UNTANGLING SPIDER BIOLOGY

Genomics begins to unravel the roots of spider diversity and the recipes of their silk and venom

By Elizabeth Pennisi
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or a display of nature’s diabolical inventiveness, it’s hard to beat spiders. Take the reclusive ogre-faced spider, with its large fangs and bulging, oversized middle eyes. Throughout the tropics these eight-legged monsters hang from twigs, an expandable silk net stretched between their front legs so they can cast it, lightning-fast, over their victims. Showy peacock spiders, in contrast, flaunt rainbow-colored abdomens to attract mates, while their outsized eyes discern fine detail and color—the better to see both strutting mates and unsuspecting prey. Bolas spiders, named for the South American weapon made of cord and weights, specialize in mimicry. By night, the female bolas swings a silken line with a sticky ball at its end while emitting the scent of a female moth to lure and nab male moths.

Among spiders, “Every group has a weird story,” says Hannah Wood, a spider researcher at the Smithsonian Institution National Museum of Natural History (NMNH) in Washington, D.C. Spiders’ universal ability to make silk helps explain their global success—an estimated 90,000 species thrive on every continent except Antarctica. This material, used for capturing prey, rappelling from high places, and building egg cases and dwellings, is itself fantastically diverse, its makeup varying from species to species. The same goes for venom, another universal spider attribute—each species makes a different concoction of up to 1000 different compounds.

Called ogre-faced spiders because of their looks, these gangly arachnids are also known as net-casting spiders for their hunting technique.

A milestone came in 2014, when two reports in Current Biology “turned spider evolution upside down,” says Gustavo Hormiga, a spider systematist at The George Washington University in Washington, D.C., who co-led one of the studies. Instead of using the usual small set of DNA markers, both teams compared hundreds of genes from up to 40 spider species to build a family tree that included all the web builders. Contrary to earlier studies, the analyses divided orb weavers, the many spider species that make the classic spiral webs and cobwebs, into two groupings and put them on very different branches of the tree. Orb weavers that produce fuzzy, sticky fibers called cribellate silk ended up in a part of the tree that included all the web builders. The analyses “never really worked that well,” says NMNH evolutionary biologist Jonathan Coddington. Now, more powerful genomic tools are beginning to make sense of the tangle. “After years of effort, all of a sudden pretty reasonable [family trees] are coming out,” says Linda Rayor, a behavioral ecologist at Cornell University.

Today, taxonomists recognize three spider groups. The Mygalomorphae—ground-dwelling creatures characterized by fangs that point straight down—include about 2500 species, including tarantulas and so-called trapdoor and funnel-web spiders. Another group, Liphistiidae, consists of 97 species, many of which also build trapdoors to capture prey. The third group, the Araneomorphae, includes 5500 jumping spiders, 4500 dwarf spiders, 2400 wolf spiders, and thousands of web spinners.

Within those three groups, researchers have tried to classify species by the direction of their fangs, the shape of their sexual parts, and other aspects of their appearance or behavior. They also enlisted molecular methods beginning in the early 1990s, when arachnologists identified a half-dozen short, conserved spider DNA sequences that still had enough variation between species to derive relationships. Yet those analyses “never really worked that well,” says NMNH evolutionary biologist Jonathan Coddington. Now, more powerful genomic tools are beginning to make sense of the tangle. “After years of effort, all of a sudden pretty reasonable [family trees] are coming out,” says Linda Rayor, a behavioral ecologist at Cornell University.

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That unexpected breakup has two possible explanations. Either the behaviors, body structures, and materials used in weaving webs evolved twice, or web capabilities evolved much earlier in a common ancestor.
of both branches and were lost in many species on the cribellate web weavers' branch. Bond, who led the other study, thinks it is more likely that orb weaving evolved only once, in a common ancestor. In a later study comparing almost 3400 active genes in 70 spider species, Bond's team found that mostly webless, ground-dwelling arachnids such as wolf spiders and jumping spiders diversified much more quickly than web weavers, perhaps because they were able to exploit a plethora of new opportunities once they no longer had to build and tend webs. "Once we get rid of the orb web, that's where we see some of the biggest bursts of speciation," Bond says. 

This diversification occurred about 100 million years ago, he and his colleagues reported 16 February 2016 in PeerJ, around the same time as an explosion of nonflying insects that could serve as prey for ground-based spiders. Such genetic comparisons, Bond says, "are transforming our understanding of spider evolution."

Spider biologists expect to learn still more from complete genomes. But spider genomes "have been a hard nut to crack," says Jessica Garb, an evolutionary biologist and geneticist at the University of Massachusetts in Lowell. The genomes are big—some surpass the human genome—and full of repetitive DNA. Moreover, spiders are not closely related to most of the other animals whose genomes have already been sequenced, making it difficult to use those references to piece together and analyze spider genomes. And overall, there's never been a lot of funding available for spider research. Whereas for some animal groups—such as birds—hundreds of genomes have been sequenced, only four spider genomes have been even partially deciphered.

Evolutionary biologist Trine Bilde at Aarhus University in Denmark spearheaded the first genome project as part of her research into the boom and bust lives of the African social velvet spider, Stegodyphus mimosarum. This species lives in nests, with up to 1000 individuals, mostly females, spinning dense, meter-sized webs capable of snaring 15-centimeter-long grasshoppers. The spiders are homebodies and therefore tend to breed only within their colony. That habit, plus evidence that colonies sometimes die out very quickly, suggested that they might be highly inbred, lacking the genetic variation that shields other organisms against such die-offs. Yet the species also thrives in a wide range of temperatures and humidity.

Bilde thought the velvet spider's genome would hold clues to the animal's social behavior and its odd mix of resilience and fragility, so her team and the Chinese sequencing giant BGI set out to sequence its DNA and, for comparison, that of a tarantula. The researchers expected that inbreeding—which reduces genetic variation between individuals—would make the social velvet spider's genome easier to complete. To their surprise and dismay, the genome turned out to contain long stretches of noncoding and repetitive DNA, which made it difficult to piece together the short reads produced by the sequencing machine. The tarantula genome was even worse—twice as large and even richer in duplicated regions. Drawing on significant computer power, however, they were finally able to stitch together the social velvet spider's genome, although they could not assemble a satisfying version of the tarantula's.

These genomes are only starting to be plumbed for insight on the velvet spider's social behavior and its adaptability. Bilde's team also plans to study populations of velvet spiders living in different environments to check whether changes in their microbiome or so-called epigenetic changes—chemical modifications of DNA—help the animals cope with varied and changing conditions.

MEANWHILE, THESE FIRST GENOMES—together with less ambitious molecular studies—are yielding a different payoff: They are helping break open silk and venom research. Cheryl Hayashi, a spider silk geneticist at the American Museum of Natural History in New York City, is among the researchers thrilled by what she is learning about the molecular diversity of these substances. "I feel like I am so fortunate to be working at this time," she says.

Silk genes, which code for extremely large proteins with stretches of amino acids that repeat many times, are themselves long and full of repetitive DNA that's hard to decipher. But the velvet spider genome, together with that of the orb weaver and the house spider, has exposed an unexpected variety of silk genes—"a lot more than we thought," Coddington says. Researchers had already identified two genes for the class of silk known as major ampullate, which forms the strongest dragline threads that anchor webs and are the inspiration for a major effort to make spider silk commercially (see p. 293). The social velvet spider's genome, however, revealed 10 genes just for that one kind of silk and nine other genes for additional silk proteins.

In search of more, Hayashi embarked on one of the other genome efforts. Early in
her career, she had cloned the Flag gene, which codes for flageliform silk, the elastic filament that orb weavers use in the insect-capturing spirals of their webs. The task took many months and was “not for the faint of heart,” Hayashi recalls. So she was happy to join with Benjamin Voight from the University of Pennsylvania, Ingi Aagnarsson from the University of Vermont in Burlington, and others to decipher and characterize the genome of the golden orb-weaver (Nephila clavipes).

The genome, the group reported online on 1 May in Nature Genetics, contains 28 silk genes, eight of them new to science. “In the past, we thought we could define all the silk genes in one species, that there would be a handful, and we could say, ‘This is the gene for a particular kind of silk,’” Hayashi says. “But it turns out that it’s not that simple.” Not only is there no one-to-one correlation between genes and silk types, but some silk genes seem to have gained entirely different functions. One of the orb weaver’s silk genes is even expressed in the spider’s venom gland.

Hayashi and her colleagues are now building on the genetic studies to learn how spiders make their silk. The typical orb weaving spider has many silk glands divided into seven types; each type of gland produces a specific mixture of proteins, which forms a distinctive type of silk when it is extruded through one of the spider’s spinnerets (see p. 292). Hayashi and her colleagues have recently identified which silk genes are active in each of the golden orb-weaver’s seven types of glands.

They have also looked for the activity of similar genes in the silk glands and other tissues of cobweb-weaving spiders, a subset of orb weavers that build 3D webs instead of flat ones. They discovered 209 potential silk and glue components, the team reported on 21 August in Scientific Reports. “It’s mind boggling,” Hayashi says.

The newly deciphered genes help explain the molecular basis of spider silk properties. The silk genes contain short stretches of DNA called motifs that vary between species in number and in their exact sequence. By comparing the genetic differences with differences in silk properties, Hayashi’s team has found that those motifs appear to influence strength, elasticity, and other features.

Sorting out this complexity may help bioengineers better understand and, ultimately, harness silk’s remarkable strength and flexibility. “In these sequences, there are answers to questions such as, ‘How do spiders keep the silk liquid at extremely high concentrations in the body?’” Hayashi notes. “It’s hard for biochemists to do this.” For example, she and others found that the silk glands contain nonsilk proteins that may serve as molecular chaperones to help with production of the fiber.

For researchers trying to make artificial silks, these findings are a gold mine. “All of a sudden we can do molecular genetics of silk,” Coddington says. “The door is open.”

THE DOOR HAS ALSO FLOWN OPEN for the similarly complex world of spider venoms, which may offer compounds useful for controlling insects or relieving pain. “Venom cocktails are really rich; they can have up to 1000 different chemicals and the mix varies a lot,” says Greta Binford, an evolutionary biologist at Lewis & Clark College in Portland, Oregon, who studies the unusual tissue-destroying properties of the venom of the brown recluse spider. (People who are bitten can develop gangrene so serious they can lose a limb.) The new genomes and follow-up protein studies, she notes, “give us more confidence that we’re capturing a comprehensive set of venoms.”

Even before the recent genome work, researchers had characterized some of the components of black widow venom, identifying two seemingly unique families of proteins: latrotoxins, which act on neurons; and latrodesins, whose role in venom remains unclear. (Both are named for the black widow genus, Latrodectus.) The black widow genome likely contains genes for many more toxins, but it has proven exceptionally hard to piece all of its DNA sequences together. So she and her colleagues have instead looked for venom genes in the genome of a close Latrodectus relative, the common house spider, Parasteatoda tepidariorum, which was reported on 31 July in BMC Biology by a team headed by Alistair McGregor of Oxford Brookes University in the United Kingdom.

Although a house spider’s bite is not as painful as the black widow’s, Garb and her colleagues were surprised to find that its venom is seething with latrotoxins—47 in all, they revealed on 16 February in BMC Genomics. All differ from those known in black widow venom. The finding “suggests [that spider family] is evolving in a very dynamic way,” Garb says. For example, black widows make α-latrotoxin, which specifically attacks vertebrate nerve cells, but the house spider does not. This toxin may have evolved in black widows because they are big enough to build webs capable of ensnaring small lizards and other prey with backbones, Garb suggests.

The analysis also supports the provocative idea that members of Latrodectus got their neurotoxins when a bacterium invaded their ancestor’s cells and left behind some of its DNA. Combing through a genome database revealed that the closest known matches to the house spider’s latrotoxin genes are bacterial genes. “Understanding the dynamics of venom evolution will help us refine not only our searches for new drugs and therapies, but also our understanding of how evolution generates chemical novelties,” Binford says.

Among spiders, silk and venom stand out as two key chemical novelties. But spiders have other impressive and unique adaptations. Peacock spiders and other jumping spiders use internal hydraulic pumps rather than leg muscles to leap 30 times their body length. Spitting spiders spew silky glue from their venom glands to pin down other, larger spiders for a killing bite to the leg. African sand spiders can survive a year with no food or water.

The size and complexity of spider genomes means that new sequences, and revelations about such traits, will be slow in coming. But like the patient predators they study, spider researchers are willing to wait. The rewards of genomics will come in time, they say. “Spider systematics, spider evolution and ecology, even spider behavior, have lagged for many years because [of] the genomic complexities,” Bond says. “That’s changed. Arachnology is starting to reach a level of maturity.” ■
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