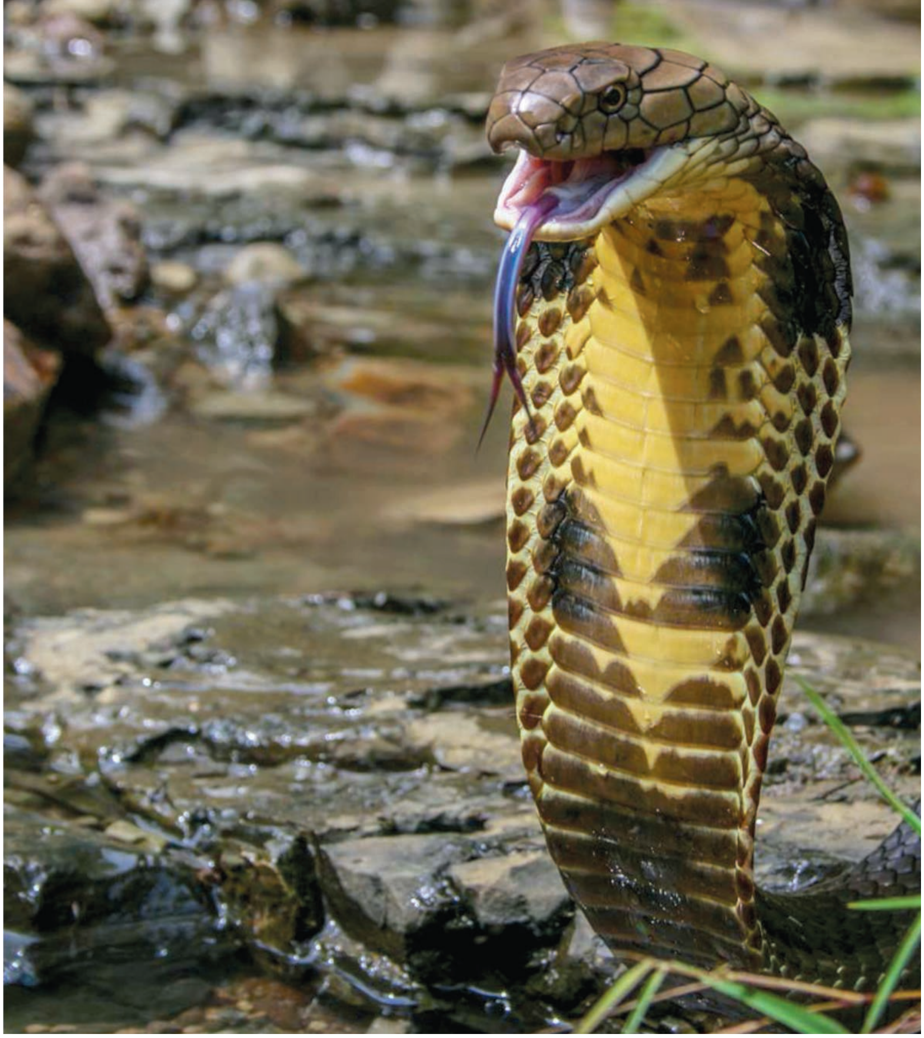

Speed kings. The king cobra (*left*) and the Burmese python (*previous page*) have evolved rapidly.

CREDIT: FREEK VONK

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 midsize 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.

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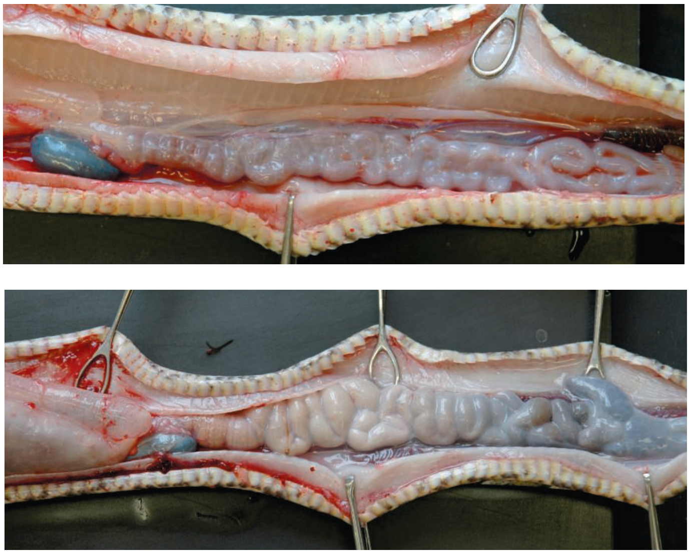
They decided that the Burmese python, which lives in Southeast Asia and recently invaded the Florida Everglades, was an appealing target because of its astonishing metabolic patterns, documented extensively by evolutionary physiologist Stephen Secor of the University of Alabama, Tuscaloosa. Pythons can 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