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Sequence Heterochrony and the Evolution of Development

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ABSTRACT One of the most persistent questions in comparative developmental biology concerns whether there are general rules by which ontogeny and phylogeny are related. Answering this question requires conceptual and analytic approaches that allow biologists to examine a wide range of developmental events in well-structured phylogenetic contexts. For evolutionary biologists, one of the most dominant approaches to comparative developmental biology has centered around the concept of heterochrony. However, in recent years the focus of studies of heterochrony largely has been limited to one aspect, changes in size and shape. I argue that this focus has restricted the kinds of questions that have been asked about the patterns of developmental change in phylogeny, which has narrowed our ability to address some of the most fundamental questions about development and evolution. Here I contrast the approaches of growth heterochrony with a broader view of heterochrony that concentrates on changes in developmental sequence. I discuss a general approach to sequence heterochrony and summarize newly emerging methods to analyze a variety of kinds of developmental change in explicit phylogenetic contexts. Finally, I summarize a series of studies on the evolution of development in mammals that use these new approaches. J. Morphol. 252:82–97, 2002.

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Of the many questions that have been asked about the relation between development and evolution, one of the most persistent involves whether there are general rules by which development and evolution, or ontogeny and phylogeny, are related. The broadest of these questions asks about the ways that developmental mechanisms bias or constrain evolution by limiting the kinds of phenotypic variation available to selection. More specific versions of this question include, for example, whether common developmental mechanisms lie behind cases of convergent or parallel evolution; the degree to which particular portions of a developmental trajectory, e.g., early development or a "phylotypic stage" are conserved relative to other parts of development; and the extent to which developmental processes are either modularized or integrated. At a more fundamental level, questions about the relation between evolution and development involve how genetic and morphogenetic changes produce taxon-specific morphological or functional differences between species. Answering questions such as these requires conceptual and analytic approaches that allow biologists to examine a wide range of developmental events in well-structured phylogenetic contexts.

For evolutionary biologists one of the dominant approaches to comparative developmental biology has centered around the concept of heterochrony. Heterochrony involves a shift in the timing of developmental processes so an event occurs earlier, later, or at a different rate in a taxon compared to its ancestor. In practice, almost all studies of heterochrony involve changes in timing among related taxa, as information on the timing of developmental events in ancestors and descendants is virtually never available. This concept became particularly prominent in the literature of evolutionary biology in the late 1970s with the publication of influential works by Gould (1977) and by Alberch et al. (1979). These works focused on one aspect of heterochrony, changes in size and shape, which I refer to as growth heterochrony. Although the work of Gould and others have introduced evolutionary biologists to many important issues of development, the emphasis on relative growth has had the effect of limiting the nature of the investigations of heterochrony and its role in evolution. This restriction has narrowed our ability to address some of the fundamental questions about development and evolution raised above.

In this article, I contrast the approaches of growth heterochrony with a broader, more traditional view of heterochrony that concentrates on developmental sequences (Smith, 2001b). I discuss a general ap-

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proach to sequence heterochrony and summarize newly emerging methods to analyze a variety of kinds of developmental change in explicit phylogenetic and comparative contexts.

HETEROCHRONY REVISITED

The definition of the term heterochrony has had a remarkable history. The term was first defined by Haeckel as one of the exceptions to recapitulation. Haeckel sought to demonstrate that the sequence of events in ontogeny and phylogeny were linked mechanistically. Heterochrony was one type of exception to this general rule. Specifically, the term heterochrony was used to describe cases in which the ontogenetic sequence of events did not recapitulate or repeat the sequence of events in phylogeny (Russell, 1916). The general issues of recapitulation and heterochrony were examined by a number of workers in the early part of the 20th century. The most attention was paid to the question of recapitulation and multiple examples of heterochrony were put forth to refute the generality of this rule (see Russell, 1916; Gould, 1977). Heterochrony began to acquire its modern definition in the work of Gavin de Beer (1930, 1940, 1951, 1958). De Beer had a number of major goals in this series of books. First, and most importantly, he aimed to divorce comparative developmental biology from the concept of recapitulation. He summarized numerous examples to demonstrate there was no general rule governing the appearance of evolutionary innovations in ontogeny. Innovation could occur at any time in an organism's life history. Further, he demonstrated that evolutionarily important innovations might or might not be reflected in the adult condition, as it was common for adaptive changes to be limited to larval or juvenile stages. De Beer thus discarded the concept of recapitulation, as well as phylogeny in and of itself, as the proper focus of comparative developmental biology.

His second major goal was to demonstrate how comparative developmental biology could be combined with the increasing understanding of developmental mechanisms and genetics. Heterochronic change became one of the centerpieces of de Beer's attempt to set an agenda for a new field of evolutionary developmental biology. To de Beer the fundamental question of evolutionary developmental biology was how developmental mechanisms produced the changes observed between species. Heterochrony, the shifting along the time-scale of development, was one such change and de Beer emphasized the importance of understanding the genetic and morphogenetic mechanisms that produced these shifts. He showed that, just as evolutionary innovations could occur at any time in ontogeny, heterochrony, or shifts in development, could also occur at any time, with no necessary relation to adult morphology. Finally, he focused on changes in the sequence of events and regarded relative size changes as only one component of heterochrony.

De Beer largely failed in his attempt to bring developmental biology into the evolutionary synthesis of the mid-20th century and, for the most part, the concept of heterochrony only entered the active vocabulary of modern evolutionary biology in 1977 with the publication of *Ontogeny and Phylogeny* by S.J. Gould. This treatment of heterochrony was enormously influential and has acted to define the way the concept is used today. However, in many ways Gould's view of heterochrony departs from ways the concept had been used by de Beer and other developmental biologists prior to its publication.

First, Gould returns to the linkage between recapitulation and heterochrony. For Haeckel heterochrony was an exception to recapitulation, the parallel between ontogeny and phylogeny. De Beer separated heterochrony from its recapitulatory implications (as he attempted to separate the issue of recapitulation from evolutionary developmental biology in general). However, Gould's overall focus in *Ontogeny and Phylogeny* was the concept of recapitulation. He redefined heterochrony as the mechanism that produces a parallel between ontogeny and phylogeny. "Parallels between ontogeny and phylogeny are produced by heterochrony. Heterochrony proceeds by acceleration or retardation" (1977:228). Acceleration produces recapitulatory patterns, while retardation leads to pedomorphosis, where the "ontogeny of the most remote ancestor goes through the same stages as a phylogeny of adult stages read in the reverse order" (Gould, 1977:215). Gould discards other cases of timing shifts as not producing parallels and therefore not qualifying as heterochrony. Thus, the concept of heterochrony began as the exception to parallels in ontogeny and phylogeny, was divorced from any particular relation to phylogeny by de Beer, and ends with Gould as the mechanism that produces parallels between ontogeny and phylogeny. Indeed, "the odyssey of heterochrony is exceedingly curious" (Gould, 1977:221).

The second major change in the concept of heterochrony arising from Gould's treatment involves the types of timing shifts considered. Both Haeckel and de Beer viewed heterochrony largely in terms of the sequence of developmental events or stages. Gould does discuss change in individual events, and one specific event, a change in the relative timing of sexual maturation, is important in his discussion. However, Gould focused his discussion of heterochrony almost entirely on rates of relative growth and changes in size and shape. This approach was furthered by Alberch et al. (1979), who model modifications in the developmental processes that produce relative changes in size and shape. The difference in Alberch et al.'s treatment is that heterochrony is defined in terms of shifts in specific

TABLE 1. Terminology of growth heterochrony taken from Gould (1977) and Alberch et al. (1979)

Heterochronic phenomenon	Gould (1977) characterization	Alberch et al. (1979) control parameter	Relative size change	Phylogenetic effect
Progenesis	Size/shape relation constant, early maturation	Early growth offset	Pedomorphosis (less growth)	Reverse recapitulation
Neoteny	Shape slowed relative to size and maturation	Decrease shape growth rate	Pedomorphosis (less growth)	Reverse recapitulation
Postdisplacement		Later growth onset	Pedomorphosis (less growth)	Reverse recapitulation
Proportional dwarfism	Size slowed relative to shape and maturation	Decrease size growth rate		Reverse recapitulation
Hypermorphosis	Size/shape relation constant, late maturation	Later offset growth	Peramorphosis (more growth)	Recapitulation
Acceleration	Shape increased relative to size and maturation	Increases shape growth rate	Peramorphosis (more growth)	Recapitulation
Predisplacement		Early growth onset	Peramorphosis (more growth)	Recapitulation
Proportionate giantism	Size increased relative to shape and maturation	Increase size growth rate		Recapitulation

Note the explicit characterization of all types of heterochrony as either recapitulatory or reverse recapitulatory phenomena, a concept that arose with Gould, and was included in the characterization of Alberch et al. (1979).

processes such as onset, cessation, or rate of growth, rather than end results, as is the case for Gould's models (Table 1). The almost exclusive focus on size and shape changes as the important heterochronic phenomenon is almost universally accepted today (for recent reviews, see McKinney, 1988; Raff and Wray, 1989; Hall, 1992, 1999a; McNamara, 1995, 1997; Raff, 1996; Zelditch and Fink, 1996; Klingenberg, 1998; Smith, 2001b).

The work of Gould (1977) and Alberch et al. (1979) generated an enormous new interest in the relation of ontogeny and phylogeny. But by focusing on size and shape changes, our ability to understand the relation between changes in timing in development and evolution has been severely limited in at least two ways. First, size is often taken as a surrogate for time. As a result, many studies of "heterochrony" are not comparisons of changes in timing but instead allometric studies. In some cases, size may be an appropriate surrogate for age, but there are cases in which this substitution obscures patterns or is theoretically questionable (e.g., Roth, 1984; Emerson, 1986; Blackstone, 1987a,b; Klingenberg and Spence, 1993; Godfrey and Sutherland, 1995a,b; Klingenberg, 1998).

Second, the analytical approaches of growth heterochrony are limited to events measured by size and shape parameters. Although many changes between closely related species may arise through patterns of relative growth, size, and shape have little bearing on many critical events, particularly those that occur early in development. Such events include the initial differentiation and patterning of the major elements of the body, appearance of segmental and regional identity, patterns of regulatory gene expression, induction and signaling cascades, cell and tissue specification and differentiation, or the differentiation of skeletal elements and organ systems. Because these events are not functions of size and shape parameters, they are excluded from the analyses of growth heterochrony, but are increasingly the kinds of events examined in comparative studies of development (e.g., Hall, 1984; Langille and Hall, 1989; Wray and McClay, 1989; Jeffery and Swalla, 1992; Swalla et al., 1993, 1994; Collazo, 1994; Richardson, 1995; Smith MM, 1995; Wray, 1995; Cubbage and Mabee, 1996; Slack and Ruvkun, 1997; Velhagen, 1997). These are precisely the kinds of events that must be understood in order to understand how changing genetic and morphogenetic processes produce evolutionary transitions. Therefore, the concept of heterochrony as promulgated by Gould (1977) and Alberch et al. (1979) eliminates many of the developmental processes that may be most important in effecting evolutionary change.

A return to a more traditional view of heterochrony returns to a focus on developmental events and a variety of shifts in the timing of development. Using the sequence of events as the criterion of standardization avoids many of the problems of comparing diverse taxa using size or time. This approach, which I will call sequence heterochrony, provides analytical tools to look at many different kinds of events in development in an explicit phylogenetic context. This method therefore provides means to test hypotheses on the fundamental questions about

evolution and development raised in the Introduction. Although a number of authors have examined sequence change in evolution (e.g., Hanken and Hall, 1984; Irish, 1989; Hufford, 1995, 1996; Richardson, 1995; Cubbage and Mabee, 1996; Dunlap and Sanchiz, 1996; Mabee and Trendler, 1996; Velhagen, 1997; Larsson, 1998), a broadly applicable analytical or conceptual approach to sequence heterochrony has not yet emerged.

SEQUENCE HETEROCHRONY

Changes in developmental sequence can arise from the same processes examined by growth heterochrony—change in onset, offset, or rate of a process (de Beer, 1958). There are, however, important differences in the way ontogeny is ordered and standardized, the kinds of events examined, and the methods of analysis. One problem with the comparative study of development arises from the lack of an appropriate measure for interspecific comparison of developmental time (e.g., Roth, 1984; Blackstone, 1987a,b; Raff and Wray, 1989; Reiss, 1989; Hall and Miyake, 1995b). Most of the criteria of standardization that have been applied such as size, age, or landmarks of maturation present significant theoretical and practical difficulties as measures for both intraspecific, and especially interspecific, comparisons (Hall and Miyake, 1995b, and references therein). Developmental sequence analysis models a developmental trajectory as a series of morphogenetic events. As a result, the sequence itself serves as the criterion of standardization. Heterochrony is defined as a change in the sequence position of an event relative to the other events. This approach assumes that the important "clock" for the embryo is not an external or internal time base, but the completion of a series of morphogenetic events and processes (Smith, 2001b).

Because development is characterized as a series of events, changes in the events themselves are the focus of study. Further, any kind of event, from any part of a developmental trajectory, may be included in the study. One example of the kinds of shifts in timing that cannot be analyzed by traditional growth heterochrony approaches is found in a series of studies by Swalla and Jeffery (Swalla and Jeffery, 1990; Jeffery and Swalla, 1992; Swalla et al., 1993, 1994) on development in ascidian larvae. In the primitive condition in ascidians, called the urodele condition, there are two phases, a larval-dispersive phase that is nonfeeding and a sessile, feeding, adult stage. In the urodele larva, larval-specific cells develop while adult cells remain undifferentiated until after morphogenesis. In multiple lineages a tailless (anural), nondispersive larva has evolved. The process by which the anural larvae are produced is called adultation. It involves the suppression of larval traits (e.g., the notochord and tail) and the shifting forward in time the development of some adult tissue types. These heterochronies involve differential acceleration and deceleration of specific developmental events relative to the overall course or sequence of development. For example, Swalla et al. (1994) show that in ascidians exhibiting adultation, the differentiation of certain mesenchymal cells is advanced relative to other events in development. This shift is mediated in part by the heterochronic expression of adult muscle actin genes in the larva. Other examples of the importance of sequence shifts in relation to important evolutionary changes are provided in a series of studies by Wray (e.g., Wray and McClay, 1989; Wray and Raff, 1991; Wray, 1995; Wray and Lowe, 1997) on genetic, molecular, and morphogenetic events in echinoderm development.

Misconceptions About Developmental Sequence

In general, sequence heterochrony approaches have been overshadowed by growth heterochrony studies. In part, this may be because the cases presented by Gould and Alberch et al. are intuitive and compelling. Further, comparative analyses of relative size and shape changes are straightforward, given established allometric and morphometric techniques. In contrast, until quite recently there have been few techniques to examine sequence heterochronies and many authors studying the evolution of developmental sequences have attempted to fit these studies into the models and terminology of growth heterochrony (i.e., Irish, 1989). In addition, and perhaps more importantly, discussions of sequence heterochrony have been dominated by preconceptions and misunderstandings that have diverted attention from the issue of developmental sequence evolution.

First, a sequence of developmental events is commonly confused with a sequence of developmental stages; this confusion has, like the concept of heterochrony itself, roots in the work of Haeckel. The notion that development proceeds as a series of discrete, conserved stages in which an embryo possesses a number of specific characters is longstanding and pervasive. Developmental stages may be the best criterion of standardization for intraspecific comparisons; however, stages can only roughly be compared across taxa. Virtually all extensive comparative developmental studies show that a regular progression of stages with detailed equivalence across taxa at higher levels simply does not exist (e.g., Richardson, 1995; Richardson et al., 1997, 1998). Developmental sequence analysis compares individual events and makes no a priori assumption about the linkage of events within conserved stages.

A second major preconception is that most discussion about the evolution of developmental sequences has focused on whether developmental sequences are, or should be, recapitulatory (see Alberch, 1985;

Raff and Wray, 1989, for discussion). Again, this idea can be traced back to Haeckel and the pervasiveness of the idea of recapitulation. Recently, there has been a great deal of attention paid to the use of ontogeny in phylogenetics and in particular the use of ontogenetic data to determine character polarity in phylogeny reconstruction (e.g., Nelson, 1978; Fink, 1982; Alberch, 1985; de Queiroz, 1985; Kluge, 1985, 1988; Kluge and Strauss, 1985; O'Grady, 1985; Mabee, 1989, 1993, 1996; Rieppel, 1990; Patterson, 1996; Meier, 1997, and references therein). With some exceptions (e.g., Mabee, 1993; Hufford, 1995, 1996; Meier, 1997), this literature largely discusses the issue in principle or by providing general examples, with little detailed testing of the data. Rigorous analysis of sequence heterochrony, without any a priori assumption can test hypotheses about the conservation of developmental sequences, the correlation between phylogenetic sequence and ontogenetic sequence, or the frequency of terminal additions

For example, Larsson (1998) compares the relation of sequence changes in ontogeny and phylogeny in the evolution of palatal structures in crocodilians. First he uses specimens in the fossil record to derive the phylogenetic sequence of the emergence of taxon-specific characters. After the phylogenetic sequence has been defined, an ontogenetic series of the study taxon is obtained. The appearance of each of the phylogenetically diagnostic characters is mapped in this ontogeny. The characters in both the phylogenetic and the ontogenetic sequences are given a sequence rank from 1 to n. Bivariate plots and Spearman rank coefficients are used to test the association of ontogenetic and phylogenetic sequence (see Larsson, 1998, for details). If the sequences in ontogeny and phylogeny are conserved, the ranks of the specific characters in the two sequences will by highly correlated. Larsson used this method to identify features that may be developmentally integrated, because it has been predicted that such characters would retain particular patterns of association in phylogeny and ontogeny (e.g., Alberch, 1985; Wimsatt, 1986). His data suggested that the premaxilla and maxilla are developmentally independent from a complex involving the palatine, pterygoid, and ectopterygoid bones. The characters of the maxilla and premaxilla exhibited little correlation in ontogeny and phylogeny. In contrast, at least two complexes—one within the pterygoid–palatine complex and another involved with the choanae—exhibited a positive, statistically significant correlation in ontogeny and phylogeny. Larsson hypothesized that these two complexes were to some extent developmentally integrated. As pointed out by Larsson, this method provides a precise means to test hypotheses such as von Baer's law, Wimsatt's (1986) hypothesis of generative entrenchment, or general hypotheses on the relation between the phylogenetic appearance and ontogenetic appearance of characters.

EVOLUTION OF DEVELOPMENT IN MAMMALS

The way that sequence heterochrony may be studied on a number of levels to address a variety of questions about development and evolution may be illustrated with an extended example, which addresses the evolution of development in mammals (see Smith, 1996, 1997, 2001a,c; Nunn and Smith, 1998). Marsupial and placental mammals are characterized by distinct reproductive and developmental modes; in particular, they differ in the means of maternal nutrient provision to the young. Both use fully the two important innovations of mammalian reproduction, placentation and lactation, but emphasize different strategies. Marsupials are considered lactational specialists, where a relatively short intrauterine period of maternal–fetal interchange is followed by an extended period of lactation (e.g., Renfree, 1983, 1993, 1995). In contrast, eutherians are characterized by relatively longer periods of intrauterine development, with extensive fetal–maternal interchange, and variable reliance on lactation. In marsupials, gestation as a whole, and in particular the period of organogenesis, is exceedingly short. For example, the period of organogenesis (roughly the period of primitive streak to birth) ranges from 3–4 days in many dasyurids to approximately 10 days in the larger macropodids (Tyndale-Biscoe and Renfree, 1987). In contrast, in *Mus*, one of the fastest-developing placental mammals, this period is approximately 10 days, and in cats, a medium-sized, highly altricial eutherian, it is around 50 days (Noden and de Lahunta, 1985). Because of the very short organogenic period, the marsupial neonate shows minimal development of most systems and is always highly altricial. Unlike placental mammals there is minimal relation between maternal size and gestation length, or between maternal size and size of either the neonate or total litter (Eisenberg, 1981; Tyndale-Biscoe and Renfree, 1987). Marsupial neonates are always small, rarely weighing more than 100 mg and often weighing much less than 50 mg. Placental neonates exhibit a range of development from altricial to highly precocial. However, even the most altricial eutherian is far more developed than the most precocial marsupial. The marked difference in life history has led to a major debate on the evolutionary consequences of these reproductive modes (e.g., Lillegraven, 1975; Kirsch, 1977a,b; Parker, 1977; Lillegraven et al., 1987; Hayssen et al., 1985; Tyndale-Biscoe and Renfree, 1987). However, these broad issues of life history evolution are unresolved, in part because a number of more specific questions remain about reproduction, development, and their evolution in mammals.

It has long been recognized that, relative to eutherians, marsupials accelerate the development of certain structures such as the tongue, the bones around the oral apparatus, and the bones and muscles of the forelimb (e.g., Hill and Hill, 1955; Lee and Cockburn, 1985; Klima, 1987; Maier, 1987, 1993, 1999; Tyndale-Biscoe and Renfree, 1987; Hughes and Hall, 1988; Nelson, 1988; Cockburn, 1989; Filan, 1991; Clark and Smith, 1993; Gemmell and Selwood, 1994, and references therein). This advancement is interpreted as an adaptive response to the functional requirements placed on the neonate by the marsupial life history. The extremely altricial neonate must independently travel to, identify, and enter the pouch or teat region and recognize and attach to the teat. The neonate must have sufficient functional maturity to suckle and process food while it completes its development. This is a fundamental issue of heterochrony: specific morphological adaptations are produced by the accelerated development of particular structures and the modification of certain developmental processes.

Although there are numerous studies of individual systems in one or a few taxa, there have been few detailed analyses of the specific heterochronies of multiple events across therian mammals. (In this article I use the term therian to refer to extant marsupial and placental mammals.) The approaches of growth heterochrony have not been useful to analyze the shifts in development in marsupial and placental mammals for a number of reasons. First, there is a clear mosaic of processes—some are accelerated and others are delayed. Second, the most interesting patterns involve shifts in the early differentiation of structures and not size and shape changes. Third, overall development in marsupials and placentals is so different that no appropriate time- or size-based criterion of standardization can be defined to compare development across these clades. Finally, the specific questions involve the interaction of elements, which cannot be addressed by existing growth heterochrony methods.

Smith (1996, 1997, 2001a,c, in press-b; Nunn and Smith, 1998) examined the comparative development of the craniofacial region of a range of placental and marsupial mammals. These studies detailed changes in the sequence of development of a number of structures of the cranial skeleton, musculature, and central nervous system (CNS) in the period between the early differentiation of the forebrain to the onset of ossification of the last bone in the cranium. Three overarching questions were the foci of these studies. First, which specific elements appear relatively accelerated in marsupials as a consequence of the necessity for independent function at an embryonic state? Second, how does the overall pattern of craniofacial development differ in these animals, e.g., are elements relatively delayed as a result of the advancement of some structures? Finally, what does the pattern of differential acceleration and delay of craniofacial elements reveal about the mechanisms of craniofacial development?

These questions are fundamentally phylogenetic, so methods were devised to analyze change in the sequence position in phylogenetic contexts (see Smith, 1997; Nunn and Smith, 1998, for details on the methods). These analyses revealed that 11 of the 28 specific events analyzed had sequence shifts that distinguished the two clades (Fig. 1). In eutherians the following events had an early overall sequence position: the evagination of the telencephalon, contact between the olfactory bulb and the olfactory epithelium, layering in the cortex, the differentiation of the thalamus and hypothalamus, filling of the lens vesicle by primary lens cells, and the meeting of the dermal bones over the cranial roof. In marsupials, the initial ossification of the dentary, maxillary, premaxillary, and exoccipital bones and the closure of the secondary palate occurred early in the sequence relative to placentals.

This list of specific differences may be translated into a more general view of heterochrony in therian mammals. Craniofacial development in marsupials and placentals is distinguished by major shifts in the relative timing of the differentiation of the muscular-skeletal structures of the head relative to the differentiation of the central nervous system (Fig. 2). There are two major components of these shifts in sequence. First, in eutherians the onset of morphogenesis of the CNS begins long before the appearance of any cranial skeletal or muscular tissues, whereas in marsupials some cranial skeletal and muscular tissues begin development early relative to CNS differentiation. Events that are particularly accelerated are structures in the face and cartilages of the basicranium. Second, in eutherians CNS development is relatively rapid as the events examined completed their development before most of the skeletal-muscular structures began differentiation. In contrast, in marsupials morphogenesis of these same elements extends long into the period of cranial skeletal development. By focusing on sequence changes we see that the most important heterochrony involves the relative timing of the two major craniofacial systems: the skeletal muscular system and the CNS. These patterns would be difficult to discern if analyzed by the methods of growth heterochrony, as the most important events involve the first differentiation of specific structures, rather than subsequent size or shape changes.

Heterochronies in Early Development

The analyses discussed above focus on the differentiation of tissues (bone cartilage, muscle, etc.) from their cellular condensations. However, in general, most critical patterning events occur before tissues and structures emerge (e.g., Hall and Miyake, 1992, 1995a) and earlier events must be examined to identify the developmental mechanisms

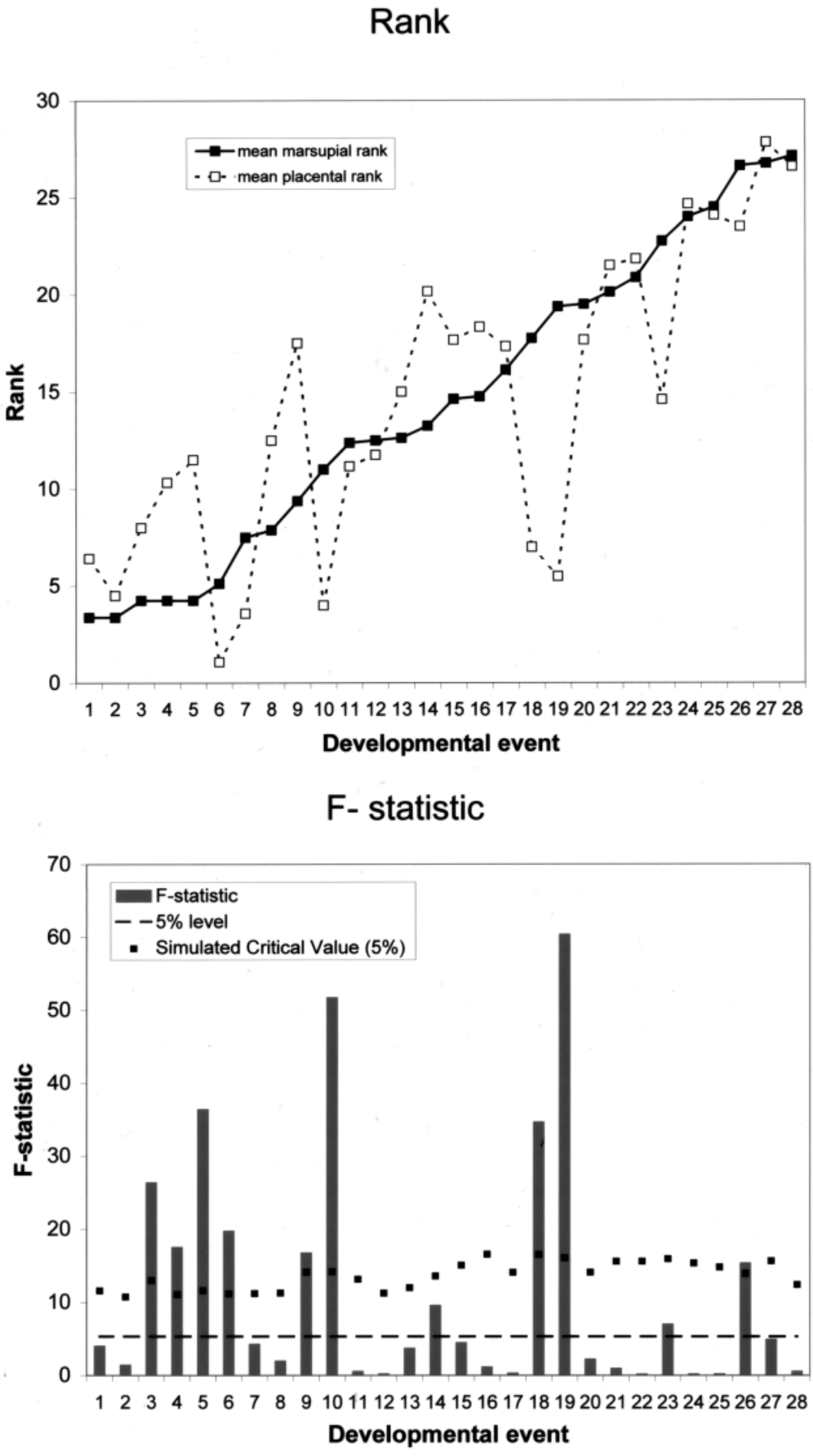

Fig 1. Results of the analysis of sequence shifts in marsupials and placentals. **A**: Mean rank of four marsupials (solid line) and six eutherians (dotted line). The events are arranged by the mean rank of marsupials. If the placental rank is higher than the marsupial rank, it occurs relatively late in placentals relative to marsupials. A lower rank indicates it occurs earlier in development in placentals. **B**: Results of an analysis of variance (ANOVA) of the rank order differences between events in the two clades (marsupials and placentals). Vertical bars represent the F-statistic; dotted line the statistical calculation of $P < 0.05$; boxes indicate $P < 0.05$ resulting from simulation to correct for phylogenetic nonindependence. See Nunn and Smith (1998) for discussion of methods. The combination of the two charts allows the identification of the events that are significantly different in the two clades and also the polarity of the shift. It is important to note that even though the mean rank may be shifted (i.e., events 1, 7, 8, 15), the difference between the two groups may not be statistically significant, given the variance within the groups. Note that significant shifts occur early, late, and in the middle of the sequence. Key to events: 1, cartilage in the basicranium; 2, alignment of myoblasts in the tongue; 3, ossification in the dentary 4, ossification in premaxilla; 5, ossification in maxilla; 6, evagination of telencephalon; 7, pigment in retina; 8, striations in muscles; 9, secondary palate closes; 10, olfactory nerve contacts bulb; 11, tooth buds; 12, cartilage on condyle; 13, frontal bone ossifies; 14, exoccipital ossifies; 15, jugal ossifies; 16, craniofacial muscles organized; 17, squamosal ossifies; 18, primary lens cells fill lens vesicle; 19, thalamus and hypothalamus; 20, parietal ossifies; 21, alsiphenoid ossifies; 22, basioccipital ossifies; 23, layering in cortex; 24, basisphenoid ossifies; 25, malleus and incus separate from Meckel's cartilage; 26, membrane bones meet over skull roof; 27, periotic bone begins ossification; 28, joint capsule forms. See Smith (1997) for more detail on the events.

that are involved in producing these heterochronies. In the case of the heterochronies discussed above, the neural crest is of particular interest. The neural crest is a set of cells derived from the neural tube that gives rise to the bones and connective tissues of the face. It is of particular interest because it originates in the tissue that is most delayed in marsupials, the neural tube, but contributes to the regions that are most advanced, the bones and connective tissues of the facial region. Other than the unpublished studies cited in Hill and Watson (1958), there are no studies of neural crest migration in any marsupial.

Neural crest migration has been studied extensively in a number of nonmammalian vertebrates, particularly in the quail-chick system (e.g., Le Douarin, 1982; Noden, 1983, 1987, 1991; Hall and Hörstadius, 1988; Hall, 1999b; Le Douarin and Kalcheim, 1999). The studies of mammals thus far have indicated essential similarity with other vertebrates, although a few important differences exist (see, for example, Nichols, 1981, 1986, 1987; Tan and Morriss-Kay, 1985, 1986; Serbedzija et al., 1992; Morriss-Kay et al., 1993; Trainor and Tam, 1995; Peterson et al., 1996). One difference is that in most vertebrates migration is typically after neural tube closure, with a distinct rostral–caudal gradient in the timing of migration (e.g., Hall, 1999b). In the placental mammals studied, neural crest migration begins relatively early, when the anterior part of the neural tube is still open. Nichols (1981, 1986) shows that neural crest cells begin to differentiate at the 3–4 somite stage in the mouse. The first indication of cells is in the area of the midbrain–rostral hindbrain. At the 4–6 somite stage in mice the neural folds begin to approach each other in the cervical region and the forebrain–midbrain flexure appears. At this stage, neural crest cells in the midbrain–rostral hindbrain begin migration. These cells will fill the first arch region. By approximately the 8 somite stage the neural tube is closed in the cervical region and caudal parts of the hindbrain and the neural crest is beginning to migrate from the region caudal to the otic placode. This postotic (third and fourth arch) crest begins migration before the hyoid (second arch) crest (Tan and Morriss-Kay, 1985). Thus, the general rostral–caudal sequence of crest migration seen in chickens is disrupted in rodents, where the sequence is first arch, postotic crest (3rd and 4th arches), and the preotic (second arch) crest (Tan and Morriss-Kay, 1985; Morriss-Kay et al., 1993).

Preliminary results from a study of neural crest migration in marsupials suggest that in marsupials the differentiation and migration of neural crest, relative to the neural tube, is even earlier in marsupials than placentals (Smith, 2001c). In *Monodelphis*, the neural crest begins to migrate from the rostral regions of the neural plate before any somites differentiate (stage 23). At the time of the first crest migration there is no morphological differentiation anterior to the preotic sulcus (roughly the site of the third rhombomere). Because there is no folding in the neural plate at this time, crest migration consists of simple movement from the ventral surface of the neural plate into the region between the neural plate and the endoderm overlying the yolk sac (Fig. 3). Second arch crest begins migration at the 4 somite stage and postotic crest at around the 6 somite stage. In a 5–6 somite embryo (stage 24) of *M. domestica* (Fig. 4), significant neural crest migration has already occurred into the first arch region and has started in the second arch region. However, there is still no contact of the neural folds and differentiation within the neural plate is minimal. The neural crest that will contribute ectomesenchyme to the first arch and future frontonasal regions appears to migrate as a single mass from the region anterior to the preotic sulcus, which includes the first two rhombomeres, midbrain, and forebrain. The hindbrain region appears to be well differentiated, as very clear preotic and otic sulci are present and indications of all rhombomeres exist. As in mice and the chick, rhombomeres 3 and 5 appear to be crest-free. In *Monodelphis* it appears that there is a clear rostral–caudal gradient in the timing of cranial crest migration (unlike reports for rodents), so that first arch and frontonasal crest has virtually completed migration while the second arch migration is underway and postotic crest migration has not yet begun. Confirmation of these patterns requires further work, but several tentative results may be presented. First, like placentals, in marsupials crest migration begins before neural tube closure. However, in marsupials neural crest migration is virtually the first event in neural plate differentiation. It begins migration before somites differentiate and when there are no neural divisions except the preotic and otic sulci. Migration of the three major cranial streams is under way before there is any contact of the neural folds. Second, fore- and midbrain differentiation, relative to neural crest differentiation, is particularly delayed in marsupials. Third, unlike placentals, marsupials appear to maintain a distinct rostral–caudal sequence in the timing of cranial crest migration (see Smith, 2001c).

These data suggest that the heterochrony we see in the differentiation of the tissues of the face in marsupials may be traced back to heterochronies in the differentiation and migration of the neural crest from the neural plate. Neural crest migration is well under way at the neural plate stage and crest cells make up a much larger proportion of the cranial tissue in marsupials than in placentals at early stages. A number of specific sequence shifts, therefore, can be identified as important heterochronies in initiating the patterns that differentiate development in marsupials and placentals. These shifts include the relative sequence of neural crest and neural tube differentiation, the order of the

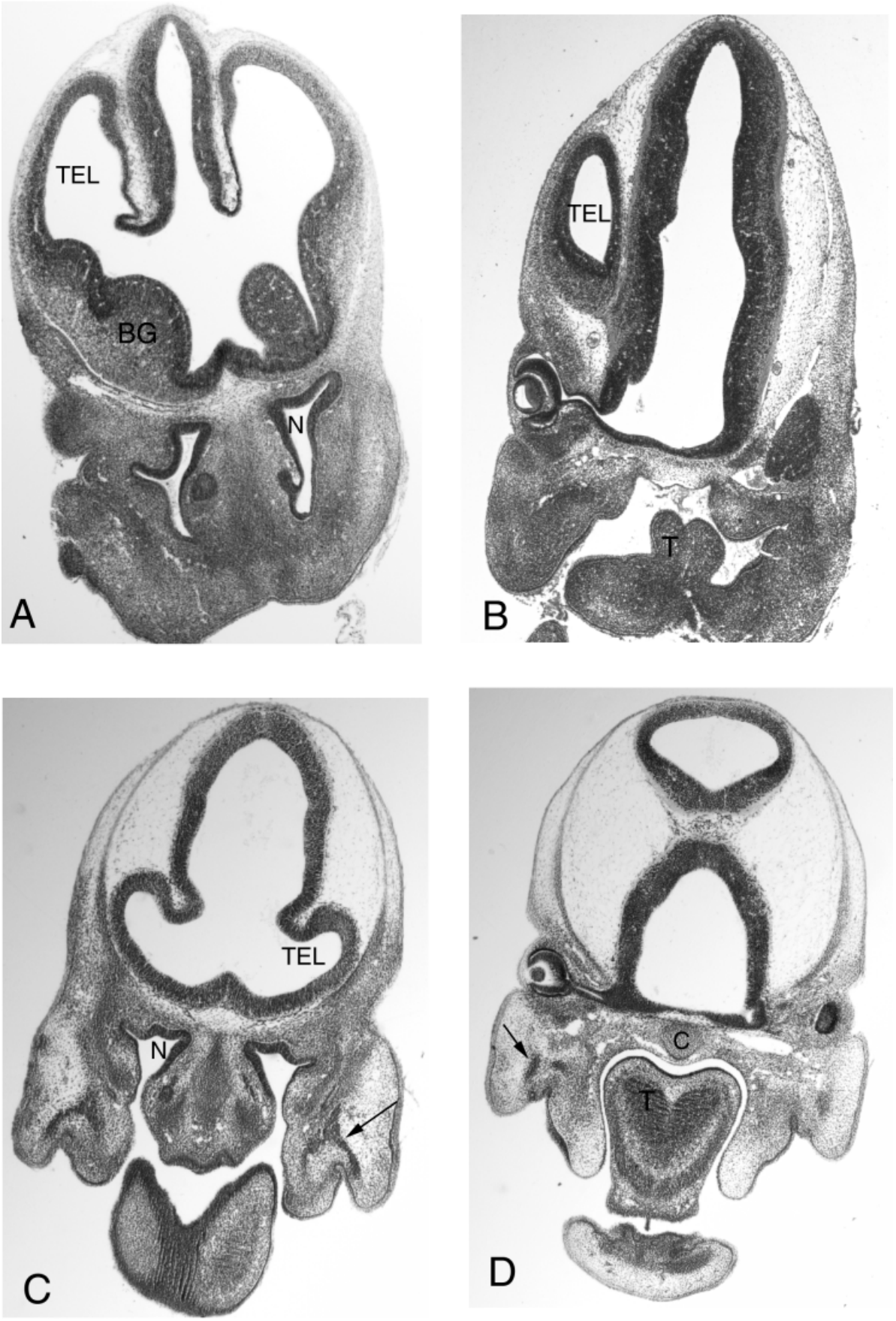

Figure 2

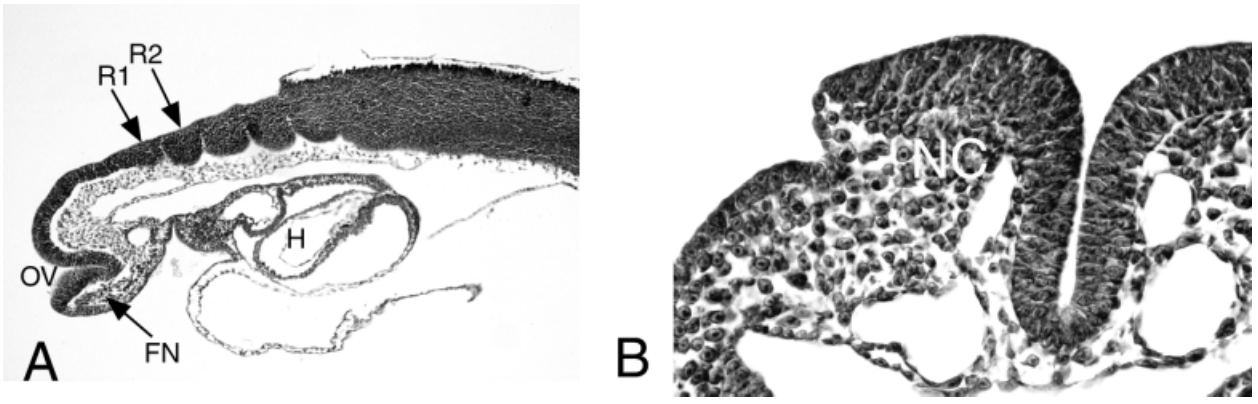

Fig. 3. Photomicrographs of paraffin sections of embryos of *Monodelphis domestica.* **A**: Stage 25 embryo, parasagittal section near midline. Anterior is to the left. Note rhombomeres, ventral invagination of optic vesicle, and accumulation of mesenchyme in frontonasal region. **B**: Stage 25 embryo cut in cross section, through the region of the first or second rhombomere. Note that mesenchyme is leaving the neuroepithelium from a broad region of the ventral neural plate. FN, frontonasal processes; H, heart; NC, migrating neural crest; OV, optic vesicles; R1, R2, first and second rhombomeres. Specimens fixed in Carnoy's fixative, and prepared for paraffin histology using techniques detailed in Smith (1994).

differentiation of relative regions of the neural tube, and the order in which various populations of neural crest migrate. Studies in progress are aimed at examining how these changes in cellular processes are related to changes in gene expression patterns in particular populations of cells in order to continue to extend this study of sequence heterochrony to a variety of morphogenetic levels (Smith, 2001c).

PHYLOGENETIC ORIGINS OF HETEROCHRONY

Discussion thus far has compared the sequence of development in two clades: marsupials and placentals. Placing these relative sequences in a broader context helps identify the polarity of these two conditions, just as any other character may be polarized by examining the distribution in a phylogenetic context.

Fig. 2. Sections through the heads of: (**A,B**) *Mus* (approximately 11 days embryonic); (**C,D**) *Monodelphis* (one-half day before birth). **A** and **C** are anterior sections through the nasal region; **B** and **D** are through diencephalic region, showing the development of the eye. Specimens were chosen for approximate match in the relative development of the eye and demonstrate the relative acceleration of craniofacial skeletal and muscular structures, relative to CNS structures in marsupials. In *Monodelphis* the neural tube is at an early stage with no significant proliferation of the neural epithelium. However, at this time the maxillary, dentary, and premaxillary bones have begun ossification, cartilage is present in the basisphenoid and basioccipital regions and muscle has differentiated in the tongue. In *Mus* the telencephalon is evaginated and there is significant proliferation of neural epithelium in all regions of the brain. For example, there is significant proliferation in the region of the basal ganglia. However, no cartilages, bones, or muscles have begun differentiation. BG, basal ganglia; C, cartilage in the basicranium; N, nasal epithelium; T, tongue; TEL, telencephalic evagination; arrow points to ossification in the maxillary bone.

Relative to placentals, marsupials are characterized by at least three major sets of sequence shifts. First, there is a relative delay in differentiation of elements of the CNS and in particular in the forebrain region. In eutherians, all regions of the brain are present before any differentiation of skeletal or muscular tissues in the face exists. Second, the differentiation of the branchial arch and facial regions is advanced. In marsupials, there are massive accumulations of mesenchyme in the first and second arches as well as the frontonasal region at a very early stage of development. Third, not discussed in detail above, is the existence in marsupials of an extreme rostral–caudal gradient of development. Although to some degree a rostral–caudal gradient exists in eutherians, this gradient is exaggerated in marsupials. The most striking expression of this gradient is the relative development of the fore- and hindlimb buds. In marsupials, the forelimb bud is massive at a time when the hindlimb bud is not yet present.

These three features may be defined as three character complexes (each of which contains a multitude of individual characters) that may be examined in a broader phylogenetic context. In Figure 5 early embryos of a chicken (*Gallus*) and snapping turtle (*Chelydra*) are compared with embryos of *Monodelphis* and *Mus*. This comparison only allows a static view of relative developmental events, but is informative. Eutherians share with the nonmammalian amniotes an advancement of the neural tube and the relatively small branchial arches relative to marsupials. In the mouse, chick, and turtle embryo the telencephalon, diencephalon, midbrain, and hindbrain regions are well differentiated at this stage. However, in marsupials the subdivisions of the midbrain and forebrain have not appeared and there are no telencephalic vesicles. The branchial arches are

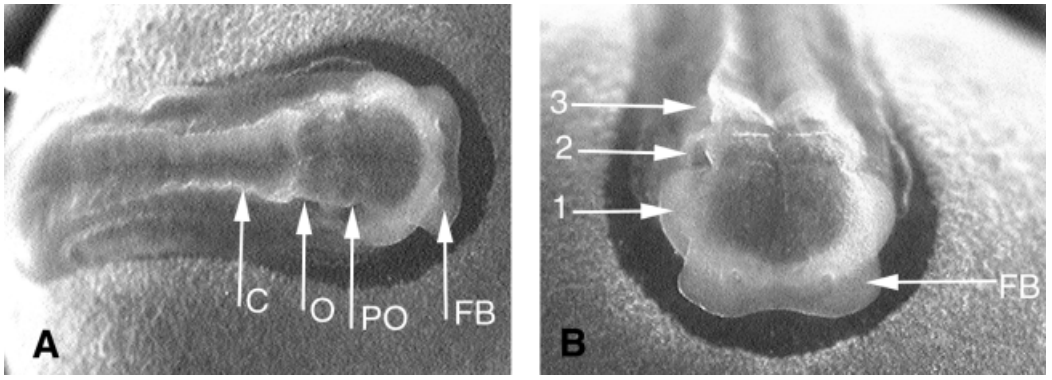

Fig. 4. Photographs of a 10.5-day gestation embryo *Monodelphis domestica* (approx. 6 somites, stage 24). **A**: Dorsal view. **B**: Anterior–dorsal view of same specimen. This embryo demonstrates that neural crest migration occurs early relative to neural tube differentiation in marsupials. Although there is no closure of the neural tube, streams of neural crest have migrated into the first arch region, are migrating into the second arch region, and appear to be about to migrate into the third and fourth arch regions. Further, at this time the hindbrain is fairly well differentiated, with recognizable rhombomeres, yet there is little or no development of midbrain or forebrain regions. This is quite different from the pattern seen in eutherians. c, cervical region; o, otic sulcus (region of rhombomeres 5 and 6); po, preotic sulcus (between rhombomeres 2 and 3); FB, region of forebrain; 1, 2, 3, neural crest streams of first, second, and third arches, respectively.

relatively larger in eutherians than in the nonmammalian amniotes; however, they do not possess the massive accumulation of mesenchyme seen in *Monodelphis*. The face is particularly advanced in *Monodelphis* and the olfactory pit and frontonasal processes are particularly well differentiated. At this

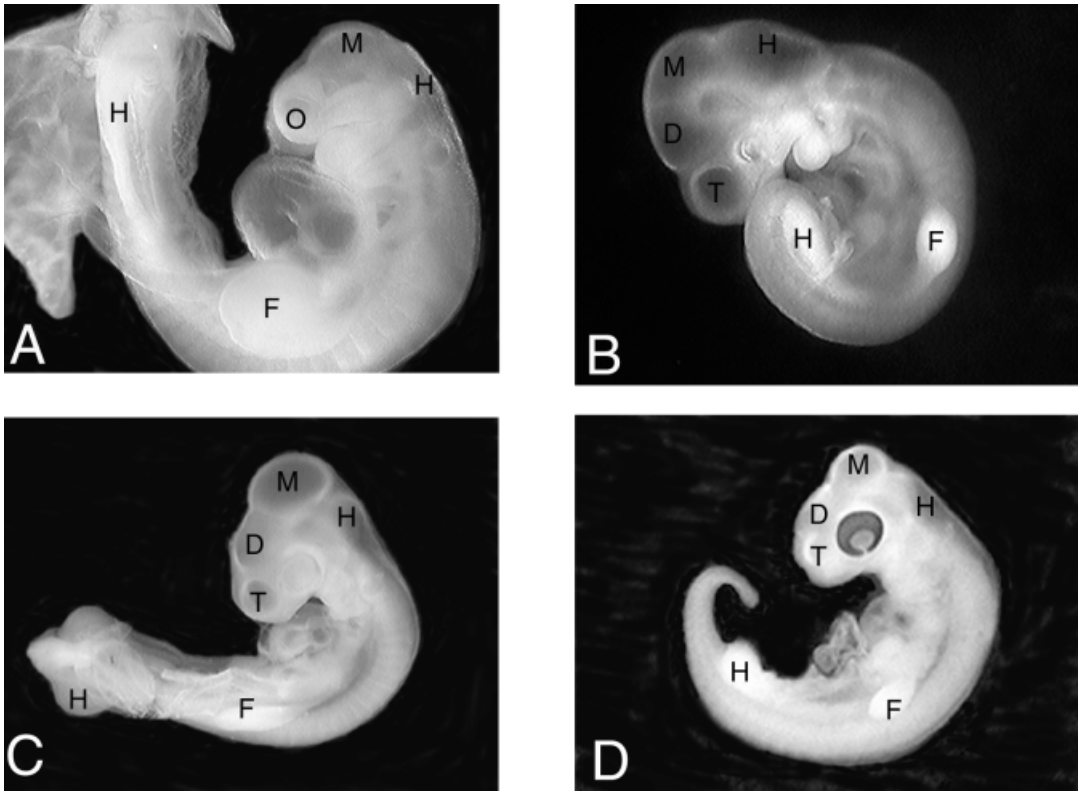

Fig. 5. Embryos of (**A**) *Monodelphis*, (**B**) *Mus*, (**C**) *Gallus*, and (**D**) *Chelydra*. Note that in **B–D** the forelimb bud (F) and hindlimb bud (H) are approximately the same size; in **A** the forelimb bud is massive, while the hindlimb is not yet at the bud stage. Further note that in **B–D** the telencephalon, diencephalon, mesencephalon, and hindbrain are recognizable as distinct swellings; no such divisions are yet present in *M. domestica*. In particular, the telencephalic vesicle has not yet differentiated. Finally, note that the branchial arches and olfactory pit are massive in **A**, and relatively small in the other taxa. D, diencephalon; H, hindbrain; O, olfactory pit; T, telencephalon, M, midbrain.

stage in *Monodelphis* the maxillary process has started fusion with the frontonasal process; in the other taxa the maxillary process is just beginning differentiation. Eutherians and nonmammalian amniotes share the relative similarity of the fore- and hindlimb buds. In *Monodelphis* the forelimb has reached the paddle stage, in which a distinct manus has differentiated, while the hindlimb is still in the early parts of the bud stage. In each of the major complexes the marsupial condition is quite distinct and must be interpreted as derived, whereas placentals possess a condition that is much closer to the primitive amniote condition.

Information on monotremes, the third major clade of extant mammals, is needed to assess the condition at the node Mammalia. Clear possession by monotremes of the derived elements of marsupial development would be parsimoniously interpreted as a shared derived resemblance. On the other hand, resemblance of monotremes to the eutherian condition (which appears to be shared with nonmammalian amniotes) would further highlight the derived and specialized nature of marsupial development and reproduction.

Few monotreme embryos are available for study; however, preliminary evaluation of some of this material indicates that monotremes exhibit a mosaic of marsupial-like and placental-like developmental characters. Monotremes share with marsupials and nonmammalian amniotes many primitive characteristics of the earliest embryo. For example, all develop as a flat blastodisc on a large yolk, in a manner that is quite distinct from eutherians (Hughes, 1993). In addition to these shared primitive characters of early development, monotremes and marsupials share some derived characters. For example, monotremes appear to share with marsupials the early migration of the first arch neural crest (e.g., Wilson and Hill, 1907).

However, although the monotreme neonate is relatively altricial, it does not fully share the set of morphological conditions associated with marsupials. In particular, monotremes do not have the steep gradient between the development of the neural tube and the musculoskeletal structures of the face. For example, Figure 6 shows sections of a prehatching *Ornithorhynchus* (platypus) embryo. These sections may be compared with those shown in Figure 2. In all species the eye is at a relatively similar stage of development. It was seen that in *Mus* the telencephalon is differentiated as distinct hemispheres and cell proliferation is well under way in both the telencephalon and diencephalon. However, the cells that will form the cartilages, bones, and muscles of the face show little or no evidence of condensation or differentiation. In contrast, in *Monodelphis* the telencephalon has just begun evagination but there is little or no proliferation of cells in either the telencephalon or diencephalon. Yet at this stage cartilage is fully differentiated and present in the nasal and basicranial regions, bone is present in the dentary, premaxilla, and maxilla, and the tongue musculature has differentiated (see Smith, 1994, 1997). The *Ornithorhynchus* embryo is intermediate between these conditions, although it is more similar to the eutherian than metatherian condition. The major subdivisions are present in the neural tube and proliferation of the neuroepithelium is well under way in both the telencephalon and diencephalon, yet like eutherians, no cartilage, bone, or muscle is present. Therefore, monotremes do not exhibit the same degree of advancement of cranial musculoskeletal tissues as marsupials.

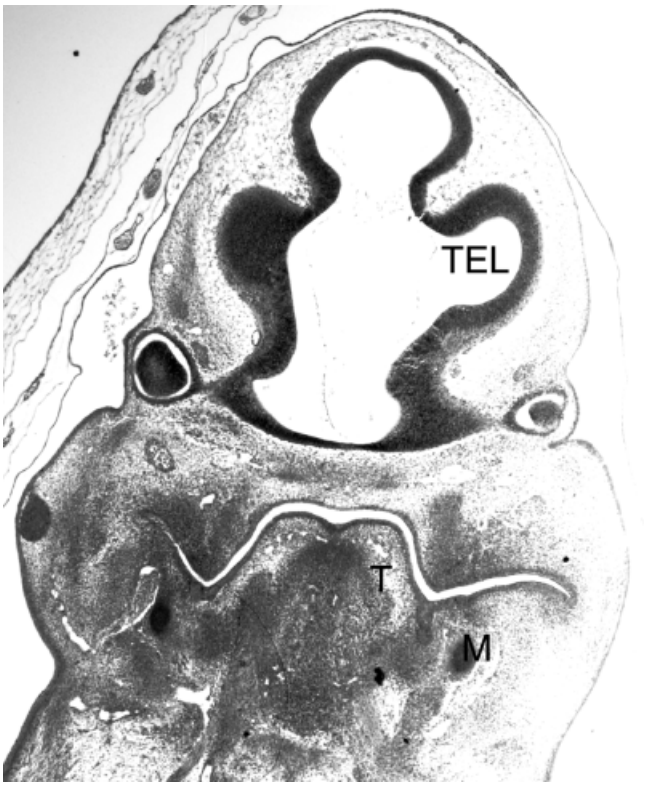

Fig. 6. *Ornithorhynchus* embryo at approximately the same stage as embryos in Figure 4. Note that in many ways *Ornithorhynchus* resembles *Mus*: there is no bone, muscle, or cartilage, yet the neural epithelium has started proliferation. Unlike *Mus*, however, it appears that condensations for bones, muscles, and cartilages have been initiated. M, precartilaginous condensation of Meckel's cartilage; T, condensation of tongue myoblasts; TEL, telencephalic evagination.

Until more monotreme material is obtained and analyzed, the issue of the condition at the node Mammalia is obscure. The fact that in most respects eutherians share the primitive amniote condition implies that the eutherian development pattern is either primitive or represents an evolutionary reversal. Monotremes appear to share many primitive characters with marsupials, as well as some derived features of early development. This pattern supports the hypothesis that the eutherian condition represents a reversal. Alternatively, it is possible to hy-

pothesize a sister-group relation between monotremes and marsupials. This relation is supported by some molecular evidence, but unsupported by the vast majority of morphological and paleontological evidence (e.g., Gregory, 1947; Crompton, 1980; Rowe, 1988; Jenkins, 1990; Hopson and Rougier, 1993; Maier, 1993, 1999; Wible and Hopson, 1993; Zeller, 1993, 1999; Janke et al., 1996, 1997; Penny and Hasegawa, 1997; Kirsch and Mayer, 1998). Further, it is undoubtedly true that each lineage contains a number of derived characters that have appeared since the last common ancestor (e.g., Zeller, 1999). Understanding the details of comparative developmental patterns is essential to our efforts to model the origins of mammalian development.

This example demonstrates that developmental sequence characters can be used in the assessment of phylogenetic and evolutionary issues. The use of developmental sequence in this example differs from the traditional use of ontogeny in that the developmental sequence is not used to polarize traits, but instead the sequence serves as a set of characters that are mapped, like any other set of characters, on a phylogeny in order to reconstruct the evolution of development. Because to some extent developmental patterns reflect reproductive patterns (Smith, 1997, 2001a), the reconstruction of development may eventually aid in the reconstruction of the evolution of reproduction in mammals.

DISCUSSION

In the Introduction, I argued that an examination of sequence heterochrony would illuminate issues not accessible through growth heterochrony studies, and that such issues touched on the most important general issues in development and evolution. These general issues include the relation of ontogeny and phylogeny, the degree to which elements are developmentally integrated, the importance of developmental modules, the ways that morphological changes are produced by developmental changes, and whether a conservative or phylogenetic stage exists. Although growth heterochrony approaches can contribute to these questions, sequence heterochrony approaches, because they examine many stages of development and many different kinds of events, may be more suited for the study of these questions. Further, I argue, as evidenced by the studies of mammal development discussed above, that because sequence heterochrony is not limited to size and shape changes, the components and mechanisms of heterochrony may be traced on multiple levels—morphology, tissue, cell, and gene.

Larsson (1998) provides an example of a means to rigorously study the parallels between ontogeny and phylogeny in his study of the sequence of the evolution and development of the palatal complex in crocodilians. In that study, he utilized data from the fossil record to reconstruct the evolutionary sequence, although a hypothesis on the evolutionary sequence might also be constructed from a well-corroborated phylogeny of extant organisms. This evolutionary sequence was statistically compared with the ontogenetic sequence. The approach can test the general hypotheses that parallels do or do not exist in ontogeny and phylogeny, that certain parts of a developmental sequence are conserved or can be used, as Larsson shows, to test hypotheses of integration or developmental modules.

Smith (1996) also used developmental sequences conservation to test hypotheses of integration. It has been proposed that the first arch in mammals exhibits particular developmental integration because of the enormous amount of parallelism exhibited by multiple lines during the evolution of mammals and mammal-like reptiles (e.g., Alberch, 1980; Kay, 1986). However, the development of the characters of the first arch across therian mammals does not provide evidence for any particular developmental conservation. In marsupials, relative to placentals, there is a mosaic of patterns of acceleration and delay of elements in the first arch. For example, skeletal elements in the anterior parts of the first arch (premaxilla, maxilla, and dentary) are among the most accelerated elements in marsupials. However, other events, including the development of a definitive dentary-squamosal joint and the regression of postdentary bones into the ear, are greatly delayed. Therefore, these data do not support a hypothesis of particular integration of the first arch, but suggest local regions of the first arch belong to different developmental and adaptive modules. These data demonstrate the way that sequence data can provide information on the possible integration of a wide variety of events.

Richardson (1995) tests the generality of the hypothesis of a conserved phylotypic stage and shows that there is enormous variation in the sequence of events at this stage across vertebrates and argues that there is little evidence for a conserved stage. Currently, Richardson and colleagues (personal communication) are studying the conservation of sequence in a wide variety of taxa throughout early development. Again, they use the relative timing of a number of events at very early stages in a broad phylogenetic comparison to test the fundamental assumption of a conserved phylotypic stage. Such tests would simply not be possible with more typical growth heterochrony approaches.

CONCLUSIONS

I have argued that our view of heterochrony has been dominated by a focus on patterns of relative growth for the past 20 years. This view was put forth in the highly influential works by Gould (1977) and Alberch et al. (1979), but represents a departure from more common and traditional views of hetero-

chrony. Here, I provide an alternative view of heterochrony, which focuses on changes in the relative sequence of events in development. This approach, called sequence heterochrony, does not suggest that different mechanisms of change are in operation, but merely provides an alternative conceptual and analytical context in which to examine changes in the relative timing of events in development. The major advantages of this approach are that it serves as a means of interspecific standardization that circumvents many of the problems arising from using size or time, and also that it allows the analysis of changes in the timing of events not characterized by size and shape parameters, and therefore not accessible to the methods of growth heterochrony. I do not intend to argue that time and size are not important; for many phenomena, they are critical, and when appropriate data are available time and size as well as relative rates of growth may be combined with sequence heterochrony approaches. The intent of this article is to broaden our view of heterochrony and encourage studies that are broad-based phylogenetically and detailed developmentally in order to address many of the outstanding questions in the relation of development and evolution.

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LITERATURE CITED

Alberch P. 1980. Ontogenesis and morphological diversification. Am Zool 20:653–667.

Alberch P. 1985. Problems with the interpretation of developmental sequences. Syst Zool 34:46–58.

Alberch P, Gould SJ, Oster GF, Wake DB. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Blackstone NW. 1987a. Allometry and relative growth: pattern and process in evolutionary studies. Syst Zool 36:76–78.

Blackstone NW. 1987b. Size and time. Syst Zool 36:211–215.

Clark CT, Smith KK. 1993. Cranial osteogenesis in Monodelphis domestica (Didelphidae) and *Macropus eugenii* (Macropodidae). J Morphol 215:119–149.

Cockburn A. 1989. Adaptive patterns in marsupial reproduction. Trends Ecol Evol 4:126–130.

Collazo A. 1994. Molecular heterochrony in the pattern of fibronectin expression during gastrulation in amphibians. Evolution 48:2037–2045.

Crompton AW. 1980. Biology of the earliest mammals. In: Schmidt-Nielsen K, Bolis L, Taylor CR, editors. Comparative physiology: primitive mammals. Cambridge, UK: Cambridge University Press. p 1–12.

Cubbage CC, Mabee PM. 1996. Development of the cranium and paired fins in the zebrafish *Danio rerio* (Ostariophysi, Cyprinidae). J Morphol 229:121–160.

de Beer GR. 1930. Embryology and evolution. Oxford: Clarendon Press.

de Beer GR. 1940. Embryos and ancestors. Oxford: Clarendon Press.

de Beer GR. 1951. Embryos and ancestors, revised ed. Oxford: Clarendon Press.

de Beer GR. 1958. Embryos and ancestors, 3rd ed. Oxford: Oxford University Press.

de Queiroz K. 1985. The ontogenetic method for determining character polarity and its relevance to phylogenetic systematics. Syst Zool 34:280–299.

Dunlap KD, Sanchiz B. 1996. Temporal dissociation between the development of the cranial and appendicular skeletons in *Bufo bufo* (Amphibia: Bufonidae). J Herp 30:506–513.

Eisenberg JF. 1981. The mammalian radiations: an analysis of trends in evolution, adaptation, and behavior. Chicago: University of Chicago Press.

Emerson SB. 1986. Heterochrony and frogs: the relationship of a life history trait to morphological form. Am Nat 127:167–183.

Filan SL. 1991. Development of the middle ear region in *Monodelphis domestica* (Marsupialia, Didelphidae): marsupial solutions to early birth. J Zool Lond 225:577–588.

Fink WL. 1982. The conceptual relationship between ontogeny and phylogeny. Paleobiology 8:254–264.

Gemmell RT, Selwood L. 1994. Structural development in the newborn marsupial, the stripe-faced dunnart, *Sminthopsis macroura*. Acta Anat 149:1–12.

Godfrey LR, Sutherland MR. 1995a. Flawed inference: why size-based tests of heterochronic processes do not work. J Theor Biol 172:43–61.

Godfrey LR, Sutherland MR. 1995b. What's growth got to do with it? Process and product in the evolution of ontogeny. J Hum Evol 29:405–431.

Gould SJ. 1977. Ontogeny and phylogeny. Cambridge, MA: Belknap Press of Harvard University Press.

Gregory WK. 1947. The monotremes and the palimpsest theory. Bull AMNH 88:1–52.

Hanken J, Hall BK. 1984. Variation and timing of the cranial ossification sequence of the oriental fire-bellied toad, *Bombina orientalis* (Amphibia, Discoglossidae). J Morphol 182:245–255.

Hall BK. 1984. Developmental processes underlying heterochrony as an evolutionary mechanism. Can J Zool 62:1–7.

Hall BK. 1992. Evolutionary developmental biology. London: Chapman and Hall.

Hall BK. 1999a. Evolutionary developmental biology, 2nd ed. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Hall BK. 1999b. The neural crest in development and evolution. New York: Springer.

Hall BK, Hörstadius S. 1988. The neural crest. London: Oxford University Press.

Hall BK, Miyake T. 1992. The membranous skeleton: the role of cell condensations in vertebrate skeletogenesis. Anat Embryol 186:107–124.

Hall BK, Miyake T. 1995a. Divide, accumulate, differentiate: cell condensation in skeletal development revisited. Int J Dev Biol 39:881–893.

Hall BK, Miyake T. 1995b. How do embryos measure time? In: McNamara KJ, editor. Evolutionary change and heterochrony. New York: John Wiley & Sons. p 3–20.

Hayssen V, Lacy RC, Parker PJ. 1985. Metatherian reproduction: transitional or transcending? Am Nat 126:617–632.

Hill JP, Hill WCO. 1955. The growth stages of the pouch young of the native cat (*Dasyurus viverrinus)* together with observations on the anatomy of the new born young. Trans Zool Soc London 28:349–453.

Hill JP, Watson KM. 1958. The early development of the brain in marsupials. Preliminary communication. J Anat 92:493–497.

Hopson JA, Rougier GW. 1993. Braincase structure in the oldest known skull of a therian mammal: implications for mammalian systematics and cranial evolution. Am J Sci 293:268–299.

Hufford L. 1995. Patterns of ontogenetic evolution in perianth diversification of *Besseya* (Scrophulariaceae). Am J Bot 82:655–680.

Hufford L. 1996. Ontogenetic evolution, clade diversification, and homoplasy. In: Sanderson MJ, Hufford L, editors. Homoplasy: the recurrence of similarity in evolution. New York: Academic Press. p 271–301.

Hughes RL. 1993. Monotreme development with particular reference to the extraembryonic membranes. J Exp Zool 266:480–494.
Hughes RL, Hall LS. 1988. Structural adaptations of the newborn marsupial In: Tyndale-Biscoe CH, Janssens PA, editors. The developing marsupial. Models for biomedical research. Berlin: Springer. p 8–27.
Irish FJ. 1989. The role of heterochrony in the origin of a novel bauplan: evolution of the ophidian skull. Geobios memoire special 12:227–233.
Janke A, Gemmell NJ, Feldmaier-Fuchs G, Vhaeseler A, Paabo S. 1996. The mitochondrial genome of a monotreme—the platypus. J Mol Evol 42:153–159.
Janke A, Xu X, Arnason U. 1997. The complete mitochondrial genome of the wallaroo (*Macropus robustus*) and the phylogenetic relationship among Monotremata, Marsupialia and Eutheria. PNAS 94:1276–1281.
Jeffery WR, Swalla BJ. 1992. Evolution of alternate modes of development in ascidians. BioEssays 14:219–226.
Jenkins FA Jr. 1990. Monotremes and the biology of Mesozoic mammals. Neth J Zool 40:5–31.
Kay E. 1986. The phenotypic interdependence of the musculoskeletal characters of the mandibular arch in mice. J Emb Exp Morphol 98:123–136.
Kirsch JAW. 1977a. Biological aspects of the marsupial-placental dichotomy: a reply to Lillegraven. Evolution 31:898–900.
Kirsch JAW. 1977b. The six-percent solution: second thoughts on the adaptedness of the Marsupialia. Am Sci 65:276–288.
Kirsch JAW, Mayer GC. 1998. The platypus is not a rodent: DNA hybridisation, amniote phylogeny, and the palimpsest theory. Philos Trans R Soc Ser B 353:1221–1237.
Klima M. 1987. Early development of the shoulder girdle and sternum in marsupials (Mammalia: Metatheria). Berlin: Springer.
Klingenberg CP. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. Biol Rev 73:79–123.
Klingenberg CP, Spence JR. 1993. Heterochrony and allometry: lessons from the water strider genus *Limnoporus*. Evolution 47:1834–1853.
Kluge AG. 1985. Ontogeny and phylogenetic systematics. Cladistics 1:13–27.
Kluge AG. 1988. The characterization of ontogeny. In: Humphries CJ, editor. Ontogeny and systematics. New York: Columbia University Press. p 57–81.
Kluge AG, Strauss RE. 1985. Ontogeny and systematics. Annu Rev Ecol Syst 16:247–268.
Langille RM, Hall BK. 1989. Developmental processes, developmental sequences and early vertebrate phylogeny. Biol Rev 64:73–91.
Larsson HCE. 1998. A new method for comparing ontogenetic and phylogenetic data and its application to the evolution of the crocodilian secondary palate. N Jb Geol Palaont Abh 210:345–368.
Le Douarin NM. 1982. The neural crest. Cambridge, UK: Cambridge University Press.
Le Douarin NM, Kalcheim C. 1999. The neural crest, 2nd ed. Cambridge, UK: Cambridge University Press.
Lee AK, Cockburn A. 1985. Evolutionary ecology of marsupials. Cambridge, UK: Cambridge University Press.
Lillegraven JA. 1975. Biological considerations of the marsupial-placental dichotomy. Evolution 29:707–722.
Lillegraven JA, Thompson SD, McNab BK, Patton JL. 1987. The origin of eutherian mammals. Biol J Linn Soc 32:281–336.
Mabee PM. 1989. An empirical rejection of the ontogenetic polarity criterion. Cladistics 5:409–416.
Mabee PM. 1993. Phylogenetic interpretation of ontogenetic change: sorting out the actual and artefactual in an empirical case study of centrarchid fishes. Zool J Linn Soc 107:175–291.
Mabee PM. 1996. Reassessing the ontogenetic criterion: a response to Patterson. Cladistics 12:169–176.
Mabee PM, Trendler TA. 1996. Development of the cranium and paired fins in *Betta splendens* (Teleostei: Percomorpha): intraspecific variation and interspecific comparisons. J Morphol 227:249–287.
Maier W. 1987. The ontogenetic development of the orbitotemporal region in the skull of *Monodelphis domestica* (Didelphidae, Marsupialia), and the problem of the mammalian alisphenoid. In: Kuhn H-J, Zeller U, editors. Morphogenesis of the mammalian skull. Hamburg: Verlag Paul Pary. p 71–90.
Maier W. 1993. Cranial morphology of the therian common ancestor, as suggested by the adaptations of neonatal marsupials. In: Szalay FS, Novacek MJ, McKenna MC, editors. Mammal phylogeny — mesozoic differentiation, multituberculates, monotremes, early therians and marsupials. New York: Springer. p 165–181.
Maier W. 1999. On the evolutionary biology of early mammals — with methodological remarks on the interaction between ontogenetic adaptation and phylogenetic transformation. Zool Anz 238:55–74.
McKinney ML. 1988. Heterochrony in evolution. New York: Plenum.
McNamara KJ. 1995. Evolutionary change and heterochrony. New York: John Wiley & Sons.
McNamara KJ. 1997. Shapes of time: the evolution of growth and development. Baltimore: Johns Hopkins University Press.
Meier R. 1997. A test and review of the empirical performance of the ontogenetic criterion. Syst Biol 46:699–721.
Morriss-Kay G, Ruberte E, Fukiishi Y. 1993. Mammalian neural crest and neural crest derivatives. Ann Anat 175:501–507.
Nelson G. 1978. Ontogeny, phylogeny, paleontology, and the biogenetic law. Syst Zool 27:324–345.
Nelson JE. 1988. Growth of the brain. In: Tyndale-Biscoe CH, Janssens PA, editor. The developing marsupial. Berlin: Springer. p 86–100.
Nichols DH. 1981. Neural crest formation in the head of the mouse embryo as observed using a new histological technique. J Emb Exp Morphol 64:105–120.
Nichols DH. 1986. Formation and distribution of neural crest mesenchyme to the first pharyngeal arch region of the mouse embryo. Am J Anat 176:221–231.
Nichols DH. 1987. Ultrastructure of neural crest formation in the midbrain/rostral hindbrain and preotic hindbrain regions of the mouse embryo. Am J Anat 179:143–154.
Noden DM. 1983. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev Biol 96:144–165.
Noden DM. 1987. Interactions between cephalic neural crest and mesodermal populations. In: Maderson PFA, editor. Developmental and evolutionary aspects of the neural crest. New York: Wiley-Interscience. p 89–119.
Noden DM. 1991. Vertebrate craniofacial development: the relation between ontogenetic process and morphological outcome. Brain Behav Evol 38:190–225.
Noden DM, de Lahunta A. 1985. The embryology of domestic animals. Baltimore: Williams and Wilkins.
Nunn CL, Smith KK. 1998. Statistical analysis of developmental sequences: the craniofacial region in marsupial and placental mammals. Am Nat 152:82–101.
O'Grady RT. 1985. Ontogenetic sequences and the phylogenetics of parasitic flatworm life cycles. Cladistics 1:159–170.
Parker P. 1977. An ecological comparison of marsupial and placental patterns of reproduction. In: Stonehouse B, Gilmore D, editors. The biology of marsupials. London: Macmillan Press. p 273–286.
Patterson C. 1996. Comments on Mabee's "empirical rejection of the ontogenetic polarity criterion." Cladistics 12:147–167.
Penny D, Hasegawa M. 1997. The platypus put in its place. Nature 387:549–550.
Peterson PE, Blankenship TN, Wilson DB, Hendrickx AG. 1996. Analysis of hindbrain neural crest migration in the long-tailed monkey (*Macaca fascicularis*). Anat Embryol 194:235–246.
Raff RA. 1996. The shape of life. Chicago: University of Chicago Press.
Raff RA, Wray GA. 1989. Heterochrony: developmental mechanisms and evolutionary results. J Evol Biol 2:409–434.

Reiss JO. 1989. The meaning of developmental time: a metric for comparative embryology. Am Nat 134:170–189.
Renfree MB. 1983. Marsupial reproduction: the choice between placentation and lactation, In: Finn CA, editor. Oxford reviews of reproductive biology, vol. 5. Oxford: Oxford University Press. p 1–29.
Renfree MB. 1993. Ontogeny, genetic control, and phylogeny of female reproduction in monotreme and therian mammals. In: Szalay FS, Novacek MJ, McKenna MC, editors. Mammal phylogeny — mesozoic differentiation, multituberculates, monotremes, early therians and marsupials. New York: Springer. p 4–20.
Renfree MB. 1995. Monotreme and marsupial reproduction. Reprod Fertil Dev 7:1003–1020.
Richardson MK. 1995. Heterochrony and the phylotypic period. Dev Biol 172:412–421.
Richardson MK, Hanken J, Gooneratne ML, Pieau C, Raynaud A, Selwood L, Wright GM. 1997. There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development. Anat Embryol 196:91–106.
Richardson MK, Allen SP, Wright GM, Raynaud A, Hanken J. 1998. Somite number and vertebrate evolution. Development 125:151–160.
Rieppel O. 1990. Ontogeny — a way forward for systematics, a way backward for phylogeny. Biol J Linn Soc 39:177–191.
Roth VL. 1984. How elephants grow: heterochrony and the calibration of developmental stages in some living and fossil species. J Vert Paleon 4:126–145.
Rowe T. 1988. Definition, diagnosis and origin of Mammalia. J Vert Paleon 8:241–264.
Russell ES. 1916. Form and function. London: John Murray.
Serbedzija GN, Bronner-Fraser M, Fraser SE. 1992. Vital dye analysis of cranial neural crest cell migration in the mouse embryo. Development 116:297–307.
Slack F, Ruvkun G. 1997. Temporal pattern formation by heterochronic genes. Annu Rev Gene 31:611–634.
Smith KK. 1994. The development of craniofacial musculature in *Monodelphis domestica* (Didelphidae, Marsupialia). J Morphol 222:149–173.
Smith KK. 1996. Integration of craniofacial structures during development in mammals. Am Zool 36:70–79.
Smith KK. 1997. Comparative patterns of craniofacial development in eutherian and metatherian mammals. Evolution 51: 1663–1678.
Smith KK. 2001a. The evolution of mammalian development. Bull MCZ 156:119–135.
Smith KK. 2001b. Heterochrony revisited: the evolution of developmental sequences. Biol J Linn Soc 73:169–186.
Smith KK. 2001c. Early development of the neural plate, neural crest, and facial region of marsupials. J Anat 199:121–131.
Smith MM. 1995. Heterochrony in the evolution of enamel in vertebrates. In: McNamara KJ, editor. Evolutionary change and heterochrony. New York: John Wiley & Sons. p 125–150.
Swalla BJ, Jeffery WR. 1990. Interspecific hybridization between an anural and urodele ascidian: differential expression of urodele features suggests multiple mechanisms control anural development. Dev Biol 142:319–334.
Swalla BJ, Makabe KW, Satoh N, Jeffery WR. 1993. Novel genes expressed differentially in ascidians with alternate modes of development. Development 119:307–318.
Swalla BJ, White ME, Zhou J, Jeffery WR. 1994. Heterochronic expression of an adult muscle actin gene during ascidian larval development. Dev Genet 15:51–63.
Tan SS, Morriss-Kay BM. 1985. The development and distribution of the cranial neural crest in the rat embryo. Cell Tiss Res 240:403–416.
Tan SS, Morriss-Kay GM. 1986. Analysis of cranial neural crest cell migration and early fates in postimplantation rat chimeras. J Emb Exp Morphol 98:21–58.
Trainor PA, Tam PPL. 1995. Cranial paraxial mesoderm and neural crest cells of the mouse embryo: co-distribution in the craniofacial mesenchyme but distinct segregation in branchial arches. Development 21:2569–2582.
Tyndale-Biscoe H, Renfree M. 1987. Reproductive physiology of marsupials. Cambridge, UK: Cambridge University Press.
Velhagen WA. 1997. Analyzing developmental sequences using sequence units. Syst Biol 46:204–210.
Wible JR, Hopson JA. 1993. Basicranial evidence for early mammal phylogeny. In: Szalay FS, Novacek MJ, McKenna MC, editors. Mammal phylogeny — mesozoic differentiation, multituberculates, monotremes, early therians and marsupials. New York: Springer. p 45–62.
Wilson JT, Hill JP. 1907. Observations on the development of *Ornithorhynchus*. Philos Trans R Soc Ser B 199:31–168.
Wimsatt WC. 1986. Developmental constraints, generative entrenchment and the innate-acquired distinction. In: Bechtel W, editor. Integrating scientific disciplines. Dordrecht, The Netherlands: Martinus Nijhoff Publishers. p 185–208.
Wray GA. 1995. Causes and consequences of heterochrony in early echinoderm development. In: McNamara KJ, editor. Evolutionary change and heterochrony. New York: John Wiley & Sons. p 197–223.
Wray GA, Lowe CJ. 1997. Radical alterations in the roles of homeobox genes during echinoderm evolution. Nature 398: 718–721.
Wray GA, McClay DR. 1989. Molecular heterochronies and heterotopies in early echinoid development. Evolution 43:803–813.
Wray GA, Raff RA. 1991. The evolution of developmental strategy in marine invertebrates. Trends Ecol Evol 6:45–50.
Zelditch ML, Fink WM. 1996. Heterochrony and heterotopy: stability and innovation in the evolution of form. Paleobiology 22:241–254.
Zeller U. 1993. Ontogenetic evidence for cranial homologies in monotremes and therians, with special reference to *Ornithorhynchus.* In: Szalay FS, Novacek MJ, McKenna MC, editors. Mammal phylogeny — mesozoic differentiation, multituberculates, monotremes, early therians and marsupials. New York: Springer. p 95–107.
Zeller U. 1999. Mammalian reproduction: origin and evolutionary transformations. Zool Anz 238:117–130.