

Palaeontology

Inside the head of an ancient vertebrate

Zhikun Gai & Philip C. J. Donoghue

Analysis of a 458-million-year-old fossil fish reveals anatomical insights about the vertebrate skull and how skull organization evolved from that of ancestral early vertebrates to that of jawed vertebrates. **See p.782**

The skull is one of the most complex structures in the vertebrate skeleton, showcasing incredible diversity in anatomy and shape. On page 782, Dearden *et al.*¹ provide evolutionary insights with a report of the earliest known fossil of a vertebrate skull.

Despite a skull's functional and anatomical integrity, it is perhaps surprising that it is composed of a number of components that have distinct embryological and evolutionary origins². These components include dermal skeleton, which comprises much of the human face; the neurocranium or braincase, which encompasses and protects the brain and sensory organs; and the visceral skeleton, which includes the jaw and ear bones. Pinpointing the evolutionary origins of these components is challenging because the two lineages of living vertebrates, the jawed (crown gnathostomes) and jawless (cyclostomes) vertebrates, have such different head structures.

All the principal skeletal components are present in living gnathostomes (including sharks, bony fishes and humans), but the living cyclostomes (lampreys and hagfishes) possess only what seem to be simple versions (either rudimentary structures or those simplified

from a more-complex ancestral state) of the neurocranium and visceral skeleton. In lampreys, the neurocranium has only side walls connected by 'bars' composed of unmineralized cartilage (Fig. 1a and Fig. 2). In hagfishes, the brain is surrounded by a fibrous sheath supported by a complex network of cartilaginous bars that bears no more resemblance to the skeleton of a lamprey's head than it does to the mineralized cartilaginous braincase of sharks or to the bony braincases of bony fishes.

Lampreys were long considered to be the first cousins, and hagfishes to be the second cousins, of gnathostomes. Attempts, therefore, to explain the origin of the vertebrate skull had to be rationalized on what was assumed to be the ancestral vertebrate form preserved in these cyclostomes. However, overwhelming molecular and anatomical evidence now indicates that lampreys and hagfishes are each other's closest relatives, equally distantly related to gnathostomes and unequally modified from the ancestral state that they share with gnathostomes³. Consequently, the chimeric combination of ancient and modern (derived) characteristics of lampreys and hagfishes make it difficult to

gain insights into the evolution of the vertebrate skull.

However, another possible research avenue arises because the evolution of mineralization of the skeleton means that there is a rich fossil record of jawless and jawed vertebrates known as ostracoderms (Fig. 2). These are characterized by their extensively developed bony dermal armour. The ostracoderms are related by degrees of kinship, as successively more-distant cousins, to the living jawed vertebrates that effectively document the sequential evolution of the gnathostome body plan, including the skull⁴ (Fig. 2).

The oldest known fossil representatives of the vertebrate lineage are from the Late Cambrian period (between approximately 518 million and 510 million years ago) and although the fossils preserve evidence of a head and jointed visceral skeleton (in the fossils of *Haikouichthys* and *Metaspriggina*)^{5,6} (Fig. 2), they were entirely soft bodied. The ostracoderms flourished in the Silurian and Devonian periods (from 444 million to 358 million years ago), although there are sparse records that extend into the earlier Ordovician period (around 458 million years ago) (Fig. 2).

The ostracoderms clearly provide evidence of the early evolution of a mineralized dermal skeleton, but mineralization of the visceral skeleton and neurocranium occurred much later, in the gnathostome lineage². Indeed, it is not until the emergence of the closest extinct jawless relatives of the jawed vertebrates, known as galeaspid and osteostracans (Fig. 2), that direct insights into the evolution of the brain and sensory organs can be obtained. Those studies revealed, for example, the fundamental reorganization of the vertebrate head that both preceded and facilitated the origin of jaws⁷. However, the structure of the skull in the oldest known ostracoderm lineages, such as the heterostracans (Fig. 2), are limited to lumps and bumps on the underside of the dermal skeleton, providing only

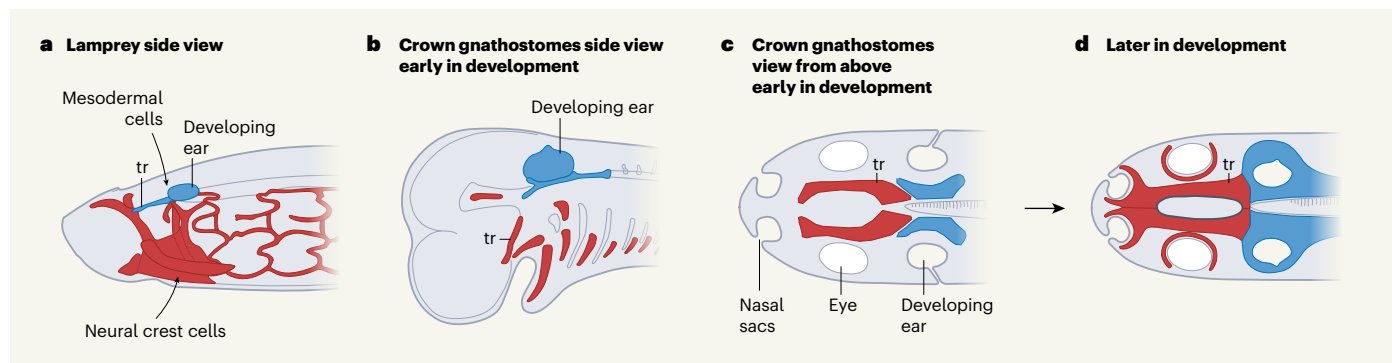


Figure 1 | The skull of living vertebrates. Living vertebrates can be divided into jawless vertebrates called cyclostomes, which include fish called lamprey and hagfish, and jawed vertebrates called crown gnathostomes. The neurocranium or braincase evolved into a structure in gnathostomes that surrounds and protects the brain and sensory organs. How this evolutionary process occurred has been difficult to determine, but Dearden *et al.*¹ present fossil evidence that

offers some fresh perspectives. **a**, The neurocranium of a developing lamprey larva¹⁰, has a rudimentary neurocranium with a structure called the prechordal cranium or trabeculae cranii (tr) made from mesodermal cells that does not protect all the sensory organs. **b–d**, A gnathostome¹⁰ early (**b**, **c**) and later (**d**) in development. Its trabeculae cranii is made from neural crest cells and protects its eyes and nose.

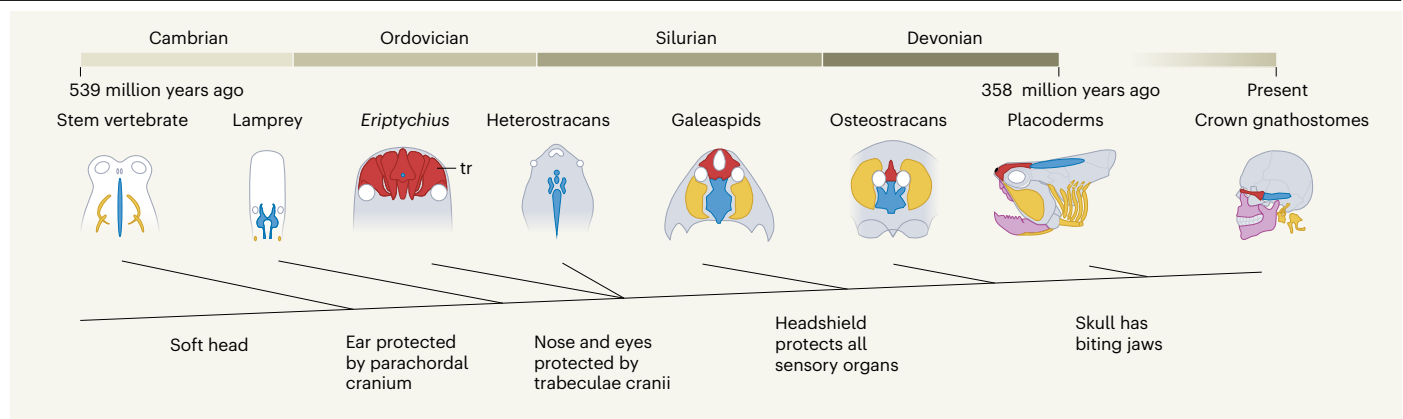


Figure 2 | Vertebrate-skull evolution. The earliest known extinct vertebrates, termed stem vertebrates, such as *Metaspriggina*, were soft bodied and lacked a mineralized skull to protect the brain and sensory organs. By contrast, living jawed vertebrates (crown gnathostomes) have a notably different skull, which is mineralized and contains components that include the dermal bone (grey), jaw bones (pink) and the neurocranium (red and blue), which protects the brain and sensory organs, and includes structures made from neural crest cells (red, yellow and grey) and those made from mesodermal cells (blue). Their neurocranium consists of parachordal cranium (blue) and prechordal cranium

(red), which is also known as the trabeculae cranii (tr). Living jawless vertebrates called cyclostomes (such as lampreys) evolved a neurocranium that protects the ears. Dearden *et al.*¹ analysed a 458-million-year-old fossil of *Eriptychius*. This vertebrate's neurocranium has a trabeculae cranii, which was probably formed from neural crest cells, and might have protected the nose and eyes. This key evolutionary innovation provides context to understand creatures such as galeaspids and osteostracans, during the transition through which the skull evolved to include dermal bone, neurocranium and branchial arches (yellow) in the formation of a headshield to protect all sensory organs.

the vaguest of impressions of the brain and sensory organs⁸.

Dearden and colleagues' report of a mineralized neurocranium associated with the enigmatic Ordovician jawless ostracoderm *Eriptychius americanus* is astonishing¹. *Eriptychius* was first described in specimens from the Late Ordovician (between 458.4 million and 453.0 million years ago) and is mainly known by its distinctive, broadly ridged fragments of dermal armour. However, a single specimen demonstrates the presence of a braincase formed from mineralized cartilage⁹.

Using the technique of X-ray computed tomography, Dearden and colleagues get inside this oldest known three-dimensionally preserved braincase. They show that the part of the head between the snout and the eyes contains a set of cartilages that might represent the earliest known fossil record of the part of the braincase called the prechordal cranium or trabeculae cranii (Fig. 1). These structures, also called trabecular cartilages, are formed in jawed vertebrates from neural crest cells (Fig. 1b–d). The neural crest origin of this part of the braincase is considered to be a major developmental and evolutionary innovation compared with the case for cyclostomes, whose trabecular cartilages are formed from mesodermal cells¹⁰ (Fig. 1a).

In living jawed vertebrates, the prechordal cranium derived from the neural crest is incorporated into another part of the cranium (the mesodermal-derived parachordal cranium) that forms a joint with components of the visceral skeleton (the paired mandibular and hyoid arches) to form the skull. Galeaspids and osteostracans show an evolutionary precursor form in which all the neural-crest-derived prechordal cranium and structures

called gill arches (including mandibular and hyoid arches) are incorporated into the parachordal cranium to form a single massive cartilaginous skull^{7,11,12} (Fig. 2).

In jawed vertebrates, the mandibular arch expands backwards to form the jaw, with the hyoid arch hinging on the braincase, whereas all other gill arches are positioned more posteriorly, independent of the braincase. Clearly, there was a lot of reorganization of the head associated with the origin of jawed vertebrates. This raises the question of what was the ancestral vertebrate condition.

Dearden and colleagues interpret the separate cranial cartilages of *Eriptychius* as being distinct from those of other ostracoderms and jawed vertebrates, representing a different way of organizing a vertebrate head. However, the shape of the braincase and the extent of its mineralization might not have been the same, because it is not clear whether the mineralized cartilages represent the full distribution of cranial cartilage or just its mineralization. As such, it remains possible that the brain was enclosed more completely than the mineralized braincase might suggest, by a cartilaginous neurocranium that was incompletely mineralized. Even so, *Eriptychius* might provide a unique insight into the otherwise unmineralized, and therefore unfossilized, neurocranium of its relatives. But who were they? Much depends on the evolutionary-tree position of *Eriptychius*, which has hitherto defied classification and jumped between proposed branches because its anatomy has been so poorly known.

The authors ally *Eriptychius* with the jawless ostracoderm *Astraspis*, arandaspids and the heterostracans (although this hypothesis is only weakly supported by data, as the authors

acknowledge). The nature of the braincase in *Eriptychius* is not incompatible with the condition in heterostracans, as inferred from impressions of the brain and sensory organs on the inside of the back (dorsal) dermal skeleton¹³, which, presumably, formed the roof of the braincase that was otherwise encased by unmineralized cartilage.

It seems unlikely that the braincase of *Eriptychius* and its heterostracan kin incorporated the gill arches, as is the case in galeaspids and osteostracans. This is not least because the chamber enclosing the gills extends much further back along the body than in these other groups. As such, the nature of the braincase in *Eriptychius* and heterostracans is clearly different from what has been known before, but was it a newly evolved feature of the group or is does it reflect the ancestral condition from which galeaspids, osteostracans and jawed vertebrates developed? Dearden and colleagues propose the latter, arguing, reasonably, that an extensive neurocranium and visceral skeleton must be an ancestral condition for the gnathostome lineage. Indeed, it might reflect the nature of the ancestral vertebrate from which cyclostomes evolved through descent with simplification and transformation.

Either way, Dearden and colleagues' study demonstrates how fossil insights into comparative anatomy can help to evaluate the utility of comparative-developmental model systems, such as cyclostomes, in attempts to infer the nature of the ancestral vertebrate. Surely, many more such insights remain to be uncovered. The specimen examined in this study was described in exquisite detail more than half a century ago⁹. X-ray tomography has, yet again, provided clues that would otherwise remain buried in rock, breathing new life into these old

bones. Although palaeontologists often like the idea of being swashbuckling excavating explorers, many of the best discoveries can be made in existing museum collections.

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newborn babies and gradually diminishes with age, but nonetheless persists well into adulthood. Craniosynostosis is a common condition (affecting 4 to 5 children in 10,000)^{2,3} caused by premature fusion of these sutures, which restricts the expansion of the cranium, leading to increased pressure on the brain that results in cognitive and learning difficulties.

Numerous studies have revealed that sutures house stem cells – often referred to as calvarial stem cells (CSCs) – and their progeny, which contribute to the formation of cranial bone by giving rise to cells known as osteoblasts. CSCs have been identified by various markers, including expression of the genes *Gli1* (ref. 4), *Axin2* (ref. 5) and *Prrx1* (ref. 6). However, many of the findings of these studies have been inconsistent, and key questions remain unresolved because of differences in the selectivity and rigorosity of the approaches used to identify CSCs.

It has generally been assumed that depletion of CSCs is the main cause of craniosynostosis^{4,7}, and that repopulating the sutures with these cells would therefore restore their function and allow cranial expansion^{7,8}. Bok *et al.* question this assumption in their study. They argue that, because fusion of sutures requires the formation of new bone, depletion of CSCs would actually result in sutures remaining open.

To test this hypothesis, the authors generated mice in which CSCs, identified on the basis that they express the *Ctsk* gene⁹, were genetically modified. More specifically, these cells (known as CTSK⁺ CSCs) were altered so that they did not express the *Twist1* gene; loss of function of the TWIST1 protein in humans is associated with Saethre–Chotzen syndrome, a condition that results in craniosynostosis. The authors observed that these CTSK⁺ CSCs underwent a form of programmed cell death,

Regenerative biology

Dual stem-cell populations interact in the skull

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The discovery that the skull has two groups of stem cell that produce similar types of descendant cell has big implications for the field of stem-cell research – and casts light on a developmental disorder that affects many children. **See p.804**

During adult life, the differentiated cells required to maintain the function of a tissue are generated from stem cells located within that tissue, generally from a single population of stem cells. But on page 804, Bok *et al.*¹ report that the skull harbours two distinct populations of stem cells that have complementary functions under physiological conditions, but

might work in opposing directions under conditions that cause disorders.

The cranium houses and protects the brain, and comprises eight bones interconnected by bands of cells. These bands, known as sutures, have a crucial role in enabling growth of the cranium to accommodate the developing brain. Such development is pronounced in

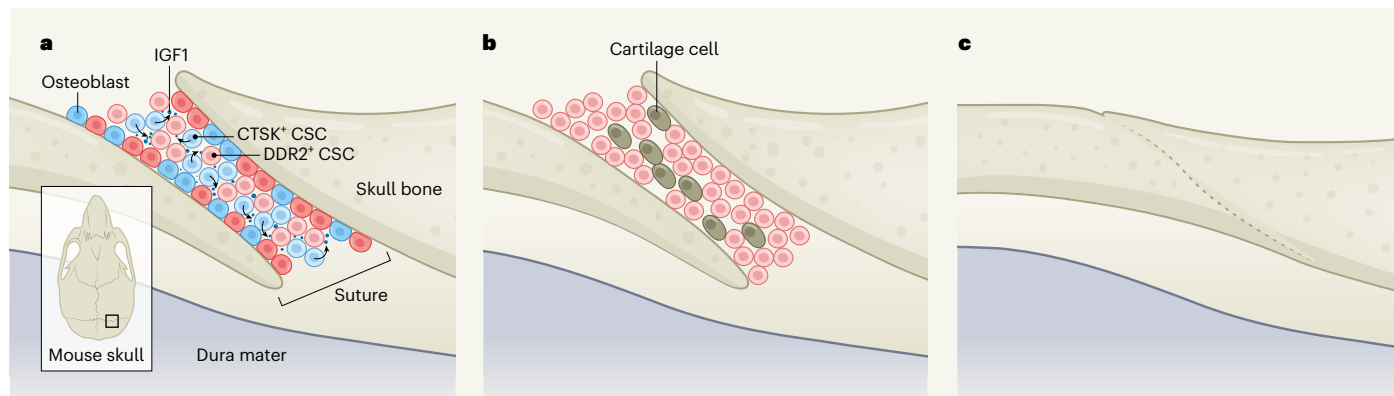


Figure 1 | The behaviour of distinct stem-cell populations in skull sutures under different conditions. **a**, The bones of the skull are connected by sutures (bands of cells), which include calvarial stem cells (CSCs). Bok *et al.*¹ report the unexpected finding in mice that sutures contain two distinct pools of CSCs: those that express the *Ctsk* gene (CTSK⁺ CSCs, shown in light blue) and those that express the *Ddr2* gene (DDR2⁺ CSCs, shown in pink). Both types of CSC self-renew and differentiate to produce bone cells (osteoblasts; those shown in red derive from DDR2⁺ CSCs, those in darker blue from CTSK⁺ CSCs). The

CTSK⁺ CSCs secrete the protein IGF1, which signals to DDR2⁺ CSCs and promotes their normal osteoblast-forming activity. The dura mater is the membrane that underlies the skull. **b**, However, in the absence of CTSK⁺ CSCs (and therefore of IGF1), the DDR2⁺ CSCs differentiate to produce cartilage cells. **c**, The cartilage is then converted into bone through a process known as endochondral ossification, thereby causing fusion of the suture. The findings cast light on the mechanism of a disorder called craniosynostosis – a common condition in humans, caused by the premature fusion of sutures in children.