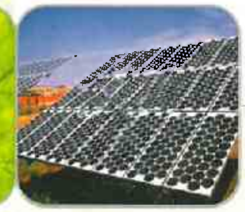
"Provocative, and could well provide one viable answer to the wake-up call that Rachel Carson sounded . . . in *Silent Spring*."

—*SAN FRANCISCO CHRONICLE*

BIOMIMICRY



Innovation Inspired by Nature

JANINE M. BENYUS

Now a two-hour public television special on *The Nature of Things* with David Suzuki

In a way, this made sense. Globular molecules floating in water would be a good way to store the protein in the gland. When the spider twisted and scurried through its days, the globules would simply roll with the punches, and the spider didn't have to fear "becoming constipated with its own silk" if the liquid protein somehow sheared into fiber form. But if there were *only* globular molecules, thought Viney, why was the polarizing light microscope showing undeniable evidence of rodlike structures?

"The mystery unraveled for me when I attended a lecture by one of my colleagues in the bioengineering department," he says. The speaker was talking about actin, a protein that self-assembles to help form our muscles. Actin is essentially a globular protein, but the balls hook up to one another—like the baubles in a kid's pop-bead necklace—to form a chain. As Viney looked at the cartoon graphic, something breached and leaped from his subconscious.

"There was my rod!" he said.

Viney turns on his computer and we look at cartoon depictions of his evolving theory of spider silk formation. He now hypothesizes that the raw liquid silk leaves the gland and travels through a thin duct just before entering the spinneret. As it squeezes through the duct, water is wrung out of the protein and calcium is added. (Calcium is what allows actin globules to hook up, so Viney thinks it may also be at work here.) The globules hook up in a pop-bead necklace, making the solution one thousand times less viscous, because the rodlike assemblies can now slide past one another. It's analogous to putting lanes of traffic on a highway sliding past one another, versus the mess that is a laneless, lawless Manhattan jam.

Connected, aligned molecules are not only easier to push through the spinneret, they are also more susceptible to the shearing action that turns liquid protein into fiber. Because the globes are unable to roll out of the way, the squeeze through the spinneret disrupts the water-loving residues on their periphery, exposing their water-fearing parts.

"These hydrophobic parts go 'ARRRGG!' and cluster together as tightly as they can," says Viney. They assume a zigzag shape, folded accordion-style into pleats. One pleated sheet stacks on top of another, as close as they can get to lock out the water. The water-loving portions of the proteins remain loose and curly at the edges, forming the springy matrix that the accordion crystal parts are embedded in.

Viney's model has a pleasing simplicity and completeness: The

globular proteins line up into a pop-bead necklace, which squeezes through the spinneret to become a silk fiber. The final product is partly flexible and partly rigid, like a reinforced Slinky. The amorphous part gives, but the stiff crystalline domains don't give. When the fiber becomes notched, a crack or tear gets interrupted by the crystalline regions and can't propagate. The model also explains why the material goes from being a soluble liquid to an insoluble fiber. Once the water-fearing portions of the proteins crowd together, they resist water, ensuring that the silk won't fall apart.

Nevertheless, it's only a model, and some, like silk researcher Randy Lewis, don't agree with it. Lewis feels he has evidence that there are actually two proteins rather than just one that make up spider silk. "In the two-protein hypothesis, Viney's pop-bead model doesn't make sense," says Lewis. But other researchers, including Viney, are still not convinced of the existence of two proteins. While the jury is out and the debate is lively, all the investigators in spider silk research encourage one another to keep theorizing. When you think about what it could mean in terms of sustainable fiber manufacture, this research, tough as it is, is definitely worth it.

Consider: The only thing we have that comes close to silk in quality is polyaramid Kevlar, a fiber so tough it can stop bullets. But to make Kevlar, we pour petroleum-derived molecules into a pressurized vat of concentrated sulfuric acid and boil it at several hundred degrees Fahrenheit in order to force it into a liquid crystal form. We then subject it to high pressures to force the fibers into alignment as we draw them out. The energy input is extreme and the toxic byproducts are odious.

The spider manages to make an equally strong and much tougher fiber at body temperature, without high pressures, heat, or corrosive acids. Best of all, says Viney, spiders don't have to drill offshore for oil to produce the silk. They take flies and crickets at one end and process a high-tech material at the other end.

If we could learn to do what the spider does, we could take a soluble raw material that is infinitely renewable and make a superstrong water-insoluble fiber with negligible energy inputs and no toxic outputs. We could apply that processing strategy to any number of fiber precursors. Imagine what it would do to our fiber industry, which is now heavily dependent on petroleum, both for raw material and processing! To break that dependency, says Viney, we have to become spider's apprentices. "If we want to manufacture something that's at least as good as spider silk, we have to duplicate

the processing regime that spiders use. We have to mix up a batch of precursor and duplicate the physical journey from the glands out to the spinnerets. It's that journey that helps impart a certain microstructure to the fibers.

"When we scale this journey up for industrial use, we have to be able to give the manufacturing crew exact specifications: what concentration of protein they should use, how big the rods in the liquid crystal should be, how much calcium they will need, how much water they should squeeze out, and how fast they should spin out the fibers to obtain a silk with desired properties. By tweaking any one of those variables, we may be able to customize the silk for different uses. For me, the processing is the really intriguing part of this story."

While Viney works on scaling up the process, there are other scientists examining the protein precursors that will make all of this possible. Silk is after all a biological material—a protein that self-assembles, under a gentle shear force, into a fiber. The protein hunters I visited are deep in the gland of the orb weaver, hoping to characterize the source of silk and find a way to produce it without Tiny's help.

Silk Maneuvers

When I first laid eyes on David L. Kaplan, it was in a photo, one of those rare shots that captures a person's essence. He was standing behind a glass case, peering not at the camera but at a six-inch-long orb weaver spider. His eyes absolutely shone with entrancement—like the eyes of a child at the zoo, staring through a window at an animal that is staring back at him.

Kaplan is entranced for nearly fourteen hours a day, arriving at the U.S. Army's Research, Development and Engineering Center in Natick, Massachusetts, long before his employees arrive, and leaving long after they leave. "It's never a dull moment," he tells me. "You learn one thing about nature, and you come up for air with ten more things to pursue. We are right on the verge of so much, so much that has no precedent."

Kaplan is always on the move, usually trailing a person or two who wants to see him. He directs forty-five people in all (those I talked to rave about him) and is responsible for overseeing the technical aspects of every study going on in the biomolecular materials department at Natick. One of his favorite projects is the quest to synthesize a gene for a silklike protein.

The army wants a fiber that protects better than Kevlar, and is willing to look to nature for it. They want it to be ethereally light, yet strong enough to bundle into cables for suspension bridges or for bungees that hook fighter planes as they scream onto aircraft carriers. And yes, they suppose it would be nice if its manufacturing process was environmentally friendly to boot. Kaplan explains how his team plans to deliver.

"We first had to find a way to efficiently remove the liquid protein from spider glands without it turning to silk, and we did, but this gave us only an infinitesimal amount. If we ever hoped to commercially produce this stuff, we knew we would have to take a genetic approach.

"Our first shot out of the barrel was to isolate the full-length natural gene—the native gene. It's a whopping nine to ten kilobases [a kilobase is a thousand DNA subunits strung together] and a nightmare to work with because it's highly repetitive and prone to deletions and recombinations when it's expressed by *E. coli*. We're still doing some work on the wild gene, but we realized we'd increase our chances of producing some kind of silk precursor if we also tried to synthesize a simpler gene on our own.

"The techniques are not there yet to synthesize a DNA fragment as long as the natural one. Instead, we could only hope to synthesize a small DNA fragment [with an oligio machine], multiply it, and glue those fragments together with ligases [enzymes that help the fragments to combine]. Deciding *which part* of the long genetic sequence to choose for synthesizing—that was a very educated guess. You learn what you can, and ultimately you go with your gut, and that's where science turns to art. We were also guessing when it came to the different ways we glued the DNA fragments together. We matched the codon preferences for *E. coli* as best we could and then we started the arduous part—trying to coax *E. coli* to accept our homemade DNA and make the protein for us.

"To make a long story short, it worked. *E. coli* expressed the protein, giving us something to test and learn from. We want to see what properties it has, and then figure out why: What is it about this amino acid sequence that might have given rise to these properties? What we're searching for is some correlation between the structure of the protein—how its amino acids are arranged—and its function. We're after some rules of thumb. Ultimately, we'd like to be able to tell a fiber designer, if you want resilience, try this repeating sequence of amino acids followed by that repeating se-

quence. Slowly but surely, we're building an information infrastructure—a knowledge base that will allow us to make materials the way nature does. What we learn from spiders will be helpful for any polymer processing. We're not the only ones by any means. Randy Lewis is working with the wild gene out your way," he tells me.

In the windy town of Laramie, Wyoming, Randolph V. Lewis has the sequences to what he believes are two proteins at work in the spider's gland. His team at the University of Wyoming used genetic engineering "probe" techniques like those used on abalone shell to isolate portions of the two genes that code for the thread-producing proteins. They then inserted these gene fragments (each representing only about one third of the real genes) into *E. coli* and successfully expressed proteins. "We even processed them into fibers, but they didn't match the qualities of dragline silk. Our truncated genes were obviously missing something important." Now Lewis's team, like Kaplan's, is working to synthesize a gene that may come closer to the qualities materials scientists are looking for.

Lewis is applying for grants that will allow him to analyze different kinds of silk from different kinds of spiders, hoping to learn more about the structure-function relationships Kaplan talked about. He, too, is trying to come up with the ultimate cookbook that will allow fiber manufacturers to look up the properties they want in a silk protein, then find the amino acid recipe for those properties. Want a better fiber? Start with a better protein, and template your fibers to taste.

Lewis's foray into different spiders and different silks makes me wonder whether we are studying the best possible models. All of our current knowledge comes from studies of only two kinds of threads spun by fewer than fifteen species of orb weavers, a subset that makes up only one third of all thirty thousand described spider species. Is an even better prototype waiting out there somewhere?

Back in Seattle, I pose this question to Christopher Viney, whose normally cheerful, mischievous face clouds. He thinks carefully. As in all of biology, model systems are chosen because they are easy to work with, he explains, and to some extent because other people have already set the track for you. But yes, there probably is a stronger, tougher, stiffer fiber being produced right this minute by a

spider that we know nothing about. A spider whose habitat may be going up in smoke.

"And do I feel an urgency to learn what I can before these models go extinct?" he asks. He looks around his office, out the window, and after a while, back at me, as serious as I've yet seen him. "Well," he says, in that way the British have of retiring from a topic. "I suppose it won't hurt to have my metallurgy wait a few more years." He stands and I stand, and for the first time all day, we check the clock.

Horns for the Rhino's Dilemma

With species like the rhinoceros, the countdown to extinction is not just speculation—it's an ongoing spectacle. There are only 2,300 black rhinos in all of Africa, down from 65,000 as recently as 1970. Zimbabwe's wild population, thought to be 1,400 in mid-1991, is down to a shocking 250 animals. Asian rhinos are faring no better. The Sumatran rhino population has been cut in half in the last ten years, with numbers now totaling fewer than 600.

The reason rhinos are in decline is because of the five to ten pounds of protein that shapes itself into a horn (or two) protruding unicorn-style from the rhino's head. Poachers risk being shot on sight to kill a rhino, but if they can get the horn and get away, they earn an amount equal to a year's wages. The horn lords reap the real money, though—they sell the horns on the black market for tens of thousands of dollars each. Half of the horns used to go to the Middle East, where they were crafted into dagger handles that Yemeni men strapped on during their ritual initiation into manhood. A single dagger may have cost $30,000, but the status conferred on the man who wore it was deemed worth the price. These days, most horns find their way into Eastern medicines. Rhino horn powder is thought to cure stomachaches, skin blemishes, a lackluster libido, and even, purportedly, a lousy singing voice.

Although legal sales have diminished since the 1977 international ban on sale of rhino horn (CITES), rhinos continue to be killed and the horns bled off slowly to the black market. Poaching has gotten so bad in Namibia that officials began a dehorning program, sawing off the trophy as a way to spare the animals' lives. Perversely, the slaughter continues, this time with dehorned rhinos. "What we now think is that the horn lords are hoping to see the rhinos go extinct, which will

increase the value of their stockpiled horns," says Joe Daniel, a rhino researcher at Old Dominion University in Virginia.

I traveled to Old Dominion because I had heard that Daniel, a zoologist by training, had teamed up with a metallurgist named Ann Van Orden, and they had a plan to help stop the slaughter. It would be biomimicry at its best.

What the world needs, say Daniel and Van Orden, is a facsimile rhinoceros horn that is inexpensive to make. "Flooding the market with this horn, identifying it as a facsimile and hoping to get other cultures to accept it—that may be our only option. Or rather, the rhino's only option. If we work it out so that horn lords are able to make a profit in volume selling, they may decide it is no longer worth their while to risk the poaching."

History bears them out. Whenever we have given people convincing substitutes for a coveted material, it has helped to conserve the original. Rubber trees were not so heavily tapped, for instance, nor pearls so voraciously fished, after artificial substitutes became available. The key is to offer a duplicate material that is almost as lustrous, almost as rubbery as the real thing. The lower price speaks for itself, and in the process, native organisms are freed from our hungry grasp.

But rhino horn is an especially tough case for mimickers. Haloed as it is with magical and medicinal qualities, consumers are loath to accept any substitutes. When I asked what the horn is made of, Ann Van Orden drummed the table with her fingernails. "It's keratin—the same tough, fibrous protein that's in your fingernails and your hair. There's absolutely no proof that rhino horn can do what it's touted to do, no more than your ground-up fingernails could. It's not the keratin itself, however, but the unique way that it's structured that gives rhino horn its coveted strength and luster. If we could induce keratin to self-assemble into that structure, we'd have the viable substitute we need."

The two collaborators who hope to pull this off met serendipitously when Van Orden's husband, a physicist at Old Dominion, came to Daniel's brown-bag seminar on infrasound and rhinos. When Daniel mentioned he needed some help preparing samples of rhino horn for the microscope, Ann's husband suggested her for the job. Van Orden picks up the story: "I was working at Langley Research Center at the time studying corrosion, and needless to say, rhinos were not in my annual work plan. So I code-named my folder

Rufus (the name of the bull at Virginia Zoological Park who had donated a broken-off piece of his horn) and kept it to myself."

At lunch, Van Orden lowered an unpolished chunk of Rufus's horn into my palm. "Be careful," she joked. "What you're holding is worth about ten thousand dollars." At the fractured edge, I could see fibers called spicules sticking out. They were tipped with points at each end, like porcupine quills. "This is an ingenious design. As one of those spicules tapers to a point, it makes room for another tapering spicule to take off. In this way, the fibers are interdigitated, and that's why you see a zigzag break when the horn breaks—some points are sticking out, some holes are left behind."

But where's the hair? I ask her. In almost every book I'd read (and one that I'd written), rhino horn had been described as bundles of hair tightly packed together. She smiled. "I know, but that's not what I saw when I sectioned it." Chances are, no biology department had ever sectioned and prepared the horn for microscopy in exactly the way Van Orden did. She treated it as she might a piece of metal that had corroded. She sawed a cross-sectional slice and sanded it, starting with 300-grit sandpaper, working up to 1,200-grit, and finally polishing it to a scratch-free finish with a diamond slurry and an alumina polish. She then examined it under a polarizing light microscope (the type Viney used on spider silk) as if it were a piece of metal. A color photo of her cross section was hanging in her office with a blue ribbon on it, having won first prize in Polaroid's science photography contest.

The horn was indeed beautiful in cross section. It was as if someone had taken a bundle of solid, copper-colored quills and cut across them, leaving a landscape of what looked like cells. The softer centers of the spicules had given way under the sander, leaving little concave depressions in the middle of each cell. As Van Orden explained, the concave depression is the central core of the spicule; that core is a fiber that grows from a follicle at the base of the rhino's horn. Around this core, keratin-producing cells—now dead, flattened, and cornified like skin cells—lay in concentric fashion, looking like growth rings on a tree trunk. They produce what amounts to a hard, multilayered keratin sleeve around each fiber. Around the outside of this sleeve, there's another kind of keratin, also fibrous, that serves as the matrix or mortar between the spicules. Despite what all the textbooks said, the horn was not hair at all; it was a composite made of two forms of keratin in the same material.

When Van Orden looked at the magnified horn slice with her materials scientist eyes, she immediately recognized the pattern: "It looked just like the graphite–fiber-reinforced composite we use for the skin of the Stealth bomber! You take graphite fibers, which are very stiff and inflexible—they break before they bend—and encase them in a resin which is flexible—it bends before it breaks. You wind up with something rigid but very tough to break. That's why composites are so wonderful; they add up to something more than the sum of their parts."

Besides the Stealth bomber, graphite-reinforced composites are also used in the masts on America's Cup sailboats, the bodies of Formula One race cars, high-end guitars, tennis rackets, and Boeing's new lightweight 777 airplane, which will fly farther and faster on less fuel. "In engineering this composite, it seems we've coevolved," says Van Orden. "We've invented something nature has already been using for sixty million years."

Next she showed me a picture of another kind of composite—silicon carbide fibers embedded in aluminum oxide (a ceramic matrix)—and pointed out the differences. "We make this composite by laying fibers down by hand and then putting a block of ceramic on top. We combine the two under pressure and heat, so the ceramic resolidifies in and around the fibers. We have to be careful to keep the fibers a certain distance apart, though, because if they diffuse into one another under those extreme conditions, the resulting composite is not as resistant to breakage."

Because our heat-and-beat processing doesn't lend itself to that level of control, we can't optimize the way nature can. The rhino's horn, which self-assembles from within, has densely packed spicules that are carefully spaced and not touching. This better "packing density" makes for a tougher horn. Matrix and fiber are also chemically similar, and consequently are able to bond well at the interfaces.

Another difference between our composite and nature's is in the shape of the fibers. In cross section, the synthetic fibers in the carbon-graphite composite are uniformly round, while the rhino fibers vary in size and shape. What *does* remain uniformly thick throughout is the mortar—the keratin matrix.

"Again, this makes great sense from a materials science point of view," says Van Orden. "It may be that a certain thickness of matrix has to be there as a buffer against insults. If the fibers were abutted together without a buffer, and you broke one, you could break them all." This way, the keratin matrix acts like the mortar in abalone shell; it interrupts cracks coming from the side, and the stress is redistributed, giving the horn torsional strength.

But what does this design buy the rhino? Daniel tells me that cow rhinos wave, joust, and stab with their horns to protect their calves from attack. Bulls use it to drive off interlopers in territorial disputes, and all rhinos use it for digging in the ground. To be able to do all this, rhino horn has to possess strength when pushed from the tip as well as from the side. This head-on strength is called compressive, and building a spicule shaped like a porcupine quill is a great way to achieve it. Van Orden shows me a close-up of a horn fracture, in which the tips of the quill-like spicules are all bent. "Here's where the compressive strength comes in—instead of being flat topped, and taking all the pushing energy directly, it's tipped with a point. The point simply bends or breaks, but doesn't transmit the load all the way down. There's a lesson we should apply right now to our composites, which are rarely blessed with both compressional and torsional strength."

What has Daniel and Van Orden really excited is a third trait of rhino horn that our materials don't possess—the ability to heal. The evidence of self-healing was hiding in the beautiful Polaroid picture. "If you look closely, you'll see a crack that has infilled with polymer, essentially healing over," says Daniel. "But as a biologist, I considered this impossible, because as far as we know, there are no living cells in the horn—only dead tissue. Or so we thought. The idea that there might be something alive in the horn opened the possibility that we could take a sample of those cells and try growing, through tissue culture techniques, a horn in vitro."

In search of living cells, Daniel went to an exotic-animal breeding facility in Texas to perform a needle biopsy on a rhino. "The veterinarian agreed to call me when he was going to do a checkup, because they don't like to anesthetize the animals very often. I flew down there, took the horn sample, and then placed it into a liquid growing medium. If keratinocytes [cells] had been present, they would have grown out into that medium. Unfortunately, that didn't happen. Now I'm waiting for another rhino to need its shots so I can fly down for another biopsy."

In the meantime, Daniels and Van Orden are investigating other options for making a horn facsimile. "Say the healing isn't caused by living cells," says Daniels. "Say instead that the material for the infilling is cannibalized from somewhere nearby. Say a portion of the

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horn depolymerizes [breaks down into its building blocks], flows to the crack, then repolymerizes to fill in the gap. That got us thinking—maybe we could do something similar. Maybe we could depolymerize rhino keratin and induce it to reassemble around a core of rhino hair."

Daniel's idea is to practice on something like horsehair. Horses have two types of hair—the rough tail hair that is used for violin bows, and the softer hair of the coat. First he would depolymerize the coat hair into a liquid, then lay the tail hairs side by side in the liquid solution and put it under pressure. The keratin would, it's hoped, polymerize around the nucleus of the larger fibers, forming connectors that gather all the hairs together.

The same sort of technique is already being done with bone, says Van Orden. "A dental surgeon can take bone and treat it so that just the hydroxyapatite is left. To build up the jawbone foundation beneath an implant, for instance, they'll cut open a patient's jaw and put in this hydroxyapatite. When the person's bone cells come in contact with this hydroxyapatite, they say, 'Hey, we forgot to calcify this!' and they'll make new bone in that spot. We're hoping that our liquefied horsehair cells might see the tail hairs and say, 'Oh, hair tissue. We forgot to gather all this together.' If it works with horsehair, we'll do the same thing with rhino keratin—we'll provide the hair, the keratin, and the right conditions and say, 'Structure yourself!' "

Although it seems like a blue-sky idea to grow rhino horns from scratch this way, it may not be. "Heck," says Van Orden with her trademark enthusiasm. "If you told us thirty years ago we'd be putting graphite fibers in a resin matrix, it would have seemed far-fetched. Today we're playing doubles tennis with composites like this."

Thirty years ago, there were many, many more rhinos than there are today. No matter how far-fetched or whatever else may come from this research in terms of composite innovations, any attempts to stop rhino slaughter will be well worth the effort. In fact, it's one of the best uses of biomimicry I can imagine. This time, we're learning to imitate an animal not to save ourselves (directly) but to save another species from "an end to birth." It's biomimicry come full circle, a glimpse of the good we could do with this new science if we choose to.

That sets me thinking about which agencies, or which foundations, have had the foresight to sponsor this type of research. When

I ask Daniel and Van Orden, they lock eyes across the table and simultaneously make the hand sign for zilch. Their rhino horn work has received no official funding. For now, their quest is a labor of love and conscience, a pro bono for Rufus and the thinning herds whose horns alone, strong as they are, cannot protect them now.