IS RATTLESNAKE VENOM EVOLVING?

by STEVE GRENARD

Few creatures, except perhaps the armadillo or the wild turkey, are as emblematic of the New World as the rattlesnake. Before Columbus s voyage, Europeans had never seen one. Rattlers are not found in Europe, Africa, or Asia, but almost every state in the Union (Maine, Alaska, Hawaii, and Delaware are the exceptions) has at least one species. Arizona boasts eleven. A total of seventy species and subspecies--ranging from gigantic diamondbacks, which may exceed seven feet, to an eighteen-inch subspecies native to only a few mountains in Arizona--are found in North, Central, and South America.

The most distinctive feature of this reptile, of course, is its rattle, made of two to ten hollow interlocking segments of a light, fingernail-like material. When the rattlesnake vibrates its muscular tail, each separate segment bounces against the adjoining ones at fifty cycles per second, creating a buzzing sound that signals sensible folks to stay away. Unfortunately, not everyone does.

Most rattlesnakes are peaceable, retiring animals that flee for the underbrush when they encounter humans. Unless they are hunting rodents, rattlers strike only in self-defense. But if you step on one or try to capture it, a rattler will retaliate with a rapid strike that can be debilitating or even lethal. In the United States, about 8,000 people a year are bitten by rattlers or their cousins in the pit viper subfamily, which includes copperheads and water moccasins. In 1988 two doctors at the University of Southern California Medical Center analyzed 227 cases of venomous snakebite, covering more than a decade, and found that 44 percent occurred during accidental contact, such as stepping on the animal. More than 55 percent, however, resulted from the victim's grabbing or handling the creatures, and in 28 percent of these cases, the victims were intoxicated. The doctors' conclusion was that the typical snakebite victim is male and under thirty, with a blood-alcohol concentration of more than 0.1 percent at the time he is bitten. Yet only 0.2 percent of all snakebite victims die each year, and most of them receive no medical treatment or first aid.

Rattlesnake venom is not a simple poison. The snake's venom glands; located at the rear of the upper jaw and connected by ducts to its pair of hollow fangs, produce a complex brew of toxic peptides, polypeptides, and enzymes. In the venom, these toxins are combined in differing proportions that vary throughout a species' range and even during an individual snake's lifetime. Rattlesnakes harbor so many biochemical mixtures for venom that toxinologists who analyze the stuff confront a range of variations rather than a standard formula for each species. Some of this variability seems to reflect recent changes in the venom of certain rattlesnakes, from the hemotoxic and proteolytic type (which affects blood and other tissues) to the neurotoxic type (which attacks the nervous system). The first type hasn't changed into the second; rather, the proportion of neurotoxins in the mix appears to have increased in some areas of the country. Consequently, victims may now receive a significant dose of both types of poison from a single bite.

Matters seemed a bit simpler a few decades ago. Scientists knew that pit vipers produced a hemotoxic venom that was rarely deadly to humans. Except in Arizona and parts of Texas and California--home to the deadly, neurotoxic Mojave rattlesnake--most humans bitten in the United States could expect to survive. But they did experience depressed blood pressure associated with shock, destruction of tissue near the bite, massive swelling of the affected area, and hemorrhaging both near the bite and internally (caused by anticoagulants in the venom). If untreated, the area around the bite would become gangrenous and turn black. Sometimes the venom would also attack the kidneys. People lost fingers or toes, but few died--particularly after the introduction in the 1930s of an antivenom made from horse serum. In the worst cases, a bite victim usually had an hour or two to get to a hospital before the situation turned dire.

Neurotoxic venom, on the other hand, doesn't allow for such leisure, because it blocks nerve impulses to muscles, including those in the diaphragm that are used in breathing. Usually associated with members of the cobra family, a neurotoxic bite can cause immediate, shortness of breath, weakness or paralysis of the lower limbs, double vision, inability to speak or swallow, drooping eyelids, and involuntary tremors of the facial muscles. Death can occur in as little as ten minutes, usually due to abrupt cessation of respiration. In the 1970s, researchers at the Veterans Administration Hospital in Salt Lake City, Utah, identified the Mojave toxin that makes this little reptile the most deadly rattler in the United States--even when its victims have been treated with antivenom.

Over the past few years, however, neurotoxic symptoms have appeared in several people who apparently were bitten by other species of rattler. In 1999 in Hesperia, California, an eighteen-year-old reptile hobbyist received a bite on the hand while trying to grab a local rattlesnake with his bare hands. The species was believed to be a southern Pacific rattlesnake, a subspecies of the prairie, or western, rattler. Within minutes, the young man developed

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general weakness, had difficulty breathing, and showed the classic neurotoxic symptoms of double vision, facial twitches, and an inability to swallow or talk. He recovered only after being treated with thirty-five vials of antivenom. The doctors who treated him, Sean Bush and Eric Siedenburg, of the Loma Linda University Medical Center, published a report of the episode, calling it the first known case of neurotoxicity associated with a suspected southern Pacific rattlesnake envenomation. Yet the victim also showed several classic symptoms of hemotoxic poisoning, such as hemorrhaging and swelling of the hand and arm. The doctors observed that even if the snake had been misidentified and was really a Mojave rattlesnake, the case would still be noteworthy "because envenomation demonstrating both venom A [neurotoxic] and venom B [hemotoxic] effects has not been reported previously from southern California."

Do all populations of Mojave rattlesnakes have neurotoxic venom? While doing their work a quarter century ago, the Salt Lake City researchers found that they didn't. In the western and southern parts of the species' range in Arizona and southeastern California, many individuals had the more virulent Mojave A, whereas populations in other parts of Arizona and Texas had the nonneurotoxic Mojave B toxin. But it wasn't long before populations with both A and B surfaced. Some herpetologists thought those results suggested the likelihood of interbreeding among local populations of the same species.

Of the fifteen species of rattlesnake found only in the United States, at least ten have been verified as having neurotoxins in their venom. Until recently, however, the low levels of these chemicals in the overall mix were not considered much of a threat to humans. The southern California case, along with a scattering of recent clinical reports from far-flung parts of the country, raises the possibility that the situation is changing. In 1998 in Alabama, the minister of a snake-handling sect died within ten minutes of being bitten by a timber rattlesnake during a church service. And last year in Florida, an army ranger on maneuvers in the Florida Panhandle stopped breathing only thirteen minutes after being bitten by a timber rattler. Fortunately, he had already managed to reach the hospital at Eglin Air Force Base, where he was resuscitated and successfully treated with forty vials of antivenom--four times the usual dose.

Are the genes for Mojave A toxin moving from Arizona westward and across the prairies to the East and Southeast? If so, one would have to consider the possibility that contiguous populations of rattlesnakes are interbreeding, creating hybrids at the borders of their ranges. Rattlesnakes have been known to produce such hybrids in captive situations. A captive-bred Mojave-diamondback hybrid is on exhibit at the Reptile World Serpentarium in Saint Cloud, Florida, and similar hybrids (some of which escaped into the surrounding countryside) were bred at the San Diego Zoo in California about fifty years ago. Mojave A toxins have been identified in the venom of some populations of prairie rattlers, western diamondbacks, timber rattlers (but not northern timbers), and eastern diamondbacks, even though researchers have not yet detected any direct evidence of their interbreeding.

Some scientists are convinced that they have found proof of rapid molecular evolution in the venoms of related rattlesnake populations. Others have difficulty believing that significant evolutionary change could be occurring within the space of a few decades. Another mechanism that might be capable of driving the development of rattlesnake venom to more lethal levels is the continual escalation of an evolutionary "arms race" between predators and prey. Texas A&M researcher John C. Perez and colleagues have studied forty species of mammals that are the natural prey of rattlesnakes in Texas, and they found that sixteen had substances in their blood serum that blocked the hemorrhagic effects of western diamondback venom. Selection may thus be favoring rattlesnakes with a more powerful venom that can subdue animals endowed with these chemical blockers.

Supporting this hypothesis is the work of James Biardi, Richard Coss, and David Smith, all from the University of California, Davis, who recently demonstrated that the California ground squirrel (Spermophilus beecheyi) suffered little after being bitten by its traditional nemesis, the northern Pacific rattlesnake. A factor in the blood serum of this squirrel actively inhibits enzymes (or proteases) in the venom that cause local tissue destruction, rupture of capillaries, and hemorrhage. The researchers found that the blood serum of squirrels in habitats where the northern Pacific rattler is abundant combats venom more effectively than does the blood serum of squirrels from locations where these rattlers are rare. Nevertheless, a good many squirrels (probably younger ones, with less resistance) still manage to get eaten by the snakes.

As an alternative to the arms race and the hybridization hypotheses, James Biardi has advanced a third explanation for the possible changes in rattlesnake venom. Such a shift, he suggests, could simply be a by-product of changes in snake demography. For some years, researchers have known that juvenile rattlers often have stronger venom than that of their larger, more mature counterparts--a difference that may have arisen because small snakes inject much less venom than adults and may go after different or faster prey. In some species, young

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snakes have a higher proportion of neurotoxins in their venom than do older individuals.

Because humans often kill, capture, or intentionally run over larger snakes when they encounter them, Biardi argues, we may be affecting the age of the overall rattlesnake population. One need only look at the annual "rattlesnake roundup" in Sweetwater, Texas, where in 1997 more than 18,000 pounds' worth of rattlers were killed during the weekend hunt. Prizes go to those who bring in the largest and heaviest ones. To qualify for the competition, a hunter must submit at least 100 pounds of rattlesnakes. According to Biardi, if humans continue to selectively eliminate older rattlesnakes, it will be mostly younger ones--with the neurotoxic venom--that remain in the wild.

Whether the apparent shift to more neurotoxic venom in rattlesnakes is attributable to snake demographics, to hybridization and gene flow, or to the coevolution of predator and prey, doctors must now use much more antivenom to treat bites. Whereas five to ten vials used to suffice, patients today don't seem to improve until they have been injected with between thirty and seventy. This is not simply a question of using a more massive dose of a known cure: like the venom itself, the antivenom is also a complex mixture.

Made by injecting horses (or rabbits or sheep or goats) with small, sublethal doses of particular venoms, antivenom is a biological concoction of antibodies. It can combat only the specific venom that was injected into the animals, however. In the United States, the only rattlesnake antivenom now available is made from the serum of horses injected with the venoms of several kinds of pit vipers. While this preparation does not specifically include anti-Mojave antibodies, these may be present if Mojave A or B toxins are constituents of some of the venoms used. The presence of antiMojave antibodies in the current U.S. antivenom formula may be just as variable as it is in wild snake populations, and this unpredictability may explain why many vials are often needed to counteract neurotoxic venom. By administering vials from a number of batches, a physician may eventually find one with enough of the right kinds of antibodies to combat Mojave neurotoxins. Meanwhile, a British company is awaiting U.S. Food and Drug Administration approval of a new antivenom made from the serum of sheep that have been injected with Mojave toxins as well as with the venoms usually injected into horses.

As the search for effective antivenom goes on, the rattlers continue in their propensity for remaining placid until disturbed. If we don't bother them, they won't bother us. It isn't hard to see why rebellious eighteenth-century American colonists placed a rattlesnake across the thirteen stripes of the first Navy Jack flag, along with the warning "DONT TREAD ON ME."

Steve Grenard ("Is Rattlesnake Venom Evolving?") is an authority on the medical management of venomous snakebites. He published his first herpetological article (on a marsupial frog in his own collection) at age fourteen. Grenard's Medical Herpetology (Reptile and Amphibian Magazine, 1994) was the first comprehensive survey of amphibians' and reptiles' importance to medicine and is also a compendium of information on treating envenomation by snakes and lizards. Currently clinical coordinator of the Sleep Apnea Center at Staten Island University Hospital, he has directed critical care for respiratory emergencies at both Mount Sinai and Lenox Hill hospitals in New York City.