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Time, pattern, and heterochrony: a study of hyperphalangy in the dolphin embryo flipper

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SUMMARY The forelimb of whales and dolphins is a flipper that shows hyperphalangy (numerous finger bones). Hyperphalangy is also present in marine reptiles, including ichthyosaurs and plesiosaurs. The developmental basis of hyper-phalangy is unclear. Kükenthal suggested that phalanx anlagen split into three pieces during cetacean development, thereby multiplying the ancestral number. Alternatively, Holder suggested that apical ectodermal ridge (AER)-directed limb outgrowth might be prolonged by a timing shift (heterochrony), leading to terminal addition of extra phalanges. We prepared a series of whole mounted and serially sectioned embryonic flipper buds of the spotted dolphin *Stenella attenuata.* This cetacean shows marked hyperphalangy on digits II and III. We confirm previous reports that the proximodistal laying down of phalanges is prolonged in digits II and III. Histology showed that the apical ectoderm was thickened into a cap. There was a weak ridge-like structure in some embryos. The cap or ridge formed part of a bud-like mass that persisted on digits II and III at stages when it had disappeared from other digits. Thus the dolphin differs from other mammals in showing a second period of limb outgrowth during which localized hyperphalangy develops. New phalanges only formed at the tip of the digits. These findings are consistent with a model in which heterochrony leads to the terminal addition of new phalanges. Our results are more easily reconciled with the progress zone model than one in which the AER is involved in the expansion of a prepattern. We suggest that patterning mechanisms with a temporal component (i.e., the "progress zone" mechanism) are potential targets for heterochrony during limb evolution.

INTRODUCTION

Cetaceans (whales, dolphins, and porpoises) show many specializations to aquatic life (Coffey 1977). The propulsive tail has horizontal flukes, the hindlimbs are rudimentary (Ogawa 1953), and the forelimbs are modified as flippers that function as rudders. In comparison with the forelimb of terrestrial mammals, the flipper shows features that may contribute to its hydrofoil properties (strength and slight flexibility). Thus, the arm and forearm bones are shortened, the digits are webbed, there are syndesmoses not synovial joints, musculature is reduced, and the skeleton is poorly ossified (Kunze 1912). A particularly striking feature in the forelimb or flipper is hyperphalangy—an unusually high number of finger bones or phalanges (Kükenthal 1889, 1893). Although terrestrial mammals commonly have a phalangeal formula (i.e., the number of phalanges on digits I–V, respectively) of 2-3-3-3-3 (Flower 1870), a formula of 1-9-7-4-1 was recorded by Tomilin (1967, p. 522) for what was probably an adult specimen of the dolphin *Stenella coeruleoalba.* Furthermore, the long-finned pilot whale (*Globicephala melas*) has up to 17 phalanges on digit II (Kükenthal 1889). Hyperphalangy (polyphalangy) is typically more marked in toothed whales (including dolphins) than in baleen whales. Thus, within the cetaceans there is wide variation between species in the forelimb phalanx number (Kükenthal 1889, pp. 55–56; Felts 1966, p. 259). Hyperphalangy is shared convergently with some members of extinct aquatic reptile groups (ichthyosaurs, plesiosaurs, and mosasaurs; see Carroll 1987).

The developmental basis of hyperphalangy is unclear, partly because cetacean embryos are rarely studied by scientists. Furthermore, molecular and experimental studies are impossible in these protected species. Classical morphologists published monographs on cetacean development, based on limited series of embryos (Guldberg and Nansen 1894; Kükenthal 1889, 1893; Müller 1920). More recent work has used the Frankfurt collection, which is studied here (Buhl and Oelschläger 1988; Oelschläger and Kemp 1998; Sedmera et al. 1997a,b; Štěrba et al. 2000).

Intercalation and terminal addition models of hyperphalangy

Kükenthal proposed that the cetacean digit initially develops three phalangeal rudiments (Fig. 1A). Each one was thought

to split, during development, into three subelements (Kükenthal 1889). This could multiply the ancestral phalanx count from three to nine (Kükenthal 1893). We characterize this model as "intercalation," because the new phalanges develop within an ancestral spatial sequence of elements (Fig. 1A). One problem with Kükenthal's model is that it can generate no more than nine phalanges per digit, yet toothed whales may have more than this number (Kükenthal 1889). Kükenthal got around this problem by suggesting that numbers above nine are formed by budding at the tip of the digit. We characterize this budding as "terminal addition," because the new phalanges develop only at the distal end of the existing series (Fig. 1B). See Leboucq (1896) for further discussion of these models, Weber (1967, pp. 369–370) for a variant form of the intercalation model, and Holder (1983) for a form of terminal addition.

Phalanx number, developmental timing, and heterochrony

Terminal addition can be explained by the progress zone model of limb development (Summerbell et al. 1973). According to this model, skeletal elements are specified in strict proximodistal sequence during limb "outgrowth." (Limb outgrowth is used here in the specific context of pattern formation and not to describe the growth of skeletal elements after they have been specified.) Experiments on chicken embryos show that outgrowth depends on reciprocal interactions between the undifferentiated mesenchyme ("progress zone") at the limb tip and the apical ectodermal ridge (AER) overlying it (Saunders 1948; Niswander et al. 1993; Dahn and Fallon 1999). For our purposes the key point about the progress zone model is that it is a temporal one: The positional value of cells along the proximodistal axis correlates with the length of time spent in the progress zone (Summerbell et al. 1973). This relationship has been shown to apply to the development of phalanges (Summerbell 1974).

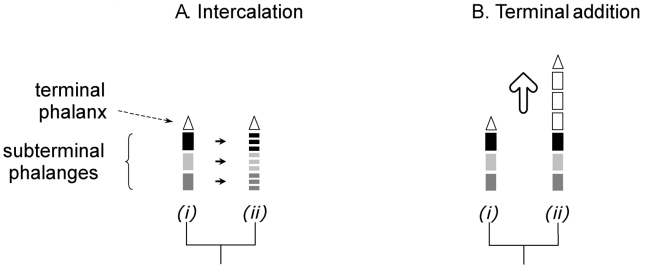


Fig. 1. Schematic diagram comparing (A) intercalation and (B) terminal addition models of hyperphalangy, both resulting in a hypothetical total of nine permanent phalanges. In each case a single adult digit is represented at the right-hand side with distal at the top. The hypothetical species shown are (i) a euphalangious terrestrial mammal and (ii) a hyperphalangious cetacean sister species. The figures can also be viewed as though (i) is an earlier stage in the development of taxon (ii), in which case the arrows indicate the polarity of ontogenetic transformations. A shows the splitting up of the three primary phalanges into three permanent phalanges each. The "terminal addition" model (B) includes a component of intercalation because the phylogenetically new phalanges (white boxes) have appeared proximal to the terminal phalanx.

A mechanistic link between the duration of outgrowth and the numbers of phalanges might be provided by the cyclical nature of cartilage specification in the limb (Lewis 1975). If outgrowth is permissive for this cyclical process, prolonged outgrowth would allow more cycles of phalanx specification to take place. Periodicity is also seen in patterns of expression of *Wnt14* and *Gdf5* genes (reviewed by Spitz and Duboule 2001), which appear to be involved in events downstream of outgrowth. Although a reduction of phalanx number can be produced rather easily by AER ablation and other manipulations, hyperphalangy is rarely reported in the experimental literature. An interesting exception is the production of six phalanges in the chick foot digit by modulating bone morphogenetic protein (BMP) signaling (Dahn and Fallon 2000).

However, new data argue against the progress zone model and show that a modified prepattern model is consistent with many experimental findings, including reaggregate experiments (Sun et al. 2002; Dudley et al. 2002). These new models include a temporal component in their formulation (Sun et al. 2002; Dudley et al. 2002). For example, it may be that a prepattern only becomes irreversibly fixed or determined over time. The determination may take place such that skeletal precursors become progressively restricted to distal fates (Dudley et al. 2002). This form of prepattern model is all but indistinguishable, at least using our data set, from the progress zone model: both behave as temporal mechanisms that link timing with proximodistal positional value. In this article therefore, our discussion of heterochronic shifts may apply to the new prepattern mechanisms with a temporal component, as well as to progress zone mechanisms.

A "prolonged outgrowth" hypothesis has been proposed to account for cetacean hyperphalangy (Holder 1983, p. 406; see also Sedmera et al. 1997b). Holder thought that an extended life of the AER would add more cells to the limb tip and might be able to generate extra skeletal elements in combination with other patterning mechanisms. In principle we believe that patterning mechanisms with a temporal component, such as the progress zone mechanism, are likely to be potential targets for heterochrony (timing shifts during evolution). Because *time* correlates with *pattern* in the progress zone model, heterochrony should lead to a change in morphology. Heterochrony has been invoked as an important mechanism in the evolution of the vertebrate limb and other systems (Gould 1977; Shubin and Alberch 1986; McKinney and McNamara 1991; Dolle et al. 1993; Richardson 1999). To examine these issues we prepared and studied a develop-

mental series of embryonic dolphin flippers using standard morphological techniques.

MATERIALS AND METHODS

The material examined is summarized in Table 1. Embryos were from a unique and well-preserved developmental series of the pantropical spotted dolphin *Stenella attenuata* (Gray 1846; see also Rice 1998 for an account of cetacean systematics). The phalangeal formula in this species is recorded as 2-6-6-4-2 (in digits I–V, respectively, for a 193-mm fetus; Štěrba et al. 2000, p. 104).

The embryos and early fetuses came from females found dead in tuna nets in the 1970s by US National Marine Fisheries Service and subsequently transferred to Frankfurt (Institute of Anatomy III [Dr. Senckenbergische Anatomie], J. W. Goethe University of Frankfurt am Main, Germany). The specimens had been stored in formalin or alcohol. All appeared to be formalin fixed, judging from the presence of formalin pigment in the blood vessels. They were staged and assigned an estimated gestational age, according to Štěrba et al. (2000). Part of this series (with "SA" catalogue numbers) has been described by Sedmera et al. (1997b).

In view of the great rarity of this material, only a few embryos could be selected for staining and sectioning. The remainder were viewed as unstained whole mounts, in 70% alcohol, to examine the external form of the limb and the apical ectoderm (Table 1). Crown–rump lengths were measured using *Image Tool* software (developed by The University of Texas Health Science Centre, San Antonio, USA) from digital images of embryos photographed next to a millimeter scale. Crown–rump length was defined according to Štěrba et al. (2000, Fig. 1). Measurements from histological sections were made with a calibrated eyepiece graticule.

Stained and cleared whole mounts

Left flippers were detached and stained overnight in 0.03% Alcian blue in acid alcohol (1% concentrated hydrochloric acid in 70% ethanol). They were then dehydrated in a graded ethanol series to 100% and cleared in three changes of methyl salicylate. Staining of cartilage was weak, as has been noted by previous workers for this material (Sedmera et al. 1997b). However, the cartilaginous skeleton could be seen clearly with dark-field illumination.

Table 1. Summary of *Stenella attenuata* embryos and early fetuses examined in this study

Specimen No.	Stage	Mean Est. Age (days)[1]	Crown–Rump Length (mm)	Whole Mounting	Section Plane	Form of Forelimb
LES021	3	24	8.08	70% ethanol	—	Bud as long as wide
CW0813	3		n.d.	70% ethanol	—	Digital plate
SA2[1]	3		n.d.[2]	70% ethanol	—	Digital plate
SA3[1]	3		8	(Existing histological series)	?	
SA8[1]	4	30	8.57	Stain/clear	a → p	Digital plate
SA10[1]	4		10.95	70% ethanol	—	Digital plate
MEH027	4		10.34	70% ethanol	—	Digital plate
FCL011	4		12.90	70% ethanol	—	Digital plate
JRH022	5	38	16.83	70% ethanol	—	Digital plate
SA23[1]	5		17.59	70% ethanol	—	Digital plate
SA26[1]	5		16.44	Stain/clear	d → v	Digital plate
SA13[1]	5		13.99	70% ethanol	—	Digital plate angular
PLT023	5		20.68	Stain/clear	a → p	Digital plate angular
MWD092	5		n.d.	Stain/clear	d → v	Digital plate angular
AXP206	5		16.40	70% ethanol	—	Digital plate angular
SA38[1]	6	46	23[3]	70% ethanol	—	Digital plate angular
SA37[1]	6		23[3]	70% ethanol	—	Chisel-shaped over digits II–III
RDL078	6		25.40	Stain/clear	a → p	Flipper, crenated margin
WKI160	6		n.d.	Stain/clear	a → p	Flipper, crenated margin
SA41[1]	6		29[3]	70% ethanol	—	Flipper, crenated margin
SA47[1]	6		30[3]	70% ethanol	—	Flipper, crenated margin
RLW052	6/7	46–57	30.78	70% ethanol	—	Flipper, crenated margin
MEH040	6/7		33.13	Stain/clear	d → v	Flipper, crenated margin
JRH025	6/7		34.52	Stain/clear	a → p	Flipper, crenated margin

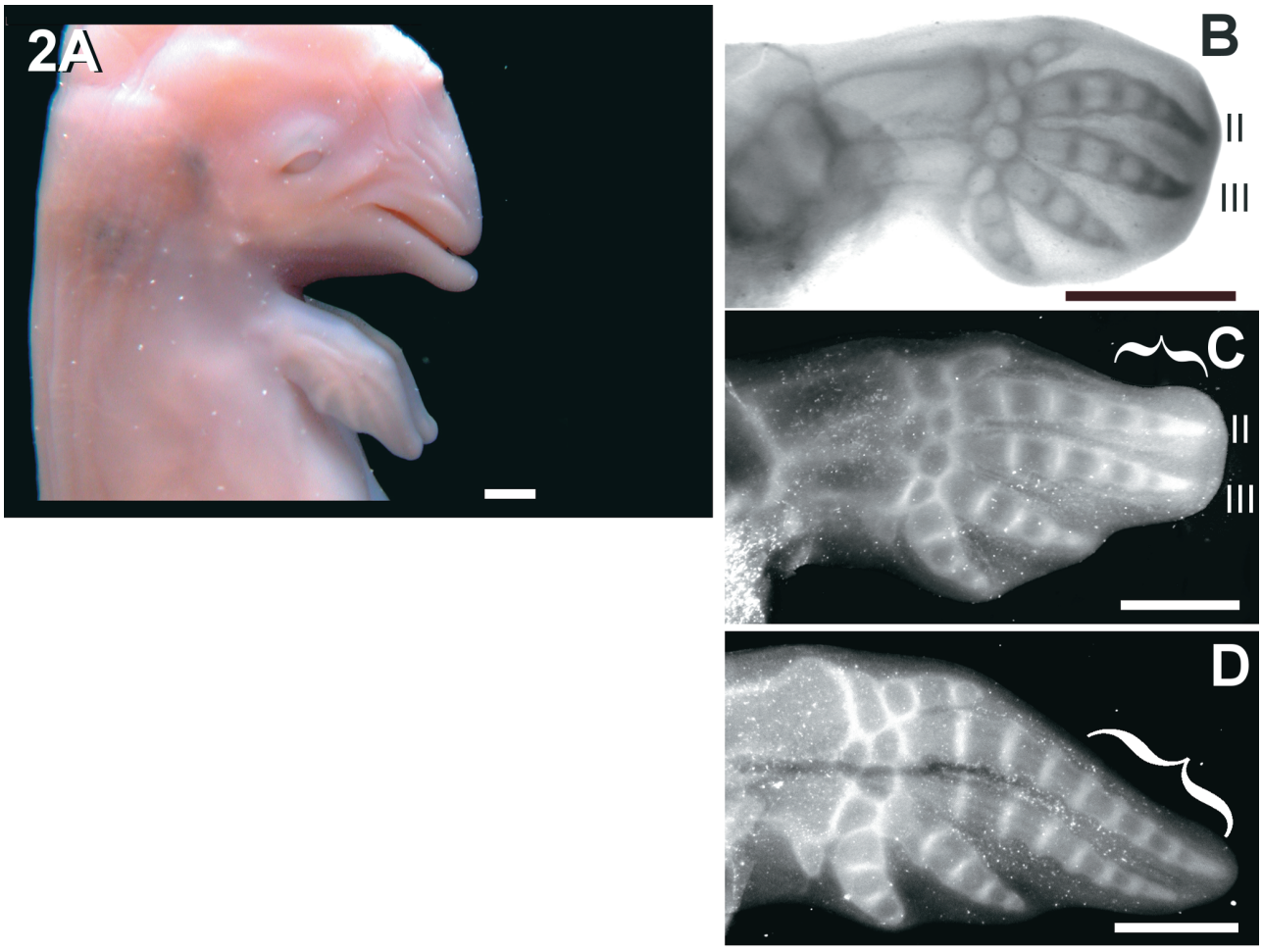
Stages and estimated gestational ages are according to Štěrba et al. (2000). Whole mounting: "70% ethanol" specimens were unstained; stain/clear indicates Alcian blue stain followed by dehydration in alcohol and clearing in methyl salicylate.

[1] Specimen described in Štěrba et al. (2000); a → p is a series in which the first section passes through digit I and the last through digit V; d → v is a series in which the first section is in the plane of the dorsal surface of the flipper and the last is in the plane of the volar surface.

[2] Head missing.

[3] Crown–rump length from Table 1 in Štěrba et al. (2000).

n.d., not determined.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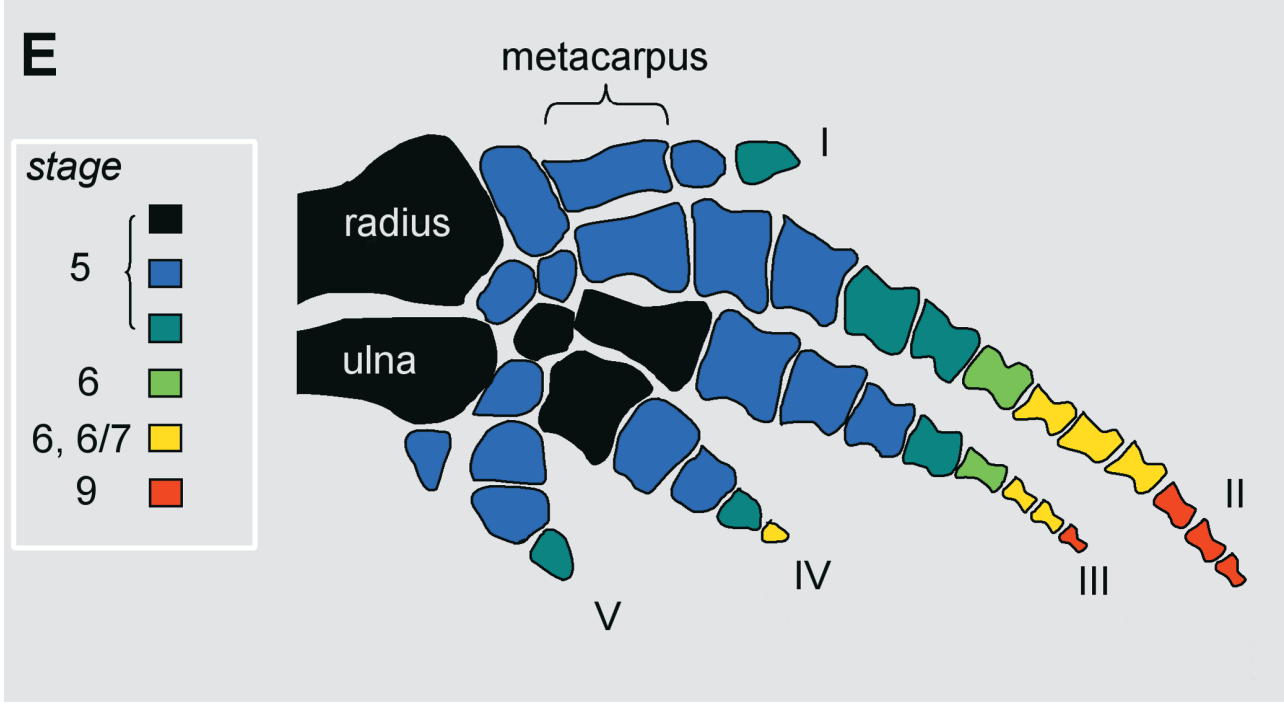
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Histology

Whole-mounted flippers (see above) were transferred from methyl salicylate into three changes of paraffin wax and then serially sectioned at 5–20 μm. Sections were de-waxed, triple stained with Alcian blue (1.0% in 1.5% acetic acid) and hematoxylin and eosin, and cover slipped. The plane of sectioning is indicated in Table 1.

RESULTS

Specimen numbers are listed in the figure legends or in the text below. For descriptions of external features of the forelimb at different stages, see Sedmera et al. (1997b) and Štěrba et al. (2000).

External form

The main external features are shown in Figure 2, A–D. Briefly, the limb was as long as wide at stage 3 and showed a digital plate at stages 3 or 4. The margins of the digital plate became angular as limited excavation between the digital rays took place (stage 5, Fig. 2B). After this, digits II and III became conspicuously elongated, giving the flipper a chisel-shaped outline at the tip (stage 6, Fig. 2C). Finally, a true flipper shape was established as digits II and III became deviated toward the ulnar side (between stages 6 and 7, Fig. 2D); the ulnar margin of the flipper at these stages started to show scalloping, due to shallow indentations between the digits (Fig. 2, C and D). The digits never become free, however.

Development of the cartilaginous skeleton

Consistent with previous reports for *Stenella* and other cetaceans (Kükenthal 1893; Sedmera et al. 1997b), we found that the development of phalanges in digits II and III continued after most of the flipper skeleton has already formed (Fig. 2, B–E) and followed a strict proximodistal sequence (Fig. 2E). The limb bud had no cartilage condensations at stage 3 and was not conspicuously elongated along the proximodistal axis (Fig. 3A). After this, the flipper bud elongated and a series of phalanges was added to the digital plate (Fig. 3, B–G). Histology showed that mesenchymal condensations for new phalanges only formed distal to the last-formed phalanx and never proximal to it (Fig. 3, B, D, and G, arrows). In other words, there was a smooth gradient of shape, differentiation, and size of the cartilaginous elements, indicating a strictly proximodistal gradient in phalanx segregation (Figs. 2 and 3D; this is difficult to show in microslides because the digits are curved; indeed this is a possible source of Kükenthal's misinterpretations). The newest (and therefore most distal) mesenchymal condensations appear as triangular structures in Figure 2, B–D and Figure 3, B, D, and G. They give the spurious appearance of terminal phalanges (which, in fact, only develop at later stages).

Thickened apical ectoderm

The essential findings relating to the apical ectoderm are shown schematically in Figure 3H. A rather complicated biphasic pattern was seen. Before describing this pattern in detail, we summarize it as follows. An apical ectodermal thickening was prominent around the margin of the digital plate at stages 3 and 4. There was a low ridge-like structure in a few embryos. In stage 5 embryos, when the margin of the digital plate became angular, the AER became more inconspicuous and was localized to the central digits. At later stages, when digits II and III were becoming significantly longer than the others, the ridge became distinct once more but was localized to the tips of those two digits. The ridge overlaid a conspicuous bud-like blastema on the tips of those two digits.

Histological series in two orthogonal planes showed that the apical ectoderm was thickened in comparison with the adjacent non-apical ectoderm (Table 2, Fig. 3, B–G). The thickened ectoderm overlaid the marginal sinus and was organized as a low AER in some specimens (Fig. 3, A, E, and G). At stages 3 and 4 the thickened ectoderm spanned the distal flipper margin. It was organized as a thickened cap in its peripheral parts and as a distinct AER over the central digits. At stage 5 the AER was still present over digits II and III in one embryo (PLT23) but was more weakly organized. On digits I, IV, and V, the apical ectoderm became thinned after stage 5 (mean, 11.0 μm thick; Fig. 3, B and C) toward the close of phalanx segregation. The apical thickening on digits II and III, and ultimately II alone, formed part of a bud-like blastema from late stage 5 onward (Fig. 3, F and G).

The only difference seen between whole mounts and the histological series was that in the former no AER was seen projecting from the surface of any flipper. This presumably reflects the generally poor development of the dolphin flipper AER. Interestingly, an AER was visible on the hindlimb of one whole-mounted embryo (LES021, stage 3). In PLT023 (stage 5) the ectodermal thickening spanned the flipper margin but was prominently thickened over the tips of digits II and III, which were beginning to project further than the other digits. In WKI160 and RDL078 (stage 6) the flipper was now strongly asymmetrical due to the elongation of dig-

Fig. 2. Showing *S. attenuata.* (A) Right lateral view of head and flippers, stage 6 (estimated mean age from Štěrba et al. 2000, 46d). Bar, 2 mm. (B–D) Cleared flippers, stages 5 (PLT023), 6 (RDL078), and 6/7 (JRH025) respectively, anterior to top. Bars, 1 mm. Extra phalanges are added to digits II and III (white braces). Roman numerals represent digit numbers. (E) Summary of our data on the first appearance of cartilaginous elements in the relevant stages (5–9).

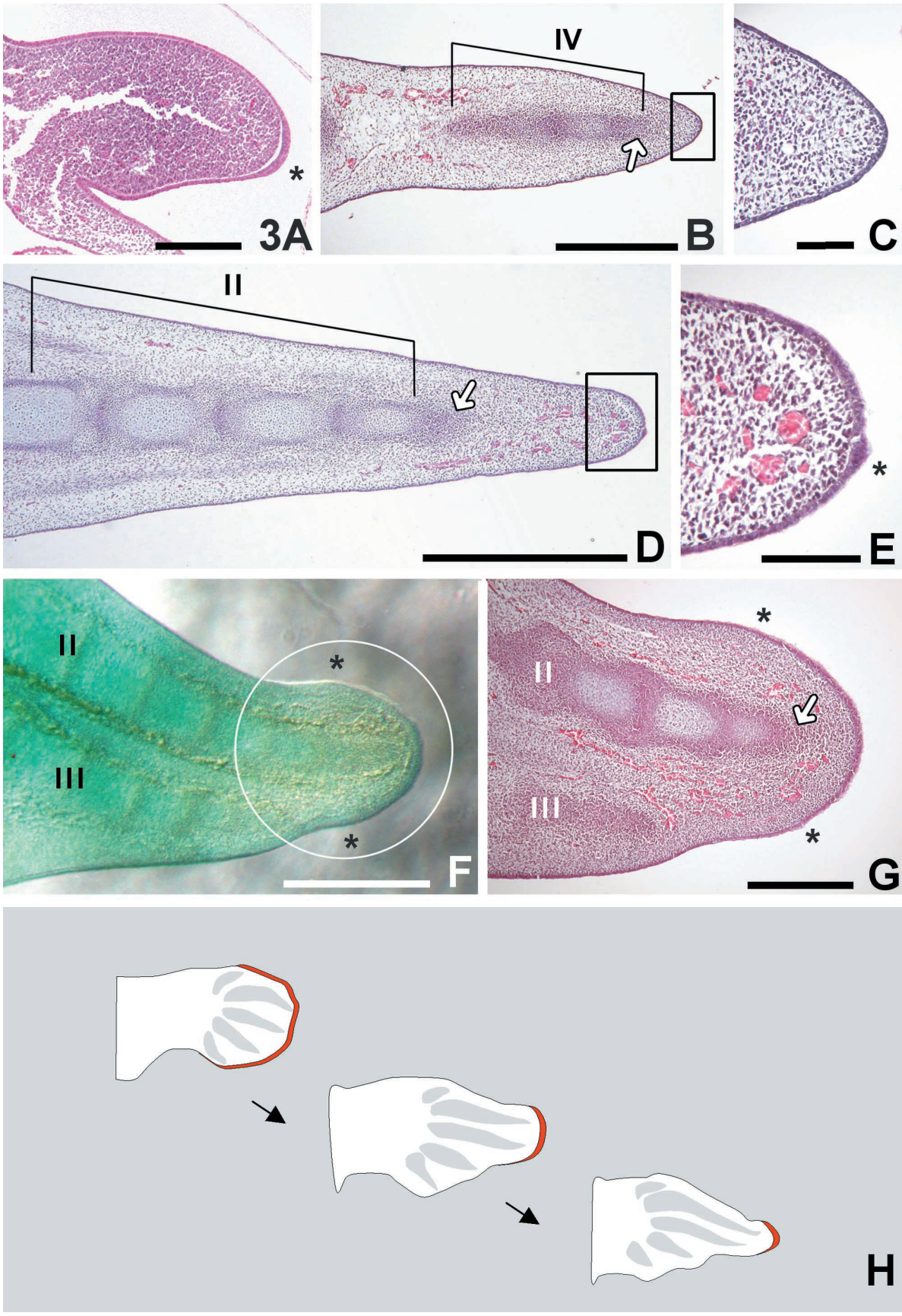
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Table 2. Measurements of thickness of apical ectoderm at tip of the flipper (i.e., in the region of digits II or III) or a non-apical region (i.e., over digit IV) measured from sections

Specimen No. (Slide No.)	Stage	Region Measured	Counts of Thickness of Apical Ectoderm (μm) on Adjacent Sections				Mean (μm)
SA8 (4)	4	Apical	22.05	17.5	22.05	22.05	20.95
SA8 (6)	4	Apical	14.7	17.5	14.7	14.7	15.4
PLT23 (10)	5	Apical	9.8	12.25	14.7	12.25	12.25
PLT23 (11)	5	Apical	13.47	13.47	12.25	12.25	12.86
JRH25 (4)	5	Apical	9.8	12.25	12.25	9.8	11.025
RDL078 (10)	6	Apical	19.6	24.5	22.05	22.05	22.05
RDL078 (12)	6	Apical	17.1	19.6	17.15	17.15	17.75
RDL078 (27)	6	Non-apical	8.57	7.35	4.9	6.125	6.73

In each individual, mean values are based on four measurements in adjacent sections and show a potential biphasic thickening of the apical ectoderm.

its II and III. The ectodermal thickening was confined to the tips of these digits II and III, tapering away to unthickened ectoderm along the radial and ulnar margins of the flipper. In MEH040 (stage 6/7), digits II and III were capped by a bud-like mesenchymal blastema (Fig. 3, F and G), which was covered with a weak AER.

DISCUSSION

We prepared and examined a normal series of dolphin flipper buds using standard morphological techniques. We found that phalanges are laid down in strict proximodistal sequence. Furthermore, this process continued into relatively late developmental stages in digits II and III, which have the highest phalanx count. These two findings are consistent with previous reports for *Stenella attenuata* and other cetaceans (Kükenthal 1893; Sedmera et al. 1997b). Further, we found that mesenchymal condensations for developing phalanges only appeared at the tip of the digit, never more proximally. Also, the most distal (terminal) phalanx formed on each digit was always the last to appear in development. Finally, and perhaps most significantly, we found that a thickened apical ectoderm persisted over the tips of digits II and III until relatively late stages, forming part of a persistent bud-like outgrowth.

Terminal addition of new phalangeal elements

Our findings are not compatible with Kükenthal's influential intercalation model. We found no evidence consistent with the secondary splitting of primary phalangeal rudiments. If proximal phalanges were splitting to form new ones, then there would be a series of irregularly sized and shaped phalanges along each digit; this was not seen, however. Rather, there was a regular gradient of phalanx size, shape, and degree of cytodifferentiation along each digit. Furthermore, no preformed phalanges were seen splitting into two. Thus, we believe that Kükenthal accurately described the pattern of cetacean phalanx development but could not identify the correct mechanism. We suggest that he was handicapped by an incomplete series of stages (although his work is nonetheless of extremely high quality). Our results show that phalanges in *Stenella* are only added terminally—in the sense that the proximodistal position of phalanges correlates with time of differentiation. Taken together with the spatial pattern of chondrogenesis, our results support the idea that digits II and III show an extension, into relatively late developmental stages, of the normal process of proximodistal phalanx specification.

Prolonged "outgrowth"

In an advance on previous studies, we showed that the terminal addition of phalanges correlated spatially and temporally with persistent limb outgrowth, suggesting that these two

Fig. 3. Showing *S. attenuata.* (A–E) Sections are each parallel to the proximodistal axis of the flipper. (A) Stage 3 flipper bud (SA3) with thickened apical ectoderm (*), dorsal at top. Bar, 200 μm. (B–E) Stage 6 flipper bud (RDL078), dorsal at top. (B) Digit IV phalanges (brace) with a new condensation of mesenchyme forming distally (arrow). Because the digits in the flipper lie at different angles, the phalanges in this digit are cut obliquely and therefore only mesenchyme is seen. Bar, 400 μm. (C) Boxed area in B; the apical ectoderm is not thickened. Bar, 75 μm. (D) Digit II phalanges (braces) and distal condensed mesenchyme (arrow). Bar, 700 μm. (E) Boxed area from D. *, thickened apical ectoderm ridge in cross-section; this ridge is also present in adjacent sections spanning digit II. Bar, 100 μm. (F, G) Stage 6/7 flipper (MEH-040), anterior at top. Whole mount (F) and section (G) are from the same specimen and are in the same orientation. Apical ectoderm of digit II (between asterisks), but not digit III, is thickened. Bar, 300 μm. (F) Cleared whole mount. (G) Section from specimen in F showing digit II phalanges (brace) with condensing mesenchyme (arrow) distally. (H) Hypothesis of the distribution of thickened apical ectoderm (red) at stages 5, 6, and late 6, respectively, shown as a schematic reconstruction. The possible thinning of the ectoderm in late stage 5 has not been rendered here.

phenomena are causally linked. Thus, there is an apparently biphasic pattern of limb outgrowth, first across all digits, as in other tetrapods that have been studied. Next, however, outgrowth and the thickened apical ectoderm became localized to digits II and III and then to digit II only. The apical ectoderm appeared to become thinned between these two phases. These findings are easily explained by the progress zone model but not by a model in which the AER simply directs the growth of a prespecified pattern. Under such a preformation model, duration of AER activity should correlate with the *size* of phalanges, whereas with the progress zone model it correlates with *number* of phalanges. We observed more phalanges where the ridge was persistent, but not larger ones.

Heterochrony and limb evolution

Many workers have argued that heterochrony plays a role in morphological evolution (reviewed in Klingenberg 1998). One mechanism that may link timing with pattern is growth; thus, shifts in growth rates may lead to allometric changes in form (Gould 1977). We believe that another key group of mechanisms underpinning heterochrony may be embryonic patterning mechanisms. The progress zone-AER system, which has a temporal component in the patterning process, is a particularly interesting case. It is reasonable to expect that a timing shift, affecting a temporally based patterning mechanism, could lead to a change in form. Unlike allometric heterochrony, patterning heterochrony in the embryo may produce changes in body plan, including changes in the number and pattern of skeletal elements.

If there is a causal link between limb outgrowth and the number of skeletal elements in the dolphin, does such a link exist in other taxa? In some limbless tetrapods, AER-directed outgrowth appears to terminate early, relative to quadrupedal sister-group taxa (Raynaud 1985; Cohn and Tickle 1999). This early termination is suggested to lead to the loss of distal elements. In extant cetaceans external hindlimbs are ab-

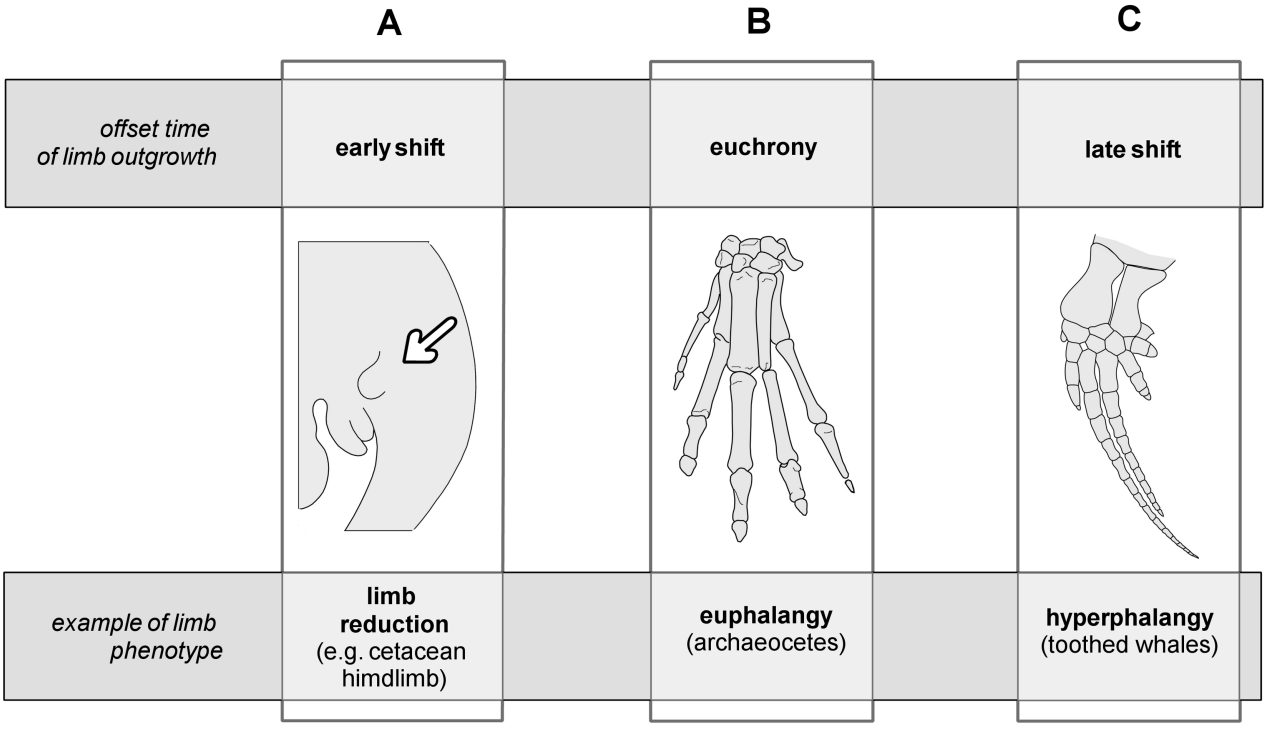

Fig. 4. Hypothesis that the duration of limb outgrowth is related to the extent of proximodistal patterning in the limb skeleton (the linear arrangement of the figures does not reflect phylogenetic relationships). This hypothesis needs to be confirmed by developmental studies in a wider sample of species. Nonetheless, it is consistent with some experimental and evolutionary evidence, discussed in the text. The hypothesis is that early termination of outgrowth, relative to euphalangious species, will produce loss of distal structures (A; arrow indicates the reduced hindlimb in a cetacean embryo). Termination of outgrowth at a later stage would be associated with euphalangy (B). More extreme delay in the offset of outgrowth would lead to hyperphalangy (C). Sources of figures: (A) Caudal region of *Stenella attenuata* embryo, stage 5, from Figure 5 in Štěrba et al. (2000). Caudal is to the bottom, ventral is to the left. After stage 5 the small hindlimb bud of this cetacean disappears, a process associated with degeneration of the AER (Sedmera et al. 1997a). A similar phenomenon of regression is seen in the python hindlimb (Cohn and Tickle 1999). (B) Left manus of the fossil whale *Rodhocetus balochistanensis.* Digit I is to the left, distal is to the bottom. After Figure 2 in Gingerich et al. (2001). (C) Forelimb of an immature pilot whale (*Globicephala melas*), distal is to the bottom, digit I is to the left. Modified (inverted) from Figure 14 (Taf. III) in Kükenthal (1889).

sent at postnatal stages (although vestigial proximal hindlimb elements may be present in the adult; see Adam 2002). This limb reduction may be due to premature termination of outgrowth. Thus, the AER in *S. attenuata* disappears from the early hindlimb bud, and necrotic cells appear in the mesenchyme at the same time (Sedmera et al. 1997a). As we have argued, the findings reported here support previous suggestions that prolonged limb outgrowth is a likely mechanism underlying hyperphalangy (Holder 1983; see also Sedmera et al. 1997b).

Piecing this fragmentary evidence together, we make the following suggestions. There is evidence that outgrowth terminates early in some limbless species, relative to euphalangious sister taxa. Equally, we presented evidence suggesting that outgrowth terminates late in the hyperphalangious dolphin flipper. One obvious hypothesis, based on these findings, is that a spectrum of limb morphologies may be attributable, at least in part, to heterochrony affecting the offset time of limb outgrowth (Fig. 4).

This hypothesis can only be confirmed by developmental studies in a phylogenetically informative sample of species. It also assumes that the progress zone model, and not a preformation model, is correct. Furthermore, the picture may be more complicated than implied by the simple morphocline in Figure 4. Lande (1977) suggested that factors other than early termination of outgrowth may contribute to limb reduction. He cited, as an example, the early necrosis of limb mesenchyme in some reptiles with limb reduction. However, it is not clear whether mesenchymal necrosis is the primary evolutionary event or whether it follows the truncation of outgrowth. Indeed, in *Python molurus* hindlimb outgrowth terminates early but can be rescued by transplantation of a functional AER from a chicken embryo. Furthermore, python limb bud mesenchyme is competent in AER-induction assays (Cohn and Tickle 1999). These results suggest that early termination of limb outgrowth, and not mesenchymal necrosis, is the primary factor in limb reduction in this snake. In the cetacean hindlimb AER regression and mesenchymal cell death are apparently simultaneous (Sedmera et al. 1997a).

Whatever its phylogenetic distribution and mechanistic basis, the change leading to hyperphalangy was an important feature of flipper evolution. In dolphins, hyperphalangy contributes considerably to the physical properties of flippers; these act as multicomponent hydrofoils that help give dolphins their exceptional maneuverability. Hindlimb reduction and forelimb hyperphalangy—both features of the fast-swimming body plan in dolphins—may represent two opposite extremes of embryonic pattern heterochrony in limb development.

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