Evolution of the Skeleton

Modern-day skeletal tissues can be traced back to the earliest fossil vertebrates

by James Hanken and Brian K. Hall

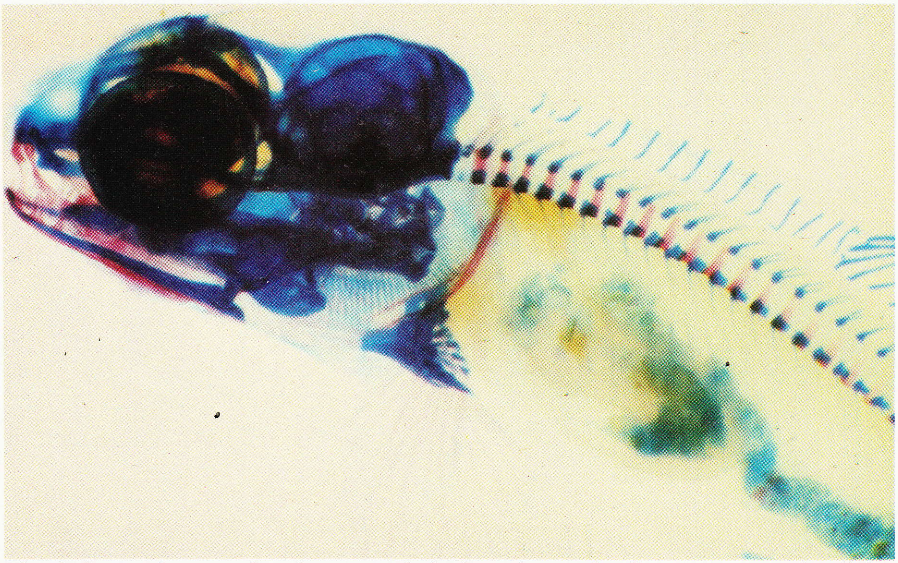
The vertebrate skeleton provides an unusual opportunity to study evolution: living vertebrates come in a variety of shapes and sizes, and the fossil record yields a historical perspective that is unavailable for most organ systems. In the case of some extinct vertebrates, not only overall skeletal architecture but even details of internal anatomy and tissue structure are preserved. Anatomists, paleontologists, and embryologists have researched and debated skeletal evolution for several centuries, and there is general agreement regarding its basic features. But the field remains quite active for there are always new questions to be answered.

The skeleton in vertebrates consists of the relatively rigid structures that support the muscles, nerves, skin, blood vessels, and other "soft" tissues that would otherwise collapse into a helpless mass. Much of the vertebrate skeleton is internal, although many vertebrates have outer skeletal structures as well. The internal skeleton usually includes a central, linear series of interlocking blocks, the vertebrae (from Latin for "joints"). All vertebrate skeletons are combinations of four basic tissues: bone, cartilage, dentine, and enamel. The notochord, a stiffened rod of cells wrapped in fibrous connective tissue that differs from the other four, forms in all vertebrate embryos in the region where the vertebrae will later develop but is absent from most adult vertebrates.

In most vertebrates, bone makes up most of the skull, ribs, vertebrae, and limb supports. Bone also turns up in the base of shark teeth; in bird bills, where it provides an inner supporting structure beneath the horny outside; in turtle shells, where it forms a series of smooth plates below the outer, often ornate horny scutes; and in deer antlers (but not in the horns of a few ungulates, such as rhinos, which are made of hardened keratin, a skin derivative that also forms the bulk of hair and fingernails). Bone is formed by cells that secrete around themselves a matrix of connective tissue fibers (mostly collagen) in which the cells become embedded. Generally, the cells remain alive after depositing the matrix, which is permeated by blood vessels that bring oxygen and nutrients and remove wastes. In some vertebrates, however, most notably many fishes, the cells die after depositing the matrix.

Cartilage, typically a resilient and pliable tissue, is found in all vertebrates and also in many invertebrates, such as squid, octopus, and horseshoe crabs. Like bone, cartilage is formed by cells that secrete an extracellular matrix in which they become embedded. This matrix lacks blood vessels, however, so the cells depend on fluids that bathe the tissue surface; this limits the thickness that cartilage structures can attain. The particular type of collagen that makes up the cartilage matrix also differs from that of bone, and this accounts in large part for the different chemical and mechanical properties of cartilage. Cartilage is found mostly at the ends of many bones, especially near joints where it covers articulating surfaces. In addition, cartilage provides flexible internal support for many things, for example, ears and noses. Although cartilage often represents only a small portion of the adult skeleton, it is the first skeletal tissue to appear and predominates in the embryo.

Enamel and dentine together form teeth: as a rule, a relatively thin layer of hard enamel surrounds a thicker core of soft dentine. (Material at the base of the



The skeleton of a young brook trout includes bone (stained red) and cartilage (stained blue).

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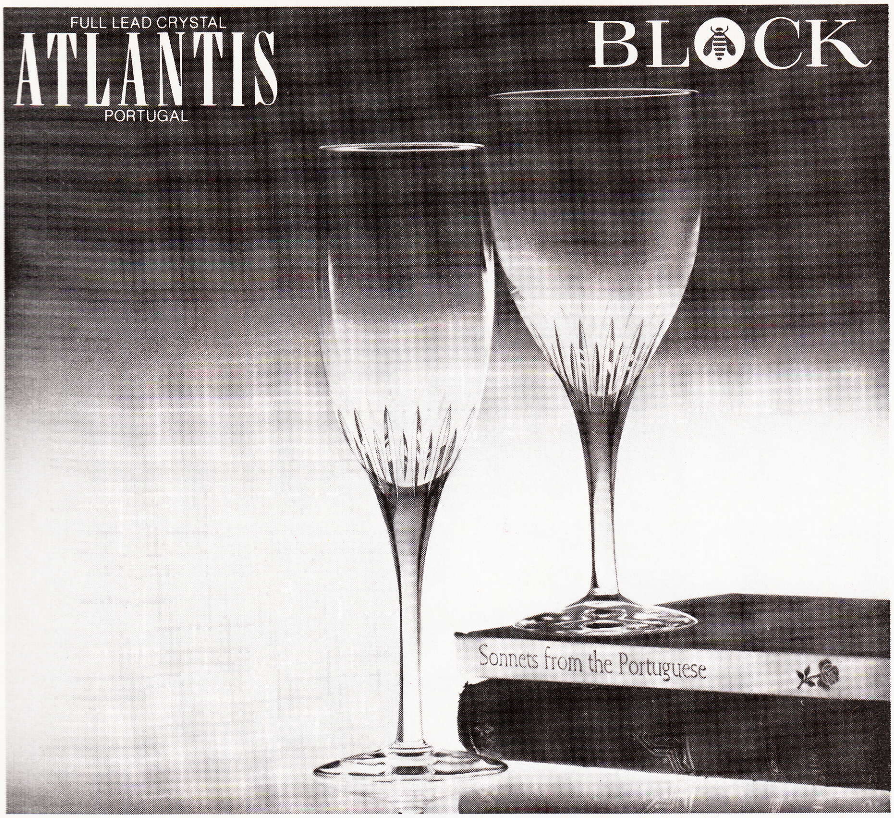


tooth is sometimes distinguished as a fifth type of skeletal tissue, cementum. But unlike dentine and enamel, cementum hasn't figured significantly in arguments concerning skeletal evolution.) Reflecting the basic similarity of tooth and scale formation, both enamel and dentine are seen in scales of sharks and a few primitive fishes. Again, enamel and dentine are each formed by cells that secrete a characteristic surrounding matrix. The specific components of the matrix distinguish the two tissues, both from each other and from bone and cartilage.

Of the four skeletal tissues, three—bone, enamel, and dentine—are calcified; that is, a good portion of the extracellular matrix is made up of inorganic deposits of calcium salts, usually calcium phosphate. This gives bone, enamel, and dentine their hardness. Cartilage is most frequently uncalcified, giving it greater pliability than the three other tissues. However, in some cases cartilage is calcified and, as in many sharks, may actually contain as much calcium as is found in bone.

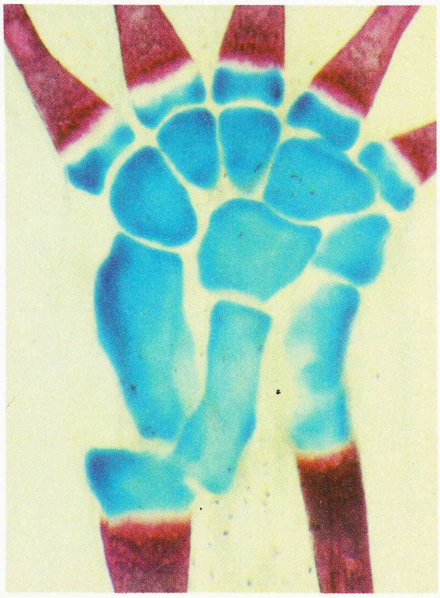
During the nineteenth and early twentieth centuries, the predominant view of skeletal evolution was that the first vertebrates possessed, as adults, an entirely cartilaginous skeleton; the various calcified tissues, including calcified cartilage, were presumed to have appeared much later in vertebrate evolution. Evidence for this view came primarily from two areas: the comparative anatomy of adult living forms, particularly fishes, and embryology.

Among living vertebrates, the most primitive are the eellike cyclostomes—the hagfish and lampreys—of the class Agnatha (from the Greek: *a* = lacking; *gnathos* = jaw). Although they are now believed to be divergent descendants of the earliest fishes, cyclostomes nevertheless have many characteristics that were typical of primitive vertebrates, including the absence of jaws and of well-developed paired fins. All modern cyclostomes possess a solely cartilaginous skeleton, and for a long time this was also presumed to be a primitive trait.

Among living jawed fishes, the elasmobranchs—sharks, skates, rays, and their relatives—also exhibit many features of early vertebrates. Most important are the particular type of jaw suspension (that is, the arrangement of skeletal elements, muscles, and ligaments that hold the lower jaw to the skull proper) and the arrangement of the paired limbs and the tail. Because of these characteristics, elasmobranchs were long considered an archaic group with a morphology little changed from that of the earliest jawed fishes. The skeleton of elasmobranchs is primarily

cartilage, and for this reason they are placed in the class Chondrichthyes (from the Greek: *chondros* = cartilage; *ichthyes* = fishes). Although calcified tissues are present—including teeth, denticles (tiny toothlike bumps that occur over the entire outer skin and give shark skin its sandpaperlike texture), dorsal spines, and calcified cartilage—bone is absent except for small deposits at the base of each tooth and along the vertebral column. As with cyclostomes, the virtual absence of bone in the "primitive" elasmobranchs was taken to substantiate claims of the evolutionary precedence of cartilage. Accordingly, the theory went, only with the advent of the so-called higher fishes did bone first appear as, in effect, the culmination of skeletal evolution in vertebrates.

This classical view derived additional support from the first embryological studies that attempted to document the development of the skeleton of living forms. In all vertebrate embryos, cartilage develops prior to the appearance of any calcified tissue. In particular, many skeletal elements that are bony in the adult—just about any limb bone, for instance—are first formed as a cartilaginous precursor in the embryo. As growth proceeds, much of the cartilage is gradually eroded and replaced by bone-forming cells that deposit bone matrix; in this way, the late-appearing bone assumes the size and shape of the precursor. By themselves these embryological observations do not necessarily support the classical view of skeletal evolution. The necessary logical ingredient was provided by recapitulationism, a view current in the nineteenth century and propounded most vociferously by the Ger-



Bone (red) and cartilage (blue) compose an arboreal salamander's left rear foot.

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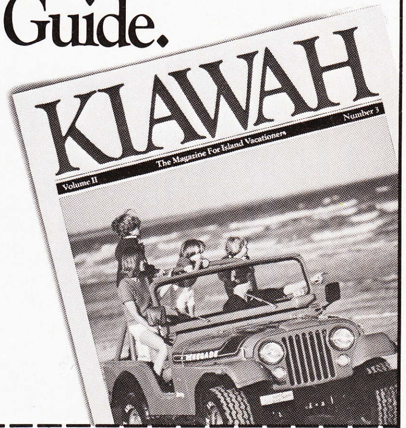
man biologist Ernst Haeckel. In its extreme form, the recapitulationist doctrine, or biogenetic law, held that the embryological development of a given structure or organism repeats the sequence of appearance of adult ancestors; in other words, ontogeny recapitulates phylogeny (a concept that is familiar to readers of *Natural History* who follow Stephen Jay Gould's columns).

Surprisingly, paleontology contributed relatively little to resolving early debates concerning the evolution of the skeleton, even though some fossil remnants of early fishes had to be explained away if the classical view was to be defended. For example, the earliest known fossil elasmobranchs, which, according to the classical view, preceded the higher, bony fishes, actually are preceded in the fossil record by many lineages of bony fishes. This problem was resolved by invoking the convenient excuse of the imperfection of the record. Much of the elasmobranch skeleton, it was argued, is cartilage, and might not be expected to fossilize well; therefore, one couldn't rely on the fossil record to show when the group actually first appeared.

Even more challenging to the classical view were the earliest fossil fishes, the ostracoderms. These lacked jaws and thus resembled the modern-day cyclostomes (particularly the lamprey); for that reason they are often placed with them in the same class, Agnatha. The ostracoderms, however, possessed a well-developed *calcified* skeleton, including bone. This suggests that the present-day cyclostomes are descendants of primitive fishes that had skeletons composed of all four basic tissues, and that their cartilaginous, uncalcified skeleton is not a retained primitive feature after all. For a long time, the evidence provided by the ostracoderms received little attention. Early in this century, however, the Swedish paleontologist Erik A. Stensiö examined fine details of cell and tissue structure of fossil skeletons microscopically, demonstrating conclusively the presence of a well-developed skeleton made up of bone, enamel, dentine, and cartilage in the earliest known vertebrates.

More recent anatomical and paleontological studies have confirmed that all four major skeletal tissue types appear more or less simultaneously in the fossil record. In many early vertebrates the structure of these tissues—for example, the three-dimensional arrangement of bone-forming cells and blood vessels within the surrounding matrix—is as complex as that seen in many recent vertebrates, such as mammals. It appears,



then, that skeletal evolution following the appearance of these basic tissues has involved only minor changes to produce the diversity of skeletal types and configurations we see today. For instance, we now believe that evolution of the elasmobranch skeleton was regressive, involving the reduction and loss of most of the bone, enamel, and dentine that were present in ancestral forms. That is, the shark's cartilaginous skeleton does not represent retention of a primitive vertebrate feature.

How can it be that the skeletons of the earliest fossil vertebrates were as diverse and complex, at least in terms of basic structure, as those we see today? Part of the answer is provided by modern research in developmental biology, particularly the discovery that the development of all of the skeletal tissue types is very similar and only the final outcome differs. For instance, studies using chick embryos suggest that many bone and cartilage cells in the adult are descendants (via numerous cell divisions) of common pluripotent (from the Latin: *pluri* = several; *potens* = potential) "stem" cells in the embryo. Whether a particular daughter cell gives rise to bone or cartilage or, more appropriately, whether it secretes a matrix that is characteristic of bone or cartilage is a function of the particular environment in which it later finds itself.

Perhaps the most widespread process by which the cellular environment influences skeletal development is what embryologists have termed inductive tissue interactions. Early in the development of all vertebrate embryos, most cells can be considered as belonging to one of two basic types: regularly arranged cells that form thin flat sheets called epithelium or more loosely arranged masses of cells called mesenchyme. Epithelium covers the surface of the embryo and lines the gut, whereas the mesenchyme occupies most of the space in between and often is in direct contact with the overlying epithelium. Embryologists have discovered that much of the skeleton, although not all of it, forms as a result of a complex interaction along the common boundary between epithelium and mesenchyme. Each skeletal tissue is derived from only one or the other of these cell populations, but this interaction is essential for proper development.

For example, a limb forms as an outgrowth from the flank, growing progressively longer with time and gradually assuming the shape characteristic of the species. On the leading edge of the growing limb sits a ridge of epithelium that "directs" the formation of the limb from a mass of mesenchyme cells that lies imme-

diately beneath it. Nearly thirty years ago, Edgar Zwilling, then at the University of Connecticut, demonstrated that if the ridge of epithelium is removed, no further limb development occurs, even though the mesenchyme—from which the skeleton would otherwise be formed—is still intact.

The epithelium and mesenchyme share control of limb skeletal development to a remarkable degree. When the epithelium from the forelimb (such as a chick wing) is grafted to the mesenchyme of a hind limb (a chick leg) early in development, a limb forms that distinctly resembles a leg. When instead the epithelium from the hind limb is grafted to the mesenchyme of a forelimb, the resultant limb skeleton is most definitely that of a wing. These and other experiments by Zwilling and others have demonstrated that in development of the limb skeleton, the epithelium determines whether the underlying mesenchyme will form an element that is typical of the base or the tip of the limb—for example, an upper arm or a hand—while the mesenchyme determines whether the skeletal element will be that of a forelimb or hind limb.

Epithelial–mesenchymal interactions characterize the development of many other parts of the skeleton in addition to the limbs, including teeth, which are composed of epithelium-derived enamel and mesenchyme-derived dentine; the hyoid skeleton, which supports, among other things, the gill arches of fish and larval amphibians; and much of the skull and lower jaw. Furthermore, the type of skeleton formed, such as bone or cartilage, as well as where and when it is formed, may be specified by epithelial–mesenchymal interactions. These interactions, at present unknown in invertebrates, also typify the development of a number of other unique vertebrate tissues, including bird feathers and reptile scales. As has long been proposed by Melvin Moss of Columbia University, the early rapid evolution of the vertebrate skeletal tissues may have been made possible by the appearance and elaboration of this basic process.

Epithelial–mesenchymal interactions generally seem to possess enough flexibility that only minor alterations in the normal sequence of developmental events, such as might be contributed by a genetic mutation, are enough to effect a dramatic change in the resultant skeleton. This was recently demonstrated by Edward J. Kollar and Christopher Fisher of the University of Connecticut in what has come to be known as the "hen's teeth" experiment. By artificially combining epithelium from the mouth of a chick embryo with mouse embryo mesenchyme from the lower jaw, Kollar and Fisher initiated an interaction between these two tissues that often resulted in the development of teeth, complete with root, crown, enamel, and dentine. Most important, they established that the enamel matrix was derived from the chick epithelium, even though chickens, as has been typical for all birds for more than a hundred million years, lack teeth and normally never manufacture enamel. They concluded that evolution of toothlessness in birds has not involved a loss of the ability to manufacture enamel; rather, the complex series of tissue interactions by which teeth are produced has been altered.

Finally, the evidence that pluripotent stem cells give rise to all the different skeletal types harmonizes with the views of Karl Ernst von Baer, the great nineteenth-century German biologist and ardent critic of recapitulationism. Among von Baer's outstanding contributions to the nascent field of embryology was the suggestion that development was a process of increasing differentiation and specialization, not a reenactment of a series of ancestral stages.

Current thinking is that the timing of the appearance of different skeletal tissues during maturation is best understood in terms of the appropriateness of each tissue to fulfill certain needs or functions. For example, cartilage is generally capable of more rapid growth than bone, and thus is more appropriate for the skeleton of the early embryo. Likewise, many calcified structures—teeth, for instance—function only after birth, and their development often is delayed until relatively late in the embryonic period. In some regions of the body, however, most notably at many joints within the skull and lower jaw, cartilage appears after bone. Invariably, the appearance of this secondary cartilage is associated with movement about the joints as a result of the involuntary muscle twitches that are so characteristic of embryonic development in vertebrates. In fact, when the movement is artifically prevented in experiments, the secondary cartilage fails to form.

An interesting exception to the norm of late development of teeth demonstrates the extent to which functional demand affects skeletal development. Caecilians are an intriguing group of limbless amphibians of tropical regions. Unlike most amphibians, which lay eggs, many caecilians retain the developing embryo in the oviduct and give birth to a fully formed juvenile. Marvalee Wake of the University of California at Berkeley has shown that in many such species the developing embryo actually feeds while inside its mother by using its jaws to scrape a milky secretion off the lining of the oviduct. Wake discovered that among the first calcified tissues to appear in the embryo were highly specialized comb-shaped teeth that are used to scrape the oviducal wall. The fetal teeth, which are shed at birth, appear before most of the rest of the calcified skull, at precisely the time when the embryo exhausts its minimal yolk reserves and becomes dependent on the mother for food.

Despite all we have learned about skeletal evolution, important questions remain. For example, earlier we mentioned that cartilage is found in some invertebrates. Yet epithelial–mesenchymal interactions, by which most vertebrate cartilage is produced, are not known to occur in invertebrates. We suspect that cartilage development in invertebrates will prove to be greatly different from that in vertebrates, and that cartilage has arisen independently in the two groups, but evidence to confirm or deny this hypothesis is not yet available. Only a few groups of invertebrates have so far been examined with these issues in mind. Another question concerns the elasmobranchs, whose predominantly cartilaginous skeleton is what remains of the bony skeleton of a distant ancestor. They retain the capacity to produce some dentine, enamel, and bone; one wonders why these tissues are so reduced. And finally, more information is needed about the cyclostomes. In this article, we've treated the hagfish and lampreys together, yet recent evidence suggests that these two groups have had quite distinct pasts. While there is some evidence that the lamprey's skeleton is secondarily cartilaginous, the hagfish remains an enigma. These bizarre fishes are highly specialized and cannot in general be considered the prototype of an early vertebrate. But so little is known about their ancestry or their embryological development that no one can say for sure whether their skeleton, which is entirely uncalcified, is a derived characteristic or a truly primitive feature.

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