Evolution of ontogeny in the hippopotamus skull: using allometry to dissect developmental change

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Allometry describes the effect of size change on aspects of an organism's form and can be used to summarize the developmental history of growing parts of an animal. By comparing how allometric growth differs between species, it is possible to reveal differences in their pathways of development. The ability to compare and categorize developmental change between species is demonstrated here using morphometric methods. This involves the interspecific statistical comparison of a large number of bivariate relationships that summarize ontogenetic trajectories. These linear ontogenetic trajectories can be modified as they evolve in any of three ways: ontogenetic scaling indicative of change in the duration of growth, lateral shifts indicative of changes in prenatal development, and directional change indicative of novel modes of postnatal growth. I apply this analysis to skulls of the common hippopotamus (*Hippopotamus amphibius*) and the pygmy hippopotamus (*Hexaprotodon liberiensis*). The number of allometric changes falling into each category was statistically determined and Jolicoeur's multivariate generalization of simple allometry was used to provide an overview of cranial variation. For these skulls, directional change was not found to be statistically significant, but ontogenetic scaling and lateral shifts were both common. This indicates that conserved patterns of growth covariance (ontogenetic scaling) can be separated from novel or derived patterns (directional change and/or lateral shifts). This study demonstrates that *He. liberiensis* is not simply an ontogenetically scaled version of its larger relative. The evolutionary implications of allometric growth variation are discussed in the light of these findings and those of other studies. © 2003 The Linnean Society of London, Biological Journal of the Linnean Society, 2003, 80, 625–638.

In any study of allometry, it is the effect of size change on an organism’s form (or shape) that is being measured and here, more specifically, it is the covarying relationship between size and shape within a postnatal growth series. This clarification of approach and its biological application is not trivial as a vast amount of literature advocating alternative morphometric approaches exists (see Marcus et al., 1996; Klingenberg, 1998). Many such studies involve some form of geometric or isometric size correction, and it is the biological relevance of such procedures specifically to the problem of growth that is questionable (Shea, 1995). Unless all components of an animal grow isometrically, ontogenetic changes are unlikely to result in a geometrically similar animal (see McKinney & McNamara, 1991). Furthermore the purpose of this analysis was not to treat size and shape as fundamentally different and separable entities as it is unlikely that such a separation reflects a corresponding dichotomy of underlying biological processes.

Gould (1975a) initially proposed the use of growth allometry (sensu Huxley, 1932) as a ‘criterion of subtraction’ to remove the pervasive influence of size from any comparison. Essentially, variation between species that results purely from differences in size will map onto a common ontogenetic trajectory. This phenomenon is referred to as ontogenetic scaling (changes in form that are usually the consequence of the extension or truncation of growth). Conversely, any variation that does not map onto a common ontogenetic trajectory implies changes in the development of a descendant trait relative to that of an ancestral trait. Although this is an example of heterochrony (sensu Gould, 1992; heterochrony defined as any change in the timing and/or rate of development) there remains an important distinction between allometric patterns or analyses and heterochronic processes (Klingenberg, 1998). Klingenberg & Spence (1993) used a simple graphical model to show that allometric patterns alone cannot be used to infer underlying heterochronic processes. It has also been demonstrated that even when ontogenetic scaling of an organism’s proportions within and/or between species occurs, the underlying shifts in rate or timing of growth may differ (Shea, 1985a). Finally, Raff (1996) pointed out that heterochronic results (or observed patterns) do not necessarily involve any kind of timing mechanism.

However, all changes to allometric patterns between species or sexes do involve a change in development even if it is a change in the number of cells of a certain kind produced as opposed to a change in the rate or timing of growth. This point is clarified here so as not to allow confusion of the allometric changes described in the Methods of this paper with the plethora of terms used to describe heterochronic processes (see Gould, 1977; Alberch et al., 1979; McKinney, 1988; Reilly, Wiley & Meinhardt, 1997; Gould, 2000). Another potential source of confusion is the usage of the term ‘ontogenetic trajectory’, a concept introduced by Alberch et al. (1979). An ontogenetic trajectory is said to depict the growth of an organism as it moves through size-shape space. However, much of the formalism devised by Alberch et al. (1979) is also based on considerations of geometric similarity and the apparent decoupling of isometric size from shape. In this context the scaling of body size is viewed as proportional dwarfism or proportional gigantism. It is ambiguous as to where size-related shape changes (or allometric differences) fit into this framework. I hope to demonstrate how allometric patterns of change are in themselves informative both in terms of recognizing developmental variation (not limited to changes in timing or rate) and in predicting size-related structural variation.

The quest to relate patterns of allometry to underlying developmental processes is still in its infancy but this issue is beginning to receive more attention (Shea, 1992; Klingenberg, 1998; Stern & Emlen, 1999). Stern & Emlen’s study of the developmental basis for allometry in insects provided a glimpse of one mechanism by which evolutionary shifts in allometries could result from alterations in direct or indirect communication among imaginal discs and body size.

The two species compared here, *Hi. amphibius* and *He. liberiensis*, constitute the only extant members of the family Hippopotamidae. These mammals provide a good model for comparison, partly because of the large difference in their body size (2–4 tons vs. 200–300 kg) and partly because the skulls undergo some remarkable transformations during growth (Fig. 1). In *Hi. amphibius* the muzzle lengthens disproportionately and diverges anteriorly, and in the adult the orbits become elevated above the level of the braincase. In contrast, in *He. liberiensis* the muzzle does not lengthen and diverge anteriorly to the same extent and the orbits do not become elevated. I aim to resolve what proportion of the differences between the species constitutes size-related shape changes and what constitutes changes in the underlying rules of development that relate size and shape.

In comparative growth studies primarily concerned with allometry, ontogenetic trajectories are represented linearly (e.g. Shea, 1985a; Masterson, 1997). These types of study document (within or between species) changes in the direction or position of linear trajectories. In particular, this approach has been used to test hypotheses of ontogenetic scaling both in giants (as in the case of giant transgenic mice; Shea et al., 1990) and in dwarfs (as in the case of human pygmies; Shea & Bailey, 1996). However, it has not yet been established how often these ontogenetic trajectories change, or what types of change are more likely to.
Figure 1. Schematic drawings of newborn (A & C) and adult (B & D) hippopotamus crania to illustrate the shape changes that occur during postnatal ontogeny. The left column denotes the common hippopotamus *Hippopotamus amphibius* (the larger species) and the right column denotes the pygmy hippopotamus *Hexaprotodon liberiensis* (the smaller species). A & B illustrate the lateral aspect of the skull. C & D illustrate the anterior aspect of the skull. In *Hi. amphibius* the muzzle lengthens disproportionately (A & B left) and diverges anteriorly (C & D left) and the orbits become elevated in the adult above the level of the braincase (A & B left). In contrast, in *He. liberiensis* the muzzle does not lengthen and diverge anteriorly to the same extent and the orbits do not become elevated (right column). Scale bars = 5 cm.
occur. Identifying and classifying these changes could have the potential to shed new light on evolutionary relationships across different taxa. Emerson & Bramble (1993) in a review of scaling, allometry and skull design remark that not all previous studies of cranial allometry are comparable with each other. For example recent studies of cranial ontogeny use methods based on geometric similarity (e.g. Ponce de León & Zollikofer, 2001; Lieberman, McBratney & Krovitz, 2002). In these studies allometric influences are assessed through correlations of shape variables with centroid or isometric size (Stand Vioarsdóttir, O’Higgins & Stringer, 2002). Centroid size is not the most appropriate size measure for many tests of allometry; therefore it is not possible to compare meaningfully the results of classical allometric studies with those of geometric studies (Klingen, in press). This appears to be the major reason why the seminal work by Alberch (1985) and Wake (1989), who recognized that a major challenge in systematics would be to incorporate ontogenetic trajectories into a methodological framework, has not been satisfactorily achieved. Here I statistically partition allometric growth covariance into three categories of change (outlined in the results) in an attempt to illuminate morphological differences between species from a developmental perspective.

MATERIAL AND METHODS

Skulls from the two extant species of hippopotamus, *Hy. amphibius* and *He. liberiensis*, were examined. The specimens were from the University Museum of Zoology, Cambridge, the Natural History Museum, London, the Royal Museum of Scotland, Edinburgh, the National Museums of Kenya, Nairobi, the Muséum National d'Histoire Naturelle, Paris, the National Museum of Natural History (Smithsonian Institution), Washington, the American Museum of Natural History, New York and the Field Museum of Natural History, Chicago. The *Hy. amphibius* sample included 48 crania/79 mandibles and the *He. liberiensis* sample included 42 crania/37 mandibles. The data set comprised skulls of mixed age, sex and locality. The age of the specimens was determined using dental criteria devised by Laws (1968). Due to the difficulty of obtaining neonate and immature skulls the sample was biased towards adults. However, all age groups were represented. Distance measures were obtained manually with callipers and 11 cranial and six mandibular traits were analysed (see Appendix).

Ontogenetic trajectories can usually be represented as linear, for example after log transformation \((\log_{10})\), allowing the direction (slope) and position (y-intercept) to be calculated. Hence the slope, or growth coefficient, and y-intercept of bivariate plots of logarithmically transformed, cross-sectional (from different individuals) ontogenetic data were estimated using Model II regression techniques. Model II regression or symmetrical line-fitting techniques (e.g. major axis or reduced major axis) are generally preferred when estimating the functional relationships among biological variables (Wolff, 1985; Martin & Barbour, 1989). The major axis method was favoured here over the reduced major axis one because of its direct relation to Jolicoeur’s (1963) multivariate generalization of simple allometry. All possible bivariate relationships were estimated for the traits studied. However, the cranium and mandible were analysed separately.

To compare major axis slope and intercept estimates between species, bootstrap tests (Efron & Tibshirani, 1993) for differences in the slope and in the intercepts were performed. For each test, 100 000 bootstrap runs were performed under the respective null hypothesis (identical slopes, or identical slopes and intercepts), the major axis regressions were computed, and the slope and intercept values were compared with those estimated for the original samples. These tests provided a non-parametric assessment of the slopes and intercepts capable of accommodating the unavoidable bias towards adult specimens in the sample. Given the large number of comparisons involved, a sequential Bonferroni correction (Rice, 1989) was employed to determine the statistical significance.

Another approach adopted in this study was Jolicoeur’s multivariate generalization of simple allometry (Jolicoeur, 1963). This approach provides an overview of the changes in proportion evident in growing skulls. Jolicoeur (1963) suggested that principal component analysis of log-transformed ontogenetic data could be utilized to determine patterns of relative growth. The calculation of principal components is directly related to the major axis methodology, the axes being determined for \(n\) dimensions (variables) as opposed to two dimensions (Sokal & Rohlf, 1995). The coefficients of the first principal component (PC 1) extracted from the covariance matrix of logarithmic values reflect changes in relative proportion. When applied to measurements made on individuals of a single species differing in age, PC 1 approximates the ontogenetic trajectory. The PC 1 coefficients are roughly proportional to the slopes obtained in bivariate allometric regressions of the traits on a measure of overall size (Shea, 1985b). In consequence, the comparison of PC 1 coefficients can give an indication of a trait’s deviation from or concordance with isometry, effectively summarizing the shape change accompanying allometric growth. In multivariate allometry the isometric value at which all the PC 1 coefficients are equal can be calculated by dividing 1 by the square root of \(p\), i.e. \(1/\sqrt{p}\), where \(p\) equals the number of vari-
ables (traits) in the analysis (Jolicoeur, 1963; Klingenberg, 1996). However, the analysis of PC 1 coefficients alone does not reveal changes in the y-intercept. In this study, PC 1 coefficients were calculated separately for each species and then compared between species to detect any dissociations from common patterns of ontogenetic allometry.

Previous allometric analyses of these skulls have revealed that *Hi. amphibius* sexual dimorphism is entirely the result of ontogenetic scaling (Weston, 2003). Therefore, specimens of different sex and origin (zoo vs. wild) were analysed together; the growth coefficients and y-intercept values calculated for each sex were the same. In contrast, *He. liberiensis* males and females, although similar in size, still exhibit sexual dimorphism. These differences in the orbit, the constriction of the muzzle and the skull width can all be attributed to significant differences in the position (or y-intercept) of ontogenetic trajectories, the postnatal growth coefficients remaining the same between sexes (see Weston, 2003, table 10.3). To reduce the possible influences of sexual dimorphism, traits characterizing the orbit and muzzle constriction (width between the infraorbital foramina) were omitted. In addition, the latter characters tend to be more variable (plastic). Similarly, growth coefficients were not found to differ between zoo and wild individuals so it seemed justifiable to analyse these data together.

**RESULTS**

**BIVARIATE ANALYSIS**

A comparison of the ontogenetic trajectories of the two species showed three different types of change (see Fig. 2).

1. They could be extended or truncated, implying a change in the duration or rate of growth without a change in the underlying patterns of covariation (e.g. the length of the ventral braincase in relation to total skull length; Fig. 2A). This category of ontogenetic (or allometric) variation is defined here as ‘ontogenetic scaling’: the major axis slope and y-intercept are unchanged from species to species; both the direction and position of the ontogenetic trajectory is conserved.

2. They could shift laterally, implying a change in development that takes place prior to the postnatal growth period, the change being amplified during subsequent development according to the same rules for each species (e.g. the width across the upper incisors in relation to total skull length; Fig. 2B). This category of ontogenetic variation is defined here as ‘lateral shift’: the direction of the trajectories is the same but the position is different. The position of the ontogenetic trajectory is altered but the direction is conserved.

![Figure 2](image)
3. They could change direction, implying a change in the rate of growth of one part of the organism relative to other parts (e.g. the width across the upper canines in relation to interorbital width; Fig 2C). This category of ontogenetic variation is defined here as ‘directional with or without lateral change’: the major axis slope and y-intercept are changed from species to species; both the direction and the position of the ontogenetic trajectory are altered.

In the hippopotamus cranium, from the 11 traits analysed it was possible to calculate slope and y-intercept values for a maximum of 55 trait relationships or ontogenetic trajectories for each species. In the hippopotamus mandible, from the six traits analysed it was possible to calculate slope and y-intercept values for a maximum of 15 ontogenetic trajectories per species. The correlation coefficients for all the ontogenetic trajectories (pairs of traits) were highly correlated, i.e. $r > 0.9$.

All of the ontogenetic trajectories were compared between species. The most effective way to provide an overview of this comparison of ontogenetic trajectories across species is to report the $P$-values from the bootstrap tests comparing the major axis slope and y-intercept estimates for the ontogenetic trajectories of each species (see Tables 1 and 2). The $P$-values for the slope tests are recorded above the diagonal of the matrix and those for the intercepts below the diagonal. Significant $P$-values after sequential Bonferroni correction (cranium $P \leq 0.00119$, mandible $P \leq 0.0125$) are indicated by *. The significance level was adjusted to take into account the number of comparisons carried out in the cranium and mandible, respectively. $P$-values $\leq 0.05$ are indicated † and no indication $P$-values $> 0.05$.

The reason for highlighting $P$-values $\leq 0.05$ is that though these comparisons were not significantly different after sequential Bonferroni correction it cannot be assumed that the quantities being compared were identical. Figure 2 illustrates this point well. Plot B is an example of laterally shifted ontogenetic trajectories that after sequential Bonferroni correction did not have significantly different y-intercept values even though $P \leq 0.00117$ (see Table 1 below the diagonal 1 SL/10 UIW). In this example the strict adherence to a significance level of 0.00119 failed to detect actual differences that exist between species. Bonferroni correction appears to be overly conservative and there is growing evidence within biological disciplines that a different approach to multiple comparison testing could be warranted (e.g. see false discovery rate: Benjamini & Hochberg, 1995; Curran-Everett, 2000).

Tables 1 and 2 indicate the specific regions of the skull that expressed each category of ontogenetic change. In the cranium the $P$-values $> 0.05$ below the diagonal in Table 1 indicate regions of the skull that were ontogenetically scaled, size alone constituting any differences in form between the species. For example, the length of the braincase relative to skull length (1 SL/2 BVL) and the width of the mastoid relative to the height of the occipital (6 OCH/7 MW) that denotes the back of the skull, scaled ontogenetically. If significant values beneath the diagonal in Table 1 are considered, these indicate regions of the skull that differed ontogenetically between the species; these differences expressed as lateral shifts of ontogenetic trajectories. The majority of the skull components exhibited this form of variation suggesting a change early in development prior to the onset of postnatal growth. These resulting differences in form were evi-

### Table 1. Cranial $P$-values for bootstrap tests comparing the major axis slope and y-intercept estimates between species of hippopotamus

<table>
<thead>
<tr>
<th></th>
<th>1 SL</th>
<th>2 BVL</th>
<th>3 BDL</th>
<th>4 ZYW</th>
<th>5 IOW</th>
<th>6 OCH</th>
<th>7 MW</th>
<th>8 BOW</th>
<th>9 UCW</th>
<th>10 UIW</th>
<th>11 OCW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 SL</td>
<td>0.6960</td>
<td>0.2858</td>
<td>0.9411</td>
<td>0.5548</td>
<td>0.0697</td>
<td>0.0771</td>
<td>0.4944</td>
<td>0.2227</td>
<td>0.8057</td>
<td>0.5516</td>
<td></td>
</tr>
<tr>
<td>2 BVL</td>
<td>0.8706</td>
<td>0.5446</td>
<td>0.5882</td>
<td>0.3926</td>
<td>0.0420†</td>
<td>0.0320†</td>
<td>0.2761</td>
<td>0.2972</td>
<td>0.9229</td>
<td>0.8029</td>
<td></td>
</tr>
<tr>
<td>3 BDL</td>
<td>0.6088</td>
<td>0.6064</td>
<td>0.3729</td>
<td>0.2963</td>
<td>0.0514</td>
<td>0.0489†</td>
<td>0.1367</td>
<td>0.6770</td>
<td>0.8259</td>
<td>0.8219</td>
<td></td>
</tr>
<tr>
<td>4 ZYW</td>
<td>0.0012†</td>
<td>0.0041†</td>
<td>0.0461†</td>
<td>0.4617</td>
<td>0.0793</td>
<td>0.3222†</td>
<td>0.3868</td>
<td>0.1603</td>
<td>0.7542</td>
<td>0.6499</td>
<td></td>
</tr>
<tr>
<td>5 IOW</td>
<td>0.0476†</td>
<td>0.0554</td>
<td>0.0890</td>
<td>0.5395</td>
<td>0.6274</td>
<td>0.7694</td>
<td>0.8893</td>
<td>0.168†</td>
<td>0.1458</td>
<td>0.4144</td>
<td></td>
</tr>
<tr>
<td>6 OCH</td>
<td>0.0010*</td>
<td>0.0002*</td>
<td>0.0063†</td>
<td>0.0042†</td>
<td>0.0192†</td>
<td>0.5522</td>
<td>0.4815</td>
<td>0.1307</td>
<td>0.2672</td>
<td>0.0695</td>
<td></td>
</tr>
<tr>
<td>7 MW</td>
<td>0.0000*</td>
<td>0.0003*</td>
<td>0.0018†</td>
<td>0.0000*</td>
<td>0.0300†</td>
<td>0.0771</td>
<td>0.5727</td>
<td>0.0831</td>
<td>0.2138</td>
<td>0.1484</td>
<td></td>
</tr>
<tr>
<td>8 BOW</td>
<td>0.0259†</td>
<td>0.0304†</td>
<td>0.0094†</td>
<td>0.6953</td>
<td>0.6169</td>
<td>0.0185†</td>
<td>0.0165†</td>
<td>0.0297†</td>
<td>0.1958</td>
<td>0.4555</td>
<td></td>
</tr>
<tr>
<td>9 UCW</td>
<td>0.0242†</td>
<td>0.0406†</td>
<td>0.0085†</td>
<td>0.0102†</td>
<td>0.0000*</td>
<td>0.0148†</td>
<td>0.0083†</td>
<td>0.0002*</td>
<td>0.0768</td>
<td>0.8230</td>
<td></td>
</tr>
<tr>
<td>10 UIW</td>
<td>0.0017†</td>
<td>0.0111†</td>
<td>0.0003*</td>
<td>0.0098†</td>
<td>0.0000*</td>
<td>0.8759</td>
<td>0.1064</td>
<td>0.0003*</td>
<td>0.0000*</td>
<td>0.7773</td>
<td></td>
</tr>
<tr>
<td>11 OCW</td>
<td>0.0007*</td>
<td>0.0007*</td>
<td>0.0055†</td>
<td>0.0034†</td>
<td>0.0234†</td>
<td>0.8341</td>
<td>0.1342</td>
<td>0.0137†</td>
<td>0.0027†</td>
<td>0.9131</td>
<td></td>
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</table>

$P$-values for the slope tests are recorded above the central diagonal of the matrix and those for the intercepts below the diagonal. *Significant $P$-values after sequential Bonferroni correction (cranium $P \leq 0.00119$; mandible $P \leq 0.0125$). †$P$-values $\leq 0.05$. For abbreviations see Appendix 1.
dent in the neonatal skulls of the two species. If the significant values above the diagonal in Table 1 are considered, a small number of ontogenetic trajectories exhibited directional change ($P \leq 0.05$ is utilized to determine difference in slope). The two traits that indicated novel growth parameters and dissociated between species were the width between the upper canines and the width of the mastoid, these characters reflecting the maximum anterior and maximum posterior breadths of the skull (e.g. Table 1: 9 UCW/5 IOW; 7 MW/2 BVL). This indication of directional change in a few ontogenetic trajectories was not found to be statistically significant after sequential Bonferroni correction. However, a similar finding was produced by the results of the multivariate analysis (see below).

In the mandible the regions that scaled ontogenetically were indicated by the $P$-values $>0.05$ beneath the diagonal in Table 2. Both the height of the ramus and the height of the ascending ramus scaled ontogenetically in relation to the total length of the ramus (Table 2: 12 RL/15 RH; 12RL/16 ARH). The width across the lower canines also scaled ontogenetically in relation to the length of the symphysis (Table 2: 13 SYL/14 LCW). Growth duration in these regions of the mandible has either been truncated or extended in one species relative to the other. In contrast, the significant $P$-values beneath the diagonal in Table 2 indicate regions of the mandible that have been further developmentally modified in one species relative to the other. This is indicated by significant differences in the position of the ontogenetic trajectories (see Table 2).

Table 3 provides a summary of the number of changes that fell into each category of ontogenetic variation. The results before and after sequential Bonferroni correction are compared. In the cranium 25% (14 of 55) of comparisons were attributable to ontogenetic scaling and 23% (13 of 55) were attributable to lateral shifts (see Table 3). In contrast, without Bonferroni correction ($P$-value $\leq 0.05$), 64% (35 of 55) of the changes were attributable to lateral shifts, 25% of the changes were attributable to ontogenetic scaling.

<table>
<thead>
<tr>
<th>Change</th>
<th>Cranium</th>
<th>Mandible</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$P \leq 0.00119^*$</td>
<td>$P \leq 0.05$</td>
</tr>
<tr>
<td>Ontogenetic scaling</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Lateral shifts</td>
<td>14</td>
<td>25.5</td>
</tr>
<tr>
<td>Directional change with or without lateral shifts</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Indeterminate variation</td>
<td>28</td>
<td>50.9</td>
</tr>
<tr>
<td>Total N</td>
<td>55</td>
<td>55</td>
</tr>
</tbody>
</table>

$N =$ number of comparisons in each category.
and a small number of changes (11%) were attributable to directional and lateral change (see Table 3). In the mandible 73% of the changes were attributable to lateral shifts and the remainder (27%) were attributable to ontogenetic scaling. However, if a $P$-value $\leq 0.05$ was utilized to partition the allometric variation, two of the 15 changes indicated directional as well as lateral change.

**MULTIVARIATE ANALYSIS**

The multivariate analysis showed nearly all the ontogenetic variation within the species to be size-related or allometric in nature (Table 4). The eigenvalues of PC 1 summarized 98.5% of the total variation in *Hi. liberiensis* and 96–97% of the total variation (mandible and cranium, respectively) in *Hi. amphibius*. In contrast, the second principal component (PC 2) summarized as little as 0.5% of the total variation in *Hi. liberiensis* and 1.4% of the total variation in *Hi. amphibius*. The PC 1 coefficients were not equal in value (Table 4). This indicates that the PC1 ‘size’ axis is allometric as opposed to isometric in nature.

The cranial and mandibular allometry characterized PC 1 is summarized in Figure 3. A trait growing isometrically undergoes no proportional change, i.e. its shape does not change with increased size (see Fig. 3A: zygomatic width 4 ZYW). In a multivariate context, the isometric value represents a measure of overall cranium/mandible size. In multivariate allometry (as opposed to bivariate allometry where a slope equal to 1 signifies isometry) the isometric value is where all PC 1 coefficients are equal and can be calculated by dividing 1 by the square root of the number of variables analysed (see Methods). Figure 3A illustrates that most of the PC 1 or growth coefficients deviated from isometry; the width between the upper canines (9. UCW) expressed positive allometry and the length of the braincase (3. BDL) expressed negative allometry. The same trend can be seen in the mandible, the width across the lower canines (14. LCW).

![Figure 3. Comparison of first principal component coefficients for the cranial traits (A) and the mandibular traits (B) of Hippopotamus amphibius and Hexaprotodon liberiensis. The plots illustrate how the allometric relationships of different traits vary in relation to isometric growth. They also illustrate the dissociation and coincidence of growth coefficients between the species. Isometric values ($A = 0.3015; B = 0.4082$) were calculated by the formula $1/p$, where $p$ equals the number of variables (traits) in the analysis.](image)
grow with positive allometry and the width across the mandibular condyles (17. MCW) growing with negative allometry (Fig. 3B). In the hippopotamus skull it is these differing allometric relationships of the various skull parts that generate the notable shape changes evident in the animal's postnatal ontogeny (Fig. 1).

The PC 2 summarized a tiny fraction of the total variation (Table 4). In Hi. amphibius a single neonate specimen had a relatively high PC 2 score. The extreme trait loadings (PC 2 coefficients indicated by *, Table 4) denote the occipital condyles in the cranium and the length of the symphysis and the height of the ramus in the mandible. In Hi. amphibius skull growth, with the exception of that of the occipital region, generally does not alter after birth (Weston, 1998). In consequence, foetal specimens were excluded from this analysis. However, the inclusion of neonate specimens in this study (N = 4 Hi. amphibius and N = 6 He. liberiensis) may have influenced slightly the shape of the occipital condyles. This is because it was impossible to gauge whether or not new-born individuals were premature. In the case of the mandible the neonate specimens had unfused symphyses and lacked dentition. Both these factors might influence the measurement of neonatal individuals and contribute to the non-allometric variation summarized in the second principal component.

**DISCUSSION**

In vertebrates in general, the neurocranium grows with negative allometry, becoming proportionately smaller as the animal increases in size and the muzzle (part of the viscerocranium) grows with positive allometry becoming proportionately larger as the animal increases in size. This difference in scaling coefficients between the brain and sensory capsules on the one hand, and jaw related anatomy on the other, offers verification of the functional division of the skull into facial and cerebral components (Radinsky, 1981; Shea, 1985a). This distinction broadly correlates with the embryonic origins of the two cranial regions (Hanken & Thorogood, 1993). The viscerocranium is almost entirely derived from neural crest, whereas a substantial part of the neurocranium comes from paraxial mesoderm. However, the location of the interface between these regions of the skull derived from the two different embryonic sources is suspected to vary among taxa (Kuratani, Matsuö & Aizawa, 1997).

The classic functional interpretation of this general pattern of cranial growth observed across vertebrates is that structures which experience very rapid size increase early in development, such as the brain and sensory capsules, must later shift to isometric or negative allometric growth if undesirable distortions of the adult cranium are to be avoided (Emerson & Bramble, 1993). Ravosa (1991) also pointed out that selection for increased overall size apparently targets postnatal systemic growth. Therefore changes in brain size, a structure which develops prenatally, is predicted to be minimal as compared with facial structures, which are influenced primarily by postnatal growth.

Here, the multivariate analysis also showed some of the PC 1 (growth) coefficients to differ between species (Fig. 3). As the PC 1 coefficients were roughly proportional to the slopes obtained in bivariate allometric regressions of the traits (see Material and Methods) these differences are an indication that a degree of directional change exists between ontogenetic trajectories in the two species. In the cranium, the directional change was related to the breadth across the upper canines (9. UCW) and the back of the skull (7. MW); in the mandible, it was related to the breadth across the lower canines (14. LCW) and the height of the ramus (16. ARH). If the bivariate results are compared, directional change is indicated in the same traits (see values indicated † above the diagonal of the matrices in Tables 1 and 2). The importance of such derived patterns is that they potentially highlight targets of selection that could indicate adaptations for specific behaviours or functions.

This analysis revealed some unexpected functional differences between the two species of hippopotamus. One of the most striking examples was within the mandible. He. liberiensis is a forest browser with much of its morphology characteristic of such a lifestyle (Weston, 2000). In contrast Hi. amphibius is a grazer, emerging from the water at night to feed on grass, with aquatic vegetation hardly contributing to the animal’s diet (Eltringham, 1993). In addition to the modifications one would expect to craniodental anatomy associated with different modes of feeding, both species of hippopotamus exhibit a wide gape which has also been shown to be a primary determinant of jaw form (Herring & Herring, 1974; Herring, 1975). In comparative studies of ungulates it is generally found that grazing species have deeper lower jaws than do browsing species (Janis, 1995). However, He. liberiensis has a proportionately deeper lower jaw at the level of the M, and the ascending ramus relative to the larger Hi. amphibius (see Fig. 3B). Even if gape is taken into consideration, where one might expect a deep ascending ramus to increase the length of the masseter muscle and permit stretching during yawning, the same determinant of jaw form should be common to both species with such a gape. However, the lower canines of He. liberiensis are much larger relative to the size of the skull compared with the canines of Hi. amphibius (Weston, 1998). This suggests that the increased depth of the lower jaw in He. liberiensis could be correlated with the possession of relatively
larger canines. However, the precise functional role of these enlarged anterior teeth is not clear.

Another anomaly revealed by the allometric analysis was the different relationship between the upper and lower anterior jaws in these species of hippopotamus. The broad muzzle of *Hippopotamus amphibius*, characterized by the laterally displaced lower canines, is the result of the lower jaw expanding with a higher degree of positive allometry relative to that of *He. liberiensis* (see Fig. 3B; compare coefficients depicting the width between the lower canines). However, comparison of the anterior upper jaw of these animals shows the opposite to be true. The distance between the upper canines increases with a lower degree of positive allometry relative to that of *He. liberiensis* (see Fig. 3A). Essentially, the anterior lower jaw of *H. amphibius* is broad relative to its upper jaw whereas the anterior lower jaw of *He. liberiensis* is narrow relative to its upper jaw. Herring (1972) noted that in the heads of pigs and peccaries a relatively large number of skeletal differences could be related to the morphology and function of the canine teeth. Hippos appear to be similar in this respect, with the variation in the anterior jaw being associated with canine function. This is probably a reflection of the differences in social behaviour of these species. In *H. amphibius* the large canines function for both intraspecific combat and threat displays during yawning (Klingel, 1991). However, little is known about the role of the caninestyle in the more solitary *He. liberiensis*.

The other region of the hippopotamus skull that exhibited dissociated patterns of growth allometry (specifically directional change in ontogenetic trajectories being considered here) was the back of the braincase. Although the back of the cranium grows with negative allometry in both species, getting proportionately smaller as the hippos grow, this condition is intensified in *He. liberiensis* (see Fig. 3A: the mastoid and occipital PC 1 coefficients are dissociated between species). Essentially, the back of the skull or the occipital plate is relatively larger in *H. amphibius* than in *He. liberiensis*. The functional significance of the relative size of the occipital plate could be linked to increased neck musculature needed to support a large head. However, whatever the explanation, this is a further example of how the teasing out of cranial parts distinguished by novel modes of growth can isolate interesting questions relating to functional morphology. In short, knowledge of allometric variation should be a prerequisite to any study of functional morphology providing ontogenetic data is available.

However, in this species comparison, it must be borne in mind that all of the changes that could be statistically verified (in light of the Bonferroni correction), resulted either from ontogenetic scaling or lateral shifts. Only one quarter of the allometric changes between species was attributable to ontogenetic scaling. Notably, characters that define the back of the skull or occipital plate scaled ontogenetically relative to each other and the brain case scaled ontogenetically relative to the length of the skull. Growth in these regions of the skull has been extended or truncated in one species relative to the other. The direction of change (extension or truncation of development) cannot be inferred from an allometric trajectory without additional knowledge of hippopotamus ancestor/descendent relationships.

In the family Hippopotamidae, *He. liberiensis* is considered as being more basal than is *H. amphibius* and basal taxa tend to be smaller in size (Weston, 2000). In heterochronic terminology (see Gould, 1977; Alberch et al., 1979; McKinney, 1988; Reilly et al., 1997; Gould, 2000) *H. amphibius* would be regarded as being pseudomorphic (development extended) as opposed to *He. liberiensis* which would be regarded as being pedomorphic (development truncated). Body size variation among extinct species of hippopotamus is vast and the living *He. liberiensis* is often mistakenly assumed to be dwarfed (Gould, 1975b). More pertinently, regardless of the direction of size change, ontogenetic scaling can only explain some of the variation between these two species of hippopotamus.

The majority of the allometric changes were attributable to lateral shifts and this was demonstrated best in the mandible where all such changes were statistically significant (Table 2). This implies that a change or changes early in development in one or other species affects large areas of the skull. However, this type of developmental modification has not altered the direction of the ontogenetic trajectories (the ratio of specific or logged growth rates stays constant). A small number of slope comparisons in both the cranium and the mandible had relatively low P-values. Although it is recognized that these slopes were not statistically different (in the context of multiple comparisons) they are interpreted here as being evidence for directional change in a small number of ontogenetic trajectories. The same interpretation can be drawn from the multivariate results. The rules of postnatal development in these regions of the skull potentially differ in the two species, but these differences constitute only a small fraction of the total ontogenetic variation between the species (10.9%, see Table 3).

The methodological approach taken here utilizes the comparison of a large number of bivariate relationships among traits. This permits the dissection of ontogenetic variation within the skull into its component parts. It also permits determination of the type of change that an ontogenetic trajectory has undergone. Multivariate approaches such as those of Jolicoeur (1963) and Burnaby (1966) are unable to partition ontogenetic variation in the same way. Jolicoeur’s
approach omits change due to lateral shifts and Burnaby’s approach omits change due to direction.

When multiple bivariate comparisons are made a change to a single trait can affect multiple trait relationships. However, in this study, each trait ‘relationship’ was treated as an independent character for comparison. The focus was not on changes in single traits but on changes in the functional relationship between traits. The count of trait relationships falling into each category of change can approximate which category of change prevails. Crucially, this breakdown of information was able to indicate which parts of the skull have been modified in the same way irrespective of each skull component’s relationship with overall skull size.

Creighton & Strauss (1986) recognized that this kind of ontogenetic variation could be of value to phylogenetic studies, pointing out that similarity of adult morphology does not necessarily reflect similarity in the underlying patterns and processes of development. They utilized growth curves as characters in an analysis of cricetine rodent relationships and obtained results that were in agreement with other phylogenetic studies. However, in their example rather general measures of size and shape were used and they did not attempt to dissect the ontogeny of an organism into separate components equivalent to those described here. In contrast, Gomez (1992) analysed relative growth in five species of loris (family Lorisidae) and focused on the dichotomy between shared vs. dissociated patterns of growth (allometry), recognizing these patterns as important for the understanding of evolutionary change. It was shown that two patterns of body size reduction occurred during lorisid evolution, ontogenetic scaling and dissociated growth reduction.

The assertion made in the studies cited above is that evolutionary size increase or decrease is most ‘easily’ accomplished through maintaining ancestral growth patterns. In other words, it is the changes that conserve the direction of ontogenetic trajectories that will be most likely to occur and those that alter the direction of ontogenetic trajectories will be least likely to occur. My data are consistent with this assertion.

Developmental modifications that preserve the direction of ontogenetic trajectories (i.e. ontogenetic scaling and lateral shifts) are more likely to constrain evolutionary change and lead to evolutionary convergence. Since convergent change amongst lineages tends to obscure evolutionary relationships, this allometric information could play a vital role in evaluating characters used in phylogenetic reconstruction. Various authors have advocated the use of ontogenetic data over that of adult morphological data with regard to the detection of homoplasy (Creighton & Strauss, 1986; Wake, 1989, 1991) but very few studies have actually attempted this. Shea (1995) pointed out (as demonstrated here) that a traditional morphometric model provides a very powerful means to tease apart and measure the inherited patterns of allometric growth covariance and the derived dissociations probably associated with novel adaptive shifts.

Shea (1983, 1985a) conducted a similar study comparing the skulls of chimpanzees and gorillas and established that all three types of ontogenetic variation occurred. However, in these earlier studies dissociations from ontogenetic scaling (lateral shifts and changes in direction of trajectories) were not statistically evaluated. Nevertheless, the general conclusion was that most of the interspecific variation in craniofacial form among the African apes resulted from ontogenetic scaling.

Several more recent primate studies do permit a more precise comparison of allometric variation between species. Masterson (1997) compared the cranial form of two species of Capuchin monkey (genus *Cebus*) and revealed that all three types of ontogenetic variation occurred in approximately equal amounts. However, the same study also revealed that changes in the direction of ontogenetic trajectories were relatively common in between-sex comparisons, i.e. growth coefficients differed between sexes. Ravosa (1991), in a comparison of the crania of two Old World monkeys from different subfamilies (Cercopithecinae, Colobinae), revealed that the greatest proportion of the allometric changes (54%) could be attributed to lateral shifts of trajectories and 18% could be attributed to directional change. In this example, practically all the morphological variation between sexes of each species was attributable to ontogenetic scaling, and hence sexual variation did not influence the interspecific results. In a further study by Ravosa & Ross (1994), the cranial allometry of two congeneric species of Howler monkey (genus *Alouatta*) was analysed. In this case no directional change of ontogenetic trajectories was apparent, with all of the allometric changes between species being attributable to ontogenetic scaling (73%) and lateral shifts (23%). Finally, in an allometric analysis of the external body proportions in human pygmies and non-pygmyes, Shea & Bailey (1996) demonstrated that the morphological differences attributed to pygmyes were all allometric correlates of ontogenetic scaling.

In general, studies of vertebrate ontogenetic allometry that adopt comparable methodologies are scarce. Klingenberg & Ekau (1996) analysed the morphological differences between nine Antarctic fish species (family Nototheniidae). This morphometric study, although not directly comparable with the work presented here, did recognize that lateral transpositions of growth trajectories were the main source of morphological variation between the fish species. Bjök-
lunds (1994, 1996) studied certain allometric relations between different species of bird. It was shown that morphological variation in blue and great tits (genus Parus) appears to result mainly from ontogenetic scaling and lateral transpositions whereas the variation across three species of finch from two subfamilies (Carduelinae, Fringillinae) also involves directional change of ontogenetic trajectories.

The studies cited above indicate that differences across species exist: directional change is evident in some examples but absent in others. It appears that the more distant the comparison (in terms of evolutionary relationship), the more likely it is to see directional change. There are only two extant species of hippopotamus but there are several fossil species with ontogenetic series well enough preserved to examine this type of variation (Weston, 2000). However, the analytical approach taken in each case would need to be standardized, and factors such as sexual dimorphism controlled for, if the biological implications of such variation are to be explored further. This study is an attempt to do that, comparing for the first time all possible trait relationships for a given number of variables. The inclusion of a large number of comparisons can be problematic with the use of the Bonferroni correction, which appears to be overly conservative when used in this context. Nevertheless, even in the present study many of the trajectories have been shown to be significantly different. The morphological differences between the two extant species of hippopotamus do not just result from ontogenetic scaling. The living He. liberiensis is not, as previously assumed, a classic example of a dwarfed species.

This paper serves to highlight the biological importance of ontogenetic allometric variation and to bring the associated methodological difficulties under fresh scrutiny. Here it is shown how ontogenetic trajectories can be used to detect different mechanistic changes that underlie morphological differences. Allometric variation of this kind can be broken down into biologically meaningful categories that reflect either conserved or derived (novel) patterns of growth. If the evolutionary significance of allometric variation in ontogeny is to be fully appreciated, comparable studies across different taxa need to be carried out. Such a treatment of allometry permits us to reinterpret the morphological variation that distinguishes adult species and brings us a step closer to integrating development into evolutionary studies.

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REFERENCES


APPENDIX

List of biometric traits (see Weston, 1998 for details).

1. Skull length (SL)
2. Ventral length of braincase (BVL)
3. Dorsal length of braincase (BDL)
4. Zygomatic width (ZYW)
5. Interorbital width (IOW)
6. Occipital height (OCH)
7. Mastoid width (MW)
8. Biorbital width (BOW)
9. Width across upper canines (UCW)
10. Width across upper second incisors (UIW)
11. Width across occipital condyles (OCW)
12. Ramus length (RL)
13. Symphyseal length (SYL)
14. Width across lower canines (LCW)
15. Ramus height (RH)
16. Height of ascending ramus (ARH)
17. Width across mandibular condyles (MCW)