

Why five fingers? Evolutionary constraints on digit numbers

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Evolutionary changes in the number of digits and other limb elements appear to be severely constrained, probably as a result of a low level of modularity during limb development. Reduced limb structures typically develop through a process of construction followed by destruction and amniotes have evolved many digit-like structures rather than actual extra digits. In amniotes, limb development occurs during the crucial phylotypic stage, when many inductive interactions are occurring throughout the body. As a result, changes in limb development usually engender changes in other body parts. Thus, mutations that change the number of limb bones are expected to have many pleiotropic effects, which severely reduces the chance of such mutations being successful. In amphibians with aquatic larvae, limb development occurs after the phylotypic stage and limb development is decoupled from the interactivity of the phylotypic stage. The constraint of pleiotropic effects is, therefore, expected to be weaker. This expectation agrees with the larger variability in the number of hand and foot structures in amphibians, with frogs even occasionally possessing six toes. These facts once again emphasize the importance of pleiotropic effects as constraints to evolutionary change, including their role in the conservation of body plans.

Reductions in the number of digits have occurred frequently during tetrapod evolution. Further reductions in the number of limb bones have also occurred, including the complete elimination of limbs in, for example, snakes and caecilians. Strikingly, an evolutionary reversal of limb reduction has never occurred^{1,2}. This is puzzling when one considers that mutations for POLYDACTYLY (see Glossary) are particularly common. In humans, for example, the most common anomaly at birth is the presence of an extra toe or finger^{3,4} (0.1–0.2%). The extra digit can be rudimentary, or fully formed and functional. Polydactyly, either as an evolutionary novelty, or as a result of ATAVISM, is a common phenomenon in other tetrapods^{1,5} (Fig. 1). The horses of Alexander the Great and Julius Caesar, for example, were reported to have three toes and to be of legendary excellence. The absence of polydactyly in evolution is clearly not an absence of heritable variation for the trait. Therefore, there must be selection against mutations causing polydactyly or other reversals of limb reduction.

Lande¹ and Wright² have hypothesized that polydactyly is not selected against directly. They argue that selection against mutations causing polydactyly occurs because of negative PLEIOTROPIC effects. Lande¹ argues that any change in the number of limb bones is usually selected against. For decreases there is, however, an evolutionary way around the constraint: construction followed by destruction. In the initial evolutionary steps of limb

reduction, the original number of digits appears as ANLAGE (Fig. 2a). The development of the Anlage of one digit, usually the last one formed, is then followed by regression in its size, resulting in only a rudimentary digit (Fig. 2b; Box 1). This can still be observed in most species with a reduced number of digits, such as the Anlage of the postminimus (a sixth finger) in humans⁶. When limb reduction is continued during evolution, the development of Anlagen of both bone and muscle, is slowly reduced. As far as we know, continued limb reduction always follows the process of construction and destruction. In reptiles, the regression of Anlagen is the result of cell death^{1,7} once the limb has started to develop. For example, in the limbless lizard *Ophisaurus apodus*, the limb buds develop, and myotomes that normally develop into limb muscles extend from the somite into the limb bud, where they distribute cells. Necrosis then begins, first in the ridge, followed by the myotomal cells and then the mesenchymal cells. At first, the limb bud continues to grow during the necrosis, but it then regresses and forms only tiny rudiments of most of the limb bones⁸.

This mechanism of evolutionary limb reduction suggests that the construction phase cannot easily be missed during normal development. The most probable reason for this is that the inductive interactions of the construction phase of the limb cannot be missed out of the chain of inductive interactions of overall development. This conclusion is in agreement with the extremely slow speed of evolutionary limb reduction¹.

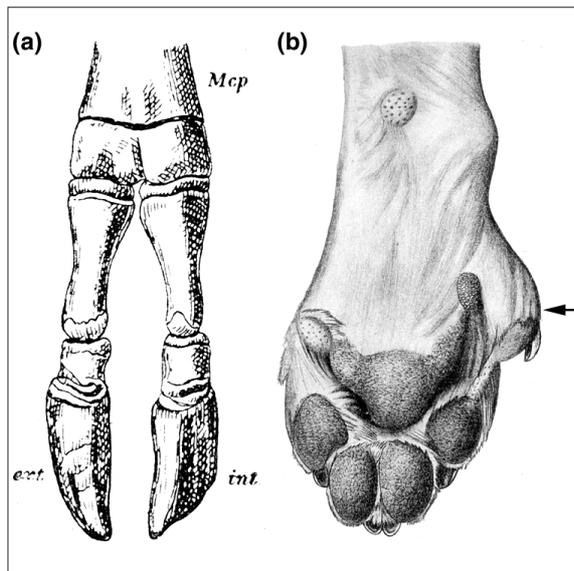
Selective advantage of changes in digit number

Under certain circumstances, there are selective advantages to having fewer toes (e.g. for running⁹), or even completely reduced legs. The reduction in the number of toes of ostriches parallels that seen in ungulates. Similarly, in burrowing organisms from different vertebrate groups, the limbs have become reduced independently many times in association with body elongation (e.g. in snakes), or hands with special digging devices have evolved, as in moles *Talpa europaea*.

Extra digits on the hand give further support in swimming and digging for some animals, and a better grasp of branches in tree-dwelling species. In amniotes, extra digit-like structures have evolved in association with these functions many times (Fig. 3). However, these extra structures are never 'true'

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Fig. 1. Examples of polydactyly. (a) Foot of horse with two toes. Reproduced from Ref. 5. (b) Foot of St Bernard dog with five toes, including an atavistic first digit (arrow). In wolves and most other canids, the first toe is reduced, and they only have four toes. Reproduced from Ref. 61.



digits but are, for example, modified wrist bones or extra phalanges. Ichthyosaurs were long thought to have evolved true polydactyly, possessing up to nine digits. However, recent insights suggest that they never had more than five digits and that there were many SUPERNUMERARY PHALANGES that spread out over many rays, possibly by bifurcations of those rays^{10,11}. In moles, there is an impressive digit-like structure that functions in digging (Fig. 3a). Pandas *Aeluropus* spp. even have two extra digit-like structures: the well-known thumb that is not a 'true' digit, and an extra digit-like structure on the side of the little finger¹². The 'thumb' is a sesamoid bone and the extra little finger an enlarged wrist bone, the pisiform. In mosasaurs, whales and turtles, the

paddle is strengthened by an extra digit-like structure, which, as in pandas, is an enlarged pisiform (Fig. 3b). Another evolutionary solution for extra digits for swimming or grasping is a change of the backward-pointing toe into a forward-pointing toe, or vice versa (Box 2).

The repeated selection for extra digit-like structures across a range of animals shows that extra digits can have a strong functional advantage; however, this does not translate into a selective advantage for polydactyly. As already observed by Lande¹ and Wright², the constraint on polydactyly cannot be explained by negative selection against the extra digits themselves. There must, therefore, be strong negative pleiotropic effects associated with polydactyly that indirectly pose an evolutionary constraint.

The recent increase in molecular biology data and the emergence of evolutionary developmental biology provide new means with which to study the pleiotropic effects associated with polydactyly.

Polydactyly, modularity and the phylotypic stage

In amniotes, the period of limb patterning occurs mainly in the phylotypic stage, which is the stage at which vertebrates most resemble each other¹³. This stage begins with neurulation and ends when the majority of somites have been formed. We propose that the constraint on changes in the number of digits is part of the constraint on changes in this stage.

Pleiotropy and the phylotypic stage

The phylotypic stage is characterized by intense INDUCTIVE SIGNALING between different parts of the embryo, including somites, notochord, neural tube, lateral plate mesoderm and limb buds. The presence of inductive interactions between different body parts causes mutational changes in one part of the body to have pleiotropic effects in other parts of the body. Raff¹⁴, refining a hypothesis of Sander¹⁵, hypothesized that this widespread global INDUCTIVE INTERACTIVITY is the cause of the conservation of the phylotypic stage. The pleiotropic effects that Lande¹ and Wright² proposed as the cause of the conservation of the number of limb bones could, thus, be part of the pleiotropic effects that appear to constrain mutational changes during this stage in general. Lande's¹ and Wright's² hypotheses would then become more specific: the evolutionary constraint on polydactyly and other reversals of limb reduction is caused by the high interactivity among body parts (MODULES) during the phylotypic stage.

Global inductive interactions constrain mutational changes

Galis and Metz¹⁶ recently tested the hypotheses of Sander¹⁴ and Raff¹⁵ by analyzing teratological studies that reported effects of phenocopies of mutational changes (i.e. induced phenotypic changes that mimic mutations). The analysis

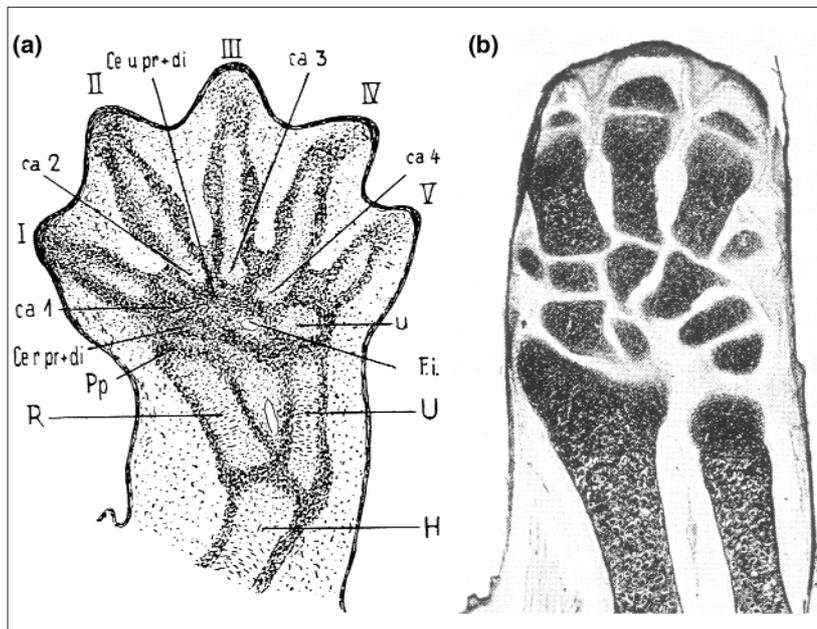


Fig. 2. Evolutionary reduction by construction and destruction. (a) Embryonic hand of the opossum *Didelphis marsupialis*, showing full-sized Anlagen of all five digits. Abbreviations: H, humerus; R, radius; pp, prepollex; U, ulna; u, ulnare; I-V, digits). (b) Hand of juvenile *D. marsupialis*, showing a reduced first and fifth digit. Reproduced, with permission, from Ref. 6.

Box 1. Polydactyly in dogs

In general, large dogs frequently have extra rudimentary or fully formed toes^a, a high rate of locomotory diseases and high mortality rates^b (often not living beyond six years of age, whereas large mammals generally die at a higher age than small mammals). In St Bernard dogs and Bernese mountain dogs, the first toe, commonly reduced in canids, is usually present in the hindlimb (Fig. 1b) and is often double in St Bernards because of a history of artificial selection for this character^{c–e}. The death rate of St Bernards and Bernese mountain dogs is very high^b. Locomotory diseases (elbow and hip joint problems), as well as tumors and heart disease, are a common cause of death in these dogs. The rare and small Norwegian Lundehunds, which are used for hunting puffins, are required to have a minimum of six toes on their hindlimbs, according to the breed standard. The extra toes presumably prevent them from slipping off the rocks. These dogs have extremely high rates of gastritis and intestinal lymphangiectasia. In addition, 30% of 14 autopsied dogs in a 13-year study suffered from gastric carcinoma^f. The

serious health problems in dogs with polydactyly are in agreement with the assumption that the pleiotropic effects of mutations for polydactyly are likely to be serious in other parts of the limb and in other parts of the body. Further research is necessary to test which of these problems in dogs are, indeed, linked to polydactyly and which are independent by-products of inbreeding.

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provides strong support for the assumption that the many global inductive interactions during the phylotypic stage lead to negative pleiotropic effects of mutational changes acting during that stage. These effects are expected to constrain evolutionary change. This is in agreement with current ideas on the importance of MODULARITY in evolutionary change^{17–19}.

The timing of limb development relative to inductive interactivity

Do these results on the strong coupling between modules during the phylotypic stage help us to understand the evolutionary constraint on polydactyly? To answer this, we need to know whether digit development of tetrapods takes place during the time when there is intense interactivity among different parts of the embryo. In mice, the period of high vulnerability resulting from global inductive interactions is from embryonic day (E) 7 to 11, and vulnerability sharply decreases thereafter (Fig. 4). Therefore, in mice, the phylotypic stage can be specified as being from E7 to E11. In these animals, the development of the limb bud starts on E8, and mesenchymal digit condensations appear on E10 and E11, in the forelimb slightly before the hindlimb²⁰. The patterning of the limb up to the determination of the number of digits therefore occurs during a stage that is characterized by global inductive interactivity. Hall and Myake²¹ emphasize that most of the mutations affecting skeletal development act on the early condensation stage^{21,22}. The staging tables of Keibel²³ show that limb development occurs at roughly comparable stages in lapwings, sand lizards, deer, mice, humans and chickens. Therefore, in amniotes, unlike in the majority of amphibians, most of the limb patterning

occurs during the phylotypic stage. However, limb development might not form part of the general interactivity of the phylotypic stage: it could instead form a semi-independent module.

Is limb development in amniotes integrated in the general interactivity of the phylotypic stage?

The self-organizing capacity of the limb is very limited in amniotes. The success of transplanting limb buds in amniotes is low, in contrast to amphibians, where even transplantations to the head can be successful²⁴. Explantation experiments in mice show that, up to E12, limb buds do not develop successfully unless somitic material is also explanted. On E12 (when the vulnerability to inductive interactions has sharply decreased) the self-differentiating capacity increases markedly, and the results are considerably better on E13 and E14 (Ref. 25). Similar results were obtained for chickens²⁶. Therefore, the transplantation and explantation results show that the developing limb is not an independent module.

Molecular pathways of limb and digit development

The recent progress in identifying molecular pathways involved in limb patterning has revealed many dependent inductive interactions. For example, there is a complex interplay between signaling centers in the limb, the zone of polarizing activity (ZPA), the apico-epidermal ridge (AER) and the progress zone^{27,28}. In addition, genes elsewhere play important roles^{29,30}. If the constraint on polydactyly is due to strong coupling between modules during the phylotypic stage, it is particularly important to know which genes are involved in patterning the digits and, in particular, which are the most upstream genes that have to be

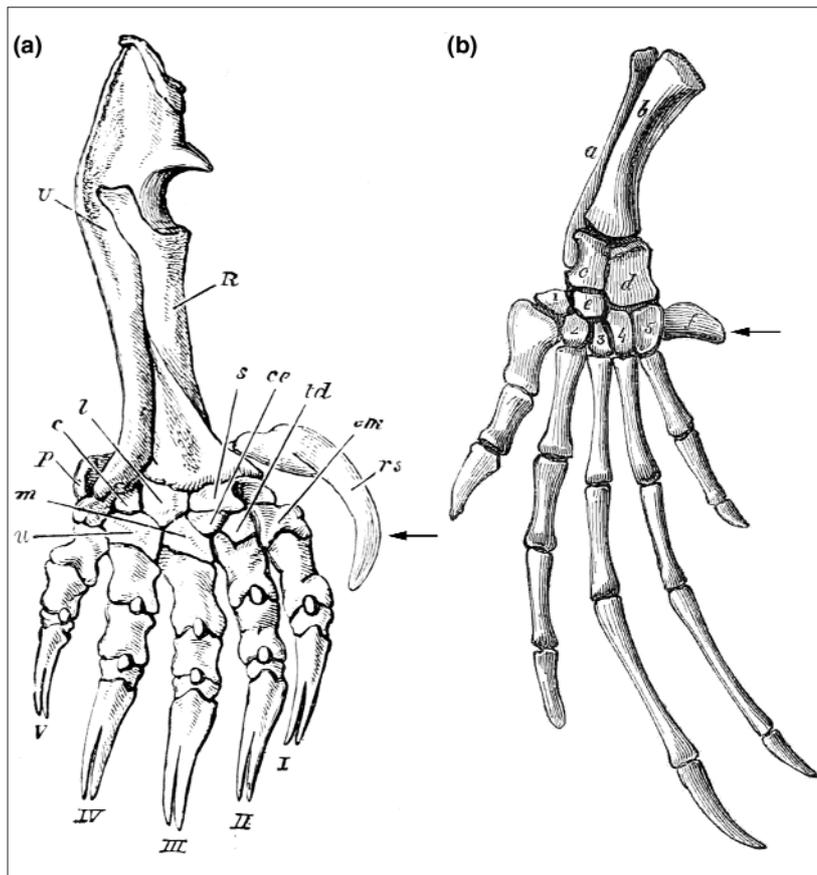


Fig. 3. Extra digitlike structures in amniotes. (a) Mole *Talpa europea* with extra digit-like structure (arrow). This structure is a sesamoid bone. Reproduced from Ref. 62. (b) The extra digit-like structure that contributes to the paddle of the sea turtle *Chelone* is an enlarged pisiform (arrow). A similar solution has evolved in whales and mosasaurs. Reproduced from Ref. 63.

affected for polydactyly to occur. The more upstream these genes are, the more likely it will be that changing their activity also affects other parts of the embryo.

Many genes are involved in digit development and specification, including members of the Hedgehog, Bmp, Fgf and Hox families, which are all relatively upstream genes. These genes, or at least a combination of them, are always involved in the development of polydactyly and oligodactyly in mice, chickens and *Xenopus*^{31,32}. A crucial role seems to be played by the Sonic hedgehog gene (*Shh*). Ectopic expression of *Shh* in the anterior part of a developing limb bud is nearly always involved in polydactyly, and when this is not the case, ectopic anterior expression of the related Indian hedgehog gene (*Ihh*) is suspected^{30,32}. In all cases, genes directly downstream from *Shh* are expressed ectopically. Recent data³² emphasize the importance of *Shh* in the determination of both digit number and identity. Drossopoulou *et al.*³² suggest that *Shh* probably acts first over a long range to control digit number and then later over a short range to induce expression of Bonemorphogeneticprotein genes (*Bmp*), the morphogenetic action of which specifies digit identity.

Expected pleiotropic effects of polydactyly for other parts of limb patterning

Members of the Hedgehog, Bmp, Fgf and Hox families play important roles in proximo–distal, dorso–ventral and antero–posterior patterning. Multiple molecular interactions link the development along these three axes to generate the integrated system of limb development²⁸. For example, the signaling of *Shh* in the ZPA that is involved in the specification of digit number (part of the antero–posterior patterning of the limb) is also involved in the proximo–distal outgrowth of the limb²⁸. The same signals might be involved in patterning bones, tendons and muscles, because the grafting of an extra *Shh* expressing ZPA in the limb bud causes the development of an extra set of all these elements. This fits with the observation that *Shh* is involved in keeping muscle precursor cells in a proliferative state, thereby increasing the size of the muscle masses of the limb³³.

Expected pleiotropic effects of polydactyly for other parts of the embryo

It is likely that the upstream genes mentioned above are also involved in interactions between the limb and other parts of the embryo. There are several processes that necessarily involve such interactions: (a) the migration of muscle progenitor cells into the limb; (b) the migration of neural crest cells (precursors of pigment cells) into the limb; (c) the invasion of axons into the limb; and (d) the invasion of blood vessels into the limb. The molecular basis of these processes has not been investigated as thoroughly as that of the patterning ones. However, the presence of inductive signals from the somites toward the limb bud, and vice versa, has been established in the migration of muscle precursor cells^{34–36}. The migration of axons into the limb provides a probable case for the involvement of digit patterning gene activation in processes that occur in the lateral ridge. The *Robo1* gene and members of the *Slit* gene family appear to be involved in axon guidance in mice, *Xenopus* and *Drosophila*. The three *Slit* genes and *Robo1* are expressed in the mouse in unique and complementary patterns in the front and hind limb bud and in the lateral ridge tissue between the limb buds. In *Xenopus*, *Slit1* and *Robo1* interact with each other, but otherwise the regulation is unknown. Yuan *et al.*³⁷ infer from the expression pattern of *Slit3* (at E10.5 and E11.5) that interactions with the antero–posterior-acting molecules, such as *Shh*, are probable and, from the expression pattern of *Slit2* and *Slit3*, that interactions with *Shh* and *Bmp* genes are likely. If this were the case, it would provide a link between axonal guidance and digit patterning.

Evidence for negative pleiotropic effects

The interdependent molecular pathways operating during the phylotypic stage lead to the expectation of many pleiotropic effects associated with changes in the number of digits or other limb bones, both within

Box 2. Homeotic changes of digit identity

In aquatic birds, an increase in paddle stiffness is not obtained via polydactyly. In many such birds, the paddle consists of three forward-pointing digits and one backward-pointing toe. This is the ancestral position of toes in birds. In the pelican family (pelicans, boobies and cormorants), the

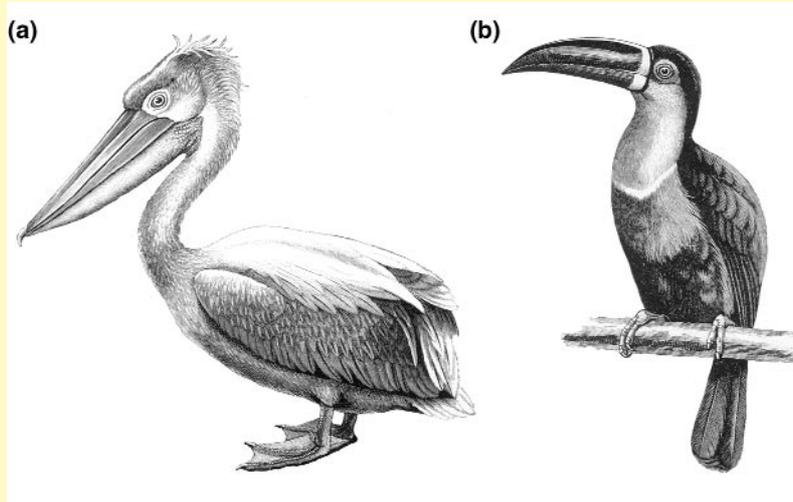


Fig. 1. Position shifts of toes in birds. (a) Pelican, with four toes included in the paddle. The fourth toe has moved forward. (b) Toucan, with two forward-pointing toes and two backward-pointing ones. Reproduced from Ref. b.

one backward-pointing toe has moved forward and is incorporated in the paddle (Fig. 1a). The absence of a backward-pointing prop for the foot could partly explain the poor walking ability of these birds, indicating a tradeoff between swimming and walking.

In tree-dwelling birds and mammals, there is an advantage in having an extra opposed digit for grasping branches firmly. In all cases, the extra opposed digit has been obtained by changing the form of the second digit into that of the first, opposable, digit. For example, parrots, cuckoos and woodpeckers have two forward-pointing toes and two backward-pointing toes (Fig. 1b). In mammals, the ancestral number of toes is five, and so koalas have three forward-pointing toes and two backward-pointing ones. The extra backward-pointing toe is never the result of an extra digit, whereas a mutation for an extra thumb occurs, at least in humans, with a frequency of one in 5000 (Ref. a).

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the limb itself and in other parts of the embryo. Indeed, mutations for changes in the number of digits are associated with many serious abnormalities in mice, chickens and humans^{22,38–40}. Polydactyly is a characteristic of many human syndromes, such as Ellis van Crefeld, Bardet–Biedl, trisomy 13 and Down syndromes^{4,41}. In 15% of babies born with an extra toe, other congenital abnormalities are also detected⁴, although in 85% of the babies with polydactyly, there are no associated anomalies. However, the problem in interpreting these data is that later medical problems are not usually recorded. A review on limb reduction in humans, recording abnormalities at birth with a follow-up a year later, revealed an incidence of limb reduction at birth of 0.06% ($n = 1\ 213\ 913$), of which 12.9% had associated abnormalities⁴². Of the children with associated abnormalities, 16% ($n = 348$) died in the first year, and of the children without associated anomalies, 4% ($n = 311$) died in the first year. This indicates that very serious abnormalities are missed during birth checks (in the general population mortality in the first year is ~0.8%; Ref. 43). No information was given on later problems.

An additional problem is that syndromes commonly have variable expression^{44–46}, which interferes with an objective evaluation of anomalies associated with polydactyly mutations. It would be useful if the longevity and fertility of patients were included in genetic studies of families with such abnormalities.

In spite of these problems, there is abundant evidence for the importance of negative pleiotropic

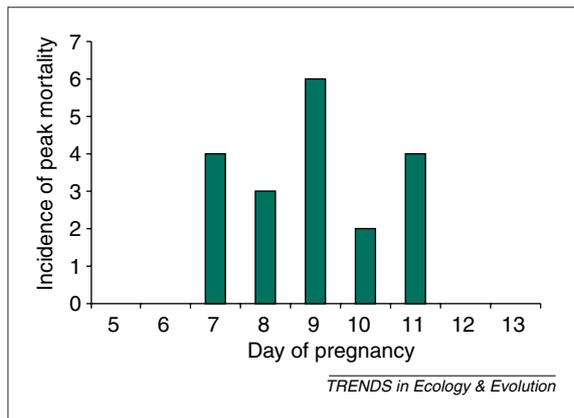
effects in birds and mammals. In mice, the occurrence of oligodactyly was always associated with pleiotropic effects, both in the appendicular and axial skeleton²². In chickens, oligo- and polydactyly are also associated with other problems. In these birds, continued selection for polydactyly, for example, led to malformations of the radius²². There are the famous polydactylous monsters described by Sewall Wright⁴⁷, who showed how guinea pigs that are heterozygous for the *Px* mutation are polydactylous without any apparent abnormalities, suggesting that this gene might act only locally on the footplates. However, individuals that are homozygous for the mutations have anomalies in almost every organ in the body. The well-established cases of polydactyly occurring in various dog breeds and the indications for pleiotropic effects are discussed in Box 1.

Overall, therefore, there appears to be sufficient evidence for the negative pleiotropic effects that are expected as a result of molecular interactions during limb development. Further studies are necessary to unravel precisely the relevant interacting molecular pathways.

The exceptional position of amphibians

In amphibians with aquatic larvae, limb development occurs later than in amniotes and generally occurs after the phylotypic stage. Limb development is especially late in anurans that have an extreme mode of metamorphosis. This suggests that limb development cannot take part in the interactivity of the phylotypic stage, a drastic

Fig. 4. The vulnerability of the phylotypic stage to induced changes (phenocopies of mutations). Vulnerability to teratogenic treatments in rodents is highest during the phylotypic stage [embryonic day (E) 7–11 in mice]. This vulnerability is caused by dependent inductive interactions. Peak sensitivity to the induction of mortality occurs on a particular day during pregnancy, always within the phylotypic stage, usually on E9. Reproduced, with permission, from Ref. 16.



difference compared with amniotes. This is in agreement with the extremely high self-organizing capacity of the limb buds in many amphibians compared with those in amniotes²⁴. Amphibian limb buds can be grafted to very different places, such as the head, and still successfully develop into limbs, which are even capable of movement⁴⁸. Limb development can proceed almost as an independent module, with few interactions with other parts of the body. This reduces the possible number of pleiotropic effects of mutations that affect limb development, at least in other parts of the body. However, the pleiotropic effects within the limb are not expected to be lower than those in amniotes. Such effects will probably constrain changes in the number of limb elements; reversals of evolutionary limb reduction are indeed rare and mainly limited to an increase in the number of phalanges (hyperphalangy)⁴⁹. Evolutionary reductions in digit number have occurred frequently in amphibians,

but as in amniotes, these also seem constrained. This follows from the presence of Anlagen of reduced fingers early on during development. For example, the fifth finger that has disappeared in the hand of frogs and salamanders is still visible as Anlage in some frogs and salamanders (Fig. 5a), and the postminimus is still formed as Anlage in some salamanders, just as in humans (Fig. 5b). The mechanism of evolutionary limb reduction, therefore, appears similar to that in amniotes, as is the loss of limb bones in approximately the reverse order that they are developed, suggesting once again a developmental constraint on limb reduction (Box 3).

In spite of the apparent presence of an evolutionary constraint on changes in the number of digits, the absence of inductive interactions with other parts of the amphibian embryo suggests that the constraint will be weaker in amphibians than in amniotes. Is there support for such an expectation? Variation, including intraspecific variation in the number of phalanges, is more common in amphibians than in amniotes^{50–54}. Variation in the number of carpal and tarsal bones is also remarkably high^{50–55}. In particular, hyperphalangy occurs frequently, and this represents the only common form of evolutionary reversal of limb reduction. Although hyperphalangy also occurs in amniotes, it is considerably less common than in amphibians and occurs only in extant species of whales and geckos. The number of phalanges and carpal and tarsal bones is determined very late during limb patterning, when most of the patterning has been completed (including the number of digits). Changes in these distal elements are, therefore, expected to be less constrained than in those elements that are patterned earlier.

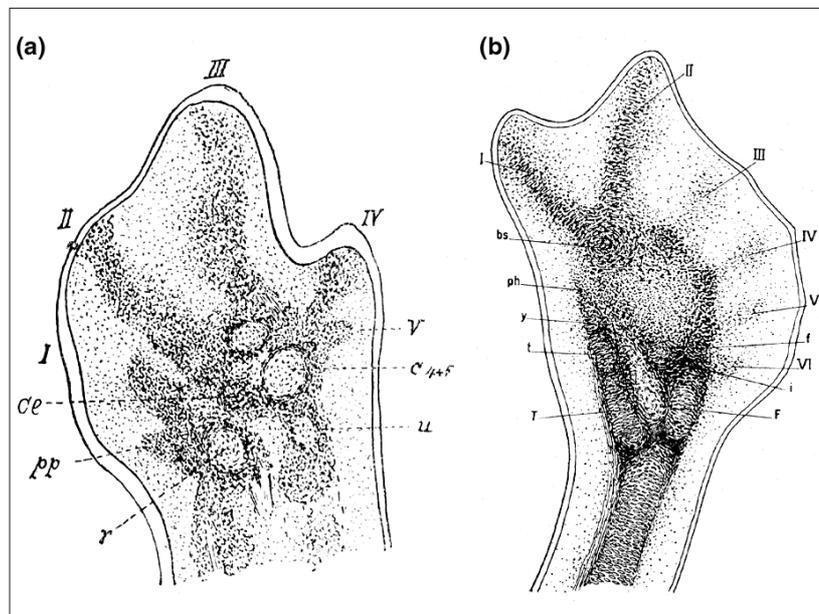


Fig. 5. Embryology of digits in amphibians. (a) Embryonic hand of the anuran *Pelobates fuscus*, showing still Anlagen of all five digits, and the prepollux as possible sixth digit. The Anlage of the fifth digit will not develop into a finger. Abbreviations: pp, prepollux; r, radius; u, ulna; I–V, digits. Reproduced from Ref. 64. (b) Embryonic foot of the urodele *Ambystoma tigrinum*, showing Anlagen of six digits and a trace of the prehallux (possibly the 7th digit). Abbreviations: F, fibula; ph, prehallux; T, tibia; t, tibiale; I–VI, digits. Reproduced from Ref. 65.

Are there polydactylous frogs?

The presence of a PREHALLUX and PREPOLLEX in frogs could represent cases of polydactyly at a species level. Many frogs have a prepollux (Fig. 6) in their hand and a prehallux in their foot. These are small digit-like structures anterior to the 'first digit'. Based on the available evidence in the literature it seems best to treat the prepollux and prehallux in the same way as the other digits (Box 4).

The presence of a prepollux and prehallux is distributed mosaically over the more advanced frog taxa. There is large inter- and intraspecific variation in the number of phalanges of the prepollux and prehallux, varying from zero in many species to an exceptional five in the leptodactylid *Telmatobius hautholfi*⁵⁵. A striking example of intraspecific variation is the presence of a prepollux in males and its absence in females, seen in several leptodactylid frogs⁵⁶ (Fig. 5). This pattern is explained by the function of the prepollux: males use it during mating to clasp the females⁵⁷. The mosaic distribution of the prepollux and the high variability within genera and

Box 3. The developmental constraint that determines the order of limb reduction

The digit reduced first during evolution is usually the one formed last^{a-c}. We propose the following explanation for this phenomenon: modification of the last digit formed during development will be subject to the least interference by inductive interactions. Most of the limb has already been formed and, in amniotes, the extent of its interactions with the rest of the embryo has also seriously declined. In addition, the regression in size of the limb bud by apoptosis or necrosis will be easiest with the smallest digit being formed, which is usually the last one.

The mechanism proposed by Alberch and Gale^e for the evolutionary constraint of this process is not supported by evidence from processes in

evolutionary limb reduction. They hypothesize that a smaller size of the mesenchymal condensation leads evolutionarily to the disappearance of the last-formed digit. This is in contrast with the described evidence that evolutionary limb reduction proceeds by a process of construction followed by destruction (Fig. 2 for mammals; Fig. 5 for amphibians).

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species suggests that both increases (polydactyly) and decreases in the number of phalanges must have occurred many times.

The variation in presence and size of the prepollex and prehallux in frogs, and the high variation in phalanges and carpal and tarsal elements in amphibians in general, suggests that the constraint against changes in the number of more distal elements is weaker in amphibians than in amniotes. Moreover, the differences in hand and foot shapes in amphibians are small compared with those of amniotes (e.g. bat wings and bird feet). In addition, in amphibians, there are no examples of the extra digit-like structures that have evolved many times in amniotes. Thus, although the selective force on changes in limb bones as apparent from changes in limb morphology appears smaller in amphibians than in amniotes, the variability in the number of limb elements is greater. Amniotes have drastically modified hand and foot bones, have a low variability

in the number of hand and foot bones, have evolved extra digit-like structures and do not display polydactyly, whereas amphibians have no drastically modified hand and foot bones, have a high variability in the number of hand and foot bones, have no extra digit-like structures and probably show polydactyly. These data support the hypothesis that the decoupling of limb development from the interactivity of the phylotypic stage owing to later limb development has relaxed the negative selection pressure against changes of limb patterning. Such relaxation in selection pressure is all the more remarkable, because it is in striking contrast to the extreme conservation of the overall body plan of amphibians, in particular that of frogs.

Conclusion

There is new support in the literature for the hypotheses of Lande¹ and Wright², that polydactyly and other changes of limb patterning are constrained by negative pleiotropic effects. These effects not only explain the absence of polydactyly and other atavisms, but can also explain the extreme conservation of the number of bones more proximal to the hand: the one proximal bone (femur or humerus), followed by two more distal ones (radius and ulna or tibia and fibula). The number of wrist bones and anklebones is more variable, but most of the variability is the result of fusions and not of changes in the number of bone Anlagen. Changes have occurred during the evolution of tetrapod limbs, most strikingly in amniotes. Changes in size and shape of elements are apparently easy to realize evolutionarily, as are changes in attachment of muscles and ligaments. Genetic changes occur at a high frequency, as do plastic changes in response to changed functional demands. Changes during later stages of development that influence the form and function of structures are, therefore, considerably easier to realize in evolution than are changes during the phylotypic stage.

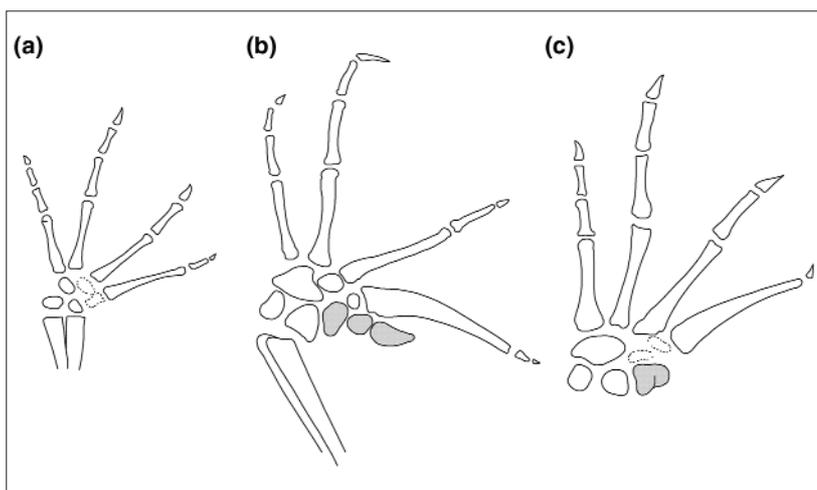


Fig. 6. Intraspecific variation in the prepollex of the leptodactylid frog *Limnodon tasmaniensis*. (a) Females do not have a prepollex. (b) and (c) Males usually have three elements (presumably two phalanges), but can have fewer elements, as shown in (c). The prepollex is indicated in grey. The broken lines are enhanced. Reproduced, with permission, from Ref. 57, by courtesy of The Natural History Museum, London.

Box 4. Do frogs have six toes?

The confusion about whether the prepollex and prehallux are digits seems to be caused mainly by the once-powerful paradigm of an archetypal tetrapod hand and foot with five digits. Frogs usually have four digits on their hand, in addition to the prepollex. Very often, the prepollex has been hypothesized to be digit I, with a total number of five digits on the hand. The problem arises when one considers the foot, because most frogs have five digits in addition to the prehallux. This has led to the conclusion that the prehallux can thus not be a toe, as this would lead to six toes^a. It is now well known that early tetrapods had more than five digits, and that there is no archetypal hand with five digits. Interestingly, embryologists at the end of the 19th century and beginning of the 20th century concluded, on the basis of developmental data, that the ancestral condition of the tetrapod hand was at least seven digits, including the usual five digits, prepollex/prehallux and the postminimus^{b-d}. Additional confusion comes from the model of Shubin and Alberch^e, which assumes that all other digits are formed from the so-called digital ray, whereas the prepollex and prehallux are formed from a different condensation. The presumed difference in development in this model could be a reason to decide against serial homology of the prepollex/prehallux with the digits of the digital ray. Shubin and Alberch do not explicitly decide on the nature of the prepollex and prehallux. In addition, there is evidence from several groups of tetrapods that cannot be explained by the model, especially the digits of the extinct amphibians with up to eight digits. Wagner *et al.*^f have concluded that, by contrast to amniotes, the model of Shubin and Alberch^e is not helpful in explaining digit development of urodeles. In mammals, a careful developmental series of the foot of the rodent *Cavia porcellus* and the monkey *Microcebus myoxinus* clearly shows that not only the prepollex emerges from a different condensation from the other digits, but so does digit one (Ref. g). In addition, explantation and extirpation experiments have suggested that already before the condensation phase of bone development there exists a rather fine-scaled mosaic prepattern with the possibility of the anterior part developing independently of the posterior part and vice

versa (e.g. Ref. h). This argues against the validity of the digital arch model. The published data on anurans are not conclusive for or against the model^{i,j}. Therefore, the support for the model of the digital arch is insufficient to contradict the digital nature of the prehallux and prepollex.

The fossil data on *Ichthyostega* and *Eryops* show that the prepollex and prehallux are very digit-like^{d,k} in these early amphibians. The similarities in shape, position and construction are striking, not only in the fossil remains of the earliest amphibians, but also in the early developmental stages. The early developmental stages of the hand of the anurans *Pelobates fuscus* and *Bombina pachypus* show that in the condensation phase the development of the prepollex and prehallux proceeds in the same way as in the other digits (Fig. 6). The remarkable similarity of the prepollex/prehallux and digits in both the fossil and developmental records leads us to consider the prehallux and prepollex as digits, equal to the other digits.

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It appears that the negative pleiotropic effects causing the evolutionary constraint in amniotes and amphibians primarily result from the many inductive interactions occurring during limb development. In amniotes, limb development is integrated into the inductive interactivity of embryonic development as a whole, and these interactions increase the number of negative pleiotropic effects associated with changes of limb patterning. There are many interactions occurring among modules, and therefore modularity is limited. In amphibians, the limb develops as a semi-independent module, and therefore pleiotropic effects of limb changes will mainly be limited to the limb itself. We conjecture, therefore, that the constraint in amniotes is stronger than that in amphibians. The higher variability in the number of phalanges, anklebones and wrist bones in amphibians supports this conjecture. The probable

cases of polydactyly in frogs and the absence of extra digit-like structures in amphibians provide further support for the hypothesis.

Medical and veterinary relevance

We suggest that these findings are relevant to human and veterinary medicine. The presence of extra toes is generally seen as a rather innocuous variation (as is the presence of cervical ribs, see below). However, the general presence of negative pleiotropic effects suggests the possibility of long-term effects. For humans, the diagnosis of extra digits should alert doctors to the possibility of medical problems in later life. In addition, prenatal checks for anomalies appear prudent. In dogs and other domestic animals, selecting for a normal number of toes might help eradicate anomalies occurring later in life.

Conservation of body plans

The strong evolutionary conservation of the number of digits and limb bones in amniotes provides an example of the conservation of a character determined during the phylotypic stage. Therefore, not only is the stage itself conserved, but also certain characters that are determined during that stage. A case has already been made for the seven cervical vertebrae in mammals^{16,58}. The conservation of the number of limb bones in amniotes can be added as an example. The crucial importance of global inductive interactions for the development of the entire organism during the phylotypic stage can be further deduced from the pattern of reduction of other structures of which the Anlage appears during this stage. As in limb reduction, the eye and PRONEPHROS are evolutionarily reduced via the laborious process of construction followed by destruction^{59,60}, suggesting that we have two eyes for the same reason that we have five digits: that is, the chain of inductive interactions cannot be changed easily.

Glossary

- Anlage:** primordium, the earliest state in the development of structure.
- Atavism:** evolutionary reversal to the ancestral state.
- Inductive interactivity:** the presence of mutual or network-like inductive signaling.
- Inductive signaling:** the process in which one or more cells induce a response in one or more other cells.
- Modularity:** absence of interactions between modules.
- Module:** unit of development characterized by a relative absence of inductive interactions toward the module from the outside and a much larger interactivity within the module.
- Pleiotropy:** the production of several effects by one gene.
- Polydactyly:** increase in digit number.
- Prehallux:** digit, or digit-like structure, anterior to the hallux (big toe).
- Prepollex:** digit, or digit-like structure, anterior to the pollex (thumb).
- Pronephros:** kidney-like structure functioning as a kidney in fish and amphibian larvae. In mammals and birds, it appears as a transitory structure during embryology before other kidney-like structures (mesonephros and metanephros) and is not thought to be active as a kidney.
- Supernumerary phalanges:** increased number of phalanges.

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Vive la différence: plant functional diversity matters to ecosystem processes

Sandra Díaz and Marcelo Cabido

The links between plant diversity and ecosystem functioning remain highly controversial. There is a growing consensus, however, that functional diversity, or the value and range of species traits, rather than species numbers *per se*, strongly determines ecosystem functioning. Despite its importance, and the fact that species diversity is often an inadequate surrogate, functional diversity has been studied in relatively few cases. Approaches based on species richness on the one hand, and on functional traits and types on the other, have been extremely productive in recent years, but attempts to connect their findings have been rare. Crossfertilization between these two approaches is a promising way of gaining mechanistic insight into the links between plant diversity and ecosystem processes and contributing to practical management for the conservation of diversity and ecosystem services.

Diversity is being lost at an unprecedented rate at the global level, as a consequence of land use, biotic exchanges and changes in atmospheric composition and climate, potentially threatening major

ecosystem processes and the ECOSYSTEM SERVICES (see Glossary) that humans derive from them¹. The issue of whether plant diversity influences ecosystem processes has received increasing attention in the past five years, as a consequence of the publication of several groundbreaking theoretical developments and experiments^{2–13}.

There is now general agreement that diversity (a synonym of biodiversity and biological diversity) includes both number and composition of the genotypes, species, functional types and landscape units in a given system. However, diversity is often equated to SPECIES RICHNESS, and other components of diversity have frequently been underestimated. In particular, FUNCTIONAL DIVERSITY¹⁴, which has received much less attention in the literature, is now emerging as an aspect of crucial importance in determining ecosystem processes.