EVOLUTION & DEVELOPMENT 4:6, 445–458 (2002)

Limbs in whales and limblessness in other vertebrates: mechanisms of evolutionary and developmental transformation and loss

Lars Bejder* and Brian K. Hall[1]

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

*Author for correspondence (e-mail: lbedjer@is2.dal.ca)

[1] Both authors contributed equally to this article.

SUMMARY We address the developmental and evolutionary mechanisms underlying fore- and hindlimb development and progressive hindlimb reduction and skeletal loss in whales and evaluate whether the genetic, developmental, and evolutionary mechanisms thought to be responsible for limb loss in snakes "explain" loss of the hindlimbs in whales. Limb loss and concurrent morphological and physiological changes associated with the transition from land to water are discussed within the context of the current whale phylogeny. Emphasis is placed on fore- and hindlimb development, how the forelimbs transformed into flippers, and how the hindlimbs regressed, leaving either no elements or vestigial skeletal elements. Hindlimbs likely *began* to regress only after the ancestors of whales entered the aquatic environment: Hindlimb function was co-opted by the undulatory vertical axial locomotion made possible by the newly evolved caudal flukes. *Loss* of the hindlimbs was associated with elongation of the body during the transition from land to water. Limblessness in most snakes is also associated with adoption of a new (burrowing) lifestyle and was driven by developmental changes associated with elongation of the body. Parallels between adaptation to burrowing or to the aquatic environment reflect structural and functional changes associated with the switch to axial locomotion. Because they are more fully studied and to determine whether hindlimb loss in lineages that are not closely related could result from similar genetically controlled developmental pathways, we discuss developmental (cellular and genetic) processes that may have driven limb loss in snakes and leg-less lizards and compare these processes to the loss of hindlimbs in whales. In neither group does ontogenetic or phylogenetic limb reduction result from failure to initiate limb development. In both groups limb loss results from arrested development at the limb bud stage, as a result of inability to maintain necessary inductive tissue interactions and enhanced cell death over that seen in limbed tetrapods. An evolutionary change in *Hox* gene expression—as occurs in snakes—or in *Hox* gene regulation—as occurs in some limbless mutants—is unlikely to have initiated loss of the hindlimbs in cetaceans. Selective pressures acting on a wide range of developmental processes and adult traits other than the limbs are likely to have driven the loss of hindlimbs in whales.

INTRODUCTION

We use approaches from paleontology, morphology, development, genetics, and molecular biology to seek an integrated explanation for whether highly specialized phenotypes, such as limb loss in vertebrates that are not closely related (snakes and whales), could result from similar genetic and/or developmental mechanisms. We address the origin and evolution of cetaceans—whales, dolphins, and porpoises—especially the series of morphological changes that facilitated the progressive reduction and ultimate loss of hindlimbs in whales. A robust phylogeny is required to be able to infer the direction and sequence of any evolutionary changes (Hall and Olson 2002; Hall 2002a). Hence, before discussing limb development, progressive reduction of the hindlimbs, and the forces that drove hindlimb reduction, we discuss whale phylogeny, the major selective forces acting on whale evolution, and the origin of modern cetaceans. To begin to understand whether hindlimb loss in lineages that are not closely related could result from similar genetically controlled developmental pathways, we evaluate the genetic and developmental bases for limb loss in snakes and leg-less lizards and ask whether similar mechanisms could have driven loss of hindlimbs in whales. To what degree are genetic/developmental mechanisms shared? To what degree have such mechanisms diverged in the evolution of the similar evolutionary trends shown by limb loss?

CETACEANS

Aristotle knew that whales were covered with hair, breathed air, and suckled their young. The British naturalist John Ray formally recognized whales as mammals in 1693. Modern whales, dolphins, and porpoises (Order Cetacea), all aquatic and almost all marine, evolved some 40–50 million years ago (Ma) from terrestrial tetrapod ancestors during some 10–12 million years of the Eocene epoch. All living whales lack hindlimbs and have forelimbs modified as flippers. Their limbed ancestors, such as the Eocene *Ambulocetus natans*, used paraxial locomotion powered by limbs (Thewissen and Fish 1997). Once in water, locomotion was by a combination of tail undulation and paddling using the pelvic limbs. Modern cetaceans use oscillatory locomotion.

Marine mammals rely much less on vision and olfactory senses than terrestrial mammals. Consequently, a major selective pressure during the transition to the aquatic environment was adaptation to underwater hearing (Thewissen and Hussain 1993; Wartzok and Ketten 1999; Spoor et al. 2002). Cetaceans use acoustics for communication, orientation, and detecting, recognizing, and localizing companions, competitors, mates, predators, and prey. External ears (pinnae) have been lost, further reducing hydrodynamic resistance during swimming.

Like so many organisms cetaceans displayed mosaic evolution; organ systems evolved at different rates as their ancestors lost traits that fitted them for existence on land and gained traits that facilitated marine life. Consequently, changes in hearing, for example, need not have been correlated with transformations in other portions of the skull or with transformations of the limbs or tail. Mosaic evolution complicates both the search for ancestors of modern whales and their allies and our ability to identify a fossil form as a whale rather than a member of an ancestral group (Hall in press). Complementary changes in the morphology of all cetaceans acquired through selective pressures on a wide range of traits—a streamlined body, loss of fur, backward shift of the nostrils, transformation of forelimbs into flippers, evolution of flukes for swimming, and loss of the hindlimbs—all enhanced swimming and optimized energy utilization and detection of prey and predators (O'Leary and Uhen 1999; O'Leary 2001).

Functional criteria are important when we consider the forces that drove changes in cetaceans limbs and the evolution of flukes. Cetaceans evolved a thick subcutaneous layer of blubber that acts as an effective heat insulator and streamlines hydrodynamic shape. The streamlined shape minimizes drag, increases performance, and reduces transport energy costs. Concurrently, cetacean skulls became more telescoped as the nasal openings migrated dorsally (Miller 1923). This morphological shift allows modern cetaceans to breathe while partly horizontal and while moving and, more importantly, minimizes time spent at or above the surface. Such time slows forward progress enormously, increasing drag by up to three and a half times in sea otters, for example (Williams 1989), and increasing energy expenditure. Drag also was reduced through loss of the external hindlimbs, further reducing energy expenditure while improving thrust production and efficiency. Hydrodynamic, kinematic, and biomechanical studies show that swimming efficiency in modern cetaceans (as in the true seals and teleost fishes) is optimized by axial (vertical, dorsoventral) oscillation, which takes advantage of a lift-based oscillating hydrofoil (Fish 1996; Thewissen 1998).

Origin of modern cetaceans

The origin of cetaceans and their transition from land to water have long been the focus of studies. The first cetaceans, the archaeocetes, originated in the early to middle Eocene, 52–42 Ma. For some 30 years (Van Valen 1968; Szalay 1969) interpretations of the fossil record held that cetaceans originated from a now extinct group of small terrestrial (presumably furred) ungulates (hoofed mammals), the mesonychian condylarths, which lived some 65 Ma. Evidence used was skull morphology, patterns of dentition, and vestigial hindlimbs of the early archaeocetes whose limb morphology bears a striking resemblance to mesonychians and to extant artiodactyls.

Until recently there was not unanimous agreement between fossil, morphological, and molecular data, although a consensus was building that cetaceans were closely related to the extant artiodactyls (even-toed ungulates), including camels, pigs, hippos, and ruminants (Milinkovitch et al. 1993; Milinkovitch 1995; Milinkovitch and Thewissen 1997; Shimamura et al. 1997; O'Leary 1999; O'Leary and Geiser 1999; Luo 2000; Gatesy and O'Leary 2001; Thewissen and Bajpai 2001). Evidence from mitochondrial, nuclear, and chromosomal DNA (Graur and Higgins 1994; Milinkovitch and Thewissen 1997; Shimamura et al. 1997), along with a recent total evidence analysis that incorporated several hundred new molecular characters (O'Leary 2001), suggests that cetaceans arose deep *within* the artiodactyls, a conclusion substantially reinforced by two recent descriptions of four new fossils of early whales with morphologies characteristic of artiodactyls; cetaceans and artiodactyls are monophyletic (Gingerich et al. 2001; Thewissen and Bajpai 2001; Thewissen et al. 2001; see below).

A unique feature of all extant artiodactyls is a greatly modified mobile ankle joint that enables rapid locomotion (Milinkovitch and Thewissen 1997; Thewissen et al. 1998). Absence of this feature from all other mammals suggests that the trait is derived in artiodactyls, supporting a monophyletic artiodactyl clade. Mesonychians did not have this joint. Archaeocetes did. As modern cetaceans have only vestigial hindlimbs, researchers have looked for evidence of this ankle structure in fossils of ancient whale species that still possessed hindlimbs. They found it in *Rodhocetus balochistanensis* and in *Artiocetus clavis*, from the Early Eocene (47 Ma) of Pakistan (Gingerich et al. 2001).

Progressive reduction of hindlimbs and changes in locomotion during the evolution from archaeocetes to modern cetaceans

As discussed above, several concurrent morphological and physiological changes occurred with the transition from land to sea. During the evolution from archaeocetes to modern cetaceans, there was a gradual reduction of the hindlimbs concurrent with a change in the mode of locomotion and elongation of the body (Figs. 1 and 2). The mode of locomotion went from quadrupedal paddling to pelvic paddling to dorsoventral undulation of the tail to caudal oscillation of the tail in synchrony with body movements.

One prominent change was progressive loss of the hindlimbs and hindlimb skeleton, which began with the transition from an amphibious to a fully marine archaeocete. The loss—initially of the most distal elements (the digits), proceeding through loss of more and more proximal elements (tibia/fibula, femur, pelvic girdle)—is documented by a sequence of intermediate forms that span 10–12 million years of the Eocene (Uhen 1998).

The series begins with *Pakicetus inachus*, an archaeocete from the early Eocene of Pakistan. *Pakicetus* is interpreted as having been terrestrial (it is preserved in deposits in association with land mammals), with teeth, limbs, and ears not yet modified for life in water (Thewissen et al. 2001). The series continues with *Ambulocetus natans*, *Rodhocetus balochistanensis*, *Basilosaurus isis*, *Chrysocetus healyorum*, and *Ancalecetus simonsi* (Gingerich et al. 1983, 1990, 1994, 2001; Thewissen et al. 1994, 2001; Gingerich and Uhen 1996; Uhen 1998; Bajpai and Thewissen 2000; Thewissen and Bajpai 2001; Uhen and Gingerich 2001). See the appendix in Thewissen and Bajpai (2001) for a description of these fossils, their discovery, and an overview of the major groups (families) of whales (Pakicetidae, Ambulocetidae, Remingtonocetidae, Protocetidae, Basilosauridae, and Dorudontidae; some regard the Basilosaurinae and Dorudontinae as subfamilies of the Family Basilosauridae). For more general articles on whale evolution, see Chadwick (2001) and Wong (2002).

One of the earliest amphibious archaeocetes, *Ambulocetus natans* (47–48 Ma), had well-developed fore- and hindlimbs. The forelimbs were small and probably did not aid in propulsion; *Ambulocetus* is thought to have swam by pelvic paddling using the hindlimbs rather than by using the tail as a hydrofoil. The hindfeet were large, with elongated flattened toes suggesting webbed feet and the ability to walk on land (Thewissen et al. 1994), although feet alone might not provide an adequate basis for inferring locomotion on land. Sea otters have large and specialized hindfeet. They may use their hindlimbs like a fluke but power the hindlimbs and swim using vertical axial undulations of the spine (Kenyon 1969; Williams 1989). They can walk on land, but more of-

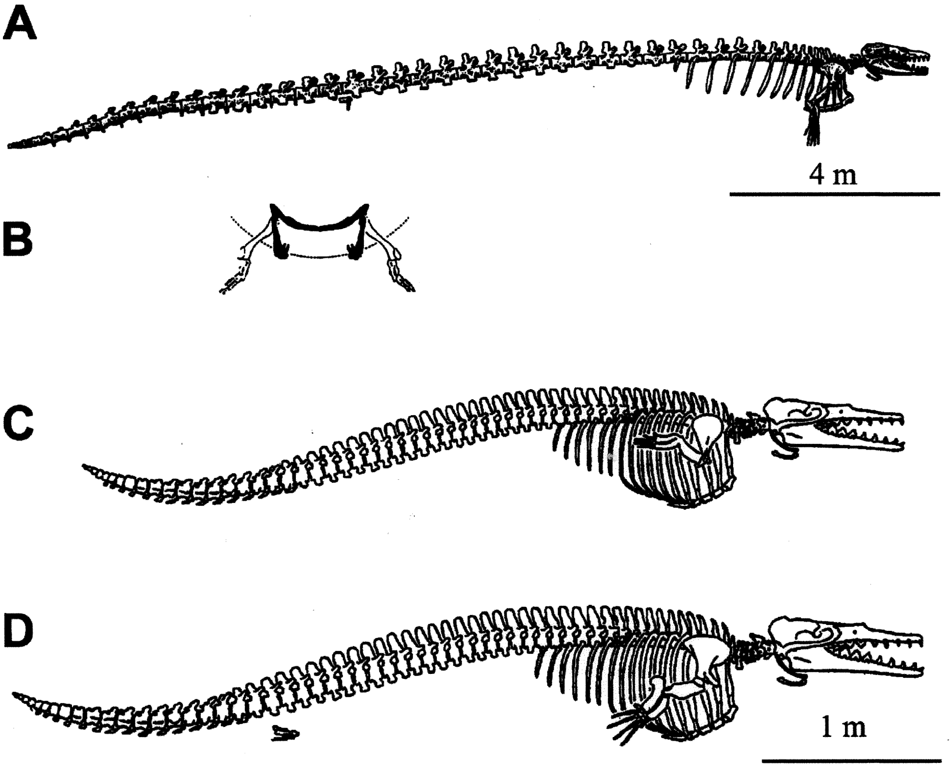

Fig. 1. Eocene whales. (A) A reconstruction of the skeleton of *Basilosaurus* indicating body elongation and proportions and the relative position and size of the hindlimbs. (B) The pelvic girdles and hindlimbs of *Basilosaurus* in resting and functional position as seen in cross-section. (C, D) Two species from the Dorudontidae family, (C) *Ancalecetus simonsi*, which no longer shows evidence of hindlimbs (no hindlimbs were discovered, but other dorudontids do possess hindlimbs), and (D) *Dorudon atrox*, a dorudontid with greatly reduced hindlimbs. The 1-m scale bar is for C and D. A and B adapted from Berta and Sumich (1999) from an original in Gingerich et al. (1990), with permission. C and D reproduced from Berta and Sumich (1999) from an original in Gingerich and Uhen (1996), with permission.

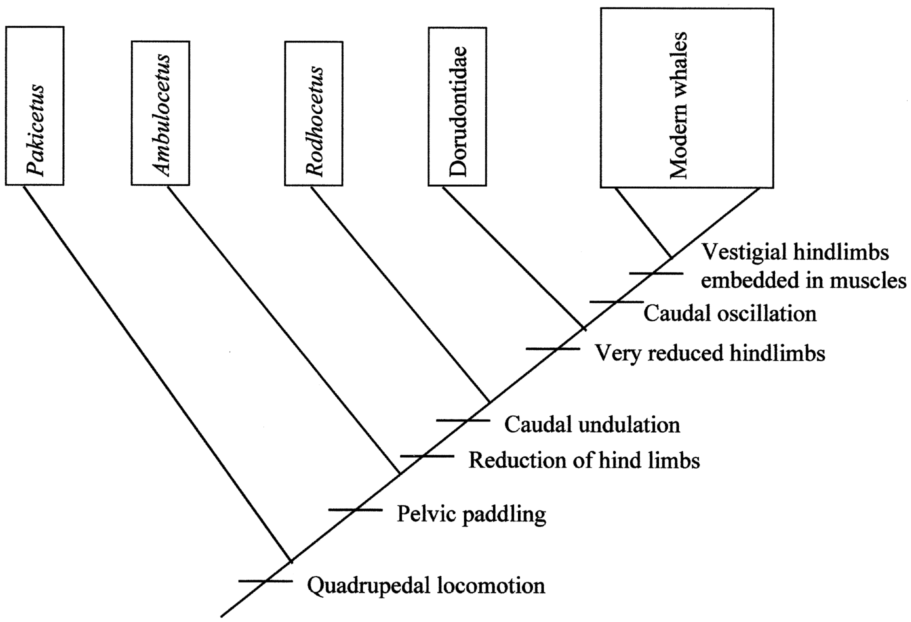

Fig. 2. Evolution of the changes in swimming mode during cetacean evolution. Modern whales comprise baleen and toothed whales. Modified from Berta and Sumich (1999) and Thewissen and Fish (1997).

ten only drag their feet (W. Olson, personal communication). Bajpai and Thewissen (2000) suggested that the diminutive *Kutchicetus minimus* from the middle Eocene of western India had a swimming mode resembling that of river otters such as *Pteronura brasiliensis*, the giant river otter.

Later archaeocetes, such as *Rodhocetus balochistanensis* (47 Ma), had smaller hindlimbs than *Ambulocetus.* The forelimbs were webbed with short hoof-bearing distal phalanges, suggestive of limited ability for locomotion on land. The structure of the feet also suggests a predominantly aquatic lifestyle (Gingerich et al. 2001). Locomotion on land probably resembled that of today's otariids (eared seals). At the water surface, *Rodhocetus* likely swam by pelvic and quadrupedal paddling. Based on interpretations of the robustness of the tail, caudal undulation was important during subsurface locomotion.

The Basilosauridae, a late-diverging family of archaeocetes (44–40 Ma), are regarded as the most highly derived of the ancient cetaceans (Uhen 1998; Thewissen and Bajpai 2001). Discovered in the early nineteenth century, *Basilosaurus* ("King Lizard") was recognized by Richard Owen (1842) as a mammal with affinities to the cetaceans. A range of features suggests that members of this family were adapted to a completely aquatic lifestyle, although elongation of the body (approaching 25 m in some species), increase in body size, and increase in vertebral numbers were already underway (Fig. 1A). A 16-m-long *Basilosaurus isis* from Egypt had a hindlimb skeleton of some 35 cm in length (Fig. 1, A and B), which would not have been robust enough to support the body mass of such a large creature (Gingerich et al. 1990; Uhen and Gingerich 2001). Although all skeletal elements were present, the hindlimbs had fused tarsals and only three digits. The absence of any articulation with the vertebral column makes a locomotory function for the hindlimbs unlikely or inefficient. The limbs may have served a grasping function during copulation or may have been vestigial and without function (Gingerich et al. 1990).

A closely related family, the Dorudontidae, were smaller dolphin-sized animals (Fig. 1C) with even more reduced hindlimbs (Fig. 1D). Skeletal morphology of the caudal region suggests that flukes had evolved and that swimming was by axial undulation of the body axis and caudal oscillation of the tail (Uhen 1998; Thewissen and Bajpai 2001), the latter being the swimming mode of all modern cetaceans (Fig. 3A).

LIMBS

To provide a basis with which to compare hindlimb bud development, we analyze forelimb bud initiation, development of the forelimbs, their transformation into flippers, and the concomitant increase in the number of phalanges. An analysis of the initiation of hindlimb buds is followed by discussion of when and how those hindlimb buds cease developing and regress. We then evaluate the presence of vestigial (rudimentary) skeletal elements in the pelvic regions of cetaceans and briefly comment on atavistic hindlimb skeletal elements in whales. For a history of studies on embryonic development of whales, see Guldberg and Nansen (1894). For an overview of staging and ageing dolphin embryos, see Sterba et al. (2000).

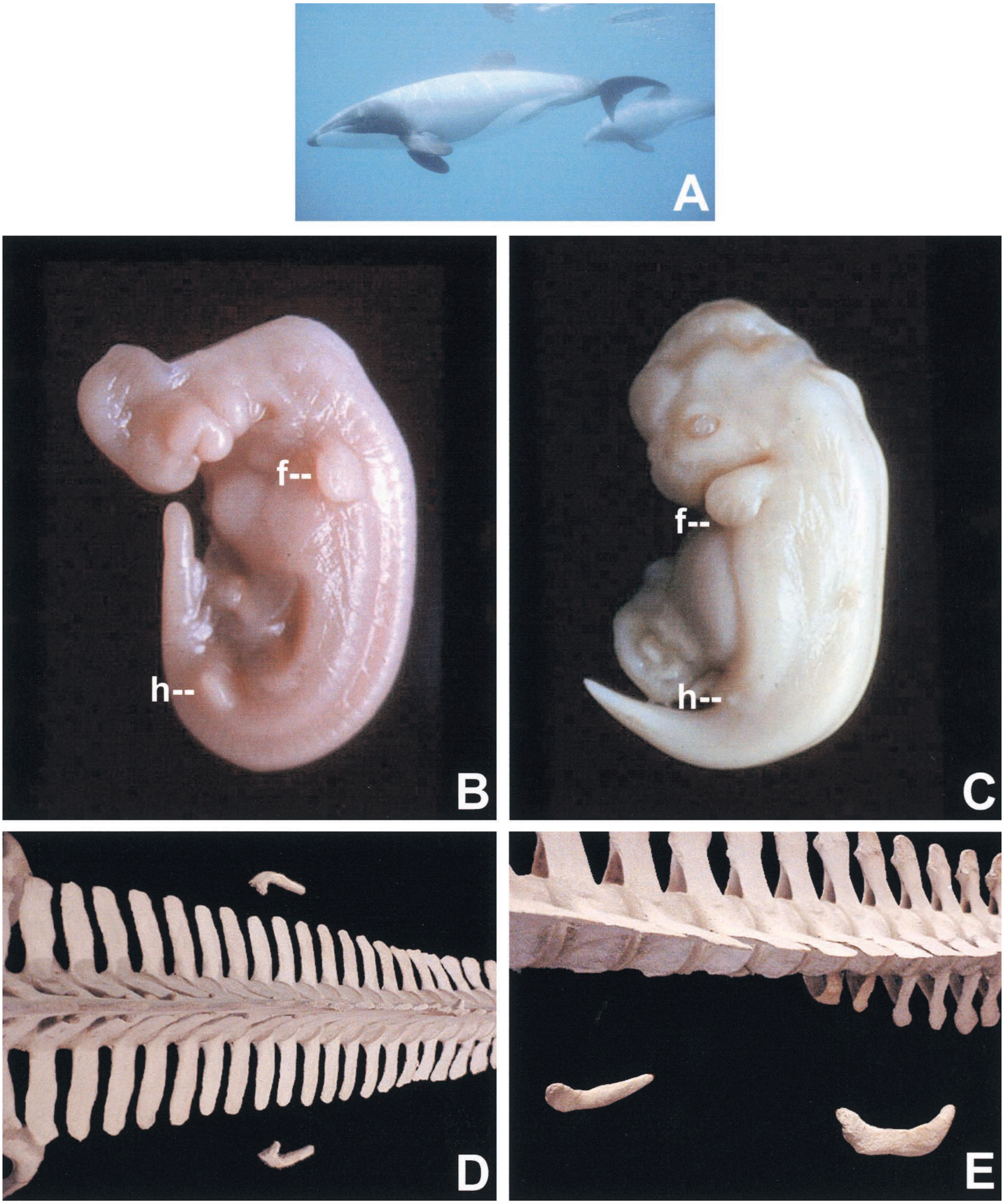

Fig. 3. (A) Hector's dolphin, *Cephalorhynchus hectori* (average adult length, 1.4 m), to show the fluke in caudal oscillation. Photographed off the South Island of New Zealand by Steve Dawson. (B, C) Embryos of the spotted dolphin, *Stenella attenuata*, of 24 (B) and 48 (C) days of gestation to show well-developed forelimb buds (f; note the digital primordia in C), a well-developed early hindlimb bud (h) at 24 days of gestation (B), and a regressing hindlimb bud (h) at 48 days (C). Reproduced from Sedmera et al. (1997a) from images kindly provided by Ivan Misek, Brno, Czech Republic. (D, E) Rudimentary pelvic bone in a pilot whale, *Globicephala* sp. on display in the Nova Scotia Museum of Natural History, Halifax. (D) Dorsal view, anterior below, with the last rib shown. (E) Side view of the pelvic bones and vertebral column. Note the general size (25 cm long) and orientation of the pelvic bones and their lack of connection to the vertebral column.

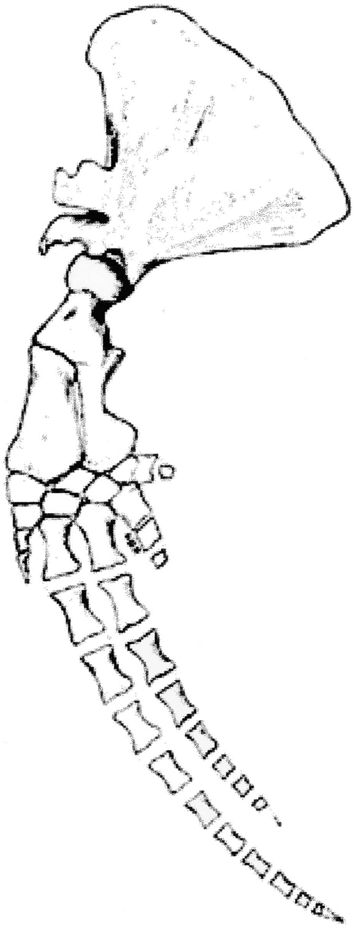

Fig. 4. Polyphalangy of digits II and III of the flipper of the long-finned pilot whale, *Globicephala melas.* Reproduced from Howell (1970).

Forelimbs

Whale embryos develop forelimb buds (Fig. 3, B and C) that develop into flippers (Fig. 3A) associated with reduced clavicles and a reduced sternum (Klima 1990; Sedmera et al. 1997a,b; Hall 2001). Two stages of forelimb bud development are shown in Figures 3B and 3C, the former (at 24 days of gestation) with an early conical bud, the latter (48 days of gestation) with well-developed digital primordia. Forelimbs served roles in locomotion and body support in the terrestrial, partly amphibious, ancestors of whales. In modern cetaceans flippers have acquired a new stabilizing and steering function; they do not generate any propulsive movements. Various morphological changes facilitated these functional changes: The only moveable joint in the flipper is the shoulder joint, the joints at the elbow, wrist, and phalanges being fixed. In archaeocetes the elbow joint was flexible and capable of rotation. Modern cetaceans have many more phalanges (polyphalangy) in the digits of their flippers than other mammals have in their forelimbs (Howell 1970), as many as 14 phalanges in digits II and III in the pilot whale *Globicephala melas* (*melaena*) (Fig. 4). Polyphalangy creates a skeleton composed of many small parts that stabilizes the strong, flexible, yet rigid flippers (Felts and Spurrell 1966).

Unusually, the number of phalanges in at least some of the digits varies between individuals and within the ontogeny of single individuals, the maximal number of phalanges per digit being found in late fetal life, not in adults; 17 in fetal pilot whales and 14 in adults (Fig. 4) (Kükenthal 1893; Coates 1991). The change in phalangeal formula during embryonic life of the spotted dolphin, *Stenella attenuata*, ranges from 2-3-3-3-0 at 41 days of gestation to 3-7-7–5-3 at 87 days, the numbers from left to right representing the number of phalanges in digits I to V (Table 1). Reduction in phalangeal number is attributed to “regression” of the most distal phalanges as a consequence of the forces acting on the leading edge of the flippers (Klima 1992). However, whether regression is by erosion of individual phalanges or through fusion of adjacent phalanges is unclear. Polyphalangy is a recent evolutionary change not found in Eocene archaeocetes (see character 59 in Uhen 1998). In an interesting example of convergent evolution in which “the influence of aquatic life . . . manifests itself in the same direction” (Kükenthal 1891, p. 161), polyphalangy occurs in other animals that secondarily returned to an aquatic environment, for example, ichthyosaurs (Coates 1991; Klima 1992) and plesiosaurs (Carroll 1988; Caldwell 1997). Polyphalangy is also found in the amphisbaenian genus *Bipes* (Pena 1967).

Table 1. Changes in phalangeal formulae during embryonic life of the spotted dolphin, *Stenella attenuata*

Age[1] (days of gestation)	Stage	Crown-Rump Length (mm)	Phalangeal Formula[2]
41	36	22	2-3-3-3-0
48	44	30	3-4-4-3-1
60	54	44	3-5-5-4-2
87	75	101	3-7-7-5-3

[1] Total gestation time is 280 days (Sterba et al. 1994).
[2] Digit I on the left, digit V on the right; see Padian (1992) for phalangeal formulae.
From Sedmera et al. (1997b).

Hindlimbs

In contrast to the forelimbs, hindlimbs did not maintain or acquire a new function during the evolution of modern cetaceans. The switch to vertical axial undulation was enhanced by the evolution of flukes for propulsion (Fig. 3A). Flukes are not vestiges of hindlimbs (Fish 1998; Sterba et al. 2000). They are secondarily acquired structures, outgrowths of skin and connective tissue of the tail that arise in embryos (Slijper 1979; Meyer et al. 1995). Given that the metabolic cost of drag-based swimming is up to five times higher than the lift-based swimming for animals of similar body mass (Weihs 1989; Fish 1996), development of flukes was a key innovation in the evolution of cetaceans. The reduction and elimination of less-functional drag-inducing hindlimbs also improved hydrodynamic swimming efficiency.

Limb reduction and limb loss have occurred repeatedly in representatives of the four classes of tetrapods, viz. cetaceans, flightless birds (the moa, *Diornis*, and the kiwi, *Apteryx australis*; the wings of *all* birds have undergone some reduction, in that all have only three not five digits), snakes, amphisbaenians, caecilians, and salamanders (Gans 1975; Lande 1978; Greer 1987; Fong et al. 1995; Cubo and Arthur 2001).

All limb-less tetrapods are descended from limbed ancestors. Most possess limb buds at some stage during their development. Limblessness thus represents arrest of limb bud development rather than absence of limb initiation or regression of a fully formed limb. Estimates of the time over which limbs were lost range from several million years for lizards (Van Valen 1973; Lande 1978) to 10–12 or up to 20 million years for whales (Lande 1978).

Although modern cetaceans lack external hindlimbs, embryos do form rudimentary hindlimb buds (Fig. 3, B and C) (Sedmera et al. 1997a,b and references therein). These buds persist longer in the humpback whale, *Megaptera nodosa*, than in odontocetes, a persistence that correlates with, and in part explains, why baleen whales retain elements of the hindlimb skeleton more often than odontocetes (Andrews 1921; Howell 1970).

Sedmera et al. (1997a) examined hindlimb development in embryos of the spotted dolphin, *Stenella attenuata.* Hindlimb buds at 24 and at 48 days of gestation are shown in Figure 3, B and C. The rather well-developed limb bud seen at 24 days is considerably smaller than the forelimb bud in the same embryo (Fig. 3B). By 48 days the hindlimb bud has regressed to a nubbin, whereas the forelimb bud has well-formed digital primordia (Fig. 3C). Whale hindlimb buds possess an apical ectodermal ridge (AER), which is the ectodermal signaling center responsible for both limb bud outgrowth and proximodistal patterning of the limb skeleton in limbed tetrapods. Sedmera and colleagues described the early hindlimb buds of *Stenella* as having an underdeveloped AER. As in snakes and leg-less lizards (see below), the AER subsequently regresses and hindlimb formation ceases.

Skeletogenesis is initiated in condensations of cells. Condensation, the process by which cells of similar fates (e.g., chondrogenic, osteogenic, or myogenic cells) aggregate or accumulate, represents the first phase of specific cell differentiation, by which we mean that this is the phase when tissue specific genes (e.g., collagen type II in chondrogenic cells) are first up-regulated. Condensation therefore represents a fundamental stage in skeletogenesis (Atchley and Hall 1991; Hall and Miyake 1995, 2000). Because whales do not develop hindlimbs, it is of interest to known whether the limbs buds progress to the condensation stage, which they do. Vascular plexuses outline the condensations for the digits, and nerves grow into the limb buds, that is, the buds initially develop normally (Sedmera et al. 1997a). Whether specific genes such as type II collagen are up-regulated in these condensations has not been studied. The limb buds then begin to regress. As the limbs deteriorate some mesenchymal cells die, whereas others are incorporated into the body wall.

Because condensations form but do not normally go on to form a skeletal element, we can ask whether the potential exists in whale hindlimb buds for individual skeletal elements to reappear as atavisms (see the following section) or as ectopic elements after gene knockout (Hall and Miyake 1995; Smith and Schneider 1998). The presence of vestigial skeletal elements in some whales (also see below) increases the possibility that such atavisms could appear.

Vestigial skeletal elements

Rudimentary skeletal elements of the limb skeleton fall into two categories: vestigial elements, which are found in all members of a species and represent reduced elements of the *pelvic girdle*, and atavistic *appendicular* skeletal elements, which are found only occasionally in single individuals. Pabst et al. (1998) and van der Schoot (1995) regarded the pelvic bones as rudiments representing arrested embryonic development, rather than vestiges, implying reduction from an ancestral condition (Rommel 1990). For a discussion of the differences between rudiments, vestiges, and atavisms, see Hall (in press).

Vestiges

Loss of the hindlimbs in whales was accompanied by loss of the pelvic girdle. Within the pelvic region, odontocetes and mysticetes possess a few small cartilages and/or bones, imbedded in musculature and unconnected to the vertebral column. At their fullest development, they consist of three bones joined by a ligament but without a symphysis or acetabular cavity. Establishing the homology of vestiges or rudiments is not a trivial problem (Hall in press). To quote from an analysis of cetacean thermoregulation and reproduction: "To date, the exact identity and development of the elements of the pelvic vestige of extant cetaceans [i.e., are they ischium, ilium or pubis] have not been established. Such identification is critical to fully understanding the events underlying the evolution of the cetacean pelvis" (Pabst et al. 1998, p. 393). These pelvic "bones" remain cartilaginous long after birth (Arvy 1979), although this conclusion was based on their failure to appear in x-rays of older embryos, an absence that may indicate lack of mineralization at that stage rather than a persistent cartilaginous nature. They do mineralize later in life (Fig. 3, D and E). Each bone has only one ossification center and is supported by the superficial cutaneous muscles that support the urogenital orifice and penis, although, according to Howell (1970), it is almost impossible to homologize cetacean cutaneous muscles with those of terrestrial mammals, in part because the nerves (from which muscle homology is usually most reliably obtained) are so greatly altered in cetaceans. The bones are sexually dimorphic, being heavier in males but slightly longer and more slender in females. They are thought to function in mating and in birth (Howell 1970).

Atavisms

Most mysticetes have a rudimentary femur. *Balaenoptera musculus*, the blue whale, has a bony femur with a ligamentous connection to the pelvic bones. In a study of an 72 individuals from the Antarctic population of the minke whale, *B. acutorostrata*, Omura (1980) found that 37% had an ossified femoral rudiment. The bowhead whale, *Balaena mysticetus*, has the least reduced hindlimbs, with a bony femur 10–22 cm in length, a cartilaginous tibial head, and a synovial joint between the femur and the pelvic rudiment (Struthers 1881; Howell 1970). Living baleen whales retain rod-like vestigial pelvic bones or cartilages, femora, and occasionally tibiae, fibulae, tarsals, and metatarsals that fail to project beyond the body wall (Howell 1970). The sperm whale (*Physeter macrocephalus* [*catodon*]) is the only odontocete with vestiges of the hindlimb skeleton (a cartilaginous or poorly ossified femur), the vestiges sometimes presenting as external appendages with digits (Berzin 1972; Yablokov 1974; Deimer 1977).

Limblessness is polygenic, involving genes with pleiotropic effects (Lande 1978). Surprisingly, genes involved in limb development also function on other developing systems, such as the jaws, teeth, and genitalia (Duboule and Wilkins 1998; Schneider et al. 1999; Rosa-Molinar and Burke 2002). We might therefore expect genes associated with limb bud development to be retained after limb buds are lost, providing the potential for partial or even complete reappearance of limb elements; recall that condensation can be present even when the skeletal element is not.

An atavism is the reappearance of an ancestral character in an individual within a descendant population (Hall 1984, 1995, 2002, in press, b). Atavistic skeletal elements, distinguishable from the rudiments of the pelvic girdle, have been documented in adult sperm and humpback whales (Andrews 1921; Berzin 1972; Hall 1984, 1999), the incidence in sperm whales being 1:5000 adults. Atavistic skeletal elements can be surprisingly complete; 79 cm long bones in 125 cm long left and right "hindlimbs" in a female humpback whale, *Megaptera novaeangliae*, when normally only a cartilaginous femur is present (as in the sperm whale; Deimer 1977). Estimates of the time by which mammals could no longer redevelop a lost limb element are of the order of 10^7 generations, assuming a rate of mutation for major genes involved in limb development of 10^{-7} mutations/locus/generation (Lande 1978; see Marshall et al. 1994 for reactivation of developmental programs after some half million years of gene silencing).

DEVELOPMENT OF LIMB BUDS IN SNAKES AND LEG-LESS LIZARDS

As demonstrated by three fossil snakes (*Pachyrhachis problematicus, Haasiophis terrasanctus*, and *Podophis descouensi*) with hindlimbs, snakes, like whales, evolved from limbed ancestors (Haas 1980; Rieppel 1988; Lee and Caldwell 1998; Coates and Ruta 2000; Greene and Cundall 2000; Rage and Escuillié 2000; Tchernov et al. 2000). Despite the discovery of limbed snakes with hindlimbs, the precise group that gave rise to snakes remains uncertain and hotly contested. How the limbs were lost is equally enigmatic. As in whales, limb loss in snakes is associated with body elongation, which in snakes occurred by increasing the length of the entire body (including the tail) or by increasing body length but not tail length (Gans 1974, 1975). In either case the number of vertebrae increased significantly in limb-less forms (Camp 1923; Stokeley 1947; Hofstetter and Gasc 1969). That said, precisely how snakes lost their limbs remains enigmatic. Jean Baptiste Lamarck presented one of the most novel scenarios:

> Snakes, however, have adopted the habit of crawling on the ground and hiding in the grass; so that their body . . . has acquired a considerable length, quite out of proportion to its size. Now, legs would have been quite useless to these animals and consequently unused. Long legs would have interfered with their need of crawling, and very short legs would have been incapable of moving their body, since they could only have had four (cited from Lamarck 1984, pp. 117–118).

Patterns of limb and girdle loss vary considerably within squamate reptiles. The most extensively studied limb-less forms are the slow worm (*Anguis fragilis*) and its relative, the leg-less lizard or glass snake (*Ophisaurus apodus*), the snakes *Python reticulatus* and *Tropidonotus tessellata*, and two South African skinks, *Scelotes brevipes*—in which only the femur is present as an ossified element—and *S. gronopii*, in which femur, tibia, and fibula are ossified but reduced

(Hall 1978, 1999; Raynaud 1990; Raynaud and Kan 1992). Those with the greatest limb loss lost the forelimbs and pectoral girdle before the hindlimbs and pelvic girdles, a pattern also seen in amphisbaenians. *Pseudobranchus* and *Siren* (salamanders of the family Sirenidae) lose their hindlimbs first and may retain vestiges of the pectoral girdles or retain the forelimbs. Some genera that retained their forelimbs, such as the amphisbaenian genus *Bipes*, developed additional digits in the forelimbs (Pena 1967). Because lizards show many intermediate stages of limb loss they represent excellent candidates for study (Shapiro and Carl 2001; Shapiro in press). Limb loss in lizards results from arrested limb development (Camp 1923; Essex 1927; Gans 1975; Presch 1975). The limb buds that form in slow worms and in the green lizard (*Lacerta viridis*) develop an AER that then regresses (Raynaud et al. 1974; Raynaud 1990).

Adult extant snakes lack forelimbs, and their embryos lack forelimb buds. As in whale embryos, hindlimb buds form in embryonic stages of primitive snakes but subsequently regress (Cohn and Tickle 1999). More advanced snakes lack hindlimb buds, that is, a progressive reduction in hindlimb bud development is seen during snake phylogeny.

In limbed tetrapods the somites that lie adjacent to the limb buds send mesenchymal cells into the developing limb buds, reaching the limb buds after the AER has been established. These mesenchymal cells differentiate into the muscles of the limb (Chevallier et al. 1977; Christ et al. 1977; Hall 1978). Somites send these mesenchymal cells into the developing limb buds of green lizards, but the mesenchymal cells fail to survive. Cellular degeneration, beginning in the tips of the extensions that somites send into the limb bud, is followed by degeneration of the AER, decreased proliferation, and finally degeneration of the limb bud mesenchyme (Raynaud and Kan 1992). In *Ophisaurus apodus*, a species from the same family (Anguidae) as *Anguis fragilis*, three somites send extensions into the future hindlimb buds. An AER forms, and the developing limb buds become vascularized and innervated. The AER, the somitic extensions, and the mesenchyme all necrose, reducing the limb buds to a vestige in which a rudimentary skeletal rod develops (M-Z Rahmani 1974). In *Python reticulatus* and in the skinks, similar patterns of somite extensions, formation of an AER (in the skinks), and subsequent regression are seen (Raynaud and van den Elzen 1976).

The implication from these studies of snakes and leg-less lizards is that the somitic extensions are required to maintain the lateral plate mesoderm from which the skeletogenic limb mesenchyme arises (Hall 1978). Although this interesting idea has yet to be tested in snakes or leg-less lizards, Vassé (1974,1977) demonstrated that removal of somites from early stage embryos of the turtle *Emys orbicularis* or implanting barriers between the somites and the lateral plate mesoderm prevented limb bud development. Pinot (1970) and Kieny (1971) demonstrated a similar dependence of limb development on somites in chick embryos, showing that somitic cells do promote proliferation of future limb bud mesenchyme. There is then a close and dependent developmental connection between developing somites and developing limb buds in both limbed and in limb-less tetrapods.

LIMB-LESS MUTANTS

Mutant limb-less chick embryos are important model for evolutionary limb loss. In both *wingless* and *limbless* mutant chick embryos, an AER forms normally but the wing buds do not continue to develop. Wings fail to form because of an inability of limb bud mesenchyme to maintain the AER (Hinchliffe 1977; Hall 1978; Lyons et al. 1991). *Dlx-5*, which plays a role in maintaining the AER in wild-type embryos, is only expressed transiently in the ectoderm of limb buds in *limbless* embryos, implicating changes in *Dlx-5* in failure of *limbless* embryos to maintain their limb buds (Ferrari et al. 1999).

During limb bud development in wild-type avian embryos, *Msx-1* and *Msx-2* are expressed in early lateral mesenchyme and then only in distal limb mesenchyme. Grafting an additional AER to a limb bud induces a new site of *Msx* gene expression, that is, the AER "controls" *Msx* expression. Neither *Msx-1* nor *Msx-2* are expressed in limb buds of *limbless* mutants, implicating these two genes in the limb-less condition and suggesting that the AER fails to induce their expression (Robert et al. 1991).

Cohn and Tickle (1999) showed that even though an AER is not present during normal development of python embryos, python embryonic body mesoderm could induce chick ectoderm to form an AER. The fact that python mesoderm does not induce an AER in vivo and in situ is therefore not the basis for failure of hindlimb development in python embryos, although inability of python ectoderm to respond to the mesodermal signal is important, as is the inability of python mesenchyme to maintain an AER.

Clearly, abnormal epithelial–mesenchymal interactions can disrupt limb development. The preaxial shortening of the AER seen in the hindlimb buds of mouse embryos carrying the mutation *Dominant hemimelia* (*dh*), which results in severe loss of preaxial limb elements (Johnson 1986), was attributed by Milaire (1981) to the hindlimb buds being shifted anteriorly relative to the somites. This has now been substantiated and a molecular basis for loss of anterior hindlimb bud mesenchyme found in altered expression of members of the fibroblast growth factor (FGF) gene family (Lettice et al. 1999). FGF-4 is normally expressed only in the posterior domain of the AER in murine embryos (adjacent to where posterior digits will develop), whereas FGF-8 is expressed throughout the AER along the entire distal face of the developing limb bud (Fig. 5). Normally, anterior limb-bud mes-

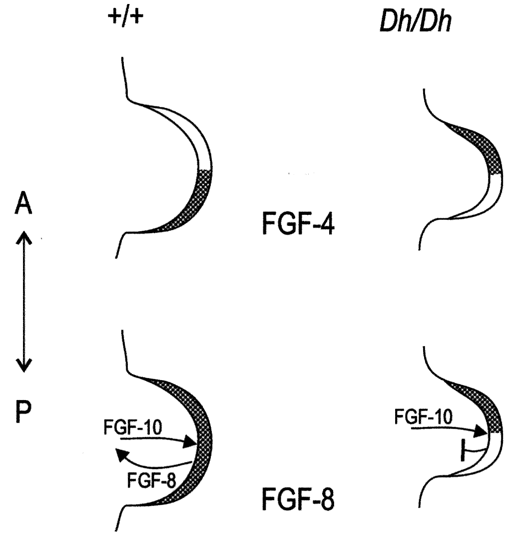

Fig. 5. Distribution of FGF-4 and FGF-8 in wild-type (++/++) and *dominant hemimelia* (*dh*) mouse embryos to show the shift in expression to the anterior border of the apical ectodermal ridge in *dh* embryos and the consequent effect on the FGF-10– FGF-8 cycle (→) which is blocked in *dh* embryos. A ↔ P, anterior–posterior body axis. See text for details.

enchyme is maintained by a regulatory loop between FGF-8 in the AER and FGF-10 in the mesenchyme (Fig. 5). FGF-4 uncouples this regulatory loop. Because there is no FGF-4 expression in the anterior domain of the AER in normal embryos, anterior mesenchyme is maintained and anterior (preaxial) digits form. *dh* embryos show normal distribution of FGF-8 throughout the AER but express FGF-4 anteriorly in the AER rather than posteriorly (Fig. 5). Anterior expression of FGF-4 uncouples the regulatory loop required to maintain anterior mesenchyme, resulting in selective loss of anterior mesenchyme and consequent severe preaxial defects in embryos carrying the *dh* mutation.

MECHANISMS OF HINDLIMB LOSS IN WHALES AND SNAKES

Hox genes and loss of hindlimbs

The mechanisms responsible for limb loss in cetaceans may be as subtle as those in snakes and lizards, if not quite so amenable to analysis. Cetaceans form both fore- and hindlimb buds during embryogenesis (Fig. 3, B and C). As demonstrated in embryos of the spotted dolphin, *Stenella attenuata*, hindlimb buds arise, form an AER (admittedly hypoplastic), but fail to develop further (Sedmera et al. 1997a), presumably due to an inability to maintain the AER, as in leg-less lizards and snakes.

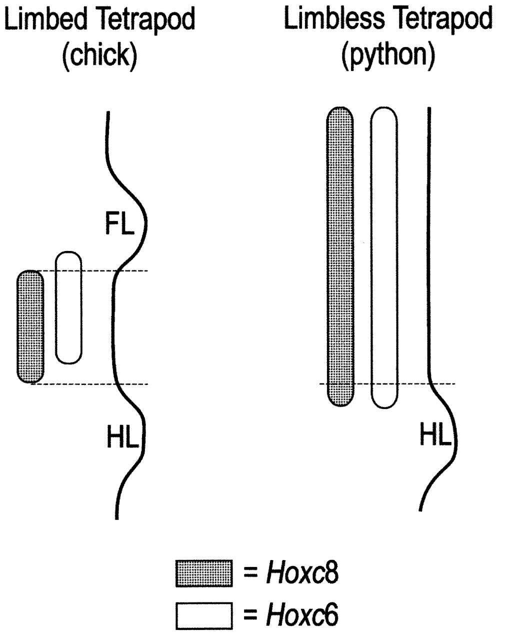

Fig. 6. The distribution of *HoxC-8* and *HoxC-6* in a limbed tetrapod (embryos of the common fowl) and a snake (embryos of the python). The expression boundaries are extended slightly more posteriorly and much more anteriorly in python than in chick embryos. FL, forelimb buds; HL, hindlimb buds.

Hox genes specify axial patterning and limb position during tetrapod embryonic development (Burke et al. 1995; Carroll 1995; Shubin et al. 1997). In both teleost fish and tetrapods, *Hox* genes are involved in specifying where along the body pectoral fins or forelimbs will form; the anterior expression boundaries of *HoxC-6* and *HoxC-8–10* coincide with the localization of fore- and hindlimb buds, respectively. This is so, even though anterior expression boundaries of *HoxC-6* terminate at different somite levels in different species; somites 11–12 in mice, 18–19 in chicks, 20–21 in geese, and 3–4 in *Xenopus* (Burke et al. 1995). *Hox* genes are also differentially expressed in the different tissues that contribute to the limb. *Hox-9* genes are differentially regulated in lateral plate mesoderm—from which the limb skeleton develops—and in paraxial mesoderm—which provides myogenic cells to the limb buds (Burke et al. 1995; Cohn et al. 1997; Burke 2000).

HoxC-6 and *HoxC-8 are* differentially expressed in flank mesoderm in the chick, and presumably in other limbed tetrapods, if the chick is representative (ibid, Fig. 6). Although their patterns of expression overlap, *HoxC-6* expression begins further anteriorly (overlapping the forelimb territory) and stops further anteriorly (before the hindlimb territory) in chick embryos (Fig. 6). Embryonic pythons have an expanded expression of *HoxB-5*, *HoxC-6*, and *HoxC-8* along the body axis (Fig. 6). *HoxC-6* and *HoxC-8* both have posterior limits of expression that are slightly more posterior than those seen in chick embryos, extending into the hindlimb territory, but extend much further anteriorly, indeed, to the anterior limit of the trunk (Fig. 6).

In limbed tetrapods, cervical vertebrae form in areas anterior to the most anterior expression of these *Hox* genes, whereas thoracic vertebrae form in areas within the expression boundaries. In correlation with the expression boundaries in python embryos, thoracic vertebrae extend to the anterior tip of the vertebral column and cervical vertebrae fail to form.

The extreme anterior extent of the expression of *HoxC-6* and *HoxC-8* in python embryos means that no boundary conditions are established for forelimbs to form. Loss of forelimb development in pythons (and, by extension, in other snakes) could, then, be due to developmental processes flowing from the progressive anterior expansion of *Hox* gene expression domains along the body axis (Cohn and Tickle 1999; Graham and McGonnell 1999). However, during development of the *hindlimb* buds in python embryos (as in tetrapods in general), expression of HoxC-8 protein occurs at the level where hindlimb buds form. Failure of the hindlimbs to develop fully must therefore be due to changes other than modification of boundaries of expression of the particular *Hox* genes that position limb buds along the body axis.

cis-Regulation

cis-Regulation is increasingly being seen as an important way in which evolution of gene regulation relates to the evolution of diverse morphologies (Carroll 2000; Tautz 2000). Was limb loss in whales related to the evolution of *cis*-regulation?

Belting et al. (1998) examined expression of *HoxC-8* and evolution of the *cis*-regulatory elements of *HoxC-8* in chick and mouse in relation to the different vertebral morphologies in these two species. In both species, *HoxC-8* is expressed in the mid-thoracic mesoderm and in the brachial region of the neural tube, the anterior limit of expression correlating with the site of origin of the brachial nerves that innervate the forelimbs (Fig. 7, A and B). Activation of *HoxC-8* is temporally delayed in the chick, with the consequence that expression in chick embryos is more posterior and over a smaller area of mesoderm than in murine embryos (Fig. 7C). Belting and colleagues argued that these differences in timing and patterns of expression between chick and mouse embryos explain the shorter thorax in chicks compared with mice. The chicken and mouse *HoxC-8* early enhancers, a potential homeodomain binding site, differ by only a few nucleotides, a difference that is consistent with changes in *cis*-regulation of the gene between the two species.

The relevance of this study for whale evolution is that five species of mysticetes—*Balaenoptera physalus* (the fin whale), *B. borealis* (the sei whale), *B. acutorostrata* (the minke whale), *Balaena mysticetus* (the bowhead whale), and *Megaptera novaeangliae* (the humpback whale)—lack 4 base pairs in element C of the *HoxC-8* early enhancer, five other *cis*-acting elements being conserved (Shashikant et al. 1998). When expressed in transgenic mice, the baleen whale enhancer directs gene expression to more posterior regions of the murine neural tube—the anterior expression boundary is four somites more posterior—but does not elicit expression of *HoxC-8* in the posterior mesoderm from which limb buds arise (Fig. 7C). A potential interpretation is that expression of *HoxC-8* is required to initiate hindlimb bud development and that changes in the *cis*-regulatory element in the baleen whale correlate with lack of *HoxC-8* expression and failure of hindlimb buds to develop further. Becuase other artiodactyls surveyed (cow, camel, llama, hippopotamus, and giraffe) showed no significant changes in *HoxC-8* early enhancer sequences, it is tempting to correlate the *cis*-regulatory changes in *HoxC-8* in mysticetes with the derived axial morphology seen in mysticetes and other whales, reduction in the hindlimbs being secondary to body elongation. Recall from the discussion above that the HoxC-8 protein *is*

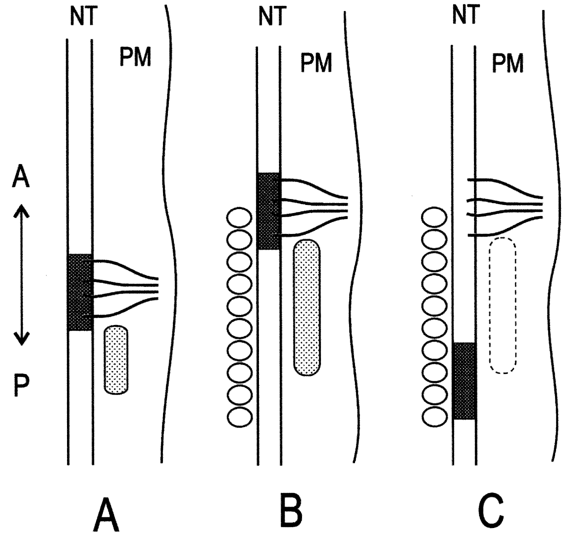

Fig. 7. Expression boundaries of *HoxC-8* in (A) chick and (B) murine embryos to show the more anterior expression in the neural tube of the mouse than in the chick and the anterior extension of expression in the mesoderm of the mouse in comparison with the chick. In both species, the anterior extent of expression coincides with the brachial nerves that innervate the limbs (shown as black lines from the neural tube). (C) Expression in mouse embryos of *HoxC-8* driven by the *cis*-regulatory enhancer of baleen whale *HoxC-8.* Expression in the neural tube is shifted the equivalent of four-somite lengths posteriorly (cf. B with C) and so is out of phase with the site of the brachial nerves. No expression is initiated in the mesoderm; the normal expression boundary is shown by the dotted oval (cf. B). A ↔ P, anterior-posterior body axis; NT, the neural tube; PM, paraxial mesoderm. See text for details.

expressed in the region where the hindlimbs would develop in python embryos, so that an explanation linked to *HoxC-8* would imply different developmental mechanisms of limb loss in pythons and baleen whales. Shashikant and colleagues, however, could not correlate the genetic change with any specific morphological trait in the mysticetes, and, more significantly, the sequence is conserved in the 12 other cetaceans surveyed, all of which, of course, have elongated bodies and lack hindlimbs.

CONCLUSION

A simple evolutionary change in *Hox* gene expression or *Hox* gene regulation is unlikely to have driven loss of the hindlimbs in cetaceans, which occurred concurrent with various other morphological and physiological changes associated with the transition from a terrestrial to an aquatic environment and adaptation to that aquatic environment. Selective pressures acting on a wide range of traits (developmental and adult) are likely to have driven the changes in cetacean morphology. Given what is known of loss of limbs in snake, lizard and limb-less mutants, and the existence of vestigial skeletal elements in whale hindlimb buds, changes *subsequent to initiation* of limb bud development, changes involving *maintenance* of the limb buds, are more likely to have been causal factors in loss of the hindlimbs and pelvic girdles in whales. Our evaluation of the secondary role of *Hox* genes in the structural changes in the hindlimbs of whales fits current revisionist thinking on the origins of body plans and of phyla (Budd and Jensen 2000; Conway Morris 2000). This thinking envisages a process of homeotic takeover; changes in *Hox* genes that are evolutionarily late in organ transformation provide sources of variation rather than initiating that variation. Such a conclusion fits both the developmental and the evolutionary data on limb bud development and limb loss.

Acknowledgments

We gratefully acknowledge research support from the Natural Sciences and Engineering Research Council of Canada, grant A5056 (to B. K. H.) and scholarship support from the Danish Research Academy (to L. B.). We thank Steve Dawson for the photograph used in Figure 3A, Ivan Misek for providing the images used in Figure 3B and 3C, and Andrew Hebda, Curator of Zoology, Nova Scotia Museum of Natural History, for assistance with photographing the specimens shown in Figure 3D and 3E. We are grateful to Annie Burke, Marty Cohn, Richard Connor, Larry Dill, Tim Fedak, Wendy Olson, Jon Stone, Matt Vickaryous, Hal Whitehead, and two anonymous reviewers for their helpful comments on the manuscript and to June Hall both for comments and for editorial expertise. Wendy Olson provided insightful comments on sea otters and on aquatic locomotion. Deborah Grantham, Wendy Olson, and Andrea Ottensmeyer helped with the preparation of some of the figures. Tim Fedak prepared Figures 5–7.

REFERENCES

Andrews, R. C. 1921. A remarkable case of external hind limb in a humpback whale. *Am. Mus. Novitates* 9: 1–9.

Arvy, L. 1979. The abdominal bones of cetaceans. In G. Pilleri G (ed.). *Investigations on Cetacea.* Vol. 10. Brain Anatomy Institute, Berne, Switzerland, pp. 215–227.

Atchley, W. R., and Hall, B. K. 1991. A model for development and evolution of complex morphological structures. *Biol. Rev. Camb. Philos. Soc.* 66: 101–157.

Bajpai, S., and Thewissen, J. G. M. 2000. A new, diminutive Eocene whale from Kachchh (Gujarat, India) and its implications for locomotor evolution of cetaceans. *Curr. Sci.* 79: 1478–1482.

Belting, H.-G., Shashikant, C. S., and Ruddle, F. H. 1998. Modification of expression and *cis*-regulation of *Hoxc8* in the evolution of diverged axial morphology. *Proc. Natl Acad. Sci. US* 95: 2355–2360.

Berta, A., and Sumich, J. L. 1999. *Marine Mammals: Evolutionary Biology.* Academic Press, San Diego.

Berzin, A. A. 1972. *The Sperm Whale.* Keter Press, Jerusalem.

Budd, G. E., and Jensen, S. 2000. A critical reappraisal of the fossil record of the bilaterian phyla. *Biol. Rev. Camb. Philos. Soc.* 75: 253–295.

Burke, A. C. 2000. *Hox* genes and the global patterning of the somitic mesoderm. *Curr. Top. Dev. Biol.* 47: 155–180.

Burke, A. C., Nelson, C. E., Morgan, B. A., and Tabin, C. 1995. *Hox* genes and the evolution of vertebrate axial morphology. *Development* 121: 333–346.

Caldwell, M. W. 1997. Limb osteology and ossification patterns in *Cryptoclidus* (Reptilia: Plesiosauroidea) with a review of Sauropterygian limbs. *J. Vert. Paleontol.* 17: 295–307.

Camp, C. L. 1923. Classification of the lizards. *Bull. Am. Mus. Nat. Hist.* 48: 298–481.

Carroll, R. L. 1988. *Vertebrate Paleontology and Evolution.* W. H. Freeman & Co., New York.

Carroll, S. B. 1995. Homeotic genes and the evolution of arthropods and chordates. *Nature* 376: 479–485.

Carroll, S. B. 2000. Endless forms: the evolution of gene regulation and morphological diversity. *Cell* 101: 577–580.

Chadwick, D. H. 2001. Evolution of Whales. *Natl. Geog.* 200: 654–677.

Chevallier, A., Kieny, M., and Mauger, A. 1977. Limb-somite relationship: origin of the limb musculature. *J. Embryol. Exp. Morphol.* 41: 245–258.

Christ, B., Jacob, H. J., and Jacob, M. 1977. Experimental analysis of the origin of the wing musculature in avian embryos. *Anat. Embryol.* 150: 171–186.

Coates, M. I. 1991. New palaeontological contributions to limb ontogeny and phylogeny. In J. R. Hinchliffe, J. M. Hurle, and D. Summerbell (eds.). *Developmental Patterning of the Vertebrate Limb.* Plenum Press, New York, pp. 325–337.

Coates, M. I., and Ruta, M. 2000. Nice snakes, shame about the legs. *TREE* 15: 503–507.

Cohn, M. J., Pate, K., Krumlauf, R., Wilkinson, D. G., Clarke, J. D. W., and Tickle, C. 1997. *Hox9* genes and vertebrate limb specification. *Nature* 387: 97–101.

Cohn, M. J., and Tickle, C. 1999. Developmental basis of limblessness and axial patterning in snakes. *Nature* 399: 474–479.

Conway Morris S. 2000. Nipping the Cambrian "explosion" in the bud? *BioEssays* 22: 1053–1056.

Cubo, J., and Arthur, W. 2001. Patterns of correlated character evolution in flightless birds: a phylogenetic approach. *Evol. Ecol.* 14: 693–703.

Deimer, P. 1977. Der rudimentäre hintere Extremitätengürtel des Pottwals (*Physeter macrocephalus* Linnaeus, 1758), seine Variabilität und Wachstumallometrie. *Z. Saügertier.* 42: 88–101.

Duboule, D., and Wilkins, A. S. 1998. The evolution of "bricolage." *Trends Genet.* 14: 54–59.

Essex, R. 1927. Studies in reptilian degeneration. *Proc. Zool. Soc. Lond.* 59: 879–945.

Felts, W. J. L., and Spurrell, F. A. 1966. Some structural and developmental characteristics of cetacean (odontocete) radii. A study of adaptive osteogenesis. *Am. J. Anat.* 118: 103–134.

Ferrari, D., Harrington, A., Dealy, C. N., and Kosher, R. A. 1999. *Dlx-5* in limb initiation in the chick embryo. *Dev. Dynam.* 216: 10–15.

Fish, F. E. 1996. Transition from drag-based to lift-based propulsion in mammalian swimming. *Am. Zool.* 36: 628–641.

Fish, F. E. 1998. Biomechanical perspective on the origin of cetacean flukes. In J. G. M. Thewissen (ed.). *The Emergence of Whales: Evolutionary Patterns in the Origin of Cetacea.* Plenum Press, New York, pp. 303–324.

Fong, D. W., Kane, T. C., and Culver, D. C. 1995. Vestigialization and loss of nonfunctional characters. *Annu. Rev. Ecol. Syst.* 26: 249–268.

Gans, C. 1974. *Biomechanics: An Approach to Vertebrate Biology.* J. B. Lippincott, Philadelphia.

Gans, C. 1975. Tetrapod limblessness: evolution and functional corollaries. *Am. Zool.* 15: 455–467.

Gatesy, J., and O'Leary, M. A. 2001. Deciphering whale origins with molecules and fossils. *TREE* 16: 562–570.

Gingerich, P. D., Smith, B. H., and Simons, E. L. 1990. Hind limbs of Eocene *Basilosaurus*: evidence of feet in whales. *Science* 249: 154–157.

Gingerich, P. D., Wells, N. A., Russell, D. E., and Shah, S. M. I. 1983. Origin of whales in epicontinental remnant seas: New evidence from the early Eocene of Pakistan. *Science* 220: 403–406.

Gingerich, P. D., Raza, S. M., Arif, M., Anwar, M., and Zhou, X. 1994. New whale from the Eocene of Pakistan and the origin of cetacean swimming. *Nature* 368: 844–847.

Gingerich, P. D., and Uhen, M. 1996. *Ancalecetus simonsi*, a new dorudontine archaeocete (Mammalia, Cetacea) from the early late Eocene of Wadi Hitan, Egypt. *Contrib. Mus. Paleont. Univ. Mich.* 29: 359–401.

Gingerich, P. D., Haq, M., Zalmout, I. S., Khan, I. H., and Malkani, M. S. 2001. Origin of whales from early artiodactyls: hands and feet of Eocene Protocetida from Pakistan. *Science* 293: 2239–2243.

Graham, A., and McGonnell, I. 1999. Developmental evolution: this side of paradise. *Curr. Biol.* 9: R630–R632.

Graur, D., and Higgins, D. G. 1994. Molecular evidence of the inclusion of cetaceans within the order Artiodactyla. *Mol. Biol. Evol.* 14: 537–364.

Greene, H. W., and Cundall, D. 2000. Limbless tetrapods and snakes with legs. *Science* 287: 1939–1941.

Greer, A. E. 1987. Limb reduction in the lizard genus *Lerista.* I. Variation in the number of phalanges and presacral vertebrae. *J. Herpetol.* 21: 267–276.

Guldberg, G., and Nansen, F. 1894. On the development and structure of the whale. *Bergens Mus.* 5: 1–70.

Haas, G. 1980. *Pachyrhachis problematicus* Haas, snakelike reptile from the Lower Cenomanian: ventral view of skull. *Bull. Mus. Natl. Hist. Nat. Paris* 2: 87–104.

Hall, B. K. 1978. *Developmental and Cellular Skeletal Biology.* Academic Press, New York.

Hall, B. K. 1984. Developmental mechanisms underlying the formation of atavisms. *Biol. Rev. Camb. Philos. Soc.* 59: 89–124.

Hall, B. K. 1995. Atavisms and atavistic mutations: evolutionary conservation of genetic and developmental information. *Nat. Genet.* 10: 126–127.

Hall, B. K. 1999. *Evolutionary Developmental Biology.* 2nd Ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hall, B. K. 2001. Development of the clavicles in birds and mammals. *J. Exp. Zool.* 289: 153–161.

Hall, B. K. 2002. Atavisms. In M. Pagel (ed.). *Encyclopedia of Evolution.* Vol. 1. Oxford University Press, New York, pp. 86–87.

Hall B. K. Palaeontology and evolutionary developmental biology: a science of the 19th and 21st centuries. *Palaeontology* 45: 647–649.

Hall, B. K. Descent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. *Biol. Rev. Camb. Philos. Soc.* In Press.

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

Hall, B. K., and Miyake, T. 2000. All for one and one for all: condensations and the initiation of skeletal development. *BioEssays* 22: 138–147.

Hall, B. K., and Olson, W. M. (eds.). 2002. *Key Concepts and Approaches in Evolutionary Developmental Biology*. Harvard University Press, Cambridge, MA.

Hinchliffe, J. R. 1977. The development of winglessness (ws) in the chick embryo. *Colloq. Int. C. N. R. S. Paris* 266: 175–182.

Hofstetter, R., and Gasc, J. P. 1969. Vertebrae and ribs of modern reptiles. In C. Gans, A. d'A. Bellairs, and T. S. Parsons (eds.). *Biology of the Reptilia.* Vol. 1, No. 5. Academic Press, London, pp. 201–301.

Howell, A. B. 1970. *Aquatic Mammals. Their Adaptation to Life in the Water.* Dover Publications, New York. (a reprint of the 1930 edition).

Johnson, D. R. 1986. *The Genetics of the Skeleton. Animal Models of Skeletal Development.* Clarendon Press, Oxford.

Kenyon, K. W. 1969. The sea otter in the Eastern Pacific Ocean. *North American Fauna*, No. 68. US Bureau of Sport Fisheries and Wildlife, pp. 1–352.

Kieny, M. 1971. Les phases d'activité morphogènes du mésoderm somatopleural le développement précoce du membre chez l'embryon de poulet. *Ann. Embryol. Morph.* 4: 281–298.

Klima, M. 1990. Rudiments of the clavicle in the embryo of whales (Cetacea). *Z. Saugetierkunde* 55: 202–212.

Klima, M. 1992. Schwimmbewegungen und Auftauchmodusi bei Walen und bei Ichyhyosurien. *Nat. Mus.* 122: 1–17, 73–89.

Kükenthal, W. 1891. On the adaptation of mammals to aquatic life. *Ann. Mag. Nat. Hist.* Ser. 6, 7: 153–179.

Kükenthal, W. 1893. *Vergleichend anatomische und entwicklungsgeschichtliche Untersuchung an Waltieren.* Denkschridt der Medizinish-Naturwissenschaftlichen Gesellschaft zu Dritter Band, Jena.

Lamarck, J. B. 1984. *Zoological Philosophy. An Exposition with Regard to the Natural History of Animals.* Translated by Hugh Elliot, with introductory essays by D. L. Hull and R. W. Burkhardt, Jr. The University of Chicago Press, Chicago.

Lande, R. 1978. Evolutionary mechanisms of limb loss in tetrapods. *Evolution* 32: 73–92.

Lee, M. S. Y., and Caldwell, M. W. 1998. Anatomy and relationships of *Pachyrhachis problematicus*, a primitive snake with hindlimbs. *Phil. Trans. R. Soc. Lond.* Ser B 352: 1521–1552.

Lettice, L., Hecksher-Sørensen, J., and Hill, R. E. 1999. The dominant hemimelia mutation uncouples epithelial-mesenchymal interactions and disrupts anterior mesenchyme formation in mouse hindlimbs. *Development* 126: 4729–4736.

Luo, Z. 2000. In search of whale's sisters. *Nature* 404: 235–239.

Lyons, G., Krabbenhoft, K., Simandl, B. K., Buckingham, M., Fallon, J., and Robert, B. 1991. *Hox-7*: temporal patterns of expression in normal and *limbless* chick embryos. In J. R. Hinchliffe, J. M. Hurle, and D. Summerbell (eds.). *Developmental Patterning of the Vertebrate Limb.* Plenum Press, New York, pp. 55–56.

Marshall, C. R., Raff, E. C., and Raff, R. A. 1994. Dollo's law and the death and resurrection of genes. *Proc. Natl Acad. Sci. USA* 91: 12283–12287.

Meyer, W., Neurand, K., and Klima, M. 1995. Prenatal development of the integument in Delphinidae (Cetacea: Odontoceti). *J. Morphol.* 223: 269–287.

Milinkovitch, M. C. 1995. Molecular phylogeny of cetaceans prompts revision of morphological transformations. *TREE* 10: 328–334.

Milinkovitch, M. C., Orti, G., and Meyer, A. 1993. Revised phylogeny of whales suggested by mitochondrial ribosomal DNA sequences. *Nature* 361: 346–348.

Milinkovitch, M. C., and Thewissen, J. G. M. 1997. Even-toed fingerprints on whale ancestry. *Nature* 388: 622–624.

Milaire, J. 1981. Les répercussions morphologiques du gène *Dh* (Dominant hememelia) sur les bourgeons de membres postérieurs et les constituants adjacens du tronc chez l'embryon de souris hétérozygote de 10 jours (stades de 32 à 43 métamères). *Arch. Biol.* 92: 95–138.

Miller, G. S. 1923. The telescoping of the cetacean skull. *Smithsonian Misc. Coll.* 76: 1–67.

M-Z Rahmani, T. 1974. Morphogenesis of the rudimentary hind-limb of the glass snake (*Ophisaurus apodus* Pallas). *J. Embryol. Exp. Morphol.* 32: 431–443.

O'Leary, M. A. 1999. Parsimony analysis of total evidence from extinct and extant taxa and the Cetacean-Artodactyl question (Mammalia, Ungulata). *Cladistics* 15: 315–330.

O'Leary, M. A. 2001. The phylogenetic position of Cetaceans: Further combined data analyses, comparisons with the stratigraphic record and a discussion of character optimization. *Am. Zool.* 41: 487–506.

O'Leary, M. A., and Geisler, J. H. 1999. The position of Cetacea within Mammalia: phylogenetic analysis of morphological data from extinct and extant tax. *Syst. Biol.* 48: 455–490.

O'Leary, M. A., and Uhen, M. D. 1999. The time of origin of whales and the role of behavioral changes in the terrestrial-aquatic transition. *Paleobiology* 25: 534–556.

Omura, H. 1980. Morphological study of pelvic bones of the minke whale from the Antarctic. *Sci. Rep. Whales Res. Inst.* 32: 25–37.

Owen, R. 1842. Observations on the *Basilosaurus* of Dr. Harlan (*Zeuglodon cetoides*, Owen). *Trans Geol. Soc. Lond.* 6: 69–79.

Pabst, D., Rommel, S. A., and Mclellan, W. A. 1998. Evolution of thermoregulatory function in cetacean reproductive systems. In J. G. M. Thewissen (ed.). *The Emergence of Whales: Evolutionary Patterns in the Origin of Cetacea.* Plenum Press, New York, pp. 379–390.

Padian, K. 1992. A proposal to standardize phalangeal formula designations. *J. Vert. Paleont.* 12: 260–262.

Pena, M. R. C. 1967. Contribucion al conocimiento de la osteologia apendicular de *Bipes* (Reptilia: Amphisbaenia). *Tesis Profesional*, Inst Politec. Nac. Mexico.

Pinot, M. 1970. Le Rôle du mésoderm somitique dans la morphogenèse précoce des membres de l'embryon de Poulet. *J. Embryol. Exp. Morphol.* 23: 109–151.

Presch, W. 1975. The evolution of limb reduction in the teiid lizard genus *Bachia. Bull. So. Calif. Acad. Sci.* 74: 113–121.

Rage, J.-C., and Escuillié F. 2000. Un nouveau serpent bipède du Cénomanien (Crétacé). Implications phylétiques. *C. R. Acad. Sci. Paris* 330: 513–520.

Raynaud, A. 1990. Developmental mechanisms involved in the embryonic reduction of limbs in reptiles. *Int. J. Dev. Biol.* 34: 233–243.

Raynaud, A., and Kan, P. 1992. DNA synthesis decline involved in the developmental arrest of the limb bud in the embryo of the slow worm, *Anguis gragilis* (L.). *Int. J. Dev. Biol.* 36: 303–310.

Raynaud, A., Okuzumi, H., and Kouprach, S. 1974. Morphologie externe des stades précoces du développement des ébauches des membres du Lézard vert "*Lacerta viridis* Laur" et de l'Orvet "*Anguis fragilis* L", etudié au moyen de la microscopie électronique à balayage. *Bull. Soc. Zool. France* 99: 149–153.

Raynaud, A., and van den Elzen, P. 1976. La rudimentation des membres chez les embryons de *Scelotes gronovii* (Daudin), reptile scincidé Sud-Africain. *Arch. Anat. Microsc. Morphol. Exp.* 65: 17–36.

Rieppel, O. 1988. A review of the origin of snakes. *Evol. Biol.* 22: 37–130.

Robert, B., Lyons, G., Simandl, B. K., Kuroiwa, A., and Buckingham, M. 1991. The apical ectodermal ridge regulates *Hox-7* and *Hox-8* gene expression in developing chick limb buds. *Genes Dev.* 5: 2363–2374.

Rommel, S. A. 1990. Osteology of the bottlenose dolphin. In S. R. Leatherwood and R. R. Reeves (eds.). *The Bottlenose Dolphin.* Academic Press, San Diego, pp. 29–49.

Rosa-Molinar, E., and Burke, A. C. 2002. Starting from fins: parallelism in the evolution of limbs and genitalia: the fin-to-genitalia transition. *Evol. Dev.* 4: 124–126.

Schneider, R. A., Hu, D., and Helms, J. A. 1999. From head to toe: conservation of molecular signals regulating limb and craniofacial morphogenesis. *Cell Tissue Res.* 296: 103–109.

Sedmera, D., Misek, I., and Klima, M. 1997a. On the development of cetacean extremities. I. Hind limb rudimentation in the spotted dolphin (*Stenella attenuata*)**.** *Eur. J. Morphol.* 35: 25–30.

Sedmera, D., Misek, I., and Klima, M. 1997b. On the development of cetacean extremities. II. Morphogenesis and histogenesis of the flippers in the spotted dolphin (*Stenella attenuata*). *Eur. J. Morphol.* 35: 117–123.

Shapiro, M. D. Developmental morphology of limb reduction in *Hemiergis* (Squamata: Scincidae): Chondrogenesis, osteogenesis, and heterochrony. *J. Morphol.* In Press.

Shapiro, M. D., and Carl, T. F. 2001. Novel features of tetrapod limb development in two nontraditional model species: a skink and a direct-developing frog. In M. L. Zelditch (ed.). *Beyond Heterochrony. The Evolution of Development.* Wiley-Liss, New York, pp. 337–361.

Shashikant, C. S., Kim, C. B., Borbély, M. A., Wang, W. C. H., and Ruddle, F. H. 1998. Comparative studies on mammalian *Hoxc8* early enhancer sequence reveal a baleen whale-specific deletion of a cis-acting element. *Proc. Natl Acad. Sci. USA* 95: 15446–15451.

Shimamura, M., Yasue, H., Ohshima, K., Abe, H., Kato, H., Kishiro, T., Goto, M., Munechika, I., and Okada, N. 1997. Molecular evidence from retroposons that whales form a clade within the even-toed ungulates. *Nature* 88: 666–670.

Shubin, N., Tabin, C., and Carroll, B. B. 1997. Fossils, genes and the evolution of animal limbs. *Nature* 388: 639–648.

Slijper, E. J. 1979. *Whales.* Cornell University Press, Ithaca.

Smith, K. K., and Schneider, R. A. 1998. Have gene knockouts caused evolutionary reversals in the mammalian first arch? *BioEssays* 20: 245–255.

Spoor, F., Bajpai, S. T., Hussain, K., and Thewissen, J. G. M. 2002. Vestibular evidence for the evolution of aquatic behaviour in early cetaceans. *Nature* 417: 163–166.

Sterba, O., Klima, M., and Schlidger, B. 1994. Proportional growth of dolphins during the prenatal period. *Funct. Dev. Morphol.* 4: 282–283.

Sterba, O., Klima, M., and Schlidger, B. 2000. Embryology of dolphins. Staging and ageing of embryos and fetuses of some cetaceans. *Adv. Anat. Embryol. Cell Biol.* 157: 1–133.

Stokeley, P. S. 1947. Limblessness and correlated changes in the girdles of a comparative morphological series of lizards. *Am. Midl. Nat.* 38: 725–754.

Struthers, J. 1881. On the bones, articulations, and muscles of the rudimentary hind-limb of the Greenland Right-whale (*Balaena mysticetus*). *J. Anat. Physiol.* 15: 141–176, 302–321.

Szalay, F. 1969. The Hapalodectinae and a phylogeny of the Mesonychidae (Mammalia, Condylartha). *Am. Mus. Nov.* 2361: 1–26.

Tautz, D. 2000. Evolution of transcriptional regulation. *Curr. Opin. Genet. Dev.* 10: 575–579.

Tchernov, E., Rieppel, O., Zaher, H., Polcyn, M. J., and Jacobs, L. L. 2000. A fossil snake with limbs. *Science* 287: 2010–2012.

Thewissen, J. G. M. (ed.). 1998. *The Emergence of Whales: Evolutionary Patterns in the Origin of Cetacea*. Plenum Press, New York.

Thewissen, J. G. M., and Bajpai, S. 2001. Whale origins as a poster child for macroevolution. *BioScience* 51: 1037–1049.

Thewissen, J. G. M., and Fish, F. E. 1997. Locomotor evolution in earliest cetaceans: functional model, modern analogues, and paleontologic evidence. *Paleobiology* 23: 482–490.

Thewissen, J. G. M., and Hussain, S. T. 1993. Origin of underwater hearing in whales. *Nature* 361: 444–445.

Thewissen, J. G. M., Hussain, S, T., and Arif, M. 1994. Fossil evidence for the origin of aquatic locomotion in archaeocete whales. *Science* 263: 210–212.

Thewissen, J. G. M., Madar, S. I., and Hussain, S. T. 1998. Whale ankles and evolutionary relationships. *Nature* 395: 452.

Thewissen, J. G. M., Williams, T. M., Roe, L. J., and Hussain, S. T. 2001. Skeletons of terrestrial cetaceans and the relationship of whales to artiodactyls. *Nature* 413: 277–281.

Uhen, M. 1998. Middle to late Eocene basilosaurines and dorudontines. In J. G. M. Thewissen (ed.). *The Emergence of Whales: Evolutionary Patterns in the Origin of Cetacea.* Plenum Press, New York, pp. 29–61.

Uhen, M. D., and Gingerich, P. D. 2001. New genus of dorudontine Archaeocete (Cetacea) from the middle-to late Eocene of South Carolina. *Mar. Mamm. Sci.* 17: 1–34.

van der Schoot, P. 1995. Studies on the fetal development of the gubernaculum in cetacea. *Anat. Rec.* 243: 449–460.

Van Valen, L. 1968. Monophyly or diphyly in the origin of whales. *Evolution* 22: 37–41.

Van Valen, L. 1973. A new evolutionary law. *Evol. Theory* 1: 1–30.

Vassé, J. 1974. Etudes expérimentales sur le rôle des somites au cours des premiers stades du développement du membre antérieur chez l'embryon du chélonien, *Emys orbicularis* L. *J. Embryol. Exp. Morphol.* 32: 417–430.

Vassé, J. 1977. Etudes expérimentales sur les premiers stades du développement du membre antérieur chez l'embryon du chélonien, *Emys orbicularis* L: détermination en mosaïque et régulation. *J. Embryol. Exp. Morphol.* 42: 135–148.

Wartzok, D., and Ketten, D. R. 1999. Marine Mammal Sensory Systems. In J. E. Reynolds and S. A. Rommel (eds.). *Biology of Marine Mammals.* Smithsonian Institution Press, Washington, DC, pp. 117–175.

Weihs, D. 1989. Design features and mechanics of axial locomotion in fish. *Am. Zool.* 29: 151–160.

Williams, T. M. 1989. Swimming by sea otters: adaptations for low energetic cost locomotion. *J. Comp. Physiol. A* 164: 815–824.

Wong, K. 2002. The mammals that conquered the seas. *Sci. Am.* 286: 70–79.

Yablokov, A. V. 1974. *Variability of Mammals.* Amerind Publishing Co., New Delhi.