DEVELOPMENTAL BIOLOGY

Tracing Backbone Evolution Through a Tunicate's Lost Tail

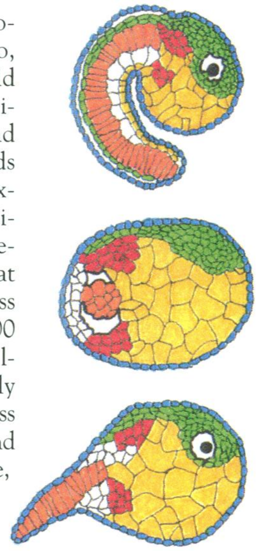
For years, researchers studying the evolutionary origins of the backbone have bypassed the world's furred, feathered, and finned creatures in favor of leathery little bundles of tissue called tunicates. Adult tunicates are sedentary sea-dwellers with no sign of a backbone, and they live like mussels, attached to a shell or rock and filtering food through chimneylike siphons. But larval tunicates are something else entirely: They're tadpoles, complete with a dorsal nerve, a flexible rod of support cells called a notochord, a tail, and skeletal muscles that power them through shallow tidal flats. As they develop into adults, the larvae shed these vertebratelike traits, reverting back to the ancient ways of the invertebrates—and in effect turning back the evolutionary clock.

Now, as reported on page 1205, it seems that a single gene may guide the development of the tunicate larvae's suite of vertebratelike characters. Developmental biologists William Jeffery and Billie J. Swalla of the University of California studied a tunicate species that lacks even its embryonic backbone and traced this loss to the disruption of a single gene, named *Manx* after a tailless cat. Without *Manx*, the tunicate larva "seems to lose the whole [group] of characters at once," says Swalla, now at Vanderbilt University in Nashville, Tennessee.

It's an impressive result, biologists say. "I can't think of a single case when such a dramatic phenotypic difference can be traced to one gene," marvels developmental biologist Sean Carroll at the University of Wisconsin, Madison. *Manx* "is going to strike people as a very powerful master regulatory gene ... probably something very basic [to] setting up vertebrate architecture," agrees developmental biologist Rudolf Raff of Indiana University in Bloomington.

Evolutionary biologists are intrigued as well. By turning *Manx* off in specially bred tailed embryos, the team has experimentally re-created an evolutionary change that likely led to the tailless tunicates—a landmark achievement for researchers trying to piece together evolutionary history, says Raff. It's even possible that *Manx* was central to the evolution of chordates, the umbrella phylum of organisms that includes both vertebrates and tunicates and is defined by the presence of a notochord, spinal cord, and other characters.

Jeffery and Swalla, then a postdoctoral fellow, began probing this problem almost a decade ago, theorizing that if they could find two closely related tunicate species with tailed and tailless larvae, the hybrids could serve as an excellent experimental system for identifying genes crucial to tail development. They found that there are about a dozen tailless species among the some 3000 tunicate species, and that taillessness arose independently five times. But only one tailless species, *Mogula occulta*, found off the city of Roscoff, France, turned out to have a close enough tailed relative, *Mogula oculata*, that the two could interbreed.



Turning tail. Embryos of tailed tunicate species *(top)*, tailless species *(middle)*, and hybrids *(bottom)* differ in expression of the *Manx* gene.

In 1990, Jeffery and Swalla found that hybrid embryos of these two species looked identical to the tailed embryos, although their tails were somewhat shorter. That suggested that one or more genes were lost or disrupted in the tailless species, and that tails could be partially restored with only one copy of the gene. And in 1993, Jeffery, Swalla, and Niriyuki Satoh and Kazuhiro Makabe, now at the University of Kyoto in Japan, described a candidate gene—*Manx*—that was active in both the hybrid and in the tailed species but not in the tailless one. Suspicions that *Manx* was the crucial gene were further bolstered by the fact that *Manx* RNA shows up early in the development of cells destined to become notochord, tail muscle, and dorsal nerve cord.

W. JEFFERY AND B. SWALLA

Twin tunicates. As adults, the tailed and tailless tunicate species look identical.

Now Jeffery and Swalla have taken the next crucial step, demonstrating experimentally that *Manx* plays a vital role in the formation of tails and other chordate traits, and that it was altered in the evolution of *M. occulta*. They treated hybrid embryos with antisense DNA, which binds to *Manx* RNA and prevents the production of *Manx* protein. In the hybrids in which *Manx* was effectively turned off by antisense, the tail never developed. (They tried this experiment in embryos of the tailed species too, but the antisense could not block the maternal *Manx* protein in the egg, and so *Manx* activity could not be completely stopped.)

It appears that *Manx* may act by turning on a cascade of other genes that control many chordate traits. Once the notochord formed in the hybrids, for example, it apparently coaxed nearby tissues to develop into chordate features such as skeletal muscles and a sensory organ in the head. And the team found that the gene's sequence contains a region that codes for a "zinc finger"—a feature of proteins that bind to DNA and act as transcription factors, regulating other genes. Another DNA segment codes for a nuclear localization signal, which helps get the *Manx* protein into the nucleus, says Jeffery.

Those findings leave researchers wondering what other genes interact with *Manx*. "*Manx* is just a dumb transcription factor. It's being regulated upstream by something," Carroll says. "What are the other things in the *Manx* pathway?" Jeffery adds that he and Swalla haven't ruled out the possibility that other pathways must also be disrupted to create tailless tunicates: "We need to find out if [*Manx* is] sufficient as well as required." He suspects that the answer may be yes, because taillessness has arisen independently so many times. "This sort of suggests there may be a simple switch," he says.

He and Swalla are now trying to re-create the details of the genetic events that could have flipped that switch and led to the evolution of *M. occulta*. For example, as they sequenced *Manx*, they noticed that a seemingly noncoding segment of the gene actually contains a second gene, which codes for a protein called p68, whose precise function is unknown; this protein is found in much larger amounts in the tailless species than in the tailed one. Swalla speculates that taillessness arose when a mutation changed the splicing of the *Manx* gene, so that cells in the tailless embryo make p68 when they should be making *Manx* protein. Once the tailless, sessile larvae appeared, natural selection might have favored these mutants in some environments, for example in areas where sand flats are patchy and mobile larvae might be swept into deep water.

Meanwhile, everyone is interested in whether *Manx* played a role much earlier in evolution, 550 million years ago when the chordates first evolved. Many biologists think developmental genes may have been involved in this event, for mutations in such genes offer a plausible way to dramatically alter an organism's body plan, and certainly *Manx* is now a leading candidate. Raff and Carroll expect researchers to start scrambling to find *Manx* equivalents in model species such as mice and frogs, to see whether the gene governs notochord and tail development in these true vertebrates.

If so, then it's possible that one of the classical divides in animal life could be traced back to a single genetic change. Of course, "what we don't know, and what [our] studies cannot tell us, is what the true genetic event was," says Nipam H. Patel, developmental biologist at the University of Chicago. "You can't run the tape of time backward, so all you can do is make good guesses."

–Elizabeth Pennisi

PALEONTOLOGY

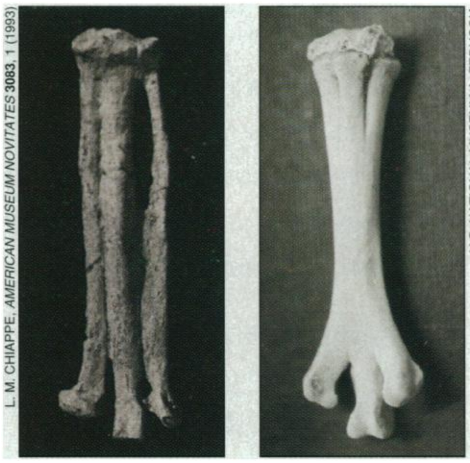
Early Birds Rise From China Fossil Beds

Paleontologists have been arguing about the ancestry of modern birds ever since 1861, with the discovery of the first indisputable bird, *Archaeopteryx*, in Bavaria. With conspicuous teeth, a lizardlike tail, and feathers draped over a dinosaurlike body, this 150-million-year-old fossil has been touted as a "missing link" between birds and dinosaurs. But the bones of modern birds look different, and researchers have been unable to agree on whether *Archaeopteryx* or its close cousins did indeed lead to modern birds; some ornithologists even doubt that modern birds descended from dinosaurs. On page 1164, Chinese and American scientists present new fossils sure to take the debate to new heights.

The bones represent what may be the oldest modern-looking bird, or ornithurine. If the dating is confirmed, it lived at the same time as a primitive, *Archaeopteryx*-like bird—and just an instant, geologically speaking, after *Archaeopteryx* itself. That could shove *Archaeopteryx* and another large group of primitive birds off the evolutionary branch that led to modern birds; it could even imply an earlier origin for all birds, perhaps before their putative dinosaurian ancestors. "It shows that there was a dichotomy, and that *Archaeopteryx* and most of the other early birds were a side line of avian evolution," says University of North Carolina ornithologist Alan Feduccia, co-author of the paper with paleontologists Lianhai Hou and Zhonghe Zhou of the Chinese Academy of Sciences, and Larry Martin of the University of Kansas.

But other paleontologists think Feduccia is out on a limb. Although few argue that *Archaeopteryx* itself led directly to modern birds, many think that a related primitive group called the opposite birds are close kin to modern birds. What's more, says Yale University paleontologist John Ostrom, "the dating [of the Chinese fossils] is controversial." If the new fossils are substantially younger than *Archaeopteryx*, as other dating work suggests, then they may offer little new insight into the origins of birds, says paleontologist Luis Chiappe of the American Museum of Natural History in New York.

Reconstructing the bird family tree has been difficult because of a dearth of fossils after *Archaeopteryx*. But new finds in the past 5 years have revealed unexpected diversity in early birds starting at about 135 million years ago. Most of these, the dominant birds of the Mesozoic, were enantiornithurines, or "opposite" birds, so named because three bones



L. M. CHIAPPE, *AMERICAN MUSEUM NOVITATES* 3083, 1 (1993)

U.S. NATIONAL MUSEUM/A. FEDUCCIA

Which feet first? Modern birds have the foot bones fused from the bottom up *(right)*; in opposite birds, the fusion is top down *(left)*.

of their feet are partially fused from the top down, rather than from the bottom up as in modern birds (see figure). In contrast, the first few fragmentary remains that might be ornithurines didn't appear in the fossil record until later, about 120 million years ago.

Until recently, many paleontologists thought that *Archaeopteryx* itself gave rise to opposite birds, which in turn gradually evolved into modern birds. That view has faded, but Chiappe and others still hold that opposite and modern birds are closely related sister taxa, with a recent common ancestor that lived at about the time of *Archaeopteryx* or a bit earlier; this scenario allows enough time for birds to descend from Jurassic dinosaurs.

Feduccia and his colleagues now challenge that view with fossils of a bird the size of a sparrow, called *Liaoningornis*. The specimen, unearthed by a farmer in the Yixian formation in northeastern China's Liaoning Province, lacks a skull but includes a nearly complete skeleton with foot bones and a keeled sternum that resemble those of modern birds. Yet the Chinese scientists cite radiometric dates of 137 million to 142 million years for the volcanic rock of the Yixian formation, which would make the bird almost as old as *Archaeopteryx*. And the same beds also yielded a magpie-sized primitive bird called *Confuciusornis*, which shares many traits with both *Archaeopteryx* and opposite birds. Indeed, these rich beds also produced a controversial "feathered" dinosaur (*Science*, 1 November 1996, p. 720).

According to Feduccia and Martin, the discoveries imply that by the time of *Archaeopteryx*, birds had already diverged into two lineages and had a rich history that is missing from the fossil record. One lineage led to modern birds. Another led to *Archaeopteryx* and the opposite birds, which they view as sister taxa, closely related to each other but distinct from the line that led to modern birds. And both these bird lineages must have descended from a much earlier ancestral bird. Feduccia reckons that the first bird must have lived about 76 million years before the birdlike dinosaurs of the Cretaceous —a fact that he says raises questions about the dinosaurian origins of birds.

However, not everyone agrees with those dates. New argon-argon dates on the volcanic rock and sediment in the Yixian formation, presented at the recent Society of Vertebrate Paleontology meetings in New York, date the birds at only about 121 million years, says University of Toronto physicist Derek York, a co-author on the poster at the meeting. If so, then it's possible that Chiappe is right: Some unknown bird that lived about the time of *Archaeopteryx* underwent rapid evolution and gave rise to both opposite and modern birds, as represented by *Confuciusornis* and *Liaoningornis*.

Feduccia's co-author Martin questions the new argon-argon date, noting that arthropods and pollen in the Yixian formation are characteristic of the Late Jurassic about 140 million years ago. Feduccia simply says that the dating controversy is overblown: "Whatever the date is, we're getting both types of birds shortly after *Archaeopteryx*"—making the stunningly successful modern birds early birds indeed.

–Ann Gibbons