.E., Denny, M.W., 1986. The structure and properties of spider silk. *Endeavour N.S.* 10, 31–43.
- Griffiths, J.R., Salanitri, V.R., 1980. The strength of spider silk. *J. Mater. Sci.* 15, 491–496.
- Grubb, D.T., Jelinski, L.W., 1997. Fiber morphology of spider silk: the effects of tensile deformation. *Macromolecules* 30, 2860–2867.
- Guerette, P., Ginzinger, D., Weber, B., Gosline, J., 1996. Silk properties determined by gland-specific expression of a spider fibroin gene family. *Science* 272, 112–115.
- Hayashi, C., Shipley, N., Lewis, R., 1999. Hypotheses that correlate the sequence, structure and mechanical properties of spider silk proteins. *Int. J. Biol. Macromol.* 24, 271–275.
- Hayashi, C.Y., Lewis, R.V., 2000. Molecular architecture and evolution of a modular spider silk gene. *Science* 287, 1477–1479.
- Hinman, M.B., Lewis, R.V., 1992. Isolation of a clone encoding a second dragline silk fibroin. *J. Biol. Chem.* 267, 19320–19324.
- Hinman, M.B., Stauffer, S.L., Lewis, R.V., 1994. Mechanical and chemical properties of certain spider silks. In: Kaplan, D., Adams, W.W. et al. (Eds.), *Silk Polymers. Materials Science and Biotechnology*. American Chemical Society, Washington, pp. 222–233.
- Hijirida, D.H., Do, K.G., Michal, C.S.W., Zax, D., Jelinski, L.W., 1996. C-13 NMR of *Nephila clavipes* major ampullate silk gland. *Biophys. J.* 71, 3442–3447.
- Heslot, H., 1998. Artificial fibrous proteins: a review. *Biochimie* 80, 19–31.
- Kaplan, D.L., Lombardi, S.J., 1990. Isolation, cloning, and physicochemical characterization of spider silk from the golden orb-weaver, *Nephila clavipes*. *Polymer Preprints* 31, 195–196.
- Kaplan, D.L., Adams, W.W., Viney, C., Farmer, B.L., 1994. *Silk Polymers: Materials Science and Biotechnology*. American Chemical Society Books, Washington.
- Knight, D.P., Vollrath, F., 1999a. Liquid crystals in a spider's silk production line. *Proc. R. Soc.* 266, 519–523.
- Knight, D.P., Vollrath, F., 1999b. Liquid crystals in the cells secreting spider silk feedstock. *Tissue Cell* 31, 617–620.
- Knight, D.P., Knight, M., Vollrath, F., 2000. Beta sheet formation and the toughness of spider silk. *Int. J. Biol. Macromol.* 27, 205–210.
- Köhler, T., Vollrath, F., 1995. Thread biomechanics in the two orb weaving spiders *Araneus diadematus* (Araneae, Araneidae) and *Uloborus walckenaerius* (Araneae, Uloboridae). *J. Exp. Zool.* 271, 1–17.
- Kümmerlen, J., Vanbeek, J., Vollrath, F., Meier, B., 1996. Local-structure in spider dragline silk investigated by 2-dimensional spin-diffusion nuclear-magnetic-resonance. *Macromolecules* 29, 2920–2928.
- Lehmensick, R., Kullmann, E., 1956. Über den Feinbau der Spinnenfäden, *Proceedings of the Stockholm Conference on Electron Microscopy*, pp. 307–309.
- Lewis, R., 1999. Hypotheses that correlate the sequence, structure and mechanical properties of spider silk proteins. *Int. J. Biol. Macromol.* 24, 271–275.
- Lombardi, S.J., Kaplan, D.L., 1990. The amino acid composition of major ampullate gland silk (dragline) of *Nephila clavipes* (Araneae, Tetragnathidae). *J. Arachnol.* 18, 297–306.
- Li, S.F.Y., McGhie, A.J., Tang, S.L., 1994. New internal structure of spider dragline silk revealed by atomic force microscopy. *Biophys. J.* 66, 1209–1212.
- Lin, L., Edmonds, D., Vollrath, F., 1995. Structural engineering of a spider's web. *Nature* 373, 146–148.
- Lubin, Y.D., 1986. Web building and prey capture in the Uloboridae. In: Shear, W.A. (Ed.), *Spiders: Webs, Behavior and Evolution*. Stanford University Press, Stanford, pp. 132–171.
- Madsen, B., Shao, Z., Vollrath, F., 1999. Variability in the mechanical properties of spider silks on three levels: interspecific, intraspecific and intraindividual. *Int. J. Biol. Macromol.* 24, 301–306.
- Madsen, B., Vollrath, F., 2000. Mechanics and morphology of silk drawn from anaesthetised spiders. *Naturwissenschaften* 87, 148–153.
- Mahoney, D.V., Vezie, D.L., Eby, R.K., Adams, W.W., Ka-

- plan, D., 1994. Aspects of the morphology of dragline silk of *Nephila clavipes*. In: Kaplan, D., Adams, W.W. et al. (Eds.), *Silk Polymers. Materials Science and Biotechnology*, Washington: American Chemical Society, pp. 196–210.
- Maynes, E., Mann, S., Vollrath, F., 1998. Preparation and mechanics of magnetic spider silk. *Adv. Mater.* 10, 801–805.
- McNamee, S.G., Ober, C.K., Jelinski, L.W., Ray, E., Xia, Y., Grubb, D.T., 1994. Toward single-fiber diffraction of spider dragline silk from *Nephila clavipes*. In: Kaplan, D., Adams, W.W., Farmer, B., Viney, C. (Eds.), *Silk Polymers. Materials Science and Biotechnology*. American Chemical Society, Washington, pp. 176–184.
- Mello, C.M., Senecal, K., Yeung, B., Vouros, P., Kaplan, D., 1994. Initial characterization of *Nephila clavipes* dragline protein. In: Kaplan, D., Wade, W.W., Farmer, B., Viney, C. (Eds.), *Silk Polymers. Materials Science and Biotechnology*, Symposium Series, 544. American Chemical Society, Washington, pp. 67–79.
- Miller, L., Putthananat, S., Eby, R., Adams, W., 1999. Investigation of the nanofibrillar morphology in silk fibers by small angle X-ray scattering and atomic force microscopy. *Int. J. Biol. Macromol.* 24, 159–165.
- Newman, J., Newman, C., 1995. Oh what a tangled web – the medicinal uses of spider silk. *Int. J. Dermatol.* 34, 290–292.
- Opell, B., 1982. Cribellum, calamistrum and ventral comb ontogeny in *Hyptiotes cavatus* (Hentz) (Araneae: Uloboridae). *Bull. Br. Arachnol. Soc.* 5, 338–343.
- Opell, B.D., 1993. What forces are responsible for the stickiness of spider cribellate threads. *J. Exp. Zool.* 265, 469–476.
- Opell, B.D., 1994. Factors governing the stickiness of cribellar prey capture threads in the family Uloboridae. *J. Morphol.* 221, 111–119.
- Opell, B.D., 1995. 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., 1997. The material cost and stickiness of capture threads and the evolution of orb weaving spiders. *Bio. J. Linn. Soc.* 62, 443–458.
- 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., 1987. Fine structure and function of capture threads. In: Nentwig, W. (Ed.), *Ecophysiology of Spiders*. Springer, Berlin, pp. 187–202.
- Riekel, C., Müller, M., Vollrath, F., 1999. In-situ X-ray diffraction studies during forced silking of a *Nephila senigalensis* spider. *Macromolecules* 32, 4464–4466.
- Rhisiart, A., Vollrath, F., 1994. Design features of a spider's orb web. *Behav. Ecol.* 5, 280–287.
- Samu, F., Matthews, G., Lake, D., Vollrath, F., 1992. Spider orb webs as selective collectors of pesticides. *Pesticide Sci.* 36, 131–137.
- Schildknecht, H., Munzelmann, P., Krauss, D., Kuhn, C., 1972. Über die Chemie der Spinne. I. *Naturwissenschaften* 59, 98–99.
- Shao, Z., Vollrath, F., 1999. The effect of solvents on the contraction and mechanical properties of spider silk. *Polymer* 40, 1799–1806.
- Shao, Z., Vollrath, F., Sirichaisit, J., Young, R.J., 1999a. Analysis of spider silk in native and supercontracted states using Raman spectroscopy. *Polymer* 40, 2493–2500.
- Shao, Z., Wen Hu, X., Frische, S., Vollrath, F., 1999b. Heterogeneous morphology in spider silk and its function for mechanical properties. *Polymers* 40, 4709–4711.
- Shao, Z., Young, R.J., Vollrath, F., 1999c. The effects of solvents on spider silk studied by mechanical testing and single-fibre Raman spectroscopy. *Int. J. Biol. Macromol.* 24, 295–300.
- Shultz, J.W., 1987. The origin of the spinning apparatus in spiders. *Biol. Rev.* 62, 89–113.
- Simmons, A., Michal, C., Jelinski, L., 1996. Molecular-orientation and 2-component nature of the crystalline fraction of spider dragline silk. *Science* 271, 84–87.
- Simmons, A., Ray, E., Jelinski, L., 1994. Solid-state c-13 NMR of nephila-clavipes dragline silk establishes structure and identity of crystalline regions. *Macromolecules* 27, 5235–5237.
- Sirichaisit, J., Young, R.J., Vollrath, F., 1999. Molecular deformation in spider dragline silk subjected to stress. *Polymers* 40, 2493–2500.
- Sun, Y., Shao, Z., Hu, P., Tu, T., 1997a. Hydrogen bonds in silk fibroin-poly(acrylonitrile-co-methyl acrylate) blends: FT-IR study. *J. Polymer Sci. B: Polymer Phys.* 35, 1414–1415.
- Sun, Y., Shao, Z., Ma, M., Hu, P., Liu, Y., Tu, T., 1997b. Acrylic polymer-silk fibroin blend fibers. *J. Appl. Polymer. Sci.* 65, 959–966.
- Termonia, Y., 1994. Molecular modeling of spider silk elasticity. *Macromolecules* 27, 7378–7381.
- Thiel, B.L., Kunkel, D., Guess, K., Viney, C., 1994a. Composite Microstructure of Spider (*Nephila clavipes*) Dragline. In: Alper, H.B.M., Kaplan, D., Navia, M. (Eds.), *Biomolecular Materials by Design, MRS Symposium*. Materials Research Society, Pittsburgh, pp. 21–30.
- Thiel, B.L., Kunkel, D., Viney, C., 1994b. Physical and chemical microstructure of spider dragline: a study by analytical transmission electron microscopy. *Biopolymers* 34, 1089–1097.
- Tillinghast, E.K., 1981. Selective removal of glycoproteins from the adhesive spiral of the spiders orb web. *Naturwissenschaften* 68, 526–527.
- Tillinghast, E.K., Sinohara, H., 1984. Carbohydrates associated with the orb web protein of *Argiope aurantia*. *Biochem. Int.* 9, 315–317.
- Tillinghast, E.K., Townley, M., 1987. Chemistry, physical properties and synthesis of Araneida orb webs. In: Nentwig, W. (Ed.), *Ecophysiology of Spiders*. Springer, Heidelberg, pp. 203–210.
- Tillinghast, E.K., Townley, M.A., Bernstein, D.T., Gallagher, K.S., 1991. Comparative study of orb web hygroscopicity and adhesive spiral composition in three araneid spiders. *J. Exp. Zool.* 259, 154–165.

- Valluzzi, R., Gido, S., Muller, W., Kaplan, D., 1999. Bombyx mori silk, fibroin liquid crystallinity and crystallization at aqueous fibroin-organic solven interfaces. *Int. J. Biol. Macromol* 24, 227–236.
- Vollrath, F., 1992. Spider webs and silk. *Sci. Am.* 266, 70–76.
- Vollrath, F., 1994. General properties of some spider silks. In: Kaplan, D., Wade, W.W. et al. (Eds.), *Silk Polymers. Materials Science and Biotechnology*. American Chemical Society, Washington, pp. 17–28.
- Vollrath, F., 1999a. Biology of spider silk. *Int. J. Biol. Macromol.* 24, 81–88.
- Vollrath, F., 1999b. Coevolution of building behaviour and building material. In: Domenici, P. (Ed.), *Animal Behaviour and Architecture* (in press).
- Vollrath, F., Rohde-Arndt, D., 1983. Prey capture and feeding in the social spider *Anelosimus eximius*. *Z. Tierpsychol.* 61, 334–340.
- Vollrath, F., Edmonds, D., 1989. Modulation of the mechanical properties of spider silk by coating with water. *Nature* 340, 305–307.
- Vollrath, F., Tillinghast, E.K., 1991. Glycoprotein glue inside a spider web's aqueous coat. *Naturwissenschaften* 78, 557–559.
- Vollrath, F., Knight, D., 1999. The silk press of the spider *Nephila edulis*. *Int. J. Biol. Macromol.* 24, 243–249.
- Vollrath, F., Knight, D. Silk spinning in nature, in preparation.
- Vollrath, F., Madsen, B. The effect of spinning conditions on the mechanical properties of a spider's dragline. *Proc. R. Soc. Lond.* (submitted).
- Vollrath, F., Fairbrother, W.J., Williams, R.J.P. et al., 1990. Compounds in the droplets of the orb spider's viscid spiral. *Nature* 345, 526–528.
- Vollrath, F., Holtet, T., Thogersen, H., Frische, S., 1996. Structural organization of spider silk. *Proc. R. Soc. Lond.* 263, 147–151.
- Vollrath, F., Wen Hu, X., Knight, D.P., 1998. Silk production in a spider involves acid bath treatment. *Proc. R. Soc.* 263, 817–820.
- Wilson, R.S., 1962a. The control of dragline spinning in the garden spider. *Q. J. Microsc. Sci.* 103, 557–571.
- Wilson, R.S., 1962b. The structure of the dragline control valves in the garden spider. *Q. J. Microsc. Sci.* 103, 549–555.
- Wilson, R.S., 1969. Control of dragline spinning in certain spiders. *Am. Zoologist* 9, 103–111.
- Wirth, E., Barth, F.G., 1992. Forces in the spider orb web. *J. Comp. Physiol. A* 171, 359–371.
- Witt, P.N., Reed, C.F., Peakall, D.B., 1968. *A Spider's Web: Problems in Regulatory Biology*. Springer, Heidelberg.
- Work, R.W., 1977. Mechanisms of major ampullate silk fibre formation by orb-web spinning spiders. *Trans. Am. Microsc. Soc.* 96, 170–189.
- Work, R.W., 1981. A comparative study of the supercontraction of major ampullate silk fibres of orb web building spiders (Araneae). *J. Arachnol.* 9, 299–308.
- Work, R.W., 1984. Duality in major ampullate silk and percursive material from orb-web-building spiders (Araneae). *Trans. Am. Microsc. Soc.* 103, 113–121.
- Work, R.W., 1985. Viscoelastic behaviour and wet super contraction of major ampullate silk fibres of certain orb web building spiders (Araneae). *J. Exp. Biol.* 118, 379–404.
- Work, R.W., Young, C.T., 1987. The amino acid compositions of major and minor ampullate silks of certain orb-web-building spiders (Araneae, Araneidae). *J. Arachnol.* 15, 65–80.
- Xue, G., 1994. Laser Raman-spectroscopy of polymeric materials. *Prog. Polym. Sci.* 18, 337–435.
- Yeh, W.Y., Young, R.J., 1998. Deformation processes in poly(ethylene terephthalate) fibres. *J. Macromol. Sci.* 37, 83–118.
- Young, R.J., Lu, D., Day, R.J., 1991. Raman spectroscopy of Kevlar fibres during deformation — Caveat emptor. *Polymer Int.* 24, 71–76.