

Developmental Patterns in Mesozoic Evolution of Mammal Ears

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Annu. Rev. Ecol. Evol. Syst. 2011. 42:355–80

First published online as a Review in Advance on
August 25, 2011

The *Annual Review of Ecology, Evolution, and
Systematics* is online at ecolsys.annualreviews.org

This article's doi:
10.1146/annurev-ecolsys-032511-142302

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1543-592X/11/1201-0355\$20.00

Keywords

mammal evolution, developmental heterochrony, gene patterning

Abstract

Complex structures with significant biological function can arise multiple times in evolution by common gene patterning and developmental pathways. The mammalian middle ear, with its significant hearing function, is such a complex structure and a key evolutionary innovation. Newly discovered fossils have now shown that the detachment of the ear from the jaw, an important transformation of the middle ear in early mammals, has major homoplasies; the morphogenesis of these homoplasies is also illuminated by new genetic studies of ear development in extant mammals. By extrapolating the developmental morphogenesis of genetic studies into the early mammal fossil record, evolution of the middle ear in early mammals provides an integrated case study of how development has impacted, mechanistically, the transformation of a major structural complex in evolution.

INTRODUCTION

Mammals and their close relatives, the extinct mammaliaforms, have a long history of 220 million years (Kielan-Jaworowska et al. 2004). Thanks to an abundant and long fossil record, mammals can shed light on many evolutionary problems. The beginning of the three living mammal groups, monotremes (egg-laying mammals), marsupials (pouched mammals) and placentals, occurred in the Mesozoic (Benton et al. 2009; Luo 2007a,b). These living lineages are nested in diverse Mesozoic mammal lineages and some mammaliaforms. The Mammaliaformes are, in turn, the descendants from pre-mammaliaform cynodonts (hereafter referred to as cynodonts) that show the precursory conditions to derived mammalian characteristics (Hopson & Kitching 2001, Kemp 2005, Sidor & Hopson 1998) (**Figure 1**).

The origins of complex and novel structures, such as the mammalian jaw and middle ear, can be deciphered by mapping their transformation in the fossil record and phylogeny (**Figure 1**) and by characterizing their developmental morphogenesis. Recent discoveries of new fossils in the cynodont-mammal transition have revealed major convergences and reversals, also known as homoplasies, in the jaw and middle ear structure among Mesozoic mammaliaform clades. Some homoplasies in ear structures of Mesozoic mammals now can be correlated with heterochrony or timing changes in ontogeny (Luo et al. 2007a), and others show the phenotypic characteristics of gene patterning in the embryogenesis of extant mammals (e.g., Oka et al. 2007). New information from development and paleontology has added to a growing body of evidence that the

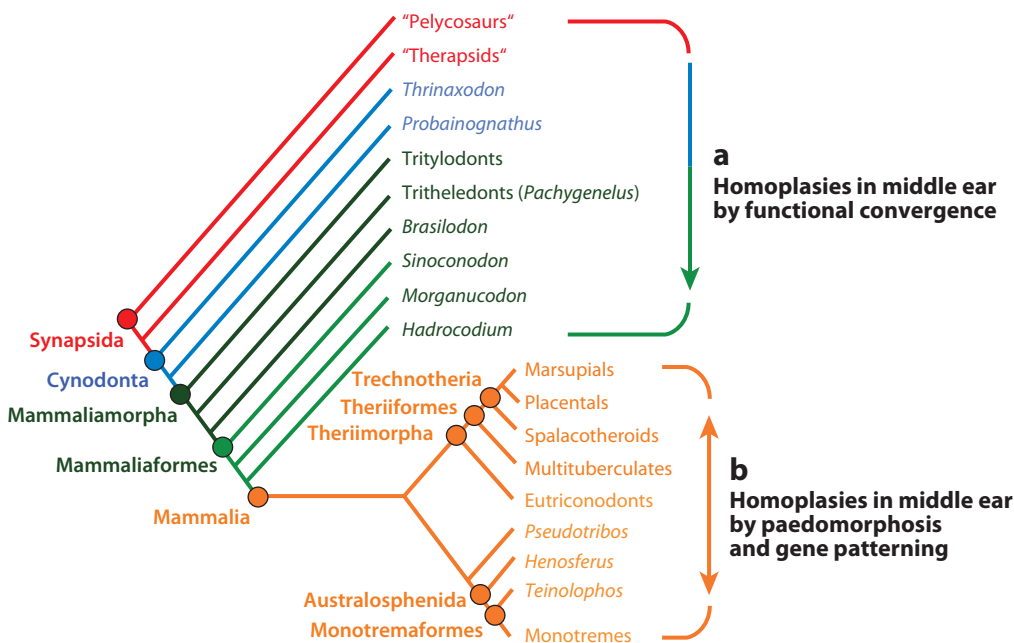


Figure 1

Phylogeny of mammals and pre-mammalian cynodonts and evolutionary patterns of the mammalian middle ear. (a) Homoplastic evolution of the greater mobility of the middle ear in the skull and the impedance-match levers for hearing in the cynodont-mammaliaform transition. (b) Homoplastic development of the partial mammalian middle ear in mammals by repetitive pedomorphosis and gene patterning of the morphogenesis of Meckel's cartilage and/or the definitive mammalian middle ear repetitively evolved in mammals by activation of modern mammal-like gene patterning of Meckel's cartilage.

seemingly labile evolution of middle ears in Mesozoic mammals was influenced, in a major way, by developmental heterochrony and gene patterning.

The key features of the jaw and ear are diagnostic of mammaliaforms as a whole, and their study is important for understanding mammalian origins (Allin 1975, Hopson & Crompton 1969). But besides their relevance to early mammals, examination of the mammaliaform jaw and middle ear offers two perspectives on evolution in general. First, would a more complex structure be less labile in evolution given the parsimony principle, and therefore less susceptible to homoplasy (Martin & Luo 2005, Novacek 1993)? Because the mammalian middle ear is so intricate, is it less labile in evolution, and therefore a more reliable character for morphological evolutionary studies of mammals (Kemp 1983, Rowe 1988)? Second, how can morphologists use the greater structural complexity of fossils from deep geological time to inform our overall evolutionary understanding of both modern and fossil groups? All extant mammals are descendants from merely three Mesozoic lineages out of more than 20 Mesozoic and extinct mammal lineages (Benton 2005, Kielan-Jaworowska et al. 2004). These Mesozoic mammals show a wide range of morphological disparity and ecological specializations that are analogous to those of modern mammals (Ji et al. 2006, Luo & Wible 2005, Martin 2006, Meng et al. 2006, Phillips et al. 2009). These analogous evolutionary experiments during the Mesozoic can provide insight into the mechanism of the recurrent pattern of evolution.

PATTERNS OF MIDDLE EARS IN CYNODONTS AND MAMMALS

Evolution and morphogenesis of the mammalian middle ear is inextricably linked to that of the jaw hinge, known as the craniomandibular joint or temporomandibular joint in medical anatomy (**Figures 2** and **3**). Depending on how the middle ear bones are attached to the lower jaw and how the jaw hinge is formed, the middle ear can be characterized by three major patterns in cynodont-mammal phylogeny.

The first and ancestral pattern is the mandibular middle ear of cynodonts (MMEC; **Figure 2a,b**) of all cynodonts and most pre-mammalian mammaliaforms. The middle ear bones are fully attached to the mandible. The plate-like angular bone has a large surface area for receiving sound (Allin 1975). The mandibular ear bones, together with the surangular bone (lost in extant mammals), are accommodated by grooves and fossae in the postdentary mandibular trough in the dentary bone (Crompton & Luo 1993, Kemp 2007, Kermack et al. 1973, Sues 1986). The delicate middle ear bones, which have a weak connection to the rest of the mandible, are frequently not preserved in fossils. In such cases, the postdentary trough and its grooves are used for inferring the presence of the MMEC for cynodonts and mammaliaforms.

The second and most derived pattern is the definitive mammalian middle ear (DMME) of modern mammals (sensu Allin & Hopson 1992) (**Figure 2d-f**), which contains the ectotympanic ring for the tympanic membrane (the eardrum) and three ossicles known as the malleus (hammer), incus (anvil), and stapes (stirrup), which have several derived structures crucial for hearing function. The malleus has a manubrium attached to the eardrum as the in-lever, and the incus has a stapedial process as the out-lever in the lever system for impedance matching and amplification in hearing (Durrant & Lovrinic 1995). The ectotympanic ring and malleus become detached from the mandible in the adult, and the incus gains greater mobility from the cranium (**Figures 2** and **3**); these changes are accompanied by size reduction of these bones. With these derived structures, modern mammals are sensitive in hearing function in general but especially for higher-frequency sound than other vertebrates (Manley 2000, Rosowski 1992). This may have facilitated adaptation of mammaliaforms to nocturnal niches and their diversification in dinosaur-dominated Mesozoic ecosystems (Luo 2007b, Manley 2000). The ear of Mesozoic

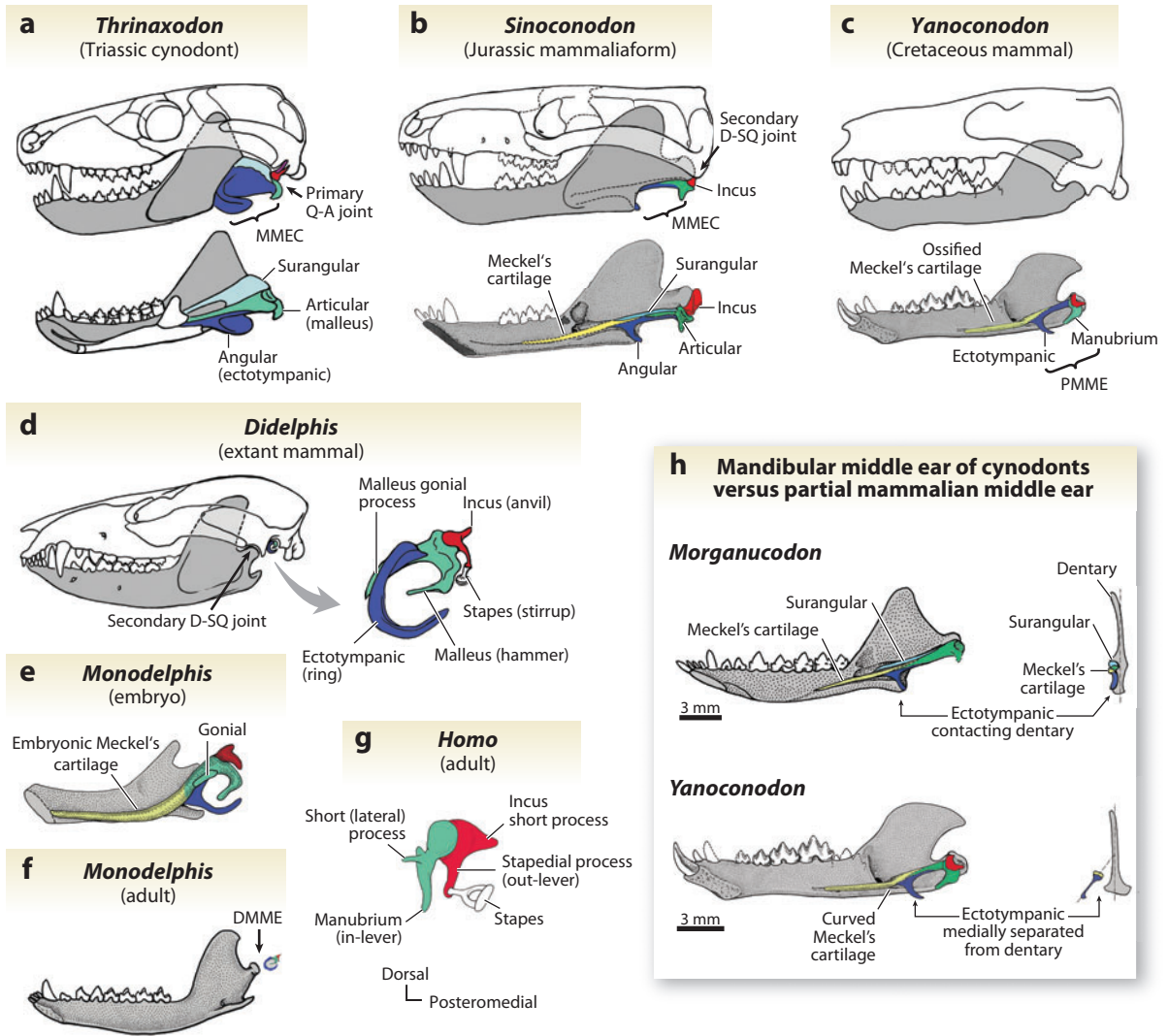


Figure 2

Patterns of middle ear evolution from pre-mammalian cynodonts to mammals. (a) *Thrinaxodon* (250 Ma) with the mandibular middle ear of cynodonts (MMEC) in lateral (top) and medial (bottom) views. (b) *Sinoconodon* (195 Ma) with a cynodont-like mandibular middle ear; the Meckel's cartilage is conjectural. (c) *Yanoconodon* (125 Ma) with a reconstructed partial mammalian middle ear (PMME) on the mandible. (d) Marsupial *Didelphis* (Virginia opossum) with a definitive mammalian middle ear (DMME, sensu Allin & Hopson 1992) separated from the mandible (lateral view). (e, f) Embryonic and adult middle ear of *Monodelphis* (short-tailed opossum). (g) Human ear bones: malleus (hammer), incus (anvil), and stapes (stirrup). (h) Differences between MMEC as retained in mammaliaforms and the PMME of some Mesozoic mammals. Abbreviations: D-SQ joint, dentary-squamosal jaw hinge; Q-A joint, quadrate-articular joint. Drawings not to the same scale.

mammaliaforms is certainly a structural precursor for the highly varied middle ears and diverse hearing adaptations of mammals (Doran 1878, Fleischer 1973), as are manifest in their great Cenozoic radiation (Rose 2006).

The third and intermediate pattern is the partial mammalian middle ear (PMME) (Figure 2c), as seen in the recently discovered Mesozoic eutriconodont and spalacotheroid mammals. The

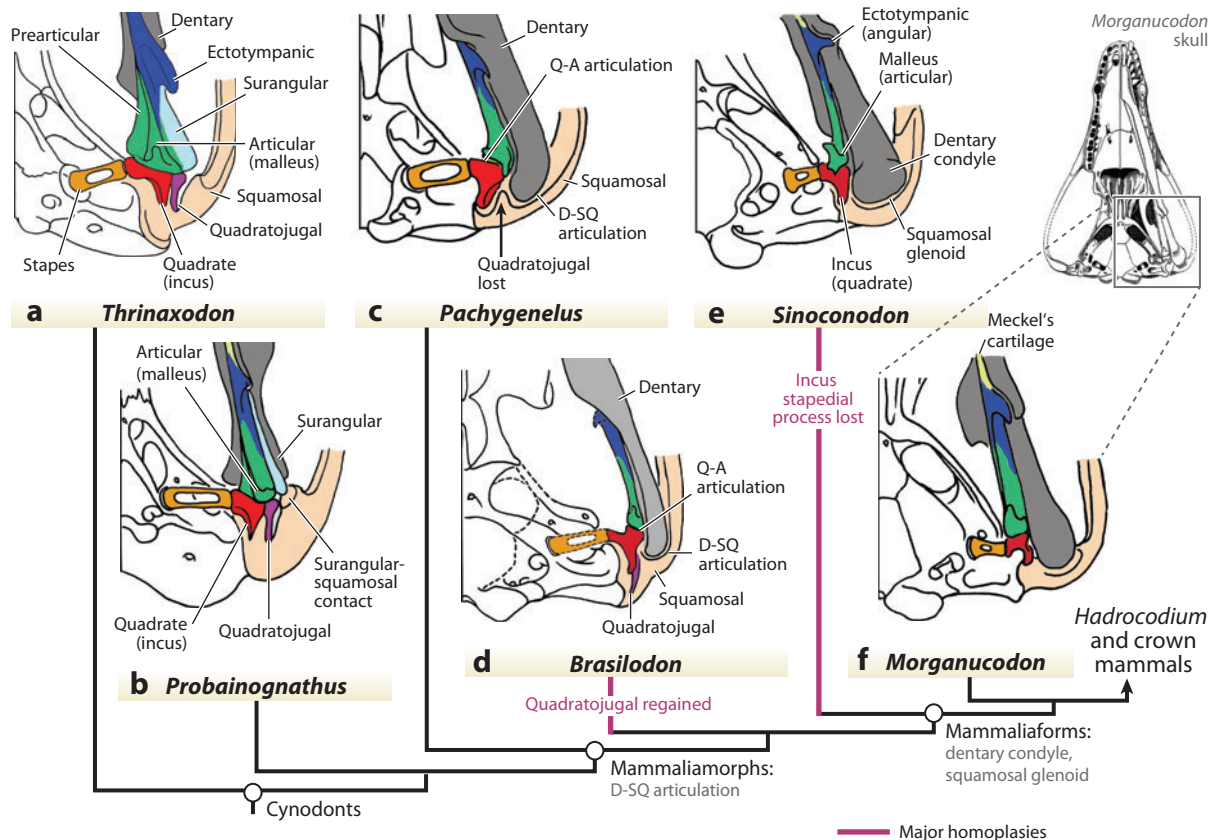


Figure 3

Major jaw hinge evolutionary patterns in premammalian cynodonts and mammaliaforms. (a) *Thrinaxodon*. (b) *Probainognathus* (Middle Triassic). (c) *Pachygenelus* (Early Jurassic). (d) *Brasilodon* (Liu & Olsen 2010), which is the same as *Brasilitherium* of Bonaparte et al. (2005) (Late Triassic; stapes from Maier et al. 2009 with permission). (e) *Sinoconodon*. (f) *Morganucodon* (Late Triassic–Early Jurassic). Both the neomorphic dentary-squamosal (D-SQ) joint and the plesiomorphic quadrato-articular (Q-A) joint are synovial joints that are mobile as jaw hinges from *Pachygenelus* through *Morganucodon* and also likely in other mammaliaforms such as *Microconodon*.

PMME is different from the ears in adults of extant mammals, although similar to their embryonic and neonatal conditions, in retaining an ossified Meckel's cartilage that permanently connects the middle ear to the dentary. However, it also differs from the MMEC in that the ectotympanic ring and the malleus have no contact with the mandible (**Figure 2b**), and the mandible's posterior part has no postdentary trough and angular fossa for receiving the middle ear. The PMME is possible because the ectotympanic ring and malleus are medially displaced and rotated away from the mandible. The transformation from the MMEC to the derived ear structure is accompanied by the formation of the neomorphic jaw hinge by the dentary-squamosal articulation in the derived mammalian cynodonts and mammaliaforms (**Figure 3**).

PATTERNS OF JAW HINGE IN CYNODONTS AND MAMMALS

In cynodonts, the quadrate and articular make up the jaw hinge (Allin & Hopson 1992, Hopson & Kitching 2001, Kemp 2005). This is basic to all gnathostome (jawed) vertebrates and known as the primary jaw joint [**Figure 2a**: primary quadrato-articular (Q-A) joint]. The Q-A jaw

Table 1 Homology of mammalian middle ear structures and their counterparts in cynodonts^a

Extant mammals		Nonmammalian cynodonts
Ectotympanic ring	=	Reflected lamina of the angular
Malleus (hammer)	=	Articular and the gonial part of the prearticular
Malleus manubrium		Mammaliaform neomorph without counterpart in cynodonts
Malleus short (lateral) process	=	Retroarticular process of the articular
Malleus anterior process	=	Prearticular (or gonial)
Incus (anvil)	=	Quadrate
		Quadratojugal (lost in extant mammals)
Incus stapedial (long) process	=	Neomorph of some mammaliaforms and most mammaliaforms without counterparts in other cynodonts
Incus short process	=	Dorsal plate of the quadrate
Meckel's cartilage	=	Postdentary rod or Meckel's element of some cynodonts and all mammaliaforms

^aThe craniomandibular joint (CMJ) is the hinge between the cranium and the mandible in jawed vertebrates or gnathostomes. The CMJ is formed by the quadrate and the articular, although other bones can contribute to this joint in some derived cynodonts. The temporomandibular joint (TMJ) is a derived pattern of the jaw hinge of mammaliaforms. It differs from the CMJ of nonmammalian vertebrates in having the dentary condyle and the glenoid of the squamosal, or the temporal bone (in medical terminology).

hinge serves both for feeding and for transmitting sound from the mandibular ear through the quadrate to the stapes and the inner ear. The angular, quadrate, and articular bones of cynodonts (**Figures 2a, 3**) are homologous to the ectotympanic ring, malleus, and incus, respectively, of mammals (**Table 1**). In mammals, the jaw hinge is formed by the condyle of the dentary and articulated with the glenoid fossa of the squamosal on the cranium [**Figure 2**: secondary dentary-squamosal (D-SQ) joint]. This jaw joint is load bearing. It represents an innovation for feeding that enabled mammaliaforms to have more powerful jaw movement and effective control of the jaw (Crompton & Hylander 1986). In adults of extant mammals, this neomorphic hinge becomes the only jaw joint and replaces the ancestral joint of the quadrate (incus) and the articular (malleus) in adult cynodonts as well as in embryonic or fetal extant mammals.

A double craniomandibular joint (**Figure 3b–d**) is present in mammaliaforms (clade defined in **Figure 1**) and mammaliaforms [clade modified by Liu & Olson (2010) from Rowe (1988)]. In these transitional taxa, the primitive but nonetheless functioning Q-A joint coexists, side by side, with the derived D-SQ articulation. In tritheledontids and brasilodontids, the dentary has a lateral ridge that contacts the ventral side of the squamosal zygoma, and this contact is functional for the action of the jaw for feeding (Bonaparte et al. 2005, Crompton 1972, Crompton & Luo 1993, Hopson 1994). However, tritheledontids and brasilodontids are primitive in that they lack a fully developed dentary condyle and a distinctive squamosal glenoid, as seen in mammaliaforms (**Figure 3**). In the derived cynodont *Probainognathus*, the surangular bone in the mandible directly articulates with a platform on the squamosal in the cranium, forming a unique surangular-squamosal contact, in addition to the typical Q-A joint in other cynodonts (Crompton 1972) (**Figure 3b**). *Microconodon* is a transitional and possibly mammaliaform taxon, and its lower jaw appears to have a well-developed lateral ridge that approaches the condition of brasilodontids (Sues 2001).

The proper function of the double joints requires that both the malleus-incus articulation and the D-SQ articulation be aligned more or less transversely. The mobile articulation of the malleus, incus, and cranium can rotate around the same transverse axis through the D-SQ joint. This is required for the mechanical function of the double joint, without which mandibular movement

would interfere with, or even disarticulate, the middle ear. This coaxial alignment of the middle ear and the surangular-squamosal or D-SQ contact is conserved in *Probainognathus* and mammalian morphs through stem mammaliaforms (**Figure 3b–f**) as well as in the Cretaceous mammals *Yanoconodon* and *Liaconodon* (Meng et al. 2011).

In living marsupials, the D-SQ joint is not yet developed at birth (Lillegraven 1975, Maier 1987). During nursing of neonates, the incus is mobile relative to the cranium and functions as an operational primary jaw joint until Meckel's cartilage is reabsorbed in later ontogeny. In the placental *Tupaia* (tree shrew), Meckel's cartilage is retained until postnatal day 14 and functions as a skeletal support for mandibular action (Zeller 1987). Homologies of the jaw hinge and middle ear of mammals are a well-supported case study in classical embryology: Meckel's and palatoquadrate cartilages of the first vertebrate branchial arch are the embryonic precursor to the malleus and incus of extant mammals as well as to the quadrate and the articular of other gnathostome vertebrates (Gaupp 1913, Takechi & Kuratani 2010) (**Figure 2e**). Shift of the functional hinge from the malleus-incus joint to the D-SQ joint during mammalian development is an example of ontogenetic recapitulation of phylogeny (Sánchez-Villagra et al. 2002).

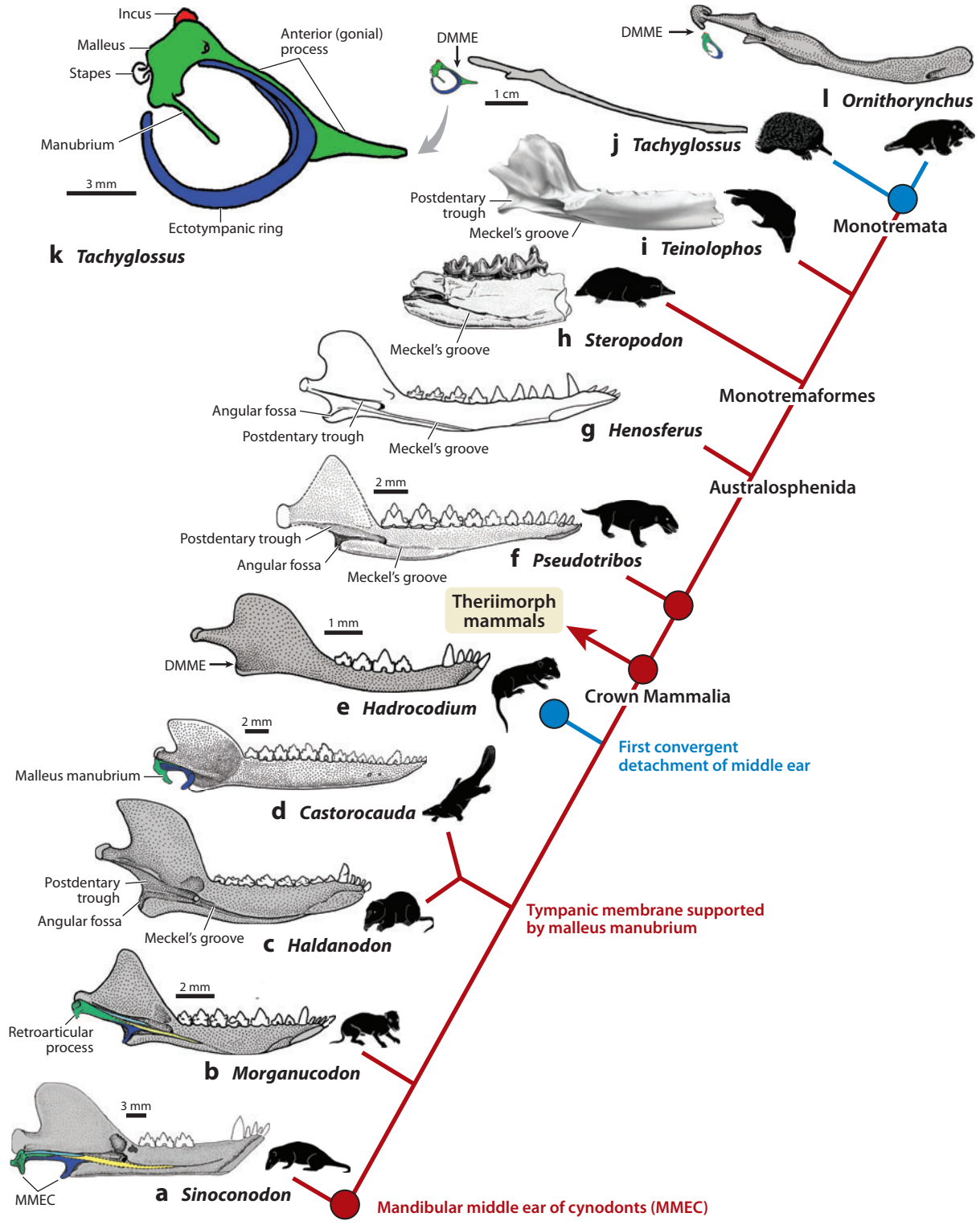
TRANSFORMATION FROM MAMMALIAMORPHS TO MAMMALS

Middle Ears in Transitional Mammalian Morphs

A highly mobile suspension of the middle ear to the cranium is indispensable for hearing sensitivity. This mechanical function is achieved in cynodont-mammal evolution by the loss of the quadratojugal of cynodonts (Luo & Crompton 1994) (**Figure 3**). In most cynodonts, the quadrate (incus) is ancestrally associated with the quadratojugal; the quadratojugal and quadrate together reinforce the jaw hinge. A trade-off is that the complex quadrate and quadratojugal association can limit the mobility of the incus, thereby reducing auditory sensitivity. The quadratojugal is present in most cynodonts, including tritylodonts and *Brasilodon* (Bonaparte et al. 2005, Sues 1986) (**Figure 3c,e**), but is lost in *Pachygenelus*, which is phylogenetically between tritylodonts and *Brasilodon* (Bonaparte et al. 2005, Kemp 2005, Liu & Olsen 2010, Martinelli & Rougier 2007), and in mammaliaforms.

Sound transmission from the tympanic membrane through the middle ear in extant mammals is facilitated by the stapedial process of the incus as an out-lever of the middle ear lever system for the impedance matching of airborne sound with the fluid-filled inner ear and for amplification of sound (Clack & Allin 2004, Durrant & Lovrinic 1995). The stapedial process is present in most mammaliaforms except *Sinoconodon* (**Figure 3e**) but is absent in most cynodonts except *Brasilodon* (**Figure 3d**). Both the mobility and the impedance matching of the incus (quadrate) are critical for hearing adaptation. However, these important apomorphies have incongruent distributions in the transitional taxa of cynodonts to mammaliaforms and conflict with each other on the current phylogenetic tree. The only scenario that can account for the conflicting distributions of these functionally important features is that separate evolutionary experiments to develop impedance-match levers and to enhance the mobility of the incus for hearing sensitivity occurred repetitively in multiple clades in cynodont-mammal evolution (**Figure 1a**).

The middle ear bones are more gracile and smaller in mammaliaforms than in cynodonts, and are presumably more sensitive for hearing (Allin & Hopson 1992, Rosowski 1992). These bones are held in the postdentary trough and still contact the mandible, as in cynodonts. Meckel's groove on the mandible accommodates Meckel's cartilage, which is continuous with the prearticular bone (MMEC in **Figures 2–4**) and partly homologous to the gonial element of the malleus in extant mammals (Zeller 1989).



A major apomorphy that appeared in some mammaliaforms is the manubrium of the malleus. *Sinoconodon* and *Morganucodon* have a short, ventrally pointed retroarticular process (**Figure 4a,b**). Restudy of well-preserved fossils confirms that *Morganucodon* certainly lacks a long retroarticular process, as putatively reconstructed by some previous studies (Kermack & Mussett 1983, Kermack et al. 1981). Rather, this structure is first seen in the Middle Jurassic mammaliaform *Castorocauda* (Ji et al. 2006).

Traditionally, the retroarticular process of cynodonts was considered to be the evolutionary precursor to the malleus manubrium (Allin & Hopson 1992, Kermack & Mussett 1983). However, recent fate-mapping studies of neural crest cells that give rise to the branchial arch structures show that the short process of the malleus (the lateral process of the human; **Figure 2g**) is derived from the second (hyoid) arch neural crest cells, in contrast to the remainder of the malleus, which is derived from neural crests of first (Meckel's) arch origin (O'Gorman 2005). In nonmammalian vertebrates, the retroarticular process of the articular is also derived from neural crest cells of hyoid arch origin (Gross & Hanken 2008, Köntges & Lumsden 1996). Because cell lineages of the retroarticular process become the short process of the mammalian malleus (O'Gorman 2005), this process in cynodonts should be homologous to the short process of the malleus, not the manubrium. The manubrium is best regarded as a neomorph in which its basal part first appeared in derived mammaliaforms (Ji et al. 2006) (**Figure 4d**). Because the manubrium is the central support for the tympanic membrane of extant mammals, its first appearance also suggests that a modern mammal-like tympanic membrane has already developed (**Figure 4d**). The presence of the malleus manubrium in *Castorocauda* is consistent with docodonts being more derived than *Sinoconodon* and *Morganucodon* in skeletal characters as well (Martin 2005).

The Early Jurassic mammaliaform *Hadrocodium* is the earliest form in which both the post-dentary trough and Meckel's groove for holding the middle ear are completely lost. Likely, its middle ear bones were already separated from the mandible (**Figure 4e**). The inferred separation of the middle ear from the mandible represents an independent acquisition of the DMME either in *Hadrocodium* (**Figure 4e**) or in the common ancestor of *Hadrocodium* and crown Mammalia (Luo et al. 2001b, Rougier & Wible 2006).

Middle Ears of Major Mesozoic Clades of Mammalia

Mammalia, or crown mammals, is a clade defined by the common ancestor of living monotremes, marsupials, and placentals (Rowe 1988). Their common ancestor is at least 170 million years old (Benton et al. 2009, Bininda-Emonds et al. 2007, Luo 2007a). More than 20 extinct orders or superfamilies of Mesozoic mammals have been recognized by distinctive, specialized dentitions. Some clades are also known to have distinctive locomotory adaptations, such as semiaquatic *Castorocauda* (Ji et al. 2006), gliding *Volaticotherium* (Meng et al. 2006), and burrowing *Fruitafossor* (Luo & Wible

Figure 4

Evolutionary transformation of the middle ear from extinct mammaliaforms through extant monotremes (mandibles in medial view unless noted otherwise). (a) *Sinoconodon*. (b) *Morganucodon*. (c) Docodont *Haldanodon* (redrawn from Lillegraven & Krusat 1991). (d) Docodont *Castorocauda* (lateral view), which is the earliest-known mammaliaform with a malleus manubrium. (e) *Hadrocodium*, the most primitive known mammaliaform without the postdentary trough and Meckel's groove, which suggest that the middle ear was detached from the mandible. (f) *Pseudotribos*. (g) Australosphenidan mammal *Henosferus* (from Rougier et al. 2007, with permission). (h) Monotremaform *Steropodon*. (i) Monotremaform *Teinolophos* (from Rich et al. 2005 with permission). (j,k) Monotreme *Tachyglossus*, which shows a mandible and a definitive mammalian middle ear (DMME) (in situ). (l) *Ornithorhynchus*, which shows a mandible separated from the middle ear (in situ, ventral view).

2005). With the exception of the monotreme, marsupial, and placental lineages that have living descendants, Mesozoic mammal clades are extinct, evolutionary dead ends (Kielan-Jaworowska et al. 2004; Luo 2007a,b), although phylogenetically nested in Mammalia by osteological and dental evidence (**Figure 1**). These Mesozoic clades, whether related to living monotremes or living therians, show the ancestral conditions of the MMEC or PMME.

The toothed monotremes *Steropodon* and *Teinolophos* of the Early Cretaceous are united with the crown Monotremata in the monotremaform clade, here defined by the common ancestor of *Steropodon* and extant monotremes (echidnas, platypus). Major groups associated with monotremaforms are the southern tribosphenic mammals and likely also pseudotribosphenidans, according to the majority of recent phylogenetic studies (Gurovich & Beck 2008, Kielan-Jaworowska et al. 2004, Luo et al. 2002, Martin & Rauhut 2005, Phillips et al. 2009, Rauhut et al. 2002, Rougier et al. 2007, Worthy et al. 2006), although some disagree (Woodburne 2004). Monotremaforms are nested in the australosphenidan clade, which, in turn, is the sister group to the pseudotribosphenic mammals on the basis of apomorphies of the mandible (Kielan-Jaworowska et al. 2002) and the shoulder girdle (Luo et al. 2007b).

Middle Ears in Monotremes and Their Kin

The earliest known monotremaforms, *Steropodon* and *Teinolophos*, have Meckel's groove, suggesting that Meckel's cartilage was connected to the mandible in some way after complete ossification of the mandible (**Figure 4b,i**). In the only fossil of *Steropodon*, the posterior part of the mandible is not preserved, so the relationship of the mandible and the middle ear per se is unknown. *Teinolophos* has a trough-like structure that likely accommodated the middle ear bones (Rich et al. 2005). However, the association of the middle ear with the mandible in *Teinolophos* has been challenged (Bever et al. 2005, Rougier et al. 2005, Rowe et al. 2008).

Three australosphenidan mammals have preserved both Meckel's groove and the postdentary trough on the mandible (Kielan-Jaworowska et al. 1998, Martin & Rauhut 2005, Rich et al. 1997, Rougier et al. 2007). Most noteworthy is that the Middle Jurassic *Henosferus* has an angular fossa on the mandible for the ectotympanic ring, as well as the postdentary trough with subdivided grooves, presumably for the discrete surangular and prearticular bones (Rougier et al. 2007) (**Figure 4g**). The pseudotribosphenic mammals *Shuotherium* and *Pseudotribos* show distinctive mandibular troughs for the middle ear (Chow & Rich 1982, Luo et al. 2007b). Overall, these monotreme-related mammals exhibit primitive, mammaliaform features of the mandible and the middle ear.

Because the middle ear attached to the mandible is ancestral for monotremaforms and their kin, the DMME of living monotremes (**Figure 4j-l**) is independently acquired (Rich et al. 2005), either after the split of extant monotremes from the stem monotremaforms *Steropodon* and *Teinolophos* or after the split of monotremaforms from the australosphenidan mammals. An alternative interpretation of the mandibular groove of *Teinolophos* (Bever et al. 2005, Rougier et al. 2005) could change where in the phylogeny of monotremaforms and their relatives this independent acquisition may have occurred, but not whether it occurred (Rougier & Wible 2006). An alternative placement of *Teinolophos* in the crown Monotremata (Rowe et al. 2008) or as a stem taxon for the extant monotremes (Phillips et al. 2009) is consistent with independent acquisition of the DMME in monotremaforms. By my own evaluation of the fossil, the current evidence favors that *Teinolophos* retained the mandibular attachment of the middle ear (also Hopson et al. 2009).

Middle Ears in Theria and Theriimorph Clade

The theriimorph clade (Rowe 1988), defined by the common ancestor of eutriconodonts, multituberculates, and spalacotheroids through living therians, has been corroborated independently

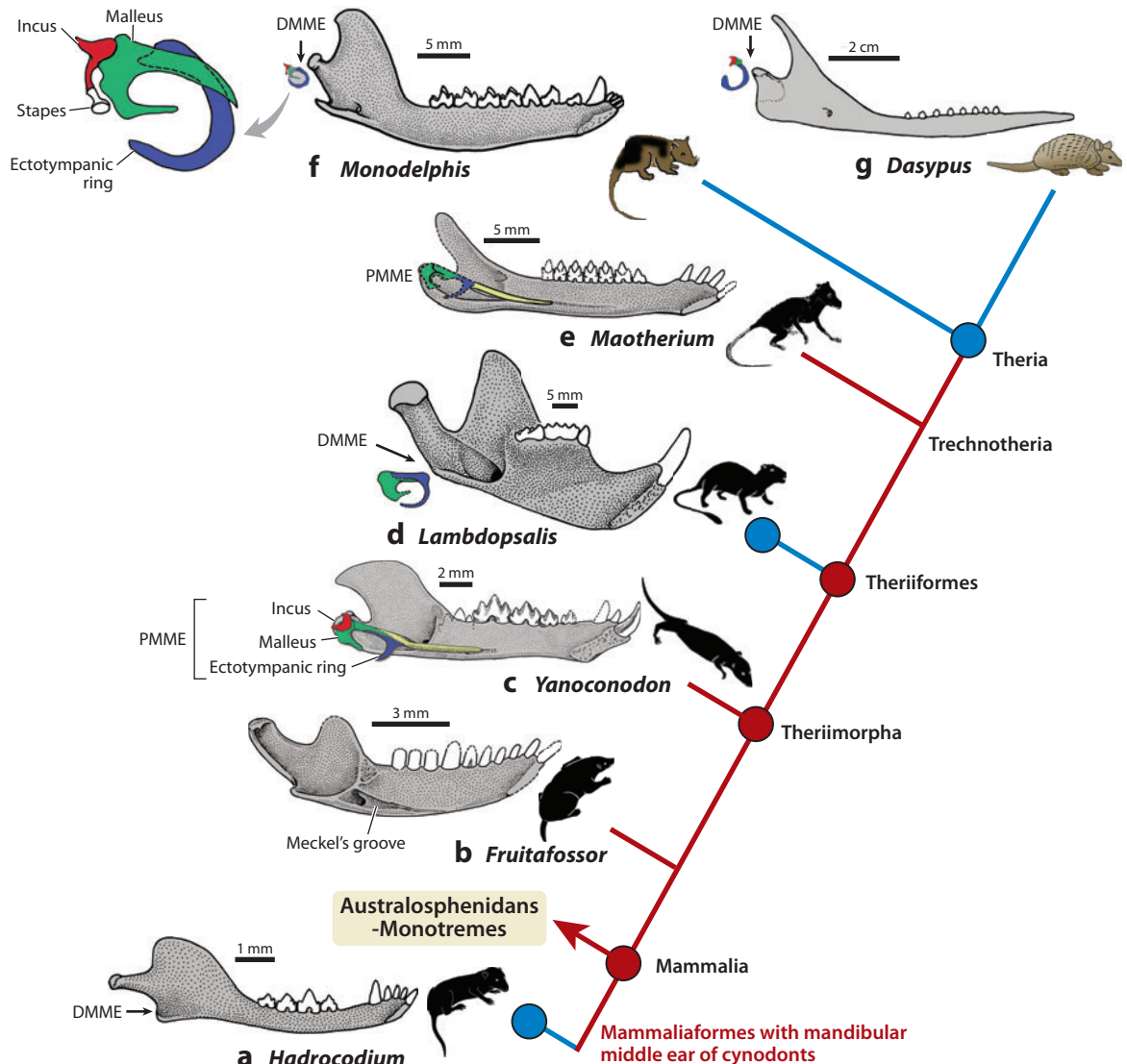


Figure 5

Mandible and middle ear of extant therians and their extinct relatives. (a) *Hadrocodium*. (b) Jurassic *Fruitafossor*. (c) *Yanacoconodon*. (d) Multituberculate *Lambdopsalis* (redrawn from Meng & Wyss 1995). (e) Cretaceous spalacotheroid *Maotherium*. (f) Marsupial *Monodelphis*. (g) Placental *Dasypus* (adapted from Wible 2010 with permission). Abbreviations: DMME, definitive mammalian middle ear; PMME, partial mammalian middle ear.

(e.g., Luo et al. 2007a,b; Rougier et al. 2007) (**Figure 5**). All groups of theriimorphs are relatively abundant in the fossil record. The putative sister group of theriimorphs is *Fruitafossor*, which has a unique pattern of a broadened Meckel's groove that also houses the mandibular foramen (**Figure 5b**) (Luo & Wible 2005).

Eutriconodonts. The eutriconodont clade (Gao et al. 2010) is a diverse Mesozoic mammal group of entirely extinct taxa from the Middle Jurassic to the Late Cretaceous

(Kielan-Jaworowska et al. 2004). Although the embryonic Meckel's cartilage is absorbed in the adults of all extant mammals, several Cretaceous eutriconodonts, members of crown Mammalia, are now known for having a Meckel's cartilage that was permanently ossified in adults (**Figure 6**), which suggests that their middle ear must have connected to the mandible, even if the middle ear itself is unknown (Li et al. 2001, Li et al. 2003, Meng et al. 2003, Wang et al. 2001). The eutriconodont *Yanoconodon* further revealed that the ectotympanic ring and the malleus are connected to the ossified Meckel's cartilage, and these elements are large relative to the mandible (**Figures 6 and 7**) (Luo et al. 2007a). The overall large size of the middle ear makes it possible for its articulation to the cranium to be coaxial, or aligned with the D-SQ joint, as is required for the middle ear to transmit sound without interference by movement at the D-SQ jaw joint. *Yanoconodon* has a Meckel's groove but no postdentary trough; nonetheless, its PMME is still

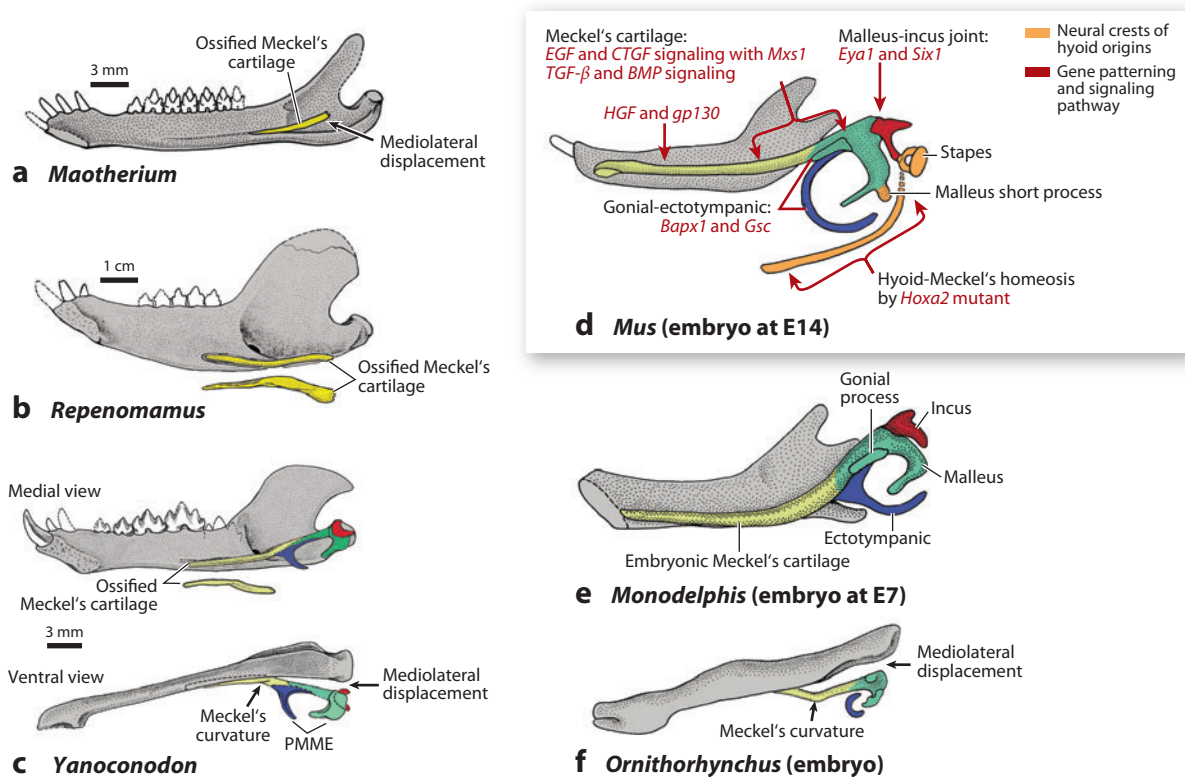


Figure 6

Paedomorphic similarity of Mesozoic mammals to embryos and neonates of extant mammals and gene patterning of the mammalian ear. (a) *Matherium asiaticus*. (b) Gobiconodontid *Repenomamus* (redrawn from Li et al. 2001). (c) *Yanoconodon*. (d) Gene patterning of morphogenesis of the mandibular arch (branchial arch 1) and the hyoid arch (branchial arch 2) in the normal development of placental *Mus* [wild type, embryonic day (E)14] and the approximate areas of phenotypic expression of genes and signaling pathways known to have impacted ear development (composite from various sources). Brown indicates the neural crest's contribution to the short process of malleus from the hyoid arch derivation. (e) *Monodelphis* (E7). (f) Monotreme *Ornithorhynchus* (ventral view, embryo). The large and ossified middle ear in *Yanoconodon* and the ossified Meckel's cartilage of *Repenomamus* and *Matherium* show paedomorphosis to the embryonic and neonatal condition of extant mammals. Drawings not to the same scale. Abbreviations: *BMP*, bone morphogenetic protein; *CTGF*, connective tissue growth factor; *EGF*, epidermal growth factor; *TGF- β* , transforming growth factor- β ; *HGF*, hepatocyte growth factor; *gp130*, glycoprotein 130.

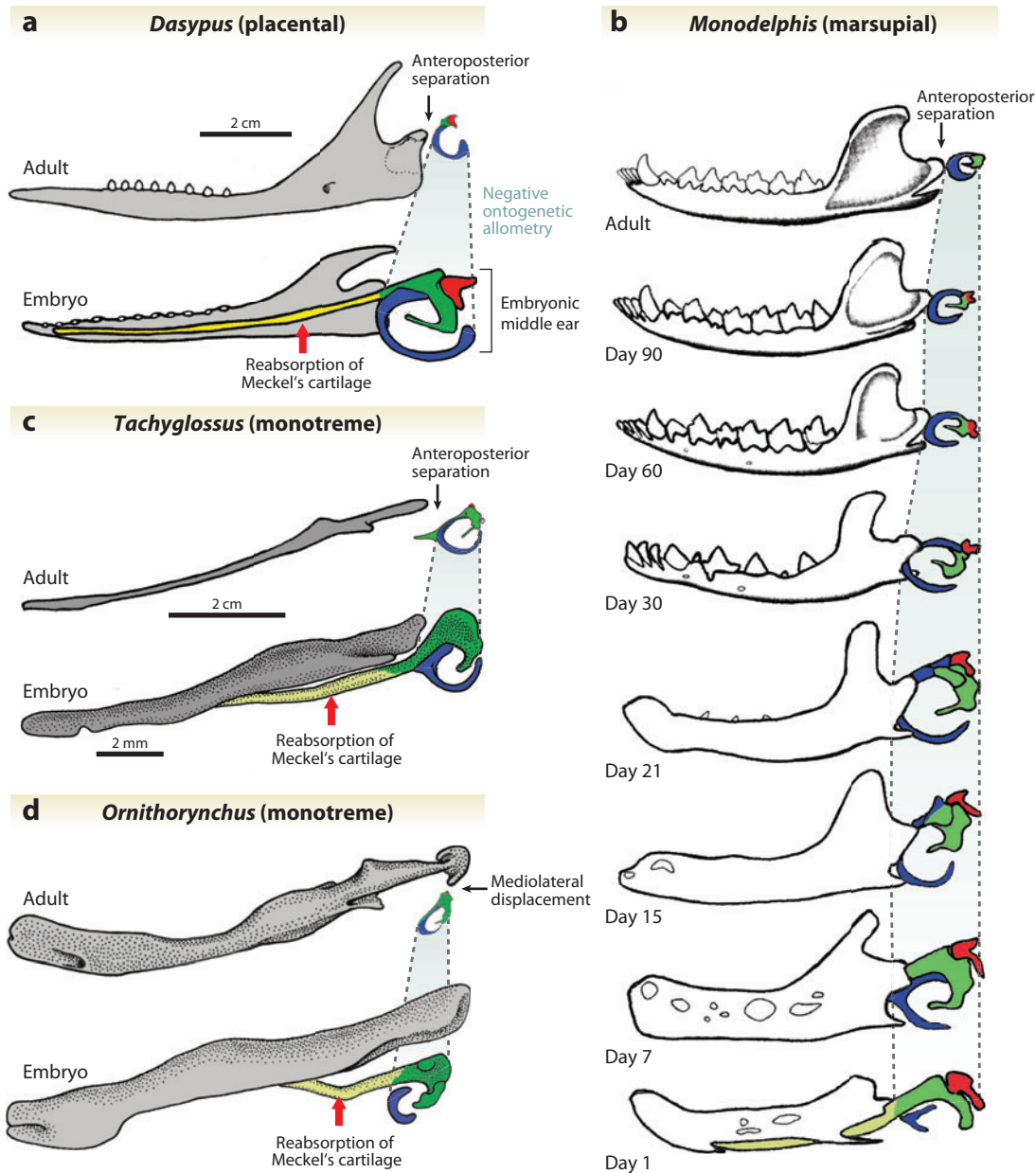


Figure 7

Allometry and displacement in development of the middle ear in extant mammals. (a) Placental *Dasyurus* mandible and middle ear in medial view [stylistic illustration of embryo (bottom) based on Reinbach 1952; adult (top) based on Wible 2010]. (b) *Monodelphis* (from Rowe 1996 with permission). (c) Monotreme *Tachyglossus* [ventral view; embryo (bottom) redrawn from Kuhn 1971]. (d) Monotreme *Ornithorhynchus* [ventral view; embryo (bottom) redrawn from Zeller 1989]. Development of the middle ear in extant mammals is facilitated by three morphogenetic processes: first, reabsorption of Meckel's cartilage connecting the middle ear to the mandible in neonatal stages; second, negative ontogenetic allometry (shading pattern) of the middle ear to the mandible, as the middle ear is much larger relative to the mandible in the embryo or neonate than in adult; and third, mediolateral displacement and/or anteroposterior separation of the middle ear from the mandible; the former occurs in therians and the monotreme *Tachyglossus* (but not *Ornithorhynchus*), whereas the latter occurs in monotremes and placentals.

connected to the mandible through the ossified Meckel's cartilage. *Jeholodens* (Ji et al. 1999), which is closely related to *Yanoconodon*, also has an ossified Meckel's cartilage, although it is loose from the mandible owing to postmortem dissociation. Implicit in many studies of the 1980s and 1990s is an assumption that absence of the postdentary trough on the mandible implies a complete separation of the middle ear from the mandible. The new eutriconodonts show that, although the presence of the postdentary trough is sufficient for inferring the presence of the mandibular middle ear, the absence of the postdentary trough is not sufficient to exclude the possibility of the middle ear attached by Meckel's cartilage to the mandible (Ji et al. 2009).

Multituberculates. Multituberculates are omnivorous to herbivorous mammals with a long history from the Middle Jurassic to the late Eocene; they are also abundant in some fossil assemblages of the Late Jurassic and the Late Cretaceous (Kielan-Jaworowska et al. 2004). The middle ear was first discovered in the Paleocene multituberculate *Lambdopsalis* (Meng & Wyss 1995, Miao & Lillegraven 1986) (**Figure 5d**). This was followed by more discoveries of the middle ear in the Cretaceous *Chulsanbaatar*, *Nemegtbaatar*, and *Kryptobaatar* (Hurum et al. 1996, Rougier et al. 1996). In multituberculates, the middle ear bones are completely separated from the mandible and are attached only to the cranium. Some characteristics of their middle ear articulation with the cranium are similar to those of monotremes (Hurum et al. 1996).

The broader evolutionary implications of the DMME in multituberculates depend on alternative hypotheses about their relationships to putative relatives. Multituberculates are more closely related to therians than to eutriconodonts, and multituberculates and therians are united in the theriiform clade (Luo et al. 2002, Rowe 1988). However, some paleontologists have contended that multituberculates are close relatives to the Triassic haramiyidans (Butler 2000). Haramiyidans have a mandibular trough and Meckel's groove for the middle ear; in these they are different from multituberculates (Jenkins et al. 1997).

On the assumption that multituberculates are nested with haramiyidans, the DMME is an independent acquisition in multituberculates that occurred after the split of the haramiyidan-multituberculate clade from other mammaliaforms. Given the current evidence, the DMME of multituberculates would be best regarded as an independent acquisition (**Figure 5d**) regardless of the putative haramiyidan-multituberculate hypothesis.

Spalacotheroids. Spalacotheroids are Mesozoic relatives of recent marsupials and placentals that are characterized by an acutely triangled molar cusp pattern for insectivorous feeding. The spalacotheroids and the crown Theria (marsupials and placentals) together are called Trechnotheria (**Figure 1, 5e**). Spalacotheroids represent the ancestral conditions from which marsupials and placentals likely evolved (Hu et al. 1997, Li & Luo 2006, Sweetman 2008). The spalacotheroid *Maotherium asiaticus* has an ossified Meckel's cartilage solidly held by Meckel's groove (**Figure 5e**) (Ji et al. 2009). The ossified Meckel's cartilage is identical to those of *Gobiconodon* and *Yanoconodon* (Li et al. 2003, Luo et al. 2007a) but more gracile than those of *Liaconodon* and *Repenomamus* (Meng et al. 2003, 2011). *Maotherium* is similar to *Yanoconodon*, which also exhibits the connection of the ossified Meckel's cartilage to ear bone; its ear is here interpreted as having an ossified connection to the mandible (**Figure 5e**). The spalacotheroid *Zhangheotherium* also has an ossified Meckel's cartilage, although displaced from its original anatomical position (Meng et al. 2003).

Maotherium shows a curvature in the middle part of Meckel's cartilage so that its posterior part is separated medially from the mandible, whereas its anterior part is nestled in Meckel's groove on the mandible (Ji et al. 2009). This is similar to the embryonic conditions of monotremes and to adult eutriconodonts (**Figure 6**). *Maotherium*, *Yanoconodon*, and *Repenomamus* all corroborate that the PMME was already medially displaced some distance from the mandible (**Figures 2 and 6**).

Homoplasies in Partial and Definitive Mammalian Middle Ears

As the above sections show, all extant members of the monotreme lineage and the marsupial-placental lineage have achieved full separation of the middle ear from the mandible (DMME); however, all basal clades of Mammalia in the Mesozoic have retained either the plesiomorphic MMEC or the PMME that is the precursor to DMME. The distribution of middle ear characters in the newly discovered fossils of these basal groups shows a much wider range of middle ear morphologies than in the extant Mammalia and reveals conspicuous cases of homoplasy.

In Hypothesis A (**Figure 8**, left nodes 2, 3), the DMME is considered to be absent in the common ancestor of monotremes, eutriconodonts, and extant therians, but DMME evolved separately in extant monotremes and then in living therians. This gains support from an interpretation that the fossil monotreme *Teinolophos* retained the cynodont-like ancestral condition of the post-dentary trough for the MMEC (Rich et al. 2005), and from the evidence of this trough in the related monotremaform taxa (Luo et al. 2007b, Martin & Rauhut 2005, Rougier et al. 2007). The theriiiform clade of multituberculates and extant therians (left node 7) has achieved the DMME from the PMME of some theriiiform ancestors (Meng et al. 2011), only to have it reverse back to the PMME in *Maotherium* inside the theriiiform clade (left node 8). These interpretations are compatible with *Hadrocodium* acquiring the middle ear separately from extant monotremes and from extant therians (left node 2).

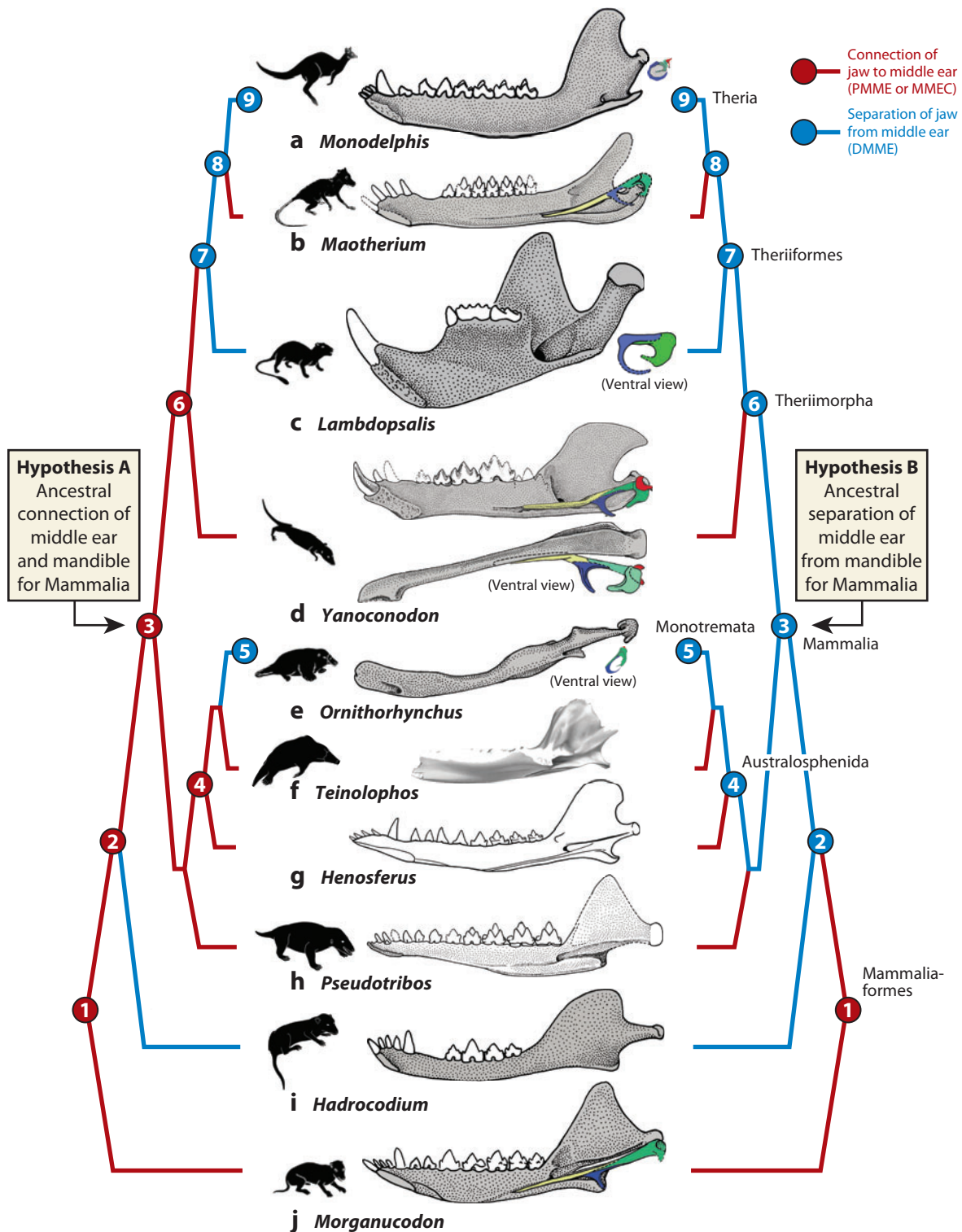
In hypothesis B (**Figure 8**, right nodes 2, 3), full separation was already accomplished in the common ancestors of *Hadrocodium* and extant Mammalia. If so, then this mammalian condition must have been reversed twice in the theriiiform lineage: first in eutriconodonts (right node 6) and then again in spalacotheroids (right node 8). It was also reversed in monotremes and their relatives at least twice: in *Pseudotribos* and in australosphenidans such as *Henosferus*, or as many as three times if *Teinolophos* had the mandibular middle ear as argued by some (Rich et al. 2005).

Both hypotheses encounter some difficulties owing to the conflicting characters in several fossil taxa occupying crucial positions in mammaliaform phylogeny. But under either hypothesis, it is abundantly clear that there have been multiple acquisitions of DMME, coupled with at least two reversals from DMME, in the fully resolved evolutionary tree by parsimonious mandate of all characteristics independent of the middle ear (**Figure 8**).

EMBRYONIC DEVELOPMENT OF MAMMALIAN MIDDLE EARS

In extant mammals, the embryonic precursors of the malleus and incus are differentiated from the posterior ends of Meckel's cartilage and the palatoquadrate cartilages, respectively. The malleus and the ectotympanic ring are connected to the mandible by Meckel's cartilage of the first branchial arch until separation from the mandible in neonatal stages (**Figure 7**) (Evans 1993; Gaupp 1913; Kuhn 1971; Maier 1993; Sánchez-Villagra et al. 2002; Zeller 1987, 1989). The embryonic middle ear shows significant morphological variability among the main groups of extant mammals (De Beer 1937), but nonetheless, four basic morphogenetic processes contributed to the separation of the middle ear in all extant mammals. First, the anlagen of ear bones are medially displaced from the mandible, as seen in monotremes (**Figure 6e**) and in most placentals (Zeller 1987), although this is less obvious in marsupials (Maier 1993). The medial displacement of the middle ear from the mandible in early embryos is a primitive pattern of extant mammals as a whole, and the lack of this in marsupials is derived (Maier 1993).

Second, the middle ear can show an anteroposterior separation from the D-SQ jaw joint during development. This displacement in the posterior direction is associated with the enlargement of the braincase and is crucial for detaching the middle ear from the mandible and from the jaw joint,



as in the embryogenesis of most mammals (**Figure 7**) (Rowe 1996), although not in the platypus monotreme (**Figure 7d**).

Third, the middle ear displays a negative ontogenetic allometry relative to the size of the mandible and the cranium (especially the braincase) (Rowe 1996, Zeller 1987). In early embryonic stages, the primordial ear bones are large relative to the mandible (**Figure 7**); they are ossified early and terminate their growth much earlier than other skull structures. With size fixed upon ossification, in subsequent development they show negative allometry to the more prolonged growth of the mandible and cranium that continues long after the middle ear is ossified. An earlier timing of ear bone ossification is critical for achieving this negative ontogenetic allometry and is fundamental to the normal formation of the DMME. It follows that, if this ontogenetic allometry is disrupted, the middle ear becomes incapable of separating from the mandible.

Displacement and negative allometry of ear bones are variable, and their impact can differ in monotremes, marsupials, and placentals. Their variability is consistent with the overall differences in the timing of craniofacial development between placentals and marsupials (Smith 1997). The negative allometry of the ear bones to the mandible is prominent in placentals and marsupials but far less manifest in the echidna monotreme (**Figure 7**). The medial displacement and posterior separation of the middle ear from the mandible also occur differently in monotremes. In *Ornithorhynchus* (the platypus), Meckel's cartilage and the primordial ear bones are displaced medially from the mandible from the start of embryogenesis. In *Tachyglossus*, the primordial ear is not only displaced medially but also positioned more posteriorly from the dentary condyle in both embryo and adult. In both monotremes, the embryonic position of the ear bones relative to the mandible remains unchanged in the adult after the reabsorption of Meckel's cartilage. In contrast, the position of the embryonic middle ear relative to the mandible is changed to a much greater extent in placentals and marsupials, especially for a greater anteroposterior separation of the middle ear in ontogeny, as convincingly demonstrated for the marsupial *Monodelphis* (Rowe 1996) (**Figure 7b**).

Fourth, Meckel's cartilage must be reabsorbed for the middle ear to be physically disconnected from the mandible. The embryonic Meckel's cartilage may be maintained by the biomechanical function of the mandible in neonatal stages. Herring (1993) proposed that its reabsorption is accompanied by the loss of this biomechanical function, as the operational jaw hinge shifts from the embryonic Meckel's cartilage to the D-SQ joint in neonates. Reabsorption of Meckel's cartilage is patterned by a network of signaling pathways and genes that control the development of vertebrate skulls (Chai & Maxson 2006, Takechi & Kuratani 2010). The mandibular mechanical function

Figure 8

Homoplastic evolution of the definitive mammalian middle ear (DMME) in Mesozoic mammals according to developmental heterochrony and gene patterning. (a) Extant therians (such as kangaroo and *Monodelphis*). (b) Spalacotheroid *Maotherium*. (c) Multituberculata *Lambdopsalis*. (d) Eutriconodont *Yanoconodon*. (e) Monotreme *Ornithorhynchus*. (f) Monotremiform *Teinolophos*. (g) Australosphenidan *Henosferus*. (h) Pseudotribosphenic *Pseudotribos*. (i) Mammaliaform *Hadrocodium*. (j) Mammaliaform *Morganucodon*. Phylogenetic nodes: ① Mammaliaformes, ② clade of *Hadrocodium* through extant therians, ③ crown Mammalia, ④ Australosphenida clade (Luo et al. 2001a), ⑤ crown Monotremata, ⑥ Theriiform clade (Rowe 1988), ⑦ Theriiformes clade (Rowe 1988), ⑧ Trechnotherian clade (*Maotherium* and other spalacotheroids through extant therians), and ⑨ crown Theria. Hypothesis A: On the assumption that the middle ear connected to the mandible represents an ancestral condition of some mammaliaforms and Mammalia (left nodes ②, ③), the acquisition of the DMME must have occurred three times. Hypothesis B: On the assumption that a full separation of the middle ear from the mandible is the ancestral condition of the clade of *Hadrocodium* (right node ②) through extant Theria (right node ⑨), the reconnection of the mandible to the middle ear must have evolved multiple times by reversal. Blue lines and nodes represent the derived evolutionary condition; the red nodes and lines are the primitive condition of the middle ear.

(Herring 1993), displacement by the growing braincase (Rowe 1996), and gene patterning all play some role in the reabsorption of Meckel's cartilage to form the middle ear, but the patterning by many genes, all of which have been characterized relatively recently, clearly impacts middle ear morphogenesis at a more fundamental level than the purely mechanical processes in the ontogeny of Meckel's cartilage. The causes for the loss of Meckel's cartilage are hierarchical.

Gene Patterning of Morphogenesis of Meckel's Cartilage and Middle Ear

Meckel's cartilage of the first branchial arch derives from cranial neural crest cells and serves as scaffolding for the development of mandibular and middle ear elements. Normal development of Meckel's cartilage includes its eventual reabsorption. Embryogenesis of its derivatives—ear bones—requires many structural and some homeobox genes (**Figure 6**). Genes expressed in the development of the middle ear include *Emx2*, *Msx1*, *Sox9*, *Bapx1*, *Gsc*, and *COL2A1* (Amin & Tucker 2006, Kuratani et al. 1999, Rhodes et al. 2003, Santagati et al. 2005). Morphogenesis of Meckel's cartilage in mammals also requires many growth factors. This complex signaling network includes *Transforming growth factor- β* (*Tgf- β*) and its receptor (Ito et al. 2002, Oka et al. 2007), *Connective tissue growth factor* (*Ctgf*) (Ivkovic et al. 2003), *Fibroblast growth factor* (*Fgf*) (Tucker et al. 2004), *Epidermal growth factor* (*Egf*) (Shum et al. 1993), and *Bone morphogenetic protein* (*Bmp*), among others (Choi et al. 2007). The normal development of the hyoid cartilage (of the second branchial arch) requires *Hoxa2*. The *Hoxa2*-null mutant shows homeotic changes of the hyoid elements into Meckel's cartilage structures (Santagati et al. 2005, Takechi & Kuratani 2010) (**Figure 6**).

Meckel's cartilage, the ectotympanic ring, and the anterior (gonial) element of the malleus are patterned by at least two signaling pathways. The first is the *Fgf8-Bmp4* pathway and the *Bapx1* gene, which mediate the ossification of the ectotympanic ring and the anterior (gonial) element as well as influence the variability of the ectotympanic ring and the malleus in size and thickness (Tucker et al. 2004). The second is by the large *Tgf- β* gene family. Oka et al. (2007) showed convincingly the impact of *Tgf- β* gene patterning of Meckel's cartilage. Meckel's cartilage and the surrounding structures are patterned by the *Tgf- β* (upstream) to *Ctgf* (downstream) pathway and the *Msx1* gene (**Figure 6**). Null mutation in any step of this developmental genetic pathway can cause a premature ossification of Meckel's cartilage in embryogenesis, resulting in permanent connection of the middle ear to the mandible. In normal chondrogenesis of Meckel's cartilage of the placental mammal *Mus* (wild type), the *Tgf- β* to *Ctgf* signaling pathway stimulates the proliferation and inhibits the terminal differentiation of chondrocytes in Meckel's cartilage (Ito et al. 2002, Ivkovic et al. 2003, Oka et al. 2007). *Tgfb β 2^{fl/fl};Wnt1-Cre* mutants (of *Tgf- β*) accelerate chondrocyte proliferation and cause ossification of Meckel's cartilage in mutant *Mus* (Oka et al. 2007).

The phenotype of ossified Meckel's cartilage in *Tgfb β 2^{fl/fl};Wnt1-Cre* mutant mice is similar to the ossified Meckel's cartilage fossilized in eutriconodonts and *Maotherium* (**Figure 8b**). This suggests that some similar developmental pathway, such as *Tgf- β* - and/or *Ctgf*-mediated control of chondrocyte proliferation, impacted the morphogenesis of the middle ear in Mesozoic eutriconodont and spalacotheroid mammals. In Mesozoic mammal evolution, the junction of Meckel's cartilage to the ectotympanic ring and the anterior part of the malleus makes a crucial difference in whether the middle ear separated from the mandible or remained connected as the PMME, as in eutriconodonts and *Maotherium*. This difference in fossils is now significantly attributable to these signaling pathways and genes (e.g., Kuratani et al. 1999, Oka et al. 2007, Tucker et al. 2004).

Genes for morphogenesis of the DMME and Meckel's cartilage are also known to control the morphogenesis of the mandible in birds and zebrafish (Eames & Schneider 2008, Tucker et al. 2004). The signaling pathway and gene regulatory network (GRN) of the branchial arches,

which include *Fgf* and *Bmp*, are ancestral and universal for all vertebrates with jaws (Chai & Maxson 2006). The distinctive pedomorphic pattern of Meckel's cartilage in eutriconodonts and spalacotheroids can be attributed to the GRN that is operative in all vertebrates. These genes and pathways belong to the "plug-in" subcircuits in hierarchical developmental GRNs (dGRNs) that are repetitively redeployed throughout metazoan evolution and serving as a recurrent mechanism for evolutionary novelties including homoplasies in mammalian middle ears (Erwin & Davidson 2009).

Knockout genetic experiments are sufficient to demonstrate that development of given phenotypic characters, some of which are seen in Mesozoic mammals, require normal function of a gene. However, most developmental genes are pleiotropic, and their mutants can have a cascade of abnormal and ectopic structures that are neither in the fossil record nor in the normal development of extant mammals (Smith & Schneider 1998). Mutation of individual genes, no matter how powerful, does not represent a recapitulation or atavistic reversal of evolution. Rather, it can show in a general way that some genes are involved in patterning of the ear in extant mammals and that such gene patterning can be extrapolated as a mechanism for the phylogenetic evolution documented by fossils.

Mapping Development to Fossil Phylogeny

On a broad scale, transformation of the mandibular ear in premammalian synapsids appears to be an orderly evolution of a major structure that sheds light on how a complex structure was assembled in phylogeny (Allin 1975, Crompton 1972, Luo 1994, Sidor 2001). However, new and well-preserved fossils from the past 10 years, beginning with the eutriconodont *Repenomamus* and the mammaliaform *Hadrocodium* (Li et al. 2001, Luo et al. 2001b), have shown clearly that the detachment of the middle ear from the mandible occurs repeatedly and should be interpreted as a frequent homoplasy within crown mammals. Recent discovery of the brasilodontid cynodonts (Bonaparte et al. 2005, Luo 2007b) also adds to the complexity in evolution toward a greater mobility of the middle ear (**Figures 1 and 3**). This assessment is fundamentally based on a robust phylogeny of all mammaliaforms (Bonaparte et al. 2005; Liu & Olsen 2010; Luo & Wible 2005; Luo et al. 2007a,b; Martin & Rauhut 2005; Rougier et al. 2007). Similarly, regardless of how patterns of ear evolution are mapped on a fully resolved evolutionary tree by parsimony of all characteristics of all Mesozoic and extant mammals, the middle ears of *Maotherium*, gobiconodontids, and *Yanoconodon* exhibit multiple acquisitions of derived conditions, plus some reversals (**Figure 8**). Labile evolution of these middle ear characteristics may be facilitated by labile developmental patterns in the hierarchy of gross morphology, cell and tissue interaction, and GRNs:

1. At the morphological level, the homoplasies of middle ears are directly correlated with pedomorphosis, the retention of fetal or juvenile characteristics of ancestors or phylogenetic relatives through developmental heterochrony (Luo et al. 2007a). Heterochrony is a common mechanism in vertebrate evolution. The relatively larger middle ear bones and ossified Meckel's cartilage of *Yanoconodon* and *Liaconodon*, for example, bear strong pedomorphic similarity with the middle ear and Meckel's cartilage of extant mammalian embryos (**Figure 6**). The premature ossification of Meckel's cartilage in *Yanoconodon*, *Liaconodon*, and *Maotherium* represents a simple change in timing in ontogeny, or heterochrony, by comparison with extant mammals.
2. At the cellular and tissue level, the labile middle ear evolution is consistent with the fundamental variability in the neural crest contribution to the branchial arches, as shown by fate mapping of osteogenic cranial neural crest cells (Gross & Hanken 2008). The relative

contributions of neural crest and mesoderm to the bony skull are variable among the major vertebrate groups and evolutionarily labile.

3. At the genetic level, the genes and pathways for mammalian ear development all belong to the subcircuit rank in hierarchical dGRNs. Changes in GRNs can impact evolution. Erwin & Davidson (2009) postulated that many signaling pathways and homeobox genes are plug-in subcircuits, a fundamental part of the architecture of the GRNs of all eumetazoans, including mammals. They have been redeployed throughout the evolution in different lineages of eumetazoan animals. These ancient subcircuits, such as the *Tgf-β*-to-*Ctgf* pathway and *Hoxa2*, must have been co-opted, repetitively, into the ossification of Meckel's cartilage, and they cause the arrest of the negative ontogenetic allometry, which is otherwise typical of mammals. This is likely a key mechanism for the homoplastic evolution in Meckel's cartilage and the mammalian middle ear.

The morphological mechanism for retaining the middle ear's connection to the mandible is simple. The paedomorphic resemblance of *Maotherium* and eutriconodonts to the embryos of modern mammals can occur by a simple change of timing in (premature) ossification of Meckel's cartilage. The reabsorption of Meckel's cartilage, as seen in the embryonic or fetal stages of extant mammals, simply did not happen owing to a change that is not unlike the experimentally altered *Tgf-β* to *Ctgf* pathway (Oka et al. 2007) or *Fgf5-Bmp4* pathway (Tucker et al. 2004). Furthermore, the middle ear bones also must have ossified late relative to the developmental timing of the mandible such that these bones never had negative ontogenetic allometry.

The homoplastic separation of the middle ear from the mandible in Mesozoic mammals appears complex on a well-resolved phylogenetic tree (**Figure 8**). But now it can be correlated with ontogenetic heterochrony, with the newly discovered variability of the neural crest precursors, and with labile changes in gene patterning, all of which are universal in vertebrate development. Extrapolating these common developmental patterns of extant mammals into the fossil record can illuminate the driving mechanism for a similar evolution to occur multiple times.

In return, the fossil record can provide phylogenetic context for developmental homoplasies. In the case of the DMME, the labile phase of evolution with multiple homoplasies to reconnect the middle ear to the mandible via ossified Meckel's cartilage is found only in eutriconodonts and spalacotheroids, both of which are basal in the mammal phylogenetic tree. No example is known after the rise of the more derived extant marsupials and placentals and their immediate relatives, presumably because the evolution of the coiled cochlea in the inner ear in advanced therians (Luo et al. 2010, Ruf et al. 2009) ended the labile evolution of the mammalian middle ear (Martin & Ruf 2009). Among living mammals, an ossified or retained Meckel's cartilage occurs only in certain mutant mice and in pathological cases among humans (Herring 1993). However, ossified Meckel's cartilage evolved at least twice in the PMME in Mesozoic mammals. The absence of ossified Meckel's cartilage in adults of extant monotremes, marsupials, and placentals represents a more canalized development of the middle ear for these living lineages. By contrast, a much more labile evolutionary development of middle ear features was made possible by the vastly wider range of evolutionary experimentations in the greater diversity of approximately 20 Mesozoic mammaliaform clades (Kielan-Jaworowska et al. 2004, Luo 2007b, Martin 2006). The developmental impact on evolution, especially its plasticity, can fully manifest only by repetitive experiments in abundant branches on an exuberant phylogenetic tree of deep history.

COMMENTS AND CONCLUSIONS

Evolution of the DMME and its related jaw hinge is homoplastic in the cynodont-mammal transition and in the diversification of major Mesozoic mammal clades. This shows that a complex

structure can be labile in evolution and no less susceptible to homoplasy (Martin & Luo 2005). The greater lineage diversity in Mesozoic mammals provided a greater possibility for evolutionary experimentation with unique structures, such as PMME, which would have been unimaginable from the more limited morphological disparity of extant mammals. The vast range of lineages in immense, deep geological time has made it possible for mammals to undergo repetitive evolutionary experiments. The repetitive, homoplastic evolution of the mammalian middle ears can now be correlated unambiguously with the phenotypic effects of changes in genes and signaling pathways in the development of extant mammals. This demonstrates that developmental changes are major mechanisms of complex morphological transformation in mammals, as is universal in animal evolution (Carroll 2005).

SUMMARY POINTS

1. New fossil discoveries show evolutionary homoplasies in structures of the mammalian jaw joint, in features related to middle ear mobility, and in independent acquisitions of DMMEs in Mesozoic mammals. This shows that complex novelties can be homoplastic and labile in mammaliaform evolution.
2. It can now be hypothesized that the labile evolution of the mammalian middle ear, as revealed by fossil discoveries of the past 10 years, is correlated with the generally labile patterns of paedomorphosis, variable contributions of neural crest cells to branchial arch precursors to the adult ear bones, and labile patterning of many genes and signaling pathways for morphogenesis of the ear.
3. Mesozoic mammaliaforms are diverse, and their family tree is an exuberant evolutionary bush. Origins and extinctions of abundant mammaliaform clades made it possible for many evolutionary experiments to occur in the long Mesozoic history of mammals and have facilitated labile developmental mechanisms to generate homoplastic structures repeatedly.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

On topics regarding evolution of the mammalian jaw hinge and middle ear, I had many discussions with Edgar Allin, Rob Asher, Chris Beard, Yang Chai, Rich Cifelli, Aaron Clauset, Fuzz Crompton, Mary Dawson, Doug Erwin, Jim Hopson, Manyuan Long, Thomas Martin, Jin Meng, Guillermo Rougier, Irina Ruf, Tim Rowe, Abigail Tucker, and John Wible. I want to thank Mary Dawson, John Wible, and Doug Futuyma for their great help in improving the manuscript and my collaborators Qiang Ji, Peiji Chen, Gang Li, and Chongxi Yuan for the opportunity to study the extraordinary fossils that stimulated the new hypotheses discussed in this paper. Support from the National Science Foundation (United States), the Senior Scientists Award by the Humboldt Foundation (Germany), and the National Natural Science Foundation (China) is acknowledged.

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