MEETING SOCIETY FOR INTEGRATIVE BIOLOGY

Rocky Mountain Rendezvous

DENVER—Researchers from all walks of biology traded tales about snakes, whales, birds, and every other organism imaginable at the annual meeting of the Society for Integrative and Comparative Biology, held here from 6 to 10 January.

How Snakes May Have Lost Their Legs

For centuries, not just scientists but artists too have speculated about the limblessness of snakes. Michelangelo thought the loss occurred in the Garden of Eden. Now, two developmental biologists offer a less fanciful explanation—one involving genes and the proteins they produce—rather than divine intervention.

At the meeting, Marty Cohn of the University of Reading in the United Kingdom reported that he and Cheryll Tickle of the University of Dundee in Scotland have linked such important changes in snake evolution as the elongation of the thorax and the loss of the forelimbs to the altered activity of several *HOX* genes, which are involved in body patterning and limb formation. In addition, the failure to develop complete hindlimbs seems to be due to an inability of the embryonic tissue to respond to the normal developmental trigger.

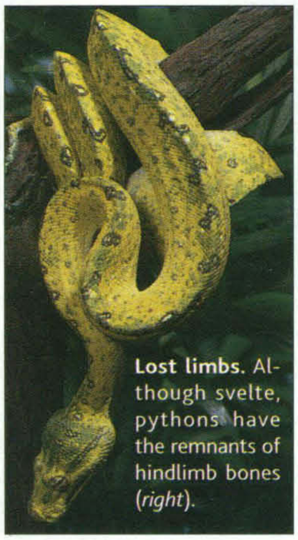
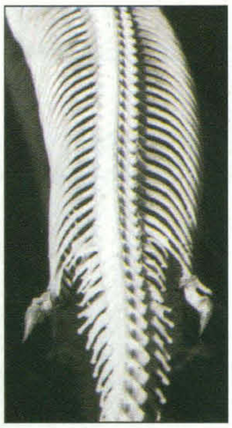
"It's a beautiful piece of work" that demonstrates how developmental biology can help explain evolution, comments Michael Coates, a vertebrate paleontologist at University College London. "He's shown us the molecular details about a large-scale evolutionary change."

Cohn decided to tackle snake evolution after he and others had demonstrated that certain *HOX* genes help prod limb development in chick and mouse embryos. He knew that *HOX* genes also control the formation of distinct neck, thoracic, lumbar, and caudal vertebrae. So, he used chemically tagged antibodies to find out which regions in the python embryo contain the proteins made from the *HOXC6*, *HOXC8*, or *HOXB5* genes. He chose those three genes because *HOXC6* and *HOXC8* are normally expressed where the ribs develop, while in mice and chickens, *HOXB5* helps define where the forelimbs sprout.

He found that the *HOXC* genes were turned on throughout the embryonic axial skeleton, thereby extending the thorax all the way from the top of the vertebral column to the hindlimb buds. And in the python embryos, *HOXB5* was active throughout the cells of the lateral mesoderm, which lie along the sides of the embryo. In limbed embryos, this gene is active in just one part of this mesoderm, thereby helping to specify the starting point for the front legs. This change in *HOXB5* expression, Cohn suggests, somehow contributes to a total loss of forelimbs.

Pythons have vestigial hindlimbs, and Cohn has linked their failure to develop normally to the fact that the embryonic hindlimb buds lack a leading edge of tissue called the apical ectodermal ridge, which prompts elongation of the leg. But no one knew whether the signal that would normally tell the ridge to develop was lacking or if the limb bud tissue just couldn't respond.

Cohn and Tickle's results point to this second possibility. For example, when the researchers grafted limb bud cells from the snake into the appropriate spot in a chick embryo, the snake cells sent the right signal, inducing ridge formation. This suggests, Cohn reported, "the python ectoderm is not competent to respond to the signal," although he doesn't yet know why.

Lost limbs. Although svelte, pythons have the remnants of hindlimb bones (*right*).

CREDITS: (LEFT TO RIGHT) E. R. DEGGINGER/ANIMALS ANIMALS; J. D. CUNNINGHAM/VISUALS UNLIMITED

If the python ectoderm could respond, however, it appears it could form hindlimbs. The researchers found that fibroblast growth factor 2, which stimulates limb growth in other organisms, did the same for the python embryo hindlimb, at least for the 24 hours they could study it. Thus, despite a few glitches, "all the other signaling networks [for limbs] are in place," he reported.

Based on these results, Cohn suggested that the *HOX* gene changes came first in snake evolution, perhaps leading to a primitive snake resembling a fossil called *Pachyrhachis problematicus*, which had hindlimbs but no forelimbs. Next, other genetic mutations cut short the full development of hindlimbs and caused the trunk to elongate, resulting in the pythonlike snakes. Finally, further mutations caused the hindlimbs to disappear altogether as in more advanced snakes.

This molecular scenario is quite pleasing to Michael Caldwell, a paleontologist with the Canadian Museum of Nature in Ottawa. "He's done the development work and put it into a phylogenetic context," Caldwell notes. "That's been very difficult to do."

Coming to Grips With Sperm Whale Anatomy

Sometimes, all it takes is a little old-fashioned biology to solve a longstanding mystery. And sometimes it takes a bit of fancy technology. At the meeting, both approaches were used to better understand the world's largest toothed mammal, the sperm whale. On the low-tech end, simple dissection has helped explain how the creatures feed. In addition, high-tech computed tomography (CT) scanning of a sperm whale head has provided a new view of how the whales generate sounds and, perhaps, even how they seek mates.

Sperm whales feed mainly on deep-dwelling ocean animals like giant squid, and how they take in their prey has been something of a mystery. Because healthy sperm whales have narrow, pointed mouths with a lower jaw that hangs down, it seemed logical that they need it to stir up and grasp prey. But many museums contain whale skeletons whose jaws are either congenitally deformed or scarred by multiple breaks that occurred during the whale's life, alterations that would have prevented their feeding that way. At the meeting, Alexander Werth of Hampden-Sydney College in Virginia reported that such whales would have been able to survive because they don't use their jaws to grasp food. Instead, he favors an alternate idea, that sperm whales use their tongues to suck in prey.

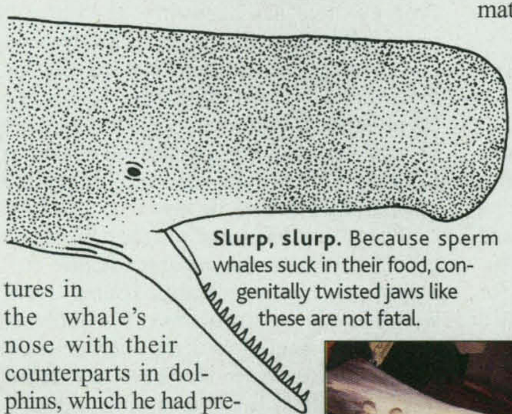
A specialist in the feeding mechanisms of toothed whales, Werth dissected the tongue of a beached sperm whale and traced its muscular connections to the mouth. Most tongues, including those of humans, have a layer of built-in muscles that help them fold and twist so that they can manipulate food to be swallowed. But Werth found that these muscles were very reduced in the whale tongue he dissected. It is, however, well equipped with muscles connecting it to jaw and skull, so that it can quickly move backward and forward over the throat and create suction. This "is a very efficient way of getting stuff into the mouth," comments Ted Cranford, a marine biologist who studies whales at California State University in San Diego. "Before this work, no one had even looked at the tongue to see if it could work this way."

Although suction feeding has not been proven experimentally, the work is still impressive because with these rarely studied deep-sea denizens, "you have to look at whatever information you can gather," points out D. Ann Pabst, a marine mammalogist at the University of North Carolina, Wilmington. "[Werth] is filling in a vacuum in knowledge."

So is Cranford, who used a CT scanner designed to look for flaws in rocket engines to view the inside of the intact, frozen head of a juvenile sperm whale. Imaging the entire head, which, at 1 meter long, was quite small for a sperm whale, took 5 days. To keep the head from defrosting and decaying during that time, Cranford encased it in foam. Some colleagues "said it would be impossible," he recalls. "But it worked perfectly." Indeed, says marine mammalogist Sentiel Rommel of the Florida Department of Environmental Protection in St. Petersburg, the scans provide "a nice clean description" of the whale's head.

For one thing, they provide a clear view of the spermaceti organ, a large sac filled with waxy oil where the clicks, pings, whistles, and other sounds of the whale's vocabulary are thought to originate. By showing the densities of the various tissues of the head, the scan may eventually help reveal how sounds are channeled and amplified in their passageway to the exterior.

In addition, Cranford compared the structures in the whale's nose with their counterparts in dolphins, which he had previously studied using a normal CT scanner. He showed that the sperm whale nose is proportionally large for its body. He speculates that this swelled nose—and the magnitude and character of sound produced from it—may be one way a male sperm whale shows off to females, or to male rivals, who like stag deer are known to fight. By listening, Cranford suggested at the meeting, sperm whales may be able to size up suitors and enemies, even in the deepest, darkest depths of the ocean. That idea still requires further investigation, Pabst notes, but nevertheless, "it's a very interesting and provocative hypothesis."



Slurp, slurp. Because sperm whales suck in their food, congenitally twisted jaws like these are not fatal.

CREDITS: (TOP, LEFT TO RIGHT) J. DUNNING/VIREO; S. BOSTWICK; (BOTTOM, LEFT TO RIGHT) A. J. WERTH/HAMPDEN-SYDNEY COLLEGE; T. CRANFORD

Snapping Wings a Manakin's Serenade

For some of the small, stocky Neotropical birds known as manakins, love knows few bounds. Many birds have evolved—in addition to melodic songs—brilliant coloration or elaborate tail feathers as part of an evolutionary race to win mates. But about half of the 40 or so manakin species have evolved an unusual ritual: The males use their wings to "sing" for their mates—clicking and rattling as the birds prance around during courtship displays. New results, described at the meeting by Kim Bostwick of the University of Kansas, Lawrence, now show that unlike most birds, which undergo rather superficial changes to appear sexier, these manakins have evolved structural modifications with potentially high costs.

Click, rattle, hum. A club-winged manakin flips its wings to serenade mates.

Based on field and anatomical studies by herself and others, Bostwick, a grad student in ornithologist Richard Prum's lab at Kansas, has found that the adaptations range from changes in feather shape to dramatic alterations in wing structure. In some species, the wing bones and muscles have bulked up to the point where the birds' flying efficiency is likely to be compromised—a testimony to the strength of the evolutionary drive to reproduce. "Almost nowhere is there nearly as much change as we see in these manakins," says Prum.

Bostwick's colleagues say her studies could provide a better understanding of how birds have evolved these extremes in their wing anatomy and how those traits relate to behavior. "She's finding some nice correlates" of anatomical change with behavior, says George Goslow Jr., an evolutionary biologist at Brown University in Providence, Rhode Island. "It's a very interesting study, and her approach is very logical."

For the past several years, Bostwick has been doing field and anatomical studies of manakins in South America. At the meeting she described the shape and size of wing bones, feathers, and muscles of several species, including one representative from each of the three groups that have apparently evolved this noisemaking capability independently.

All the clicking birds had some changes in feathers, but the most unusual plumage belongs to the club-winged manakin, which also has the most unusual wing song. This bird hums, as well as clicks and rattles, and likely uses irregularly shaped inner flight feathers—twisted at the tips—in making the humming noise, Bostwick reported.

Also, the wing bones and musculature of noisemaking manakins had surprisingly striking variations. Because flight is so difficult, the proportions of the bones and muscles are virtually indistinguishable among most birds. Take the 300 species of flycatchers, a group closely related to manakins. They all have a thin ulna, one of the wing bones, for example. But the same bone in clicking manakins is thicker and is uniquely outfitted with knobs that help support the enlarged muscles attached to feathers. This setup may help brace the feathers for sound generation, Bostwick suggested.

Other wing changes may help manakins achieve the right noisemaking posture. "When club-winged manakins display, they lean forward and flip their wings back and then forward," Bostwick explained. This requires that they rotate the elbow joint, a motion seen in few other birds. Whereas the muscle that moves that joint is small in most other birds, in the clicking manakins it is bulky and strong. In one species, the ends of the joint maneuvered by this muscle are also much more rounded, perhaps "translating into an increased mobility of that joint," Bostwick pointed out.

She hasn't worked out how these changes would affect the manakins' flying ability. But she expects that their heavier wings would slow them down, compared to their agile flycatcher cousins. Bostwick plans to extend her work to include manakins that either have secondarily lost their clicking ability or have evolved to make the clicks with either their tail or their vocal cords. "It will be interesting to see if the loss of that ability has led to more changes in the morphology," she notes.

–ELIZABETH PENNISI