Melding genes, neurons, and fossils, a new synthesis overturns long-held ideas about the rise of our favorite lineage and shows how a good theory can propel science—even if it's partly wrong

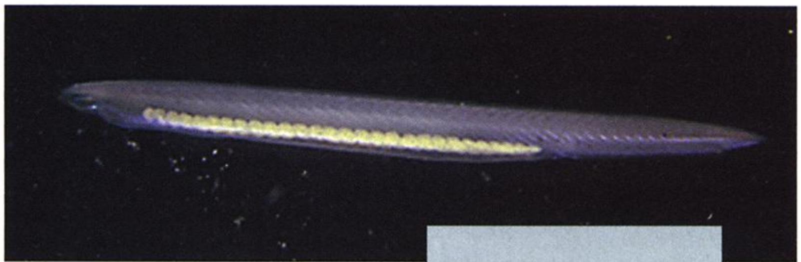
In Search of Vertebrate Origins: Beyond Brain and Bone

Walk through the halls of the American Museum of Natural History in New York City, and you will see our lopsided bias toward vertebrates on spectacular display. The history of the vertebrates unfolds in hall after hall of magnificent fossils, from dinosaurs to cave bears. Meanwhile, equally magnificent specimens of invertebrates such as *Nautilus* and giant clams are tucked away in a few smaller, less popular rooms scattered throughout the building. Most museums have a similar split, yet invertebrates make up the vast majority of Earth's animal biomass and include many millions of species, compared to only 42,000 known species of vertebrates. But museums are run by humans, who are most interested in animals like ourselves. And some of the signs of our kinship with reptiles, birds, and fish are obvious to even the most casual visitor: We all have skeletons, complete with backbone and skull, and big, complex brains.

For all the attention lavished on vertebrate evolution, however, just when and how vertebrates arose has long been a mystery. For most of the 20th century, primitive vertebrate fossils seemed a confusing mess, and paleontologists had little luck finding fossils that preserve the earliest hints of brains and bones. The basic anatomy of vertebrates' closest living relatives—which might provide clues to what our earliest ancestors looked like—was worked out over 70 years ago, and anatomical studies since then have yielded few fresh insights. "It made the field pretty stagnant," says Nicholas Holland of the Scripps Institution of Oceanography in La Jolla, California.

But in the past few years, work in several different disciplines has converged to provide a surprising new picture of the transition from invertebrate to vertebrate, a picture that upsets some previous ideas. Developmental geneticists have begun to uncover the genes that create the body plan in vertebrates and their closest living invertebrate relatives, just as neurobiologists are exploring the detailed neural connections of these seemingly simple animals. And late last year Chinese paleontologists announced the earliest fossils yet found of vertebrates and their close relatives, both dating back to 530 million years ago (*Science*, 5 November 1999, p. 1064, and 3 December 1999, p. 1829).

These studies suggest that the evolution of the vertebrate brain may have had a surprisingly early start in invertebrate ancestors, long before the evolution of the mineralized skeleton that makes most vertebrates so distinctive. What's more, the skeleton may have arisen in an unexpected form: as teeth. The true innovation that launched the lineage of fish, dinosaurs, and people seems to have been new kinds of embryonic tissue, which could form new sensory organs. That allowed protovertebrates, such as those represented by the new Chinese fossils, to embark on a new way to make a living—as predators. Vertebrate evolution is turning out to be more complex—yet more comprehensible—than scientists ever expected.



Next of kin. A developing lancelet (with eggs; embryo, inset) reveals surprising similarities to vertebrates.

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Brain and bone together

One way to track vertebrates' evolutionary history is to analyze their closest living relative. Molecular and anatomical research both give this honor to the lancelet *Amphioxus*, a 5-centimeter-long sliver of a beast that as an adult burrows in sand and filters food from the water. It has little in the way of a skeleton, and its central nervous system consists of a nerve cord with a barely swollen tip. But it does possess vertebrate traits such as gill slits, rows of muscle blocks along its flanks, and a notochord, a stiff rod of tissue that supports the nerve cord along its back. Because of these shared characteristics, biologists classify lancelets and vertebrates together in a phylum called chordates.

Paleontologists have long suspected that vertebrates diverged from a lancelet-like relative sometime in the Cambrian period, which began 545 million years ago. Meanwhile, molecular studies of gene similarities between lancelets and today's vertebrates suggest that the vertebrate lineage goes all the way back to 750 million years ago. But the fossil record provides few clues to help resolve this contradiction, because there are no animal fossils that old and no examples of an intermediate species. Until very recently, the earliest undisputed vertebrates were a mere 475 million years old.

These small, jawless fish with bodies completely covered in bony plates of armor are thought to have dined on sea-floor invertebrates and to have used their armor to defend against predators. Fossils retaining the imprint of the brain reveal that these fish had already evolved many of the major features of modern vertebrate brains, such as divisions into forebrain, midbrain, and hindbrain. "There's no question by that date a vertebrate brain had evolved," says Linda Holland, Nicholas Holland's wife and a fellow Scripps biologist.

If these armored fishes represent the earliest vertebrates, they suggest that brains and bone evolved together. Yet lampreys and hagfish, the only jawless fish alive today, are squishy creatures without a speck of armor and scant amounts of cartilage—and are far more primitive than the fossil forms.

With no obvious intermediates among either ancient or living creatures, biologists were hard put to explain the origins of the vertebrate skeleton and nervous system. Then in

1983 two researchers proposed a new theory that provided an intriguing answer to these puzzles. Their insight rested in part on evidence from another discipline: embryology. Glenn Northcutt, now of the University of California, San Diego, and Carl Gans, now at the University of Texas, Austin, argued that the key to vertebrate evolution was the invention of a head, which in turn was made possible by the evolution of a new kind of embryonic cell.

Studies of living vertebrates reveal that as an embryo forms, a sheet of cells on its surface curls up into a tube that sinks into its body. This structure, called the neural tube, eventually becomes the central nervous system, including the brain and spinal cord. Along the edges of the sheet, a special collection of cells called neural crest cells breaks away and wanders around the embryo, helping to shape many structures such as eyes, nose, nerves, head muscles, and skull bones.

Eye-opener. This 3D reconstruction of a lancelet's nerve cord reveals two rows of flask-shaped photorecepter cells (magenta and cyan) and a cup of pigment cells (red) that may resemble the early vertebrate eye.

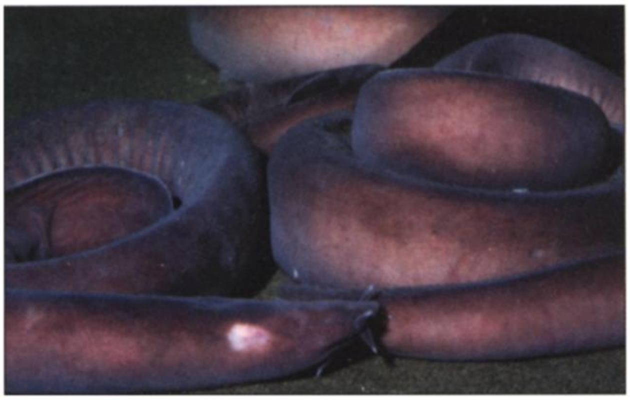
It was the neural crest, Gans and Northcutt proposed, that gave vertebrates the flexibility to build a new kind of body, one that included the complex sense organs, big brains, and powerful pumping throats seen for the first time in lampreys and fossil jawless fish. Along with the new body plan came an ecological shift, as vertebrates evolved from small, passive filter feeders to large, active predators that darted about hunting their prey. "If you're a filter feeder, there are real restraints on how big you can get," says Northcutt. "Developmental changes produced new structures and presented the opportunity to the animal to start doing something else."

This developmental revolution, Gans and Northcutt argued, also sparked the origin of bone. Neural crest cells build the electroreceptors that line the bodies of fish; once these receptors evolved, the researchers theorized, neural crest started building mineralized bone around them to insulate them from the rest of the body. Later, the bone spread out to form a protective coat of armor, as seen in the early bony fish.

This comprehensive model "was one of the most important contributions ever made to the question of the origin of the vertebrates," says Nicholas Holland, because it united disparate lines of evidence and challenged scientists to find ways to test it. It would take over a decade, however, before they had the tools to do so. Their findings partly confirm the model—but suggest some surprising revisions to it.

Raising lancelets

Researchers had long been stymied in their efforts to see whether the biology of lancelets supports the Gans-Northcutt model, because no one knew how to rear lancelets in the lab. A partial breakthrough came in the early 1990s, when Nicholas and Linda Holland figured out how to gather sexually mature wild lancelets from off the coast of Florida on summer nights. In the lab, the Hollands apply an electric current to make the lancelets shed eggs and sperm, then the researchers raise the embryos. "The progress that we make is comparatively slow, because if we're really lucky we'll have embryos on a dozen nights," says Linda Holland.



Humble beginnings. The first vertebrates may have resembled the toothless hagfish.

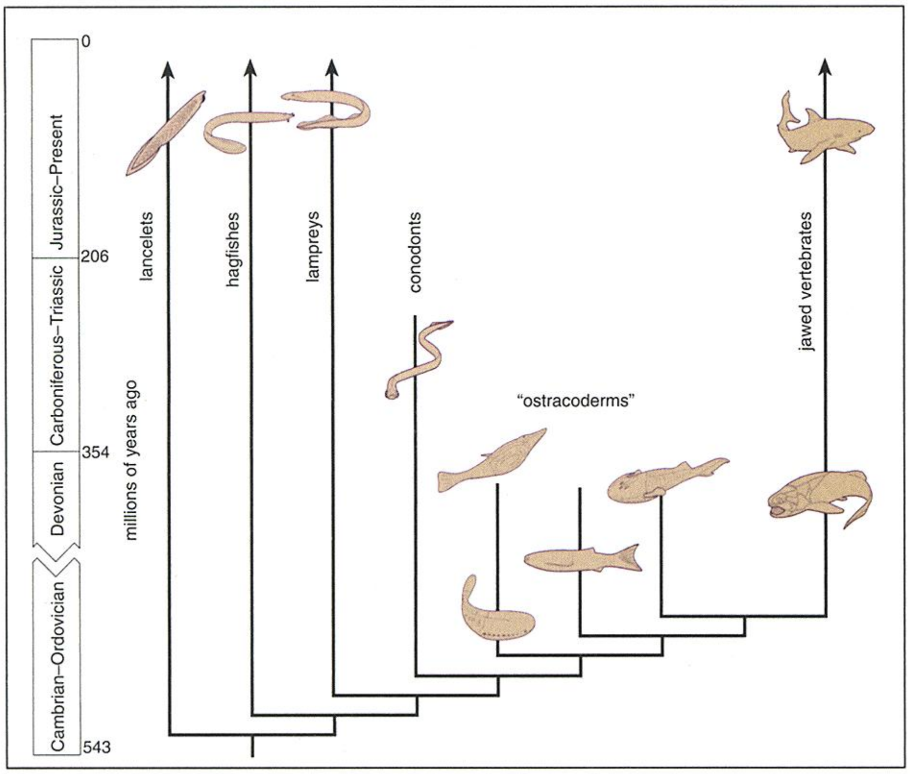
With their meager supply of developing lancelets, the Hollands and others began studying the expression of genes that build the creatures' bodies, using special tags to stain cells expressing the products of known developmental genes. After examining dozens of genes, the picture that emerges is that of a protovertebrate brain. The lancelet doesn't have a true neural crest, but it does have cells in the same position as neural crest cells, and they express some of the same genes that neural crest cells express before they begin to migrate. These cells also migrate, but only as a sheet moving on the surface of an embryo, not as small clusters traveling inside it. "They haven't managed to break loose and wander," says Linda Holland. Their work thus both confirms and refines Gans and Northcutt's notion of the importance of the neural crest. "One innovation of vertebrates is certainly the invention of the wandering neural crest," says Linda Holland. "It opened up the potential to get awfully fancy in the vertebrate head."

What's more, the Hollands and other researchers found evidence that even without a true neural crest, the swollen bud on the front end of the lancelet nerve cord bears a striking similarity to the vertebrate brain. The same genes that organize major regions of the forebrain, midbrain, and hindbrain of vertebrates express themselves in a corresponding pattern in this small cluster of cells in the lancelet's nerve cord. It would be tempting to conclude that these patterns are an atlas of the primordial vertebrate brain, but the lancelet genes may actually be performing quite different tasks from the vertebrate genes, even though they're expressed in the same place, cautions Holland.

Slicing the lancelet brain

While the Hollands probe the genetics of lancelet development, University of Saskatchewan biologist Thurston Lacalli is working on a parallel research program to uncover the animal's detailed neuroanatomy. Starting in 1991, Lacalli's technician Jenifer West photographed 2000 cross sections of the front end of the nerve cord in a lancelet larva. Lacalli began painstakingly tracing out the shapes and connections of each of the approximately 300 neurons, combining them into three-dimensional computer reconstructions. The work is slow: So far Lacalli has identified and traced only two-thirds of the neurons and published data on only half of these. For other lancelet researchers, the wait is agonizing. "It's like taking a 747 and chopping it up a millimeter at a time," says Nicholas Holland.

CREDITS: (TOP TO BOTTOM) T. LACALLI/UNIVERSITY OF SASKATCHEWAN; K. LUCAS/VISUALS UNLIMITED



Shaking the vertebrate tree. A new analysis finds conodonts to be full-fledged vertebrates, whereas hagfish are primitive members of the group.

But already Lacalli's work supports the Hollands' claims that the lancelet nerve cord is divided like a vertebrate brain. In the regions of the lancelet nerve cord where the Hollands found forebrain and midbrain genes at work, the neuronal structure matches that of the vertebrate forebrain and midbrain. "When the two lines of evidence point to the same thing, then we have a lot of confidence," says Linda Holland.

Lacalli goes even further, claiming that clusters of neurons in the lancelet brain seem to perform the same functions as their vertebrate counterparts—even though in the lancelet these clusters may be made up of only a handful of neurons. "It's a surprise that it fits into a vertebrate model so neatly," says Lacalli. For example, noting a retina-like pattern of connections near a cluster of pigment cells near the tip of the lancelet, Lacalli claims that the cluster is a single eye, homologous to the paired vertebrate eyes. The lancelet eye is too crude to form images, but Lacalli suspects it can detect moving shadows of predators. And the hairlike projections that ring the lancelet's mouth—used to accept or reject food—are connected to nerves in much the same way as cells in vertebrate taste buds, Lacalli says.

In a paper in press in *Acta Zoologica*, Lacalli presents an even more dramatic correspondence: He claims that lancelets have a rudimentary limbic system. The vertebrate limbic system, which includes the hypothalamus, monitors the body's internal state, such as its temperature and hormone levels. It then uses this information to control basic behaviors such as when to sleep, when to eat, when to flee, and when to fight. Lacalli has found lancelet neurons whose structure and organization resemble those of vertebrate limbic neurons and that are located in the corresponding parts of the midbrain and forebrain. He suggests that the common ancestor of vertebrates and lancelets used its protolimbic system to switch between its handful of behaviors, such as swimming and feeding. "They fed and they escaped; maybe they even decided to migrate at night. They had [essential] decisions to make. And the way they made these decisions is part of the limbic system," he says.

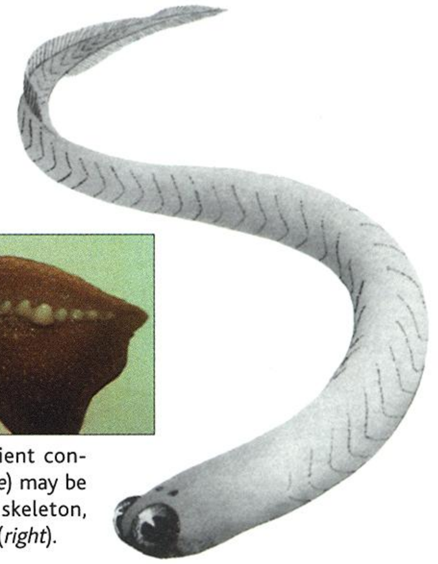
Other researchers say Lacalli makes a strong case. "Excellent work," says Rudolf Niewenhuys, an expert on the limbic system at the University of Nymegen in the Netherlands. "The very beginning of [the limbic system] is to be found here in [Lacalli's] work, because he shows that there is the precursor of the hypothalamus," a crucial part of the limbic system.

The work of Lacalli, the Hollands, and others suggests that in some basic ways, the vertebrate head is not new. Wandering neural crest may have been a key development in the evolution of the vertebrate nervous system, as Gans and Northcutt argued, but by the time the head arose, some of the fundamental structure of the vertebrate brain was already in place. "The regions of the brain that you and I think with are not there," says Lacalli. "But the regions that motivate us, to determine whether we're going to eat or run away or lie down and rest, those things we see some evidence of."

Predators on the prowl

Northcutt agrees that "there are far more similarities between lancelets and vertebrates than any of us would have believed." But to him the head of vertebrates, with its ability to integrate a host of different signals from the senses, still represents a huge evolutionary and developmental step. "One of the major areas of research that will go on for some time is how the [vertebrate and lancelet] are different. Because it's the difference that becomes critical for understanding vertebrate evolution."

Lancelets, for example, apparently have no sense of smell. One of the parts of the vertebrate brain that's missing from the lancelet nerve cord is the most forward portion of the forebrain, known as the telencephalon, which among other tasks handles signals from the nose.



The first teeth. Ancient conodont elements (*above*) may be the first mineralized skeleton, set in an eel-like body (*right*).

Such differences add further weight to Gans and Northcutt's idea that early vertebrates shifted from filter-feeding to predation. But instead of a divided brain, one of the key inventions of early vertebrates might well have been a nose. "A lancelet doesn't need to sniff out its prey, but as the early vertebrates became predators, smell became an asset," says Nicholas Holland. They would also benefit from eyes to see prey and sophisticated control of their bodies to chase prey down.

In the past 6 months paleontologists may have captured that crucial step from filter-feeding to active predation. Last November, Chinese researchers reported a trove of 300 specimens of a creature called *Haikouella*. In some ways these sliver-shaped impressions on ancient rocks look like lancelets, but they also have a few key vertebrate traits unnecessary for filter feeders, such as eyes and muscle blocks. These clues suggest that *Haikouella* is poised at the transition from invertebrate to vertebrate, closer to vertebrates than even the lancelet.

Some researchers have questioned this close kinship, noting that *Haikouella* has a few anatomical peculiarities, such as in the organization of its muscle blocks. But overall, these fossils "look like little vertebrates," says Linda Holland. That makes another feature of their anatomy significant: The fossil nerve cord has an even larger swelling than does that of the lancelet. "It has more stuff up front," says Linda Holland. "I'd say they do have a brain." If so, that pushes the origin of a vertebrate-like brain back to more than 530 million years ago. And *Haikouella* is just the sort of brain-powered, sensory-enhanced predator that Gans and Northcutt predicted 18 years ago.

Primeval predator. The 530-million-year-old *Haikouella* may be poised close to the transition between invertebrate and vertebrate.

CREDIT: NANJING INSTITUTE OF PALAEONTOLOGY AND GEOLOGY

How bone was born

If Northcutt and Gans's theories about the origin of the vertebrate brain have been borne out, their ideas about bone are taking a real body blow. In an upcoming paper in *Biological Reviews of the Cambridge Philosophical Society*, paleontologists Philip Donoghue of the University of Birmingham, U.K., Peter Forey of the Natural History Museum in London, and Richard Aldridge of the University of Leicester, U.K., create a new evolutionary tree for vertebrates that for the first time incorporates a mysterious group of animals called conodonts. These creatures left behind vast numbers of enigmatic little fossils in the shapes of cones and thorns, ranging in age from 510 million to 220 million years old. Over the years "conodonts have been attributed to almost every major phylum you can think of," says Donoghue. Finally in the 1980s new fossils began to emerge with the conodont elements lodged in soft tissue.

Now researchers envision conodonts as eel-shaped predators with a pair of giant eyes and a gaping mouth filled with the toothlike, bony conodont elements, which are made of dentine and other ingredients of the vertebrate skeleton. This new information seemed to elevate conodonts to the status of chordate predators, but paleontologists have fought over exactly what sort of chordate they might be. Donoghue and his co-workers tried to resolve the debate with a massive study of both fossil and living creatures, analyzing 103 different traits in 17 different groups of chordates, ranging from lancelets to jawed vertebrates. "With the fossils we're dealing with, it's as good as we're going to get for a long time," says Donoghue.

Their results show that after the vertebrate lineage split from lancelets, the first group to branch away were the hagfish; lampreys are only slightly less primitive. Conodonts, surprisingly, turn out to be full-fledged vertebrates, even closer to living jawed fish than to lampreys or hagfish. Only after the rise of conodonts did the armored jawless fish, the ostracoderms, appear, and from one of their ranks, the jawed fish eventually evolved (see diagram, p. 1578).

According to the new tree, hagfish and lampreys offer a good representation of what the most ancient vertebrates were like: unarmored and without mineralized skeletons. And conodonts represent the first appearance of a mineralized skeleton. "The conodont skeleton is the primitive vertebrate skeleton," says Donoghue. And it's not the sort of skeleton Gans and Northcutt predicted, notes conodont expert Mark Purnell of the University of Leicester, who calls the results "significant." Mineralization began not in the skin of fish but in the mouths of conodonts, and it presumably made them fiercer predators.

"I think they have good evidence that that is the case, and if it is the case there are some very, very important things there," says Northcutt. "Much of the story Carl [Gans] and I put together has to be wrong," at least when it comes to the evolution of bone.

The conclusion that bone was born after the rise of vertebrates is not yet certain, as more primitive chordates may turn out to have possessed the precursors of conodont mouth parts. Hagfish have "toothlets" made of keratin plus a little phosphate, which might have originated as genuine dentine-based teeth and shifted to other materials later. And Jun-Yuan Chen of the Nanjing Institute of Palaeontology and Geology in Nanjing, China, and his colleagues claim that *Haikouella* had mineralized "pharyngeal teeth" in its throat. Whether these so-called teeth have anything to do with the rise of the vertebrates will have to wait for microscopic analyses of the fossils and for an end to the debate over whether they are chordates at all.

Although these open questions remain, scientists are no longer resigned to the idea that they will never be answered. Now that the stream of data about the origin of vertebrates has started flowing, it shows no sign of slowing down. Museums may never mount a major exhibit about the neural crest, but fitting the developmental data with genetics and paleontology, as Gans and Northcutt first did years ago, is beginning to create a much more satisfying picture of how our favorite group of animals came to be. "I think we're going to see exciting new data in all three fields," says Northcutt. **–CARL ZIMMER**

Carl Zimmer is the author of *At the Water's Edge*.

ADDITIONAL READING

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