Developmental Biology 317 (2008) 389–400

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Review

Review of fate-mapping studies of osteogenic cranial neural crest in vertebrates

Joshua B. Gross *, James Hanken

Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received for publication 5 June 2007
Received 20 February 2008
Accepted 21 February 2008
Available online 7 March 2008

Keywords:
Osteocranium
Skull
Vertebrates
Homology

ABSTRACT

Recent years have witnessed renewed interest in defining the embryonic cell populations that directly contribute to the bony skull. This question lies at the intersection of several important developmental, clinical and evolutionary interests. Until recently, our collective understanding of the embryonic origin of the vertebrate osteocranium has been based on a small number of reports published solely using avian models. As data gradually accumulates from other, distantly related species (e.g., mouse and frog), we can begin to evaluate long-standing assumptions regarding the behavior of osteogenic (bone-forming) neural crest cells within a wider phylogenetic and comparative context. In this review, we summarize data collected to date in three major vertebrate taxa: amphibians, birds and mammals. We highlight three largely unexplored topics within the field of osteogenic neural crest development: 1) disagreements in bone tissue origin within and across current model systems; 2) whether the pattern of neural crest cell contribution to skull bone is evolutionarily conservative or labile; and 3) how our understanding of development and morphology will benefit from fate maps using currently unexamined animal models.

Introduction

A long-standing goal of developmental biology is to define the relation between embryonic cell populations and the adult structures to which they directly contribute. One method used to address this issue is the construction of 'fate maps' that depict long-term (i.e., adult) derivatives of embryonic tissues. For the developmental biologist, fate maps provide an essential step towards understanding morphological and genetic interactions required to generate complex structures (Clarke and Tickle, 1999). For the evolutionary biologist, comparison of fate maps may offer insight into how embryonic tissues generate species-specific morphologies (Rudel and Sommer, 2003).

In the field of craniofacial development, comprehensive fate maps have been technically difficult to obtain for a variety of reasons. Primary among these is the requirement of an indelible cell marker for labeling embryonic cell populations that give rise to late-forming tissues, such as skull bones. This topic is further complicated by the fact that the skull is comprised of multiple components, each arising from a unique combination of embryonic tissue precursors and mode of ossification, which are tightly integrated in the adult organism. These components are the viscerocranium, neurocranium, dermal skull roof and sclerotomal occipital tissues (Morriss-Kay, 2001). The viscerocranium comprises the lower jaw, its supporting structures and other elements of the branchial-arch skeleton and is regarded as derived principally from neural crest (Kuratani et al., 1997; Cerny et al., 2006). The neurocranium, which includes the floor of the braincase and associated sensory capsules (nasal, optic and otic), is regarded as derived principally from embryonic mesoderm (Kuratani et al., 1997). Cartilaginous elements of both the viscerocranium and neurocranium form first and undergo endochondral ossification in most species (Morriss-Kay, 2001; Colnot, 2005). Associated dermal bones form later (e.g., dentary, palatine).

The third component of the skull, the dermal roof (Morriss-Kay, 2001), lacks a cartilaginous precursor and instead arises via intramembranous ossification of osteogenic mesenchymal cells (Franz-Odendaal et al., 2006). The embryonic origin of these cells has been, and remains, controversial depending on the model system being explored (Kuratani, 2005; Cerny et al., 2006). Finally, the fourth and most caudal component of the skull is the occipital region, which comprises endochondral bones that are believed to be derived, either exclusively or in large part, from mesodermal somitic tissues of the occipital region (Morriss-Kay, 2001). In the present context, 'cranium' shall refer specifically to the bony adult skull, excluding the lower jaw. Both the cranium and bony jaw elements shall be described collectively as the skull.

Until the end of the last century, most knowledge regarding tissue origins of the vertebrate skull arose from research on a single species, the domestic chicken, *Gallus gallus* (Le Douarin and Barq, 1969). One consequence of the paucity of data from other species is that patterns of crest contribution to the skull have been widely extrapolated across vertebrate taxa, e.g., from avians to humans (Johnston et al., 1973; Noden, 1988; Kardong, 2002).

The assumption, common among developmental biologists, that neural crest contributions to the skull are evolutionarily conservative

* Corresponding author. Current address: Harvard Medical School, Department of Genetics, Boston, MA 02115, USA. Fax: +1 617 432 6595.
E-mail address: jgross@genetics.med.harvard.edu (J.B. Gross).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.02.046

is understandable from an historical perspective. Indeed, this assumption is validated by previous studies of the viscerocranium, for which a rich literature stretching back several decades has demonstrated consistently a nearly exclusively neural crest origin. And while early studies provide evidence primarily regarding viscerocranial cartilages in bony fishes (Langille and Hall, 1988) and amphibians (Platt, 1893; Landacre, 1921; Stone, 1926, 1929; Raven, 1931; de Beer, 1947; Hörstadius, 1950), subsequent studies in chicken demonstrate a comparable embryonic origin for viscerocranial bones (e.g., Le Lièvre, 1974).

Extrapolation of fate-mapping data across distantly related vertebrate taxa also has provided an important heuristic tool for researchers interested in the etiology of a variety of human clinical disorders. As a starting point for the study of neurocristopathies (reviewed in Hall, 1999), several human craniofacial malformations are believed to be a consequence of aberrant migration and/or development of the neural crest or of an abnormal local signaling environment that secondarily influences the neural crest. Many such predictions have been confirmed by subsequent analysis involving non-human models.

For example, arrested crest cell migration in Treacher–Collins syndrome, in which affected individuals suffer from hypoplasia of derivatives of the first branchial arch, has been linked to a defect in the gene TCOF1 (Gorlin et al., 1990; Farlie et al., 2004). Mouse models of this disorder have been produced through mutant mice that carry a heterozygous mutation for TCOF1 (Edwards et al., 1997). The mutant phenotype reveals extensive cell death in cranial neural crest (CNC) cells prior to their departure from the neural tube and migration to the first branchial arch (Dixon et al., 2000). Other neurocristopathies in humans include some forms of cleft lip, cleft palate, and frontonasal dysplasia (Beauchamp and Knepper, 1984; Poswillo, 1988; Sulik et al., 1988; Fukiishi and Morriss-Kay, 1992).

Yet, from developmental and evolutionary perspectives the extrapolation from primary literature (fate-mapping) studies to human clinical manifestations may be inappropriate, or at least premature. For example, assuming that the distribution of osteogenic neural crest in a bird (e.g., domestic chicken) is the same as those in higher primates (e.g., humans) implies that patterns of crest contribution to bone are invariant across these distantly related taxa. While this assumption may be correct, it requires more 'data points' to test its validity. Moreover, the developmental origin of bony tissues, and whether the embryonic populations that constitute these structures are evolutionary invariant or labile, remain to be investigated in a rigorous comparative framework.

To facilitate additional studies that assess the range of osteogenic potential of neural crest cells among different species of vertebrates, we summarize the results of published studies that report crest contributions to the bony skull. In particular, we focus on regions of the skull that have long been controversial with respect to their origin, including the dermal skull roof, the columella, and the otic complex. We also highlight the need for comparable data from other potential model systems, including bony fishes and reptiles, in the hope that such attention will stimulate study of these neglected taxa.

Bony fishes and reptiles

There are no published reports that directly assess contributions of neural crest cells to the ossified adult skull of bony fishes. Such data, when placed within an appropriate phylogenetic context, will be invaluable for defining the generalized condition of "crest-domains" (i.e., regions of the skull to which the neural crest directly contributes cells) in the vertebrate subphylum (Knight and Schilling, 2006). The same data would inform our understanding of the ancestral pattern of neural crest contribution to individual skull bones in vertebrates and the extent to which this pattern is retained or modified in more recently evolved fishes, amphibians, and amniotes. Finally, given the extreme cranial diversity among extant species (Schultze, 1993), comparative studies of neural crest derivation likely would provide insights into the developmental mechanisms that underlie the evolution of morphological novelty and adaptive diversification of cranial form.

Along with bony fish, there currently exists no fate map depicting long-term contributions of osteogenic cranial neural crest in a reptilian model system. The embryonic origin of postcranial bones in turtles, however, is gaining increased attention (e.g., Toerien, 1965; Meier and Packard, 1984; Clark et al., 2001; Cebra-Thomas et al., 2005, 2007; Gilbert et al., 2007), including the potential role of the neural crest. Importantly, these studies offer evidence that at least some dermal bones in the turtle shell are derived from postcranial, or trunk neural crest, a claim that contradicts the widely accepted principle that osteogenic neural crest is limited to the cranial region in all vertebrates (see below).

Gilbert et al. (2001), using molecular markers to assess cell lineage (e.g., HNK-1, pdgfβ), provides indirect evidence of neural crest derivation of dermal bones of the turtle shell (both carapace and plastron). In this study, HNK-1 expression is used to assess embryonic origin because it is commonly expressed in early migrating neural crest cells (Bronner-Fraser, 1986). While previous work by Clark et al. (2001) demonstrated unequivocally that cells of the developing turtle shell are HNK-1-positive, neither this report nor the one by Gilbert et al. (2001) involves fate-mapping as typically defined, insofar as the associated experiments did not apply an extrinsic label to migratory cells to follow their fate into later developmental time points.

More recently, Cebra-Thomas et al. (2007) and Gilbert et al. (2007) provide additional evidence of a neural-crest origin of the HNK-1-positive cells by co-labeling with other known molecular markers of the neural crest, including *p75* and *FoxD3*. Furthermore, by using DiI labeling they demonstrate migration of these cells from the dorsal neural tube in stage 17 embryos to the developing plastron in the ventral shell, where they differentiate into bone via intramembranous ossification.

Fate-mapping studies and studies in which the ability of trunk neural crest to form skeletal tissues have been investigated in other vertebrates document a highly conserved difference between cranial and trunk neural crest with respect to their skeletogenic potential (e.g., Lumsden, 1988; Graveson et al., 1997; Hall, 1999). The apparent capacity of trunk neural crest cells to differentiate into bones of the postcranial skeleton in turtles is an obvious exception to this general rule. Indeed, it may represent a novel developmental feature in turtles that facilitated the evolution of their protective shell. Alternatively, osteogenic potential of trunk neural crest may be more widely distributed among vertebrates than is currently recognized (e.g., alligators; Gilbert et al., 2007), reflecting the limited taxonomic sampling of relevant studies to date.

Amphibians

Most early studies that examined the fate of the CNC in the vertebrate head skeleton utilized amphibian models because of their experimental tractability. Amphibian embryos are simple to obtain and, owing to their large size and external development, make a variety of experimental approaches tractable and straightforward (e.g., tissue ablations and grafting).

Classical studies, however, did not focus on the derivation of skull bones in model amphibians. Rather, most reports focused on the contribution of the neural crest to the larval cartilaginous skull. The protracted life history of metamorphic amphibians (particularly anurans) requires a cell label that can persist through the extended developmental time before cranial ossification commences in larvae, shortly before or during metamorphosis (Trueb and Hanken, 1992),

and cell labels with these characteristics have been developed only in the last few years (see below). Nevertheless, several studies attempted to trace the long-term derivatives of CNC through histological observations, tissue ablation, chromatic and radioactive label applications, and heterospecific grafting (reviewed in Gross and Hanken, 2004). As an example, Stone (1926) performed ablation experiments in which a small portion of premigratory neural crest and overlying neural ectoderm was removed from developing embryos. After 2 or 3 days, the larval animal was assessed for altered morphology of the cartilaginous skull and hyobranchial skeleton. Results provided the basis for one of the first fate maps of premigratory neural crest in amphibians. Other researchers have used a combination of tissue ablation and wild type-to-albino chimeric grafting techniques as a means of demonstrating the neural crest origin of much of the body pigmentation in amphibian embryos in both classical (DuShane, 1935; Twitty and Bodenstein, 1939) and contemporary investigations (Barlow and Northcutt, 1995; Barlow and Northcutt, 1997).

By the middle of the last century, two techniques led to reports that neural crest contributes to skull bone as well as cartilage. By inferring patterns of crest-cell migration from histological sections of embryos of the salamander *Ambystoma mexicanum* (based on intrinsic features in the cytoplasm between the neural crest and other cells, e.g., yolk), de Beer (1947) proposed an "ectomesenchymal" (i.e., neural crest) contribution to the intramembranous splenial bone of the lower jaw (Table 1). This is the first claim of neural crest derivation of the osteocranium in any vertebrate. Wagner (1949) and Andres (1949) subsequently applied a technique of heterospecific grafting of portions of the embryonic neural fold (which includes the neural crest and overlying ectoderm) between a frog (*Bombina*) and a newt (*Triturus*) and reported donor neural crest-derived cells within several bones of the late-larval skull of the host, including the premaxilla, dentary, splenial and vomeropalatine (reviewed in Noden, 1983a; Table 1). These studies also took advantage of the fact that these bones form at different times in frogs and salamanders (before vs. after metamorphosis) and assume characteristically different shapes in the two groups.

There are at least two potential problems associated with these early experiments with amphibians, which, until very recently, had not been validated with more contemporary techniques for tracing cell derivatives. First, histology alone may not be a reliable fate-mapping technique because of the difficulty tracking migration of unlabeled cells. Secondly, because individual grafts in these experiments included the entire neural fold, as opposed to grafts comprised exclusively of neural crest tissue, one cannot conclude that donor-derived cells came from neural crest and not from overlying ectoderm. While the overlying epithelium is not predicted to form hard tissues of the skull, it is likely that migratory multipotent cells derived from placodal tissue reside in the transplanted surface epithelium. Placode-derived cells are known to interact with neural crest cells later in development (Barlow, 2002) and could be interpreted incorrectly as neural crest cells, thereby obscuring results obtained using this methodology. These concerns, combined with the potential for experimental artifacts (e.g., aberrant migration in non-native environments owing to different developmental time-frames), may render these data unrepresentative of neural crest development *in vivo*.

Newer labeling methods have enabled studies that assess the long-term fate of the neural crest in models that historically have been inaccessible (e.g., applied labels, such as fluorescent dextran and DiI—Gross and Hanken, 2004; transgenic approaches—Gross et al., 2006). Many also provide more definitive and robust results than earlier methods. One potential approach is "genetic fate mapping," which utilizes site-specific recombinases (e.g., Cre and flp) in combination with neural crest-specific promoters. Although site-specific recombinases have been used successfully in *Xenopus* (Gargioli and Slack, 2004), to date they have not been applied specifically to fate-mapping studies of the neural crest in any model system other than the mouse (Jiang et al., 2002).

Every cell labeling technique, be it contemporary or classical, has its own set of potential artifacts and limitations. Sophisticated extrinsic cellular labeling techniques, for example, have recently allowed for the reliable tracking of the CNC through metamorphosis and into adult skull bone in frogs (Carl et al., 2000; Gross and Hanken, 2004). Only five bones, however, have been assessed for CNC origin to date—frontoparietal, nasal, parasphenoid, squamosal and dentary (Table 1; Gross and Hanken, 2004, 2005; Gross et al., 2006)—and there still is no comprehensive neural crest fate map for the adult osteocranium in any amphibian. One caveat to these studies is that they did not assess other possible tissue contributions. Thus, whereas a neural crest contribution to five skull bones has been demonstrated unequivocally, it has not been possible to determine if neural crest is the sole tissue contributing to a given bone, or if one or more additional tissues (e.g., mesoderm) also contribute to the same bone.

Table 1
Summary of cranial neural crest (CNC) contributions to skull bones in amphibians

Bones derived from the CNC	Reference
Premaxilla, dentary, vomeropalatine	Wagner, 1949[a]
Splenial	de Beer, 1947; Wagner, 1949[b]
Frontoparietal	Carl et al., 2000; Gross and Hanken, 2004, 2005[c]
Nasal, parasphenoid, squamosal	Gross and Hanken, 2004
Dentary	Gross et al., 2006

[a] Wagner (1949) performed grafting studies between newts (Triturus) and frogs (Bombina).
[b] de Beer (1947) utilized the Mexican salamander, *Ambystoma mexicanum*.
[c] Carl et al. (2000), Gross and Hanken (2004, 2005) and Gross et al. (2006) utilized grafting studies in the clawed frog, *Xenopus laevis*.

Domestic chicken, *Gallus gallus*

During the 1960s, fate-mapping studies that investigated the derivation of cranial tissues from the CNC shifted largely from amphibian to avian models following the advent of radioactive (thymidine) tissue-labeling and quail-chick chimeric grafting techniques (Weston, 1963; Le Douarin and Barq, 1969). Direct experimental evidence that neural crest contributes to skull bone in vertebrates was first reported in the early 1970s by using tritiated thymidine labeling in the domestic chicken, *G. gallus* (Johnston et al., 1973). This discovery, however, was confined to a single bone in the lower jaw, the dentary. More comprehensive data regarding adult derivatives of the osteogenic cranial neural crest followed the advent of the quail-to-chick chimeric grafting technique (reviewed in Le Douarin and Kalcheim, 1999). This technique was the first to offer a stable and indelible marker of embryonic tissue grafts that would last through early development until cranial bones have fully differentiated and ossified. In addition, chimeric grafting obviated the need to apply harmful radioactivity (i.e., thymidine) to embryonic tissues.

Several research groups have since used quail-chick chimeras to derive fate maps for particular bones, for specific regions of the skull (e.g., the mandibular skeleton), or even for the entire skull. Yet while these maps are substantially in agreement, they differ in several important aspects. These differences may be, to some extent, a function of different research goals and experimental protocols and reflect the potential artifacts of different transplantation methods. Le Lièvre (1978), for example, utilized whole neural tube fragments as a 'coarse' means of grafting the entire neural crest, whereas subsequent workers utilized shorter explants of the neural tube (Noden, 1978; Couly et al., 1993). The latter grafts, while easily freed of mesoderm at the midbrain level, are susceptible to contamination with mesodermal tissues at hindbrain levels.

It remains to be seen which of the conflicting claims of neural crest derivation are accurate. A universally accepted, definitive fate map for the bony skull of birds is yet to be produced. In this section we review the landmark studies authored by four groups. Each reports the long-term fate of premigratory CNC in the adult skull of the domestic chicken.

Fate maps of Le Lièvre (1974, 1975, 1978)

Three pioneering reports by Le Lièvre led the way for many subsequent investigations using the quail-to-chick chimera technique to examine regional fate mapping of neural crest in the head skeleton. The first report describes Meckel's cartilage as derived from both mesencephalic and anterior rhombomeric neural crest (Le Lièvre, 1974; see below). The other reported contributions from mesencephalic crest include the dentary, opercular, supra-angular and angular bones.

This work also was the first to define an important phenomenon related to crest contributions to the head skeleton: discrete crest populations that originate from different regions of the neural tube may jointly participate in the development of a single skeletal element, while retaining regional identity. Specifically, the articular region of Meckel's cartilage is derived from both "mesencephalic" and "anterior rhombencephalic" crest, whereas its distal region is derived solely from "mesencephalic" crest (Table 2; Le Lièvre, 1974). This observation would be expanded many years later in a more detailed study of the bony lower jaw, which documented retention of "cryptic" segmental boundaries between adjacent embryonic crest populations in adult tissues (Köntges and Lumsden, 1996; see below).

In a second report on crest contributions, Le Lièvre and Le Douarin (1975) characterized mesenchymal derivatives, including the dermis, portions of striated cranial muscle, vasculature and other connective tissues. All but one of these proposed derivatives have been confirmed by subsequent analyses. With the exception of specialized ciliary muscles in the eye (Couly et al., 2002), Le Lièvre's claim of a neural-crest origin of skeletal myocytes has not been confirmed. Rather, the direct contribution of neural crest cells to myogenesis appears to be limited to their serving as a source of tendinous and other muscular connective tissues (Noden, 1983a; Köntges and Lumsden, 1996; Matsuoka et al., 2005).

The third report contains Le Lièvre's (1978) most thorough description of neural crest contributions to the bony skull, including an assessment of the origin of bones of the facial skeleton, cranial vault and cranial base (Table 2). Contributions are organized according to the region(s) from which neural crest initially migrates from the neural tube, viz., prosencephalon, mesencephalon and rhombencephalon. Neural crest emigrating from the prosencephalon was detected in the anterior maxilla, palatine, nasal, premaxilla and vomer bones. Cells derived from mesencephalic neural crest contribute to the largest amount of skull tissue, including the rostral parasphenoid, a small portion of the frontal bone, the jugal, quadratojugal, pterygoid, maxilla and entoglossum bones, as well as Meckel's cartilage (described previously). Cells derived from the mesencephalon and anterior rhombencephalon contribute to the columella and squamosal bones and the articular region of Meckel's cartilage.

This report also described a mixed contribution of both neural crest and mesoderm in the formation of the "orbital skeleton," viz., the region of the skull that includes the rostral portion of the frontal bone (Le Lièvre, 1978; p. 26). With regard to the rest of the cranial vault, Le Lièvre observed no contribution from grafted crest cells and therefore attributed its origin to mesoderm. Beginning with this claim and continuing to the present day, the cranial vault has remained the most disputed and controversial region with respect to embryonic origin.

Fate maps of Noden (1978, 1982, 1984), Noden and Trainor (2005) and Evans and Noden (2006)

Beginning in the late 1970s, another series of studies focused on osteogenic neural crest in the domestic chicken were performed using the quail-chick chimeric method. The principal aim of the first report by Noden (1978) was to investigate whether cells derived from premigratory neural crest were restricted in their developmental potential. Small portions of embryonic crest corresponding to different axial levels, or "populations," were reciprocally grafted between regions and assessed with respect to their effects on subsequent skeletal morphology. Results indicated that the extracellular environment mediates the adult morphology of crest-derived skeletal structures after crest cells migrate from the neural tube.

Patterns of neural crest derivation outlined in this study largely confirm previous work (Tables 2 and 3), even though the particular regions of the neural ridge from which grafts were removed differ somewhat between studies. Le Lièvre (1974), for example, removed successively overlapping regions of the neural tube that extend along the rostrocaudal axis, from the developing prosencephalon to the posterior rhombencephalon. Similarly, Noden (1978) utilized overlapping regions from the caudal prosencephalon through the metencephalon and periotic regions (with the exception of the third rhombomere). The primary distinction between these two grafting protocols centers on the use of longer grafts of the neural tube (Le Lièvre, 1974) versus shorter grafts of the neural folds (Noden, 1978).

Notes to Table 2:
Several additional bones and cartilages were reported as not derived from the neural crest in these reports, including the parietal bone, "occipital" bones (supraoccipital, exoccipital, basioccipital), bones of the sphenoid complex (basisphenoid, alisphenoid, orbitosphenoid), acrochordal cartilages, and polar cartilages (Le Lièvre, 1978); the basisphenoid, alisphenoid, and the otic complex (Noden, 1978); the basioccipital, exoccipital, pars canalicularis, supraoccipital, basipostsphenoid, orbitosphenoid, and pleurosphenoid (Couly et al., 1993).
"n/s": bones and cartilages derived from neural crest in which the source of the neural crest is not specified. "ant."=anterior; "post."=posterior.
[a] Couly et al. (1993) report the following structures as crest-derived: sutures between membranous bones of the cranial vault, dermis of the scalp, meninges of the forebrain, dermis of the parietal bones.
[b] Le Lièvre (1978) describes only the anterior maxilla and the anterior palatine as derived from CNC originating from the prosencephalon. The posterior palatine and the rostral parasphenoid are reported as derived from mesencephalic crest.
[c] "Forebrain" grafts of neural crest include the posterior diencephalon and the anterior mesencephalon. "Hindbrain" grafts of neural crest include the posterior mesencephalon and the metencephalon.
[d] Described in Le Lièvre (1978) as "mixed" in origin, presumably indicating contributions from both neural crest and mesoderm.
[e] Noden (1978) describes the frontal bone as "mixed" in origin, presumably indicating contributions from both neural crest and mesoderm.
[f] Noden (1978) describes the proximal portion of the quadrate bone as derived from metencephalic crest; distal portions are derived from posterior mesencephalic crest (p. 300).
[g] Noden did not describe an embryonic origin of the columella bone in his 1978 study. His later reports, however, describe the columella as a composite structure derived from neural crest and mesoderm (Noden, 1982, 1984).
[h] Couly et al. (1993) report the pars canalicularis as mixed in origin, presumably indicating contributions from both neural crest and mesoderm.
[i] Köntges and Lumsden (1996) report the pterygoquadrate as derived from rhombomere 1 neural crest, with dorsal articulations and ventral articulations derived from midbrain and rhombomere 2 neural crest, respectively. The articular bone is reported as derived from rhombomeres 1+2, the retroarticular process (proximal articular bone) is reported as derived from rhombomeres 3–5.

Table 2
Summary of CNC contributions to skull bones in the chicken, *Gallus gallus*, according to Le Lièvre (1974, 1978), Noden (1978), Couly et al. (1993) and Köntges and Lumsden (1996)

Bones and cartilages derived from the CNC	Embryonic origin of the CNC			
	Le Lièvre (1978)	Noden (1978)	Couly et al. (1993)[a]	Köntges and Lumsden (1996)
Maxilla[b]	Prosencephalon	Forebrain[c]	Diencephalon + ant. mesencephalon	Midbrain
Vomer	Prosencephalon		Diencephalon + ant. mesencephalon	
Premaxilla	Prosencephalon	Forebrain + mid-mesencephalon	Diencephalon + ant. mesencephalon	
Nasal	Prosencephalon	Forebrain + mid-mesencephalon	Diencephalon + ant. mesencephalon	
Concha nasalis	Prosencephalon + mesencephalon			
Nasal capsule	Prosencephalon + mesencephalon			
Orbital cartilage[d]	Prosencephalon + mesencephalon			
Sclerotic cartilage[d]	Prosencephalon + mesencephalon		Diencephalon + ant. mesencephalon	
Entoglossum	Prosencephalon + mesencephalon			
Parasphenoid[b]	Mesencephalon	Forebrain	Diencephalon + ant. mesencephalon	
Sphenoid		Forebrain		
Temporal		Forebrain	n/s	
Prefrontal		Forebrain + mid-mesencephalon		
Frontal[d,e]	Mesencephalon	Forebrain + mid-mesencephalon	Diencephalon + ant. mesencephalon	
Parietal			Post. mesencephalon + metencephalon	
Jugal	Mesencephalon		Diencephalon + ant. mesencephalon	Midbrain
Quadratojugal	Mesencephalon		n/s	Rhombomeres 1 + 2
Pterygoid	Mesencephalon	Hindbrain[c] + mid-mesencephalon	Diencephalon + ant. mesencephalon/ Post. mesencephalon + metencephalon	
Quadrate[f]	Mesencephalon	Hindbrain	Diencephalon + ant. mesencephalon	Rhombomeres 1 + 2
Pterygoquadrate				Rhombomere 1, midbrain, rhombomere 2
Quadratoarticular			n/s	
Maxilla	Mesencephalon			
Palatine[b]	Prosencephalon + mesencephalon	Hindbrain + mid-mesencephalon	Post. mesencephalon + metencephalon	Midbrain
Meckel's cartilage	Mesencephalon	Hindbrain	Post. mesencephalon + metencephalon	Midbrain
Columella[g]	Mesencephalon + anterior rhombencephalon		n/s	Rhombomeres 3–5
Squamosal	Mesencephalon + anterior rhombencephalon	Hindbrain	Diencephalon + ant. mesencephalon/post. mesencephalon + metencephalon	Rhombomeres 1 + 2
Otic capsules[d]	Mesencephalon + anterior rhombencephalon		Post. mesencephalon + metencephalon	
Metotic cartilages[d]	Mesencephalon + anterior rhombencephalon			
Pars cochlearis[d]	Mesencephalon + anterior rhombencephalon		n/s	
Pars canalicularis[d,h]	Mesencephalon + anterior rhombencephalon			
Basihyal	Rhombencephalon			
Basibranchial	Rhombencephalon			
Meckel's cartilage (articular)	Rhombencephalon			
Interorbital	n/s	Forebrain + mid-mesencephalon	Diencephalon + ant. mesencephalon	
Internasal septum	n/s			
Supraorbital cartilage	n/s		Diencephalon + ant. mesencephalon	
Antorbital cartilage	n/s			
Postorbital cartilage		Forebrain		
Lacrymal	n/s			
Articular[i]	n/s			Rhombomeres 1 + 2/rhombomeres 3–5
Angular	n/s	Hindbrain	Post. mesencephalon + metencephalon	Rhombomeres 1 + 2
Supraangular (Surangular)	n/s	Hindbrain		Rhombomeres 1 + 2
Dentary	n/s	Hindbrain	Post. mesencephalon + metencephalon	Midbrain
Opercular		Hindbrain	Post. mesencephalon + metencephalon	
Splenial	n/s			Midbrain
Mentomandibular	n/s			
Ceratobranchial	n/s			
Epibranchial	n/s			
Basipresphenoid			Diencephalon + ant. mesencephalon	
Hyoid cartilage			Post. mesencephalon + metencephalon	
Ethmoid			n/s	
"Mandibular"			n/s	

Noden has since published fate maps based upon additional quail-to-chicken tissue-grafting experiments (Noden, 1982, 1984; Fig 1A; Table 2) and, more recently, using retroviral labeling (Noden and Trainor, 2005; Evans and Noden, 2006). These studies ascribe a crest origin to the entire facial and mandibular skeleton (Table 2). The results, while largely congruent with those of earlier maps, differ from those maps with respect to the derivation of the skull vault and the columella bone.

With respect to the skull vault, Noden (1978, 1982, 1984) differs from Le Lièvre's (1978) account in deriving only the most rostral portion of the frontal bone from neural crest, whereas the remainder of the frontal bone is derived from mesoderm. Additionally, Noden (1982, 1984) claims that the columella is a composite bone derived from both neural crest and mesoderm, whereas according to Le Lièvre (1978) the columella is derived only from mesencephalic and anterior rhombomeric neural crest. More recently, Köntges and

Table 3
Summary of CNC contributions to skull bones in the mouse, *Mus musculus*

Bones and cartilages derived from the CNC	Reference
Meckel's cartilage, "mandible"[a], temporomandibular joint, palatine	Chai et al., 2000
Squamosal, frontal	Morriss-Kay, 2001; Jiang et al., 2002
Nasal, alisphenoid, interparietal, "viscerocranial bones"[b], premaxilla, maxilla, zygomatic, squamosal, dentary, tympanic bones	Jiang et al., 2002
Malleus (processus brevis)[c], Otic capsule (pars canalicularis)	O'Gorman, 2005

Several additional bones are reported as not derived from neural crest, including the parietal, interparietal (lateral portion), and basioccipital (Morriss-Kay, 2001; Jiang et al., 2002). Bones of the "viscerocranium" are assumed to be derived from CNC (Jiang et al., 2002).

[a] Chai et al. (2000) do not include an analysis of the bones comprising the lower jaw. "Mandible" refers to cartilaginous tissues exclusively, e.g., the articulating joint of the temporomandibular joint.

[b] Jiang et al. (2002) report "viscerocranial bones" as derived from crest, although explicit evidence regarding individual bones is not provided.

[c] O'Gorman (2005) reports the malleus as a composite of first- and second arch-derived neural crest.

Lumsden (1996) describe the columella as a crest-derived bone that receives contributions only from rhombomeres 3–5 (Table 2; see Discussion).

In two recent reports, Noden and others reexamine the origin of the cranial vault in chicken using replication-incompetent retroviral labeling of both embryonic neural crest and mesoderm (Noden and Trainor, 2005; Evans and Noden, 2006). The results localize the boundary between neural crest- and mesoderm-derived regions to the junction between the supraorbital and calvarial regions of the frontal bone. The report of Evans and Noden (2006), in particular, provides the only other thorough fate-mapping analysis (along with Couly et al., 1993) that is based on labeling both neural crest and mesodermal tissues. Utilizing focal injection sites, they demonstrate in dissected wholemount and sectioned bony tissues that the majority of the frontal bone and all of the parietal bones are labeled in chicken embryos following injections into cranial mesoderm. The same bones are not labeled following comparable injections into neural crest.

The results of this latter study may offer some resolution to the long-standing controversy surrounding the developmental origin of the cranial vault in chickens. As the authors point out, most chicken fate-mapping studies attempt to follow large populations of grafted neural crest or mesodermal cells. By instead performing focal labelings without grafts, the authors identified regions of cells derived solely from neural crest in the rostral, supraorbital portion of the frontal bone. In contrast, paraxial mesodermal cells (adjacent to the mid-mesencephalic region) were shown to populate the larger calvarial portion of the frontal bone, consistent with earlier reports (Noden, 1978). Focal cell labeling is technically superior to the use of larger grafts of the entire neural crest, as it offers finer control over cell labeling as well as higher resolution of the cellular migrations of crest and mesodermal populations throughout development.

Evans and Noden (2006) demonstrate a complementary "interface" between neural crest and mesodermally derived cells at the junction between the supraorbital portion of the frontal bone (derived from the neural crest) and the larger, calvarial portion of the frontal bone and entirety of the parietal bone (derived from mesoderm). In chickens, the two anatomical domains of the frontal bone arise via

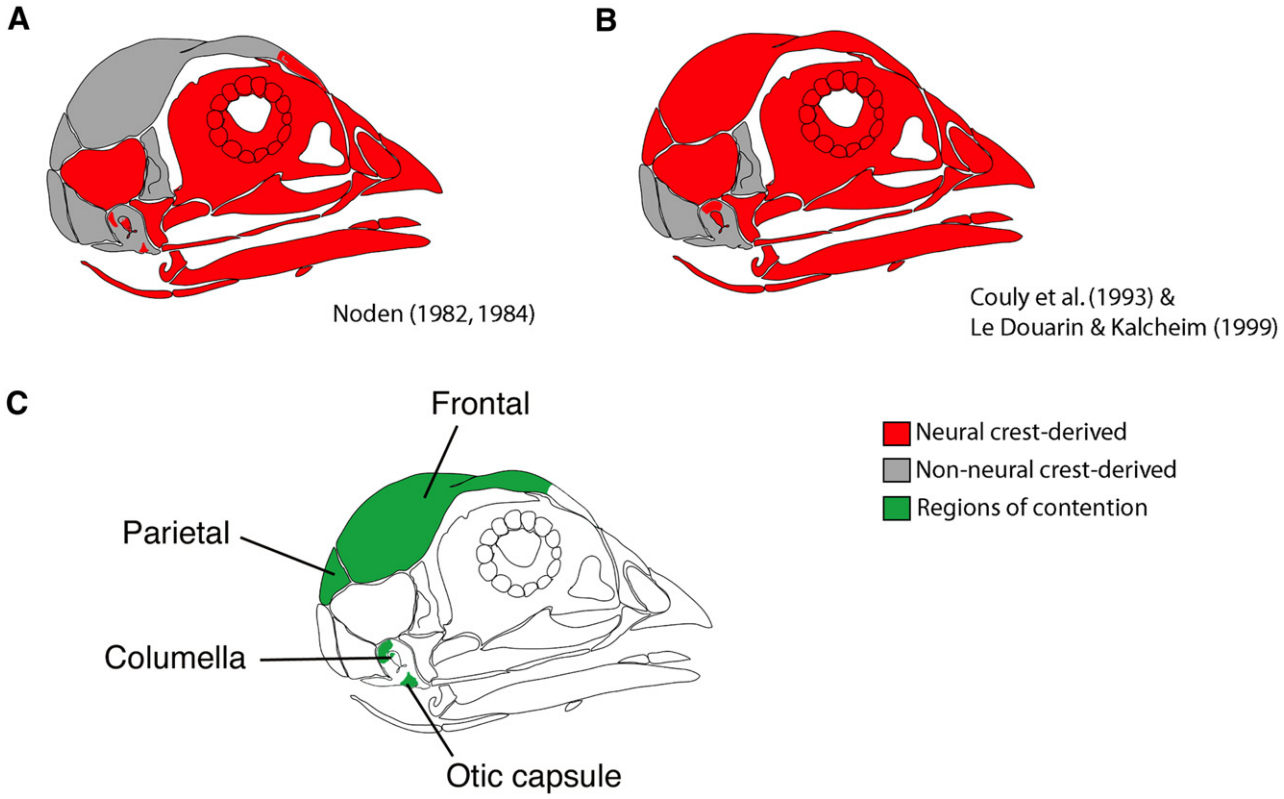

Fig. 1. Comparison between the two published cranial fate maps of osteogenic neural crest in the domestic chicken, *Gallus gallus*. (A) Schematic drawing of the adult chicken skull (redrawn from Noden, 1982, 1984) illustrating the extent of crest contribution to skull bones (red). Mesodermal or "non-neural crest" regions are shaded grey. (B) Alternate fate map redrawn from Couly et al. (1993) and Le Douarin and Kalcheim (1999). (C) The principal regions of disagreement between these two maps (green) include the columella, otic capsule and the cranial vault (i.e., caudal aspect of the frontal bone and the parietal bone).

separate centers of ossification (Erdmann, 1940). It is, therefore, quite plausible that each center arises from an embryonically distinct subpopulation of osteogenic cells, one derived from mesoderm, the other from neural crest, which later fuse together to form the single frontal bone.

Fate maps of Couly et al. (1993), Le Douarin and Kalcheim (1999) and Köntges and Lumsden (1996)

Later reports of osteogenic derivatives of CNC in the chicken were obtained as part of comprehensive analyses of the composite origin of the entire skull and head from three discrete embryonic cell populations: cranial neural crest, somitic mesoderm, and cranial paraxial mesoderm (Couly et al., 1993; Table 2). These results yielded another fate map of CNC contributions to the adult bony skull (Fig. 1B).

Couly et al. (1993) explores 11 different grafted regions assayed across multiple time points from embryonic days 9 to 14. The resulting fate map describes the most extensive crest-derived domains in the adult chicken skull to date. These domains comprise the "prechordal skeleton," which includes both cartilage-replacement bones (interorbital septum, basipresphenoid, scleric ossicles, ethmoid, pterygoid, quadrato-articular,[1] hyoid and pars cochlearis of the otic capsule) and membrane bones (frontal, parietal, squamosal, columella, nasal, maxilla, vomer, palatine, quadratojugal and mandibular[2]).

Köntges and Lumsden (1996) focuses on premigratory crest cell fate in the adult avian skull by careful examination of the derivation of the jaw skeleton (Table 2). This study investigates whether boundaries between premigratory crest populations located between the midbrain and hindbrain rhombomere 7 coincide with borders of anatomical structures derived from those cells. The authors conclude that these neural crest boundaries do not correspond with adult anatomical borders, but rather traverse adult anatomical structures, a phenomenon they term "cryptic segmentation."

Overall, the pattern of crest derivation reported in this study is congruent with those of Le Lièvre (1978), Noden (1978) and Couly et al. (1993). One especially important finding is the contribution of hyoid stream crest (i.e., crest grafted from rhombomeres 3, 4, 5) to the retroarticular process of the articular bone in the lower jaw, whereas the remainder of the articular bone and lower jaw are derived from mandibular crest (grafted from rhombomeres 1 and 2). This finding illustrates that neural crest originating from different regions of the neural tube and populating different migratory streams (e.g., mandibular and hyoid) may nevertheless combine to form a single bone. A similar result was reported earlier by Le Lièvre (1974) regarding the cartilaginous precursor of the adult lower jaw. Whereas most of Meckel's cartilage is derived from mesencephalic crest, the 'articular region' is derived from more posterior rhombomeric neural crest.

Another key finding by Köntges and Lumsden (1996) is the identification of a constrained pattern of cranial skeletomuscular connectivity, whereby rhombomeric populations of crest cells giving rise to muscular connective tissue attach to sites of crest-derived neuro- and viscerocranium that arise from the same population. This 'code of connectivity' has been extended recently to neural crest and mesodermal populations in the scapular region of mammals (Matsuoka et al., 2005), yet it also is debated as a meaningful tool for the determination of skeletal homologies based on patterns of muscle attachment (Sánchez-Villagra and Maier, 2006; Ahlberg and Köntges, 2006). Indeed, genetic fate-mapping of middle ear bones in *Hoxb1-Cre* mice reveals unlabeled connective tissues within the tensor tympani, a muscle expected to bear connective tissues derived from second (hyoid) arch neural crest (O'Gorman, 2005).

Collectively, the principal fate maps of osteogenic neural crest in the head of the domestic chicken are congruent in most respects. For example, by consensus among all research groups the entire facial skeleton, upper jaw and lower jaw bones are derived from embryonic neural crest. There also are, however, important differences among the fate maps (see Discussion). These include Noden's claim of a composite origin of the columella from neural crest and mesoderm (Noden, 1982, 1984), which differs from claims by other groups of an exclusively neural crest derivation of this bone.

A second difference concerns the extent of neural crest contribution to the calvarial bones of the cranial vault, specifically the frontal and parietal. Noden (1982) ascribes a neural crest origin to only the supraorbital portion of the frontal (Fig. 1C), whereas Couly et al. (1993) report the entire frontal and parietal as derived from neural crest.

Finally, comparisons among the various schematic fate maps discussed above reveal apparent discrepancies with respect to the derivation of the occipital region (Fig. 1C; Noden, 1982; Couly et al., 1993; Le Douarin and Kalcheim, 1999). Because each group utilizes slightly different anatomical nomenclature, however, it is difficult to specify the exact nature of these differences (Fig. 1C). Recent studies in mouse demonstrate a close similarity between the contributions of neural crest to the mammalian otic capsule (O'Gorman, 2005) and those depicted in Noden's (1983a) study of crest contributions in chicken. Comparable reports in anamniotes (fishes and amphibians) will help to clarify the embryonic and evolutionary origin of this puzzling region of the vertebrate skull.

The mouse, *Mus musculus*

Attempts to assess neural crest contributions to the skull of mammals confront serious technical challenges, including the general inaccessibility of embryos for grafting procedures and many other experimental techniques. While early neural crest transplantation studies have been performed successfully (Jaenisch, 1985; Tan and Morriss-Kay, 1986), this approach has not yielded a long-term, viable method of tracking embryonic cell movements. Instead, the source and fate of osteogenic crest have been assessed through the use of a

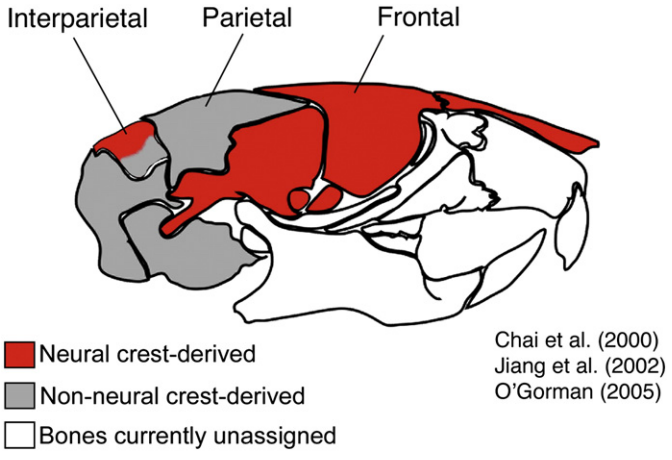

Fig. 2. Partial fate map of neural crest contribution to the adult skull in the mouse, *Mus musculus*, collected from study of the Wnt1-Cre/R26R mouse transgenic line (Chai et al., 2000; Jiang et al., 2002). Neural crest-derived bones are shaded red; mesoderm- or other "non-crest-derived" bones are shaded grey. Note the dual origin of the cranial vault, in which the frontal bone (rostral) is crest-derived but the parietal bone (caudal) is derived from mesoderm. Similarly, the interparietal bone is derived from neural crest medially, with the lateral component derived from mesoderm. The "bones of the viscerocranium" (unshaded) are reported as crest-derived by Jiang et al. (2002), but a study explicitly focused on this region of the skull has not yet been published.

[1] We interpret the 'quadrato-articular' bone, as reported here, as representing the quadrate and articular bones.

[2] We interpret the 'mandibular' bone, as reported here, as comprising the bones associated with the adult lower jaw in chickens, i.e., the endochondral articular and the intramembranous dentary, angular, surangular, splenial and opercular (Table 2).

double-heterozygous transgenic mouse, Wnt1-Cre/R26R (Chai et al., 2000; Jiang et al., 2002).

In this system, the enzyme Cre recombinase catalyzes the removal of a stop codon upstream of the ubiquitous R26R reporter allele, which allows sustained expression of β-galactosidase. Because expression of Cre recombinase is under the control of the Wnt-1 promoter, which is expressed in the dorsal portion of the neural tube (including premigratory neural crest), skull bones derived from these cells express β-galactosidase (Morriss-Kay, 2001; Jiang et al., 2002). β-Galactosidase, in turn, is easily visualized in serial sections and whole mounts.

Two reports have used this method to trace the cranial neural-crest origin of bones and cartilages of the viscerocranium and cranial vault in *M. musculus* (Chai et al., 2000; Jiang et al., 2002; Table 3). The following skull bones are ascribed a crest origin: palatine, squamosal, frontal, nasal, alisphenoid and interparietal (medial portion). Several other bones are assigned a mesodermal origin: parietal, interparietal (lateral portion) and basioccipital (Table 3, Fig. 2). Results of these genetic studies have so far only been assessed as crest vs. non-crest contributions to particular bones. The respective contributions of individual crest migratory streams have not been evaluated. Furthermore, the embryonic cellular origins of the several remaining bones of the adult skull, including those of the upper and lower jaws, are not addressed experimentally, although bones of the viscerocranium are assumed to be derived from neural crest (Jiang et al., 2002).

It would be very informative to apply an intersectional fate-mapping approach (Awatramani et al., 2003; see below) to explore the stream-level derivation of the viscerocranial bones. Such an analysis would, presumably, confirm the neural-crest derivation of these bones. More importantly, it would reveal if the relative contributions of different neural crest streams to the lower jaw of mammals, which comprises a single (paired) bone, the dentary, resemble those of other vertebrates, which have one or more additional bones in the adult jaw.

Two results from fate-mapping studies in the mouse are unexpected when compared to fate-mapping results from avian and amphibian studies. First, derivation of the bones of the cranial vault shows a surprising distribution that does not agree completely with any published report in chicken or frogs. Specifically, the frontal bone was demonstrated as derived from the CNC, while the parietal bone is derived (demonstrated using DiI labeling) from cranial mesoderm (Jiang et al., 2002). This report, when compared to studies in chicken and frogs, illuminates variation at the species level in the embryonic origin of the cranial vault (see Discussion).

Secondly, "genetic fate mapping" in the mouse reveals a mixed distribution of both labeled and unlabeled cells within Meckel's cartilage, the primary cartilaginous core of the vertebrate lower jaw (Jiang et al., 2002; Table 3). Initially, these data might appear inconsistent with the exclusively neural crest derivation of each skeletal element in the lower jaw, including Meckel's cartilage, as reported in both chicken and amphibian models. Unlabeled cells present in Meckel's cartilage of these mice, however, may still be derived from neural crest cells, but cells that for whatever reason failed to express the Wnt-1 transgene. Such premigratory neural crest cells would not drive sufficient expression of Cre recombinase in these transgenic mice, as a result of which their progeny would not appear labeled, despite their neural crest derivation. In the absence of additional data demonstrating a direct contribution of other embryonic cell populations to Meckel's cartilage (e.g., mesoderm), these results do not provide sufficient evidence to conclude that the murine lower jaw is derived from any embryonic source other than neural crest.

A recent study by O'Gorman (2005) applies site-specific, recombinase-mediated lineage tracing of mammalian second-branchial (hyoid) arch mesenchyme using a doubly heterozygous, *Hoxb1-Cre* mouse. The study aims to evaluate several longstanding assumptions regarding the homology of mammalian middle ear bones based on their tissue of origin (Reichert, 1837). By using the *Hoxb1-Cre* transgenic line, neural crest cells derived exclusively from rhombomere 4 could be traced to their final position in the middle ear. Potential contributions from neural crest cells that emerge from other rhombomeres, however, could not be assessed. In non-mammalian tetrapods, the middle ear comprises a single bone, the columella, which is considered homologous to the mammalian stapes (Romer and Parsons, 1977). Two additional bones of the middle ear in Recent mammals, the malleus and incus, are considered homologous to the articular and quadrate bones, respectively, of other vertebrates. The malleus and incus are regarded as components of the first branchial (mandibular) arch, whereas the columella (stapes) is considered a second-arch component (Reichert, 1837).

This study, however, reports second branchial-arch crest contributions to the malleus (specifically, the processus brevis) and to portions of the cartilaginous otic capsule (O'Gorman, 2005; Table 3). While initially surprising in light of conventional assignments of arch identity for middle ear bones, this result regarding the malleus agrees with Köntges and Lumsden's (1996) demonstration of second-arch neural crest derivation of the retroarticular process of the articular bone in chicken (see above). Thus, these two homologous bones in mammals and birds, which traditionally are assigned to the first arch on anatomical criteria, nevertheless are associated with the second arch on developmental features.

Because contributions of second-arch neural crest to the otic capsule in *Hoxb1-Cre* mice appear identical to those reported for chicken (Noden, 1983a), O'Gorman further concludes that incorporation of neural crest cells into the otic capsule likely predated the evolutionary divergence of birds and mammals. Comparable studies in anamniotes are needed to determine if contributions of neural crest to the cartilaginous otic capsule are a shared trait among all vertebrates, among only tetrapods, or exclusively among amniotes.

More detailed studies that assess neural crest contributions from different axial levels (e.g., by following crest migrations from individual rhombomeres) are yet to be performed. Such studies are possible with the application of "intersectional fate mapping," which allows anatomically defined regions of embryonic tissues (defined by intersections of coordinately expressed genes) to be fate mapped using a recombinase-based method (Awatramani et al., 2003). This approach already has been applied to mapping the fate of embryonic rhombic lip contributions to the choroid plexus, hindbrain roof plate and brainstem cochlear nuclear complex (Awatramani et al., 2003; Farago et al., 2006). Successful application of this technique would yield a higher resolution map of crest contributions to the skull in the mouse and allow stream-level comparisons of crest contributions to skull bones among chickens, frogs and mice.

Discussion

Neural crest contributions to the bony skull in vertebrates

In this review we provide a reference tool that summarizes the primary literature reporting direct contributions of premigratory CNC to the bony skull. Many shared patterns of embryonic derivation emerge when comparing fate-mapping studies performed to date in chicken, mouse and frog. For example, there is broad agreement, in the model organisms so far examined, that the neural crest gives rise to all the bones comprising the facial skeleton (e.g., nasal and maxillary) and the mandibular skeleton (e.g., dentary). Similarly, there is consistent evidence of a neural crest contribution to the rostral-most region of the cranial vault (i.e., the rostral frontal bone). Finally, bones at the base of the skull (e.g., the occipital complex) consistently lack a neural crest contribution and instead are derived from cranial or somitic mesoderm.

Several regions of the skull, however, remain controversial, or at least inconsistent, with respect to embryonic origin. These include the

columella, bones of the otic complex, and the dermal bones that comprise the remainder of the cranial vault. Critical differences regarding the derivation of these regions are apparent in comparisons among species and even, in at least one instance, among accounts for the same species from different laboratories.

For example, three different research groups offer contrasting accounts of the embryonic derivation of the avian cranial vault based on detailed studies of the domestic chicken. These accounts range from complete derivation of frontal and parietal bones from neural crest; to mesodermal derivation of the parietal and composite derivation (neural crest and mesoderm) of the frontal; to an exclusively or largely mesodermal derivation of both bones (Table 2; Fig. 1).

The topography of ossification of the cranial vault may offer valuable insights into this dispute and help clarify main points of contention. Whereas endochondral bones of the skull arise through a cartilaginous template that is later replaced by bone, membranous bones arise directly through ossification of cranial mesenchyme. Importantly, the membranous avian frontal bone arises initially from two separate pairs of ossification—a rostral 'supraorbital' pair and a caudal 'postorbital' pair—which later fuse to form the adult bone (Lillie, 1908; Erdmann, 1940; de Beer, 1947; Jollie, 1957).

There is consensus in chicken supporting a neural crest origin for the rostral region of the frontal bone, which is derived from the supraorbital center of ossification. There is no complete consensus, however, regarding the embryological origin of the remaining caudal region. The calvarial region of the frontal bone, which arises from the postorbital ossification center, is reported as mesodermally derived in most studies (Le Lièvre and Le Douarin, 1975; Le Lièvre, 1978; Noden, 1978; Noden, 1983a; Noden, 1983b; Evans and Noden, 2006), with only two accounts claiming a neural crest origin (Couly et al., 1992, 1993). While it would be speculative for us to assign the "correct" account(s) of calvarial origin in chickens, we find it compelling that the majority of reports, which utilize two different labeling techniques (i.e., chimeric grafting and focal retroviral labeling), assign a mesodermal origin to the postorbital region of the cranial vault. Definitive determination and acceptance of the embryonic origins of the postorbital pair of ossifications likely would resolve the current disagreement regarding the derivation of the avian cranial vault.

Data from the frog, *Xenopus laevis*, when compared to other models, provide an example of interspecific variation in the neural crest origin of individual bones. The cranial vault in adult *Xenopus* comprises a single, median bone, which arises from laterally paired ossification centers that fuse in the midline. This bone, unique to anurans, has long been recognized as the "frontoparietal," representing the fusion of separate frontal and parietal bones of early tetrapods (Parker, 1871, 1876; de Beer, 1937). Not all authors, however, have accepted this evolutionary scenario. Eaton (1942), for example, argued for homology of the anuran frontoparietal with only the frontal bone of other tetrapods because in some anuran species the frontoparietal forms from only a single pair of ossification centers (Eaton, 1939; Sedra, 1948). More recently, this proposal has been largely dismissed based on patterns of ossification in many additional frog species, which show two distinct rostral and caudal centers of ossification on each side of the head (Griffiths, 1954; Trueb, 1973).

In *Xenopus*, the frontoparietal receives contributions from all three migratory streams of cranial neural crest along its entire length (Hanken and Gross, 2005). Assuming that this bone is homologous to the frontal and parietal bones in amniotes, this pattern of derivation most closely resembles the account of the avian cranial vault offered by Couly et al. (1993) yet contrasts sharply with the account of the murine cranial vault offered by Morriss-Kay (2001), in which only the frontal bone is derived from neural crest and the parietal bone is derived from mesoderm. If, instead, one assumes that the anuran frontoparietal is homologous only to the amniote frontal (Eaton, 1942), then its observed pattern of derivation most closely resembles that reported for the mouse but differs from two of the three alternative fate maps of the cranial vault offered for birds.

Published fate-mapping studies of a small number of diverse model organisms indicate that 1) patterns of neural crest contribution to the vertebrate osteocranium are variable among species, and 2) the relative contributions of neural crest and mesoderm to the bony skull are evolutionarily labile. As discussed below, however, these inferences rely critically on current hypotheses of skeletal homology and represent a very narrow sampling of vertebrate cranial and developmental diversity. At best, they represent working hypotheses in need of further testing and confirmation.

Derivation of the columella

There are at least three differing accounts of the embryonic derivation of the middle ear skeleton (columella) in birds. All are derived from studies of the domestic chicken (Table 2). Noden (1982, 1984) reports that the columella is a composite bone derived from both neural crest and mesoderm. Le Lièvre (1978) and, more recently, Köntges and Lumsden (1996) describe the columella as exclusively crest-derived, although the source of neural crest along the neural tube differs between these two accounts: mesencephalic and anterior rhombomeric crest (Le Lièvre, 1978) vs. crest derived from rhombomeres 3–5 (Köntges and Lumsden, 1996). Couly et al. (1993) similarly report the columella as CNC-derived, but they do not assign its derivation to any specific axial level along the neural tube.

Köntges and Lumsden's claim is most consistent with established anatomical convention, which regards the columella as a second (hyoid) arch derivative (Reichert, 1837). Indeed, there is no other published report of mesencephalic neural crest migrating into the second arch in any vertebrate, as proposed by Le Lièvre. We suggest that her claim of mesencephalic/anterior rhombencephalic crest contribution to the columella likely is a consequence of the longer grafts used in her experiments (see above). Presumably, the neural crest cells that contributed directly to the columella were derived from the caudal, rhombencephalic portion of the grafts, although this detail is not addressed in her report. Importantly, in neither of these reports is it clear if neural crest contributions were evaluated in both the columellar footplate and shaft (Le Lièvre, 1978) or if the bone itself, versus only the associated muscular connective tissues, was evaluated in detail (Köntges and Lumsden, 1996). Both features were evaluated in Noden's studies.

The only empirical data from any other vertebrate groups regarding the embryonic derivation of the columella (or its mammalian homolog, the stapes) reported a second arch-contribution to the stapes in *Hoxb1-Cre* mice (O'Gorman, 2005).

Origin of the mammalian interparietal bone

The interparietal bone in the mouse has a dual origin: its medial portion is crest-derived, whereas the lateral portion is mesoderm-derived (Jiang et al., 2002; Table 3; Fig. 2). The developmental history of this bone is especially intriguing considering that it is absent from all other vertebrate species in which fate-mapping studies have been performed. The mammalian interparietal is generally regarded as homologous to the postparietal bone of archaic reptiles, a dermal bone that overlies the cerebellum and articulates posteriorly with the endochondral supraoccipital bone (Morriss-Kay, 2001). Among Recent reptiles, the postparietal has been observed to date only in the American alligator (Klembara, 2001), and it will be very interesting to learn if this putative homolog of the mammalian interparietal is similarly derived from both neural crest and mesoderm once a fate map of this species is produced.

Alternatively, the interparietal bone is regarded by some as homologous to paired parietal bones. Evidence for this interpretation

comes from the skull of the fallow deer, *Dama dama*. Instead of having a discrete interparietal, the bone's characteristic position within the cranium is occupied by expanded parietal bones (Kierdorf and Kierdorf, 1992). Similarly, the location of the interparietal within the caudal portion of the cranial vault in the mouse corresponds closely to the location of the parietal in the avian skull. As discussed above, the avian parietal is reported to have an exclusively mesodermal origin by some authors (Le Lièvre, 1978; Noden, 1982) and an exclusively neural crest origin by others (Couly et al., 1993). Unlike the interparietal, no one has yet reported a dual embryonic origin for the parietal bone.

Unresolved issues in the osteogenic neural crest literature

Given the variation among model systems examined so far, how can we hope to clarify primitive versus derived patterns of neural crest contribution to the skull bones of vertebrates? For example, which, if any, of the contrasting patterns of derivation of the cranial vault reported from *Xenopus*, chicken and mouse is characteristic of bony fishes, or do these vertebrates display unique patterns? From comparative and developmental perspectives, only by construction of additional, long-term fate maps of the CNC across a wider phylogenetic framework (e.g., bony fishes, additional amphibians, reptiles) may we resolve which features are primitive and which are derived, and, more generally, determine the full extent to which patterns of crest cell contribution to the bony skull are invariant (i.e., rigidly conserved across vertebrates) or evolutionarily labile.

Representing extant organisms as surrogate "primitive" data points can be problematic. Individual species may not accurately replicate either the primitive or the generalized condition of the broader taxonomic group to which they belong (Hanken, 1993). This includes model organisms, which may display species-specific traits, including unique features of life history and developmental mode. By amassing comparable data from a wider array and number of vertebrates, however, we can more readily identify unique features of model species and more confidently resolve how, and to what extent, crest contributions to the adult skull have changed over the course of evolution (Helms et al., 2005).

It is reasonable to assume that homologous skull bones among different vertebrates are derived from the same embryonic tissue(s), yet this assumption remains largely untested. Moreover, the limited comparative data available at this time suggests that this relationship does not always hold true. Since the same principal migratory crest streams (viz., mandibular, hyoid and branchial) are all present in amphibians, birds and mammals, it is possible to directly test such *a priori* hypotheses for the stream-level derivation of bones and cartilages of the skull, at least among these tetrapods. These comparisons would answer the question of whether embryonic tissue of origin is a valid criterion for evaluating the homology of individual cranial elements. Existing data suggest that embryonic derivation—mesoderm vs. neural crest—of at least some skull bones is evolutionarily labile, and that shared features of development is not an appropriate criterion of homology for these elements in all interspecific comparisons. Alternatively, contrasting patterns of embryonic derivation of what is regarded as the same (homologous) bone among distantly related species may indicate that the corresponding homology statement itself is incorrect. Accumulation of additional detailed information regarding the embryonic derivation and developmental biology of cranial bones in diverse species may eventually force a reevaluation of the traditional assessment of specific cranial bone homologies among vertebrate classes, which was codified largely in the absence of such data and before the advent of experimental and molecular biology.

Future studies must extend fate-mapping research to additional model and non-model species in order to document more widely the patterns of embryonic derivation and the extent to which these patterns correlate with different, taxon-specific morphologies and life histories. This avenue of research, which aims to define the full scope of variation in the pattern of neural crest derivation of the osteocranium, would facilitate unification of the fields of vertebrate morphology, development and neural crest biology. Moreover, additional studies in diverse organisms will help define the ancestral pattern of cranial development at key "junctures" in vertebrate evolution, such as the origin of jaws and the evolution of tetrapods. Finally, a synthetic and comprehensive evaluation of neural crest derivatives in additional species will better characterize the role and behavior of this intriguing embryonic tissue in the development and evolution of the vertebrate skull, and especially in the origin of novel features.

Why are there disagreements among neural crest fate mapping studies?

An important but largely unresolved question within the field of neural crest biology is why differences in derivation schemes and fate maps for a given species arise among different research groups. Some discrepancies in the literature may be due to the fact that different chicken fate-mapping studies have utilized slightly different grafting procedures at slightly different developmental stages. Compare, for example, grafts of "mesencephalic" neural crest at the 9-somite stage (Le Lièvre, 1974) to grafts of the entire rostrocaudal axis of the developing neural tube at embryonic days 8 and 14 (Couly et al., 1993). Indeed, Couly et al. (1993) suggest that differences in the timing of grafts and their subsequent recovery (following initiation of bone development) explain conflicting results with respect to CNC contribution to the frontal bone reported in earlier experiments by Le Lièvre (1978).

A second potential source of variability may be contamination of purportedly pure grafts of one embryonic cell type with other cells. Schneider (1999) performed experiments in which embryonic tissues that give rise to the lateral wall of the braincase (a strictly mesoderm-derived structure *in vivo*) were replaced with grafts of neural crest. The resulting braincase appeared morphologically normal and contained cells derived from the neural crest, demonstrating that both tissues can respond in the same way to cues that mediate skeletogenesis and morphological patterning. Since neural crest is able to form at least some skeletal structures that normally are derived from mesoderm, even slight contamination of neural crest in grafts of mesoderm—and vice versa—may significantly skew fate-mapping results that are based on analysis of chimeric embryos. This underscores both the importance of minimizing contamination of grafts in such studies and the need to verify specific fate-mapping results with several alternate methods in order to control for potential experimental artifacts associated with any particular one.

A third potential explanation of discrepancies in the literature is the intriguing possibility of significant variation in craniofacial development among inbred strains of domestic chicken. If this explanation were true, then the contrasting claims offered by different research groups may not conflict, but instead reflect real differences among source populations on which the studies are based.

Perhaps different chicken strains, or even the same strain bred and reared in different countries, evince characteristic differences in the embryonic derivation of the craniofacial skeleton. Published fate maps for the domestic chicken are based on studies of several inbred strains, including White Plymouth Rock (Noden, 1978), White Leghorn (Couly et al., 1993; Evans and Noden, 2006) and Rhode Island Red Hen (Köntges and Lumsden, 1996). Strain type was not reported by Le Lièvre (1978). Presumably, even slight differences in the timing and pattern of neural crest migration among strains could alter the derivation of craniofacial tissues. This potential explanation, however, remains to be explored adequately.

How can we resolve issues and differences in fate maps among species? One way might be to apply the same methodological approach to pursuing a fate map across different model systems. For example, would application of non-invasive, recombinase-based genetic fate mapping based on the same early genetic driver produce the same patterns of derivation in other vertebrate model systems as it has in the mouse? This approach would obviate the need for painstaking grafting studies that are susceptible to human error and other experimental artifacts. As with any methodological approach, however, genetic fate-mapping studies carried out in multiple vertebrate models would also be susceptible to experimental artifacts. For example, the presence of both labeled and unlabeled cells in individual tissues, as reported for Meckel's cartilage in Wnt1-Cre mice (Jiang et al., 2002), would require additional direct-labeling studies to determine if this is an artifact of the labeling method or an indication of tissue derivation from other embryonic sources in addition to neural crest.

Additionally, this approach would require a suitable Cre recombinase-driver (e.g., the Wnt1 promoter) that accurately and exclusively labels early cranial neural crest in all species. At this time, no such early marker, which maintains the same expression pattern in early cranial neural crest across all vertebrates, has been identified. Interestingly, the only report to date that utilizes genetic fate mapping to assess neural crest contributions to the middle ear does not exclusively label neural crest, but rather cells derived from only a single rhombomere, which includes some neural crest (O'Gorman, 2005).

Alternatively, the chimeric grafting approach could be extended to a wider array of organisms. Using this strategy, the same regions of neural crest in each model could be excised and grafted into a host embryo, as has been applied in chicken and frogs. The advantage of this approach is that it would reveal patterns of crest contribution to the skull in a wider assortment of animals, e.g., reptiles, cartilaginous fish, bony fish. The disadvantage is that it will not resolve long-standing disagreements that apply to a single species (e.g., domestic chicken).

In light of current disagreements among fate maps produced by different laboratories, some studies have begun to utilize more fine-grained experimental procedures as an additional means of resolving long-standing questions regarding the contribution of neural crest to cranial structures. For example, Cerny et al. (2004) and Lee et al. (2004) combined molecular marker analysis and vital dye (DiI) staining of highly specific sub-populations of late-emerging neural crest cells to determine the distinct origins of the palatoquadrate, Meckel's cartilage and trabecular cartilage condensations. Additional use of highly focused crest cell labeling procedures such as these may help to resolve existing disagreements regarding neural crest contributions in the chicken skull.

Contemporary neural crest research must aim to better define the significance of boundaries and interfaces between the neural crest and other embryonic tissues (e.g., mesoderm) throughout development. For example, is the embryonic origin of cells or their local interactions post-migration more important in defining the eventual fate of embryonic contributions to the skull? In order to better understand the nature of evolutionary alterations to cranial fate maps, it will be critical that additional studies be carried out in both model and non-model systems. Ultimately, as patterns of neural crest contribution to the skull are better characterized in the coming years, so too will the details of how diversity in cranial form arose among vertebrates.

Acknowledgments

This study was supported by the U.S. National Science Foundation, grant no. EF-0334846 to J.H. The authors wish to thank two anonymous reviewers for their helpful comments on earlier drafts of this manuscript.

References

Ahlberg, P.E., Köntges, G., 2006. Homologies and cell populations: a response to Sánchez-Villagra and Maier. Evol. Dev. 8, 116–118.
Andres, G., 1949. Untersuchungen an Chimären von *Triton* und *Bombinator*. Genetica 24, 387–534.
Awatramani, R., Soriano, P., Rodriguez, C., Mai, J.J., Dymecki, S.M., 2003. Cryptic boundaries in roof plate and choroid plexus identified by intersectional gene activation. Nat. Genet. 35, 70–75.
Barlow, LA., 2002. Cranial nerve development: placodal neurons ride the crest. Curr. Biol. 12, R171–R173.
Barlow, L.A., Northcutt, R.G., 1995. Embryonic origin of amphibian taste buds. Dev. Biol. 169, 273–285.
Barlow, L.A., Northcutt, R.G., 1997. Taste buds develop autonomously from endoderm without induction by cephalic neural crest or paraxial mesoderm. Development 124, 949–957.
Beauchamp, G.R., Knepper, P.A., 1984. Role of the neural crest in anterior segment development and disease. J. Pediatr. Ophthalmol. Strabismus 21, 209–214.
Bronner-Fraser, M., 1986. Analysis of the early stages of trunk neural crest migration in avian embryos using monoclonal antibody HNK-1. Dev. Biol. 115, 44–55.
Carl, T.F., Dufton, C., Vourgourakis, Y., Klymkowsky, M., Hanken, J., 2000. Green fluorescent protein used to assess cranial neural crest derivatives in the frog, *Xenopus laevis*. In: Jacobson, C.-O., Olsson, L. (Eds.), Regulatory Processes in Development. Portland Press, London, pp. 167–172.
Cebra-Thomas, J.A., Tan, F., Sistla, S., Estes, E., Bender, G., Kim, C., Riccio, P., Gilbert, S.F., 2005. How the turtle forms its shell: a paracrine hypothesis of carapace formation. J. Exp. Zool. B. Mol. Dev. Evol. 304, 558–569.
Cebra-Thomas, J.A., Betters, E., Yin, M., Plafkin, C., McDow, K., Gilbert, S.F., 2007. Evidence that a late-emerging population of trunk neural crest cells forms the plastron bones in the turtle *Trachemys scripta*. Evolut. Develop. 9, 267–277.
Cerny, R., Lwigale, P., Ericsson, R., Meulemans, D., Epperlein, H.H., Bronner-Fraser, M., 2004. Developmental origins and evolution of jaws: new interpretation of "maxillary" and "mandibular". Dev. Biol. 276, 225–236.
Cerny, R., Horácek, I., Olsson, L., 2006. The trabecula cranii: development and homology of an enigmatic vertebrate head structure. Anim. Biol. 56, 503–518.
Chai, Y., Jiang, X., Ito, Y., Bringas Jr., P., Han, J., Rowitch, D.H., Soriano, P., McMahon, A.P., Sucov, H.M., 2000. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127, 1671–1679.
Clark, K., Bender, G., Murray, B.P., Panfilio, K., Cook, S., Davis, R., Murnen, K., Tuan, R.S., Gilbert, S.F., 2001. Evidence for the neural crest origin of turtle plastron bones. Genesis 31, 111–117.
Clarke, J.D., Tickle, C., 1999. Fate maps old and new. Nat. Cell Biol. 1, E103–E109.
Colnot, C., 2005. Cellular and molecular interactions regulating skeletogenesis. J. Cell. Biochem. 95, 688–697.
Couly, G.F., Coltey, P.M., Le Douarin, N.M., 1992. The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development 114, 1–15.
Couly, G.F., Coltey, P.M., Le Douarin, N.M., 1993. The triple origin of 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. Clarendon Press, Oxford. 552 pp.
de Beer, G.R., 1947. The differentiation of neural crest cells into visceral cartilages and odontoblasts in *Amblystoma*, and a re-examination of the germ-layer theory. Proc. R. Soc. London, Ser B. Biol. Sci. 134, 377–398.
Dixon, J., Brakebusch, C., Fassler, R., Dixon, M.J., 2000. Increased levels of apoptosis in the prefusion neural folds underlie the craniofacial disorder, Treacher Collins syndrome. Hum. Mol. Genet. 9, 1473–1480.
DuShane, G.P., 1935. An experimental study of the origin of pigment cells in Amphibia. J. Exp. Zool. 72, 1–31.
Eaton Jr., T.H., 1939. Development of the frontoparietal bones in frogs. Copeia 1939, 95–97.
Eaton Jr., T.H., 1942. Are 'frontoparietal' bones in frogs actually frontals? J. Wash. Acad. Sci. 32, 151–153.
Edwards, S.J., Gladwin, A.J., Dixon, M.J., 1997. The mutational spectrum in Treacher Collins syndrome reveals a predominance of mutations that create a premature-termination codon. Am. J. Hum. Genet. 60, 515–524.
Erdmann, K., 1940. Zur Entwicklungsgeschichte der Knochen im Schädel des Huhnes bis zum Zeitpunktdes Ausschlüpfen aus dem Ei. Z. Morphol. Okol. Tiere Berlin 36, 315–400.
Evans, D.J., Noden, D.M., 2006. Spatial relations between avian craniofacial neural crest and paraxial mesoderm cells. Dev. Dyn. 235, 1310–1325.
Farago, A.F., Awatramani, R.B., Dymecki, S.M., 2006. Assembly of the brainstem cochlear nuclear complex is revealed by intersectional and subtractive genetic fate maps. Neuron 50, 205–218.
Farlie, P.G., McKeown, S.J., Newgreen, D.F., 2004. The neural crest: basic biology and clinical relationships in the craniofacial and enteric nervous systems. Birth Defects Res. C 72, 173–189.
Franz-Odendaal, T., Hall, B.K., Witten, P.E., 2006. Buried alive: how osteocytes become bone. Dev. Dyn. 235, 176–190.
Fukiishi, Y., Morriss-Kay, G.M., 1992. Migration of cranial neural crest cells to the pharyngeal arches and heart in rat embryos. Cell Tissue Res. 268, 1–8.
Gargioli, C., Slack, J.M., 2004. Cell lineage tracing during *Xenopus* tail regeneration. Development 131, 2669–2679.

Gilbert, S.F., Loredo, G.A., Brukman, A., Burke, A.C., 2001. Morphogenesis of the turtle shell: the development of a novel structure in tetrapod evolution. Evol. Dev. 3, 47–58.
Gilbert, S.F., Bender, G., Betters, E., Yin, M., Cebra-Thomas, J.A., 2007. The contribution of neural crest cells to the nuchal bone and plastron of the turtle shell. Int. Comp. Biol. 47, 401–408.
Gorlin, R.J., Cohen Jr., M.M., Levin, L.S., 1990. Syndromes of the Head and Neck. Oxford Univ. Press, New York. 977 pp.
Graveson, A.C., Smith, M.M., Hall, B.K., 1997. Neural crest potential for tooth development in a urodele amphibian: developmental and evolutionary significance. Dev. Biol. 188, 34–42.
Griffiths, I., 1954. On the nature of the fronto-parietal in Amphibia, Salientia. Proc. Zool. Soc. Lond. 123, 781–792.
Gross, J.B., Hanken, J., 2004. Use of fluorescent dextran conjugates as a long-term marker of osteogenic neural crest in frogs. Dev. Dyn. 230, 100–106.
Gross, J.B., Hanken, J., 2005. Cranial neural crest contributes to the bony skull vault in adult *Xenopus laevis*: Insights from cell labeling studies. J. Exp. Zool. B Mol. Dev. Evol. 304, 169–176.
Gross, J.B., Hanken, J., Oglesby, E., Marsh-Armstrong, N., 2006. Use of a *ROSA26:GFP* transgenic line for long-term *Xenopus* fate-mapping studies. J. Anat. 209, 401–413.
Hall, B.K., 1999. The Neural Crest in Development and Evolution. Springer-Verlag, New York. 313 pp.
Hanken, J., 1993. Model systems versus outgroups: alternative approaches to the study of head development and evolution. Am. Zool. 33, 448–456.
Hanken, J., Gross, J.B., 2005. Evolution of cranial development and the role of neural crest: insights from amphibians. J. Anat. 207, 437–446.
Helms, J.A., Cordero, D., Tapadia, M.D., 2005. New insights into craniofacial morphogenesis. Development 132, 851–861.
Hörstadius, S., 1950. The Neural Crest: Its Properties and Derivatives in the Light of Experimental Research. Oxford Univ. Press, London. 111 pp.
Jaenisch, R., 1985. Mammalian neural crest cells participate in normal embryonic development on microinjection into post-implantation mouse embryos. Nature 318, 181–183.
Jiang, X., Iseki, S., Maxson, R.E., Sucov, H.M., Morriss-Kay, G.M., 2002. Tissue origins and interactions in the mammalian skull vault. Dev. Biol. 241, 106–116.
Johnston, M.C., Bhakdinaronk, A., Reid, Y.C., 1973. An expanded role of the neural crest in oral and pharyngeal development. In: Bosma, J.F. (Ed.), Fourth Symposium on Oral Sensation and Perception: Development in the Fetus and Infant. U.S. Dept. of Health, Education, and Welfare, Bethesda, pp. 37–52.
Jollie, M.T., 1957. The head skeleton of the chicken and remarks on the anatomy of this region in other birds. J. Morphol. 100, 389–436.
Kardong, K.V., 2002. Vertebrates: comparative anatomy, function, Evolution, 3rd ed. McGraw-Hill, Boston. 762 pp.
Kierdorf, U., Kierdorf, H., 1992. Studies on the parietal region of the cervid skull. II. The parietooccipital region in the skull of the fallow deer (*Dama dama* L.). Anat. Histol. Embryol: J. Vet. Med. Series C 21, 373–379.
Klembara, J., 2001. Postparietal and prehatching ontogeny of the supraoccipital in *Alligator mississippiensis* (Archosauria, Crocodylia). J. Morphol. 249, 147–153.
Knight, R.D., Schilling, T.F., 2006. Cranial neural crest and development of the head skeleton. Adv. Exp. Med. Biol. 589, 120–133.
Köntges, G., Lumsden, A., 1996. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 122, 3229–3242.
Kuratani, S., 2005. Craniofacial development and the evolution of vertebrates: the old problems on a new background. Zool. Sci. 22, 1–19.
Kuratani, S., Matsuo, I., Aizawa, S., 1997. Developmental patterning and evolution of the mammalian viscerocranium: genetic insights into comparative morphology. Dev. Dyn. 209, 139–155.
Landacre, F.L., 1921. The fate of the neural crest in the head of Urodeles. J. Comp. Neurol. 33, 1–43.
Langille, R.M., Hall, B.K., 1988. Role of the neural crest in development of the cartilaginous cranial and visceral skeleton of the medaka, *Oryzias latipes* (Teleostei). Anat. Embryol. (Berl.) 177, 297–305.
Le Douarin, N., Barq, G., 1969. Use of Japanese quail cells as “biological markers” in experimental embryology. C. R. Acad. Sci. Hebd. Seances Acad. Sci. D 269, 1543–1546.
Le Douarin, N.M., Kalcheim, C., 1999. The Neural Crest, 2nd ed. Cambridge Univ. Press, Cambridge. 445 pp.
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.
Le Lièvre, C.S., 1974. Rôle des cellules mésectodermiques issues des crêtes neurales céphaliques dans la formation des arcs branchiaux et du squelette viscéral. J. Embryol. Exp. Morphol. 31, 453–477.
Le Lièvre, C.S., 1978. Participation of neural crest-derived cells in the genesis of the skull in birds. J. Embryol. Exp. Morphol. 47, 17–37.
Le Lièvre, C.S., Le Douarin, N.M., 1975. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J. Embryol. Exp. Morphol. 34, 125–154.
Lillie, F.R., 1908. The Development of the Chick. An Introduction to Embryology. Henry Holt and Co., New York. 472 pp.
Lumsden, A.G., 1988. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development 103, 155–169.
Matsuoka, T., Ahlberg, P.E., Kessaris, N., Iannarelli, P., Dennehy, U., Richardson, W.D., McMahon, A.P., Köntges, G., 2005. Neural crest origins of the neck and shoulder. Nature 436, 347–355.
Meier, S., Packard Jr., D.S., 1984. Morphogenesis of the cranial segments and distribution of neural crest in the embryos of the snapping turtle, *Chelydra serpentina*. Dev. Biol. 102, 309–323.
Morriss-Kay, G.M., 2001. Derivation of the mammalian skull vault. J. Anat. 199, 143–151.
Noden, D.M., 1978. The control of avian cephalic neural crest cytodifferentiation. I. Skeletal and connective tissues. Dev. Biol. 67, 296–312.
Noden, D.M., 1982. Patterns and organization of craniofacial skeletogenic and myogenic mesenchyme: a perspective. In: Sarnat, B.G. (Ed.), Progress in Clinical and Biological Research: Factors and Mechanisms Influencing Bone Growth. Alan R. Liss, Inc., New York, pp. 167–203.
Noden, D.M., 1983a. The role of the neural crest in patterning avian cranial skeletal, connective, and muscle tissues. Dev. Biol. 96, 144–165.
Noden, D.M., 1983b. The embryonic origins of avian craniofacial muscles and associated connective tissues. Am. J. Anat. 168, 257–276.
Noden, D.M., 1984. The use of chimeras in analyses of craniofacial development. In: Le Douarin, N.M., McLaren, A. (Eds.), Chimeras in Developmental Biology. Academic Press, London, pp. 241–280.
Noden, D.M., 1988. Interactions and fates of avian craniofacial mesenchyme. Development 103 Suppl., 121–140.
Noden, D.M., Trainor, P.A., 2005. Relations and interactions between cranial mesoderm and neural crest populations. J. Anat. 207, 575–601.
O'Gorman, S., 2005. Second branchial arch lineages of the middle ear of wild-type and *Hoxa2* mutant mice. Dev. Dyn. 234, 124–131.
Parker, W.K., 1871. On the structure and development of the skull of the common frog. Philos. Trans. R. Soc. Lond. 161, 137–211.
Parker, W.K., 1876. On the structure and development of the skull in Batrachia—Part II. Philos. Trans. R. Soc. Lond. 166, 601–669.
Platt, J.B., 1893. Ectodermic origin of the cartilages of the head. Anat. Anz. 8, 506–509.
Poswillo, D., 1988. The aetiology and pathogenesis of craniofacial deformity. Development 103 Suppl., 207–212.
Raven, C.P., 1931. Zur Entwicklung der Ganglienleiste. I: Die Kinematik der Ganglienleisten Entwicklung bei den Urodelen. Roux' Arch. Entwicklungsmech. Org. 125, 210–292.
Reichert, C., 1837. Über die Visceralbogen der Wirbelthiere im Allgemeinen und deren Metamorphosen bei den Vögeln und Säugethieren. Archiv. Anat., Physiol. Wiss. Med. 120–222.
Romer, A.S., Parsons, T.S., 1977. The Vertebrate Body, 5th ed. Saunders College Publishing, Philadelphia. 624 pp.
Rudel, D., Sommer, R.J., 2003. The evolution of developmental mechanisms. Dev. Biol. 264, 15–37.
Sánchez-Villagra, M.R., Maier, W., 2006. Homologies of the mammalian shoulder girdle: a response to Matsuoka et al. (2005). Evolut. Develop. 8, 113–115.
Schneider, R.A., 1999. Neural crest can form cartilages normally derived from mesoderm during development of the avian head skeleton. Dev. Biol. 208, 441–455.
Schultze, H.-P., 1993. Patterns of diversity in the skull of jawed fishes. In: Hanken, J., Hall, B.K. (Eds.), The Skull. Patterns of Structural and Systematic Diversity, vol. 2. University of Chicago Press, Chicago, pp. 189–254.
Sedra, S.N., 1948. On the homology of certain elements in the skull of *Bufo regularis* Reuss (Salientia). Proc. Zool. Soc. Lond. 119, 633–641.
Stone, L.S., 1926. Further experiments on the extirpation and transplantation of mesectoderm in *Amblystoma punctatum*. J. Exp. Zool. 44, 95–131.
Stone, L.S., 1929. Experiments showing the role of migrating neural crest (mesectoderm) in the formation of head skeleton and loose connective tissue in *Rana palustris*. Wilhelm Roux Arch. Dev. Biol. 118, 40–77.
Sulik, K.K., Cook, C.S., Webster, W.S., 1988. Teratogens and craniofacial malformations: relationships to cell death. Development 103 Suppl., 213–231.
Tan, S.S., Morriss-Kay, G.M., 1986. Analysis of cranial neural crest cell migration and early fates in postimplantation rat chimaeras. J. Embryol. Exp. Morphol. 98, 21–58.
Toerien, M.J., 1965. Experimental studies on the columella–capsular interrelationship in the turtle *Chelydra serpentina*. J. Embryol. Exp. Morphol. 14, 265–272.
Trueb, L., 1973. Bones, frogs, and evolution. In: Vial, J.L. (Ed.), Evolutionary Biology of the Anurans: Contemporary Research on Major Problems. University of Missouri Press, Columbia, pp. 65–132.
Trueb, L., Hanken, J., 1992. Skeletal development in *Xenopus laevis* (Anura: Pipidae). J. Morphol. 214, 1–41.
Twitty, V.C., Bodenstein, D., 1939. Correlated genetic and embryological experiments on *Triturus*. III. Further transplantation experiments on pigment development. IV: The study of pigment cell behavior *in vitro*. J. Exp. Zool. 81, 357–398.
Wagner, G., 1949. Die Bedeutung der Neuralleiste für die Kopfgestaltung der Amphibienlarven: Untersuchungen an Chimären von *Triton* und *Bombinator*. Rev. Suisse Zool. 56, 519–620.
Weston, J.A., 1963. A radiographic analysis of the migration and localization of trunk neural crest cells in the chick. Dev. Biol. 6, 279–310.