How the cheetah got its stripes—a genetic tale

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A house cat with the "blotched" coat pattern. Credit: Helmi Flick

Feral cats in Northern California have enabled researchers to unlock the biological secret behind a rare, striped cheetah found only in sub-Saharan Africa, according to researchers at the Stanford University School of Medicine, the National Cancer Institute and HudsonAlpha Institute for Biotechnology in Huntsville, Alabama. The study is the first to identify a molecular basis of coat patterning in mammals.

The scientists found that the two felines share a biological mechanism responsible for both the elegant stripes on the tabby cat and the cheetah's normally dappled coat. Dramatic changes to the normal patterns occur when this pathway is disrupted: The resulting house cat has swirled patches of color rather than orderly stripes, and the normally spotted cheetah sports thick, dark lines down its back.

"Mutation of a single gene causes stripes to become blotches, and spots to become stripes," said Greg Barsh, MD, PhD, emeritus professor of genetics and of pediatrics at Stanford and an investigator at the HudsonAlpha Institute.

The differences are so pronounced that biologists at first thought that cheetahs with the mutated gene belonged to an entirely different species. The rare animals became known as "king cheetahs," while affected tabby cats received the less-regal moniker of "blotched." (The more familiar, striped cat is known as a mackerel tabby.)

The study will be published Sept. 21 in *Science*. Christopher Kaelin, PhD, a senior scientist in the Barsh laboratory, is the co-first author; Xiao Xu PhD, from the National Cancer Institute-Frederick National Laboratory for Cancer Research and the Sichuan Key Laboratory of Conservation Biology on Endangered Wildlife in Sichuan, China, is the other co-first author. Marilyn Menotti-Raymond, PhD, of NCI-Frederick is the senior author.

Barsh and his lab members have spent decades investigating how traditional laboratory animals such as mice develop specific coat colors. His previous work identified a variety of biologically important pathways that control more than just hair or skin color, and have been linked to brain degeneration, anemia and bone marrow failure. But laboratory mice don't display the pattern variation seen in many mammals.

"We were motivated by a basic question," said Barsh of the turn to the study of big (and little) cats. "How do periodic patterns like stripes and spots in mammals arise? What generates them? How are they maintained? What is their biological and evolutionary significance? It's kind of surprising how little is known. Until now, there's been no obvious biological explanation for cheetah spots or the stripes on tigers, zebras or even the ordinary house cat."

The research relied primarily on DNA samples from feral cats in Northern California captured for sterilization and release, on tissue samples provided by the City of Huntsville Animal Services group, and on small skin biopsies and blood samples from captive and wild South African and Namibian cheetahs. It also hinged on the recent availability of the whole-genome sequence of the domestic cat. (Menotti-Raymond's research focuses on the genomic analysis of the domestic cat to better understand many human diseases.)

The spots displayed by a typical cheetah (standing), compared with the blotched pattern of king cheetahs. Credit: Ann van Dyk Cheetah Preserve

"The Laboratory of Genomic Diversity at the National Cancer Institute has long championed the cat as an animal model of human disease," said Menotti-Raymond. "Studying color variation in cats provides the opportunity to uncover new principles of gene action and interaction that may have unexpected applications to understanding developmental and morphologic variation in natural populations, including humans."

Comparing gene sequences of feral cats with different patterns allowed Kaelin and Xu to identify mutations in a gene they dubbed Taqpep associated with the blotched tabby markings: 58 of 58 blotched tabbies had a mutation in each of its two copies of Taqpep, while 51 of 51 mackerel tabbies had a least one unmutated version.

Taqpep encodes a protease normally found in the cell membrane, but that can also be cleaved to allow it to diffuse outside the cell. This ability to float freely and interact with other molecules in the extracellular soup is a key component of a principle called reaction diffusion proposed by the famous computer scientist Alan Turing, PhD, in 1952 as a way to explain how periodic patterns (like stripes and spots) can arise out of randomness.

"Turing realized that, under specific conditions, diffusible 'activator' and 'inhibitor' molecules can self-organize into a variety of periodic patterns," said Barsh. "We are excited about the idea that Taqpep might be an entry point to understand if, and how, reaction-diffusion mechanisms can explain 'how the leopard got its spots.'"

After nailing down Taqpep's role in tabby stripes (and analyzing its sequence in more than 350 other cats of 24 distinct breeds), Kaelin wondered if it might play a similar role in generating and maintaining the spots on wild and captive cheetahs. He obtained blood samples from a king cheetah named Kgosi, a resident of a wildcat education and conservation program in Northern California, and found that Kgosi also had a mutation in Taqpep.

Kaelin next contacted Ann van Dyk, who maintains a cheetah conservation center in South Africa from which all captive king cheetahs, including Kgosi, originate. (Van Dyk was the first to learn, though meticulous breeding records, that the king cheetah pattern is due to a recessive genetic mutation.) Van Dyk obtained DNA samples from all her cheetahs, allowing confirmation that a Taqpep mutation is responsible for the king cheetah pattern.

Mammals aren't the only animals with patterned hair or skin, obviously. Fish, salamanders and some invertebrates also have stripes and spots. However, there is an essential difference. While the non-mammals simply add stripes or spots as they grow to adulthood, mammals keep the same number and pattern by increasing the surface area of the contrasting colors.

"Somehow, cells in the black stripes know they are in a black stripe and remember that fact throughout the organism's life," said Barsh. "We were curious about what's happening at the boundary between light and dark stripes and spots. How do these spots know to grow with an animal?"

When Kelly McGowan, MD, PhD, a senior scientist in Barsh's group, studied fetal cat skin after seven weeks of gestation, she found that the tabby pattern begins to arise only when the hair begins to grow. In other words, there are no apparent differences between the cells themselves—only in the color of hair they produce. That suggested that the changes in color are due to differences in the levels of expression of certain genes within the cells.

Lewis Hong, a former graduate student in Barsh's lab, used a technique he developed called EDGE to identify changes in gene expression levels between black and yellow areas of cheetah skin (obtained under anesthesia). He found several differences, many associated with a pathway influencing the expression of a gene called Edn3. McGowan found that Edn3 mRNA was produced at the base of the follicles making the black hairs. To test their theory, the researchers collaborated with a group at Florida International University to study a yellow-colored laboratory mouse that had been engineered to express Edn3. The coats of the resulting animals were much darker than their unmodified peers.

"This is very strong evidence that Edn3 is a critical regulator of black versus yellow hair in animals," said Barsh. The researchers hypothesize that expression of Taqpep is required to establish a pattern of stripes or spots in early feline development that is then carried out by Edn3 as the hair grows.

Clearly, not all cats are patterned. In particular, some big adult cats like African lions and mountain lions are distinctive for their lack of color variation even though their cubs are striped. Furthermore, Taqpep mutations are surprisingly common in some non-striped domestic cat breeds like the Abyssinian and the Himalayan.

"We know there's a mutation that suppresses pattern formation in some cats," said Barsh. "We'd like to investigate that mechanism as well."

More information: The research is described in the paper "Specifying and sustaining pigmentation patterns in domestic and wild cats" and will be published in the 21 September issue of *Science*.

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