REVIEW ARTICLE

Michael K. Richardson · James Hanken
Mayoni L. Gooneratne · Claude Pieau
Albert Raynaud · Lynne Selwood · Glenda M. Wright

There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development

Abstract Embryos of different species of vertebrate share a common organisation and often look similar. Adult differences among species become more apparent through divergence at later stages. Some authors have suggested that members of most or all vertebrate clades pass through a virtually identical, conserved stage. This idea was promoted by Haeckel, and has recently been revived in the context of claims regarding the universality of developmental mechanisms. Thus embryonic resemblance at the tailbud stage has been linked with a conserved pattern of developmental gene expression – the zootype. Haeckel's drawings of the external morphology of various vertebrates remain the most comprehensive comparative data purporting to show a conserved stage. However, their accuracy has been questioned and only a narrow range of species was illustrated. In view of the current widespread interest in evolutionary developmental biology, and especially in the conservation of developmental mechanisms, re-examination of the extent of variation in vertebrate embryos is long overdue. We present here the first review of the external morphology of tailbud embryos, illustrated with original specimens from a wide range of vertebrate groups. We find that embryos at the tailbud stage – thought to correspond to a conserved stage – show variations in form due to allometry, heterochrony, and differences in body plan and somite number. These variations foreshadow important differences in adult body form. Contrary to recent claims that all vertebrate embryos pass through a stage when they are the same size, we find a greater than 10-fold variation in greatest length at the tailbud stage. Our survey seriously undermines the credibility of Haeckel's drawings, which depict not a conserved stage for vertebrates, but a stylised amniote embryo. In fact, the taxonomic level of greatest resemblance among vertebrate embryos is below the subphylum. The wide variation in morphology among vertebrate embryos is difficult to reconcile with the idea of a phygenetically-conserved tailbud stage, and suggests that at least some developmental mechanisms are not highly constrained by the zootype. Our study also highlights the dangers of drawing general conclusions about vertebrate development from studies of gene expression in a small number of laboratory species.

Key words Morphogenesis · Developmental biology · Comparative anatomy · Comparative study · Embryology

M.K. Richardson (✉) · M.L. Gooneratne
Department of Anatomy and Developmental Biology,
St. George's Hospital Medical School, Cranmer Terrace,
London SW17 ORE, UK
Fax: 0181 725 3326; e-mail: m.richardson@sghms.ac.uk

J. Hanken
Department of Environmental, Population,
and Organismic Biology, University of Colorado,
Boulder, CO 80309–0334, USA

C. Pieau
Département Dynamique du Génome et Evolution,
Institut Jacques Monod, 2 Place Jussieu – Tour 43,
F-75251 Paris Cedex 05, France

A. Raynaud
Laboratoire de Zoologie, Université Paul Sabatier,
118 Route de Narbonne, F-31062, Toulouse Cedex, France

L. Selwood
School of Zoology, La Trobe University, Bundoora,
Victoria, 3083, Australia

G.M. Wright
Department of Anatomy and Physiology,
Atlantic Veterinary College, University of Prince Edward Island,
550 University Avenue, Charlottetown, Prince Edward Island,
C1A 4P3, Canada

Introduction

There is no stage of development in which the unaided eye would fail to distinguish between them (vertebrate embryos) … a blind man could distinguish between them. (Sedgwick 1894).
Embryos of different species (of vertebrate) pass through identical embryonic stages before acquiring their specific features. (Butler and Juurlink 1987).

Raff (1992) has pointed out that developmental biologists tend to emphasise the similarities among species,

whereas evolutionary biologists emphasise the differences. The result is a long history of disagreement over the extent to which embryonic development is conserved during evolution (reviewed by McKinney and McNamara 1991; Hall 1992; Raff 1992, 1996; McNamara 1995; Richardson 1995). This is partly because, with a few exceptions (e.g. Slack et al. 1993; Burke et al. 1995), many of our current ideas about the molecular basis of vertebrate development and evoltuion come from studies of a small number of laboratory species. The assumption that these findings are generally applicable to all vertebrates is not necessarily a valid one (Raff 1996).

A prevalent idea in developmental evolution is that intermediate embryonic stages are resistant to evolutionary change, and that differences among species arise through divergence at later stages of development. As a consequence, all vertebrates are often said to pass through a common stage when they look virtually identical (Haeckel 1874; Butler and Juurlink 1987; Wolpert 1991; Alberts et al. 1994; Collins 1995). The conserved stage is called the *phylotypic* stage because it is thought to be the point in development when there is maximum resemblance among members of a phylum or comparable higher taxon (Slack et al. 1993). Conservation of embryonic form is thought to be associated with the conservation of patterns of developmental gene expression across a wide range of animal clades (Slack et al. 1993).

One puzzling feature of the debate in this field is that while many authors have written of a conserved embryonic stage, no one has cited any comparative data in support of the idea. It is almost as though the phylotypic stage is regarded as a biological concept for which no proof is needed. This has led to many problems, not least of which is the lack of consensus on exactly which stage is conserved (Richardson 1995). The phylotypic stage in vertebrates has been defined as the *pharyngula* stage, after the series of pharyngeal pouches seen in embryos (Ballard 1981). However it is not clear precisely which stage of development this represents, since pharyngeal pouches appear over an extended period of development. Slack et al. (1993) suggest that the tailbud stage may be a time of maximum resemblance among species. Wolpert's (1991) definition of a conserved stage is much earlier and corresponds to an early somite stage, just after neurulation. Duboule (1994) takes a broader view and regards the period between the head fold and tailbud stages as a time of high morphogenetic resemblance.

According to recent models, not only is the putative conserved stage followed by divergence, but it is preceded by variation at earlier stages, including gastrulation and neurulation. This is seen for example in squamata, where variations in patterns of gastrulation and neurulation may be followed by a rather similar somite stage (Hubert 1985). Thus the relationship between evolution and development has come to be modelled as an "evolutionary hourglass" (Fig. 1; Elinson 1987; Duboule 1994; Collins 1995).

The idea of a conserved embryonic stage arose in the nineteenth century. Pioneers in the field of embryology
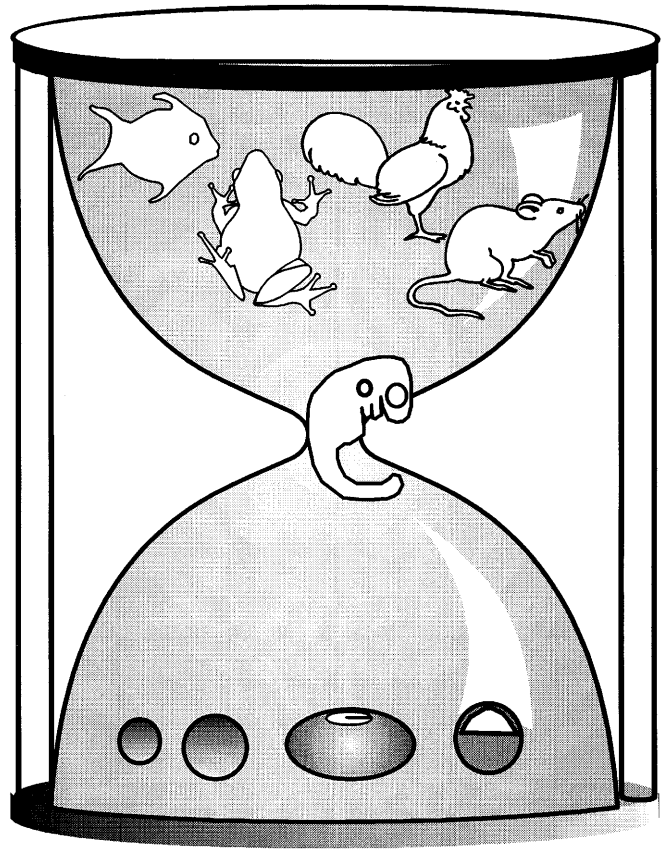

Fig. 1 The hourglass model of the relationship between evolution and development. This model implies divergence at earlier and later stages from a conserved intermediate stage. Horizontal distance represents morphological divergence during evolution, and vertical distance represents developmental stage. Based on Elinson (1987), Duboule (1994), and Collins (1995)

in particular stressed the similarities among the embryos of different vertebrates. For example, von Baer (1828) argued that the embryos of different amniotes often appear strikingly similar, and that many differences among the adults of different species arise at later stages of development. The gradual appearance during development of differences among embryos is known as divergence or deviation (de Beer 1951). Haeckel took this idea further and suggested that essentially *all* differences among species arise at late stages, and that embryos are therefore virtually identical. This was famously depicted in his drawings (Fig. 2) which purport to show different species arising from a conserved embryonic stage (Haeckel 1874). The drawings were intended to demonstrate Haeckel's recapitulation theory or biogenetic law. A conserved stage was a necessary part of this theory because evolution was claimed to progress principally by the terminal addition of new adult stages to the end of ancestral developmental sequences.

Haeckel's ideas soon came in for strong criticism. His drawings are also highly inaccurate, exaggerating the similarities among embryos, while failing to show the differences (Sedgwick 1894; Richardson 1995; Raff
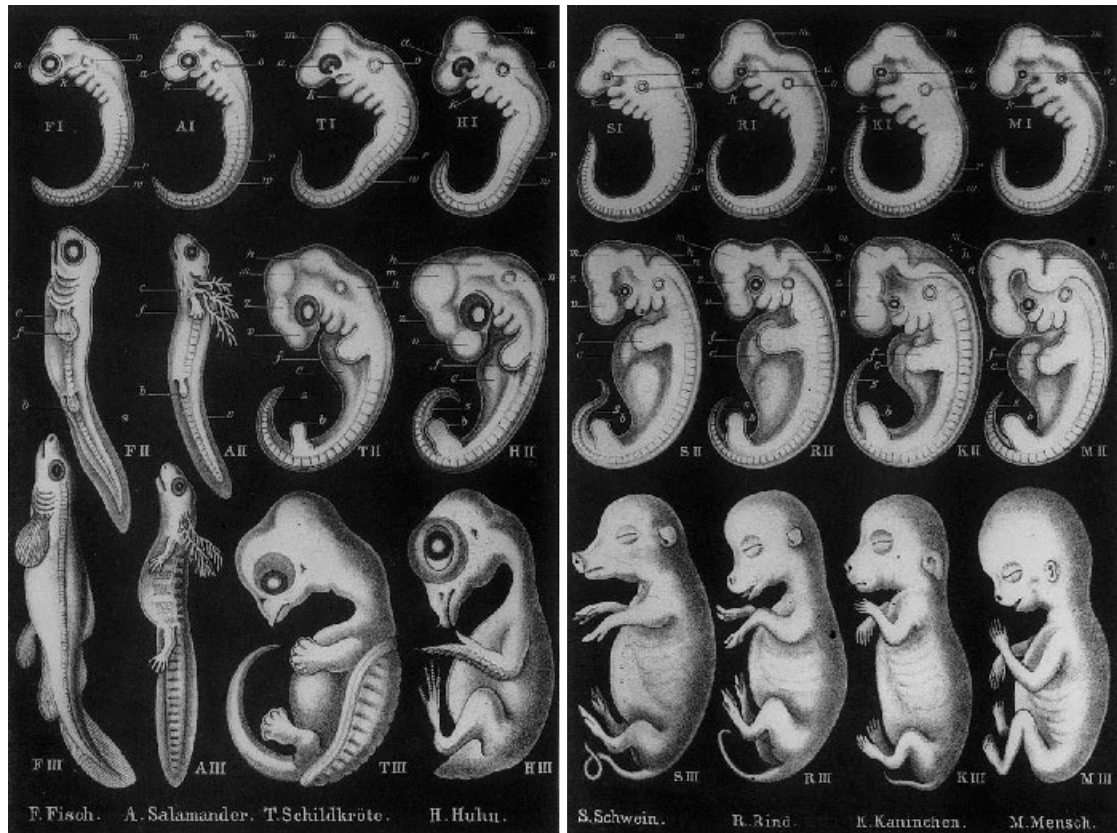
Fig. 2 Haeckel's (1874) view of a conserved stage. Successive stages in the development of each species are read from top to bottom. The top row represents Haeckel's view of a stage at which all species look virtually identical. Species from left to right: fish, salamander, turtle, chicken, pig, cow, rabbit, human. Courtesy of The Bodleian Library, University of Oxford, Plates 4 and 5 from 18917d.25

1996). Sedgwick (1894) argued that even closely related species of vertebrates can be told apart at all stages of development, but that the distinguishing characters are not necessarily the same as those used to distinguish among adults. Lillie (1919) argued that embryos show as much proportional variation as adult stages, but that adults look more divergent because of differential growth and an increase in size and complexity of organisation. The idea that embryonic stages are more resistant than other stages to evolutionary change was criticised by de Beer (1951). Summarising more than a century of comparative embryology studies he concluded:

… there are no grounds for the view that an organism as it develops passes through systematic categories of differing 'values', or of differing degrees of estrangement from organisms of other groups. (de Beer 1951).

The idea of a phylogenetically conserved stage has regained popularity in recent years. It has been claimed that all vertebrate embryos pass through a conserved stage when they are the same size (Collins 1995). Furthermore it has been argued that the existence of a phylotypic stage is consistent with the concept of a universal positional field, whereby the embryos of all species use the same mechanisms to encode positional value (Wolpert 1989). Recent findings of a conserved pattern of developmental gene expression (the zootype) in a wide range of vertebrates support this view (Slack et al. 1993). Furthermore, it has been suggested that the zootype is most clearly expressed at the tailbud stage, and that this in turn corresponds to the conserved stage for vertebrates (Slack et al. 1993). Nevertheless, the idea remains controversial. Richardson (1995) has recently shown that embryonic stages are subjected to shifts in developmental timing during evolution (heterochrony) so that different organs develop at different times in different species. This makes it impossible to define a single conserved stage when all species will have the same body plan.

Thus, more than a century after von Baer's (1828) pioneering work in comparative developmental biology, there remains considerable uncertainty over the true extent of morphological variation in vertebrate embryos at the putative phylotypic stage. The debate is hindered by the scarcity of comprehensive comparative studies of vertebrate embryos, and the great practical difficulties in obtaining embryos for study from a wide range of species. Keibel (1906) provided figures, redrawn from published studies, of embryonic development in a wide range of vertebrates. However, with a few notable exceptions (Bellairs 1971) modern textbooks rarely consider species other than the common laboratory animals. There has been no textbook of descriptive comparative embryology in English, covering all the major vertebrate

Table 1 Specimens examined in this study. Hill and Hubrecht collections are both housed in the Netherlands Institute for Developmental Biology (*coll.* collection, *St.* stage)

Species		Source	Stage
Petromyzontoidea			
Sea lamprey	*Petromyzon marinus*	Dr. G.M. Wright	St. 14 (Piavis 1971)
Elasmobranchii			
Electric ray	*Torpedo ocellata*	Hill coll. Fi50/4	C.f. St. 21 in Scammon 1911
Actinopterygii			
Sterlet	*Acipenser ruthenus*	Hubrecht coll. *A. ruthenus* 28	Tailbud
Flying fish	*Exocetus sp.*	Hubrecht coll. *Exocetus* sp. 2	Tailbud
Zebrafish	*Danio (Brachydanio) rerio*	Photo courtesy Dr. C.B. Kimmel, Univ. of Oregon	Tailbud
Salmon	*Salmo salar*	Hubrecht coll. *S. salar* 34	Tailbud
Dipnoi			
African lungfish	*Protopterus sp.*	Hill coll. Fi154a	St. 26 (Kerr 1909)
Queensland lungfish	*Neoceratodus forsteri*	Hubrecht coll. *Ceratodus* 4	St. 31–32 (Kemp 1982)
Caudata			
Mudpuppy	*Necturus maculosus*	Hubrecht coll. *N. maculosus* 76	St. 23 (Eycleshymer & Wilson 1910)
Hellbender	*Cryptobranchus alleghceniensis*	Hill coll. AM61-3	St. 18–19 (Smith 1912).
Mexican axolotl	*Ambystoma mexicanum*	Hill coll. AM70-4	St.29–30 (Schreckenberg and Jacobson 1975)
Sharp-ribbed salamander	*Pleurodeles waltl*	Hubrecht coll. *P. waltlii* 2.4	Tailbud
Alpine newt	*Triturus alpestris*		C.f. St. 21 (Anderson 1943)
Anura			
Common toad	*Bufo bufo*	Hubrecht coll. *B. bufo* 72–91	Tailbud
Common frog	*Rana temporaria*	Hubrecht coll. *R. fusca/temporaria* 70–73	St. 11 (Kopsch 1952)
Puerto Rican treefrog	*Eleutherodactylus coqui*	Dr. J. Hanken	St. 4–5 (Townsend & Stewart 1985)
Striped chorus frog	*Pseudacris triseriata*	Hubrecht coll.	Tailbud
Gymnophiona			
Blind worm	*Siphonops sp.*	Hill coll. AM104-3	C.f. St. 21 (Sammouri et al. 1990)
Testudinata			
European pond terrapin	*Emys orbicularis*	Dr. C. Pieau	C.f. St. 10 (Yntema 1968)
Lepidosauria			
Green lizard	*Lacerta viridis*	Dr. A. Raynaud	33 Somites (C.f. no. 77, Peter 1904)
Australian skink	*Sphenomorphus quoyi*	Hill coll. RE43 (7)	Tailbud
African skink	*Mabuya sp.*	Hill coll. RE34	Advanced tailbud
Crocodilia			
Alligator	*Alligator mississippiensis*	Photo courtesy Prof. Mark Ferguson, Univ. of Manchester.	Stage 3 (Ferguson 1985)
Aves			
Duck	*Anas platyrhynchos*	Hubrecht coll. *A. platyrhynchos* 1	Advanced tailbud
House sparrow	*Passer domesticus*	Hill coll. AV23	Advanced tailbud
Moorhen	*Gallinula chloropus*	Hubrecht coll. *G. chloropus* 1	Advanced tailbud
Chicken ("Ross White")	*Gallus gallus*	Dr. M.K. Richardson	Advanced tailbud
Japanese quail	*Coturnix coturnix*	Dr. M.K. Richardson	Advanced tailbud
Monotremata			
Echidna, spiny anteater	*Tachyglossus aculeatus*	Hill coll. MOF 142c	Early forelimb bud
Marsupialia			
Eastern Australian native "cat"	*Dasyurus quoll*	Hill coll. MA195d	Digital plate
Brush-tailed possum	*Trichosurus vulpecula*	Dr. L. Selwood	25 somites, C.F. St. 27–28 (McCRady 1938)
Eutheria			
Domestic cat	*Felis catus*	Hill coll. CA236c	Tailbud st. (Evans & Sack 1973).
Domestic dog	*Canis familiaris*	Hill coll. CA39	Tailbud st. (Evans & Sack 1973).
Domestic sheep	*Ovis aries*	Hubrecht coll. *O. aries* 27a	Tailbud st. (Evans & Sack 1973); C.f. 20d. (Bryden et al. 1972).
Scaly anteater or Malayan pangolin	*Manis javanica*	Hubrecht coll. *M. javanica* 136	St. 10 (Huisman & de Lange 1937)
Norway rat	*Rattus norvegicus*	Dr. N. Brown, Univ. London	St. 22 (Christie 1964)
Rabbit	*Oryctolagus cuniculus*	Hill coll. LA16	St. 10 (Minot & Taylor 1905); St. 5 (Edwards 1968)
Hedgehog	*Erinaceus europaeus*	Hubrecht coll. *E. europaeus* 150a	Embryo 13 (Jacobfeuerborn 1908)
Human	*Homo sapiens*	Photo courtesy Prof. R. O'Rahilly	St. 13 (O'Rahilly and Müller 1987)

Table 2 Taxonomic classification of specimens examined

Traditional groupings				Current grouping	Figure
Jawless vertebrates	Fishes	Anamniotes	Agnathans	Petromyzontoidea (lampreys)	3a
Jawed vertebrates			Cartilaginous fishes	Elasmobranchii (sharks, skates and rays)	3b
			Bony fishes	Actinoptreygii (rayed-fin fishes)	
				Acipenseriformes (sturgeons and paddlefish)	3c
				Teleostei (teleosts)	3d-f; 4a,b
				Dipnoi (lungfishes)	3g-i
	Tetrapods		Amphibians	Amphibia	
				Caudata (salamanders	5a-e)
				Anura (frogs and toads)	5f-i
				Gymnophiona (caecilians)	5j
		Amniotes	Reptiles	Testudinata (turtles)	6b
				Lepidosauria (lizards, snakes, tuatara)	6c-e
				Crocodilia (alligators and crocodiles)	6a
			Birds	Aves	
				Anseriformes (ducks)	7a
				Galliformes (game birds)	7d,e
				Gruiformes (rails)	7c
				Passeriformes (perching birds)	7b
			Mammals	Mammalia	
				Monotremata (monotremes)	8a
				Marsupialia (marsupials)	8b,c
				Eutheria ("placental" mammals[a])	8d-k

[a] Although the term "placental" mammals is commonly used for eutherians, it is unsatisfactory because marsupials also have a placenta

groups, for over 70 years (Jenkinson 1913; Kerr 1919). Huettner's (1941) book, purporting to be a comparative vertebrate embryology text, is typical of the textbooks available to the modern reader. It only covers Amphioxus, which is not a vertebrate; and the frog, chick and "the mammal". To compound problems, developmental biologists use just a small number of laboratory species as model systems, and are therefore unfamiliar with the diversity of embryonic form in vertebrates (Hanken 1993; Bolker 1995; Raff 1996).

Our aim in this paper is to examine the idea that embryos from all or most vertebrate clades pass through a highly conserved stage; and that at this stage their external form is virtually identical. Haeckel's drawings of embryos at tailbud stages are widely used in support of this hypothesis. We have therefore examined the external form of embryos from a wide range of vertebrate species, at a stage comparable to that depicted by Haeckel. A significant problem in this study has been to define a common reference stage that is applicable to all species. Heterochronic variation makes it impossible to define a conserved stage at which all vertebrate embryos have the same combination of organ primordia present (Richardson 1995). Chosing a fixed somite number would be meaningless because of the wide variation in the rate of somitogenesis across the vertebrates, and the total number of somites formed. Nevertheless the tailbud stage, proposed by Slack et al. (1993), is a useful concept because it corresponds approximately to the end of somite segregation in the trunk region.

However, even the tailbud stage is problematic because in marsupials and monotremes anterior structures are accelerated and are more advanced at the tailbud stage than they are in other species. In this review we have chosen to compare embryos at a stage when somite segregation in the trunk is advanced, but the tailbud has not yet become elongated and segmented. In all species this corresponds to a stage when many organ primordia are present, but have not differentiated (Slack et al. 1993). Because of heterochronic variation, it is not meaningful to try to be more precise than this with the staging.

Materials and methods

Species, sources and stages studied are listed in Table 1. The classification of these species is outlined in Table 2. Many embryos are from the Hill and Hubrecht collections assembled earlier in this century, or in the late 19th century, and now housed in The Netherlands Institute of Developmental Biology (formerly the Hubrecht Laboratory), Uppsalalaan 8, Utrecht, The Netherlands. The reader is referred to the catalogues held in Utrecht for details of the origin, age and fixation of these embryos. The other embryos were either collected by the authors, or are shown as photographs supplied by collaborators (for details see Table 1). Embryos were fixed in conventional histological fixatives and photographed in 70–80% alcohol through a dissecting microscope using reflected light. The brush-tailed possum and rat were fixed in formaldehyde and are semi-translucent. All other specimens were opaque except the zebrafish, which was photographed as a live specimen.

The extra-embryonic membranes were either missing or were removed by us. However the allantois was preserved where present. All measurements give greatest length (including, where appropriate, the tailbud), or crown-rump length in the case of curved embryos. Left-lateral views, in line with zoological convention, are used, except for the treefrog, which was easier to display in

oblique (dorsolateral) view. As indicated in the figure legends, negatives have sometimes been inverted so that the printed image conforms to the standard left view. This proved to be particularly necessary for the birds, where a left view would show only the ventral surface of the trunk because of torsion. Each plate shows embryos at the same magnification to facilitate size comparisons. For most plates this was ×10. Because many fish embryos analysed were extremely small, some have been enlarged (see figure legends for details). Species for which an illustration is provided are indicated in **bold type** at their first mention.

Results

Anamniotes

Jawless fishes

The jawless fishes (agnathans or "cyclostomes") include lampreys and hagfishes. It is regrettable that so few descriptive studies have been made of the embryology of these animals. Their development shows a number of interesting features. The **sea lamprey** (*Petromyzon marinus*) embryo has a serpentiform body resembling, in general form, that of the adult (Fig. 3a). Somites and pharyngeal pouches constitute two distinct and overlapping segmental series, with little indication of regional modification along the anteroposterior axis. In other words, each unit in the repeating series resembles its neighbours. This contrasts with embryos of many tetrapods, in which the pharyngeal arches are of different sizes and shapes. The heart in the lamprey embryo is tubular and, unusually among vertebrate embryos, lies immediately caudal to the pharynx instead of forming a ventral bulge. The head region is of similar diameter to the rest of the body and is not distinctly marked off from the trunk. The nostrils begin at this stage as a single midline invagination rather than as the two nasal pits seen in other vertebrates (Piavis 1971). The embryo begins hatching at the stage described, and shows active movements and a heartbeat. The body plan itself differs in cyclostomes from that in many other vertebrates because paired fin or limb buds never form.

Damas (1944) found that in the brook lamprey (*Lampetra fluviatilis*), the liver, lens, optic vesicle, heart tube, nasal placode, four pharyngeal pouches and 40 somites have formed by the tailbud stage. Embryos of myxinoids, which are phylogenetically distinct from lampreys (e.g. hagfish, *Bdellostoma stouti*; not shown), are extremely difficult to obtain and are rarely studied. As illustrated by Dean (1899), the hagfish embryo at the tailbud stage is highly elongated and has more than 70 somites.

Cartilaginous fish (class Chondrichthyes)

Cartilaginous fish include the most primitive living gnathostomes. They are represented here by the **electric ray**, *Torpedo ocellata* (Fig. 3b; see also Ziegler and Ziegler 1892), which at this stage strongly resembles other chondrichthyan embryos such as the dogfish,

Squalus acanthias. The characteristic dorsoventral flattening of the head in rays does not become apparent until later embryonic stages. The first gill has not begun to form the spiracle, a structure which characterises adult chondrichthyans. There are no paired fin buds at this stage, so the great enlargement of the pectoral fins seen in adults of this species must occur at later stages. What appears to be the tail is part of the trunk; the cloaca lies close to the tip of this structure (Scammon 1911). The true tailbud swelling has only just appeared, so this is an early tailbud embryo. This is an example of the caution that must be exercised in attempting to identify the tailbud stage in vertebrate embryos.

As in the lamprey, the series of pharyngeal arches and somites do not show distinct regionalisation with respect to the craniocaudal axis in the electric ray. The ray differs from the agnathan embryos at the tailbud stage in possessing a prominent rhombo-mesencephalic flexure, which may correlate with greater size and development of the brain; and in the position of the heart, which lies in a plane ventral to the pharynx. The pharyngeal clefts are elongated in the dorsoventral axis.

The detailed anatomy of a chondrichthyan at the tailbud stage is described and illustrated in Scammon's (1911) work on the dogfish. At stage 21 (comparable to Fig. 3b), 44 pairs of somites are visible externally, the dorsolateral, epibranchial and lens placodes are forming, nasal pits are present, and the otocyst is developing. Four pharyngeal arches are present and the first two clefts are becoming perforate. As in the ray, it is not until much later that the first slit becomes modified to form the spiracle. The liver diverticulum is present and the pronephric duct is becoming connected to the pronephric tubules, which are just beginning to develop. The heart loop and first two aortic arches have appeared. There is a median fin fold but paired fin buds have yet to form.

Bony fish (class Osteichthyes)

Ray-finned fishes (subclass Actinopterygii). The simple series of pharyngeal clefts seen in lampreys and chondrichthyans at the tailbud stage is not as well defined in the embryos of the bony fish (Figs. 3, 4). For example, *Lepi-*

Fig. 3a–l Fish embryos at the tailbud stage. **a–i** Are all at the same scale (×10) for comparison with each other, and with other figures. **j–l** Are details. **a–e, g, i** Are lateral views with the cranial end of the embryo at the top; **j** is a lateral view with the cranial end at the left of the figure; **f** is a dorsal view; **k, l** are dorsal views of the head, with the rostral end at the bottom. See Table 1 for further details of embryos shown (* negative inverted). **a** Sea lamprey (*Petromyzon marinus*) cleared with methyl salicylate. **b** Electric ray (*Torpedo ocellata*). **c** Sterlet* (*Acipenser ruthenus*). **d** Flying fish (*Exocetus* sp.) **e, f** Salmon (*Salmo salar*) in left lateral view (**e**) and dorsal view (**f**). **g, h** African lungfish (*Protopterus* sp.) in oblique left anterolateral view (**g**) and ventral view (**h**). **i** Queensland lungfish (*Neoceratodus forsteri*). **j** As **a**, but detail of side of head and rostral end of trunk (×47). **k** As **c**, but enlarged view of dorsum of head and rostral end of trunk (×20). **l** As **d**, but enlarged view of dorsum of head and rostral end of trunk (×50)
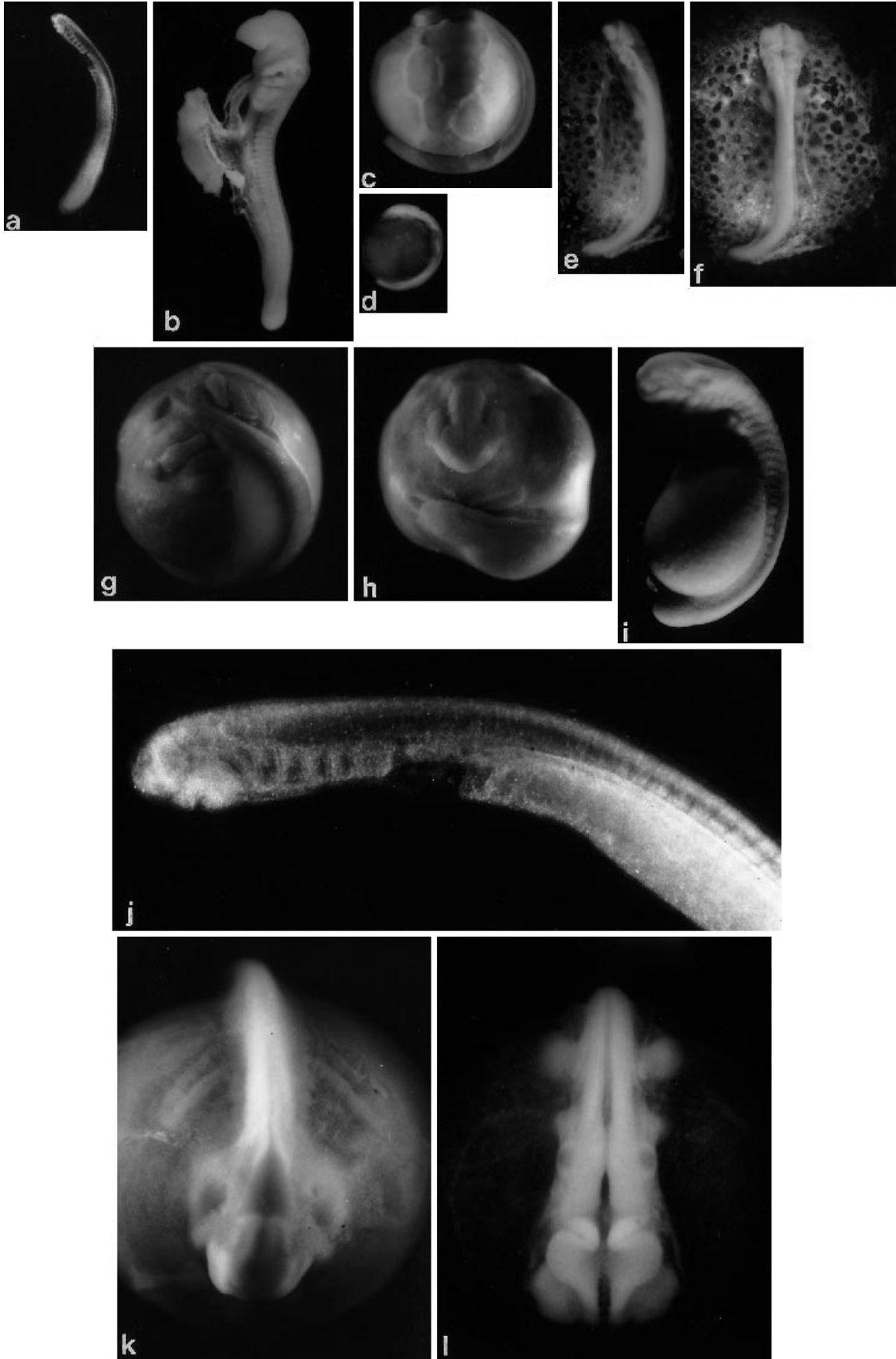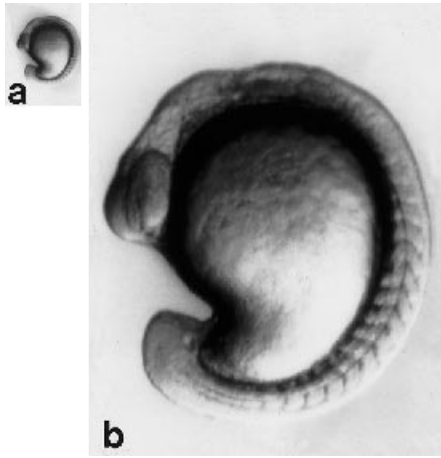
Fig. 4a,b Zebrafish (*Danio rerio*) embryo. Lateral view with the cranial end at the top. Courtesy of Dr. Charles Kimmel. Reproduced from Kimmel et al. (1995). **a** Fifteen-somite embryo. ×10. **b** As **a**, but enlarged. ×52. Copyright © Wiley-Liss, Inc., a subsidiary of John Wiley & Sons Inc.

dosteus at the tailbud stage possesses a branchial eminence with two transverse streaks that may represent the first two clefts (Balfour and Parker 1882). Teleosts (Figs. 3, 4) include the smallest embryos examined in this study. The zebrafish at 0.9 mm was the smallest embryo included in this review. The largest teleost embryo examined was the **salmon** (*Salmo salar*) at 4 mm. In addition to wide size variation, our analysis of the literature indicates that teleost embryos show a striking range of heterochronic shifts. Thus in the cunner, (*Ctenolabrus coeruleus,* Kingsley and Conn 1882), and *Fundulus* (Armstrong and Child 1965), fin buds first appear much later than the tailbud stage. However in *Symbranchus,* an unusual eel-like teleost, the pectoral fin buds are precocious and appear earlier than in the cunner and *Fundulus* (Taylor 1913). Optic vesicles start evaginating at the 6/7 somite stage in zebrafish (Schmitt and Dowling 1994) whereas in the platyfish (*Platypoecilus maculatus*) they are already well developed before any somites have segregated (Tavolga 1949).

Because many teleost embryos are so small we include an enlarged image of a tailbud stage **zebrafish** (*Danio rerio*) embryo in order to indicate the general form (Fig. 4). There are no pharyngeal arches because the pharyngula stage in the zebrafish does not occur until after the tailbud stage (Kimmel et al. 1995). This is highly unusual amongst vertebrate embryos, which typically have one or more arches differentiated by the tailbud stage. In the shad (*Alosa* sp.) heart anlagen are seen as condensations of lateral plate mesoderm at the 15-somite stage. The heart tubes start to fuse at the 34-somite stage and circulation begins at the 42-somite stage, when the dorsal aorta is completed (Senior 1909). The shad has a total somite count of 59 or 60, so heart development does not take place until relatively advanced stages of somite segregation in this species. By comparison, heart development is apparent at early somite stages in some amniotes (Mollier 1906; Mc Crady 1938).

There are surprisingly few detailed studies on the developmental anatomy of teleosts, and indeed fish are often completely ignored in textbooks of vertebrate embryology. Tavolga (1949) gives a good description of development in the platyfish (*Xiphophorus maculatus*). At the tailbud stage in this species (stage 10) there are 12/13 somites, the heart is no more than a diffuse mass of mesenchyme, and the pectoral fin buds are indicated as mesenchymal condensations. There is an open blastopore, three primary brain vesicles, optic cup, lens pit, hollow auditory vesicle without an endolymphatic duct (which never develops), and three pharyngeal pouches. It has been claimed that all organs are indicated as primordia by the tailbud stage (Slack et al. 1993) but this is not true in the case of the platyfish because the swim bladder anlage does not appear until later. Furthermore, although a pronephric duct is beginning to form, nephric tubules do not develop until after the tailbud stage and nor does a liver primordium. The anlagen of the lateral line system are present as thickened ectodermal grooves, so even at embryonic stages this species is showing a character that distinguishes it from amniote embryos. The telencephalon is small and scarcely distinguishable from the diencephalon.

Lobe-finned fishes (subclass Sarcopterygii). The **African lungfish** (*Protopterus* sp.) shows secondary neurulation (Kerr 1909). By the tailbud stage shown here (Fig. 3g, h) the neurocoele has formed in the central nervous system. Secondary cavitation is seen in many other organs as well. There are two pronephric tubules and a prominent cement gland. The heart primordium is only just beginning to develop and there are no limb, lung or liver buds yet. Thus the tailbud embryo lacks several organ primordia. A tail swelling develops at an early stage in this species, but growth in the true caudal or postcloacal region does not begin until later. This again shows the difficulty in defining precisely a tailbud stage. There are six solid pharyngeal arches, and the pharyngeal clefts are imperforate. This in contrast to the electric ray and amniotes, in which one or more clefts is perforate. The **Queensland lungfish** (*Neoceratodus forsteri*) shows primary neurulation (Kemp 1982). At the stage shown here (Fig. 3i) the embryo has 30–32 somites, a heart primordium and three pharyngeal arches; like the African lungfish, limb and lung buds do not appear until much later (Semon 1901; Kemp 1982).

Amphibians (class Amphibia)

There is great variation in embryonic morphology among the Amphibia at the tailbud stage (Fig. 5). Our series varies in size from the small embryo of the **striped chorus frog** (*Pseudacris triseriata*) at 1.5 mm, to the large embryo of the **mudpuppy** (*Necturus maculosus*)*,* which has a greatest length of 9.25 mm. This is the largest tailbud-stage vertebrate embryo examined in this study. Oral suckers are present in some species but not others. Dor-
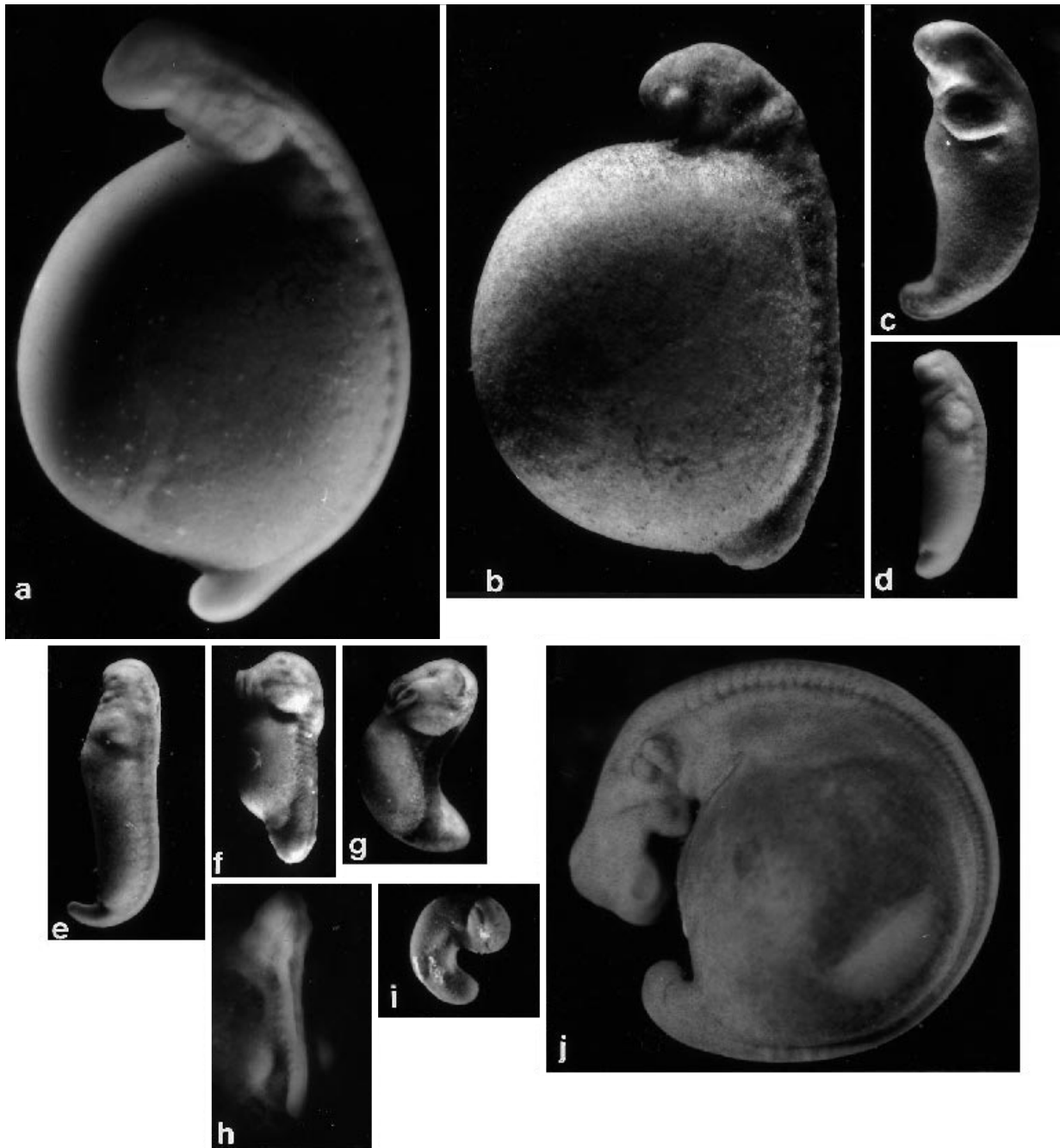
Fig. 5a–j Amphibian embryos at the tailbud stage, lateral views with the cranial end at the top (* negative inverted). See Table 1 for further details of embryos shown. **a** Mudpuppy* (*Necturus maculosus*). **b** Hellbender (*Cryptobranchus allegheniensis*). **c** Mexican axolotl* (*Ambystoma mexicanum*). **d** Sharp-ribbed sala-mander (*Pleurodeles waltlii*). **e** Alpine newt (*Triturus alpestris*). **f** Common toad (*Bufo bufo*). **g** Common frog (*Rana temporaria*). **h** Puerto Rican treefrog (*Eleutherodactylus coqui*), stained with Al-cian blue. **i** Striped chorus frog (*Pseudacris triseriata*). **j** Blind worm (*Siphonops* sp.). **a–j** ×10

sal curvature is kyphotic in the **common frog** (*Rana tem-poraria*) lordotic in the **blind worm** (*Siphonops* sp.) and straight in the **alpine newt** (*Triturus alpestris*). Pharyn-geal pouches are clearly indicated in most species but are not perforate. Somite numbers range from 11 in the **Puerto Rican treefrog** (*Eleutherodactylus coqui*) to over 60 in the blind worm.

The morphology of several urodeles is shown in Fig. 5a–e. The mudpuppy has a cranial flexure, and al-though the branchial eminence is becoming segmented there are no pharyngeal clefts yet. There is a hollow ear vesicle with a well-developed endolymphatic duct, and the heart tube has formed (Eycleshymer and Wilson 1910). The **hellbender** (*Cryptobranchus allegheniensis*), shown at an early tailbud stage, has 12 somites in exter-nal view, two pharyngeal pouches, and distinct cervical and cranial flexures. Limb buds only appear at later stag-es (Smith 1912). In **axolotls** (*Ambystoma* sp.) there are
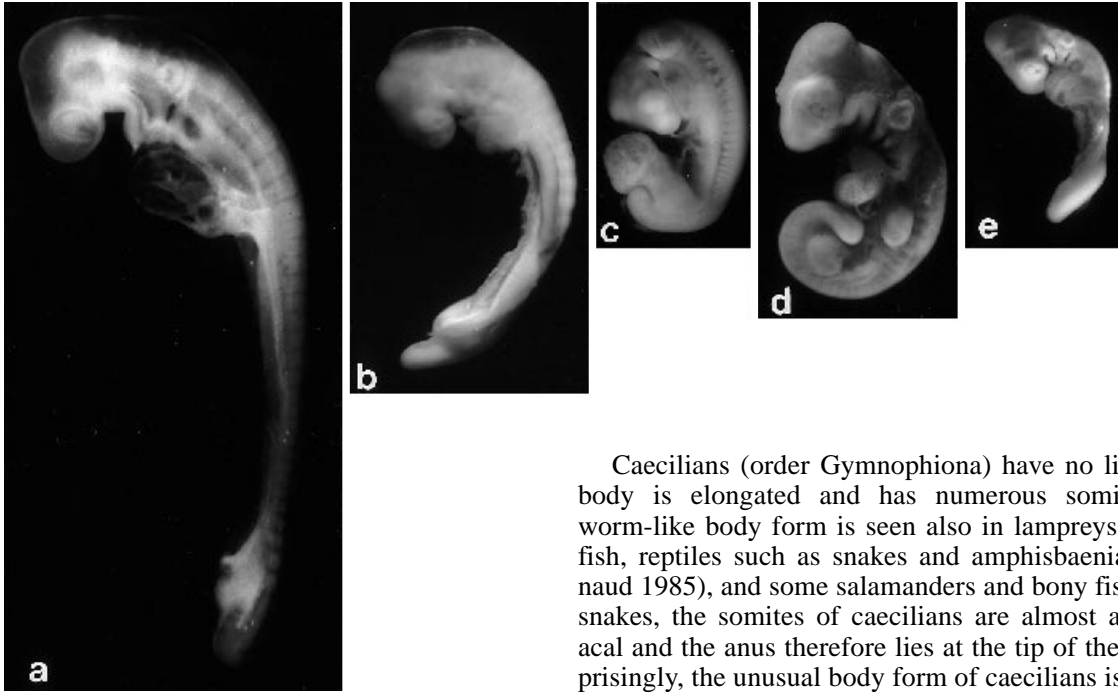
Fig. 6 Reptile embryos at the tailbud stage, lateral views with the cranial end at the top (* negative inverted). See Table 1 for further details of embryos shown. **a** Alligator (*Alligator mississippiensis*). **b** European pond terrapin (*Emys orbicularis*). **c** Green lizard* (*Lacerta viridis*). **d** African skink* (*Mabuya* sp.). **e** Australian skink (*Sphenomorphus quoyi*). **a–e** ×10

16–18 somites, fin folds, and three pharyngeal arches, but no heart tube or limb buds (Harrison 1969; Bordzilovskaya et al. 1989 for *Ambystoma punctatum*). The first three pharyngeal arches are formed from distinct cell masses, whereas the remaining arches become segregated from a common branchial eminence (Harrison 1969).

Anurans (Fig. 5f–i) that have a tadpole stage are called indirect-developing species; those that lack a tadpole stage are direct-developing species. The morphology of indirect-developing anurans is exemplified by *Xenopus laevis*. When the tailbud becomes prominent, this species has 19 somites, three pharyngeal arches, but no heart tube or limb buds (Nieuwkoop and Faber 1994). The common frog *Rana temporaria* has a similar morphology at this stage although the heart tube has formed (Kopsch 1952).

The direct-developing Puerto Rican treefrog is unusual among the species of anurans considered here in showing distinct hind limb buds at the tailbud stage. Other unusual features of development in this species are the fact that the embryo develops on top of the egg, rather like an amniote embryo, and only two pairs of complete aortic arches develop (New 1966). The branchial region shows two pharyngeal clefts (which are imperforate) and an unsegmented region posterior to the second arch (Moury and Hanken 1995); the first two arches are formed from separate neural crest streams, and the remaining arches from a common (caudal otic) stream (Moury and Hanken 1995).

Caecilians (order Gymnophiona) have no limbs. The body is elongated and has numerous somites. This worm-like body form is seen also in lampreys and hagfish, reptiles such as snakes and amphisbaenians (Raynaud 1985), and some salamanders and bony fish. Unlike snakes, the somites of caecilians are almost all precloacal and the anus therefore lies at the tip of the tail. Surprisingly, the unusual body form of caecilians is apparent at the tailbud stage and does not arise through divergence from a conserved stage (Fig. 5j). At the tailbud stage, the caecilian *Typhlonectes compressicaudus* has more than 60 somites, a heart primordium and six pharyngeal arches (Sammouri et al. 1990).

Amniotes[1]

While sharing many features with anamniotes, the embryos of amniotes (Figs. 6–8) also show characteristic differences. The heart develops early and forms a large bulge. Brain vesicles are prominent. The head region is large and distinctly marked off from the trunk. As we have pointed out, neural crest pigmentation develops much later in amniotes than in anamniotes (Richardson et al. 1989). Rotation (torsion) is a conspicuous feature of development in birds and some mammals and reptiles.

Reptiles (class Reptilia)

The appearance of some reptile embryos at the tailbud stage is shown in Fig. 6. The **alligator** embryo (*Alligator mississippiensis*) is remarkably large (8.9 mm, measured from plates in Ferguson 1985). This is larger than most other vertebrate embryos at the tailbud stage examined in this study. Like the **European pond terrapin** (*Emys orbicularis*) and **green lizard** (*Lacerta viridis*) it shows no evidence of torsion. This is in contrast to the **skinks** (*Mabuya* sp. and *Sphenomorphus quoyi*) in which there are signs of torsion. Peter (1904) made a detailed study of development of the lizard *Lacerta agilis*. At the tailbud

---

[1] We include the paraphyletic group "reptiles" in this category, together with mammals and birds, because all living reptiles have an amnion
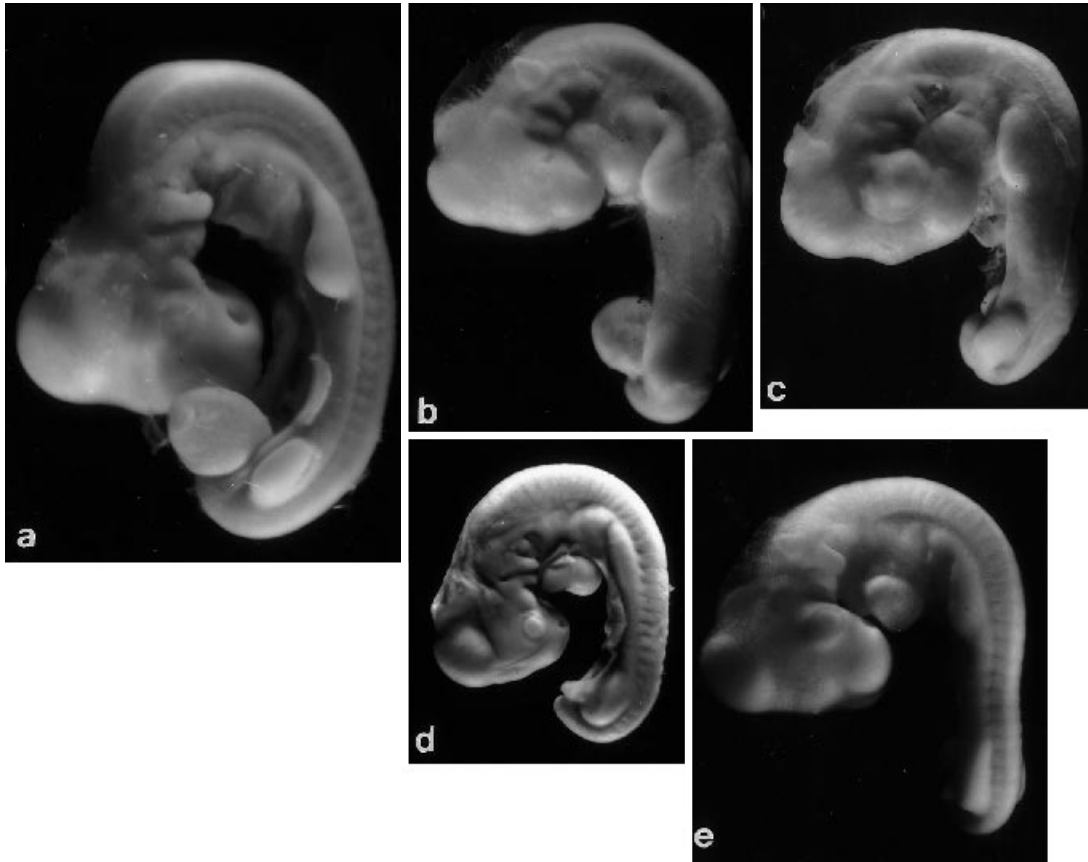
Fig. 7a–e Bird embryos at the tailbud/early limb bud stages, lateral views with the cranial end at the top (all negatives inverted). Because of limitations of the material available, embryos are all slightly more advanced tailbud stages than most other embryos in this survey. See Table 1 for further details of embryos shown. **a** Duck (*Anas platyrhynchos*). **b** Sparrow (*Passer domesticus*). **c** Moorhen (*Gallinula chloropus*). **d** Chicken (*Gallus gallus*). **e** Japanese quail (*Coturnix coturnix*). **a–e** ×10

stage this species has over 30 somites, nasal pits, liver anlage, three pharyngeal arches and two perforate clefts; but no lung bud and only faint indications of forelimb buds.

Birds (class Aves)

Birds are said to show the least embryonic variation of all groups of vertebrates (Kerr 1919), and it has been argued that specific differences among birds arise largely through the modification of later stages (Starck 1993). However, we need to study development in a greater range of birds before we can be sure that this is a general rule. Like many mammals and some reptiles, the bird embryos illustrated (Fig. 7) show rotation around the craniocaudal axis (torsion) and have a prominent cervical flexure. Another conspicuous feature of avian embryos at this stage is the prominent mesencephalon.

Mammals (class Mammalia)

The embryos of monotremes and marsupials illustrated here (Fig. 8a–c) are easily distinguished from the euthe-

rian mammals shown (Fig. 8d–k) because of the precocious development of the forelimb buds. External morphology of the **echidna** (*Tachyglossus aculeatus,* a monotreme) is strikingly similar to that of the **brush-tailed possum** (*Trichosurus vulpecula,* a marsupial). The branchial region looks similar in all of the mammalian embryos shown in Fig. 8. There are large maxillary and mandibular processes, with the size of succeeding pharyngeal arches decreasing progressively so that the posterior arches are barely distinguishable. This is in strong contrast to the cyclostomes and chondrichthyans, which have a series of rather similar arches. In mammals the arches caudal to the mandibular lie recessed in the cervical sinus (Tamarin and Boyd 1977).

Among the eutherian mammals examined, the principle variations in external form appear to be differences in flexion and rotation. For example, the **rabbit**, *Oryctolagus cuniculus* has a characteristic double flexure in the trunk, whereas the **rat**, *Rattus norvegicus* has a spiralled body. The prosencephalon is particularly prominent in the rat (compared, for example, with the prosencephalon in the human embryo; Fig. 8). Huisman and de Lange (1937) found that the **scaly anteater** (*Manis javanica*) at the tailbud stage has approximately 28 somites, an allantois and an amnion, early indications of lens and nasal placodes, a pair of early limb buds, a liver diverticulum, a looped heart, and a mesonephric duct that opens into the cloaca. This embryo shows marked differences from the embryos of anamniotes. It has a very small notochord (Huisman and de Lange 1937). In anamniotes such as the

Fig. 8a–k Mammal embryos, lateral views with the cranial end at the top (* negative inverted). All tailbud stage except **a**, in which the tailbud has not yet formed. See Table 1 for further details of embryos shown. **a** Echidna or spiny anteater (*Tachyglossus aculeatus*). Because of the extreme rarity of monotreme material we were only able to obtain this embryo, which is younger than the tailbud stage. **b** Brush-tailed possum (*Trichosurus vulpecula*). **c** Eastern Australian native "cat" (*Dasyurus quoll*). **d** Domestic cat* (*Felis catus*). **e** Domestic dog (*Canis familiaris*). **f** Domestic sheep (*Ovis aries*). **g** Scaly anteater (*Manis javanica*). **h** Rat (*Rattus norvegicus*). **i** Rabbit (*Oryctolagus cuniculus*). **j** Hedgehog (*Erinaceus europaeus*). **k** Human (*Homo sapiens*). **a–k** ×10

lamprey, the notochord is large in the embryo and remains prominent in the adult. The pronephros, which may be retained in anamniotes into larval stages or even adulthood, has already been reduced to mere traces in the scaly anteater at the tailbud stage (Huisman and de Lange 1937).

Discussion

We have reviewed the morphology of vertebrate embryos at the tailbud stage, which is generally considered to be resistant to evolutionary change, if not invariant. A wide range of clades has been considered, and the possible
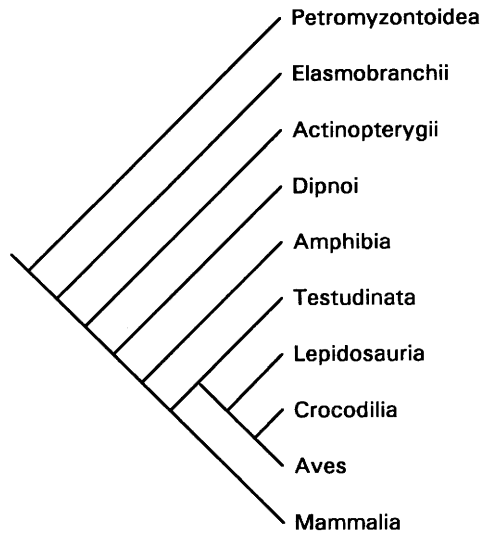
Fig. 9 Hypothesis of phylogenetic relationships among the groups of vertebrates considered in this study. Based on Pough et al. (1996)

phylogenetic relations among these clades are indicated in Fig. 9. Vertebrates show many common features at this stage. These include the presence of somites, neural tube, optic anlagen, notochord and pharyngeal pouches. However, these and other prominent features of the vertebrate body plan show considerable evidence of evolutionary modification when the embryos of different species are compared. Some examples of variation in embryonic anatomy at the putative phylotypic stage are given in Table 3.

A striking example of this variation is somite number, which differs widely in the embryos examined. According to the hourglass model, one might predict that somite number would be similar in different species at the tailbud stage, and that differences in somite number would only become apparent at later stages. However this is not the case. In the embryos examined here, somite count at tailbud stages varies from 11 in the Puerto Rican treefrog, to over 60 in the blind worm. This indicates that somite number can vary independently of conserved positional field encoded by genes of the zootype.

Differences in somite number may be related to the rate of growth of the body. Data from comparative studies support this idea. Raynaud (1994) compared somitogenesis in the lizard and the slow worm. The adult slow worm has a more elongated body and more vertebrae than the lizard. Raynaud found that the slow-worm embryo elongated more rapidly, and laid down somites more rapidly than the lizard. Furthermore these emerging differences in body form were apparent at early stages of somite segregation, and could not therefore be attributed to divergence from a conserved intermediate stage. Differential growth (allometry) is also apparent in embryos. For example, the mesencephalic vesicle is the most prominent brain vesicle in the chicken embryo, whereas the prosencephalic vesicle is the most prominent in the rat (Figs. 7d, 8h).

Size is another parameter which varies tremendously between tailbud embryos – from 700 μm in the scorpion fish to 9.25 mm in the mudpuppy. Our data do not therefore support the recent proposal that all vertebrates pass through a conserved stage when they are the same size: 7–8 mm (Collins 1995). The belief that size is necessarily conserved at the proposed phylotypic stage is a puzzling one: variations in size are not incompatible with the idea of a conserved positional field established by homeobox and other genes. Indeed size regulation is one of the predicted properties of patterns generated by positional information mechanisms (Wolpert 1989).

Heterochrony is another phenomenon that is associated with evolutionary changes in embryonic stages (Richardson 1995). The heart has not yet formed in the zebra-

Table 3 Variations in developmental anatomy in a small selection of vertebrate embryos at the tailbud stage. Even this narrow range of species and morphological criteria demonstrates the striking variability in body form between species at the tailbud stage. (✓ structure is present; ✗ structure has not yet developed; ✓/✗ structure faintly indicated)

| Species	Somites	Pharyngeal arches	Limb or paired fin buds	Lens bud	Liver tube	Heart	Reference
Brook lamprey (*Lampetra fluviatilis*)	40	4	never present	✓	✓	✓	Damas 1944
Dogfish (*Squalus acanthias*)	44	4	✗	✓	✓	✓	Scammon 1911
Platyfish (*Xiphophorus maculatus*)	12–13	3	✓	✓	✗	✗	Tavolga 1949
Queensland lungfish (*Neoceratodus forsteri*)	30–32	2	✗	✗	✗	✓	Semon 1901
African clawed toad (*Xenopus laevis*)	19	1	✗	✓/✗	✓	✗	Nieuwkoop and Faber 1994
Caecilian (*Typhlonectes compressicaudus*)	65	6	never present	✓	✗	✓	Sammouri et al. 1990
Lizard (*Lacerta agilis*)	32	3	✓/✗	✓	✓	✓	Peter 1904
Chicken (*Gallus gallus*)	23	2	✗	✓	✓	✓	Keibel and Abraham 1900
Scaly anteater (*Manis javanica*)	28	3–4	✓/✗	✓/✗	✓	✓	Huisman and de Lange 1937
Mouse (*Mus musculus*)	30–34	3–4	✓	✓	✓	✓	Theiler 1989
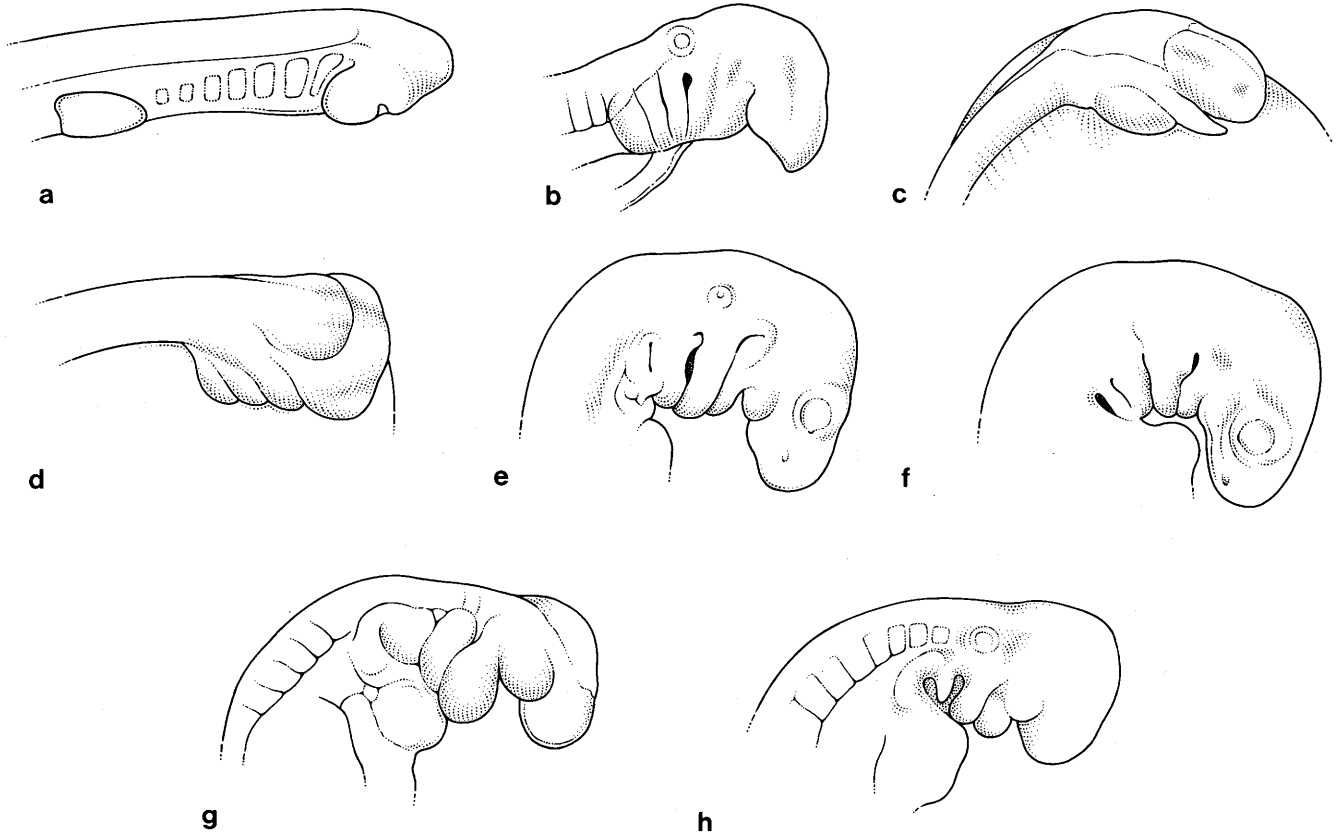
Fig. 10a–h Right lateral views of the head and cervical region of selected embryos (except Fig. 10d, which is a dorsolateral view), mostly drawn after the embryos illustrated in Figs. 3–8; those marked with an *asterisk* were drawn after other specimens from the same source. **a** Sea lamprey (*Petromyzon marinus*). **b** Electric ray (*Torpedo ocellata*). **c** Sterlet (*Acipenser ruthenus*). **d** Puerto Rican treefrog (*Eleutherodactylus coqui*). **e** European pond terrapin* (*Emys orbicularis*), 40 somite embryo. **f** Chicken (*Gallus gallus*). **g** Brush-tailed possum* (*Trichosurus vulpecula*), 25 somite embryo. **h** Domestic cat (*Felis catus*)

fish at the tailbud stage, whereas in the amniote embryos discussed here, the heart has completed looping. Forelimbs in marsupials are well-advanced compared to the other species. These and other observations, such as the lack of a swim-bladder anlage, liver bud, and nephric tubules in some osteichthyans at the tailbud stage, are hard to reconcile with the claim that most organs are present as primordia at this stage (Slack et al. 1993).

No limb or paired fin buds ever form in the lamprey, so this element of the body plan differs among vertebrate embryos. Furthermore there appears to be an inverse relationship between limb development and somite number, so that in species with large numbers of body segments, the limb buds are small or absent (Raynaud 1985). Most embryos at the tailbud stage are also "pharyngulas", defined by Ballard (1981) as embryos having a series of paired pharyngeal pouches, but in the zebrafish the branchial apparatus does not appear until after the tailbud stage (Kimmel et al. 1995). Thus it is difficult to accept that the tailbud stage and pharyngula both represent a conserved stage for vertebrates.

Another point to emerge from this study is the considerable inaccuracy of Haeckel's famous figures. These drawings are still widely reproduced in textbooks and review articles, and continue to exert a significant influence on the development of ideas in this field (Wolpert 1991; Alberts et al. 1994; Duboule 1994). Sedgwick (1894) and Richardson (1995) have argued that Haeckel's drawings are inaccurate, and we have now provided persuasive evidence that this is indeed the case. This is particularly apparent from the atypical body form of agnathan, anuran and caecilian embryos shown here (Figs. 3, 5). Haeckel inexplicably omitted these species from his figures. It is hard to believe that he could not have obtained embryos of a common anuran such as *Rana temporaria*. Furthermore he fails to give scientific names, stages or source of the specimens illustrated. These inaccuracies and omissions seriously undermine his credibility. We suggest that Haeckel's conserved embryonic stage is in fact a stylised amniote embryo. An interesting perspective was provided by Goldschmidt (1956):

The present generation cannot imagine the role he played in his time, far beyond his actual scientific performance ... Haeckel's easy hand at drawing made him improve on nature and put more into the illustrations than he saw ... one had the impression that he first made a sketch from nature and then drew an ideal picture as he saw it in his mind.

Haeckel depicted the branchial apparatus as being virtually identical in all species at his stage 1 (top row of Fig. 2). Our observations do not support this implication. We show details of the branchial apparatus, redrawn from photographs of original specimens, in Fig. 10. As

can be seen, the branchial region varies considerably between clades.

Contrary to the evolutionary hourglass model, variations in the adult body plan are often foreshadowed by modifications of early development. A good example is the aortic arch system in the rat that, even during the pharyngula stage, begins to presage the adult pattern of arteries. Thus the first arch has already broken down completely by the 25-somite stage in the rat (de Ruiter et al. 1989).

In summary, evolution has produced a number of changes in the embryonic stages of vertebrates including:

1. Differences in body size
2. Differences in body plan (for example, the presence or absence of paired limb buds)
3. Changes in the number of units in repeating series such as the somites and pharyngeal arches
4. Changes in the pattern of growth of different fields (allometry)
5. Changes in the timing of development of different fields (heterochrony)

These modifications of embryonic development are difficult to reconcile with the idea that most or all vertebrate clades pass through an embryonic stage that is highly resistant to evolutionary change. This idea is implicit in Haeckel's drawings, which have been used to substantiate two quite distinct claims. First, that differences between species typically become more apparent at late stages. Second, that vertebrate embryos are virtually identical at earlier stages. This first claim is clearly true. Our survey, however, does not support the second claim, and instead reveals considerable variability – and evolutionary lability – of the tailbud stage, the purported phylotypic stage of vertebrates. We suggest that not all developmental mechanisms are highly constrained by conserved developmental mechanisms such as the zootype. Embryonic stages may be key targets for macroevolutionary change.

References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) Molecular biology of the cell, 3rd edn. Garland, New York, p 33

Anderson PL (1943) The normal development of *Triturus pyrrhogaster*. Anat Rec 86: 59–73

Armstrong PB, Child JS (1965) Stages in the normal development of *Fundulus heteroclitus*. Biol Bull 128: 143–168

Baer KE von (1828) Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion. Borntrager, Kornigsberg

Balfour FM, Parker WN (1882) On the structure and development of *Lepidosteus*. Phil Trans Roy Soc 173: 359–492

Ballard WB (1981) Morphogenetic movements and fate maps of vertebrates. Am Zool 21: 391–399

Beer GR de (1951) Embryos and ancestors. Clarendon Press, Oxford

Bellairs R (1971) Developmental processes in higher vertebrates. Logos Press, London

Bolker JA (1995) The choice and consequences of model systems in developmental biology. Bioessays 17: 451–455

Bordzilovskaya NP, Dettlaff TA, Duhon ST, Malacinski GM (1989) Developmental-stage series of axoltl embryos. In: Armstrong JB, Malacinski GM (eds) Developmental biology of the axoltol. Oxford University Press, Oxford, pp 201–219

Bryden MM, Evans HE, Binns W (1972) Embryology of the sheep. J Morphol 138: 169–186

Burke AC, Nelson CE, Morgan BA, Tabin C (1995) Hox genes and the evolution of vertebrate axial morphology. Development 121: 333–346

Butler H, Juurlink, BHJ (1987) An atlas for staging mammalian and chick embryos. CRC Press, Boca Raton, Florida

Christie GA (1964) Developmental stages in somite and post-somite rat embryos, based on external appearance, and including some features of the macroscopic development of the oral cavity. J Morphol 114: 263–286

Collins P (1995) Embryology and development. In: Collins P (ed) Gray's anatomy, 38th edn. Churchill Livingstone, London, pp 91–341

Damas H (1944) Récherches sur le développement de *Lampetra fluviatilis* L. Contribution à l'étude de la céphalogenèse des vertébres. Arch Biol 55: 1–284

Dean B (1899) On the embryology of *Bdellostoma stouti*. In: Festschrift zum siebenzigsten Geburtstag von Carl von Kupffer. Fischer, Jena, pp 221–276

Duboule D (1994) Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate bauplan and the evolution of morphologies through heterochrony. Development [Suppl]: 135–142

Edwards JA (1968) The external development of the rabbit and rat embryo. Adv Teratol 3: 239–263

Elinson RP (1987) Changes in developmental patterns: embryos of amphibians with large eggs. In: Raff RA, Raff EC (eds) Development as an evolutionary process. Liss, New York, pp 1–21

Evans HE, Sack WO (1973) Prenatal development of domestic and laboratory mammals: growth curves, external features, and selected references. Anat Histol Embryol 2: 11–45

Eycleshymer AC, Wilson JA (1910) Normal plates of the development of *Necturus maculosus*. In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 11. Fischer, Gena, pp 1–31

Ferguson MWJ (1985) Reproductive biology and embryology of crocodilians. In: Gans C, Billett F, Maderson PFA (eds) Biology of the Reptilia, development. Wiley, New York, pp 329–491

Goldschmidt RB (1956) The golden age of zoology; portraits from memory. University of Washington Press, Seattle London, pp 31–33

Haeckel E (1874) Anthropogenie oder Entwickelungsgeschichte des Menschen. Engelmann, Leipzig

Hall BK (1992) Evolutionary developmental biology. Chapman and Hall, London

Hanken J (1993) Model systems versus outgroups: alternative approaches to the study of head development and evolution. Am Zool 33: 448–456

Harrison RG (1969) Harrison stages and description of the normal development of the spotted salamander, *Amblystoma punctatum* (Linn). In: Wilens S (ed) Organization and development of the embryo. Yale University Press, New Haven, pp 44–66

Hubert J (1985) Embryology of the squamata. In: Gans C, Billet F (eds) Biology of the Reptilia, development, B. Wiley, New York, pp 1–34

Huettner AF (1941) Fundamentals of comparative embryology of the vertebrates. Macmillan, New York

Huisman FJ, Lange D de (1937) Tabellarische Übersicht der Entwicklung von *Manis javanica*. Ontogenese der Wirbeltiere in Übersichten, vol 2. Oosterhook, Utrecht

Jacobfeuerborn H (1908) Die intrauterine Ausbildung der äußeren Körperform des Igels (*Erinaceus europaeus* L) mit Berücksichtung der Entwicklung der wichtigeren inneren Organe. Z Wiss Zool 91: 382–420

Jenkinson JW (1913) Vertebrate embryology. Clarendon Press, Oxford

Keibel F (1906) Der Entwickelung der äusseren Körperform der Wirbeltier Embryonen, insbesondere der menschlichen Embryonen aus den ersten 2 Monaten. In: Hertwig O (ed) Handbuch der vergleichenden und experimentellen Entwickelungslehre der Wirbeltiere, vol 1. Fischer, Gena, pp 1–176

Keibel F, Abraham K (1900) Normentafel zur Entwicklungsgeschichte des Huhnes (*Gallus domesticus*). In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 2. Fischer, Gena, pp 1–67

Kemp A (1982) The embryological development of the Queensland Lungfish, *Neoceratodus forsteri* (Krefft). Mem Queensl Mus 20: 553–597

Kerr JG (1909) Normal plates of the development of *Lepidosiren paradoxa* and *Protopterus annectens.* In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 10. Fischer, Gena, pp 1–26

Kerr JG (1919) Textbook of embryology, vol 2. MacMillan, London

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the zebrafish. Dev Dyn 203: 253–310

Kingsley JS, Conn HW (1882) Some observations on the embryology of the teleosts. Mem Boston Soc Nat Hist 3: 183–212

Kopsch FR (1952) Die Entwicklung des braunen Grasfrosches *Rana fusca* Roesel. Thieme, Stuttgart

Lillie FR (1919) The development of the chick. Holt, New York, p 5

McCrady E (1938) Embryology of the opossum. Am Anat Mem 16: 1–233

McKinney ML, McNamara KJ (1991) Heterochrony, the evolution of ontogeny. Plenum Press, New York

McNamara KJ (1995) Evolutionary change and heterochrony. Wiley, New York

Minot CS, Taylor E (1905) Normal plates of the development of the rabbit (*Lepus cuniculus* L). In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 5. Fischer, Gena, pp 1–27

Mollier S (1906) Die erste Anlage des Herzens bei den Wirbeltieren. In: Hertwig O (ed) Handbuch der vergleichenden und experimentellen Entwickelungslehre der Wirbeltiere, vol 1. Fischer, Gena, pp 1019–1297

Moury JD, Hanken J (1995) Early cranial neural crest migration in the direct-developing frog, *Eleutherodactylus coqui*. Acta Anat 153: 243–253

New DAT (1966) The culture of vertebrate embryos. Logos Press, London

Nieuwkoop PD, Faber J (1994) Normal table of *Xenopus laevis* (Daudin). Garland, New York

O'Rahilly R, Müller F (1987) Developmental stages in human embryos. Carnegie Inst Washington Publ no. 637

Peter K (1904) Normentafel zur Entwicklungsgeschichte der Zauneidechse (*Lacerta agilis*). In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 4. Fischer, Gena, pp 1–119

Piavis GW (1971) Embryology. In: Hardisty MW, Potter IC (eds) The biology of the lampreys, vol 1. Academic Press, London, pp 361–400

Pough FH, Heiser JB, McFarland WN (1996) Vertebrate life, 4th edn. Prentice-Hall, New Jersey

Raff R (1992) Evolution of developmental decisions and morphogenesis: the view from two camps. Development [Suppl] pp 15–22

Raff R (1996) The shape of life. University of Chicago Press, Chicago

Raynaud A (1985) Development of limbs and embryonic limb reduction. In: Gans C, Billett F (eds) Biology of the Reptilia, development. Wiley, New York, pp 61–148

Raynaud A (1994) Données préliminaires sur l'allongement du corps et la somitogenèse chez les jeunes embryons d'orvet (*Anguis fragilis*, L) et de lézard vert (*Lacerta viridis*, Laur). Bull Soc Hist Nat Toulouse 130: 47–52

Richardson MK (1995) Heterochrony and the phylotypic period. Dev Biol 172: 412–421

Richardson MK, Hornbruch A, Wolpert L (1989) Pigment pattern expression in the plumage of the quail embryo and the quail-chick chimaera. Development 107: 805–818

Richardson MK, Hornbruch A, Wolpert, L (1990) Mechanisms of pigment pattern formation in the quail embryo. Development 109: 81–89

Ruiter MC de, Gittenberger-de Groot AC, Rammos S, Poelmann RE (1989) The special status of the pulmonary arch artery in the branchial arch system of the rat. Anat Embryol 179: 319–325

Sammouri R, Renous S, Exbrayat JM, Lescure J (1990) Développement embryonnaire de *Typhlonectes compressicaudus* (Amphibia, Gymnophiona). Ann Sci Nat Zool Biol Anim 11: 135–163

Scammon RE (1911) Normal plates of the development of *Squalus acanthias*. In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 12. Fischer, Gena, pp 1–78

Schmitt EA, Dowling JE (1994) Early eye morphogenesis in the zebrafish, *Brachydanio rerio*. J Comp Neurol 344: 532–542

Schreckenberg GM, Jacobson AG (1975) Normal stages of development of the Axolotl, *Ambystoma mexicanum*. Dev Biol 42: 391–400

Sedgwick A (1894) On the law of development commonly known as von Baer's law; and on the significance of ancestral rudiments in embryonic development. Q J Microsc Sci 36: 35–52

Semon R (1901) Normentafel zur Entwicklungsgeschichte des *Ceratodus forsteri*. In: Keibel F (ed) Normentafeln zur Entwicklung der Wirbeltiere, vol 3. Fischer, Gena, pp 1–25

Senior HD (1909) The development of the heart in the shad. Am J Anat 9: 212–262

Slack JMW, Holland PWH, Graham CF (1993) The zootype and the phylotypic stage. Nature 361: 490–492

Smith BG (1912) The embryology of *Cryptobranchus allegheniensis*, including comparisons with some other vertebrates. J Morphol 23: 455–579

Starck JM (1993) Evolution of avian ontogenies. In: Power DM (ed) Current ornithology, vol 10. Plenum Press, New York

Tamarin A, Boyde A (1977) Facial and visceral arch development in the mouse embryo: a study by scanning electron microscopy. J Anat 124: 563–580

Tavolga WN (1949) Embryonic development of the Platyfish (*Platypoecilus*), the Swordtail (*Xiphophorus*) and their hybrids. Bull Am Mus Nat Hist 94: 167–229

Taylor M (1913) The development of *Symbranchus marmoratus*. Q J Microsc Sci 59: 1–51

Theiler K (1989) The house mouse. Springer, Berlin Heidelberg New York

Townsend DS, Stewart MM (1985) Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): a staging table. Copeia 1985: 423–436

Wolpert L (1989) Positional information revisited. Development [Suppl]: 3–12

Wolpert L (1991) The triumph of the embryo. Oxford University Press, Oxford

Yntema CL (1968) A series of stages in the embryonic development of *Chelydra serpentina*. J Morphol 125: 219–251

Ziegler HE, Ziegler F (1892) Beiträge zur Entwicklungsgeschichte von *Torpedo*. Arch Mikrosk Anat Entwicklungsmech 39: 56–102