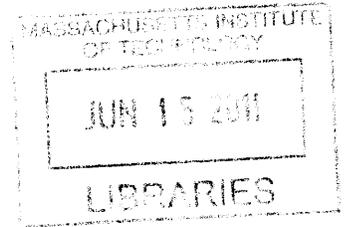


Bulletproof Gossamer: Spinning a Superfiber

by

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B.S. Humanities and Science
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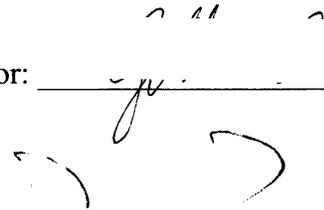
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ABSTRACT

Spider silk is a material of extraordinary beauty and utility. From the spider's perspective, it is foremost a building material, but also a safety net, a sensory organ, a weapon. From a human perspective, it is a material of extraordinary mechanical properties, an object of artistic and cultural interest, and a valuable window into the evolutionary history of spiders.

Historically, there have been a mere handful of spider silk textiles, the most recent example of which was constructed by Simon Peers and Nicholas Godley. This was an extravagant work of art, and the silk was collected entirely by hand. Though the finished article is a testament to the beauty of spider silk, it also illustrates the technical challenges associated with obtaining it in any significant quantity.

The effects of this scarcity are evident in the lab of David Kaplan at Tufts University. His group has developed a wide variety of applications for silk, but has focused mainly on silkworm silk in spite of spider silk's greater variety and superior mechanical properties.

In the wild, spiders use silk for everything from weaving webs and capturing prey to breathing underwater. Shaped by almost every environment on Earth, spider silk has evolved into endless variations and permutations, offering a vast wealth of material knowledge if we can find a way to tap it.

A visit to Cheryl Hayashi's spider silk genetics lab at the University of California, Riverside offers a look inside a spider and a glimpse of how genetic research can illuminate the evolution of silk. At the same time, it puts the limits of our knowledge into stark relief.

The scarcity of natural spider silk has helped to drive a small industry in bioengineered and synthetic silk research, and also motivated projects that seek to apply the structural principles of silk fibers to other materials. But so far, these efforts are only a pale imitation of the real thing. For now, the spider is keeping her secrets.

Thesis Advisor: Russ Rymer

Arachne was a talented weaver, and very proud. Though a mere mortal, she boasted that she was more skilled than Athena, the goddess of wisdom, war, and crafts. Athena took the form of an old woman and warned her not to provoke the gods, but Arachne scoffed and wished for a contest to prove her skills. Affronted, the goddess revealed herself and challenged the mortal woman.

They strung their looms and began. Athena created a scene of the gods triumphant, with images of the dire fates suffered by mortals who dared to trifle with them. Arachne wove the gods in scenes of undignified debauchery, seducing and deceiving mortals in all manner of ways. Her skill was impeccable, and so angered was Athena by Arachne's impudence and her own jealousy that she tore down the tapestry and turned the mortal woman into a spider, forevermore to ply her trade in small and crawly form.

Nephila madagascariensis loomed in the center of her web, waiting for prey. She was large for a spider, with a six-inch leg span and a body about the size and shape of your thumb. Her back was striped in black, white, and yellow, and her spindly legs gleamed cordovan red. She was a striking creature, beautiful and wicked in every detail, not so different from an ordinary garden spider and yet vaguely monstrous—an arachnophobe's worst nightmare. The web seemed scarcely substantial enough to support her weight, its strands bowing beneath the pressure of her gangly limbs. Its spiral lattice, a full arm-span across, its weave too fine even to stick a finger through, bespoke hours of meticulous labor.

Another *madagascariensis* lurked a few feet away, her own sprawling web like a private, parallel, two-dimensional universe. The enclosure, at the Smithsonian National Zoo in Washington, DC, was open to the air, with nothing but force of habit to constrain the spiders'

movements. With little need to compete for food, the spiders live easy lives. Indifferent to the gawking public, they stray from their webs only to occasionally deposit an egg case a few feet away, and a six-inch lip on the roof of their alcove is sufficient to discourage them from wandering further. If they are aware of each other's presence, or that of the steady trickle of zoo visitors, they seem happy enough to coexist.

The alcove is directly adjacent to the volunteer station, where zoo staff can thwart any attempts to harass the spiders or knock them from their webs. Some visitors are so certain that such large and sinister creatures must be enclosed that they've been known to lean against the absent glass and fall into the expanse of web. There's little need to confine the spiders. Despite their appearance, they're almost completely harmless to humans.

At two o'clock on a Sunday afternoon, a volunteer—Ceci, according to her name tag—arrived bearing a long pair of tweezers and a carton of crickets. She extracted a cricket, which was sluggish but alive, and after a few attempts succeeded in depositing it in a web. It remained very still, as if knowing its peril. Ceci blew on the web to make it sway, and the cricket began to thrash.

In the center of the web, *madagascariensis* stirred. The web is no simple snare—it is an extension of her nervous system, taut and attuned to vibrations. From the center where she sat, limbs poised on the strands, the spider could feel the hapless cricket's every twitch and thrash. She struck suddenly, crossing three feet of web in a blink and seizing her prey. Gripping it in her jaws, she clambered back up to her preferred resting place and began to wrap it. Her legs moved with machinelike coordination, tugging the silk from the set of spinnerets on the underside of her abdomen and looping it around the cricket like a diabolical knitting machine, enveloping it completely in less than a minute. She anchored the swaddled bug to the web by a strand of silk,

allowing it to dangle free, then turned around to sink in her fangs and pump the cricket full of venom and digestive enzymes. All her artistry culminates in this brutal act of survival, a reminder that the beauty of the web is a mere byproduct of its finely honed function.

Silk is a building material, a weapon, and a sensory organ. Spiders use it to build homes, to wrap prey, to protect their eggs from predators, to parachute through the air. Increasingly, humans too are intrigued by the potential of this curious material. Despite its fragile appearance, silk is one of the toughest substances known to humanity—tougher by weight than steel or even Kevlar. It is also tremendously varied, as the endlessly inventive spiders can testify, with almost limitless applications.

Over hundreds of millions of years of spider history, the primeval fiber has evolved into a plethora of strand types: a few small genetic changes can make it stronger or weaker, stretchy or firm, capable of melting away in water or enduring in the environment for decades. Scientists have imagined a whole host of possible uses for this versatile stuff, from car airbags to tissue engineering and biodegradable electronics, harnessing all the natural properties of silk to do things spiders never thought to try.

There's just one problem: we don't actually know how they make it, and the spiders aren't interested in sharing. The web may be a thoughtless thing to a spider, wrought by millions of years of evolution and written in instinct, but it is a feat of engineering that has confounded scientists for decades, and confounds them still.

The tapestry is a rich yellow-gold and worked with intricate patterns from the Madagascar *Lambas* weaving tradition. It is exquisitely beautiful, its silk lustrous and light as air. It's also strong enough to stop a bullet.

In 2004, British art historian Simon Peers joined forces with American fashion designer Nicholas Godley to create a dramatic work of art: an intricate tapestry, the like of which hadn't been seen in a century, crafted from the brilliant golden silk of Madagascar spiders.ⁱ The result is a potent reminder that spider silk is *silk*, that for all its utility it is also capable of spectacular beauty.

Peers and Godley got their silk straight from the source, collecting golden orb weavers—the same *Nephila madagascariensis* on display at the National Zoo—in their native Madagascar. The impressive size of *Nephila* spiders makes it relatively easy to extract their silk, and because they are accustomed to building large, densely-woven webs, they can produce over 300 meters at a time. They're common in warm areas all over the world, making them a convenient resource for scientists and weavers alike. The very first studies of silk structure, back in the early 1990s, used dragline silk from *Nephila clavipes*, a relative of *madagascariensis* native to the southern United States.

The difficulty of acquiring a reasonable amount of spider silk seems counterintuitive. Spiders are everywhere, after all, and most people have walked into a spider web as they went out the front door in the morning. Spiders are even more familiar to most of us than silkworms, *Bombyx Mori*, which have been domesticated for thousands of years and are raised by the millions all over the world. Silkworm silk is several times thicker than spider silk, which means that you need many more strands of spider silk to produce the same mass of fiber. But spiders make up for this by being, in some ways, superior producers. A *Bombyx* caterpillar produces only one cocoon, containing 300 to 900 meters of silk, which protects it as it pupates into a moth. If the fiber is to be harvested intact, the caterpillar must be killed before it can eat its way back out and destroy the cocoon. Spider silk can be harvested without killing the spiders, which means

that spiders can be silked multiple times if given a few weeks to recover between sessions.

Spiders compensate for their advantages, though, by being all but impossible to handle. Compared with the docile and herbivorous silkworms, which happily subsist on mulberry leaves until it comes time to wrap themselves up in their cocoons, spiders are savage and unruly. Raising most spiders on an industrial scale would be impossible: carnivorous and cannibalistic, they would quickly turn a farm into a macabre gladiatorial arena. And while some spiders do form large communal webs, these are not necessarily the ones that produce the most valuable silk. Finally, spider silk is more difficult to collect. It's easy to confuse one kind of silk for another as they're coming out of the spider, which could prove troublesome for applications that require the particular properties of one type or another. While silkworms produce all their silk in one go, wound up into a convenient package, spiders produce their silks at their own discretion, which has nothing at all to do with human production schedules.

Despite all this, spider silk has been used on small scales the world over, mostly collected from existing webs. Cobwebs are an element of traditional folk medicine in many regions, and cultures in the South Pacific have used silk for fishing nets and lures. In the seventeenth century, a French aristocrat named François Xavier Bon de Saint Hilaire reportedly acquired enough silk from spiders' egg cases to make gloves, stockings, and possibly even a set of clothing for Louis XIV. Accounts of the project are unclear on the details; at least oneⁱⁱ reports that the resulting garments were so weak that they "tore in all directions."

While collecting silk from existing webs is good enough for some purposes, it's hardly a precision method. The silk it yields isn't reliable or pure enough to serve as an artistic or technical material. Instead, Peers and Godley did what many others had done before them: they went straight to the source.

It's the sort of thing that an amateur naturalist might try on a whim, or a fearless and dexterous child. Burt Wilder, a Civil War surgeon from Massachusetts, described the necessary train of thought in 1865. "At the north end of Folly Island," he wrote,ⁱⁱⁱ

I found in a tree a very large and handsome geometrical spider, whose web was about three feet in diameter. While examining the insect at my tent, it occurred to me to see how much of the silken thread could be drawn from the spinners. As it was not disturbed by pulling out a few yards, I wound the thread around the quill, and then, by turning this in my fingers, reeled off silk from the body of the spider for one hour and a quarter, at the rate of six feet per minute, making one hundred and fifty yards of most beautifully shining golden silk.

Wilder went on to gather several thousand yards of silk, and kept a number of spiders in captivity to study their habits. He didn't express any particular interest in using the silk material he'd collected for anything other than scientific purposes, but his method was strikingly similar to those used in later efforts.

In the 1880s, a French missionary named Jacob Paul Camboué undertook what may have been the first attempt at larger-scale spider silk production. He became fascinated by the large orb-weaving spiders of Madagascar, both for the size of their webs and the striking golden color of the silk. He hired local girls to collect the spiders in baskets, and the head of a local technical school devised a hand-cranked apparatus to hold the spiders and extract their silk. They eventually made a set of bed-hangings that were exhibited in Europe around the year 1900, and were subsequently destroyed or lost.

Inspired by this effort, Peers and Godley resolved to make a spider-silk tapestry of their own, using very nearly the same method and technology. They employed a crew of seventy spider collectors to roam the area surrounding Madagascar's capital, Antananarivo, collecting the leggy orb weavers from telephone poles at a rate of several thousand per day. Twelve more people in a workshop harnessed the spiders up 24 at a time, braving the spiders' painful (though

not dangerous) bites, and affixed the dangling draglines to a spool that drew out the hundreds of yards of silk the spiders had stored. When the spiders had been exhausted, they were released into the wild to replenish their reserves. Given a week to recover, the same spiders could be collected again and again.

Spider silk is stronger than silkworm silk, but it's also much finer, which recommends it for some uses. In the first half of the 20th century, dragline was harvested for use as crosshairs in precision sights and optical instruments.^{iv} Each thread of the golden tapestry contains at least 96 strands of silk twisted together, and in the end Peers and Godley gathered and silked more than a million spiders for the project. The spiders only produce silk during the rainy season, so the work took four years to complete.

The result, put on display at the American Museum of Natural History in the fall of 2009, is stunning. The silk is lustrous gold, feather-light and tremendously strong. Based on a textile tradition that was originally reserved for Madagascar's royalty, the intricate design incorporates stylized birds and flowers in a geometric brocade. As a work of art, it is unique and exquisite. But for scientists who hope to develop more practical applications for silk, it presents a daunting caution. Over four years of meticulous labor, this one piece accumulated a price tag of half a million dollars. The first challenge for anyone studying spider silk is getting enough material to work with.

Cheryl Hayashi picked up a pill-bottle-sized vial from the lab bench. Inside lurked a slick black gumdrop clinging to a cloudlike puff of silk. "Her name is *Latrodectus hesperus*, the western black widow," Hayashi said. She turned to a post-doc seated at a nearby computer. "Do you need this spider?"

She didn't. Hayashi carried the spider over to the apparatus they use to knock the spiders out before playing with them, a perforated canister connected to a tank of carbon dioxide. The gas hissed as she opened the valve. She held a hand over the nozzle and adjusted the flow back down to avoid launching the spider across the lab. Once she'd reduced it to a gentle breeze, she shook the spider out of her vial.

"They're a little shy," she said, gently prodding the spider with a pair of forceps. "I kinda let them walk around a little bit first, to make sure she has a dragline sticking out of her."

Major ampullate silk, the tough structural fiber that most people think of when you mention spider silk, is called dragline for the way spiders leave a trail of it everywhere they go. They use it as a safety tether, allowing them to drop and dangle and walk on unstable footing without fear. Spiders like tarantulas that don't make dragline, are, with a few exceptions, much more likely to live close to the ground where there's nowhere to fall.

The spider appeared reluctant, but after a bit of prodding she wobbled across the dish, a glimmering thread trailing behind her. Satisfied, Hayashi replaced the lid of the container.

While we waited for the gas to knock the spider out, Hayashi retrieved a little electric spindle from the cabinet under the lab bench. It's a simple little apparatus: it has a shaft that holds the test tubes she uses for spools, and a sewing machine pedal to control the speed of the motor. Not the sort of thing you can order from a catalog—Hayashi had this cobbled together specially.

She held up a roll of Scotch tape. "A very valuable lab item," she said. The spider was now thoroughly unconscious. Hayashi tipped it out of the container and placed it on a plastic tray about the size of a salad dish. A little dab of paper was taped to the center of the tray, looking more or less like a stray spitball. It was, in fact, a spider pillow. The black widow's abdomen is

so much larger than her head that this little bit of paper is necessary to keep her propped up in the right position for silk extraction.

Hayashi positioned the unconscious spider on her back, spinnerets pointing upward. Long strips of tape secured the legs and head, leaving only the abdomen exposed. The tape, it seems, is necessary both to hold the spider steady and to prevent her from hurting herself or her handlers. “People ask if I get bitten during my work,” Hayashi noted, but did not seem terribly concerned by the prospect.

Jessica Garb, a former researcher in Hayashi’s lab, is now an assistant professor at the University of Massachusetts, Lowell. She likes working with black widows because they’re easy to find, at least in the southern US where they tend to live, and like Hayashi she prefers to collect specimens herself when she can. The black widows in her lab when I visited were the last of a batch she’d collected in Arizona, that traveled all the way back to Massachusetts in a cooler in her car trunk. In addition to studying these spiders’ silk, she extracts their venom in hopes of better understanding the potent cocktail of neurotoxins that has made these spiders infamous.

While nearly all spiders possess some form of venom, it is almost universally designed for subduing invertebrate prey and harmless or only mildly irritating to humans. Black widows are among only a few hundred species of spiders whose bites are considered medically significant to humans, a distinction they share with recluses, Australian funnel-web spiders, and South American armed spiders. Their venom glands contain a whole soup of unique neurotoxins, mostly designed to dispatch insects but at least one of which is vertebrate-specific. Since dangerous spider venoms are so unusual, Garb hopes to discover what this toxin is used for, and how and when it developed. Though it’s possible that the black widow’s vertebrate-targeted toxin is designed for defense, Garb thinks it’s more likely that she uses it to capture small prey

like lizards and mice.

Black widows are surprisingly slow to use their venom, and when approaching potential prey they prefer to immobilize it before getting too close. They look, Hayashi says, like they're attacking backwards. "If you were the prey," she said, stepping away from the lab bench, "I would run up to you and then turn around and start kicking glue on you." She sketched the gesture, her two legs approximating the motion of the spider's many more.

Only after the hapless insect is thoroughly immobilized by aggregate glue does the widow turn back around and secure the capture with a deadly bite. There are aggressive spiders, but the black widow isn't one of them. We, for obvious reasons, do not look much like food, and black widows are much more likely to flee from humans than to attack. Occasionally, though, some unfortunate soul puts on a shoe that's been sitting empty for too long, or sticks a finger into the wrong crevice, leaving the spider with no escape. The resulting bite can produce days of pain, sweating, muscle spasms, and other effects, though it is rarely fatal.

Against this prospect, Hayashi is armed with Scotch tape. Her precautions seem more than sufficient. "I anesthetize them, I tape them down, keep track of where they are."

Fully secured, the legendary menace of the woodpile looks almost pitiful. It would be difficult to imagine any creature more fragile than she appeared at that moment, swathed in almost comically large strips of Scotch tape. She worked one leg free as she began to wake up, and flailed it ineffectually—I could just imagine her waving a little white flag. "Stop that," Hayashi said, securing it with another strip of tape.

Hayashi slid the plate under the microscope to get a good look at the spinnerets. There are three pairs, two large and one smaller tucked between the other two, and tiny vestigial nubs hinting at a fourth somewhere in the spider's evolutionary history. They looked like tiny fingers

curled into a fist, and they twitched as Hayashi brushed them with a pair of tweezers to pick up the strand of silk trailing from them. She wound it around the test tube and directed me to step on the pedal and draw out the silk. I went slowly, afraid I was going to hurt the tiny creature, and the pedal wasn't quite sensitive enough to respond to my hesitant press. The spool ran in jerky starts.

How spiders produce this silk is a mechanical wonder. Inside the silk glands, of which a given spider can have hundreds, silk is a liquid. As Hayashi says, "there are no spools in there." But as it passes through the spigots and spinning ducts, the liquid solidifies automatically into the strong, tough strand, becoming thread as fast as you can reel it out.

After a few seconds of spooling the strand broke. Hayashi tried to catch the strand a few more times, but it seemed that the now-conscious spider had had enough. She'd constricted the miniscule muscles that control the flow from her spinnerets, stopping the strand. Brushing the spigots with the tweezers yielded a delicate fuzz of thin, weak silk strands and a drop of the milky glue that she uses to coat her webs and snare prey.

Hayashi didn't need this particular spider's silk, so she peeled away the tape and returned the undamaged spider to the gas container to be anesthetized again. "No spider has been harmed in this process," Hayashi said. "Yet."

The result of our efforts was a gleaming ring around the test tube, thin as a line from a technical pen. Hayashi showed me a test tube that held a few hundred yards of *Nephila* silk. It formed a gleaming ribbon no wider than a pencil lead, more precious even than the gold it resembled.

David Kaplan, a professor of biomedical engineering at Tufts University, has become

something of a silk evangelist over his decades-long career, and produced a dizzying array of high-tech biomedical applications for silks. Though he—and his lab—have done some work with spider silk, and have been part of various efforts to synthesize it over the years, they've accomplished a great deal more with ordinary *Bombyx* silk. Although weaker and less varied than spider silk, silkworm silk is useful for many of the same purposes. Thanks to a well-established sericulture industry, labs can order silkworm cocoons by the box, which has proved much more practical than raising temperamental spiders in the lab and silking them by hand.

In Kaplan's lab one afternoon last winter, Senior Technician Carmen Preda opened up a box of papery white *Bombyx* cocoons, which strongly resembled packing peanuts. She snipped one open with a pair of scissors to show me the lima-bean-sized silkworm pupa inside. After removing the silkworms, she cuts the cocoons into pieces and boils them in a sodium carbonate solution to dissolve the sericin glue that binds the strands of fibroin protein together. If the silk was being used for fibers, they'd boil these cocoons intact and unravel the entire unbroken strand. In Kaplan's lab, though, the cocoons will be dissolved into their component proteins anyway. Once the fibers are cleaned, Preda dissolves them in water or solvent to create a liquid protein solution similar to what's stored inside silk glands.

Preda pulled tubes of the prepared solution out of the refrigerator. Translucent and viscous, it varied from whitish to yellow in color. According to Preda, it's stable enough to keep for several months before it congeals into an intractable gel.

This solution is the basis for almost everything in Kaplan's lab. It can be poured it out onto flat surfaces or molds to make silk films, or spun into extremely fine fibers using powerful electric fields. Or technicians can create springy, flexible sponges by mixing the silk solution with salt, allowing it to dry and solidify, and then dissolving the salt away with water. Silk

sponges can be made into scaffolds for growing tissues or bone, which naturally dissolve away as body cells grow in to replace them. Hardened silk tubes can replace damaged blood vessels. Vaccines, blood, and other delicate substances can be sandwiched in silk film, stabilizing them so that they can be transported without refrigeration. Drugs can be embedded in silk capsules, which, implanted in the body, slowly dissolve to provide a steady, reliable dose. On a more everyday level, bottles and other containers made of silk would be cleaner to produce and biodegrade more completely than the current generation of biodegradable plastics.

In Kaplan's hands, silk seems like a wonder-material. Pure fibroin protein is biocompatible, so it can be implanted directly in living tissues without risk of irritation or rejection, and it's durable enough that it can be sterilized before implantation to reduce the risk of infection. Unlike many of the high-tech materials we see today, silk can be produced at room temperature, in a water solution with almost no hazardous chemicals, and it is completely biodegradable. Researchers can even determine how quickly it breaks down in the body or in the environment by changing the way it's processed.

Fiorenzo Omenetto is one of Kaplan's converts. An optical physicist, also at Tufts University, he calls silk "technologically cool." His research has produced mirrors, circuits, and other structures on silk films, which could lead to flexible, implantable electronics to monitor conditions within the body. Electronics suspended in silk can be poured into a brain, wrapping around structures to pick up electrical activity in the neurons, and silk-based antennas could allow sensors implanted deep within the body to communicate with the outside.

Omenetto and the others in Kaplan's lab have coaxed a wealth of applications from ordinary *Bombyx* silk. For their purposes the additional benefits of spider silk might not outweigh the difficulties in obtaining it. Spider silk is many times stronger than silkworm silk,

but silkworm silk is still impressively strong. Omenetto handed me a *Bombyx* cocoon. “Try to tear it,” he said. It was very light and looked as if it should be delicate, and I took it gingerly. But when I twisted and pulled, it felt like I was trying to tear a stack of Tyvek envelopes. My efforts produced nothing but a crackling sound, which—as Omenetto informed me, to my dismay—was the sound of the dried silkworm inside the cocoon being pulverized. The cocoon itself was unaltered.

Yet in various ways, *Bombyx* silk remains second best. The added strength of spider silk, for example, might make it a better replacement for tough tissues like tendons. It lacks an irritating sericin glue coating, which must be removed from *Bombyx* silk so that it doesn't provoke harmful immune responses. But the most promising thing about spider silk, Kaplan said, is the sheer variety of silks that spiders have to offer. He's been playing with silk for over two decades now, but spiders have been doing R&D for three hundred million years. That's a hell of a head start.

There are a few hundred species of silkworms in the world, but they pretty much all use silk for the same purpose. Over 40,000 species of spiders have been identified, and there could be several times that many still awaiting discovery. Spiders have found their way into almost every crevice of the globe, and they—and their silks—have adapted to almost every possible environment. Almost all spiders make more than one type of silk, and some make six or seven types, each from their own specialized glands.

The diversity of silk is closely tied to its genetic structure. Spider silks are composed of long, repetitive chains of amino acids, always with the same beginning and end. It's like a freight train, said Hayashi. The engine and caboose are the same each time, but the important

information is carried by the long strings of identical cars in between, which might be passenger cars, tankers of fuel, or bins of grain. Dragline silk tends to have long chains of the amino acid alanine, which folds into crystalline structures that add to the strength of the fiber. Long sequences of alternating glycine and proline, as in capture spiral silk, produce loose, springy coils that allow the silk to stretch to twice its length. The repetitive genes are one reason why silks are so strong, because the proteins fold and bond with both themselves and neighboring strands, producing a crosslinked fiber that's much stronger than a simple protein chain would be. It also offers a great deal of opportunity for errors in transcription. "Imagine if I told you to retype a sentence—a very simple sentence, 'see spot run, see spot run'—and I told you to retype it a million times," Hayashi suggested to me. "It would be very easy to lose your place. 'Is that my five hundredth? Or my five hundred first?'" The cellular machinery can stumble in much the same way, producing silk genes that are much longer or shorter than they're supposed to be. This sort of structural change can dramatically alter the properties of the silk, and the frequency with which these errors occur results in an unusually high rate of mutation in silk genes. Natural selection plays its usual part, and after a couple hundred million years of this, the result is the dizzying array of silks we observe today.

If you're interested in developing applications for spider silk, Hayashi said, then you could do worse than to see what spiders have already done with it. For silks resistant to heat and dry conditions, look to the deserts. For silks resistant to moisture and rot, there's most likely a rainforest spider that has what you're looking for. One dramatic example of this came just last year, when scientists found that major ampullate silk produced by Darwin's bark spider in Madagascar was the toughest biological material yet identified. These spiders were known locally for their colossal webs, some of them over two and a half square meters, with anchoring

threads up to 25 meters long. Because these threads bear a great deal of strain, the researchers reasoned, the furry gray spiders—a little larger than a thumbnail—must have been under great pressure to develop something special.

The orb web is the most familiar incarnation of spider silk, the one you're likely to encounter just walking around your backyard. The five-foot edifices produced by the Darwin's bark spider and *madagascariensis* are of this type, differing from the average garden spider web only in scale. Like other orb weavers, *madagascariensis* uses four different types of silk to produce an ordinary web.

Major ampullate silk comes first, a strong and moderately stretchy fiber that forms the backbone of the web. In orb weavers, the major ampullate silk is a blend of two different proteins, one of which provides most of the strength while the other provides the stretch. Major ampullate—or dragline—silk was an evolutionary breakthrough, enabling spiders to climb and dangle and do most of the things we tend to think of as characteristically spider-y. It arose around 240 million years ago, when the very first dinosaurs were appearing and the Earth's continents were still jammed together into the supercontinent Pangaea, and distinguishes the “true” spiders from their more primitive relatives. *Madagascariensis* uses it to build an outer frame for the web, and then a series of radial lines like the spokes of a wheel.

Once the foundation of the web is laid, the spider links these radial strands with a temporary spiral, a sort of construction scaffold. Starting at the center of the web, the spider circles toward the outer edge, laying a framework of minor ampullate silk that will help to support the web as she works on it. Minor ampullate silk is similar to the major ampullate silk—the glands even look like miniature versions of the major ampullate glands—but weaker. Later, she removes (and eats) the temporary spiral as she lays down a capture spiral of extremely

stretchy flagelliform silk, circling from the outer edge back toward the center. As she goes, she strings the capture spiral with beads of liquid glue from the aggregate silk gland to create a near-inescapable trap.

The flagelliform-and-aggregate-silk combination is a relatively recent innovation, at about 135 million years old. Other spiders have taken a very different approach to ensnaring prey, using cribellate silk, which gets its “stick” from a woolly, fibrous structure rather than a dedicated protein glue. Some of these cribellate silk producers even make orb webs, though they are typically oriented horizontally rather than vertically. Although at one point scientists thought that the flagelliform and cribellate silk spirals might have emerged independently, arriving at their similar structure by different evolutionary paths, in 2006 evidence from sequencing the silk genes showed that they had most likely diverged from a common ancestor about 136 million years ago.

In addition to the web-building silks, orb weavers have piriform, aciniform, and cylindrical glands. Piriform silk forms the anchors that allow spiders to attach their draglines wherever they need to. Aciniform silk is generally the toughest silk, even tougher than dragline. It’s used to wrap up prey—like the unfortunate cricket—and as a protective outer coating for egg cases, to keep them dry and safe from predators and parasites. The cylindrical glands, which only female spiders have, produce a fluffy inner layer for the egg case.

Not all spiders produce such a range of specialized silks. Mygalomorphs, a group encompassing tarantulas, trapdoor and funnel-web spiders, are sometimes said to be more primitive than the araneomorphs (the suborder that includes orb weavers and black widows), though evolution hasn’t actually left them behind. They use silk to line their nests with cobwebby masses, build triplines for prey, and protect their eggs. They also produce tarsal silk,

secreted from their feet, that allows them to walk up walls in spite of their significant bulk.

Some spiders make silks that make orb weavers look downright mundane. Ogre-faced spiders weave modified orb webs between their own legs, and use them as nets for capturing prey. Bolas spiders make a blob of glue on the end of a long string, and swing it at prey to catch them and reel them in. There are spiders that camp out in other spiders' webs to steal their prey. There are spiders that live underwater, inside air bubbles trapped in envelopes of silk. Only a tiny fraction of the world's silks have been studied.

After collecting silk from her laboratory spiders, Hayashi strings it across a hole punched in a cardboard slide. Then she can look at it through a microscope to measure its diameter, which is usually about a micron but will vary by a few tenths from spider to spider. She also has a machine that will test the silk's mechanical properties, its resilience and strength and how it fatigues over time. Some more advanced versions of this apparatus, she says, can measure the silk's performance in different humidities as well, a significant characteristic because of silk's property of "supercontraction."

As a demonstration, Hayashi offered me what looked like the world's grossest strand of dental floss. It was silk, of course, collected from regular old spider webs rolled together into a single dry, crusty-looking strand. She wrapped it around my finger and dampened it, until I could feel it begin to constrict. Unwinding, I found that the silk had changed texture entirely, becoming stretchy and floppy like an exceptionally tough rice noodle. Hayashi weighted it with a couple of binder clips when she hung it up to dry again, stretching it back to its original length so that it wouldn't constrict into a rubbery knot.

The reason for this phenomenon should be apparent if you've ever seen a spider web weighed

down by drops of rain or dew. If the silk didn't tighten when dampened, the web would sag and break under the weight of all that water. The effect is dramatic enough that Hayashi's been forced to use superglue to affix silk to cardboard slides, since the water in ordinary white glue is enough to shrink the fibers and bend the card.

The physical properties of silk are intimately linked to its evolutionary history, and knowing a little about one can tell us a great deal about the other. The Darwin's bark spider, for example, was known for its colossal, river-spanning webs long before the astounding strength of its silk was known. But not just any fiber could hold up to the strain placed on the support strands for these webs, and biologists correctly assumed that the evolutionary pressures that had created *Caerostris darwini* must have also given her a mechanically spectacular silk. When they brought samples back to the lab, they found a fiber ten times tougher than Kevlar.

It works the other way too. One of the properties that is often measured in studies of silk is birefringence, a characteristic of its crystalline makeup that causes it to bend light differently depending on what direction the light is coming from. It's possible to observe this in action, as Hayashi demonstrated. She took out one of her pre-prepared slides, a slip of cardboard with a single strand of silk suspended across a hole in the middle. Placing it under the microscope, she beckoned me over to take a look. The silk was a bright vertical line in the center of my field of vision, clearly visible. "Now rotate it," Hayashi said. As I turned the card slowly counterclockwise, the bright line of silk vanished. Then, when I'd rotated it all the way around and back to its starting position, it popped back into view.

"I'll tell you how I learned this," Hayashi said. "I was trying to mount silks on a piece of metal for the Scanning Electron Microscope, and I carefully put my fiber on there and then went to check to see if it worked. And I said 'oh darn, lost the fiber,' and went back and did it again,

and lost it again. And then by chance—I always say to do things in the exact same way every time, but I happened to put it under the microscope the other way, and there were about ten pieces of silk on there.”

Birefringence isn't just a neat party trick, though. It often occurs in materials when they're stressed, like a piece of plastic bent to near breaking. This tells us something interesting about silk: it forms under tension as it's pulled through the silk ducts. If this weren't true—if perhaps spiders squirted silk out instead of pulling it—we wouldn't be able to collect silk simply by pulling a thread, and it might not have the particular crystal structure that lends it its famous strength.

The interesting optical qualities of silk form the basis for Fiorenzo Omenetto's work at Tufts, which also draws on other natural optical materials. When I visited his office, just down the hall from Kaplan's, he had a morpho butterfly mounted on the wall. The playing-card-sized wings were vibrant blue, iridescent and jewel-like. His projects in silk optics, he said, were partly inspired by that butterfly. The brilliant sapphire color of its wings doesn't come from pigment, but from the way miniscule structures on the surface of the wing catch and reflect different wavelengths of light. The butterfly wing is made of chitin, not fibroin, but by creating similar structures in silk films Omenetto can create all kinds of optical devices. Implanted under the skin, such a structure might change color to indicate changing conditions inside the body. He can make mirrors, diffraction gratings that split light into a rainbow of colors, even holograms. Picking up a laser pointer to demonstrate, he shone it through a stamp-sized patch of silk film to project an image of the Tufts logo onto the wall.

Omenetto's work uses *Bombyx* silk because of its convenience and availability. But with spider silk, the optical story gets even more interesting. Silkworms mostly use their silk for

protection, and its beautiful sheen is more or less a happy accident. The way that spider silk catches and reflects light is a key component of its function.

There are different approaches to luring prey into a net. One strategy is to make the web as invisible as possible, so that unsuspecting insects fly into it by accident. The microscopically thin, translucent fibers of silk do this very well, and as Hayashi demonstrated, from most angles they practically disappear into the background. The sticky aggregate glue, though, poses a problem: the tiny droplets of glue scatter light, a miniature version of what you might see when a web is highlighted by morning dew. While this makes the web easier to see, and might allow an alert insect to identify the danger and turn back, that isn't quite how it works out in nature. Many spider webs reflect a great deal of ultraviolet light, which is a major component of sunlight. To insect eyes, this might suggest a forest clearing or opening in the underbrush, offering an unobstructed flight path. In the case of golden orb weavers, the bright yellow color of the webs may resemble light reflecting off leaves, which suggests to insects that they can find food there. So insects are actually *attracted* by the play of light on the sparkling web, drawn by the promise of food or a clear path to the sky, and many of them only realize their danger when it's too late to turn back.

For Cheryl Hayashi, everything starts with the spiders. She began her career in spider silk as an undergraduate, when she accompanied a field ecology professor to Panama and got up before dawn every day to spend hours watching spiders spin webs. Now a professor of biology at the University of California, Riverside, she studies the genetics and mechanical properties of silk. In 2007, she received a MacArthur Foundation grant for her work.

By decoding the genes that have produced the myriad flavors of silk in the world today,

Hayashi hopes to “unravel history,” tracing the evolution of spiders back through the millennia to discover where they came from and how they fit together. Her affection for her creepy-crawly subjects is evident, and she speaks with delighted wonder of the first time she dissected a spider and saw the silk glands for herself. “It’s the most amazing thing.”

Hayashi first worked on the genetics of spider silk in the lab of Randy Lewis, a molecular biologist at the University of Wyoming who was the first person to sequence a spider silk gene. He achieved this feat in 1990, with a major ampullate protein from *Nephila clavipes*. In 2001 he provided genetic material to a Canadian biotech company, Nexia, which they used to create a herd of transgenic goats that produced spider silk proteins in their milk. Nexia hoped to make commercial production of spider silk— or “biosteel”—viable, but ultimately failed to produce a satisfactory product. When the company went under, the goats made their way back to Lewis’s lab, where they produce spider-protein-laden milk to this day. In addition to ongoing work with a variety of silk genes, he’s joined efforts to produce silk proteins in alfalfa and silkworms, continuing to chase the dream of mass-producible spider silk.

When Hayashi started doing research in silk genetics, as a post-doc in Randy Lewis’s lab, most of the work in the field was still focused on dragline silk, and concentrated on a handful of species including golden orb weavers. For people who were mostly interested in applications for silk, this was all very sensible. Dragline is both incredibly tough and relatively easy to extract, since all araneomorphs produce it constantly and leave a trail—like an automatic safety line—everywhere they go. Golden orb weavers were also a natural choice because they’re common all over the world and their large size and large silk glands make them easy to work with.

Coming from a field ecology background, though, Hayashi wanted to look at a wider

range of silks, and to study spider species that hadn't yet been explored. She began by decoding a gene for capture spiral silk, to provide a contrast to the dragline silk Lewis had been working with. It turned out to be composed of similar building blocks, four or five basic genetic sequences arranged in long, repetitive chains to produce a variety of properties. Then she started to look at spiders that were “way out there” compared with the golden orb weavers, and realized that world of spider silk was far more complex than she'd thought. She'd hoped that silk research would give her a “nice simple lens through which to view biological diversity,” but the spiders had other plans: “I had no idea how rich the system was going to be.”

Hayashi now studies a variety of silk genes to see how this vast array came about. When she begins a new research project, her first question is always which spiders to use. With so many species to choose from, a few constraints are necessary. She makes sure to select species that aren't rare or endangered, saying “I'm sort of making the decision that they're donating their bodies to science, so we try to pick ones that are fairly abundant.” This is also important in a practical sense, as she needs to know she can get several specimens in order to make an effective study. Bigger spiders are better. Hayashi and her students spend a lot of time dissecting spiders to get at their silk glands, and with big, meaty spiders it's easier to see what you're doing. Hayashi looks for spiders that do interesting things with their silk, things that might give hints about how spiders evolved and when specific traits might have developed.

Research subjects come from a variety of sources: animal suppliers, colleagues in different parts of the world, and the researchers' own backyards. Where possible, Hayashi prefers to use local species that she can collect herself—black widows unearthed in secluded corners of the UC Riverside campus, or tarantulas from the surrounding desert. “Wherever you see a field gone wild we'll be looking for spiders there.”

She gets some funny looks, poking around dumpsters and loading docks at midnight, when black widows are most active. “We get a lot of questions—people say ‘did you lose your contact lens? Can I help you find something?’ When we tell them what we’re looking for...they think we’re crazy.”

People also bring her spiders, which explains the collection of salsa jars and other oddball spider-delivery containers that has accumulated on the bottom shelf of the rack where her spiders live. Some people even call her out to their property to retrieve the spiders herself. Unlike spider biologists, who are concerned with maintaining healthy populations, many property owners are less than enchanted with their arachnid tenants. “You’ll take them all, right?” is a typical question, to which Hayashi responds “We don’t have an army working here!”

In her lab, Hayashi prepared a little dish filled with saline solution. “This next part is not for the squeamish,” she said. Using a pair of forceps in each hand, she began her dissection, neatly severing the black widow’s head and gangly legs from her pearl-like abdomen. It happened too quickly to be stomach turning, but in a moment the intent of her warning became clearer. Placing the abdomen in the dish, she grasped the shiny black skin with her forceps and tore it open. “We peel her like a grape,” she said, stripping away the delicate outer layer in glossy chunks. In about a minute *hesperus* lost her inky skin and characteristic red hourglass, everything but a little patch right around the spinnerets that Hayashi left intact to avoid damaging the silk glands.

The solution clouded as she worked, scraping away yellowish fatty tissue and blobs of glue to reveal the abdomen’s internal structures. She replaced the solution twice, swishing the tattered spider remnant back and forth to rinse it clean. Her tweezers picked out blobs of undeveloped eggs before pulling them away to expose the silk glands buried beneath. “It’s a

never-ending battle to keep our forceps sharp enough to do this,” Hayashi said, clearing away a few more stray eggs and blobs of tissue to make the glands easier to see. This spider was an older one, she told me, and not preparing to produce an egg sac. As a result, her glands were smaller. Still, the major ampullate gland was large enough to pick out with the naked eye, maybe a quarter of an inch long. Elongated and pointy at the ends, it resembled the cells in orange pulp, and like orange pulp it gleamed translucently amid the milky, opaque body tissue.

Continuing to dig through the spider’s guts, Hayashi pointed out the other glands. The minor ampullate gland, as the name suggests, is the same shape as the major ampullate gland but smaller. The aggregate silk glands—tiny glands that form grainy-looking clusters—produce the liquid glue that snares prey. Egg-case silk is produced in long, loopy glands that look like a bowl of ramen noodles. “When we’ve been doing a series of dissections, it’s funny, everybody has the same experience—you’ll go home, and when you close your eyes you still see it. It’s burned into your retina for a while. When your project is major ampullate glands you’re going to see that shape.”

The primordial silk gland was little more than a round sack of proteins with a string hanging out of it, but a great deal of development and innovation has occurred in the intervening eons. While most spiders still have some of the ball-shaped glands, Hayashi says, “From there the morphology has gone crazy. We’ve got ones that look like party balloons, you know, the ones a clown would take and twist into a dog. You have another that has a hook on it—why? I don’t know why! I still don’t know why.”

In a way, it makes sense that a civil engineer would be interested in spider silk. Spiders, after all, are among nature’s greatest engineers. Bridge builders could do worse than be inspired

by their sturdy webs. And according to Markus Buehler, a professor in the MIT Department of Civil and Environmental Engineering, silk has a number of material properties that make it very good at what it does—sometimes in surprising ways.

He became interested in silk, he says, because he wanted to understand how nature builds things. In nature, unlike in the lab, there are no high temperatures, powerful solvents, advanced materials, or elaborate manufacturing processes. But in spite of these limitations, life has developed all sorts of highly functional building materials from a few basic building blocks. Buehler is particularly interested in structural proteins, which include collagen (found in joints and connective tissue) and keratin (responsible for hooves, fingernails, and hair) as well as silk. Silk is particularly interesting, he says, because it's very simple—its enormous array of properties emerges from just a handful of amino acids.

Unlike the biologists, who begin with spiders when trying to figure out how silk is made, Buehler built his own silk protein from the ground up. Beginning with basic tools from quantum mechanics, and theories of how atoms and molecules behave together, he designed a computer model that simulates the structure of silk proteins in the finest possible detail. His model calculates exactly how hundreds of thousands of individual atoms in a strand of silk interact, allowing him to see how the very smallest components of silk work together to form larger structures. After verifying the model by comparing its properties to those observed by silk researchers, he was able to use it to uncover some very interesting facts about silk.

To greatly oversimplify, engineers learn by breaking things. Just as one might disassemble an engine to see how it worked, Buehler tested his silk model by stretching and straining and breaking it every which way. One of the interesting things about silk is that it breaks very gracefully, stretching and failing gradually instead of snapping all at once. Another

is that, given opportunity to recover, a stretched and frayed strand can reassemble itself and return to its original state. This is possible, it turns out, because silk is mostly held together with hydrogen bonds, which are very weak. This weakness allows them to stretch and distort, certainly, and also to re-form easily once broken. But if these bonds are so weak, then how could they produce a structure that's so strong?

The beauty of biological materials is that they're designed to tolerate a degree of imperfection. Nature is chaotic, and life is a constant game of making do with bad conditions and limited resources. Cracks, blemishes, and impurities are only to be expected, not to mention disease and injury. If a bridge was built according to such standards, no one would dare set foot on it. But biological systems can heal and self-repair, and even apparently inert silk can recover from minor damage.

When several hydrogen bonds are grouped together, they reinforce and stabilize each other to become much stronger than any individual bond. Buehler found that the proteins in silk are organized into interlinked crystalline sheets that provide strength, combined with looser, less organized coils of proteins that allow the silk to stretch and give. Combining these two types of structures in the correct proportions resulted in a strand of spectacular strength and toughness, with different properties depending on the amount of each type and the size of the crystals.

What all this seems to mean is that a clever structure can overcome poor building materials and weak bonds. Buehler thinks this principle could be applied much more broadly. If simple proteins linked with feeble hydrogen bonds can outperform steel and Kevlar, perhaps other weak materials could be made strong and tough as well. Consider, for example, silica. As a chief component of sand, this mineral is common as dirt. Its brittleness, unfortunately, limits its usefulness as a structural material: glass breaks. But if Buehler is right, maybe someday we'll be

able to produce glass that performs like steel.

At the same time, human technology doesn't need to be bound by nature's constraints. Applying lessons from silk to the engineer's entire arsenal of materials and processing techniques could produce new generations of super-materials, better than anything we've imagined so far. If we can figure out how to do it, it could mean stronger, cheaper, and more environmentally friendly materials in our future.

Spiders could be the key to this future, but, for the moment, the spiders' knowledge still eludes us.

Actually assembling silk-like structures is one area where nature still has scientists stumped. The silk solution is a mixture of protein and water, but mostly protein. As Hayashi pointed out, the very best biochemists haven't been able to produce such a concentrated silk solution. "Picture a bodybuilder trying to make a protein shake—there's only so much protein they can get to dissolve in that shake, and then the rest is just undissolved powder and it's gross." It's an utter mystery how spiders keep the silk solution stable in liquid form.

The exact details of spinning silk are also a mystery to technology, and scientists have never managed to do it as well as spiders do. There's something about what happens inside that spinning duct that human efforts to spin silk from protein solution have thus far failed to match. It's easy enough to squirt out some silk and make a thread, but our best spinning methods have only produced a fraction of the strength spiders can achieve.

To return to Hayashi's freight train analogy, it's as if our efforts so far have only succeeded in putting together a train with half the cars upside down. Somewhere in the duct between silk gland and open air, the liquid silk is transformed into the complex, crystalline

strand we're familiar with—and whether the cause is the environment inside the duct or the shear forces as the silk passes through, it works some kind of magic on the jumble of liquid fibers inside the silk gland that we have yet to fully comprehend.

Nature has been working on the problem of silk for a long time. Where human progress is sporadic, focused, inspired, stumbled upon by chance or achieved through years of concerted effort, evolution is at once random and inexorable, a plodding brute-force algorithm for survival. It has ground on for hundreds of millions of years, molding and developing that primeval silk into a million specialized progeny.

Hayashi swirled the ragged scrap of black widow in her dish of saline, clearing the last remnants of tissue and eggs from the glands. At this point, it had been about ten minutes since the start of the dissection—a leisurely pace by Hayashi's standards. When she wants to identify the genes that a gland is expressing, she has to work quickly so that the RNA doesn't degrade. That means taking the live, unconscious spider, killing, peeling and cleaning it, extracting the silk glands and getting them into the freezer within five minutes. This spider was already long past deadline.

It was just as well. Hayashi's final rinse was just a little too rough, separating the silk glands from the spinnerets and rupturing the major ampullate gland. "I'm going to say I broke it on purpose so you could see the liquid silk oozing out," she said with a laugh. The liquid silk was clear just like the salt solution in which it was submerged, but much thicker and denser. It spilled out to form a viscous pool around the gland, like a puddle of molten plastic. Hayashi handed me a pointed tool. "If you poke at it and draw the needle up out of the liquid you'll actually draw up a little fiber," she said. I tried it, prodding at the submerged puddle. To my surprise, the silk had already solidified, the super-saturated protein congealing as soon as it was

released from the gland. In just a few seconds, the liquid silk solution had acquired the approximate consistency of rubber cement, confounding my attempt to turn it into a shimmering crystalline strand. Even here, dismantled in a dish of saline, the spider kept her secrets.

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- i The story of the remarkable tapestry was reported in a wide range of publications, from newspapers and science publications to textile hobbyist magazines. Among the best articles were “Gossamer Silk, From Spiders Spun” by Randy Kennedy at the *New York Times*, and “1 Million Spiders Make Golden Silk for Rare Cloth” by Hadley Leggett at Wired Science.
 - ii P.L. Simmonds, “Spider Silk.” *Journal of the Society of Arts* 35 (1886-1887): 958.
 - iii Burt G. Wilder, “On the Nephila Plumipes, or Silk Spider,” *Proceedings of the American Academy of Arts and Sciences* 7 (1865-1868): 52.
 - iv Leslie Brunetta and Catherine Craig, *Spider Silk*. New Haven: Yale University Press (2010). 57.

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