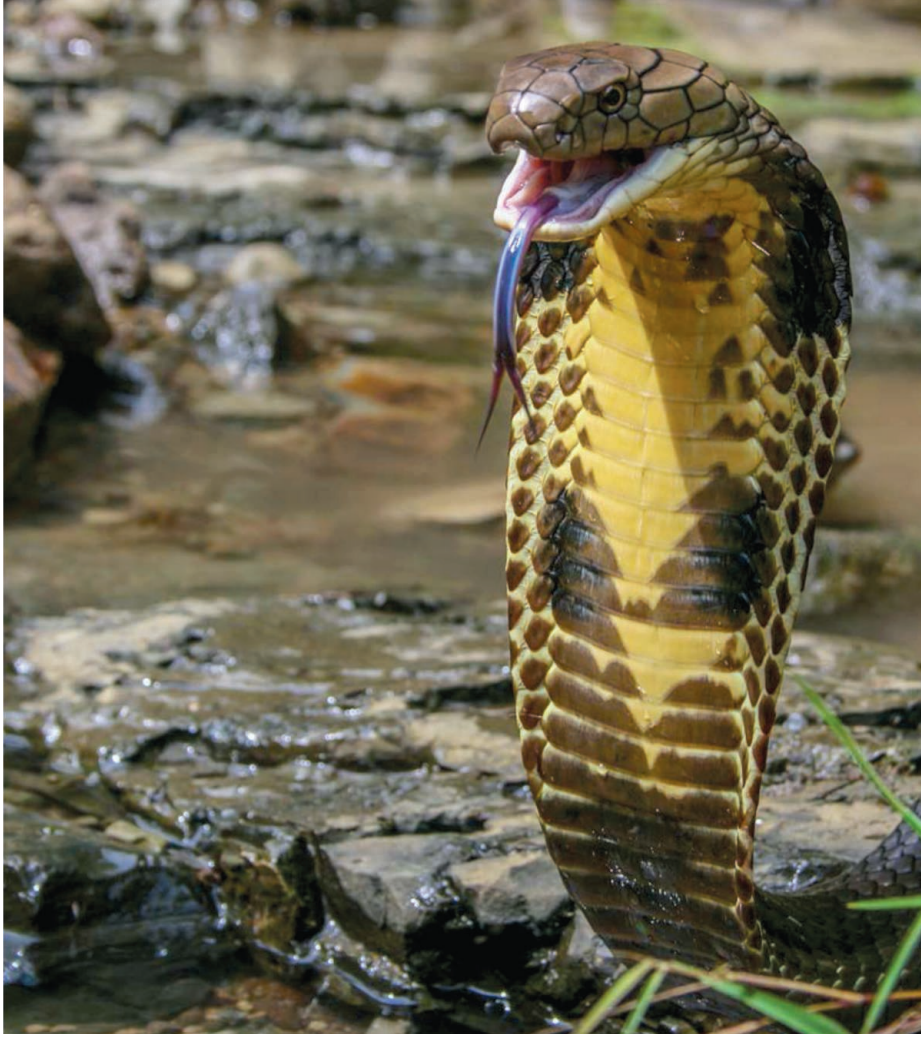
SECRETS OF SNAKES

Exotic, elusive, and dangerous, snakes have fascinated humankind for millennia. They can be hard to find, yet their 3000 species have conquered almost every corner of the planet. They play key roles in religion and mythology—and even now snakebites kill an estimated 100,000 people annually.

These days, molecular biologists, too, are falling under the spell of snakes, pursuing the secrets of their bizarre anatomy and powerful venoms. To mark the publication of the first two snake genomes, *Science* reporters take a close look at the work of scientists who were bitten—in some cases literally—by snakes.



Speed kings. The king cobra (*left*) and the Burmese python (*previous page*) have evolved rapidly.

Genes for Extremes

The first two snake genomes, published this week, reflect the amazing evolutionary tales of a prey-crushing python and a venomous cobra

Harry Greene has long been crazy about snakes—but less so about molecular biology. A veteran herpetologist at Cornell University, Greene has tracked down bushmasters, rattlers, and other snakes in 30 countries; once, in a Brazilian swamp, he brushed up against a green anaconda as long as a mid-size car (*Science*, 26 March 2010, p. 1577). But like many of his fellow snake researchers, he long eschewed molecular biology. "I'm so over that," Greene now says.

He's not the only one. Researchers have recently started delving deep into the molecular biology of venom, where some hope to find clues to important new drugs (see story on p. 1162). And in papers published online this week by the *Proceedings of the National Academy of Sciences*, two teams describe the genomes of the Burmese python and the king cobra—the first snake genomes ever published.

The two studies reveal the molecular basis for snake features that Greene and other researchers have long marveled about. The Burmese python eats three to five times a year, strangling prey 1.5 times its size. ("Imagine if I could eat a 270-pound cheeseburger, with no hands and no implements, and that made up a third of my annual energy budget," Greene says.) The king cobra, the largest venomous snake in the world at 4 meters long, has developed a fearsome venom consisting of 73 peptides and proteins that swiftly immobilize and kill its prey, mostly other snakes. Together, the papers represent "the opposite ends of the extreme evolution that has occurred in snakes," says Bryan Grieg Fry, an evolutionary biologist at the University of Queensland in Brisbane, Australia.

Greene says he was thrilled by what the genome studies turned up. "It's almost like expedition research, except it's in a genome and not in a tropical forest," he says. "I think these papers are going to lead the way for all kinds of work by younger researchers." The new genome analyses, along with studies of when and where the newly revealed genes are active, show that snakes as a group have evolved very quickly, changing the function of existing genes and coming up with additional ones to gain new abilities.

"A lot of people think of snakes as these simple tubes, but life is hard as a tube," says David Pollock, an evolutionary biologist at the University of Colorado School of Medicine in Aurora. "The bottom line is that snakes have done a lot of really impressive things in adapting at all levels": physiological, morphological—and molecular.

Forking path

Snakes have slithered their way through oceans and across all the continents save Antarctica; their 3000 species have infiltrated nearly every conceivable habitat from termite mounds to rainforest canopies. But they got their start in a specialized niche where legs were a handicap. A few researchers think snakes first evolved while living in water, but most now contend that they originated from lizards that went underground (*Science,* 8 November, p. 683). There, they acquired not just the serpentine body type, but also an economical metabolism able to deal with low oxygen levels. Eyes weren't needed, so they degenerated. When snakes surfaced again, lacking limbs for capturing prey, some species evolved venom instead. And they developed visual systems quite different from those of their lizard relatives.

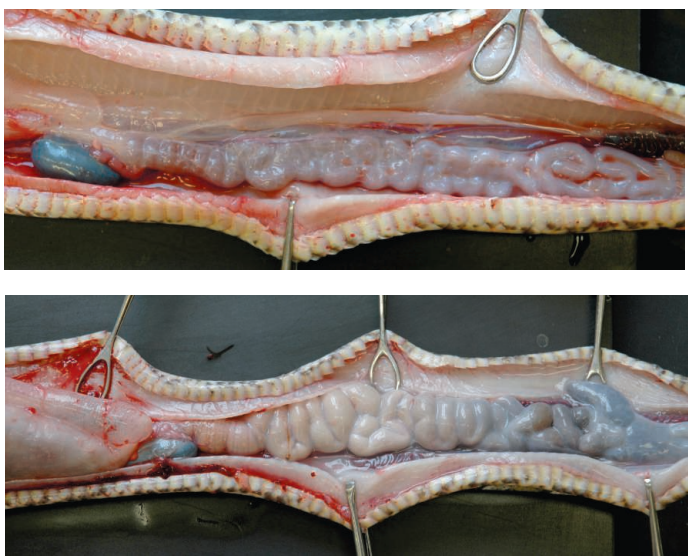
Pythons belong to a group that branched off early from these resurfaced snakes. They switched their diet from insects to larger animals and instead of biting their prey to death, started to constrict their powerful bodies around their meals to strangle them. (Pythons don't have venom.) Cobras took a different evolutionary path, developing outer teeth that move independently from their inner teeth. That way, their fangs could specialize for injecting venom while the inner teeth could help swallow prey.

The plan to sequence the python genome came from evolutionary biologist Todd Castoe of the University of Texas, Arlington. As a postdoctoral fellow working with Pollock, Castoe had studied a variety of vertebrate mitochondrial genomes. Comparisons had shown that these small genomes, found in cellular organelles, had evolved faster in snakes than in other groups. Castoe wanted to know if this was true for snakes' nuclear genomes as well.

CREDIT: FREEK VONK

They decided that the Burmese python, which lives in Southeast Asia and recently invaded the Florida Everglades, was an appealing target because of its astonishing metabolic patterns, documented extensively by evolutionary physiologist Stephen Secor of the University of Alabama, Tuscaloosa. Pythons can go without food for months at a time; when they do finally eat, organs like the kidney, liver, and gut can double in size in less than 3 days, while the snake's metabolism revs up to 40 times its usual rate. Getting to the molecular basis of this massive organ growth, Castoe hoped, might also yield some clues to how to treat cancer or heart disease.

As part of the study, the scientists checked the activity of genes in the heart, kidney, small intestine, and liver before a meal and again 1 and 4 days after eating. "The magnitude of the gene expression response really floored us," Castoe recalls. Half the python's genes changed their activity significantly within 48 hours. With the study in hand, "people are going to have a ton of new targets for looking at the genomics" of how snakes adapt physiologically, predicts Harvard University evolutionary biologist Scott Edwards.



Gut reaction. The Burmese python's small intestine shrinks (*top*) in response to fasting but expands greatly within days of a meal (*bottom*). So do its liver and kidneys.

CREDITS (TOP TO BOTTOM): IAN MCDONNELL/ISTOCKPHOTO; STEPHEN M. SECOR (2)

Toxic mix

The python's ballooning organs represent one evolutionary extreme; the venom of the king cobra is another. The cobra, which occurs in India, China, and Southeast Asia, competes with the African black mamba and Australia's inland taipan for the title of the most dangerous snake on Earth. The initiative to sequence it came from Freek Vonk of the Naturalis Biodiversity Center in Leiden, the Netherlands, who picked the king cobra in part because it happens to be his favorite species (see story on p. 1164).

Vonk teamed up with Nicholas Casewell, an evolutionary biologist at Bangor University in the United Kingdom, and a large group of scientists at 15 other institutes. They not only sequenced the genome, but also measured gene activity in the venom gland and in the so-called accessory gland, a poorly understood gland through which the venom passes before it leaves the cobra's mouth.

Vonk and his colleagues discovered that the two glands have very different gene activity patterns. The accessory gland doesn't produce toxins but makes many different lectins, a group of proteins that bind carbohydrates. In some other snake venoms, toxic lectins are part of the mix, but in the cobra, lectins are never released into the venom. The accessory gland's role may be to activate the venom somehow, but "we really don't know" what lectins do exactly, Casewell says.

The venom gland itself relies on 20 gene families for its toxins. Examining the genes, the team discovered a few toxins known from other snakes but never before seen in cobras, such as nerve growth factor and an enzyme called phospholipase B; they also identified proteins unknown in any other snake venom, such as insulin-like growth factor. Its gene was active only in the venom gland, the researchers report—but they have no idea what role the growth factor might play in venom.

The scientists found that the genes for each toxin family were also used in other parts of the body in the snake's evolutionary past, and some are used even today. "These dangerous proteins are co-opted from elsewhere in the body and [are] turned into weapons and diversified," says Frank Burbrink, an evolutionary biologist at the City University of New York. Genes involved in blood clotting, for example, may have been turned on in the venom gland through some regulatory quirk, and now they help bring down the prey's cardiovascular system.

In some cases, the gene was modified and ceased to perform its original role; more often it was duplicated, setting the new copy free to evolve toxicity. Duplication "gives you more material for selection to work on," says developmental biologist Michael Richardson of Leiden University, the last author on the paper. Often, a gene was copied more than once, allowing each copy to mutate in different ways, yielding an ever more sophisticated mix.

That gives the snake an advantage in an evolutionary arms race. The cobra's prey evolve constantly as well, developing ways to resist the toxins. For snakes, this genetic competition can be deadly, because ineffective venom can enable potential prey to turn on the snake and kill it. By analyzing how genes in the venom families had changed over time compared to matching genes in the python, other snakes, and the green anole lizard—the only lizard species sequenced so far—the researchers showed that venom genes were under intense positive selection. "It's a great demonstration of natural selection at work on the genome," Vonk says. Jimmy McGuire, an evolutionary biologist at the University of California, Berkeley, calls the paper "a stunning piece of work, just amazing."

Castoe and his colleagues also documented broader evidence of rapid evolution in snake genomes. They compared the 7442 genes found as single copies in both the cobra and the python with the same genes in all other land vertebrates sequenced so far. The bottom line: Snake genomes have changed a lot—and they have changed very fast to meet the demands of their unusual lifestyles. In snakes, about 10 times more genes are under positive selection than in other vertebrates, Castoe says, meaning that mutations in those genes were likely advantageous.

The comparison enabled the researchers to pinpoint where in the evolutionary history of snakes these changes occurred: 516 of them in the common ancestor of cobras and pythons, most of them having to do with snakelike qualities such as left-right asymmetry in their organs and shifts in metabolism; 174 changes in the cobra lineage; and 82 in the python lineage. The scientists have only just begun to milk their data. They hope to sequence more snake genomes as well; Castoe is already planning to decipher the genome of a blind snake, which looks and lives much the way the first snakes likely did. Another 10 snake genomes are likely to come out within the next couple of years, Casewell says.

Greene says he can't wait. "Natural history has all of the questions," he explains, "but molecular biology has the key to the answers."

–ELIZABETH PENNISI

Published by AAAS

Precious poison. A scientist collects venom from a Malayan pit viper.

From Toxins To Treatments

Researchers are hoping to find lifesaving drugs in the deadly venoms from snakes and other animals

SÃO PAULO, BRAZIL—If Kathleen Grego ever needs a reminder of the power of a snake's venom, she only has to look at her left thumb. A few years ago, the head of the herpetology department at the Instituto Butantan here was bitten by a jararacussu, a venomous pit viper. One of the snake's fangs sliced straight through her fingernail and discharged its deadly venom into her flesh. Grego was taken to the hospital down the road and immediately received antivenom, but her fingernail still bears a vertical scar and the top of her thumb looks like it has caved in on one side, where the toxins digested muscle and other tissue.

Grego could have lost a lot more than a bit of flesh. The peptides and proteins in jararacussu venom can latch on to molecules that regulate blood pressure and coagulation, causing a crash of the cardiovascular system and death. Other snakes produce toxins that wreak havoc in the cellular machinery of the nervous system, paralyzing the victim. Nature has had millions of years to perfect this molecular sabotage and turn snakes into accomplished killers.

But more and more researchers are studying venom's powers to heal rather than harm. Interfering in key pathways in the body is exactly what drugs are supposed to do, and hidden in the complex mixtures produced by snakes' venom glands are strings of amino acids that can dull pain, lower blood pressure, and more. Peptides yet to be found might prevent heart attacks or even treat cancer. "People have started realizing that there are a lot of very unique proteins with tremendous specificity in the venom of snakes," says Kini Manjunatha, a researcher at the National University of Singapore (NUS). "I am confident there will be more drugs from snakes."

The field has leaped ahead in recent years by combining two new technologies that help scientists quickly identify unknown peptides in venom: mass spectrometry and next-generation sequencing. And it's not just snakes that researchers are interested in. The number of venomous animals is estimated to be more than 170,000; even if the average venom contained just 250 peptides, a very conservative estimate, that's "a huge natural library" of more than 40 million compounds worth exploring, says Pierre Escoubas, a French researcher who started VenomeTech, a company that aims to produce drugs from venom.

Milking snakes

Precedents for the work go back to the 1960s, when Brazilian researchers studying the effects of venom from a lancehead viper found that it contained a range of peptides called BPFs that dramatically lowered blood pressure. Chemists at Bristol-Myers Squibb developed a small molecule, captopril, that mimics one of these peptides; it was the first of the so-called ACE inhibitors, which went on to earn billions of dollars and are used to this day.

In 1998, the U.S. Food and Drug Administration approved a blood thinner named eptifibatide, modeled on a rattlesnake peptide that binds to blood platelets and prevents them from aggregating in clots. A year later, a similar drug called tirofiban, inspired by a protein from vipers, hit the market. Several other drugs based on snake venom, including powerful painkillers, are now being tested in clinical trials.

The path to any such drug starts in a place like the Instituto Butantan, which breeds snakes and milks them for their venom. In one of the lab's rooms, three black plastic bins are set up on the floor in a neat row. Each of the bins contains several liters of CO_2, in which a snake is slowly falling into a daze. Grego's colleague Sávio Sant'Anna opens one of the containers; with a metal hook on a long wooden stick, he fishes out the snake, a gray viper with dark brown markings, and carries it over to a metal table.

With a swift movement, Sant'Anna grabs

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the snake's head, forcing its mouth wide open. Then he pushes back the protective skin covering each of the snakes' fangs with a pair of tweezers and forces the teeth through a sheet of cellophane covering a glass flask. Gently massaging the venom glands helps milk the deadly venom, a few drops of which collect at the bottom of the flask.

Sometimes, researchers already know what to look for in their harvest—but it can be hard to find. Yara Cury, another researcher at the institute, had read early 20th century reports that the venom of the South American rattlesnake (*Crotalus durissus terrificus*) had an analgesic effect, and in the 1990s, she confirmed that it reduced pain in animal studies. But identifying and purifying the peptide responsible, now called crotalphine and in development by a Brazilian pharmaceutical company, took years. "Venoms can contain several hundreds or thousands of peptides," says Glenn King, who researches venom at the University of Queensland in Brisbane, Australia. "Looking at them one by one would take you a lifetime."

To identify the individual compounds, scientists now use mass spectrometry, breaking proteins into smaller fragments and then sorting them by weight. The fragments form "ladders" differing by one amino acid each; by determining the mass difference between adjacent fragments, researchers can, in theory, identify the amino acids and infer the peptide's sequence. "But current technology only allows you to do that for the smallest toxins, about 15 to 20 amino acids long," Escoubas says. Peptides don't break after every single amino acid, and snake toxins that are 40, 50, or even 60 amino acids long leave scientists with a plethora of possible solutions.

Sequencing technology can help scientists solve this puzzle by giving them a genetic template of the snake's toxic peptides. "You milk the hell out of the animal," King says. "Then you give it 3 days." During that time, the animal starts producing new venom by activating genes encoding the various venom peptides. Researchers sequence messenger RNA from the venom gland, fishing out any typical toxin patterns. With that list of sequences, they can go back to the mass spectrometry data and interpret it.

That's quite a revolution, Escoubas says. "Before, we were taking some venom from an animal and painfully doing some biochemistry on some peptides," he says. "Now we go to the coding language of the venom."

Online
sciencemag.org
Podcast interview with author Kai Kupferschmidt (http://scim.ag/pod_6163).

Blessing in disguise. Scientists recently reported that the venom of the Chinese red-headed centipede contains a powerful painkiller.

A wider net

So far, snake toxins have been the most popular study objects, partly because snakes produce larger amounts of venom than most other animals do. But the venom of spiders, scorpions, cone snails, or centipedes is just as interesting—or even more so. "There are only 1500 species of venomous snakes, but 50,000 species of venomous spiders," Escoubas says. Spiders also have more toxins per venom, probably because they prey on insects, a hugely diverse group of animals that requires an equally diverse arsenal. Species other than snakes have already yielded drugs. Ziconotide, a peptide in cone snail venom, was approved in 2004 to treat chronic pain; exenatide, isolated from the saliva of a venomous lizard called the Gila monster, has become a blockbuster drug for type 2 diabetes.

It's time to explore the toxic treasure trove more systematically, Escoubas says. He leads a partnership that received €6 million in E.U. funding in 2011 for VENOMICS, a 4-year project aiming to produce a library of venom peptides that can be screened for their therapeutic potential. The group plans to look at some 200 different venomous creatures, which should result in about 50,000 peptides, Escoubas says—a massive step up from the 3000 to 4000 venom peptides described in the past 50 years. (Of those 50,000, the group aims to produce 10,000 in the lab.) So far, they have analyzed venom from 70 animals, including snakes, spiders, and a centipede, collected in places like Tahiti and French Guiana.

Pain therapy is a particularly promising area, venom experts say. In 2006, researchers discovered that a mutation in Nav1.7, a protein channel that lets sodium flow into human cells, makes people insensitive to all types of pain. The finding excited pharmacologists about the prospect of treating pain by disrupting that channel, but finding small molecules that block Nav1.7 without affecting eight similar channels in humans has proven all but impossible.

Venomous animals, however, seem very adept at doing exactly that. In September, King and colleagues from China reported in the *Proceedings of the National Academy of Sciences* that a string of 46 amino acids from the venom of the Chinese red-headed centipede specifically blocked Nav1.7 and acted as a strong painkiller in rodents. The molecule is "absolutely deadly to insects," King says, but in humans it could lead to a powerful pain therapy.

Winding road

Identifying a promising compound in venom is often only the beginning of a long, winding road. Used as drugs, peptides have the advantage that they can be more selective than small molecules, leading to fewer side effects, says Björn Hock, a peptide engineer at Merck, but they have several drawbacks: They're expensive to make, they sometimes elicit immune reactions, and, perhaps the biggest stumbling block, they usually need to be injected because they are broken down in the stomach when taken orally. That's why most future drugs based on snake venoms will, like captopril, be small molecules that mimic the function of the original peptide, predicts Johannes Eble, who researches the effect of snake venom on cell adhesion molecules at Frankfurt University Hospital in Germany.

But Escoubas is convinced that peptides' problems can be addressed. Peptide chemistry has greatly improved, making synthesis cheaper, and the injection pens developed for insulin show that drugs don't have to be a pill or a capsule to be successful.

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Exenatide is a case in point, King says: "Who would have thought that a 37-residue peptide drug with bad stability would become a billion-dollar drug?"

Moreover, some venom peptides may be stable enough to be taken orally. Many have multiple disulfide bonds, bridges between the sulfur atoms in the amino acid cysteine, that make the peptide stable enough to resist degradation by the enzymes in gastrointestinal juices, King says. A toxin derived from cone snails that's under investigation to treat neuropathic pain was recently shown to work when taken orally.

NUS's Manjunatha, meanwhile, has discovered an analgesic peptide developed from king cobra venom that he says is 20 to 200 times more potent than morphine—and active orally. Placed under the tongue, the 11 amino acid peptide is absorbed by the mucosal membrane, he says.

Manjunatha, who has patents on dozens of other snake peptides, was born and brought up in a small village in India surrounded by forests. "A lot of people lost their limbs or died" from snakebites, he says. When he started out as a researcher, he wanted to understand what makes toxins so dangerous: "Why are human proteins helpful and snake venom proteins so harmful?" Now, Manjunatha says he realizes an even more interesting question is which of those lethal snake peptides can help humankind most. **–KAI KUPFERSCHMIDT**

The Freek Show

Snake scientist Freek Vonk can't choose between research and starring in wildlife documentaries. And so far, he doesn't have to

LEIDEN, THE NETHERLANDS—On an October afternoon, a throng of children is waiting expectantly at the entrance of the Naturalis Biodiversity Center, a museum and research center just outside the city center. At 2 p.m. sharp, a green Land Rover pulls up, a door swings open, and a tall, blond young man jumps out. He's wearing jungle boots, khaki shorts, and a denim shirt—as if he were on safari in Africa instead of in an academic town. A platinum blonde-haired woman emerges from the passenger seat, and soon the couple is mobbed by kids screaming for autographs.

The man is Dutch biologist and snake scientist Freek Vonk, and he has just arrived at his workplace, along with his girlfriend, talk show host Eva Jinek. He's in safari gear because he is opening a new exhibition at the center. *Freek's Favorites* showcases 45 animal species that fascinate Vonk; Naturalis has invited kids and their parents to come celebrate.

At 30, Vonk has already built a respectable career studying snakes—animals he has been obsessed with for half his life. He has a couple of *Nature* papers to his name, and he is the first author on a study of the king cobra genome, published this week in the *Proceedings of the National Academy of Sciences* (*PNAS*) (see story on p. 1160). But here in the Netherlands, Vonk is known primarily as a TV celebrity. His wildlife shows air every day, and Naturalis has made Vonk its public face.

Just how Vonk—whose first name is pronounced "Frake"—manages to combine research and showmanship is baffling to some. He credits his attention deficit hyperactivity disorder, which made it hard to get through high school but now prevents him from ever doing nothing. "It's always go, go, go. It's nonstop with Freek," confirms Nicholas Casewell of Bangor University in the United Kingdom, a co-author of the new paper. In 2014, Vonk plans to further study the evolution of cobra venom, but he will also be filming abroad for 4 months.

Vonk's shows are about animals, ecology, evolution—but they're also adventures, presented with an un-Dutch touch of machismo. He wrestles with crocodiles, yanks snakes from their hiding places, and bares his torso for a bath in the Amazon after a day in the jungle. Many say he seems to be emulating one of his childhood heroes, Steve Irwin, an Australian conservationist known for his exciting wildlife documentaries. (Irwin died in 2006 after a stingray pierced his chest.)

It's a style not everybody likes. "You don't have to jump on crocodiles or show what a daredevil you are," says experimental

CREDIT: NATURALIS BIODIVERSITY CENTER

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zoologist Johan van Leeuwen of Wageningen University in the Netherlands. Vonk is "a very good biologist," Van Leeuwen adds, "but if he's trying to become like Irwin, he's on the wrong path."

But Vonk says his "hands-on approach" shows nature's exciting side and can promote conservation and raise interest in living things. During the opening ceremony at Naturalis, he puts a living snake around a boy's neck but also urges his young audience to consider a career in biology. The kids are impressed. "Freek loves animals. He isn't afraid of anything," a young fan says. "And he was bitten by a shark!"

Bitten by a passion

Two weeks later, on the terrace of a trendy cafe in Amsterdam, Vonk sets down his Heineken to show three impressive scars on his right hand. His crew was filming blacktip sharks off the South African coast earlier this year when one of the animals mistook his moving hand for a fish, he says. "Fortunately I resisted the impulse to pull back. He let go pretty quickly."

Over dinner, Vonk explains how his two disparate careers got started when, at age 15, he was enthralled by a snake belonging to a friend's brother. "They're so different than any other animal," he says, "that long limbless body, that little tongue that's always moving back and forth. … There's this cloud of mysticism surrounding them."

"If the snake is in, I'm out," his mother declared when he told his parents he wanted a snake, too. She soon gave up her opposition, however, figuring it would be best to let her son follow his passions. After high school, Vonk studied biology at Leiden University—mostly as a way to learn more about snakes. For his bachelor's degree, he wanted to do a research project at the lab of developmental and evolutionary biologist Michael Richardson. "I told him that we don't really do reptiles," Richardson recalls, "but that we'd be happy to make some room in a corner of the lab."

Vonk started comparing histological sections from the venom glands of various lizards, as part of a broader study on venom evolution in lizards and snakes by Bryan Fry of the University of Melbourne. Their paper, with Vonk as fourth author, was published by *Nature* in 2006. His master's work, also in Richardson's lab, led to another *Nature* paper, with Vonk as the first author, on the evolution of fangs. A striking picture of a Lataste's viper landed it on the cover.

Both papers triggered a wave of local media attention, and journalists discovered that the enthusiastic young researcher had charisma and a knack for storytelling. A

Cover boy. Vonk posed with one of his own pythons for an interview in a Dutch newspaper.

popular prime-time talk show contracted him to bring an animal—usually a scary one—into the studio on a regular basis and talk about it. The formula made some biologists wince but proved hugely popular. In 2009, Vonk was approached by a TV producer who wanted to take him to Africa to produce a series aimed at kids. *Freek in the Wild*, as the show is now called, became a huge success. This month, he will start filming a new prime-time series targeting adults, *Freek in Australia*.

With the fame came a stream of interview requests. The fact that Jinek, his girlfriend, is a well-known TV personality has added to Vonk's appeal. "Now the gossip press is after me as well," he says.

Mr. Science

Four weeks after its tumultuous opening, *Freek's Favorites* has sent visits to Naturalis soaring, says its director, Edwin van Huis. Van Huis hired Vonk last year, weeks after he finished his Ph.D., offering him a half-time job. Vonk also has a starring role in a new crowd-funding campaign to buy a *Tyrannosaurus rex* skeleton dug up by Dutch researchers in Montana this year. If Naturalis can raise the €5 million to €7 million it needs to buy the fossil, it will be the first *T. rex* on permanent display in Europe.

Van Huis says he hired Vonk primarily because he's a good scientist, "but we also like the way he can reach people with his enthusiasm. Freek represents what Naturalis is all about." Vonk's high-octane style may seem to clash with the museum's almost 200-year tradition, but Van Huis says he hopes it will attract a broader and younger audience. "I'm an old fart myself, I grew up with David Attenborough," says Van Huis, referring to the iconic British documentary maker. "But young people don't like that style anymore."

Managers at Dutch reptile shelters raised another worry about Vonk's work in a newspaper story 2 weeks ago: They said his shows may entice people to get reptiles they don't know how to take care of, which then get dropped at their facilities. Vonk, stung by the story, says he has always conveyed the opposite message: "Don't take a snake lightheartedly. It's not for everyone."

Vonk wants to combine his two careers as long as he can: "If I don't have to choose, why would I?" In the long run, he's hoping for an international audience. Talks with Discovery and National Geographic a few years ago didn't pan out; one problem is that his English isn't good enough. He hopes to improve it by living in the United States for a year. "I need to start thinking in American," he says.

Risky business

Vonk won't show off the collection of snakes he still keeps in his apartment in The Hague. "I don't want to talk about snakes in captivity," he says, and he's worried about being seen as a hobbyist. He's moving out soon and is now giving away most of his animals. He'll keep his king cobras, however, the species he loves best and whose genome is analyzed in this week's *PNAS* paper. "I figured we might as well choose an icon to sequence": the largest venomous snake and an endangered species. "They're very intelligent," he adds. "When they're looking at you, you can see that they're thinking, anticipating."

They're also very dangerous. A friend of Vonk's, British snake breeder Luke Yeomans, died in 2011 after being bitten by a king cobra in his snake sanctuary. Yeomans's widow donated six of his cobras to Vonk—although not the one that killed his friend.

The tragedy drove home the risks of his own work, Vonk says. He has had his share of mishaps, including two bites by venomous snakes. There was that shark, of course, and a few other close encounters. The one that most impressed him was with a Cape cobra, one of Africa's most dangerous snakes. Lying on the ground, a small camera mounted on his head, Vonk was taunting the snake to film how it responded, when suddenly it lunged at him, so close to his face that he could feel a breeze as its fangs raked the air. "If it had bitten me, I would have had a real problem," he says.

He now asks his producer to say "Cape cobra" whenever he's taking too much risk just to get a good shot. "It means: Watch out, Freek," he says. "You're going too far."

–MARTIN ENSERINK

CREDITS (TOP TO BOTTOM): GLOBALP/ISTOCKPHOTO; COURTESY OF *VOLKSKRANT*/CORNELIE TOLLENS

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Island of The Snakes

The invasive brown tree snake has devastated Guam's ecosystems. Can it be eradicated?

As the leader of a specialized team of snake hunters based on Guam, biologist James Stanford was used to getting calls at odd hours. Early in the morning on 20 March 2005, his cellphone rang. On the line was a worried official with the Division of Fish & Wildlife on Saipan, one of the Northern Mariana Islands, some 200 kilometers away. He urgently needed Stanford's help.

The previous night, just after 1 a.m., a flight attendant had glimpsed a meter-long snake slithering off the runway at Saipan International Airport. The plane had come from Guam. Alarmed, her supervisor immediately called a government hotline set up to protect Saipan's native wildlife from invasive predators. Guam is a stronghold of the invader that its island neighbors fear most: the brown tree snake.

Since arriving on Guam, a U.S. territory in the western Pacific, in the 1940s, brown tree snakes (*Boiga irregularis*) have extirpated native birds, bats, and lizards and disrupted entire ecosystems. Although harmless to adult humans, they have slipped into houses and tried to swallow infants' arms, sickening them with mild venom. By crawling onto power lines, the snakes have caused up to 200 blackouts a year, electrocuting themselves in the process.

To prevent the spread of brown tree snakes, the U.S. Department of Agriculture (USDA) inspects all cargo leaving Guam and kills snakes in the forests surrounding ports and runways. The U.S. Geological Survey (USGS) runs a rapid response team, headed by Stanford until earlier this year, to help investigate sightings on other islands.

The report from Saipan sounded credible, so Stanford didn't hesitate. He grabbed the packed suitcase he always kept at home, and after picking up some search equipment and a trained Labrador retriever, he and three other biologists with USGS caught the next flight to Saipan. Their mission: Find any snakes and kill them.

That's not easy. Even when brown tree snakes are abundant, as on Guam, it is hard to know how many there are. But years of research on Guam have provided unique and valuable insights into tracking snake populations, says J. D. Willson, an ecologist at the University of Arkansas, Fayetteville.

Monitoring the snakes will be crucial to the next challenge: beating them back. Researchers are now testing new tools. In September, USDA started dropping poisoned bait from helicopters into fenced-off forests. This approach, some say, finally raises the possibility of eradicating the brown tree snake from Guam.

Invade and conquer

In the summer of 1944, the U.S. military was amassing forces in the Pacific to advance the battle against Japanese troops. Materiel from the Admiralty Islands of Papua New Guinea was transported to large Navy and Air Force bases on Guam. The brown tree snake, native to Papua New Guinea, came along for the ride.

Away from its usual predators—thought to include large monitor

Looking up. Research has improved the ability to find tree snakes.

CREDIT: BJORN LARDNER

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lizards and feral pigs—and in a climate that allows year-round reproduction, the snake went wild. The native fauna was clueless, because the only endemic snake on the island was a blind, harmless species. By the 1970s, many bird populations began to collapse. Without birds to eat spiders, forests grew thick with webs. When USGS biologist Gordon Rodda first visited Guam's forests in 1987, "it was like being parachuted onto another planet," he recalls.

At first, most scientists blamed disease, such as avian malaria, which afflicted Hawaii. It took years of research by ecologist Julie Savidge of Colorado State University, Fort Collins, to reveal the true culprit. "I had a terrible time convincing people that tree snakes were responsible," she says. One reason was that the snakes seemed to be rare. "The number of snakes you see has little to do with the number of snakes that are there," Willson says.

When they followed up on Savidge's work in the 1980s, Rodda and other federal biologists were astounded to discover the density of brown tree snakes. Tropical forests typically contain one or two snakes per hectare; on Guam, biologists could trap 55 brown tree snakes per hectare. Having polished off native species, the snake had found other sources of food, including rats and other introduced wildlife. It is also an extraordinary generalist that has been caught scavenging soiled diapers and used tampons.

Authorities on other Pacific islands began to fear what could happen if the snakes spread. USDA's Animal and Plant Health Inspection Service started an interdiction program on Guam in 1993 and fenced off loading areas at ports and airports. Ever since, inspectors have patrolled nightly with spotlights. Every piece of cargo leaving the island is searched by teams of Jack Russell terriers, agile and energetic snake hunters. And each week, traps baited with caged, live mice are placed on the fences and in nearby forests. Some of the traps have poisoned bait: dead mice stuffed with 80 milligrams of acetaminophen, a widely used pain reliever that kills a snake in about 24 hours. Since 1993, the combined efforts have caught more than 150,000 brown tree snakes. Those that are trapped, rather than poisoned, are euthanized.

The government and university researchers who have developed these control measures have also investigated the snake's biology and improved their survey methods. A unique experimental facility has helped tremendously. In 2004, USGS enclosed a 5-hectare site with a snake-proof fence. Rodda and his colleagues then captured all 122 snakes inside and implanted each one with a transponder tag. As a backup, they also marked each animal by clipping a unique pattern of scales, a classic method in herpetology.

Defense line. Fences, traps, and constant vigilance are needed to prevent tree snakes from sneaking off the island of Guam.

The setup has allowed the team to test traps and other control tools on a real-world population. One important conclusion is that the traps rarely collect small snakes. As it turns out, young snakes don't like the mice used as bait; they prefer geckos. Unfortunately, baiting traps with geckos would be too expensive, so catching young snakes by hand is the only way to get them.

Thanks to the testing ground, scientists have honed their ability to catch snakes. During searches, teams of experts and volunteers fan out, armed with hooked staffs to pull snakes from branches. They spend hours aiming their headlights into the trees, looking for the sheen of snakeskin.

Over the years, the team has identified some two dozen factors that can help or hinder the search. For example, a moonless night increases the odds of success by 20%, as does a calm night without fluttering leaves. Even the type of headlamp makes a difference. Snake hunting still takes twice as much time per snake caught as using traps, but it has become more effective. As a result, even though Stanford's team didn't find the reported snake on Saipan after 2 weeks of nightly searching, they could be confident that the island didn't harbor a population.

Attack from above

Now, a new phase in the battle has begun. USDA wants to scale up the use of poison bait, so its officers are strapping dead rodents to pieces of cardboard attached to biodegradable streamers. When tossed from a helicopter, the mice get snagged in the tree canopy, available to tree snakes but not to animals on the ground. This could be an easier way to kill snakes in remote or rugged terrain.

Initial tests were promising. Dozens of mice were dropped on a 6-hectare site last year, some of them with tiny radio transmitters implanted. All 30 snakes that ate bait with transmitters died, the researchers reported last fall. (A toad and monitor lizard had also eaten one toxic mouse each, but did not appear to be the worse for it.) In September, USDA expanded the tests to two 55-hectare fenced sites on Andersen Air Force Base. Helicopters will drop bait there every few weeks for 16 months. Researchers will compare the snake populations to those at a nearby reference site.

The ultimate aim is to eradicate the brown tree snake from the island. "We are nowhere near that goal," says Daniel Vice, USDA's supervisory wildlife biologist in Barrigada, Guam. Among the hurdles are the island's considerable size—it's two-thirds the size of New York City—and the biology of the snake. Females often hide out for months after mating and can store sperm for years, producing new offspring even if the males have been extirpated. Visual searches alone can't confirm success; for the 5-hectare USGS plot, for example, it would take 42 searches, each requiring 36 person-hours, to be 95% certain that the last snake had been found.

But Robert Reed of USGS in Fort Collins isn't discouraged. He expects that aerial poisoning will sharply reduce the snake population across the landscape. Even without full eradication, that might allow restoration of native birds, some of which persist in zoos. "I think it will become possible at some point," says Daniel Simberloff, an invasive species biologist at the University of Tennessee, Knoxville. And as snakes' numbers dwindle, other islands need to worry a little less about suffering Guam's misfortune.

–ERIK STOKSTAD

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