

Developmental Constraints in a Comparative Framework: A Test Case Using Variations in Phalanx Number During Amniote Evolution

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Abstract Constraints are factors that limit evolutionary change. A subset of constraints is developmental, and acts during embryonic development. There is some uncertainty about how to define developmental constraints, and how to formulate them as testable hypotheses. Furthermore, concepts such as constraint-breaking, universal constraints, and forbidden morphologies present some conceptual difficulties. One of our aims is to clarify these issues. After briefly reviewing current classifications of constraint, we define developmental constraints as those affecting morphogenetic processes in ontogeny. They may be generative or selective, although a clear distinction cannot always be drawn. We support the idea that statements about constraints are in fact statements about the relative frequency of particular transformations (where ‘transformation’ indicates a change from the ancestral condition). An important consequence of this is that the same transformation may be constrained in one developmental or phylogenetic context, but evolutionarily plastic in another. In this paper, we analyse developmental constraints within a phylogenetic framework, building on similar work by previous authors. Our approach is based on the following assumptions from the literature: (1) constraints are identified when there is a discrepancy between the *observed* frequency of a transformation, and its *expected* frequency; (2) the ‘expected’ distribution is derived by examining the phylogenetic distribution of the transformation and its associated selection pressures. Thus, by looking for congruence between these various phylogenetic distribution patterns, we can test hypotheses about constraint. We critically examine this approach using a test case: variation in phalanx-number in the amniote limb. *J. Exp. Zool. (Mol. Dev. Evol.)* 296B:8–22, 2003. © 2003 Wiley-Liss, Inc.

INTRODUCTION

Constraints are factors that limit evolutionary change. Constraints may be ‘negative,’ as when they reduce the extent of heritable phenotypic variation; or ‘positive,’ as when the evolutionary history of an organism channels it along specific pathways of evolution (Gould, ’89; discussed by Schwenk, ’95; see also the ‘developmental drive’ of Arthur, 2001). There are several lines of evidence suggesting the existence of constraints (Schwenk, ’95). One is the observation that different characters diversify at different rates in a given lineage (a phenomenon known as mosaic evolution). Another line of evidence is that the range of phenotypes observed in nature is limited—a phenomenon characterised as the incomplete filling of morphospace (Alberch, ’82).

Furthermore, constraint may be indicated by the comparison of existing phenotypes to theoretical optima (reviewed in Schwenk, ’95). Finally, when embryos are treated with teratogens, or subjected to genetic manipulation, the morphological abnormalities induced tend to be limited to certain defined clusters or syndromes (Alberch and Gale, ’83; reviewed in Alberch, ’89). With all of these lines of evidence, constraints are suspected because the observed frequency of a character differs from some expected value.

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A further approach to the identification of constraints is to look at the phylogenetic distribution of characters (see “Constraints in a phylogenetic context” below). For example, Eberhard (2001, 2002) examined male sepsid flies and found that they have evolved moveable abdominal lobes, at least twice independently, in response to sexual selection. Such lobes are rare or absent in other dipterans. He concluded that the absence of such lobes in most Diptera is due to lack of selection, not constraint. However, Wagner and Müller (2002) reanalysed the dataset and concluded that abdominal lobes had failed to evolve in some lineages, despite appropriate selection pressures; this supports the hypothesis of constraint. See also Galis et al. (2001) for a non-quantitative, character-based approach to the analysis of constraint in the mid-embryonic (phylotypic) period.

There are some areas of disagreement concerning constraints. One is the relative importance of internal and external factors in limiting evolutionary change. Maynard Smith ('83) acknowledges that limitations on heritable variation can restrict evolutionary change, but argues that stabilising selection is a more likely explanation for the long-term conservation of some characters in the fossil record. On the other hand, Gould ('83) argues that special features of the developmental programme may sometimes be more important than natural selection in limiting change.

There are other areas of difficulty concerning developmental constraints. Not only is the nomenclature of constraints unclear, but there is no agreed methodology for formulating developmental constraints as testable hypotheses (Schwenk, '95). Our aim in this paper is to present some potential solutions to these problems. We begin with a simplified definition and classification of developmental constraints. This is not intended to replace other classification schemes—of which there are many (Antonovics and van Tienderen, '91)—but rather to provide a framework for this paper.

CATEGORIES OF CONSTRAINT

Many different categories of constraint have been described (reviewed by Alberch, '82; Kauffman, '83; Maynard Smith et al., '85; Wake and Roth, '89; Antonovics and van Tienderen, '91; McKinney and McNamara, '91; Schwenk, '95; Hall, '96; Raff, '96; Arthur, '97; Sarà, '99). We summarise some of these categories in Table 1.

Amundson ('94) draws a distinction between constraints on form (constraints_F) and constraints on adaptation (constraints_A). This distinction emphasises differences in approach between developmental biologists and evolutionary biologists, respectively. Developmental biologists and morphologists often see constraints as limiting the appearance of certain morphologies, whereas evolutionary biologists may see constraints as limiting 'ideal' adaptation. It is this conceptual difference that may account for some of the differences in categories outlined by different authors. This nomenclatural confusion is, to some extent, comparable to that seen in discussions of heterochrony (discussed by Gould, 2000).

Generative and selective constraints

One crucial distinction among constraints depends on whether they are due to generative or selective factors (Table 1). Generative constraints arise when a phenotype cannot be produced during a given ontogeny (Alberch, '89). Generative constraints limit the production of certain phenotypes during ontogeny, resulting in a non-random production (or non-production) of phenotypic variants (Wagner and Misof, '93; Schwenk, '95). Under the influence of generative constraints, certain phenotypes ('forbidden morphologies') will not appear at any stage of ontogeny. The question of whether a variant phenotype is adaptive or maladaptive is not relevant to the consideration of generative constraints. The only consideration is whether developmental mechanisms can produce a given phenotype. Sorting processes, such as random drift and natural selection, lead to differences among the frequencies of available phenotypes; they do not generate phenotypic novelties (Fusco, 2001). Generative constraints, by contrast, precede the sorting process, and limit the range of phenotypic variants on which sorting can act (Fusco, 2001).

Selective constraints, in the context of development, are due to natural selection acting during embryonic development. This is an example of sorting that occurs after a phenotype has been generated (Fusco, 2001). With these constraints, certain phenotypes produced in ontogeny are maladaptive, and therefore do not persist in evolution. The dividing line between stabilising natural selection, and this type of constraint, is unclear. Natural selection *sensu strictu* (i.e., external selection) is that which stems from the interactions between the organism and its

TABLE 1. Classification of constraints into generative (limited production of variant phenotypes during ontogeny) and adaptive (limited survival of variant phenotypes during ontogeny or phylogeny)

Category	Types of constraint	Explanation and selected references
Generative constraints	Historical constraints*	The evolutionary history of an organism limits the developmental pathways that it can evolve—either because of its current form (McKittrik, '93; Resnik '95; but see Schwenk, '95), or because of the patterning mechanisms* available to it (Holder, '83; Gould, '89; Hall, '96).
	Developmental buffering*	Genetic changes can take place without phenotypic effect, because of feedback loops and buffering in developmental pathways (Kauffman, '83; Duboule and Wilkins, '98).
	Genetic constraints	Bias in the appearance of certain genotypes because of the nature of the genetic system, probability of types of mutation, or recombination.
	Physical constraints*	The physical properties of the egg or the embryo, the extra-embryonic membranes and environment, limit or bias the evolutionary changes that can take place during development (Elinson, '87).
Selective constraints	Horizontal constraints (coupling; linkage)*	Some developmental pathways influence many different processes in development. Changes in any pathway are likely to have a negative selective value because of pleiotropic effects (Raff, '96).
	Vertical constraints*	Development is sometimes viewed as a temporally hierarchical process (the inverted cone model-Arthur, '97). Changes in early stages of development may have multiple effects on later stages, and are therefore constrained (Raff, '96).
	Structural constraints	Constraints that are the results of the basic laws of physics. Organisms are limited in the form they can have because exceeding certain physical boundaries will be selected against. Often referred to as 'universal constraints' (Maynard Smith et al., '85; Resnik, '95).
	Functional constraints	Functional integration of different systems is an important aspect of organismal form. The limit this places on morphological changes constitutes a constraint (Galis, '93; Schwenk, '95).

*Denotes developmental constraints. It can be seen that developmental constraints are a disparate group of generative and selective constraints. Note that historical constraints include both developmental constraints and non-developmental constraints.

environment. Selective constraints can be defined as the subset of selective forces that are a result of properties of the phenotype. In the context of development, these would be properties of the embryonic phenotype. Thus, selective constraints remain approximately constant through a broad range of environments (Arthur, '97).

The definitive example of selective constraints, which is most relevant to our discussion, is selection acting during development, resulting in embryo lethality. This type of selection has been called 'developmental selection' (Whyte, '60) or 'internal selection' (Whyte, '65; Arthur, '97; Fusco, 2001). Whyte defines 'internal selection' as: 'the internal selection of mutants at molecular, chromosomal, and cellular levels, in accordance with their compatibility with the internal co-ordination of the organism' (Whyte, '65: p. vii). Wagner and Schwenk (2000) discuss this concept in relation to 'evolutionarily stable configurations'

(ESC)—characters within the organism that show strong integration, in the sense that they function as a unit. One example they give is of the incisor teeth in gnawing rodents: there is strong selection for the upper and lower teeth to fit together accurately in order to carry out their 'proper function' (i.e., cutting). Wagner and Schwenk (2000) argue that such integration may overcome external selection pressure on individual characters comprising the ESC. Modularity of the developmental program is a manifestation of the internal co-ordination of embryogenesis that is related to character linkage (Wagner and Altenberg, '96; Galis et al., 2001).

Developmental constraints

Developmental constraints are defined as biases on heritable phenotypic variation, caused by properties of developmental systems (Maynard

Smith et al., '85). The view of developmental constraints followed in this paper is summarised in Table 1. In our terms, 'developmental constraints' are any type of constraint, selective or generative, acting during embryonic development. As can be seen in Table 1, several disparate phenomena can be grouped under our heading of 'developmental constraints,' demonstrating that the latter do not form a natural grouping. Nonetheless, the term is useful; it denotes constraints that involve, or are mediated by, morphogenetic mechanisms at some level—regardless of whether selection is involved.

SOME PROBLEMATIC ASPECTS OF DEVELOPMENTAL CONSTRAINTS

No clear line between selection and developmental constraint

Under some classifications, only generative constraints are called true constraints (e.g., Gould, '89). Thus, Alberch ('89) uses external and internal factors to mean selection and constraints, respectively. However, other workers see constraints as including both generative and selective types (Maynard Smith et al., '85; Schwenk, '95; Hall, '96; Schlichting and Pigliucci, '98). Several previous reviewers have argued that there is overlap between phenomena categorised as constraints, and the results of natural selection acting at different levels (e.g., Schwenk, '95; Hall '96). We follow this point of view, and maintain that there is no clear line separating constraints from natural selection, and that the distinction between them is problematic.

Gould and Lewontin ('79) argued that developmental constraints may play an important part in determining the form of an organism—more important than had been assumed under models that stressed the importance of selection. Gould and Lewontin's argument is often misinterpreted as meaning that certain aspects of development (i.e., those influenced by developmental constraints) are not affected by selection. This interpretation is false, since, as we have argued, many developmental constraints are in fact a result of selection acting at the embryonic level. Houston ('97) shows that Gould and Lewontin's examples of generative constraint, the spandrels of San Marco, are in fact selected for: they fulfil an important role in support of the dome in large buildings. Indeed, many of the examples cited as cases of developmental constraints can easily be explained in terms of Darwinian selection on

structures that are linked to, or affected by, changes in other structures. This highlights the fact that the distinction between developmental constraints and selection cannot easily be made, except in the case of purely generative constraints. In a sense, just as Gould and Lewontin ('79) argue that not every feature observed in nature is an adaptation, it is clear that not every example of stasis is due to developmental constraints (Schwenk, '95). According to Carroll ('87): 'On the basis of currently available evidence, it is difficult, if not impossible, to determine whether conservative developmental patterns are a constraint on evolutionary change or, conversely, reflect long-term stabilising selection' (p. 584).

Universal constraints and forbidden morphologies

Some controversies concerning developmental constraints arise from different views about the potential for evolutionary change in developmental mechanisms. One view is that developmental mechanisms obey certain universal laws (where 'universal' means that the laws are shared at a particular phylogenetic level of inclusiveness). Under the action of these laws, developmental mechanisms will present a limited menu of phenotypes for natural selection to act upon (Oster and Murray, '89). Certain 'forbidden' morphologies can never evolve (Holder, '83), and will constitute unfilled phenotypic space (Alberch, '82, '89).

This scenario is supported by experimental studies showing that teratogen treatment in the limb can cause clustered sets of phenotypes, rather than a continuous spectrum (reviewed in Alberch, '89). A further example in the tetrapod limb is the suggestion that developmental mechanisms cannot generate two humeri (Goodwin and Trainor, '83). An alternative view is that, when developmental mechanisms evolve, their associated constraints also evolve. Constraints are therefore 'local' rather than universal, and will be historically contingent (Maynard Smith, '83). In support of this view is the observation that some phenotypes, considered impossible by developmental biologists, can evolve (Carroll, '87: p. 582).

SOME PROPOSALS

In this section, we outline the arguments that will guide our methodological approach to developmental constraints.

Developmental constraints act on transformations, not morphologies

It is possible to make statements about constraints at the level of characters alone (as with forbidden morphologies or morphospace, for example). Indeed, Schwenk ('95) argues that evolutionary constraints affect characters, not clades or organisms. In the context of developmental constraints, morphological characters may not be the best parameter to consider. We suggest that constraints act not on single morphologies, but on transformations between ancestral and derived morphologies. A transformation is a change from one state to another during evolution (as opposed to an ontogenetic transformation; for further discussion of this distinction, see Kluge and Strauss, '85). Nonetheless, we assume that most, or all, evolutionary transformations in morphology are associated with underlying developmental transformations. As we define it, a transformation could include any of the suite of changes in developmental mechanisms, molecular and cellular, associated with the final morphological transformation (cf. Holder, '83: p. 400).

Ideally, this approach to constraints focuses on the process and not on the outcome, and allows a more robust discussion of the mechanisms underlying the constraints. An additional utility of considering transformations is that they allow the polarity of change to be defined. This is important in any discussion of constraints because the likelihood of a given change depends on the conditions in the ancestor (as argued below). Certain transformations are considerably less likely to occur than others. As a result, certain morphologies are less likely to appear—not because there is a constraint against these

morphologies per se, but because there is a constraint on the evolutionary transformation leading to them. A major practical problem here is that data on the actual transformations in developmental mechanisms are available for very few taxa. A phylogenetic survey will have to study morphological traits and thus uncover evidence of transformations along the history of the lineage being studied (as we find in our example below).

Constraints affect the relative frequency of transformations

If we are to analyse constraints as testable hypotheses, we need to be able to measure their effects in some way. There are two ways to do this. One is by recording the frequency of a transformation in a population (where the population may be experimental or natural). The other is to look at the phylogenetic distribution of a transformation, as we shall show later. Kauffman ('83) argued that constraints define the relative frequency of particular transformations. Schwenk ('95) talks about the probability of particular changes taking place in a given time period. We agree with these views, and envision a spectrum of probabilities of transformations, with evolutionarily plastic transformations (Chipman, 2002) lying at one end and constrained transformations at the other (Fig. 1). 'Constrained' in these terms, means the same as 'unlikely,' if one is making predictions about future transformations; or 'rare,' if one is describing the occurrence of a transformation in different species of a given taxon (see also Schwenk, '95 for discussion of a priori and a posteriori views of constraint). Fusco (2001: 285) says, in the context of generative constraints: '...constraints rank things from "impossible" to "highly probable,"

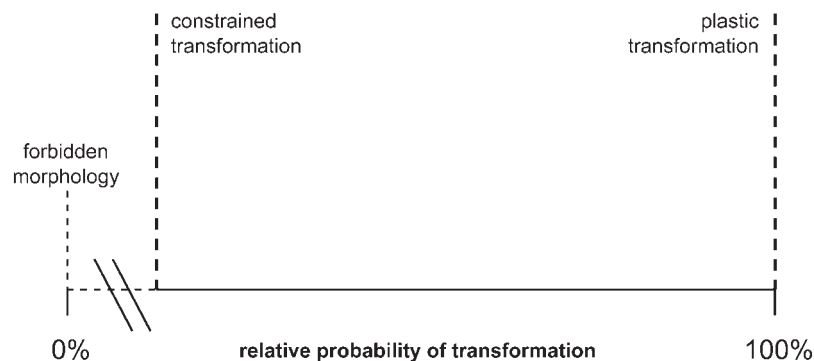


Fig. 1. Constraints are statements about the probability of a given transformation. The probability of one transformation is always relative to that of another. We show an absolute category of constraints in this figure (i.e., those with 0% probability), although on conceptual grounds, it is difficult to prove that a certain morphology will never evolve.

while sorting winnows the real world from the world of the possible.'

Constraints in a phylogenetic context

We argued above that a constraint determines the probability of a given transformation. This probability is always relative to that of other transformations. Therefore, the degree to which a particular transformation is constrained depends upon its phylogenetic context. For example, in mammals, changes in the numbers of cervical vertebrae appear to be highly constrained (Galis, '99). Thus, long-necked species such as the giraffe show differential growth of the neck region, and not increased numbers of cervical vertebrae. In other tetrapod groups, by contrast, evolutionary elongation of the neck is often achieved by an increase in the number of cervical vertebrae (Burke et al., '95). Thus the same transformation—increase in numbers of cervical vertebrae—differs in the degree that it is constrained between taxa. For these reasons, we adopt the suggestion of Antonovics and van Tienderen ('91) that any null model for the study of constraints must state which clade is being discussed.

An explicitly phylogenetic approach has been used to examine internal constraints on the lingual feeding apparatus in iguanid lizards (Wagner and Schwenk, 2000). The authors found that specific modes of lingual feeding persisted during evolution in the face of presumed changes in external selection pressures. These pressures would be expected to lead to the abandonment of this feeding mode. They conclude that internal selection—which we see as a form of constraint—may account for the unexpected persistence of the lingual feeding phenotype (Wagner and Schwenk, 2000).

Arthur and Farrow ('99) describe a method for testing hypotheses about generative constraints. It employs a null hypothesis that the phylogenetic distribution of a character is determined by selection pressures and not by generative constraints. The null model is tested by mapping the distribution of the character, and the relevant selective pressure, onto an evolutionary tree. Congruence between the distribution of the character, and the selective pressure, supports the null hypothesis. The constraint may be called a 'bias' or 'restriction' if the trait, instead of being absent, is present at reduced frequency (Arthur and Farrow, '99). In this example, constraints are treated as factors that limit the phylogenetic distribution of a transformation. See also McKi-

trick ('93) for discussion and review of phylogenetic and other approaches to constraints. There are relatively few examples of the practical use of a phylogenetic framework for analysing constraints (e.g., Wagner and Schwenk, 2000), and so we shall explore it further in this paper.

Discussions of constraints are statements about expected patterns of variation

McKittrick ('93) says (p. 309): 'Constraint in general is a check on an expected action or lack of action, and thus it implies the absence of an anticipated outcome.' A similar view was put forward by Gould ('89: 516) who says that constraints are actions not arising from the stated causes within a favoured theory. We support these ideas, and believe that constraint is diagnosed when the *observed* frequency (in teratology experiments), or distribution (in phylogenetic studies), of a transformation, differs from some *expected* value. In the work of Wagner and Schwenk (2000) discussed above, the mapping of traits onto a phylogeny, together with relevant external selection pressures, suggested an *unexpected*, and therefore constrained, pattern of phenotypic evolution (Wagner and Schwenk, 2000). In some theoretical approaches, the unobserved 'expected' variants constitute unfilled morphospace or forbidden morphologies. In experimental situations (e.g., when embryos are treated with mutagens; reviewed by Alberch, '89), a continuous spectrum of phenotypes is expected; however, only a partial set is seen, suggesting that constraints are acting (Alberch and Gale, '85). Maynard Smith ('83) argued that constraints in a fossil lineage can be analysed by examining the same trait in a modern population. The situation in extant taxa provides what, in our terms, is the expected frequency. Thus, if a character showing stasis in the fossil record is variable in a modern population, then he assumes that it was subject to stabilising selection. If constraints are analysed in a phylogenetic context, the expected phylogenetic distribution of a transformation is derived from sister-group and out-group comparison (Schwenk, '95; Arthur and Farrow, '99).

TESTING HYPOTHESES OF DEVELOPMENTAL CONSTRAINT

Our aim in this section is to examine the proposals we made above, using a specific test

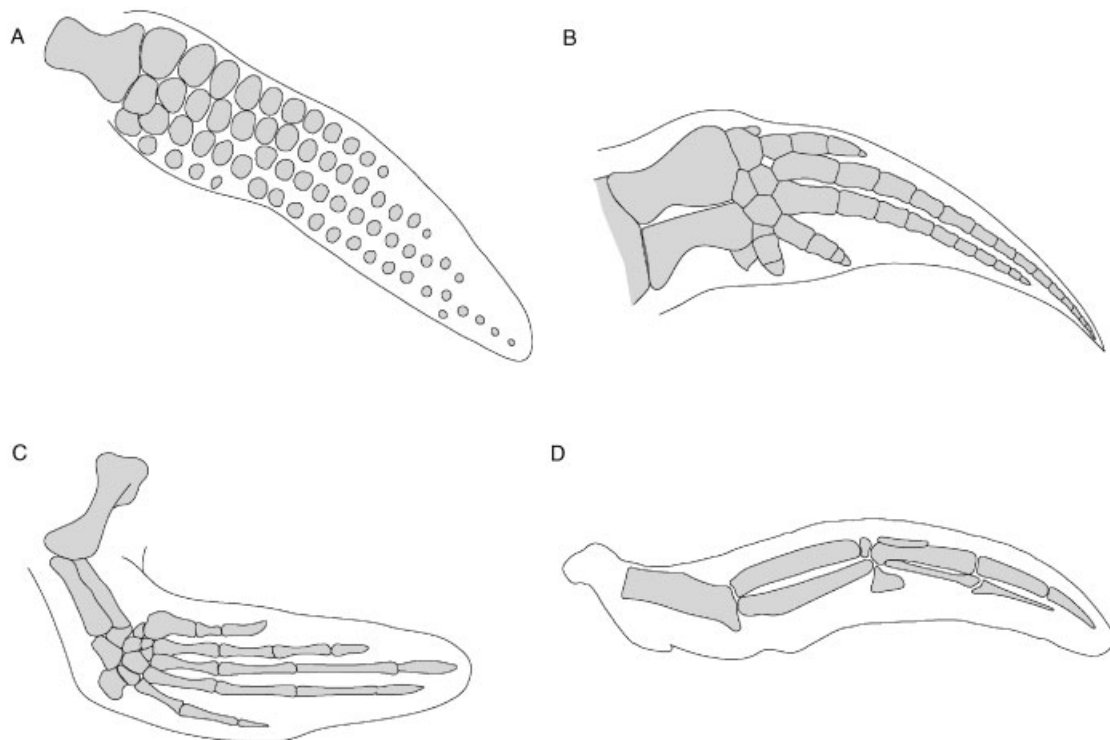


Fig. 2. Examples of relatively elongated forelimbs modified for swimming. Some species achieve elongation through hyperphalangy, others through elongation of skeletal elements. Distal is to the right, rostral to the top. A, Ichthyosaur *Eurhinosaurus longirostris*, after Figure 1, Plate III, in Huene

(’31). B, Pilot whale *Globicephala melas*, after Fig. 14, plate III in Kükenthal (1889). C, Loggerhead turtle *Caretta caretta*, after Fig. 27 in Swinton (’65). We have added a suggested flipper outline. D., Penguin *Pygoscelis* sp. For source, see note 8 to Figure 3.

case, namely a possible constraint on phalanx number in mammals. We chose this example because phalanx number is a simple quantitative trait that is well recorded in the literature. We are using the trait to map instances of a transformation: an increase in phalanx number (leading to hyperphalangy). For practical purposes, the trait is recognised as being part of a character complex (phalangeal formula) our reasoning is explained below in the section ‘The phylogenetic distribution of transformations leading to hyperphalangy.’ Our basic assumption is that a constraint on phalanx number will affect the phylogenetic distribution of a transformation—in this case, an increase in phalanx number compared to the primitive amniote condition. As noted by Schwenk (’95), phylogenetic constraint should lead to a particular pattern of character distribution on a phylogeny.

Our basic aim is to look for evidence of a phylogenetic signature in phalanx number variation (sensu Wagner and Schwenk, 2000). We will concentrate on the forelimb because the hindlimb is rudimentary in one of the key clades to be

considered (the modern cetaceans). We do not have space here to review the literature on limb form and function. Instead, we test a few simple predictions in order to illustrate general points about treating constraints as phylogenetic hypotheses. Some illustrations of the problem are given in Figure 2.

Background: the phalangeal formula of the mammalian forelimb

In mammals, the primitive number of phalanges in the manus is 2-3-3-3-3 (where the numbers represent the phalanx count on digits I-V respectively). This number evolved from the primitive reptilian formula of 2-3-4-5-3 (4) among cynodont synapsids (R. Carroll, pers. comm, and Carroll, ’87). The formula 2-3-3-3-3 is preserved in the majority of modern mammals. Notable exceptions include reduction in some bats (Walton and Walton, ’70) and some other groups (Nauck ’38: 129); and the increase in many cetaceans (whales, dolphins, and porpoises). In cetaceans, there may be a large increase in the number of phalanges on the forelimb or flipper (Flower, 1870: 255). Thus

the pilot whale *Globicephala melas* has very long flippers equipped with up to 17 phalanges on digit II (Kükenthal, 1889). This high phalangeal count is known as hyperphalangy or polyphalangy¹ and is not seen among mammals outside the Cetacea. Baleen whales typically have less pronounced hyperphalangy than toothed whales and so, within the cetaceans, there is wide variation between species in the forelimb phalanx number (Kükenthal, 1889: p. 55-56; Felts, '66: p. 259).

Deriving the 'expected' pattern of variation from sister-group and out-group comparison

To summarise our arguments, constraints are suspected where there is a discrepancy between the *observed* distribution of a transformation, and its *expected* distribution. The expected distribution comes from sister-group and out-group comparison. In our test case, we will compare the distribution of: (i) hyperphalangy (the trait under study); and (ii) selective pressures that may be linked with hyperphalangy. There are few direct data available on the transformations in the developmental pathways leading to changes in phalanx number (Richardson and Oelschläger, 2002). We are therefore mapping the occurrence of the trait (hyperphalangy), to indirectly uncover the phylogenetic transformations. We consider the potential flaws in this use of traits under "Critique of the phylogenetic approach to constraints," below.

The phylogenetic distribution of transformations leading to hyperphalangy

There is no absolute definition of hyperphalangy in terms of phalanx numbers on particular digits; it simply means 'many phalanges' at any level chosen by the investigator. For the purposes of this paper, we have chosen to define hyperphalangy as an increase in phalanx number on any digit, relative to the primitive amniote condition of 2-3-4-5-3 inferred from the Protorothyridae (Carroll, '87: 196). We have mapped instances of hyperphalangy, thus defined, onto a phylogeny (Fig. 3). We selected amniotes which show hyperphalangy, or which share selective pressures that may be related to hyperphalangy. The selection is arbitrary in the sense that we have chosen a restricted set of species for which data were readily available, and which illustrated the issues

¹Polyphalangy (a high phalanx number on a digit) should not be confused with polydactyly (the possession of more than 5 digits per limb). The two conditions sometimes occur together, as in some extinct marine reptiles.

we want to explore. Variation in phalanx number is seen to be common, and so we are not able to confirm the suggestion that hyperphalangy has a limited distribution among amniotes (Galis et al., 2001: 642). Phalanx number does not exceed 2-3-3-3-3 in any mammal in this phylogeny with the exception of the dolphin (a species chosen here to represent hyperphalangy in cetaceans). This species shows hyperphalangy relative to the basal cetacean *Rodhocetus balochistanesis* (Gingerich et al., 2001). Instances of hyperphalangy are also seen among extinct aquatic reptiles (mosasaurs, plesiosaurs and ichthyosaurs in Fig. 3).

There are several instances of hyperphalangy in the squamates (Romer, '56: 407). Greer ('92) has shown that the evolutionary loss of a phalanx is much more common than the gain of one. Nonetheless, several lineages (Fig. 4) show an increase of one phalanx compared to the primitive squamate condition (which, for the forelimb, is 2-3-4-5-3; Greer, '92). The chelonians (turtles and tortoises) also show instances of hyperphalangy, as Rabl ('10), and others have documented (Table 2).

Possible selection pressures on phalanx number

We will now consider whether hyperphalangy is co-distributed, in phylogeny, with selective pressures that might favour it.

Digging and modes of terrestrial locomotion

In squamates, hyperphalangy is selective, affecting only digits I, IV, or V (Greer, '92). The tips of these 3 digits lie at the cardinal points of the foot (i.e. at its distal and lateral extremes) and their length is therefore a major determinant of the size of the foot. This led Greer ('92) to suggest functional explanations for some cases of squamate hyperphalangy. Some of the species with hyperphalangy are burrowers, or run on loose sand, and in these situations a bigger foot could be an advantage (allowing it to function as a scoop or sand-shoe, respectively). Burrowing mammals, such as the mole, do not show hyperphalangy (Fig. 3), although this could be because they dig in heavy soils and require powerful shovels rather than broad scoops. Crocodylians dig long tunnels (Gadow, '09) but lack hyperphalangy.

Digital elongation

Romer ('56: 405) notes that the length of the toes in primitive reptiles is usually correlated with

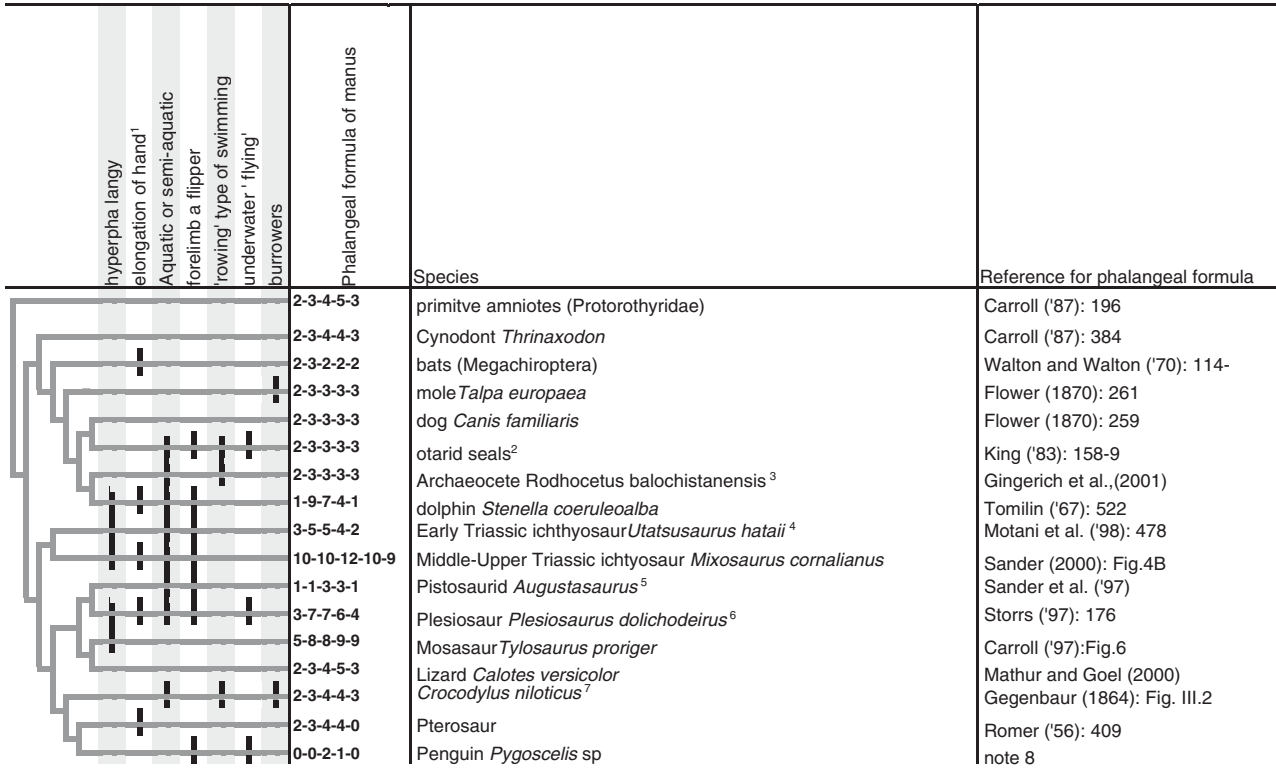


Fig. 3. Mapping of hyperphalangy (here defined as a transformation increasing the phalanx number, on any digit, above the primitive amniote condition inferred from the Protorothyridae), and other traits onto a phylogeny. For fossil forms, the preserved phalangeal formula is given. Phylogeny based on the following sources: Sander et al., '97; Shaffer et al., '97; Motani et al., '98; Murphy et al., 2001.

Notes:

- (1) Here arbitrarily defined as the manus being at least double the length of the humerus.
- (2) Otarids have cartilaginous extensions on the tips of the phalanges and there is elongation of the digit I, but in general the pinniped flipper is relatively short (King, '83: 158).
- (3) *Rodhocetus balochistanensis* was possibly amphibious, with a webbed manus, and may have swum by pelvic paddling and caudal undulation; the forelimbs may have been folded back

the number of phalanges present. One selective pressure for hyperphalangy might therefore be elongation of the manus. The pilot whale shows extreme hyperphalangy, and its flipper is greatly elongated. However, bats and pterosaurs show strong digital elongation, for support of the wing, but do not show hyperphalangy (Walton and Walton, '70; Padian, '91). Indeed some bats show a decrease from the primitive mammalian phalangeal formula (Walton and Walton, '70: 115). Of course, the biomechanics of aerial flight impose entirely different selection pressures in flying animals. The need for reduced wing weight (Vaughan, '70), and the need to fold the wing back, are examples of such pressures. In sea

turtles (Table 2), there is an elongated flipper, but the elongation has been achieved through the increase in length of bones without hyperphalangy (Walker, '73: 21; Shaffer et al., '97: 257). Indeed the phalangeal count for digit V is lower than in some other chelonians (Table 2). Furthermore, the highest phalanx counts listed among the chelonians in Table 2 are in the Trionychidae, whose forelimbs are not true flippers. Among the pinnipeds, otarids may achieve some flipper elongation by means of cartilaginous finger extensions (Flower, 1870; King, '83); and in monachine seals and otarids the flipper is elongated through increase in the length of metacarpal I. In neither case is hyperphalangy seen (King, '83).

during active swimming (Gingerich et al., 2001).
 (4) Motani et al. ('98) suggest that this species had eel-like swimming, whereas Sander (2000: 12) suggests that tail propulsion might have been important.
 (5) Sander et al. ('97) suggest that the forelimb was not used for propulsion.
 (6) Preserved formula; the number of phalanges may have been somewhat higher in life (Storrs, '97: 176).
 (7) = '*Crocodylus niloticus*' in (Gegenbaur, 1864)
 (8) This assumes that the digital formula of modern birds is II, III, IV and not I, II, III (see Burke and Feduccia, '97). We determined this count for the penguin by making a radiograph of the preserved right wing of an adult specimen in the collection of the Evolutionary Morphology, Leiden University (wing length: 33 cm). There was a shadow suggesting the presence of a rudimentary metacarpal for digit II.

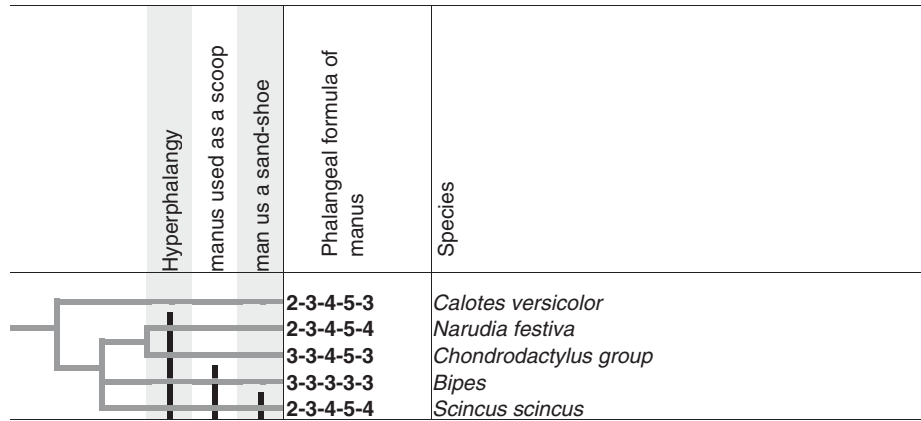


Fig. 4. Variation in phalangeal formula in the manus of selected squamates (lizards and skinks). The species were chosen to show examples of independent evolution of hyperphalangy (i.e. transformations leading to an increase in phalanx count compared to a sister taxon). Phalangeal formulas are from Greer ('92), except *Calotes* which is from

Mathur and Goel, 2000). We thank Dr. Greer for pointing out that there was an minor error in the paper (Greer, '92) such that *Lerista dorsalis* was reported as having hyperphalangy in the manus, when in fact it does not. This species is therefore omitted here.

Swimming

Is hyperphalangy an adaptation to aquatic life, or a particular mode of swimming? Before exam-

ining this question, a distinction should be noted between axial swimming, where propulsion is provided by flexion of the body axis (as in dolphins); and appendicular swimming, where

TABLE 2. Variation in phalangeal formula in the manus of selected testudines (turtles and tortoises)¹

Group	Swimming type (Rises and Frey, '91)	Species (Synonym used by Rabl, '10)	Phalangeal formula of manus	Reference for phalangeal formula (page numbers are for Rabl, '10)
Pleurodira	rowing	<i>Proganochelys</i>	2-2-2-2-2	Carroll ('97): 210
		<i>Erymnochelys</i> (= <i>Podocnemis</i>) <i>madagascariensis</i>	2-3-3-3-3	117
		<i>Pleomedusa subrufa</i> (= <i>P. galeata</i>)	2-2-2-2-2	117
		<i>Chelus fimbriatus</i> (= <i>Chelys fimbriata</i>)	2-3-3-3-3	118
		<i>Hydromedusa tectifera</i>	2-3-3-3-3	116
Chelonioidea (marine turtles) ²	flying by means of an elongated flipper	<i>Chelonia mydas</i>	2-3-3-3-2	81
		<i>Dermochelys coriacea</i>	2-3-3-3-2	83
Trionychidae	flying and rowing	<i>Caretta</i> (= <i>Thalassochelys</i>) <i>caretta</i>	2-3-3-3-2	83
		<i>Cyclanorbis senegalensis</i>	2-3-3-5-4	129
		<i>Pelodiscus sinensis</i> (= <i>Trionyx sinensis</i>)	2-3-3-4-4	128
Carrettochelidae	flying and rowing	<i>Chitra</i>	2-3-3-6-4	Walker ('73): 20
		<i>Carrettochelys</i>		
Emydidae tortoises	rowing (terrestrial)	<i>Emys orbicularis</i> (= <i>E. lutaria</i>)	2-3-3-3-2	99
		<i>Rhinoclemmys punctularia</i> (= <i>Nicoria punctularia</i>)	2-3-3-3-2	113
		<i>Geochelone</i> (= <i>Testudo</i>) <i>radiata</i>	2-2-2-2-2	124
		<i>Testudo graeca</i>	1-2-2-2-1	120
		<i>Testudo marginata</i> (= <i>T. campanulata</i>)	1-2-2-2-1	124
		<i>Psammobates geometricus</i> (= <i>Testudo geometrica</i>)	2-2-2-2-2	124
		<i>Pyxis</i> (= <i>Acinixys</i>) <i>planicauda</i>	2-2-2-2-2	125

¹Romer's ('56) statement, that 'the phalangeal count varies greatly' is amply illustrated in this sample. Because of controversies over chelonian phylogeny, we have not attempted to suggest any sister-group relationships here. For this reason, phylogenetic transformations leading to hyperphalangy cannot be mapped, and we can only look for ecological correlations. There is no obvious correlation between high phalanx number, swimming pattern or the presence of a true flipper.

²Gadow ('09: 379) gives a formula of 2-3-3-2-2 for this group.

propulsion is provided by the limbs (Riess and Frey, '91). Appendicular swimmers include 'rowers' who make paddling movements along the line of travel, and underwater fliers (e.g., *Dermochelys*, penguins), who use their flippers as hydrofoils, perpendicular to the line of travel, to generate some lift (Riess and Frey, '91).

Dolphins have axial swimming: the thrust is generated by the tail (Videler and Kamermans, '85) and the flippers function as rudders for steering and stability (Felts, '66). Ichthyosaurs (Sander, 2000: 5) and mosasaurs (Carroll, '97: 232) may also have shown axial propulsion with the flippers used as rudders, and they also had hyperphalangy. Among these groups, however, only advanced ichthyosaurs are presumed to have shown the dolphin-type of high speed (thunniform) cruising.

Other vertebrates possess elongated flippers, but do not show marked hyperphalangy. Examples include some seals, penguins and, as noted above, marine turtles. Among Sauropterygia, there was hyperphalangy; the nothosaurs may have used rowing and axial propulsion, while plesiosaurs were possibly underwater fliers (Riess and Frey, '91) although the swimming patterns of extinct marine reptiles are a matter of debate: see Carroll ('87); Massare, ('97: 246–248). In mosasaur evolution, hyperphalangy may not have been a primary adaptation to aquatic life, but a relatively late one associated with subsequent radiation (Carroll, '97).

In short, the phylogenetic distribution of hyperphalangy is not completely congruent with any of the following alone: aquatic lifestyle, the possession of a flipper, or swimming mode. What we can say is this: (i) all cases of extreme hyperphalangy are seen in the flippers of primarily aquatic species (ii) some aquatic species with flippers do not show hyperphalangy (iii) all extremely hyperphalangeous flippers are used as rudders for steering except, perhaps, those of plesiosaurs.

Discussion of the examples considered

As Wagner and Schwenk (2000) argue, evolutionarily stable configurations (ESC's) can be revealed by comparative analysis. If there is stasis of a character complex in the ESC clade, and variation in ESC character states in a sister group, then the ESC has left a phylogenetic signal. In our case, a putative ESC is revealed through study of a trait, phalangeal formula. Our mapping exercise reveals precisely the phylogenetic ESC signal for

phalanx number that Wagner and Schwenk (2000) discuss.

We find that phalanx number varies widely in non-mammalian tetrapod clades. Furthermore, selective pressures, plausibly correlated with hyperphalangy in those clades, are shared by some mammals. This creates an expectation that mammals will show comparable variation in phalanx number. However, phalanx number is relatively stable in mammals outside the Cetacea. The stasis in phalangeal formula contrasts with its variation in other amniote clades. The presence of hyperphalangy in dolphins could indicate a paraphyletic distribution of constraint (Wagner and Schwenk, 2000: p. 189), consistent with a eutherian-level constraint being broken (or the ESC disintegrating). Why is forelimb phalanx number such an evolutionarily plastic trait in cetaceans, yet apparently stable in other mammals? It seems clear that some kind of constraint is acting, because hyperphalangy is not equally distributed to all amniotes in the phylogenies studied here. One possibility is that phalangeal number is subject to a generative or internal constraint in mammals, and this constraint has been 'broken' in the cetaceans. An alternative explanation is that there is no constraint, and phalanx number in all mammals is determined instead by functional considerations, lack of genetic variation or other factors, and the existence of hyperphalangy only in cetaceans is merely due to a chance combination of selection and functional considerations.

Nonetheless, this analysis tends to favour the former explanation, and thus generates a testable hypothesis as to the nature of the factors determining phalanx number in mammals. This hypothesis can be tested in principle by studying the developmental mechanisms involved in digit differentiation in a wide range of mammals including cetaceans.

Critique of the phylogenetic approach to constraints

A possible advantage of using a phylogenetic framework is that some problematic categories of constraints are necessarily excluded from consideration. While 'universal constraints' (Maynard Smith et al., '85; Resnik, '95) and forbidden morphologies (Holder, '83) are useful in developing theoretical models, it is not easy to see how they can be tested empirically. In our scheme, universal constraints and forbidden morphologies are either present or absent, respectively, in all

taxa. This means that they do not represent synapomorphies at any level in the phylogeny and are therefore uninformative.

Our study and others have shown several potential limitations to the phylogenetic approach to constraints. A robust phylogeny is required for the species studied, and the polarity of transformations must be identifiable through three-taxon comparisons (Schwenk, '95); this may not always be possible (as in our example in Table 2). Moreover, it may be difficult to reconstruct the selection pressures acting on ancestral species (Wagner and Müller, 2002).

Character homology is a further issue in any comparative study as is the hierarchical level of homology. There is evidence that variations in phalanx number may have a common developmental origin: heterochrony in embryonic limb outgrowth (Richardson and Oelschläger, 2002). If true, this would indicate that the independent evolution of hyperphalangy in the groups studied here shares the same developmental mechanisms, but is homoplastic in the sense of being absent in the common ancestor. Although Schwenk ('95) argued that homoplasy can confound the interpretation of constraint, we feel that it does not weaken our example. Rather, it shows that the pathway, or pathways, to hyperphalangy found outside of mammals are rarely followed within the mammals. Thus, as has been argued, parallelism (independent evolution using the same mechanism) can be evidence of developmental constraint (Wake, '91; Hodin, 2000).

Another problem with the phylogenetic approach used here is uncertainty over whether a particular constraint, or character, is indeed related to a given external selective pressure. Our ability to identify constraints is therefore limited by our ability to identify relevant alternative scenarios based on selection. It may never be possible to determine, for example, whether marine turtles lack hyperphalangy because their embryos cannot generate it; or because these turtles have more readily evolved other features which serve the presumed functions of hyperphalangy (features such as the elongation of phalanges, the loss of joints between them, and flattening of the carpal elements: Shaffer et al., '97: 257). Once again, we see that the relative ease or difficulty of a transformation (i.e., its degree of constraint) may vary between different phylogenetic contexts. This is important: if transformations change their properties according to their phylogenetic context, then it may not be possible

to treat them as homologous characters when optimising them onto phylogenies.

CONCLUDING POINTS

We have argued that developmental constraints embrace a wide variety of disparate phenomena. We suggest that the most useful classification is based not on developmental *versus* other constraints, but on the distinction between generative and selective constraints. This distinction is by no means clear cut, and it is possible that many apparent generative constraints are a function of selection. Absolute constraints are difficult to prove, and so it may be easier to think of constraints in terms of the probabilities of particular transformations. Ideally, a transformation includes the entire suite of changes in developmental mechanisms leading to a morphological change, and is made as a phylogenetic statement. Because constraints are relative, we argue that a particular transformation may be either constrained or plastic, depending on its phylogenetic context. The context is set by the range of other available transformations, responding to the same selective pressure, in that taxon.

Constraints are generally considered to strongly limit the evolutionary variation of developmental systems. As we have argued, many cases of constraint represent the action of internal selection. While constraints simply reflect the lowered probability of certain transformations taking place, many examples demonstrate that even highly constrained systems can undergo radical alteration (e.g., Lowe and Wray, '97). Well-known examples are the evolution of direct development in many phyla (e.g., Raff, '92; Hanken et al., '97), in which the most basic constraints of early development are broken.

The attainment of a constrained transformation may open a new adaptive space, and allow an adaptive radiation that was not possible before (see Wagner '88; Müller and Wagner, '91 for the relation between constraint and innovation). This may indeed be the case with cetaceans. A further example is provided by the transformations leading from a reptilian scale to feathers (Prum, '99), which allowed the rapid adaptive radiation of birds in the previously unfilled niche of arboreal-aerial locomotion. Thus, we support the view that developmental constraints, sometimes thought to have a role mainly in limiting the variability of morphological forms in nature, can actually be central in facilitating the appearance of new forms

(Gould, '89; Wake, '91; Wagner and Müller, 2002 and references therein; see also Hall, '96). This perspective makes a deeper understanding of constraints crucial to our understanding of evolutionary processes in general.

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