

# Developmental Constraints Vs. Variational Properties: How Pattern Formation can Help to Understand Evolution and Development

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**ABSTRACT** This article suggests that apparent disagreements between the concept of developmental constraints and neo-Darwinian views on morphological evolution can disappear by using a different conceptualization of the interplay between development and selection. A theoretical framework based on current evolutionary and developmental biology and the concepts of variational properties, developmental patterns and developmental mechanisms is presented. In contrast with existing paradigms, the approach in this article is specifically developed to compare developmental mechanisms by the morphological variation they produce and the way in which their functioning can change due to genetic variation. A developmental mechanism is a gene network, which is able to produce patterns in space through the regulation of some cell behaviour (like signalling, mitosis, apoptosis, adhesion, etc.). The variational properties of a developmental mechanism are all the pattern transformations produced under different initial and environmental conditions or IS-mutations. IS-mutations are DNA changes that affect how two genes in a network interact, while T-mutations are mutations that affect the topology of the network itself. This article explains how this new framework allows predictions not only about how pattern formation affects variation, and thus phenotypic evolution, but also about how development evolves by replacement between pattern formation mechanisms. This article presents testable inferences about the evolution of the structure of development and the phenotype under different selective pressures. That is what kind of pattern formation mechanisms, in which relative temporal order, and which kind of phenotypic changes, are expected to be found in development. *J. Exp. Zool. (Mol. Dev. Evol.)* 306B:107–125, 2006.

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The principal way by which the evolutionary process is currently understood is based on the theoretical framework of the neo-Darwinian synthesis. This framework has been successfully used to integrate the nature of inheritance, population dynamics and many aspects of the ecology of adaptation into the theoretical framework of the Darwinian evolutionary theory. In spite of this considerable success, there have been critiques to some of the assumptions and inferences underlying the neo-Darwinian synthesis. One ongoing disagreement among evolutionary biologists is to do with the nature of morphologic variation and its importance as a factor in evolution (Gould and Lewontin, '79; Ho and Saunders, '79; Alberch, '80; Wake et al., '83; Wagner, '88; Wake, '91; Goodwin, '94; Newman and Müller, 2000; Gould, 2002). In close relationship with these disagreements is the unachieved integration of developmental biology into the

evolutionary theory (Alberch, '82; Raff, '96; Gilbert and Sarkar, 2000). In recent years, there has been an increase in the number of researchers trying to integrate developmental biology into the evolutionary theory. The reason for such a renewed interest comes from a better understanding of development, especially of its molecular basis. However, evolutionary developmental biology is, presently, a largely multidisciplinary field in which there is, yet, no theoretical framework integrating the recent advances in each sub-field.

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The basic ensemble of concepts used to approach evolution and development in most current research is borrowed either from the neo-Darwinian synthesis or from a set of theoretical works from the early eighties appearing as, more development based, alternatives to some neo-Darwinian assumptions (here loosely referred as the constraint school: Alberch, '80, '82; Oster and Alberch, '81; Stearns, '82; Wake et al., '83; Wagner, '88; Horder, '89; Wake, '91; Wagner and Misof, '93; Arthur, 2001, 2002; Richardson and Chipman, 2003). A long-lasting controversy between these two approaches is whether development constrains the direction of evolution or, on the contrary, selection is the only main player in determining the direction of evolution. Recently, some authors (Arthur, 2001; Richardson and Chipman, 2003) have suggested new concepts to bridge the gap between these two views. This article proposes a different way to integrate the research performed under these two different views. This article proposes that the understanding of the roles of development and selection in morphological evolution requires a theoretical framework able to describe two aspects of morphological evolution: (1) what are the different morphological variations possible under different hypotheses about the functioning of development? and (2) In what way can developmental functioning change due to genetic variation. The proposal of this article is, in other words, that a clear way to compare different developments in these two aspects allows transforming the "developmental constraints"/selection controversy into a less controversial discussion about types of development and their influence in evolution. This transformation can easily integrate the useful evolutionary insights achieved from the neo-Darwinian and constraint schools as well as those arising from current developmental biology.

The first part of this article discusses why the neo-Darwinian approach to morphological evolution cannot accurately describe, in its present state, these two aspects of morphological evolution. At the same time, some important assumptions in the neo-Darwinian approach to morphological evolution will be critically reviewed. Moreover, it will be discussed how the concept of developmental constraints logically requires some of those neo-Darwinian assumptions.

The second part of the article discusses why some of these assumptions are not tenable after reviewing the information accumulated about

developmental functioning since the time of the neo-Darwinian synthesis.

The third part of the article presents a new set of concepts to approach the interrelationship between development and evolution in morphological evolution. It will be discussed how these new concepts avoid the problems of the developmental constraint concept and of the neo-Darwinian assumptions it criticizes. It will also be discussed how these new concepts can be used to re-address important questions in evolution and development. Finally, some studies will be reviewed to exemplify how these concepts are used to understand the relationship between evolution and development.

### **NEO-DARWINIAN AND CONSTRAINT SCHOOL VIEWS ON MORPHOLOGICAL EVOLUTION**

Development has played a relatively small role in evolutionary discussions for a large part of the last century (with some exceptions, de Beer, '58). Indeed, among others, Fisher ('30) suggested that selection alone is sufficient to explain the evolutionary process. Others (Wright, '78) acknowledged that the mechanisms by which genetic variation produces morphological variation were unknown but may play a role in evolution. The perception that development plays no major role in evolution logically requires, as it will be explained, three assumptions about the functioning of development and variation: (1) Additive genetic variation is normally available for most traits (Barton and Turelli, '89; Falconer and Mackay, '96). (2) The relationship between the phenotype and the genotype is simple. (3) Morphological variation is gradual and normally distributed.

#### ***Genetic additive variance is abundant for most characters in populations***

If most characters exhibit additive genetic variance, selection can be the main factor determining the direction of evolution because it can act most of the time for most of the traits (Fisher, '30). This assumption is consistent with many experiments of artificial selection (Lerner, '54; Weber, '92; Cortese et al., 2002; Wijngaarden et al., 2002; Bronikowski et al., 2004) in which most populations rapidly respond to selection (although this response may saturate over generations). This shows that for the studied traits genetic variation seems to be abundant.

Additional support comes from many quantitative genetic studies which show that, in natural populations, there is plenty of heritable phenotypic variation available for selection (Wright, '78). For morphology, the bulk of those early studies deal with simple univariate traits that show continuous distributions. Notice, however, that the existence of genetic variation for most traits is not sufficient for selection to be the main force in evolution. Even if there is genetic variation for most traits, some genetic variation may occur more often than others. It is also possible, in principle, that different genetic mutations produce the same morphologic variation (as is often the case Alberch, '80; Horder, '89). In this situation, some morphological variants will appear more often even when all genetic variants are equally common. In all these cases, the direction of morphologic change in evolution will be affected both by selection and the relative frequencies of occurrence of genetic and morphological variants. In the ideal case where an optimal morphology is selected for during a long time, the optimal morphology would be achieved independent of the properties of the morphological variation produced by genetic variation. However, the properties of both the genetic and morphological variations would determine which intermediate morphologies will be found though evolutionary time before attaining the optima. As it will be carefully discussed, the properties of possible morphological variation largely depend on developmental dynamics.

### ***Relationship between phenotype and genotype***

The assumption that development is not critically important in the process of evolution implies that the relationship between phenotype and genotype is relatively simple. A simple relationship between genotype and phenotype means that genetically similar individuals (clones differing in some few DNA bases) will exhibit similar morphologies. Selection requires some correlation between phenotype and genotype to act and its speed and efficiency depends on how simple this relationship is. When the relationship between phenotype and genotype is complex, many (even "optimal") phenotypes may be unreachable (or unlikely to reach) through selection. This is because genotypes similar to the genotype of the "optimal" phenotype may produce phenotypes that are very different from the "optimal" phenotype. If fitness

is related to phenotypic similarity to the optima then these genotypes are maladaptive. As a consequence, there is not always an adaptive path leading to any adaptive morphology, and the ones existing can be very labyrinthine (Kauffman, '93). In general, the direction and speed of evolutionary change produced by selection have been extensively proved to depend on the local properties of the phenotype-genotype map (Kauffman, '83; Fontana and Schuster, '98; Salazar-Ciudad et al., 2001a; Salazar-Ciudad and Jernvall, 2004). At least in the case of morphology, the relationship between genotype and phenotype directly depends on development. Before the times of the synthesis, it was clear that simple genetic changes can produce consistent phenotypic changes (Morgan, '03). This does not imply a simple relationship between phenotype and genotype but it may have influenced a widespread perception that phenotypic characters have a more or less direct genetic "encoding" that may allow them to be selected directly. Notice that a simple relationship between phenotype and genotype is not a sufficient condition for selection to be the only main force determining the direction of evolution. A simple relationship between genetic and morphologic variation does not help selection if genetic variation is not present (assumption 1).

### ***Morphological variation is gradual and normal***

The studies which suggest that selection is the principal explanatory cause in morphological evolution assume that morphological variation is gradual. That is, between any two morphological variants there can be a genetic variation producing an intermediate morphology. The claim is not that all variations in a character or trait are possible at any given time (Fisher, '30; Charlesworth and Lande, '82; Charlesworth et al., '82). At any given time only small departures from the mean "wild-type" morphology would be possible, but it is required, for morphology to be fully plastic to selection that these can happen in any direction. In other words, there is no preferred way in which morphologies exhibit variation: variation is essentially gradual and isotropic (Fig. 1a). In addition, it has been suggested that even if mutations of non-gradual effect (macromutation) may be possible, their large phenotypic effects are likely to disrupt the whole organism functioning (Fisher, '30), and, thus are unlikely to contribute to evolution. Smaller changes (that is,

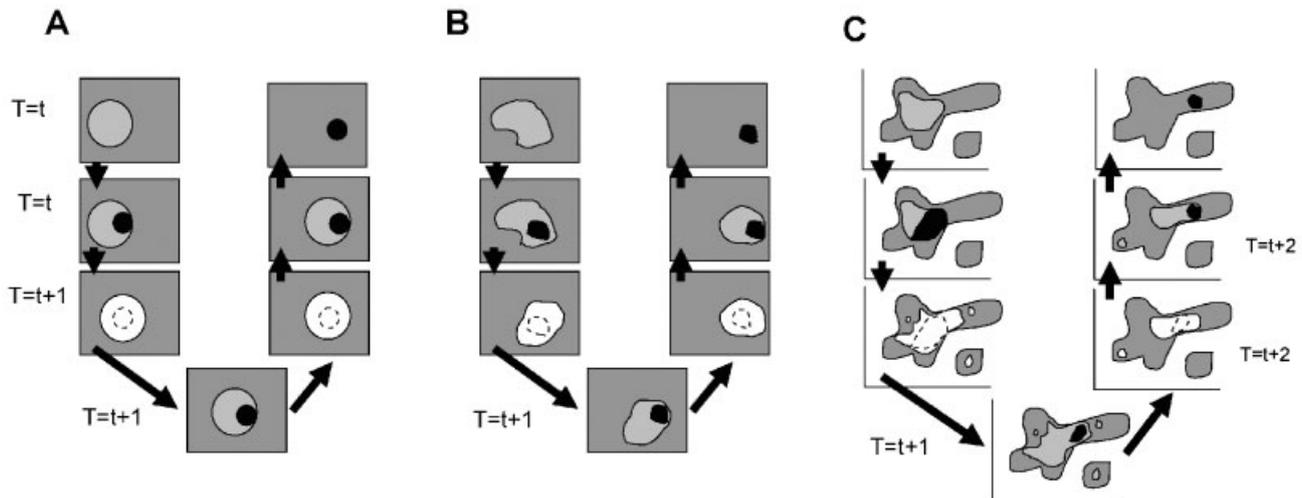


Fig. 1. The schema represents the different views about morphological evolution and variation possible in development. (A) View in which selection alone can explain the evolutionary changes in morphology (B) view in which developmental dynamics bias the possible variation (C) variational properties view. Each plot represents the distribution of an idealized two-dimensional phenotype in a population. They are arranged in a temporal order from generation  $t$  to generation  $t+2$ . The dark grey areas represent possible morphologies under existing developmental mechanisms. The light grey areas are the realized morphologies, the morphologies of individuals before selection acts. The black areas are the individuals that are fit enough to contribute to new generation. The white areas include the offspring produced from the previous generation (These individuals with the same morphology than the parents are enclosed by a dashed line.) In (C) not all variations are possible through mutational changes in the existing developmental mechanisms. Under some conditions, however, these variational properties can change (Fig. 4). Note that since in (C) it is not assumed that the relationship between genotype and phenotypes is simple, the white areas have a more complex shape.

they involve less DNA base changes) at the genetic level are by chance more probable than larger ones. If the relationship between phenotype and genotype is supposed to be simple, then the later statement implies that small gradual changes in morphology are more likely than larger ones. Thus phenotypic distributions in populations can be visualized as normal with variants being more or less frequent depending on how different they are from the mean phenotype. Measurement of morphological variation in populations shows examples of both gradual (Lerner, '54; Wright, '78; Weber, '92; Cortese et al., 2002; Wijngaarden et al., 2002; Bronikowski et al., 2004) and non-gradual variations (Bateson, 1894; Sawin and Edmonds, '49; Grüneberg, '50; Yablokov, '66; Alberch, '82, '83; Wolsan, '89; Jernvall, 2000; Szuma, 2002). At the level of genetic sequences, any change is, in principle, possible. Genetic variation is an ultimate cause of morphological variation. Taking into account that at the time of the synthesis it was not clear how genetic interactions are involved in the construction of organisms during development, that populations exhibit gradual variation in many of the traits measured at the time and that the main focus of attention of neo-Darwinism was on understanding

the kinetics of appearance and replacement between genetic variants, it may seem a useful working assumption that at least most small morphological changes can be produced by genetic mutation in populations.

The three assumptions just explained can be summarized by saying that the properties of morphological variation are assumed to be similar to those known for genetic variation. In addition, the relationship between genetic and morphologic variation is assumed to be simple. Thus any small change is possible. This view has influenced the perception that genetics is the fundamental level from which evolution at other levels can be understood (Dobzhansky, '37; Mayr, '82). Morphology has been regarded sometimes as a secondary problem that can be reduced to genetics. In other words, evolution has been reduced by some authors (Dobzhansky, '37) to the evolution of gene frequencies.

### *Developmental constraints*

The constraint school advocates for a totally different picture of variation, development and their influence on morphological evolution. (Alberch, '80, '82; Oster and Alberch, '81; Wake

et al., '83; Wagner, '88; Horder, '89; Wake, '91; Wagner and Misof, '93). According to these researchers, morphological and molecular variations have very different properties and a relationship that is far from simple. The phenotype is produced during development by the physical interactions of gene products with the microenvironment of the developing embryo, the genes in the genome and the expressed gene products (Alberch, '80; Oster and Alberch, '81). These interactions are arranged in complex spatiotemporal patterns that produce the phenotypic effects of a genetic change, which depends on the rest of the genes and on the epigenetic context of the embryo in complex ways. The variation produced in a morphological structure can be continuous but also limited to a number of discrete states. The probability that an individual with a specific morphology gives rise, by mutation, to an offspring with another specific morphology is not uniform; i.e. some morphological changes are more likely than others and some are not possible. The literature gives many examples of developmental systems where morphological variation exhibits these properties (Alberch, '80; Oster and Alberch, '81; Kauffman, '83; Wagner, '88; Wagner and Misof, '93; Kangas et al., 2004). In fact, even in the case of RNA molecules it has been suggested that the relationship between genotype (RNA sequence) and phenotype (RNA 3D conformation) is rather complex (Fontana and Shuster, '98a,b). These authors claimed that all morphologic changes in evolution imply changes in development and that development can produce, at any given time, only a limited number of morphological variants. This implies that development does strongly influence evolution (Alberch, '80, '82; Oster and Alberch, '81; Kauffman, '83; Wagner, '88; Antonovics and van Tienderen, '91; Wagner and Misof, '93; Resnik, '95; Schwenk, '95; Hall, '96; Schlichting and Pigliucci, '98; Arthur, 2001, 2002; Fusco, 2001; Richardson and Chipman, 2003). This is often stated as development constraining or limiting the evolutionary process.

A group of evolutionary biologists with considerably different backgrounds proposed a consensus definition of developmental constraints (Maynard Smith et al., '85): "A developmental constraint is a bias in the production of a variant phenotype or a limitation on phenotypic variation caused by the structure, characteristics, composition and dynamics of the developmental system". There is a considerable amount of literature concerning constraints, especially reviews focusing on the

different kinds of constraints (Schwenk, '95). Here, we focus on developmental constraints due to the dynamics of pattern formation during development. We will neither consider internal selection or related phenomena (Whyte, '65; Schwenk, '95) nor canalization (Scharloo, '91).

### *The developmental constraint/ selection debate*

The existence, exact nature and implications of developmental constraints are recurrent and controversial issues in evolutionary research (Gould, '80; Charlesworth and Lande, '82; Perrin and Travis, '92; van Tienderen and Antonovics, '94; Eberhard, 2001; Beldade et al., 2002; Wagner and Müller, 2002; Arthur, 2003). To test developmental constraints is a difficult task because developmental constraints can be understood as limits to what evolution can achieve. Development can be seen, according to the constraint school, as producing the genetic variation which can produce some morphological changes but not others. But these constraints may be breakable (Arthur, 2004) since changes that are not possible by development in a lineage may occur in closely related lineages. This does not imply that, in practice, any small change is possible, as assumed by the neo-Darwinian approach, but that the mechanisms by which morphological variation is produced during development, and the constraints it imposes, change in evolution. Development can be considered as a black box from which anything is possible (as in the neo-Darwinian approach to morphological evolution) or as a process that produces morphological variation at a given time (constraint school) and changes with evolution (changing what is possible). Without an explicit association between a constraint and a type of development, it would always be possible to claim that development exerts no strong influence in evolution because presumed developmental constraints can be broken. A type of development constrains evolution as far as it is used. Changes in development change existing constraints. This implies that to evaluate the existence of a developmental constraint with respect to evolutionary potential, it is required to have criteria to decide when development has changed and when two types of development are different. If development changes, it may be the case that the concept of developmental constraint is no more adequate to describe the influence of development on morpho-

logical evolution. Moreover, the developmental constraint concept may not be adequate to compare the types of development and their evolution. The aim of this article is to show that concepts, which are able to describe and test how development changes and which variation is possible by a type of development can efficiently, replace the concept of developmental constraint and its controversies.

The claim that development constrains morphological evolution was originally developed against the view that all small variations are possible and then selection is the main factor explaining morphological evolution (Fisher, '30; Charlesworth et al., '82). To claim that there is a constraint in a process means that it is known how that process would be without it, or it is known that the process would be different without the constraint. But is it really known how morphologic evolution would be without developmental constraints? Is it even possible to know? Traditionally, developmental constraints have been regarded as an alternative to selection to

explain the distribution of phenotypes in a population or phylogeny (Alberch, '82). But can selection and developmental constraints be alternative to each other? If the fact that morphological variation is generated by changes in developmental dynamics (produced by genetic or environmental variation) is fully taken into account, then the answer is no. Development is a process by which genetic variation can produce morphological variation (that is injected into populations) while selection is a process by which some morphological variation is filtered out (from populations). In fact, development-free evolution cannot exist. Development does not impose a limit or constraint on evolution but, on the contrary, allows it to happen through the production of morphological variation. Selection only affects the direction of evolution by carving in the phenotypic distributions produced by development (Fig. 2). Selection and development are doing different things and thus cannot be alternative explanations for a process: both jointly determine the direction of evolution.

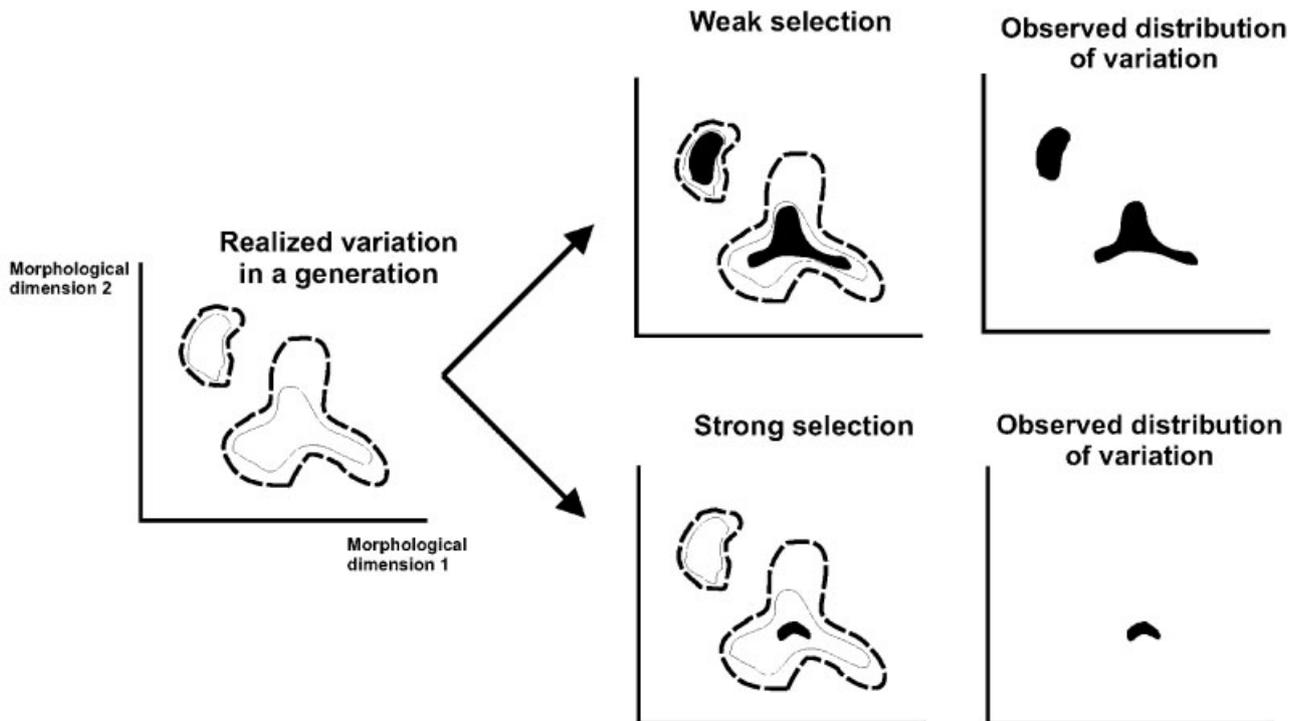


Fig. 2. The schema shows on the left-hand side a plot with the variational properties (discontinuous contour) and realized variation (continuous contour) of a developmental mechanism. To the right the same morphospace is plotted for different strengths of selection. The black shapes enclose the variation that is observed after selection. The figure shows our point that development always determines the direction of evolution though the variation produces. Selection affects the direction of evolution by carving on the distribution of realized variation.

When selection is suggested to be more important than developmental constraint (or when it is claimed that there is no developmental constraint in the evolution of some morphology), it is assumed, or, as we have seen, logically required, that unrestricted morphological variation exists as originally suggested in neo-Darwinism (Dobzhansky, '37; Fisher, '30). In other words, the selection option in the selection/development debate comes with an implicit assumption about which morphological variation is possible and how it is produced by development (Fig. 1). Thus the selection/development debate relies on two different hypotheses about how development works and how it generates morphological variation. One of them assumes that all small variations are eventually possible and that most traits have a more or less direct or explicit genetic encoding (development can be seen as producing that morphologic and genetic variation having similar properties). The alternative view is that development is a complex epigenetic process by which morphology, and its possible variation, is generated by interaction between genes and between genes and the epigenetic context of the developing embryo. Part of the controversy of developmental constraints may be due to the fact that these logical implications of the "selection" option are not always considered explicitly. Then the controversy between selection and developmental constraints can be rephrased into a controversy about how development works (and consequently which morphological variation is possible in a given time) and which are its implications for morphological evolution.

The inadequacy of the developmental constraint concept to grasp the creative nature of development has been criticized several times by people close to the constraints school (Gould, '89; Arthur, 2002, 2003; Richardson and Chipman, 2003). The alternatives proposed, bias and positive constraint, still fail to describe development. These alternatives describe the role of development as an effect (biases or tendencies) in the expected distributions of morphological variation. In other words, developmental biases and positive constraints make some variants more likely than others in respect of what would be expected without them. How these expected distributions are caused, is not explained. In any case the role of development is not to bias the morphological variation that would be produced otherwise. Development is simply the phenomenon responsible for the production of morphological varia-

tion. It determines what is possible and among it what is likely. The use of the word tendency or bias implies that in principle anything is possible and then development makes some of the possible variation more likely or unlikely. As before, the distinction between developmental biases or selection hides a distinction about how development is supposed to work.

The proposal in this article is to replace the concept of developmental constraint by a new set of concepts based on the description of what development can do. In addition, the new concepts are designed to provide a precise way to compare different kinds of development in relationship with their relative influence on morphological evolution. The aim is to compare and integrate the insights acquired by the neo-Darwinian approach to morphological evolution and the constraint school by a set of concepts that are not embedded in any of these two perspectives. In other words, it is acknowledged that selection and development are not alternatives but the alternatives are different kinds of development that produce different morphological variation, different relationships between genetic and morphologic variation and ultimately different modes of evolution. In fact, the new concepts are designed to compare all possible views about the production of morphological variation by development and its influence in evolution. These concepts are designed to transform the question of "whether development constrains evolution or not", into the question of "how different kinds of developmental functioning influence evolutionary dynamics (through the morphologic variation they can produce) and also which proportion of developmentally possible variation is filtered out by selection (how strong selection is)". The claim in this article is that this rephrasing does not imply any loss of generality in the questions addressed in evolution and development. On the contrary, these concepts avoid the problems outlined for developmental constraints and related concepts while allowing more operative concepts by which to take advantage of the diversity of ways in which development can affect evolution.

#### **RELATIONSHIP BETWEEN THE GENOTYPE AND PHENOTYPE IN CURRENT DEVELOPMENTAL BIOLOGY**

The advance of molecular techniques has produced a dramatic increase in the understanding of the molecular basis of development. First, it was

discovered that easily identifiable mutant phenotypes involve, often, transcriptional factors regulating the expression of many other genes. This leads to a number of very hierarchical views about development functioning (Davidson, '71; Garcia-Bellido, '75) that still influence developmental biology research. Among these genes, the Hox genes received special attention because their mutation produces dramatic anterior–posterior misplacement of whole morphological structures in *Drosophila* (Lewis, '78; McGinnis et al., '84a). Later, these genes were found to be widespread among metazoan, although the co-linearity and the dramatic mutant phenotypes are not always present (McGinnis et al., '84b; Hart et al., '85; Akam, '89; Schummer et al., '92; Aisemberg and Macagno, '94; Chow and Emmons, '94; Popodi et al., '96; Callaerts et al., 2002). The use of animal models other than mouse and *Drosophila* (Wedeen and Weisblat, '91; Davidson, '99; Sommer, 2001), and research in genes other than Hox, has led to the finding that, not only Hox genes but, also many other developmental genes are considerably conserved among animals (Manak and Scott, '94; Gilbert et al., '96; Raff, '96). In many cases, the interactions between genes are also conserved (Wilkins, 2001). Interestingly, the same genes and gene interactions are used over and over again in the development of unrelated morphological structures in the same embryo (Chuong et al., 2000; Huelsken and Birchmeier, 2001; Aoto et al., 2002).

A large proportion of research in developmental biology is based on disrupting gene products and reporting the morphologic effects produced (Boldogkoi, 2004; Fraser and Marcotte, 2004). Other studies identify gene interactions, and their precise spatio-temporal occurrence are necessary for proper development (Jacq et al., '97; Parker et al., '99; Meulemans and Bronner-Fraser, 2004). A large proportion of evolutionary inspired studies consist in a phylogenetic mapping of patterns of gene expression and genes known to have an important role in development (Panganiban et al., '94; Averof and Patel, '97; Wilkins, 2001; Ronshaugen et al., 2002). Then, inferences about the genetic basis of the morphological differences between species are made from what is known about these genes in model organisms (mostly *Mus musculus*, *Drosophila melanogaster* and *Caenorhabditis elegans*). While explanations based on single genes or few gene interactions accounting for the formation of whole structures might appear tempting, development may be more than changes in gene action over developmental time,

in the same way that the evolutionary process is more than changes in gene frequencies over evolutionary time.

A change of paradigm has taken place, gradually, as more developmentally important genes have been identified. Gene products act in networks. Each gene product performs a function only at the biochemical level (that can have complex consequences at the phenotypic level when altered). Gene products can specifically bind to other molecules, catalyse specific reactions, move (actively or passively) and undergo configuration changes that alter their functions or specificities. The significance of the function of each gene critically depends on the rest of the genes in the network and, specially, on how they are globally connected. Thus, the topology of the genetic networks, and not just their constituent genes, are critical for understanding how development works (Waddington, '57; Kolchanov et al., 2000; Wilkins, 2001; Hall, 2003; Rast, 2003; Boldogkoi, 2004; Fraser and Marcotte, 2004; Silver and Rebay, 2005). Thus the morphological changes produced by a new allele would change depending on the rest of the alleles existing in the other genes of the network. Then in every generation, in a sexual lineage, the same allele would produce different morphological variation depending on the rest of the alleles present. The dynamics of these networks have been repeatedly suggested to be complex enough to make theoretical or/and computational analysis (Meinhardt, '82; Goodwin and Trainor, '85; Collier et al., '96; Holloway and Harrison, '99; Gilbert and Sarkar, 2000; Salazar-Ciudad et al., 2000; Holloway et al., 2002; Hentschel et al., 2004) a valuable tool to test hypothesis about developmental mechanisms functioning.

However, this conceptual change can go beyond gene networks. Development is a process of creation or transformation of spatial information in the form of specific spatial distributions of cell types. Genetic networks act in a complex embryonic context. Often, the tissues or groups of cells sending molecular signals and the tissues receiving them are making at the same time changes in cell behaviours that change their spatial distributions. Then inductive signals have two kinds of information: chemical information which would activate specific transduction pathways, and spatial information which consists of the distribution of these signals in space. In many cases, the final spatial distributions would depend on the relative forms and distances of inducing and induced

tissues and in their changes over time due to the effect of signals on cell behaviours and mechanical interactions with surrounding cells. Thus, genes can affect each others' expression and activity not only by direct interaction but also through their influences in the intermediate phenotype. From this perspective, the key factor to understand development is not only how gene networks are organized but also how they affect cell behaviours and are affected by the environment and the changing intermediate phenotype (Alberch, '80; Salazar-Ciudad et al., 2003).

In many cases, it has been possible to describe genetic differences that are causally necessary for such morphologic differences (Panganiban et al., '94; Carrol et al., '95; Averof and Patel, '97; Ronshaugen et al., 2002). In some cases (Holloway et al., 2002; Salazar-Ciudad and Jernvall, 2002; Pribyl et al., 2003), even pattern formation as a process is understood to an extent to explain or predict morphological differences between species. In general, a point is reached where the basic cellular and molecular interactions involved in development are largely understood. Then understanding at the level of pattern formation can proceed by studying how gene networks are constructed and how they interact with the local epigenetic contexts. However, no general widely accepted picture about the relationship between genotype and phenotype has emerged. The highly interactive and complex spatio-temporal dynamics of development described here preclude a relationship between genotype and phenotype as assumed by the neo-Darwinian approach to morphological evolution (Alberch, '82; Kauffman, '83; Fontana and Schuster, '98a,b). In fact, which is the relationship between phenotype and genotype is an open question with wide implications for our understanding of morphological evolution. The new concepts introduced in the next section are designed to explore how different relationships between the genotype and the phenotype affect morphological evolution.

#### **USE OF THE VARIATIONAL AND GENETIC PROPERTIES OF DEVELOPMENTAL MECHANISMS IN EVOLUTIONARY INFERENCES**

##### ***Patterns, developmental mechanisms and variational properties***

One of the more important phenomena in development is pattern formation or transformation: the transformation of a spatial distribution of

cell types and extra-cellular components, the *previous pattern*, into another, a *pattern* or *resulting pattern*, over time. Morphology and its variation are largely produced during pattern formation and because of pattern formation by, as discussed, gene networks that affect some basic cell behaviours (like adhesion, proliferation, etc.). A developmental mechanism is, as the term used in this paper, any of these networks that can produce some pattern transformation. Thus from now on I will refer to developmental mechanisms and not to kinds or types of development. Developmental mechanisms can be composed of other developmental mechanisms as sub-networks. The development of an organism can be described as the successive transformations between patterns; each performed by one or several developmental mechanisms. The evolution of morphology can be described as the changes in these patterns over generations in a lineage while the evolution of development as the changes in the developmental mechanisms used in a lineage over time. The variational properties of a developmental mechanism are defined as the ensemble of patterns produced by it under all possible previous patterns, environmental conditions and genetic mutations that do not alter the topology of the network (from now on these mutations will be called *IS-mutations*). The genetic properties of a developmental mechanism include the genetic encoding of the gene network topology and interaction strengths between genes. These can be encoded in the DNA sequence and topology of promoter regions or in the amino acid sequence and 3D structure of the gene products involved in protein—protein, DNA—protein or RNA—protein interactions (or other). Two developmental mechanisms are considered to be different if they have different topology or if they affect different cell behaviours (this implies to affect different genes and consequently different topology). This criterion is useful because in most cases only the topology of developmental mechanisms is known. In addition, mutations changing the topology of a developmental mechanism, here called *T-mutations* (from topology mutations), are less likely to occur by chance at the genetic level and more likely produce large changes in pattern transformations (Salazar-Ciudad et al., 2000). *T-mutations* often involve changes in more bases (for example changes in the transcriptional factors that bind to a promoter due to recombination events involving the promoter region) than mutations in the interaction strengths, here called

*IS-mutations* (for example, point mutation in a promoter region affecting the binding affinity constant of a receptor). However, *T-mutations* can also be produced by single base changes and *IS-mutations* by large recombination.

### ***Inferences on morphological evolution based on development***

Natural populations can exhibit genetic, morphological and developmental variations. The *IS-mutations* and environmental conditions occurring in a generation determine the sub-set of the variational properties realized in a population using the same developmental mechanism. *T-mutations* determine variation in the developmental mechanisms themselves.

Most of the times, it can be expected that *T-mutations* are comparatively rare because they may involve larger, and then by chance less frequent, changes in DNA. Then, as suggested by the constraints school, significant insights about the evolution of morphology can be obtained by studying specific developmental mechanisms and how they produce morphologic variation. A large number of studies specifically consider pattern formation in evolution (Alberch, '80, '82; Oster and Alberch, '81; Stearns, '82; Wake et al., '83; Oster et al., '88; Wagner, '88; Horder, '89; Newman and Comper, '90; Wagner and Misof, '93; Goodwin, '94; Sanders, '94; Newman and Müller, 2000; Arthur, 2001, 2002; Richardson and Chipman, 2003). These identify aspects of the variational properties produced by a partially known developmental mechanism. The variational properties of a developmental mechanism can be inferred experimentally by studying the pattern variation produced by genetic and environmental variation in the genes and regulatory DNA sequences involved in that mechanism. Theoretical models offer the opportunity to infer variational properties by implementing hypotheses about developmental mechanism (Goodwin, '94; Holloway and Harrison, '99; Salazar-Ciudad et al., 2000; Salazar-Ciudad and Jernvall, 2002; Pribly et al., 2003). If a model correctly reproduces known pattern variation, then "genetic" *IS-mutations* and environmental changes induced in it can be used to infer all possible variation.

Quantitative genetic studies of morphology offer an easier way to approach which morphological variation is possible in an organism (Birdsall et al., 2000; Polly, 2005), especially when selection is not acting (Klingenberg and Zaklan, 2000; Klingen-

berg and Leamy, 2001). Some of these studies apply sophisticated techniques to deal with complex and multidimensional patterns of morphological variation (Bookstein, '82; Klingenberg, 2002). They do not describe the variational properties of a developmental mechanism but the joint variational properties of all the developmental mechanisms involved in the formation of a morphological structure (normally described as a set of arbitrarily defined traits). The estimations, however, depend on the frequency distribution of the alleles segregating and not just a reflection of development (Wagner and Altenberg, '96). The methodology of quantitative genetics cannot distinguish between *T* and *IS-mutations* since it does not consider how development can work or change. The matrix of additive genetic covariance can, in fact, easily change without changes in the developmental mechanisms themselves, *IS-mutations* (Steppan et al., 2002; Whitlock et al., 2002), and then inferences based on them may not be possible for large time intervals in evolution. Quantitative genetics approaches, however, provide valuable information when not too much about development is known. But, even if some quantitative genetic studies consider genes involved in development (Gibson, '99; Birdsall et al., 2000; Orr, 2003), they do not consider the dynamics of pattern formation nor its evolution. Instead of focusing on the variation produced in a set of arbitrarily chosen traits, the variational properties describe the morphological variation possible from the use of an arbitrarily chosen developmental mechanism.

The studies based on developmental dynamics or on the statistical properties of the genotype-phenotype map are useful as far as developmental mechanisms do not change. Considerations about the evolution of development become important when studying morphological evolution on long temporal or phylogenetic spans.

### ***The evolution of developmental mechanisms***

From the proposed definition of developmental mechanism, the structure of development can be described as which developmental mechanisms are used in the development of an organism and in which temporal order. A new developmental mechanism can become involved in a new or already existing pattern transformation in a part of the organism because of one or several *T-mutations*. These mutations can be in the

developmental mechanism producing that pattern transformation or in an already existing developmental mechanism that then becomes recruited in that part of the organism. Recruitment happens because one or several T-mutations connect a gene “A” expressed in the previous pattern to a gene “B” in a development mechanism. Then gene “B” leads to the activation of the rest of the genes of the mechanism in which gene “B” is wired. This can happen, for example, because gene “A” is a transcriptional factor and a point mutation occurs in the promoter region of gene “B” that allows gene “A” to bind to it. Recruitment is more likely in these mechanisms in which many of the genes can produce the activation of the whole gene network. Recruitment of already existing mechanisms allows a fast exploration of the morphospace because pattern transformations that have been proven to work in other parts of the embryo can be produced in new parts of the embryo by few genetic changes (eventually only one *T-mutation*). This may explain why not only the genes but also the whole gene networks are reused in different parts of the embryo. In this case, developmental mechanisms can be considered as modules and development as exhibiting some degree of modularity. The so-called gene process modules (Wagner and Mezey, 2004) are similar to developmental mechanisms in this sense except that they are not precisely framed into a pattern transformation paradigm.

To understand developmental mechanisms is crucial to understand which morphological variation is possible and thus how it changes in evolution. Developmental mechanisms are selected by the specific pattern transformations (phenotypic variation) they produce every time they are used. In other words, selection does not necessarily see which developmental mechanisms are used but the specific patterns they produce in each generation. This has two important consequences. First, the developmental mechanisms used in development can substantially change their genetic properties as far as the exact pattern they produce does not change (Weiss and Fullerton, 2000; True and Haag, 2001). Thus, *T-mutations* that do not change the pattern produced, but change the non-realized part of the variational properties of a developmental mechanism, can be neutral. This allows a considerable explorative capacity that can be expressed in some special conditions (for example when selective pressures relax). This implies that developmental mechanisms do not always have

the simplest, more parsimonious topology imaginable but a more complicated one that also depends on history. This may blur modularity in development by overconnecting genes between developmental mechanisms and suggests that modularity may be more easily identifiable when conservative selection has not acted for a long time on a pattern.

***Using genetic and variational properties to infer the relative frequency of different kinds of developmental mechanisms in evolution and development***

Currently, in only few cases the variational properties of developmental mechanisms are known to the extent to allow evolutionary inferences. However, powerful predictions about morphological and developmental evolution are still possible if, instead of focusing on specific developmental mechanisms, the focus is made on classes of developmental mechanisms. For this purpose it is required that the developmental mechanisms in a class share some aspects of their genetic and variational properties and a similar relationship between these properties. The genetic properties of the developmental mechanisms in a class offer the opportunity to estimate the likelihood by which T-mutations transform developmental mechanisms of one class into developmental mechanisms of another class. Comparing the variational properties of different classes, it is possible to estimate how lineages using different classes of developmental mechanisms may differently adapt to different selective pressures. Moreover, the relationship between genotype and phenotype can be used, as it is exemplified in the next section, to compare how fast adaptation can be. These comparisons allow inferring the relative involvement of each class of developmental mechanisms in different evolutionary and developmental contexts.

In a recent study, a mathematical model of pattern formation by signalling between cells has been constructed (Salazar-Ciudad et al., 2000). The model includes basic molecular interactions between genes through the binding of gene products to other gene products and to promoter regions. Some gene products diffuse in the extracellular space or are attached to the membrane and affect receptors in neighbouring cells. A large number of gene networks were constructed at random. The networks that were able to produce *pattern* were selected and studied. By this proce-

ture, the most possible developmental mechanisms were identified and their variational and genetic properties studied. Some of these developmental mechanisms had been previously proposed by both experimental and theoretical studies (see Salazar-Ciudad et al., 2000 and references therein) but none had variational properties and a relationship between genotype and phenotype as the one assumed by the neo-Darwinian approach to morphological evolution. Some topological properties of these networks allow classifying them in classes. Developmental mechanisms within a class share similar genetic and variational properties and a similar relationship between genotype and phenotype. The genetic and variational properties of these developmental mechanisms together with selection simulations suggest that some classes are more likely to be involved in the generation of different types of patterns under different selective pressures (Salazar-Ciudad et al., 2001a). For example, developmental mechanisms analogous to Turing-like reaction–diffusion mechanisms (Meinhardt, '82) produce more disparate and less gradual morphological variation. In spite of their simple genetic properties (they include less genes and genetic interactions), they can easily produce complex morphological patterns. This suggests that these mechanisms may be more often involved in the appearance of complex patterns the first time they appear in the evolution of a lineage. This approach can only be applied to these few systems in which pattern formation takes place without cells movement; like in *Drosophila* segmentation (Salazar-Ciudad et al., 2001b).

A similar analysis by a classification of the developmental mechanisms can be possible when cells not only signal to each other but also divide, move, die or change their adhesion. The developmental mechanisms in which cells both signal and use other cell behaviours (like, adhesion, proliferation, etc.) can be classified into morphostatic mechanisms when signalling takes place before changes in cell position and into morphodynamic mechanisms when signalling and cell position changes occur simultaneously (Salazar-Ciudad and Jernvall, 2002; Salazar-Ciudad et al., 2003). These morphostatic mechanisms should not be confused with those explained by Wagner and Misof ('93) that refer to a different definition of mechanisms. These two kinds of developmental mechanisms have been shown to exhibit very different variational properties even if they share similar genetic properties (Salazar-Ciudad et al., 2003). In brief, morphodynamic mechanisms

produce less-gradual, more complex and more diverse (or disparate) morphological variation. The relationship between genotype and phenotype is much more complex for morphodynamic mechanisms. Some other studies show how a morphodynamic mechanism can account for tooth development (Salazar-Ciudad and Jernvall, '02) and how morphodynamic and morphostatic mechanisms may be involved in the development of several animal organs (Salazar-Ciudad et al., '03).

The study of the variational and genetic properties of morphodynamic and morphostatic mechanisms allows exemplifying the types of inferences that the concepts presented in this article allow. The distinction between morphodynamic and morphostatic mechanisms is based on the relative timing of signalling in relationship to other cell behaviours and not in any intrinsic difference in the complexity of genetic properties. In other words, morphostatic and morphodynamic mechanisms with the same genes and gene interactions may have very different variational properties. Morphodynamic mechanisms can be expected to be more often involved in the production of a new *pattern* transformation the first time it appears in the evolution of a lineage. Of course, this does not prevent specific *patterns* to be more easily produced by specific mechanisms of any of the two kinds. But, for roughly the same genetic properties, morphodynamic mechanisms produce more diverse variation. Then there is a large proportion of possible new *patterns* that can be generated by them (Salazar-Ciudad and Jernvall, 2004). This relative genetic simplicity also allows morphodynamic mechanisms to be more easily recruited by mutation. If the newly arisen *patterns* are under conservative selection or under selection that favours only small changes from an ancestral pattern, then mechanism replacement is expected to take place because morphostatic mechanisms exhibit more appropriate variational properties. Morphostatic mechanisms produce more gradual variation that exhibits a simpler relationship with genetic variation. This allows a faster and finer tuned adaptation to these selective pressures. In other words, to produce patterns differing only slightly and gradually from an optimal ancestral pattern is more likely by morphostatic mechanisms. This replacement may take a long time because relatively few morphostatic mechanisms can produce the *patterns* produced by morphodynamic mechanisms. In other words, most morphostatic mechanism would require much tinkering in its genetic

structure for replacement to occur. This is specially the case for complex morphologies (Salazar-Ciudad et al., 2003).

If selection is weak, or if many *patterns* are either neutral or adaptive, then the higher explorative capacity of morphodynamic mechanisms would produce that lineages using morphodynamic mechanisms would find adaptive *patterns* more often. Then rapid morphological radiations can occur when lineages using these mechanisms encounter environments in which selection is relaxed (Solé et al., '99).

Lineages using morphodynamic mechanisms or morphostatic mechanisms in the development of a morphological structure are also expected to show different temporal patterns of evolution in such structure. Under equally strict selective pressures, lineages using morphodynamic mechanisms can be expected to exhibit large periods of stasis and short intense periods of change. This is because the morphological changes produced by an IS-mutation are, on average, larger for a morphodynamic mechanism than for a morphostatic mechanism. Thus the morphologic changes produced by a morphodynamic mechanism are more likely maladaptive. In lineages using morphostatic mechanisms, evolutionary rates would be relatively similar all the time (see Salazar-Ciudad and Jernvall, 2004), although real innovation may be rare and recurrent evolution common. This is because most genetic changes produce only small morphological changes that are more likely not maladaptive but less likely innovative.

### ***The interplay of developmental mechanisms and selection in the evolution of morphology and development***

This section presents an ideal description of the evolution of morphology from the concepts just presented. The developmental mechanisms used in the individuals of a population determine which morphological variation is possible in them. Assuming that all individuals in a population use the same developmental mechanisms, morphological evolution can be described as change over time in the morphologies found in a population due to selection of the morphological variation produced by IS-mutations. From time to time, T-mutations can appear. The proportion of individuals using the newly appeared developmental mechanisms would increase if the morphology they produce has some adaptive advantage with respect to the morphologies of the other indivi-

duals. Developmental mechanisms are likely to change into developmental mechanisms that are genetically similar (this is more likely because less mutational changes are then required for the transformation) and are able to produce morphologies more fitted to existing selective pressures. By knowing existing selective pressures and the genetic properties of developmental mechanisms, it may be possible to estimate how the developmental mechanisms used in a lineage change over time. The fixation over time of a new developmental mechanism affects subsequent morphological evolution by affecting which new variation will be possible through IS-mutations (and then to which selective pressures the population would be able to adapt) and which changes in development are more likely by T-mutations. This way the developmental mechanisms, the morphologies and the kind of selective pressures to which a population can adapt change over time (see Figs. 3 and 4).

The new concepts offer a different way to explain the morphological differences between two lineages sharing a common ancestor and exhibiting different morphologies. From a classical perspective four, not totally exclusive, hypotheses can be proposed to explain these morphological differences: (1) The two lineages have had a different history of selective pressures. (2) There are developmental constraints acting in one lineage but not in the other. (3) The different environments in which the two lineages live affect development in a way that the morphologies produced are different in each lineage. (4) The differences in morphology are neutral and have occurred by genetic drift. There are more or less standard procedures to distinguish between these hypotheses except for hypothesis 2. As we have seen, developmental constraints are difficult to test. Indeed, from the first part of this article it follows that developmental constraints are defined in such a way that they are difficult to test or cannot be tested at all. Instead, the concepts suggested here can be used to address the same question. From our perspective the hypothesis that can explain the morphological differences between the two lineages are: (1) Both lineages use the same developmental mechanisms. They have had a different history of selective pressures that has produced that a different part of the variational properties of the developmental mechanisms is realized in each population. (2) The developmental mechanisms used in the two lineages are different due to one or more

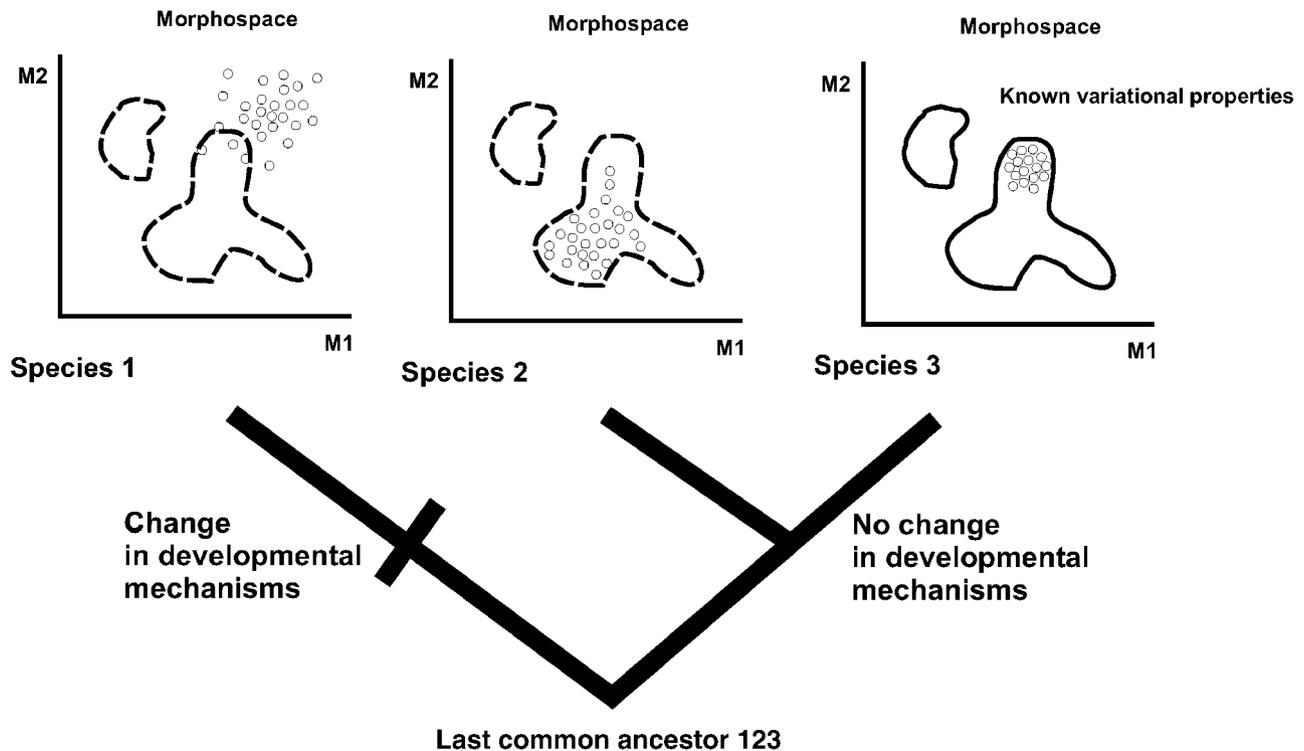


Fig. 3. The schema shows a phylogeny in which there has been changes in the developmental mechanisms used to produce a pattern. The variational properties are known from species 1. The small circles indicate the position in the morphospace of patterns found in individuals of species 1–3. The variational properties inferred from studies in species 3 are plotted in the morphospace for species 1 and 2 as a discontinuous contour. In species 2, the observed variation is included in the variational properties inferred from species 3. This suggests that the developmental mechanism has not changed from species 2 and 3. What have changed between species 2 and 3 is either environmental conditions (that affect the realized patterns) or genetic variations in the interaction strength between genes (*IS*-mutations). This last case can indicate either selection or genetic drift. There has been a change in development from the common ancestor of species 1 and 2 and 3 because the realized variation in species 1 is not included in the variational properties of the developmental mechanism known to be used in species 3. This is due to genetic variation that can be adaptive or not.

T-mutations since the common ancestor. These became fixed or not due to selection or genetic drift (as described in the section about the evolution of developmental mechanisms). (3) The different environments in which the two lineages live affect development in a way that the morphologies produced are different in each lineage. (4) Both lineages use the same developmental mechanisms. The differences in morphology are neutral and thus the part of the variational properties realized in each lineage is different due to genetic drift (affecting only *IS*-mutations). (4) The hypotheses 1, 3 and 4 are similar to hypotheses 1, 3 and 4 in the previous perspective. hypotheses 2 are quite different. In the new perspective, it is clearly described how morphological differences can be due to changes in devel-

opment itself (T-mutations). The concept of developmental mechanisms and variational properties offers the opportunity for a precise particulate description of development that allows testing hypothesis 2. This can be done by comparing the variational properties (or and estimation of them) of the developmental mechanisms used in the two lineages (Fig. 3). If the morphological variation observed in a lineage is different from the variational properties observed in the other, or in the common ancestor, hypothesis 2 is, at least partially, supported.

This perspective allows reinterpreting controversial (Beldade et al., 2002; Arthur, 2003) studies that claim to be testing developmental constraints. In a recent study (Beldade et al., 2002), artificial selection is performed in a species of butterfly to

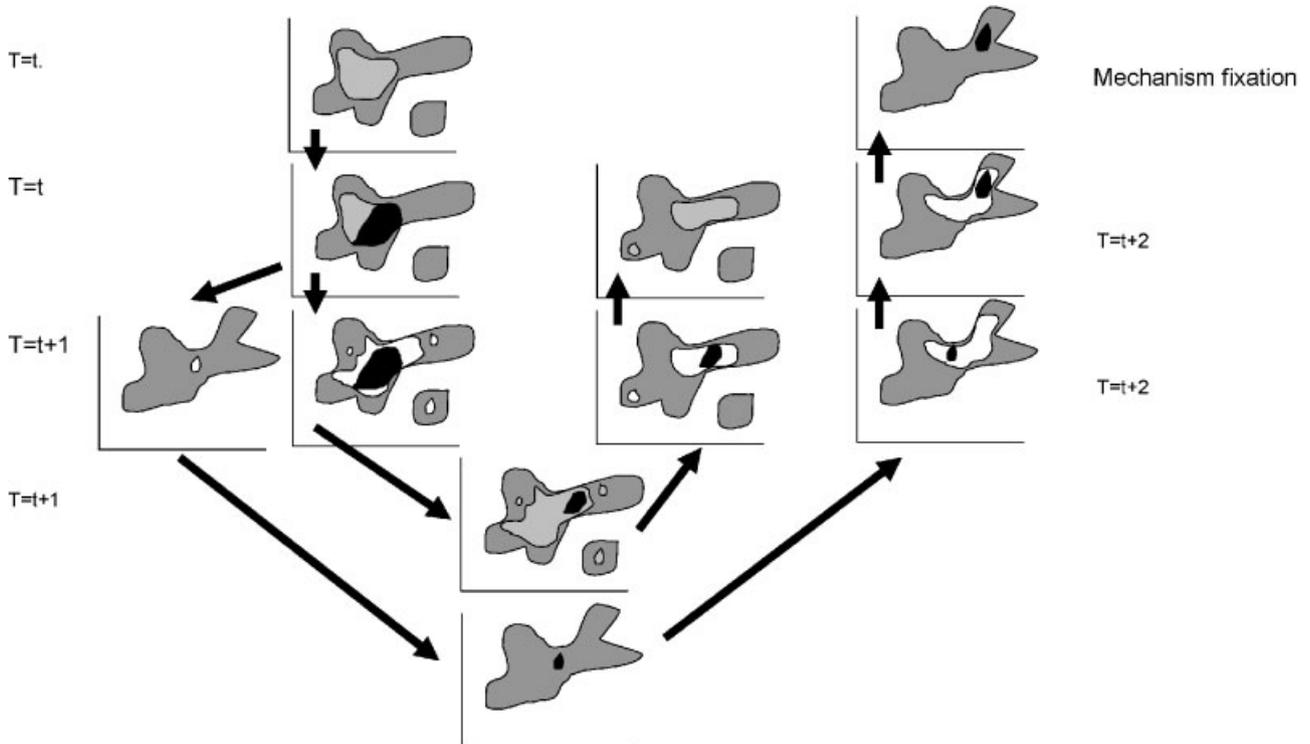


Fig. 4. The figure shows the same situation as in Fig. 1c except that in this case at generation  $t+1$  a T-mutation occurs. Then there is some variation for the developmental mechanisms used in the population. Over time, the morphology that can be produced by the new developmental mechanisms is more well adapted to existing environmental pressures and then it gets fixed in the population. This way the area of the morphospace that can be explored through IS-mutations can change but in contrast with the situation in Fig. 1a; not all variations are eventually possible, only that by developmental mechanisms are possible.

change the diameter of a specific colour spot in the forewing and hindwing. The authors report uncited developmental studies that suggest that the sizes of these two colour spots cannot be changed independently. However, the artificial selection experiments show that this is indeed not the case: butterflies with colour spot diameters, very different in the forewing and hindwing, are found after artificial selection. According to the authors, this result shows that there is no developmental constraint in the diameter of wing colour spots and, thus, the evolution of this morphological trait is governed by selection and the population genetics of this species of butterfly. These results however offer a different interpretation. First, the study shows that the developmental studies suggesting that colour spot diameters cannot change independently are wrong. These studies had a hypothesis about the variational properties of the developmental mechanisms used in wing coating that has been tested by the study of Beldade et al. The study also shows that selection is acting on the diameter of the colour

spots since they observe morphological variation that, apparently, is not found in nature for the species studied.

### ***Contrasting developmental mechanisms and developmental constraints***

This paper tries to suggest how a different conceptualization of the role and nature of development, away from constraints, can help to improve the understanding of morphological evolution. The developmental constraint concept has been very useful to highlight the importance of development in morphological evolution but offers a poor description of the diversity of types of development that can affect morphological evolution and how constraints can be modified. On the other hand, the constraints school and the neo-Darwinian approaches to morphological evolution do not offer a description of how development can change. To make predictions or explanations about how development can change in evolution, it is required to be able to describe the structure of

development and its change. The concepts of developmental mechanisms and variational properties may be useful because they provide an explicit description of what development can do, generate morphological variation from genetic and environmental conditions, and a criterion to cut development into approachable parts that can produce morphological variation. Moreover, a criterion is provided to identify two developmental mechanisms as different. Development is not described as constraining evolution but as a set of possible developmental mechanisms that produce different morphological variations from genetic and environmental variation and consequently differentially affect evolution. The approach in this article is free of any previous assumptions about how morphological variation should be or about how it relates to genetic variation. On the contrary, the aim is to be able to compare different kinds of developmental mechanisms. As a consequence, the presented framework can be used to ask which developmental mechanisms are used in a lineage for the formation of a specific morphological structure or which patterns of morphological variation are found in a lineage using a specific set of developmental mechanisms. These predictions about the relationship between development and evolution can be tested by looking if the developmental mechanisms or patterns of morphological variation found in a lineage are the ones expected. These tests, about how development works and how morphological variation is, can be regarded as more precisely defined and less prone to controversy alternatives to the testing of limitations or constraints in morphological evolution.

The perspective presented here allows for predictions about the change in the structure of development over evolution and its influence on morphological evolution. The kinds of predictions presented here on the evolution of developmental mechanisms and on the interplay between development and selection are not possible from the views and questions about variation described from the neo-Darwinian paradigm and the constraint school. The framework presented here describes the evolutionary process at any given time as the interplay between what is possible from development and what is allowed (from it) by selection. For large time scales, development changes according to a trade-off between which developmental mechanisms are more likely to be produced by mutation from existing ones and which of these are more likely to produce adaptive

morphological variation. This way development and selection affect each other historically and recursively over time.

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