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Vertebrate head development: Segmentation, novelties, and homology ☆

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Abstract

Vertebrate head development is a classical topic lately invigorated by methodological as well as conceptual advances. In contrast to the classical segmentalist views going back to idealistic morphology, the head is now seen *not* as simply an extension of the trunk, but as a structure patterned by different mechanisms and tissues. Whereas the trunk paraxial mesoderm imposes its segmental pattern on adjacent tissues such as the neural crest derivatives, in the head the neural crest cells carry pattern information needed for proper morphogenesis of mesodermal derivatives, such as the cranial muscles. Neural crest cells make connective tissue components which attach the muscle fiber to the skeletal elements. These crest cells take their origin from the same visceral arch as the muscle cells, even when the skeletal elements to which the muscle attaches are from another arch. The neural crest itself receives important patterning influences from the pharyngeal endoderm. The origin of jaws can be seen as an exaptation in which a heterotopic shift of the expression domains of regulatory genes was a necessary step that enabled this key innovation. The jaws are patterned by *Dlx* genes expressed in a nested pattern along the proximo-distal axis, analogous to the anterior–posterior specification governed by

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Hox genes. Knocking out *Dlx 5* and *6* transforms the lower jaw homeotically into an upper jaw. New data indicate that both upper and lower jaw cartilages are derived from one, common anlage traditionally labelled the "mandibular" condensation, and that the "maxillary" condensation gives rise to other structures such as the trabecula. We propose that the main contribution from evolutionary developmental biology to solving homology questions lies in deepening our biological understanding of characters and character states.

Keywords: Neural crest; Paraxial mesoderm; Segmentation; Characters; Constraints

Introduction

The structure, evolution and development of the vertebrate head is a problem as old as the study of morphology itself. In this paper we try to give an overview of some recent advances in vertebrate head development research, which is now in a state of fast and multifaceted progress. We start with a historical introduction, to give credit to the fact that this is a research area with a long and distinguished history of enquiry. The historical part ends with a brief look at the segmentalist ideas developed by members of the "Stockholm school" of palaeozoology. This is followed by a modern view of head development and segmentation. The fascinating, recently developed hypothesis that an important pre-requisite for jaw evolution was a heterotopic shift in the expression domains of certain developmental regulatory genes is described. Upper and lower jaws have quite different morphologies in most extant gnathostomes. The genetic specification of lower (as opposed to upper) jaw identity has recently been shown to be encoded in certain *Dlx* genes by a mechanism involving nested expression in the same manner as when *Hox* genes code for segment identities. We report on this exciting study and the consequences it might have for how we imagine that jaws and their identities originated as evolutionary innovations. New data on the precise developmental origin of maxillary (upper jaw) and mandibular (lower jaw) skeletal structures throw doubt on old textbook knowledge, and finally, we review the role of cranial neural crest cells for proper morphogenesis of the cranial musculature. In the last part, we discuss the importance of developmental data for a deeper understanding of characters and as a (fallible) guide to recognizing homology.

The skull as a continuation of the vertebral column

The idea that the vertebrate skull is segmented has been attributed to both the naturalist Lorenz Oken (1779–1851) and the poet and scientist Johann Wolfgang von Goethe (1749–1832), who also coined the term "Morphologie". Similar anecdotes have been attributed to both scientists, the main point being that Goethe (or Oken) saw a sheep's skull that had dried and started to break apart at the sutures. The idea

that the skull was made up of vertebrae came as a flash of insight, typical of a romantic age. Goethe wrote (as translated in Richards, 2002) that it struck him "as I lifted a battered sheep's skull from the dune-like sands of the Jewish cemetery in Venice", that not only the posterior parts of the skull were transformed vertebrae, but that "I immediately perceived that the facial bones were likewise to be traced to vertebrae". Goethe might have been the first to have the idea, as documented in letters from the 1790s (Rose-Engelberth, 1999). Oken was the first to publish it, and made it the topic of his inaugural lecture as a professor in Jena (Oken, 1807). This was an idea whose time had come, and between 1807 and 1820, in addition to Goethe and Oken, similar views were published by von Spix, E. Geoffroy St. Hilaire, d'Azyr and Carus (de Beer, 1937). However, the idea that the skull is made up of vertebrae did not go uncriticized, and was famously attacked by Thomas Henry Huxley in his Croonian lecture (Huxley, 1858). Although Huxley did not believe that the skull consisted of vertebrae, he did think it was segmented. Head segmentation ideas then developed in parallel in the English- and German-speaking worlds.

Segments in the embryonic head

In 1876, Francis Maitland Balfour (1851–1882), in his monograph on shark development, came up with a famous scheme of head segmentation in which each segment had one cranial nerve, one branchial arch and one head cavity (or "head somite"). All in all there were eight head segments. Balfour (1878) wrote "…within the last few years it has been more or less generally accepted that the head is, in part at least, merely a modified portion of the trunk and composed, like that, of a series of homodynamous [serially homologous] segments."

A major shift in emphasis from the earlier period is that the *development* rather than just the adult morphology of the head now becomes the center of attention. The British development of head segmentation research was summed up in a famous diagram (Fig. 1) by Goodrich (1930). This scheme shows simultaneous metamery of the nervous system (cranial nerves), the mesoderm ("head somites") and branchial arches in a generalized vertebrate embryo.

In Germany, Ernst Haeckel used head development to illustrate his "biogenetic law", showing the similarity of vertebrate heads in early stages of development in contrast to the diversity of adult head structures (Fig. 2). Haeckel's friend, Carl Gegenbaur saw the head as a continuation of the segmented (or metameric) trunk, much like Balfour. In his view, the skeletal elements in the branchial arches were serially homologous with ribs, and the cranial nerves with spinal nerves. His ideas developed over decades, but a late review paper gives a good overview (Gegenbaur, 1888). Gegenbaur also emphasized that the "head problem" is a phylogenetic problem. In 1888, he wrote "[…] the question of the vertebral theory of the skull becomes a problem of the phylogenesis of the entire head".[1] His method was to

[1]German original. "[Š]gestaltet sich die Frage der Wirbeltheorie des Schädels zu einen Problem der Phylogenese des gesamten Kopfes" (Gegenbaur, 1888).

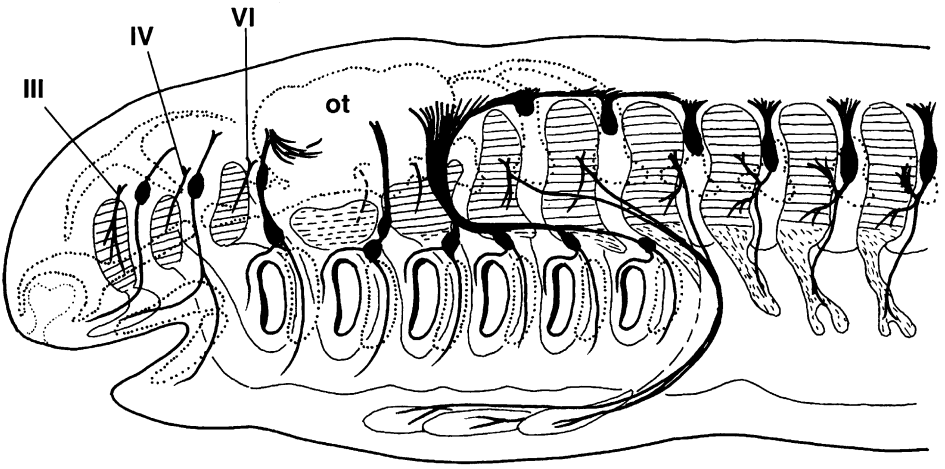

Fig. 1. Schematic (from Kuratani, 2003) of the metamery of the vertebrate head redrawn from Goodrich (1930), who based the drawing on embryos of *Scyllium canicula*. ot, otocyst; III, IV, and VI, cranial nerves.

investigate developmental head anatomy in an animal he thought was phylogenetically basal or "primitive", to get at the basic pattern of head development (Mitgutsch, 2003). Gegenbaur chose sharks as his main model animal, and many of his general statements are based on his comparative studies of cartilaginous fish.

Segmentalist palaeozoology – "The Stockholm School"

In addition to developmental biologists and comparative morphologists, palaeontologists have also taken a keen interest in the structure of the vertebrate head, its evolution and in head segmentation (see Janvier, 1996 for review). An influential school was started in Stockholm, Sweden, in the 1920s by Erik Stensiö (Olsson, 2005). Stensiö (Fig. 3) became famous for taking anatomical work on fossils to a new level of exactness. He used a method developed by Sollas, where the fossils were carefully and extremely finely sequentially ground down, and the surface drawn or photographed after each round of grinding. The drawings were magnified and transferred to wax plates, and the wax plates were put together into a three-dimensional model of the whole fossil, or of selected organ systems. In the end, the fossil was completely ground down and destroyed, but the resulting wax models

Fig. 2. A plate from Ernst Haeckel's "Antropogenie" (1874) showing two things. (1) The difference between the head shape between embryos and adults within a species and (2) the similarities between embryonic heads of different species of mammals. The species are M, human; S, sheep; F, bat; and K, cat. Courtesy of the Photo Archive in the Ernst-Haeckel-Haus, Jena, Germany.

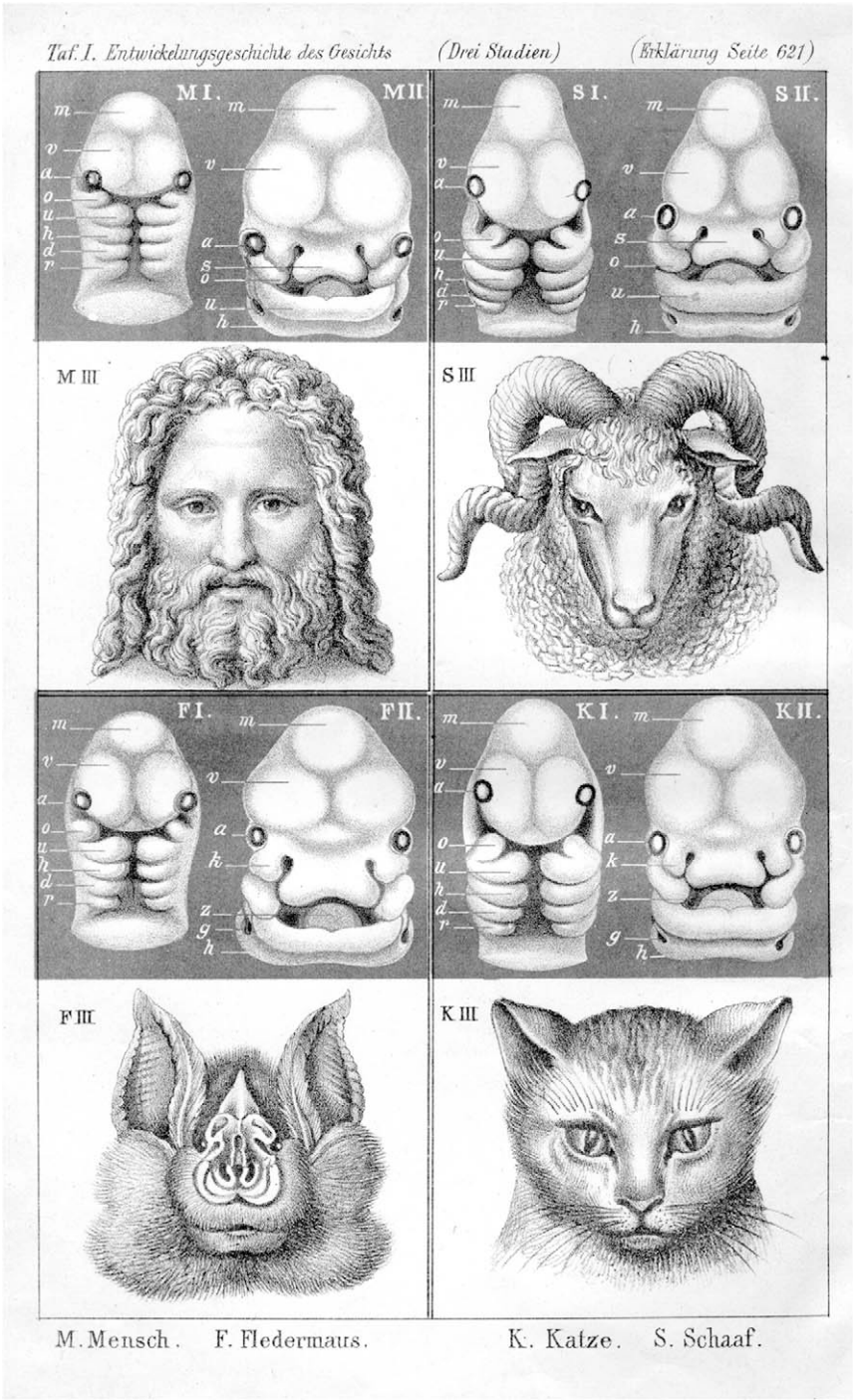
Taf. I. Entwickelungsgeschichte des Gesichts (Drei Stadien) (Erklärung Seite 621)
M I.
M II
S I.
S II.
M III
S III
F I.
F II.
K I.
K II.
F III
K III
M. Mensch. F. Fledermaus. K. Katze. S. Schaaf.

Fig. 3. Erik Stensiö (1891–1984) with drawings of prepared fossils. Portrait courtesy of the Center for the History of Science at the Royal Swedish Academy of Sciences, Stockholm.

made it possible to describe the anatomy of the fossil at a level of detail that was beyond anything seen before. A research school in palaeozoology was formed by Stensiö's assistants and visiting (sometimes for long periods) researchers.

A peculiarity of Stensiö's method, and a key to his success, was that he treated his fossils as if they had been recent animals. It was basically an ahistorical approach that has its roots in idealistic morphology. Stensiö also acknowledged how important the German anatomical tradition had been for the development of his thinking (P. Janvier, pers. comm.). Stensiö made constant reference to extant fishes when describing the anatomy of his fossils. Placoderms were compared to sharks, and ostracoderms to lampreys. Stensiö also collaborated with the Stockholm zoologist Nils Holmgren on classical questions in comparative anatomy like head segmentation.

A leitmotiv in Stensiö's research was polyphyletism; that is, the independent appearance of the same character in several groups. Thus, groups, which were generally regarded as monophyletic, such as cyclostomes, cartilaginous fishes, or four-legged vertebrates (tetrapods), on the ground of their sharing unique characters, were decomposed into several subgroups which were supposed to have acquired these unique characters independently and to be derived from separate fossil groups in which these characters were lacking. An idealistic view can easily accommodate polyphyletic evolution, because characters, which are present ideally, in the type, can

easily re-evolve, which leads to parallel or convergent evolution. They just go from a potential to an actual state. This is a very different way of arguing from that of cladistics, in which evolutionary changes are minimized using the parsimony principle.

Stensiö's student Erik Jarvik was to take over the professorship and develop the idealistic ideas further, most clearly perhaps in the second part of his great monograph "Basic structure and evolution of vertebrates" (Jarvik, 1981). The research school developed by Stensiö and continued by his younger colleagues still exists today in the work of Hans C. Bjerring, whose view of the vertebrate head clearly betrays its origins in idealistic morphology (Bjerring, 1977, 1984).

Head segmentation – a contemporary view

Interest in "the head problem" was renewed by the discovery of *Hox* genes in vertebrates, when it was found that these genes are expressed in a nested pattern along the anterioposterior (AP) axis (Hunt et al., 1991a, b). As shown in Fig. 5, *Hox* genes (and other homeobox-containing genes such as the *Otx* genes) have sharp anterior borders of expression, which correspond to morphological boundaries that only develop later. *Hox* genes are expressed from this anterior boundary and along the AP axis posteriorly. This leads to different combinations of *Hox* genes being expressed in different segments (e.g. somites, rhombomeres). It has been shown that these "Hox codes" are important for giving each segment its correct positional identity. In the head (defined as beginning just anterior to the atlas vertebra), altering the Hox code converts segment identities (by homeotic transformation) only in the pharyngeal arches and rhombomeres (the primary hindbrain segments) (Rijli et al., 1998; Grammatopoulos et al., 2000; Pasqualetti et al., 2000). There are no distinct head somites in front of the otic vesicle, although that was earlier taken for granted. If there were "head somites", i.e. if the cranial paraxial mesoderm in front of the otic vesicle was organized in the same way as in the trunk, one would expect the patterning of this mesoderm to be governed by the same mechanism via *Hox* genes – like the somites in the trunk. However, *Hox* genes are not expressed in this part of the head. Although somite-like structures ("somitomeres") have been claimed to exist in the pre-otic part of the head (Jacobson and Meier, 1984; Meier and Packard, 1984; Jacobson, 1988), most researchers describe the head mesoderm in this region as "unsegmented" (Kuratani et al., 1999; Noden et al., 1999; Cerny et al., 2004a; Ericsson et al., 2004), and the topic remains controversial.

It has been argued (Kuratani, 2003) that in the trunk, the existing segmental pattern of the paraxial mesoderm, i.e. the somites, imposes a segmental pattern on neural crest-derived structures such as the dorsal root ganglia, thereby acting as a generative constraint. If somite number is changed, the number and patterning of dorsal root ganglia changes in the same fashion. The reason is that trunk neural crest cells are forced, by the presence of tenascin and other non-permissive molecules on the posterior half of each somite, to migrate only in the anterior half. The trunk

neural crest cells are not pre-patterned, but become secondarily metameric by the imposed somite pattern. The same is true for myelomeres (neuromeres in the spinal cord) and motor neurons (reviewed in Kuratani, 2003).

In the pre-otic head, on the other hand, the mesoderm cannot act as the primary segment-forming structure, as no somites are formed here. So from where do the segmental patterns seen in structures such as rhombomeres and pharyngeal arches come? The rhombomeres do not need to be induced by mesoderm, but are formed autonomously (Källén, 1956). Moreover, the rhombomere borders seem to act as a constraint for cranial neural crest movements, and signals from the neural tube and overlying ectoderm navigate neural crest cells into specific pharyngeal arches such that crest cells from rhombomeres 1 and 2 all migrate into the mandibular arch, those from rhombomere 4 into the hyoid arch, and most cranial neural crest cells from rhombomeres 5 and beyond end up in the branchial arches. In chicken and mouse embryos, the majority of crest cells from rhombomere 3 and 5 die from apoptosis, and the rest are divided up between the neighboring arches (Birgbauer et al., 1995; Graham et al., 1996; Kulesa et al., 2000). This is important for preventing mixing of neural crest cells between streams and thereby for correct patterning of the cranial neural crest. It is not completely clear how the crest streams form in species where apoptosis in rhombomeres 3 and 5 have not been reported, such as the zebrafish teleost and the *Xenopus* frog (Schilling and Kimmel, 1994; Hensey and Gautier, 1998), but specific signaling molecules like ephrins seem to be involved (Smith et al., 1997; Holder and Klein, 1999). The pattern imposed on cranial neural crest cells by the rhombomeres from which they originate is transported by them into the rest of the head and imposed on the mesodermally derived muscles (Noden, 1983a, b, 1986; Noden et al., 1999). So, unlike in the trunk, the neural crest in the head acts as a generative constraint on the paraxial mesoderm. That cranial crest cells are carriers of patterning information to the rest of the head was shown elegantly recently by Schneider and Helms, who transplanted an anterior cranial neural crest between duck and quail embryos, and always got a donor-type morphology of the beak and associated parts of the host head in their chimaeric ''qucks'' and ''duails'' (Helms and Schneider, 2003; Schneider and Helms, 2003). They conclude that donor neural crest cells must be able to induce autonomous molecular programs and regulate gene expression in adjacent host tissues (Schneider and Helms, 2003).

The other patterning process is the formation of the endodermal pharyngeal pouches, which imposes a segmented pattern on the pharyngeal arches that is independent of patterning information from the neural crest (Graham and Smith, 2001). If the cranial neural crest is removed (the neural tube was removed before crest cell migration), pharyngeal arches form anyway (Veitch et al., 1999). As argued recently by Graham et al. (2004), the independence of pharyngeal segmentation from neural crest influences might reflect the fact that pharyngeal arches are evolutionarily older than the neural crest. The presence of pharyngeal arches is an autapomorphy for chordates, whereas only vertebrates have a neural crest. Thus, the origin of the vertebrate pharynx as a novel structure was accomplished by integrating the cranial neural crest-derived skeletal elements with an existing, segmentally organized

pharyngeal endodermal (Graham et al., 2004). That neural crest cells react to patterning information has been shown elegantly in the chicken embryo, where removal of parts of the endoderm in the pharynx leads to loss of the corresponding neural crest-derived skeletal elements which would no longer receive the proper patterning signals (Couly et al., 2002).

The view of vertebrate head segmentation that emerges from these developmental studies differs from earlier models, such as the segmentalist views developed by the "Stockholm school" of palaeozoology. As summed up in Fig. 4 (from Kuratani,

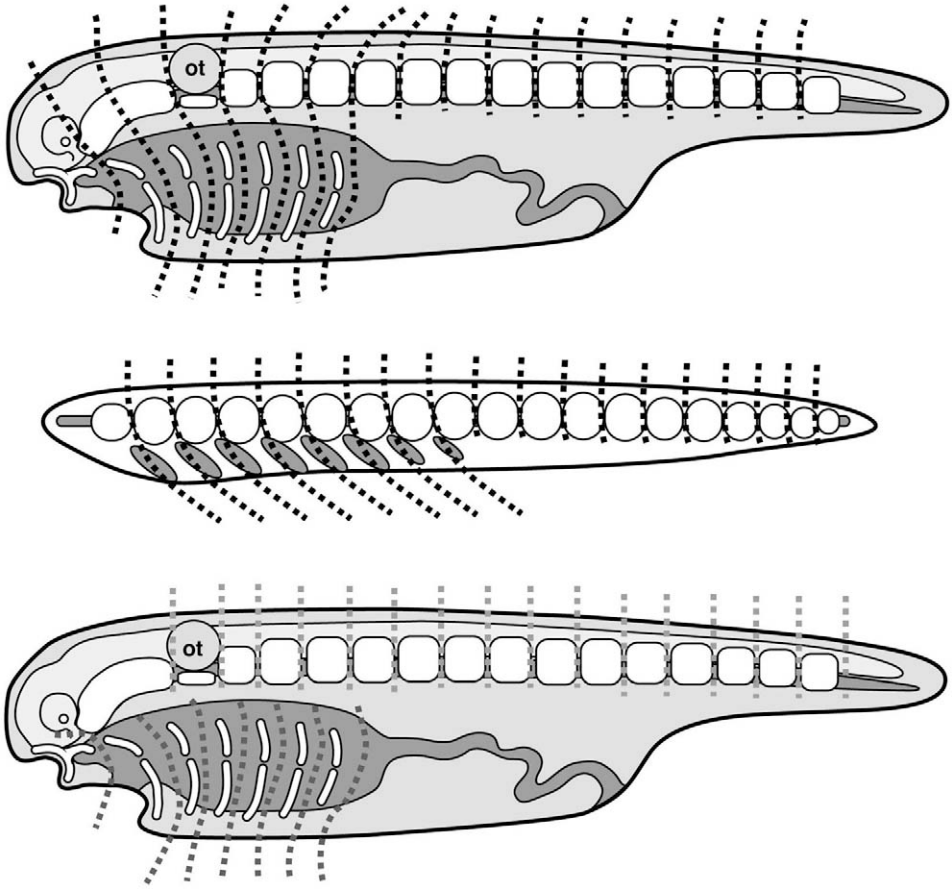

Fig. 4. Vertebrate metamery. Top: a traditional segmentalist view where head mesoderm segmentation is just a continuation of the segmented somites in the trunk, and pharyngeal arch segmentation is in register with mesoderm segmentation. Middle: schematic of *Branchiostoma* morphology. Segmentalism refers all vertebrates to the basic segmented body plan seen in *Branchiostoma*. Bottom: a non-segmental view of the vertebrate head. Branchiomerism and somitomerism are recognized as different developmental programs or morphological patterns in the head and trunk. ot, otic vesicle (from Kuratani, 2003).

2003), segmentalists see the head as a continuation of the trunk (Fig. 4, top), with the same segmentation mechanisms in both parts of the body – and in all parts of the head. This makes vertebrates comparable to *Branchiostoma* (Fig. 4. middle), with which they share the same basic body plan. However, the head is not a simple extrapolation of the trunk, but a much more complicated and fascinating structure. The segmentation of the paraxial mesoderm in the trunk into somites seems to have no equivalent in the head. In the head, the paraxial mesoderm neither contains the patterning information, nor does it impose its pattern on other structures as it does in the trunk. Instead, the pharyngeal endoderm in the head is segmented independently and imposes its pattern on the neural crest cells that stream into the pre-formed pharyngeal arches. The neural crest cells in their turn have important effects on the patterning of muscle, placodes and connective tissue in the head.

The origin of jaws – a key innovation

A very controversial topic has been whether there is a segment (or segments) in front of the mandibular arch. The presence in this region of mesodermal vesicles (Platt's vesicle, Chiarurgi's vesicle) has led to the suggestion that a pre-mandibular arch (or arches) once existed in craniates, something for which Stensiö tried to find evidence (Stensiö, 1927). Most researchers think, however, that the pre-mandibular material in the head does not (and never did) form an arch, or are extensions from the mandibular arch (Janvier, 1996).

Viewing the head as segmented logically leads to the assumption that the jaws of gnathostomes are modified gill arches. The skeleton in both gill arches and jaws is considered to be neural crest-derived, and the placement of jaws is consistent with them being serial homologs of gills. This view is the textbook ''truth'', but is not necessarily true. Alternatively, not gill elements, but the velar skeleton (as seen in lampreys) might have given rise to jaws (Smith and Coates, 2001). The embryonic development of hagfishes is too poorly known to be helpful.

Usually, the jaws are seen as being derived exclusively from the mandibular gill arch. However, Erik Jarvik proposed a variant of this idea called the ''composite theory''. He hypothesized that ten gill arches were present in the ancestor (terminal, pre-mandibular, mandibular, hyoid and six branchial arches) and postulated that parts of the jaws were derived from the pre-mandibular arch (Jarvik, 1980, 1981). There is no clear fossil evidence that a complete gill arch skeleton ever existed anteriorly to the first gill arch and in fact the fossil vertebrates contribute very little to the question of the formation of the pre-mandibular skull (for a recent review, see Janvier, 1996). Developmental data can help settle this question. Detailed fate mapping of neural crest cells would provide a conclusive test of the composite theory, but has yet to be made in relevant taxa. So far, only the cranial neural crest of the chicken embryo has been fate mapped in detail (Couly et al., 1993; Köntges and Lumsden, 1996).

The role of heterotopy in jaw evolution

Lampreys are the only agnathans available for developmental studies. Recent lamprey–gnathostomate comparative research has suggested that the evolution of jaws involved a heterotopic shift in tissue interactions during the development of the first (mandibular) arch and more anterior parts of the viscerocranum (Shigetani et al., 2002, 2005). Lampreys have prominent lower and upper lips, but whether they are homologous to gnathostome jaws is uncertain (Kuratani et al., 2001). Most developmental features in the lamprey head are very similar to what we observe in gnathostomes. This includes the migration and pattern formation of cranial neural crest cells, which divides into "premandibular" (nasal and post-optic) and "mandibular" regions. The upper lip in the lamprey receives pre-mandibular (post-optic) neural crest cells, whereas the lower lip and velum are derived from the mandibular crest. In gnathostomes, both maxillary and mandibular processes are classically considered to be derived from the mandibular crest (Richman and Lee, 2003), whereas the pre-mandibular crest seems to give rise only to the main part of the trabecular cartilage. Thus, the upper lip in the lamprey is probably not homologous to the upper jaw in gnathostomes.

If we look at the expression patterns of genes thought to be important for oral patterning, growth factors (FGF8, BMP2/4) secreted by the epidermis induce target homeobox genes (*Dlx*, *Msx1*) in the mesenchyme in both lampreys and gnathostomes. In lampreys, the expression is both pre-mandibular and mandibular, whereas in gnathostomes it is restricted to the mandibular area (Shigetani et al., 2002). So maybe the heterotopic caudal shift of gene expression patterns defined a new oral area in gnathostomes and freed the post-optic neural crest (which in lampreys becomes the upper lip) to evolve into the trabecula, which becomes an integrated part of the neurocranium (Kuratani, 2005; Shigetani et al., 2005). Because expression patterns of orthologous genes are not associated with morphologically equivalent cell populations, the shared molecular mechanisms can be viewed as exaptations for jaw evolution rather than as a guide to homology (Shigetani et al., 2002).

Specification of jaw subdivision by *Dlx* genes

In a way similar to the *Hox* code for anterior–posterior patterning in the head, *Dlx* genes were recently discovered to be expressed in a nested pattern along the proximal–distal axis in the mouse mandibular arch (Depew et al., 2002). In mammals there are six *Dlx* genes, tandemly linked to *Hox* clusters. *Dlx 1* and *2* with *HoxD*, *Dlx 5* and *6* with *HoxA*, and *Dlx 3* and *7* with *HoxB*. Lampreys have only four *Dlx* genes, which are not expressed in a nested pattern. In mouse embryos, *Dlx 1* and *2* are expressed in both proximal and distal parts of the pharyngeal arches, whereas *Dlx 5* and *6* are only expressed in the distal half (Fig. 5). Expression of *Dlx 3* and *7* is restricted to the distal (ventral) tips of the pharyngeal arches (Fig. 5). In this remarkable paper, the authors show that a double *Dlx 5/6* knock-out has multiple

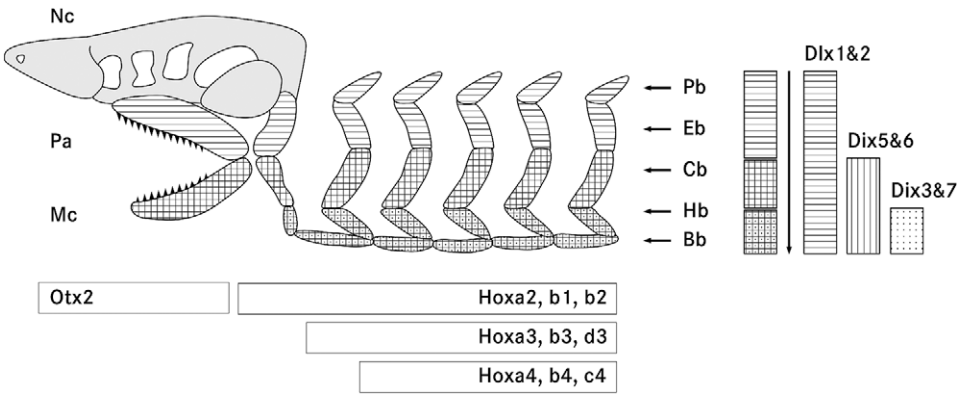

Fig. 5. Role of *Dlx* genes in proximodistal pharyngeal arch patterning. Diagram of a generalized gnathostome with neurocranium (Nc) and pharyngeal arches. The identity of pharyngeal arches along the anterior–posterior axis is regulated by *Hox* and *Otx* genes, which are expressed in a nested pattern. *Dlx* genes have a similar nested expression (and function) along the proximal–distal axis Bb, basibranchial; Cb, ceratobranchial; Ep, epibranchial; Hb, hypobranchial; MC, Meckel's cartilage; Pb, pharyngeobranchial; PQ, palatoquadrate (redrawn from Depew et al., 2002).

effects on head morphogenesis. The most interesting effect is the transformation of the mandible into a mirror image maxilla, but there are many other effects, too. The nasal capsule is nearly absent, the otic capsule distorted, the incus is duplicated as are vibrissae and rugae, and exencephaly (a condition in which the brain is located outside of the skull) is common. So the cellular identity within the mandibular arch depends on the *Dlx* genes being expressed in a properly nested pattern. Without *Dlx 5* and *6* expressed in the distal parts of the mandibular arch, the cells there take on proximal (upper jaw) identity. Thus upper jaw morphology could be the default identity, and lower jaw identity may have evolved secondarily (Kuratani, 2005). There are even fossils, such as *Peracanthodes* (Acanthodii), in which upper and lower jaws are morphologically identical mirror images of each other (Köntges and Matsuoka, 2002).

Morphogenesis of maxillary and mandibular structures

Recently, doubt has been cast on the "textbook version" of the origin of the cranial neural crest cells that give rise to the cartilage in the upper and lower jaws. It has been thought that crest cells from the first mandibular arch form a dorsal, "maxillary" and a ventral, "mandibular "condensation, which later give rise to the upper jaw cartilage (palatoquadrate) and the lower jaw cartilage (Meckel's cartilage), respectively. Now it has been shown in both the Mexican axolotl and in the chicken embryo, that this is incorrect (Cerny et al., 2004a; Lee et al., 2004). Using vital-dye labeling, cells which form the ventral or "mandibular" condensation were shown to

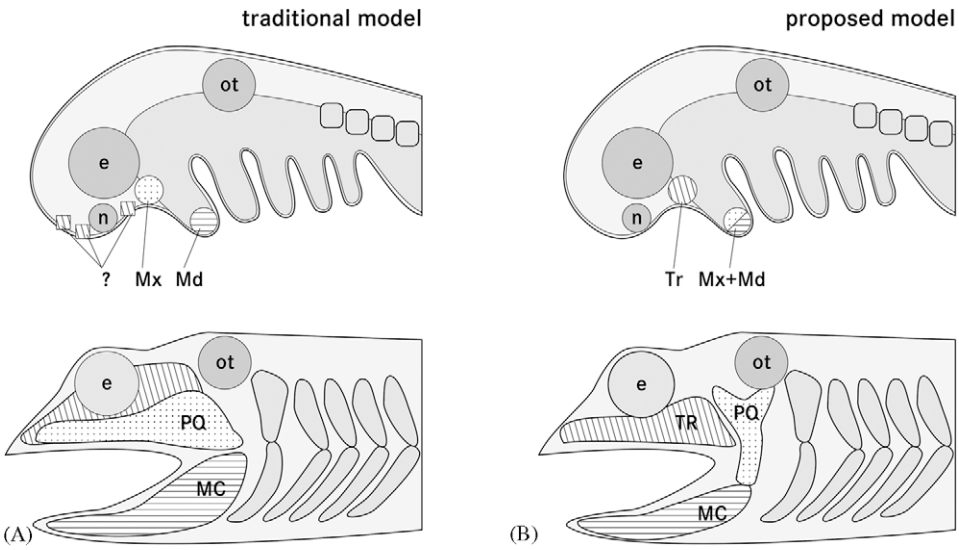

Fig. 6. (A) The traditional model of the developmental origin of jaw cartilages in gnathostomes. In this model, the dorsal (maxillary, Mx) neural crest condensation gives rise to the palatoquadrate cartilage (PQ), which is often described to form the entire upper jaw, whereas the ventral (mandibulary, Md) condensation develops into Meckel's cartilage (MC), the lower jaw element. (B) The new model. The dorsal or trabecular (Tr) condensation of neural crest cells contributes to the trabecular cartilage (TR). The trabeculae are connected to the anterior palatoquadrate, which forms the hinge of the upper jaw in modern tetrapods. The ventral condensation (probably fused maxillary and mandibular, Mx + Md) gives rise to both Meckel's and palatoquadrate cartilages. e, eye; n, nose; ot, otic vesicle. Dashed curves represent pathways of neural crest migration (redrawn from Cerny et al., 2004a).

give rise to both jaw cartilages (Cerny et al., 2004a). The dorsal or "maxillary" condensation contributes to the trabecular cartilage, but not to the jaw joints as previously assumed (Fig. 6). A study of the early development of jaw cartilages (Cerny et al., 2004a) argues for homology of the jaw cartilages of gnathostomes to the lower lip and velum in lamprey. Focusing on the development of the maxillary prominence and its skeletal derivatives in chicken, the second paper (Lee et al., 2004) disproves the classical view mentioned above that the maxillary area is derived from the mandibular arch. These new results challenge our definition of classical terms like "maxillary" and "mandibular", and urge us to broaden the scope of our fate mapping studies to find out if the results can be generalized.

Neural crest guidance of cranial muscle morphogenesis

The lower jaw is moved by cranial muscles that are derived from the mandibular and hyoid pharyngeal arches. Work with the chicken embryo, mostly by Drew

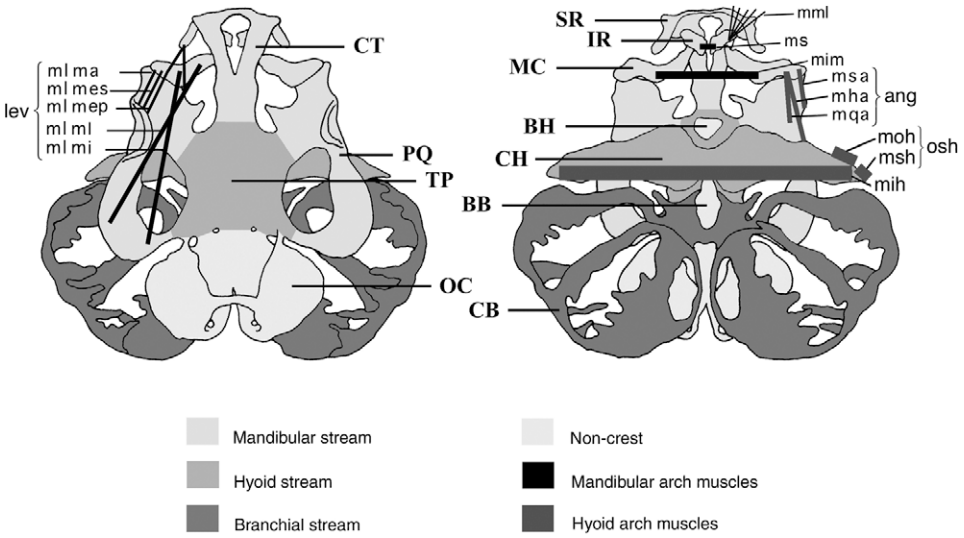

Fig. 7. Larval skull and cranial musculature of *Bombina orientalis*, depicted in dorsal (left) and ventral views. Neural crest-derived cartilages are shaded according to the migratory stream from which they originate (redrawn from Olsson and Hanken, 1996): very light gray, mandibular stream; light gray, hyoid stream; medium gray, branchial stream. The few non-crest-derived cartilages are lightly shaded. Cranial muscles are depicted schematically; only muscles of interest for the present study are shown. Mandibular (first) arch muscles are black, hyoid (second) arch muscles are dark gray. Paired muscles are depicted on one side only. Cartilages: BB, basibranchial; BH, basihyal; CB, ceratobranchials I–IV; CH, ceratohyal; CT, cornua trabecula (trabecular horn); IR, infrarostral; MC, Meckel's; OC, otic capsule; PQ, palatoquadrate; SR, suprarostral; TP, trabecular plate. Muscles: lev, levator mandibulae group – mlmi, levator mandibulae internus; mlma, levator mandibulae articularis; mlmep, levator mandibulae externus profundus; mlml, levator mandibulae longus (comprising two parts; superficialis and profundus); ang, angularis group – mha, hyoangularis; mqa, quadratoangularis; msa, suspensorioangularis; hyoideus group – mih, interhyoideus; moh, orbitohyoideus; msh, suspensoriohyoideus; osh, orbito- and suspensoriohyoideus; others – ms, submentalis; mim, intermandibularis; mml, mandibulolabialis. Redrawn from Olsson et al. (2001). Anatomical nomenclature follows Haas (2001).

Noden, has established that while the myofibers in the muscles that operate the lower jaw are mesodermal, the connective tissue component, including the muscle attachments, are of neural crest origin (Noden, 1983a, b, 1986). Because no other vertebrates have been studied in detail, the generality of this pattern remains unclear. A study using neural crest extirpation and DiI fate mapping in the frog *Bombina orientalis* (Olsson et al., 2001) indicated that connective tissue components of individual muscles of the mandibular and hyoid pharyngeal arches originate from the particular crest migratory stream that is associated with that arch. Furthermore, this relationship was maintained regardless of the segmental identity, or embryonic derivation, of associated skeletal components (Fig. 7). These developmental relations defined a pattern of segmentation in the head of larval anurans similar to that in

chicken embryos (Köntges and Lumsden, 1996). Larval frogs have a highly specialized oral region, with major evolutionary innovations in the form of novel cartilages and muscles (Svensson and Haas, 2005). Would a phylogenetically more basal amphibian such as the Mexican axolotl (*Ambystoma mexicanum*) also conform to the same pattern? Surprisingly little work (but see e.g. Epperlein et al., 2000; Cerny et al., 2004b) has been done on the embryonic development of the head in this salamander since the classical work in the first two-thirds of this century (reviewed in Hall and Hörstadius, 1988). We used DiI-labeling and GFP-mRNA injections combined with unilateral transplantations of neural folds to show that neural crest cells contribute to the connective tissues, but not the myofibers, of developing visceral arch muscles in the mandibular, hyoid, and branchial arches (Ericsson et al., 2004). Extirpations of individual cranial neural crest streams showed that the position of visceral arch musculature is *not* dependent upon the presence of neural crest cells. They are, however, necessary for normal muscle morphogenesis. Visceral arch muscles forming in the absence of neural crest cells start to differentiate at their origins but fail to extend toward their insertions and may have a frayed appearance. Our interpretation is that the cranial neural crest-derived connective tissues provide directional guidance important for the proper extension of the cranial muscles and the subsequent attachment to the insertion on the correct cartilage. In a comparative context, our data from amphibians support the view that the cranial neural crest plays a fundamental role in the development of not only the skeleton of the vertebrate head, but also in the anatomical patterning of the cranial muscles, and that this might be a primitive feature of cranial development in vertebrates.

Developmental biology and homology

Homology often refers to the common descent of parts of organisms. Ernst Mayr wrote that "a feature in two or more taxa is homologous when it is derived from the same (or corresponding) feature of their common ancestor" (Mayr, 1982). This is not the only way to define homology, in fact, a confusing multitude of definitions are in use (Hall, 1994), but Mayr's definition is the one commonly used by systematists. The main problem is to know when two characters are "the same". It is often useful to assume that characters which are produced by the same cells and developmental mechanisms are homologous, but caution is needed because there is a whole range of known examples where characters which are "the same" morphologically have been shown to arise in different ways developmentally (see Hall, 1998 for a list). These include, e.g. the induction of Meckel's cartilage by different tissues in different groups of vertebrates and the origin of primordial germ cells from different germ layers (mesoderm in salamanders, endoderm in other vertebrates). So, as Günter Wagner writes in this volume, "…the evolutionary conservation of a phenotypic character does not imply the conservation of its developmental pathway". We think that evolutionary developmental biology can contribute to several aspects of the homology discussion, but most of all the value of developmental data lies in a better

understanding of characters and character states. In one of the examples discussed in this paper, a heterotopic shift in tissue interactions in mandibular arch development is suggested to have been important for early jaw evolution (Shigetani et al., 2002, 2005). This illustrates how data from comparative morphology and evolutionary developmental biology can throw new light on the old question of how jaws, one of the defining characters of the Gnathostomata, have evolved, and give a deeper understanding of homology between characters at different hierarchical levels. Intensified work of this type is needed for other characters whose evolution constitute important novelties in organ systems other than the head, and in organisms other than vertebrates. The promise of evolutionary developmental biology is that such studies are being conducted at an increasing pace.

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References

Balfour, F.M., 1878. The development of the elasmobranchial fishes. J. Anat. Physiol. 11, 405–706.

Birgbauer, E., Sechrist, J., Bronner-Fraser, M., Fraser, S., 1995. Rhombomeric origin and rostrocaudal reassortment of neural crest cells revealed by intravital microscopy. Development 121, 935–945.

Bjerring, H., 1977. A contribution to structural analysis of the head of craniate animals. Zool. Scr. 6, 127–183.

Bjerring, H., 1984. Major anatomical steps towards craniotedness: a heterodox view based largely on embryological data. J. Vert. Palaeontol. 4, 17–29.

Cerny, R., Lwigale, P., Ericsson, R., Meulemans, D., Epperlein, H.-H., Bronner-Fraser, M., 2004a. Developmental origins and evolution of jaws: new interpretation of ''maxillary'' and ''mandibular''. Dev. Biol. 276, 225–236.

Cerny, R., Meulemans, D., Berger, J., Wilsch-Bräuninger, M., Kurth, T., Bronner-Fraser, M., Epperlein, H.-H., 2004b. Combined intrinsic and extrinsic influences pattern cranial neural crest migration and pharyngeal arch morphogenesis in axolotl. Dev. Biol. 266, 252–269.

Couly, G.F., Coltey, P.M., Le Douarin, N.M., 1993. The triple origin of the skull in higher vertebrates: a study in quail-chick chimeras. Development 117, 409–429.
Couly, G.F., Creuzet, S., Bennaceur, S., Vincent, C., Le Douarin, N.M., 2002. Interactions between Hox-negative cephalic neural crest cells and the foregut endoderm in patterning the facial skeleton in the vertebrate head. Development 129, 1061–1073.
de Beer, G.R., 1937. The Development of the Vertebrate Skull. Oxford University Press, Oxford.
Depew, M.J., Lufkin, T., Rubenstein, J.L., 2002. Specification of jaw subdivisions by Dlx genes. Science 298, 381–385.
Epperlein, H.-H., Meulemans, D., Bronner-Fraser, M., Steinbeisser, H., Selleck, M.A., 2000. Analysis of cranial neural crest migratory pathways in axolotl using cell markers and transplantation. Development 127, 2751–2761.
Ericsson, R., Cerny, R., Falck, P., Olsson, L., 2004. Role of cranial neural crest cells in visceral arch muscle positioning and morphogenesis in the Mexican axolotl, *Ambystoma mexicanum*. Dev. Dyn. 231, 237–247.
Gegenbaur, C., 1888. Die Metamerie des Kopfes und die Wirbeltheorie des Kopfskelettes. Morphol. Jahrbuch 13, 1–144.
Goodrich, E.S., 1930. Studies on the Structure and Development of Vertebrates. Macmillan, London.
Graham, A., Smith, A., 2001. Patterning the pharyngeal arches. Bioessays 23, 54–61.
Graham, A., Köntges, G., Lumsden, A., 1996. Neural crest apoptosis and the establishment of craniofacial pattern: an honorable death. Mol. Cell. Neurosci. 8, 76–83.
Graham, A., Begbie, J., McGonnell, I., 2004. Significance of the cranial neural crest. Dev. Dyn. 229, 5–13.
Grammatopoulos, G.A., Bell, E., Toole, L., Lumsden, A., Tucker, A.S., 2000. Homeotic transformation of branchial arch identity after Hoxa2 overexpression. Development 127, 5355–5365.
Haas, A., 2001. Mandibular arch musculature of anuran tadpoles, with comments on homologies of amphibian jaw muscles. J. Morphol. 247, 1–33.
Haeckel, E., 1874. Antropogenie oder Entwickelungsgeschichte des Menschen. W. Engelmann, Leipzig.
Hall, B.K., 1994. Homology. The Hierarchial Basis of Comparative Biology. Academic Press, San Diego and London.
Hall, B.K., 1998. Evolutionary Developmental Biology, second ed. Kluwer, Dordrecht.
Hall, B.K., Hörstadius, S., 1988. The Neural Crest. Oxford University Press, Oxford.
Helms, J.A., Schneider, R.A., 2003. Cranial skeletal biology. Nature 423, 326–331.
Hensey, C., Gautier, J., 1998. Programmed cell death during *Xenopus* development: a spatio-temporal analysis. Dev. Biol. 203, 36–48.
Holder, N., Klein, R., 1999. Eph receptors and ephrins: effectors of morphogenesis. Development 126, 2033–2044.
Hunt, P., Gulisano, M., Cook, M., Sham, M.H., Faiella, A., Wilkinson, D., Boncinelli, E., Krumlauf, R., 1991a. A distinct Hox code for the branchial region of the vertebrate head. Nature (London) 353, 861–864.
Hunt, P., Whiting, J., Muchamore, I., Marshall, H., Krumlauf, R., 1991b. Homeobox genes and models for patterning the hindbrain and branchial arches. Dev. Suppl. 1, 187–196.
Huxley, T.H., 1858. The Croonian Lecture – on the theory of the vertebrate skull. Proc. Zool. Soc. London 9, 381–457.
Jacobson, A.G., 1988. Somitomeres: mesodermal segments of vertebrate embryos. Development 104, 209–220.
Jacobson, A.G., Meier, S.P., 1984. Morphogeneis of the head of a newt: mesodermal segments, neuromeres, and distribution of neural crest. Dev. Biol. 106, 181–193.
Janvier, P., 1996. Early Vertebrates. Oxford University Press, Oxford.
Jarvik, E., 1980. Basic Structure and Evolution of Vertebrates, vol. 1. Academic Press, London.
Jarvik, E., 1981. Basic Structure and Evolution of Vertebrates, vol. 2. Academic Press, London.
Källén, B., 1956. Experiments on neuromery in *Ambystoma punctatum* embryos. J. Embryol. Exp. Morphol. 4, 66–72.
Köntges, G., Lumsden, A., 1996. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 122, 3229–3242.
Köntges, G., Matsuoka, T., 2002. Evolution. Jaws of the fates. Science 298, 371–373.
Kulesa, P., Bronner-Fraser, M., Fraser, S., 2000. In ovo time-lapse analysis after dorsal neural tube ablation shows rerouting of chick hindbrain neural crest. Development 127, 2843–2852.
Kuratani, S., 2003. Evolutionary developmental biology and vertebrate head segmentation: a perspective from developmental constraint. Theor. Biosci. 122, 230–251.

Kuratani, S., 2005. Craniofacial development and the evolution of the vertebrates: the old problems on a new background. Zool. Sci. 22, 1–19.
Kuratani, S., Horigome, N., Hirano, S., 1999. Developmental morphology of the head mesoderm and reevaluation of segmental theories of the vertebrate head: evidence from embryos of an agnathan vertebrate, *Lampetra japonica*. Dev. Biol. 210, 381–400.
Kuratani, S., Nobusada, Y., Horigome, N., Shigetani, Y., 2001. Embryology of the lamprey and evolution of the vertebrate jaw: insights from molecular and developmental perspectives. Phil. Trans. R. Soc. London B Biol. Sci. 356, 1615–1632.
Lee, S.H., Bedard, O., Buchtova, M., Fu, K., Richman, J.M., 2004. A new origin for the maxillary jaw. Dev. Biol. 276, 207–224.
Mayr, E., 1982. The Growth of Biological Thought. Harvard University Press, Cambridge, MA.
Meier, S.P., Packard, D.S.J., 1984. Morphogenesis of the cranial segments and distribution of neural crest in embryos of the snapping turtle, *Chelydra serpentina*. Dev. Biol. 102, 309–323.
Mitgutsch, C., 2003. On Carl Gegenbaur's theory on head metamerism and the selection of taxa for comparisons. Theor. Biosci. 122, 204–229.
Noden, D.M., 1983a. The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. Am. J. Anat. 168, 257–276.
Noden, D.M., 1983b. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev. Biol. 96, 144–165.
Noden, D.M., 1986. Patterning of avian craniofacial muscles. Dev. Biol. 116, 347–356.
Noden, D.M., Marcucio, R., Borycki, A.G., Emerson, C.P., 1999. Differentiation of avian craniofacial muscles: I. Patterns of early regulatory gene expression and myosin heavy chain synthesis. Dev. Dyn. 216, 96–112.
Oken, L., 1807. Über die Bedeutung der Schädelknochen. Ein Programm beim Antritt der Professur an der Gesamt-Universität zu Jena. Göbhardt, Bamberg and Würzburg.
Olsson, L., 2005. Alternatives to Darwinism in Sweden: Lamarckism and idealistic morphology, disbelief in mutations and the poverty of selection. Jahrbuch für Europäische Wissenschaftskultur 1, 1–14.
Olsson, L., Hanken, J., 1996. Cranial neural-crest migration and chondrogenic fate in the Oriental fire-bellied toad *Bombina orientalis*: defining the ancestral pattern of head development in anuran amphibians. J. Morphol. 229, 105–120.
Olsson, L., Falck, P., Lopez, K., Cobb, J., Hanken, J., 2001. Cranial neural crest cells contribute to connective tissue in cranial muscles in the anuran amphibian, *Bombina orientalis*. Dev. Biol. 237, 354–367.
Pasqualetti, M., Ori, M., Nardi, I., Rijli, F.M., 2000. Ectopic Hoxa2 induction after neural crest migration results in homeosis of jaw elements in *Xenopus*. Development 127, 5367–5378.
Richards, R.J., 2002. The Romantic conception of life. Science and Philosophy in the Age of Goethe. Chicago University Press, Chicago and London.
Richman, J.M., Lee, S.H., 2003. About face: signals and genes controlling jaw patterning and identity in vertebrates. Bioessays 25, 554–568.
Rijli, F.M., Gavalas, A., Chambon, P., 1998. Segmentation and specification in the branchial region of the head: the role of the Hox selector genes. Int. J. Dev. Biol. 42, 393–401.
Rose-Engelberth, M.J., 1999. Das Kopfproblem der Vertebraten – Kenntnisstand und Problematik. Unpublished Ph.D. thesis, Witten-Herdecke.
Schilling, T.F., Kimmel, C.B., 1994. Segment and cell type lineage restrictions during pharyngeal arch development in the zebrafish embryo. Development 120, 483–494.
Schneider, R.A., Helms, J.A., 2003. The cellular and molecular origins of beak morphology. Science 299, 565–568.
Shigetani, Y., Sugahara, F., Kawakami, Y., Murakami, Y., Hirano, S., Kuratani, S., 2002. Heterotopic shift of epithelial–mesenchymal interactions in vertebrate jaw evolution. Science 296, 1316–1319.
Shigetani, Y., Sugahara, F., Kuratani, S., 2005. A new evolutionary scenario for the vertebrate jaw. Bioessays 27, 331–338.
Smith, A., Robinson, V., Patel, K., Wilkinson, D.G., 1997. The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. Curr. Biol. 7, 561–570.
Smith, M.M., Coates, M.I., 2001. Evolutionary origins of teeth and jaws: developmental models and phylogentic patterns. In: Teaford, M.F., Smith, M.M., Ferguson, M.W.J. (Eds.), Development, Function and Evolution of Teeth. Cambridge University Press, Cambridge and London, pp. 133–151.

Stensiö, E.A., 1927. The Devonian and Downtonian vertebrates of Spitsbergen. 1. Family Cephalaspidae. Skrifter om Svalbard og Ishavet 12, 1–391.
Svensson, M.E., Haas, A., 2005. Evolutionary innovation in the vertebrate jaw: a derived morphology in anuran tadpoles and its possible developmental origin. Bioessays 27, 526–532.
Veitch, E., Begbie, J., Schilling, T.F., Smith, M.M., Graham, A., 1999. Pharyngeal arch patterning in the absence of neural crest. Curr. Biol. 9, 1481–1484.