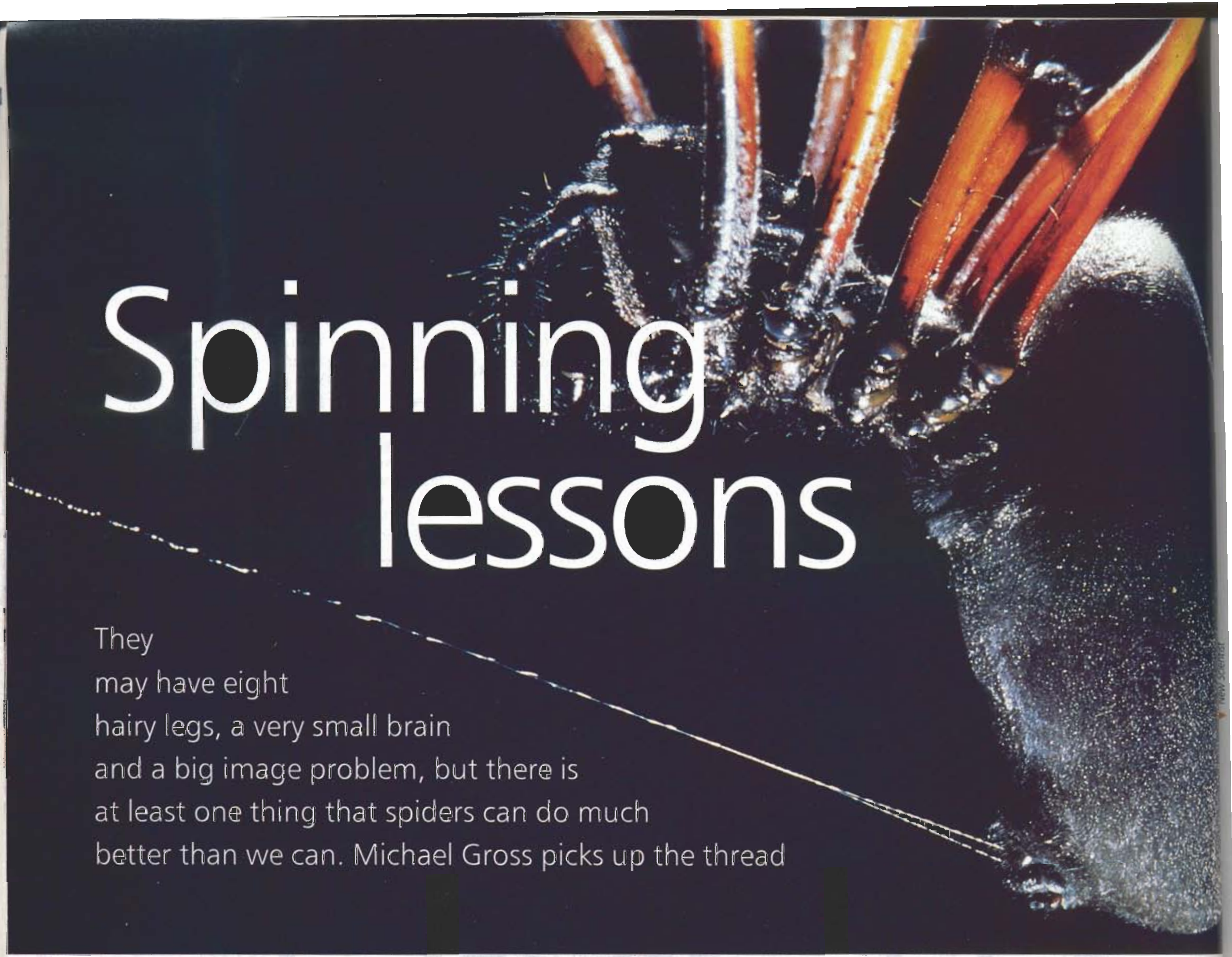


Spinning lessons

They may have eight hairy legs, a very small brain and a big image problem, but there is at least one thing that spiders can do much better than we can. Michael Gross picks up the thread

NATURE IS IN MANY WAYS A BETTER ENgineer than humankind. No matter whether you look at the ways that diatoms, mussels or snails make their shells, butterflies seem to change their colours, or trees withstand strong winds, there is always a lesson to be learnt. One of the most spectacular examples of how Nature beats our best efforts is spider silk.

Like our own hair, a sheep's wool or a silkworm's cocoon, a spider's web consists mostly of protein. But the polypeptide chains are aligned and interwoven in mysterious ways that make the product much stronger than these other materials. Able to stop an insect in full flight, spider silk is in fact the strongest material we know in strength per weight terms. If you compare a spider's thread with a steel wire of the same diameter, they will be able to support roughly the same weight. But the silk is one-sixth as heavy, so it is really six times stronger than steel, and the spider wins every time.

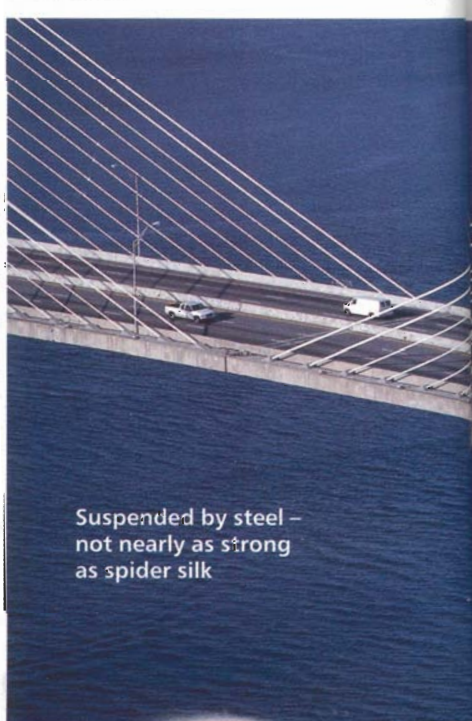
So why are suspension bridges still dangling on steel ropes rather than silken ones? The trouble is that we can't make spider silk as nicely as the spider can. Sure, we can express its proteins in other organisms (including goats) and we will soon be able to spin those into some kind of fibre. However, given our limited understanding of the processes going on in the spider's silk gland, the result may not live up to the natural product.

A small scattering of biologists in various laboratories around the world is trying to get behind the spiders' secret. First they need the right genes. Until recently, only very few DNA sequences of silk protein genes were known. Earlier this year, John Gatesy and Cheryl Hayashi with their coworkers at the University of Wyoming in Laramie presented a comprehensive overview of gene sequences from a wide variety of eight-legged silk producers. These included tarantulas and other animals that separated from the 'true spiders' more than 200 million years ago.[1] Gatesy and Hayashi showed that the amino acid sequences for which these genes code are extremely diverse between species. About the only thing they all have in common is the occurrence of unusual repetitive sequences following four simple patterns: polyalanine (A_n), alternation of glycine and alanine (GA), then combinations of glycine with a small subset of amino acids (X) with or without proline: GGX and $GPGX_n$.

These peptide motifs have been retained (or evolved convergently) over a time span of more than 200 million years. It appears likely, therefore, that their properties hold important clues about how the various silk proteins that they each code for interact to form silk. So far the only structural information that we have is about the finished silk, where it is known that the alanine-rich repeats occur in quasi-crystalline domains, while the glycine-rich repeats adopt more disorderly states.

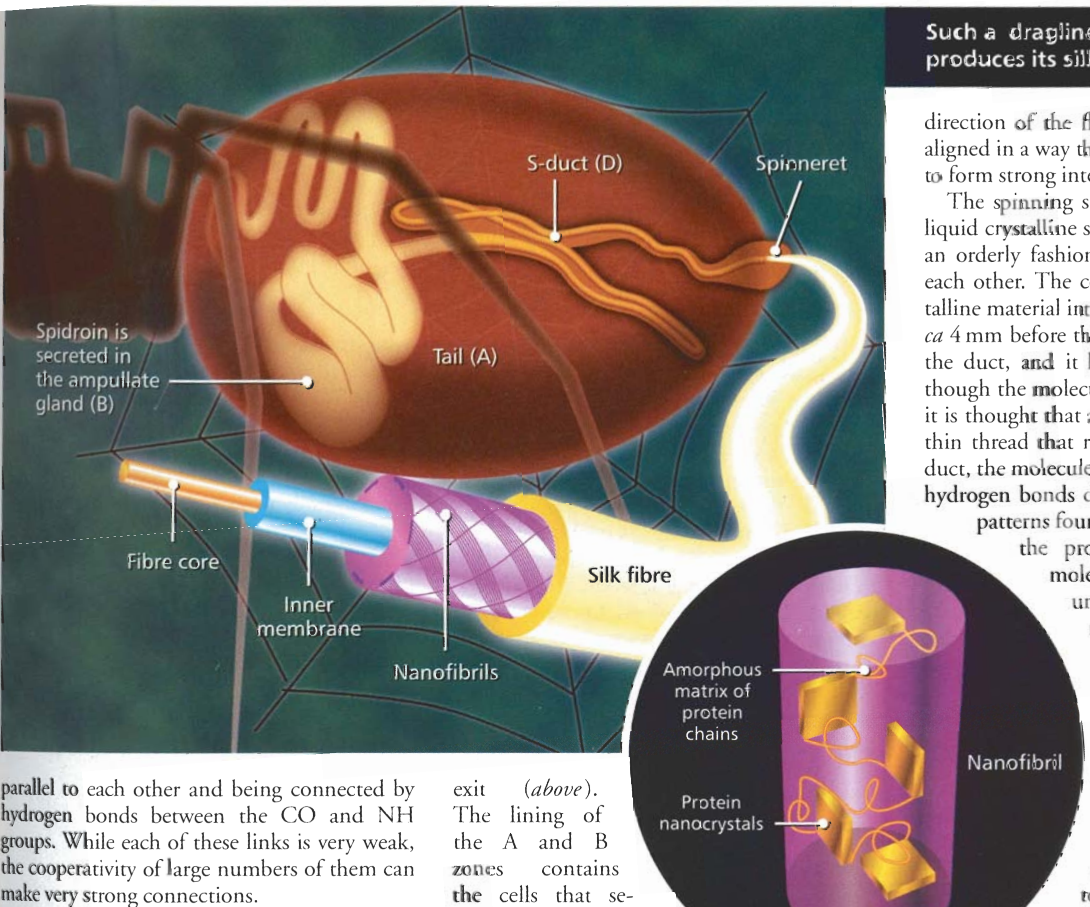
Silken threads

Each individual silk fibre consists of an inner core of what are collectively known as fibroin proteins. This is surrounded by concentric layers of glycoprotein nanofibrils running either parallel with the core fibre or corkscrewing around it. What makes it so strong is the magical transformation of tiny droplets of protein solution into silk thread that takes place in a complex structure called the silk gland. Still only poorly understood, this transformation is known to involve a substantial increase in the proportion of the the core fibroin protein chain that is arranged as a β-pleated sheet. This is a very versatile structural element found in many proteins. It involves the polypeptide backbones of different parts of the protein chain running



Suspended by steel – not nearly as strong as spider silk

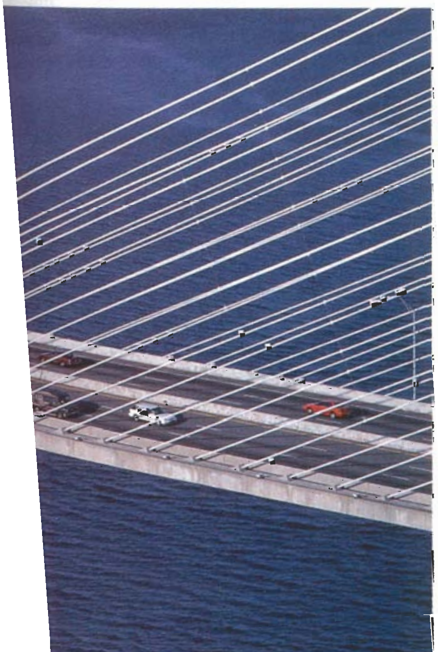
Such a dragline – how *N. clavipes* produces its silk



parallel to each other and being connected by hydrogen bonds between the CO and NH groups. While each of these links is very weak, the cooperativity of large numbers of them can make very strong connections.

Oxford zoologists Fritz Vollrath and David Knight, who have been studying silk production in the orb spider *Nephila clavipes* for many years, recently summed up the state of current knowledge.[2] Found in Central America, *N. clavipes* is an orange and black, hairy knuckled variety of spider measuring 2.5 cm long. Like most spiders, it has not one but several – in this case seven pairs – of silk glands, producing seven different types of silk for different uses; even the protein composition in these glands is significantly different. The best characterised of these glands is the major ampullate gland that produces dragline silk – the type that the spider uses to abseil from walls and ceilings.

This ampullate gland consists of three major regions: a central bag (B zone) flanked by a tail (A zone) and a duct (D) leading towards the exit (*above*). The lining of the A and B zones contains the cells that secrete the fibroin solution, the major component of which is a 275 kDa protein containing the polypeptides spidroin I and spidroin II. To be secreted from a cell, proteins must be wrapped in membrane bubbles called secretory vesicles. In the A zone cells, these vesicles contain the spidroin protein filaments that form the strong core of the thread. In the B zone vesicles, one finds liquid crystals of the glycoproteins that end up coating the thread. Liquid crystals form when highly concentrated solutions of these rod-like protein molecules align themselves to form highly ordered structures like those of solids, but which are capable of flowing like liquids. Vollrath and Knight believe that this liquid crystalline state has an important role to play in helping spiders to lower the viscosity of the concentrated protein solution, allowing them to process the silk thread more easily.



Weaving ways

Let us follow the route of a spidroin molecule from its secretion from the cell through to the finished thread. On leaving the cells of the A zone, our protein finds itself in a small spherical droplet with lots of other spidroin molecules. This highly viscous protein solution flows into the B zone, where it gets coated by glycoproteins. After exiting this bag, the liquid is funnelled into the narrow duct D. What is especially remarkable about this process is that the protein concentration in the whole gland is about 50 per cent by weight – higher than in most protein crystals. Most proteins would aggregate into insoluble lumps at much lower concentrations. This is where liquid crystals have an important part to play. As the viscous protein mass moves into the duct, the constituent spidroins and glycoproteins are slowly distorted into long thin shapes aligned with the direction of the flow. They are stretched and aligned in a way that will eventually allow them to form strong intermolecular links.

The spinning solution or dope is now in a liquid crystalline state, with proteins aligned in an orderly fashion, but still able to slide past each other. The conversion of the liquid crystalline material into a thread happens at a point *ca* 4 mm before the protein solution exits from the duct, and it happens quite suddenly. Although the molecular details are far from clear, it is thought that as the dope is drawn out to a thin thread that retracts from the walls of the duct, the molecules align even further and form hydrogen bonds defining the complex β-sheet patterns found in the finished product. In the process, the spidroin protein molecules uncurl to reveal their uncharged hydrophobic segments, simultaneously ejecting some of the solvated water that it has been carrying until this point. Finally, most of the water is stripped off the surface when the thread leaves the exit spigot, helping the spider to avoid water loss and making its thread even tougher.

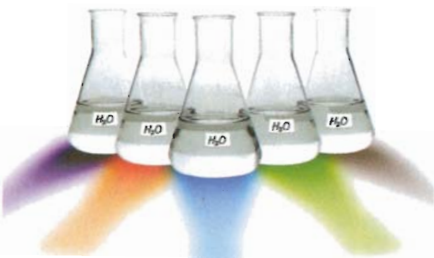
This broad picture drawn by Vollrath and Knight combines anatomical with some structural data. However, the exact details of the crucial structural transitions are far from understood. The trouble is that the most powerful tools for determining protein structures, X-ray crystallography and NMR, require a protein crystal or a homogeneous solution, respectively. As yet, there is no method that could give you the atomic detail structure of a protein molecule flowing down the duct of a spider's silk gland.

And yet, even in the absence of a full understanding in molecular terms, maybe one could copy the spider's technique on a microscopic scale, by supplying a dope with the right protein composition and passing it through a spinning device modelled on the spider's gland? The only comparable man-made material, aramid – Kevlar, the fibre used in bullet-proof vests – is spun from hot sulphuric acid (*see Box*). An ambient temperature process leading to something similar would be very attractive, even if the resulting fibre turned out only as good as Kevlar, and not quite as good

From strength to strength

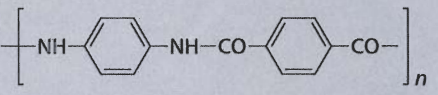
Material	*Tensile strength* MN m^{-2}*
Human hair	192
Spider silk (not dragline)	240
Nylon fibre	1050
High tensile engineering steel	1550
Kevlar 29 fibre	2700

*Tensile strength refers to absolute strength (not per weight)



Strong opposition

The strongest man-made organic fibre so far is aramid, developed by DuPont in the 1970s and marketed as Kevlar (see www.dupont.com/kevlar/). As in nylon, wool and silk, its monomers are held together by amide bonds, created by the polymerisation reaction between terephthalic acid and *p*-phenylene diamine.



It is this strong amide backbone that gives Kevlar its strength. Unlike proteins, aramid has no side chain functions, because the aryl group is incorporated in the backbone of the polymer. In addition to its high tensile strength, Kevlar is non-flammable and resistant to heat and chemicals. It is often used in composites, and applications include bullet-proof clothing, protective gear such as helmets and gloves, tyres, ropes for ships and also to secure the airbags on *Mars Pathfinder* during landing, as well as ski and other sports equipment. In future, however, Kevlar could face competition from artificial spider silk.

Spiders can produce silk over a 30 °C temperature range and can vary the speeds at which they make the threads. The only solvent required is water and it doesn't matter if the resulting silk nanocrystals contain minor imperfections. By contrast, making Kevlar involves a complex series of organic reactions in hot sulphuric acid at high pressures. Interestingly, the final spinning process entails a liquid crystalline transition similar to that for spider silk. ☐

as authentic spider silk.

First, however, you would need to produce the proteins in reasonable quantities. Unlike silk moth caterpillars ('silkworms'), spiders have an aggressive territorial behaviour, which means that they won't be cooperating with any ideas of high-throughput farming. Expressing the silk proteins in bacteria or yeasts doesn't work either. The curious repetitive nature of their sequences invites the microbes to take shortcuts and produce abridged versions of the protein chains.

Thus, if you want to use the silk to catch fighter jets rather than flies, you'd better get an animal that can produce more than a few milligrams of the precious material. Canadian company Nexia Biotechnologies (www.nexiabiotech.com), has recently bred goats that are genetically modified to secrete spidroin protein in their milk. The secretory cells of mammary glands aren't that different from those of silk glands, only there are a lot more of them in a goat, which makes milking goats a lot more economical than milking spiders.

Since the summer of 2000, Nexia has boasted the possession of two African dwarf goats, Peter and Webster, who have been shown to carry the appropriate spider gene. A couple of breeding generations later, there will be a flock of females producing spidroin in their milk by the gram. Nexia is keeping mum about how exactly it wants to spin that milk-silk protein into strong fibres on an industrial scale. As soon as it does so, however, applications ranging from surgical threads through to missile protection and aviation security will be rapidly conquered.

Although most of the envisaged applications are substantially scaled up compared with a spider's web, one group of researchers has gone the other way and scaled their threads down. In an attempt to turn a visible thread into an invisibly thin nanowire, Michael Stuke's group at the Max Planck Institute for Biophysical Chemistry in Göttingen stripped spider silk down to the core, using ultraviolet laser technology. They obtained very strong nanowires, currently with a diameter as small as 100 nm. Future plans include coating this thread in metal to make it conductive.

But even when we can copy the spider's thread and use it on various length scales, the hairy little arthropods can still do one better. As Stefan Schulz and his coworkers at the Technical University of Braunschweig, Germany, reported last year, the female tropical spider *Cupiennius salei* leaves a thread marked with sex pheromones, which induce any male of her species to vibrate excitedly. The vibrations are transmitted through the thread, which rapidly switches from a role of odour dispenser to that of a phone line. The female vibrates back, and you can figure out the rest for yourself. I wonder whether anybody wants to set up a company banking on that technology …

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