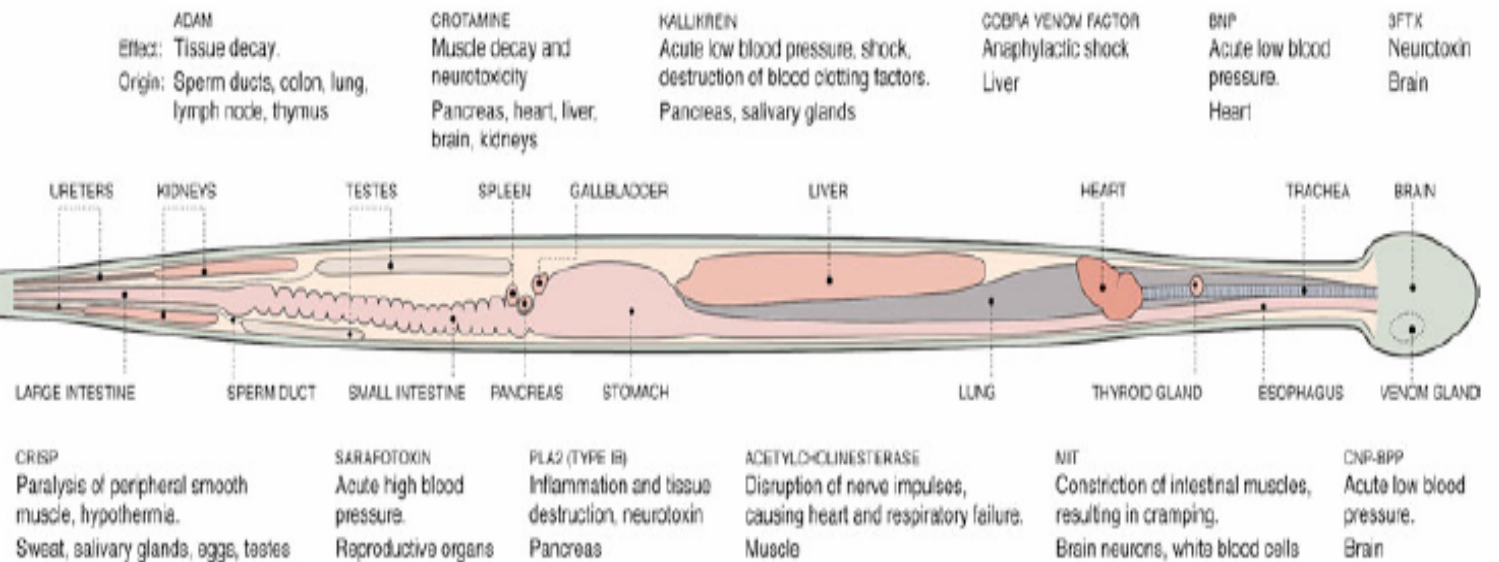


Ancient Origins of Venom

Scientists have found that the toxic proteins in some venoms have their evolutionary origins in tissues throughout the snake's body, not just the

venom glands. At right, some snake toxins, their effects and the tissues where they originated.



Source: Dr. Bevan Fry, University of Melbourne

Al Granberg/The New York Times

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Open Wide: Decoding the Secrets of Venom

By **CARL ZIMMER**

The inland taipan, a nine-foot-long Australian snake, is not the sort of creature most people would want to bother. Drop for drop, its venom is the deadliest in the world, 50 times as potent as cobra venom. Its fangs are so long they can poke through the snake's lower jaw. Its victims collapse in seconds and suffer a quick death.

Dr. Bryan Fry, a biologist from the University of Melbourne, will readily admit he is not like most people. He not only bothers inland taipans; he hunts them down in dense cane fields, pins them down and bags them. Later he grabs them by the head and squeezes venom from their fangs.

Besides inland taipans, Dr. Fry collects venom from death adders, rattlesnakes, king cobras, sea snakes and many others. He estimates that he handles 2,000 to 3,000 snakes a year.

"Working with some of these snakes is the biggest adrenaline rush you could ever do," he admitted. "I used to do extreme ski jumping and big wave surfing, but none of that can touch working with some of these animals."

Ultimately, this rush is not what drives Dr. Fry, who is 34. His goal is to decipher the evolution of snake venoms over the past 60 million years. Reconstructing their history will help lead to medical breakthroughs, Dr. Fry believes. For the past 35 years, scientists have been turning snake venoms into drugs. Just this February, Dr. Fry and his colleagues filed a patent for a molecule found in the venom of the inland taipan that may help treat congestive heart failure.

Understanding the evolution of snake venoms will speed up these discoveries immensely, Dr. Fry predicted. "You need a good road map to get your research going," he said.

Snakes produce venom in special glands on either side of their upper jaw. When they strike their prey, they squeeze the gland, causing the venom to spurt out. In some species, the venom simply pours into the wound. In other species, like cobras and inland taipans, the venom first flows into hollow fangs and then into the prey.

Once venom molecules enter a snake's prey, they become intimate assassins. Their intricate shapes allow them to lock onto particular receptors on the surface of cells or onto specific proteins floating in the bloodstream.

Some venom molecules can plug the channels that muscle cells use to receive signals from neurons to contract. Without the signals, the muscles go slack, leading to asphyxiation. Other venoms send the immune system into a tailspin, making it attack the prey's organs. Still others loosen blood vessel walls, leading to shock and bleeding. Rather than rely on one of these attacks, most venomous snakes produce a cocktail of molecules.

Dr. Fry says he has been fascinated by venomous snakes ever "since I could walk." By the time he started his dissertation research on the inland taipan in the late 1990's, he was already experienced at catching snakes and milking their venom. To find new toxins, he would weigh the molecules in the venom, and when he found molecules that were close in weight to known venoms, he would isolate them for a closer look.

As Dr. Fry discovered more venoms, he began to wonder how they had evolved. "It's been an area of great controversy," he said. Many researchers have argued that different lineages of venomous snakes, like rattlesnakes and cobras, evolved venom independently. They observed that the closest relatives of these venomous snakes were nonvenomous.

Dr. Fry discovered that they were wrong. "Most of the snakes that we think of as nonvenomous are actually venomous," he explained. Garter snakes and many other supposedly nonvenomous snakes actually produce tiny amounts of venom.

Dr. Fry is quick to point out that this does not mean that garter snakes are dangerous. "All they need to do is stun a frog or slow it down a bit, and it's enough to help them," he said.

These discoveries prompted Dr. Fry to carry out a large-scale study of the evolution of snake venom. His project would have been impossible a few years ago, because traditional methods for identifying new venoms are painfully slow. But the technology developed for the Human Genome Project has changed all that.

"Instead of spending a couple months and getting two or three protein sequences done, in a month I can get up to 2,000 sequences done," Dr. Fry said. "It's an amazing increase in efficiency."

"Fifteen years ago, this wouldn't even be thought of," said Alejandro Rooney, a molecular evolutionist at the National Center for Agricultural Utilization Research in Peoria, Ill., who has collaborated with Dr. Fry on some of his venom research.

Dr. Fry is able to identify all of the genes that are active in venom gland cells, and then read their DNA sequence. About half of the genes that are active in a venom-gland cell produce well-known "housekeeping" proteins that are essential to any animal cell. Most of the others are venoms.

After identifying new toxins, including many that represent entirely new types of venom, Dr. Fry said, "I think we've just scratched the surface."

Dr. Fry has constructed evolutionary trees of these venom genes, and his results indicate that

venom actually evolved only once in snakes. It started out being produced at low levels, as illustrated today by garter snakes. Later some lineages evolved a more deadly bite.

"It's been the most important adaptation in the history of snakes," Dr. Fry argued. The snakes that evolved venom no longer had to rely solely on constriction or other ways of physically subduing their prey. "It's freed them up from having to be big-muscled and slow-moving and killing their prey using constriction," he said. "They can be light, agile, athletic, and they can occupy any niche from the bottom of the ocean to the top of the tallest tree."

Dr. Fry's research has also shed light on the origin of venom molecules. A number of researchers have suggested that venom toxins are modified saliva proteins. They point out that ordinary saliva proteins are able to start breaking down food in the mouth. Perhaps some tinkering was all that was necessary to turn them into lethal poisons.

As Dr. Fry reports in the March issue of *Genome Research*, the DNA of venom genes goes against this idea. He constructed evolutionary trees of 24 venom genes, searching through online databases for their closest relatives among nonvenom genes. In only two cases did he find that venom genes evolved from saliva genes. In almost all the other cases, venom genes evolved from ones that were active outside the venom gland - in the blood, for example, as well as the brain and liver.

The evidence indicates that the evolution of a typical venom gene may begin with the accidental duplication of a gene that is active in another organ. In a process known as gene recruitment, one of these copies then mutates in such a way that it begins producing proteins in the venom gland.

In some cases, these borrowed proteins turn out to be harmful when injected into a snake's prey. Natural selection then favors mutations that make these proteins more lethal.

"The snakes have harnessed these proteins, changed them and thrown them right back at us, which is a pretty elegantly evil way of doing it," Dr. Fry said.

Previous research on venom had offered hints that some toxins might have evolved this way, but nothing more. For example, Dr. Elazar Kochva, a zoologist at Tel Aviv University, and his colleagues had noted some similarities between the venom of burrowing asps and certain enzymes in mammals. "We alluded to it, but Bryan Fry said it loud and clear and with a lot of evidence," Dr. Kochva said.

The concept of gene recruitment is not new, Dr. Rooney pointed out. Scientists have found evidence that it has played a role in the evolution of other organs, including the eye. "With venom, it seems to have occurred on a much grander scale," Dr. Rooney said.

As new lineages of snakes evolved, their venom evolved as well. New genes were borrowed to produce new venoms, while existing venom genes duplicated many times, producing a huge diversity of molecules.

This high-speed evolution allows snake venom to adapt to particular sorts of prey. Green mambas and black mambas, for example, are closely related species, but the green mambas live in trees while the black mambas live on the ground. "Not surprisingly, the black mamba venom is more potent against rats than against birds," Dr. Fry said, "while the green mamba venom is more potent to the birds than against rats."

This rapid evolution has produced a wealth of complex molecules that researchers have barely begun to investigate. Evolutionary trees can serve as guides, speeding the search for new venoms and shedding light on how venoms work. "Just looking by chance is very difficult and not economical," Dr. Kochva said.

The venom molecules that Dr. Fry has isolated from the inland taipan is a case in point. He has established that they evolved from a family of proteins known as natriuretic peptides. In snakes, humans and other vertebrates, these peptides relax the muscles around the heart.

In the ancestors of the inland taipan, the genes for natriuretic peptides began producing these proteins in the venom glands. Over time, their muscle-relaxing ability increased. Now they can stop their prey's aorta from contracting at all.

"They drop the blood pressure, which will knock the prey out," Dr. Fry said. "That gives the slower, but more lethal toxins a chance to exert their effects." These slower toxins create swarms of clots in a prey's bloodstream, setting off a vast number of strokes. "It's a good one-two punch."

Dr. Fry and his colleagues report their study of these toxins in the Feb. 25 issue of *Biochemical and Biophysical Research Communications*.

They are investigating the heart-relaxing toxins for treatment of congestive heart failure. By making blood vessels around the heart relax, it may be possible to increase the flow of blood out of the heart.

Understanding the evolution of these venoms helps Dr. Fry and his colleagues figure out how they work. Because they have evolved from proteins that only act on the heart, they probably will not pose a risk to other parts of the body.

"If you want to use a venom for some kind of drug, you'd better look back and see where it came from," Dr. Kochva said.

Dr. Fry knows that few people are as fond of deadly snakes as he is. But he hopes that these sorts of discoveries help lead to the protection of the world's snakes.

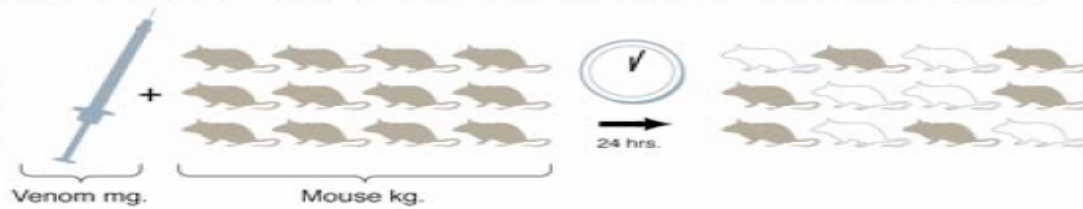
"If you kill off the snakes, you could be killing the next wonder drug," he said.

Deadlier Than a Cobra

The inland taipan of Australia is considered the most poisonous land snake. Below, how scientists compare different species.

TESTING VENOM ON MICE

One method is to inject venom into a group of mice. The amount of venom that kills 50 percent of the mice in 24 hours is called the lethal dose, or **LD50**, and is measured in milligrams of venom per kilogram of mouse.



WINNER: THE INLAND TAIPAN

Venoms that are effective at smaller doses are stronger. With an LD50 of 0.025 mg., the inland taipan is 68 times as poisonous as the king cobra and 740 times as poisonous as the western diamondback rattlesnake.



Sources: Dr. Bryan Fry, University of Melbourne; photographs by Photo Researchers Inc.

Al Granberg/The New York Times