How different types of pattern formation mechanisms affect the evolution of form and development

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SUMMARY Here we investigate how development and evolution can affect each other by exploring what kind of phenotypic variation is produced by different types of developmental mechanisms. A limited number of developmental mechanisms are capable of pattern formation in development. Two main types have been identified. In morphodynamic mechanisms, induction events and morphogenetic processes, such as simple growth, act at the same time. In morphostatic mechanisms, induction events happen before morphogenetic mechanisms, and thus growth cannot influence the induction of a pattern. We present a study of the variational properties of these developmental mechanisms that can help to understand how and why a developmental mechanism may become involved in the evolution and development of a particular morphological structure. Using existing models of pattern formation in teeth, an extensive simulation analysis of the phenotypic variation produced by different types of developmental mechanisms is performed. The studied properties include the amount and diversity of the phenotypic variation produced, the complexity of the phenotypic variation produced, and the relationship between phenotype and genotype. These variational properties are so different between different types of mechanisms that the relative involvement of these types of mechanisms in evolutionary innovation and in different stages of development can be estimated. In addition, type of mechanism affects the tempo and mode of morphological evolution. These results suggest that the basic principles by which development is organized can influence the likelihood of morphological evolution.

INTRODUCTION

Evolutionary theory states that individuals in populations exhibit heritable phenotypic variations that affect their chance of being perpetuated in the next generation. It has long been known that the ultimate cause of much phenotypic variation lies in molecular variation. But whereas molecular biology can identify genetic contributions to phenotypic variation, most phenotypic characteristics in metazoa are generated during development, not by individual genes acting alone but by networks of interacting gene products. Thus, current evolutionary theory can predict which variants are selected over time but not which phenotypic variation appears due to molecular variation. New theoretical approaches in evolutionary biology must take into account the dynamics of development. This would explain not only how ecological forces drive phenotype frequency changes, but also which variant phenotypes can appear during evolution.

Studies on phenotypic variation for which the developmental origin is known have been done for both naturally occurring (Wright 1912; Alberch 1980; Shubin et al. 1995; Jernvall 2000) and artificially occurring variation (Alberch and Gale 1983; Nijhout 1991; Streicher and Müller 1992; True et al. 1999). Such work has been complemented by the use of mathematical models that have attempted to integrate empirical data on the mechanisms that act in specific developmental processes (Oster et al. 1988; Ho 1990; Goodwin 1994; Salazar-Ciudad and Jernvall 2002).

These studies do not address the question of how developmental mechanisms themselves can be generated or change in evolution. The relevance of such studies is thus confined to the interval of evolutionary time in which the developmental mechanisms considered remain unmodified. Few previous studies directly address how developmental mechanisms themselves evolve. The developmental mechanisms used to generate very similar structures can differ even between closely related species (Wagner and Misof 1993; Müller and Wagner 1991; Felix and Sternberg 1997; Newman and Müller 2000). Moreover, within the same organism, the same spatial patterns can be generated by various independent mechanisms acting at roughly the same time (Tautz 1992; Felix 1999; Wang and Sternberg 1999). In general, a theoretical framework able to approach how developmental mechanisms appear or replace each other in evolution is missing. Consequently, how development structure and

variational properties change over time cannot be approached in a satisfactory manner.

Here we present a theoretical study of the phenotypic variation that different types of developmental mechanisms can produce and the relationship between phenotypic and molecular variation. The study is based on mathematical models that have been shown to untangle the dynamics of development of well-known morphological structures (Salazar-Ciudad and Jernvall 2002). Our results allow us to produce inferences about the relative use of these mechanisms in different developmental and evolutionary contexts. These inferences relate to the structure of development. In other words, they relate to which mechanisms are used in different stages of development. We also make inferences regarding which type of developmental mechanisms might be more often involved in the production of morphological innovations. Moreover, we make predictions about how the use of a particular developmental mechanism affects the morphological evolution of any lineage. We also try to exemplify how knowledge about developmental dynamics can be used to make predictions about the evolution of development and morphology.

MATERIALS AND METHODS

Developmental mechanisms

Development is a process by which an apparently homogeneous egg becomes an adult organism characterized by complex spatial distributions of cells and cell states (each of such distributions in a specified spatiotemporal window in development will be called a *pattern* in this article). Such complexity is attained through precise spatiotemporal coordination of cell behaviors, such as cell communication, cell division, cell adhesion (with the consequent changes in cell shape or cell migration), cell differentiation, and apoptosis. Development can be arbitrarily decomposed into a set of consecutive stages in which patterns are transformed into other patterns. Developmental mechanisms are described here as networks of interacting genes in which some of the genes directly affect these basic cell behaviors. Thus, developmental mechanisms transform one pattern into others. The development process can be seen as the recruitment of the right developmental mechanisms in the right places and times. Note that, by this definition, one developmental mechanism can be composed of other developmental mechanisms. Many mutations with important effects on pattern formation have been identified. But, in most cases, causal mechanistic information about how the global functioning of networks produce spatial patterns is very incomplete.

Types of developmental mechanisms

It has been proposed that all possible developmental mechanisms capable of producing patterns can be classified into very few types (Salazar-Ciudad et al. 2000, 2003). Mechanisms of the same type exhibit similarities in genetic structure and functioning and in the kind of patterns they produce. Inductive developmental mechanisms use only cell behaviors related to communication (and consequently change pattern by changing cell states), whereas morphogenetic mechanisms use only cell behaviors other than those implicated in communication. Morphogenetic mechanisms thus change pattern by changing form but not cell differentiation states. Often, these mechanisms act together. There are two ways to combine inductive and morphogenetic mechanisms into composite mechanisms (Fig. 1). In morphostatic mechanisms, inductive mechanisms act first to establish a set of territories (here referred as a set of contiguous cells with the same or similar patterns of gene expression) in the embryo, and then each territory interprets the signals received to activate a particular set of morphogenetic mechanisms in a precise temporal order (Wolpert 1989; Bard 1990). It has been suggested (Wolpert 1969, 1989) that after the inductive phase, the forms of territories play a small role compared with the interpretation of the received signals. However, the experimental evidence accumulated over the years has demonstrated that the reception of a signal often produces changes of genetic expression in the receiving cells that produce changes in the proliferation rates, apoptotic rates, and adhesion properties and changes in the expression of signaling molecules or receptors. In many cases, cells are interchanging signals all the time, and cell behaviors other than signaling are used at the same time. An alternative possibility is that morphogenetic and inductive mechanisms act at the same time or are closely intermixed. We call the composite mechanism morphodynamic (Salazar-Ciudad et al. 2003). As we show, the properties and functioning of morphodynamic mechanisms are very different from those of morphostatic mechanisms. These

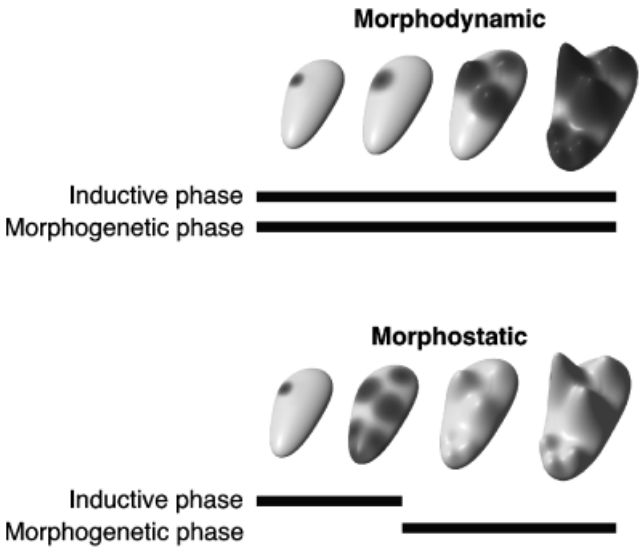

Fig. 1. Signaling and morphogenesis phases of development can be integrated in two distinct ways. In morphodynamic mechanisms, inductive and morphogenetic phases of development are concurrent, whereas in morphostatic mechanisms induction precedes temporally growth that produces final morphology. This morphostatic development change in growth will not affect the induced pattern, but in morphodynamic development growth alteration can also alter the pattern.

differences arise because the form of a territory depends on previous signaling and on how the morphogenetic mechanisms have affected where and when inductions between territories have taken place. These effects are due to the forms, relative distances, and orientations of territories and how they change in time. Thus, inductive and morphogenetic mechanisms, intermediate patterns and gene interactions, are causally interdependent in morphodynamic mechanisms. In fact, the phenotype at each stage (it is the intermediate phenotype) can be seen as a causal factor in the mechanism functioning.

Here we use a realistic mathematical model to predict the development of mammalian teeth (Salazar-Ciudad and Jernvall 2002). The model is general enough to include the development of simple systems composed of both an epithelia and mesenchyme capable of proliferating and of sending and receiving signals. The model can be implemented as either a morphodynamic mechanism or as a morphostatic mechanism. The model links genetic and morphological variation, offering a controlled way to explore differences in the variational properties of morphodynamic and morphostatic mechanisms composed by the same basic inductive and morphogenetic mechanisms. Using this model we explore such questions as (a) which category of pattern-forming mechanisms is most likely involved in the course of evolutionary change, (b) which is most closely tied to the generation of certain types of morphological outcomes, and (c) which is more likely substituted by the other in different selective contexts. Of course, which developmental mechanism is responsible for a given developmental outcome can only be answered definitively by direct experimentation. However, the strategy adopted here is highly suitable for integrating such empirical data into evolutionary scenarios that can be simulated and computationally tested. In addition, we show the different characteristics of the two types of mechanisms are mainly related to the their internal logic and are thus biologically relevant despite their necessary simplifications.

Our general conclusion is that differences between the two distinct classes of developmental mechanism are very extensive. This drastic difference allows probabilistic inferences about their relative roles in evolution and development despite the historical contingencies affecting all evolutionary processes.

The models

The morphodynamic model was originally developed to simulate and predict the three-dimensional morphology and patterns of gene expression in teeth development (Salazar-Ciudad and Jernvall 2002) and consists of a mesenchyme and an epithelium that is able to fold inside the mesenchyme. The patterns produced are in three dimension and can be interpreted as teeth or simply as phenotypic outcomes of quite general developmental mechanisms that use simple epithelial–mesenchymal interactions. The model includes four cell behaviors: cells can secrete signaling molecules, cells can receive signaling molecules (and change their behaviors in consequence), cells can divide, and cells can differentiate. It also includes a network of gene products that regulates these behaviors and interacts between them. The morphodynamic and morphostatic mechanisms differ in the relative timings of these cellular behaviors. In the morphostatic model cells divide mainly after all the signaling and differentiation has taken place.

Morphodynamic model

The epithelial growth rate is a constant (R_e) intrinsic to the cells, minus the activator concentration. Initially, all epithelial cells secrete activator at an intrinsic rate (k_3) and also in response to the local activator concentration. Next, in areas where the local activator concentration exceeds a set threshold, the epithelial cells differentiate irreversibly into nondividing knot cells. These knot cells also secrete inhibitor at a rate equal to the local activator concentration. This inhibitor counteracts activator secretion and enhances growth of the mesenchyme. Experimental data suggest that the activator may be bone morphogenetic protein and the inhibitor may be fibroblast growth factor and/or sonic hedgehog. As a result of those processes, part of the epithelium folds into the mesenchyme, leaving the knots isolated in the tips of the forming cusps. At the same time, mesenchymal growth produces localized lateral expansion affecting cusp sharpness.

Morphodynamic model implementation

Diffusion takes place inside the three-dimensional space (subdivided into a three-dimensional grid of boxes) of the growing tooth. The system has zero-flux boundary conditions in the epithelium (diffusion is not allowed in their apical side) and open boundary conditions in the mesenchyme (molecules exit the system through the borders). The mesenchyme is surrounded by the epithelium (where diffusion is allowed), except in the ventral border where lies the nondental mesenchyme (where the activator and inhibitor can diffuse out of the system). The rate of activator secretion in nonknot epithelial cells is as follows:

$$\frac{\partial A}{\partial t} = \frac{k_1[A]}{k_2[I]+1} + k_3 + D_A\nabla^2[A] \quad (1)$$

where $D_A\nabla^2[A]$ is the diffusion term and D_A is the diffusion coefficient of the activator. The k_1 and k_2 constants can be related to biochemical aspects as the affinity of each molecule for its receptor or to the signal amplification produced by its chain of signal transduction. The rate of inhibitor secretion by knot cells is as follows:

$$\frac{\partial I}{\partial t} = [A] + D_I\nabla^2[I] \quad (2)$$

where $D_I\nabla^2[I]$ is the diffusion term and D_I is the diffusion coefficient of the inhibitor. Epithelial growth is implemented by making epithelia to increase its depth into the mesenchyme. When a single epithelial cell shifts ventrally one cell length into the mesenchyme, it displaces ventrally all the underlying cells in that column, thus mimicking the down growth of valleys along with the retention of the crown base. Epithelial growth rate is $R_e - [A]$ and is at least zero. The mesenchymal growth occurs mainly in the direction offering less resistance (away from the space apical to the epithelium). Visible expansion is thus lateral, and the force producing the expansion by a column of mesenchymal cells was calculated as the sum of the concentration of inhibitor in all the cells of the column multiplied by a constant (R_m) that reflects the sensitivity of cells to inhibitor's growth effect. Specifically, the lateral force of cells in a column i is distributed into four nearest neighboring columns (the anterior, posterior, buccal, and lingual columns) by the following rules. i) Force distribution can only occur to columns shorter than column i. ii) The resistance ($1/S_j$) of

each neighboring column shorter than column i is the total number of cells that all the columns in a direction have (e.g., all the posterior columns next to the column i). This reads as follows:

$$S_j = 1/(\sum_{k=0}^{k=n(i,j)} m(k)) \quad (3)$$

where j can be any of the four directions (anterior, posterior, buccal, or lingual), $n(i,j)$ is the number of columns between column i and the border of the tooth in the direction j, and $m(k)$ is the number of cells in column k. Note that $n(i,j)$ and $m(k)$ depend on tooth shape at each time point and are not external functions or fixed parameters of the model. iii) The force of column i is distributed to its neighbors in inverse proportion to their resistance. This is defined as follows:

$$R_j(i) = D_j R_m \sum_{k=0}^{k=m(i)} [I]_{ik} \quad (4)$$

where $D_j = S_j/(S_p+S_a+S_b+S_l)$ for j [p,a,b,l].

$R_j(i)$ is the rate of growth of column i in direction j. $[I]_{ik}$ is the concentration of the inhibitor in cell k in column i, and R_m is the rate constant of mesenchymal growth. S_j is the inverse of the resistance and $(S_p+S_a+S_b+S_l)$ is the overall inverse of the resistance in all directions. The lateral expansion is mimicked by adding new cells when lateral force on a cell exceeds a unit corresponding to a cell size in a given direction. For a column that is not in the border of the tooth, the neighboring column of cells increases its height by one unit and a new cell is added at the bottom of the column, and for a column in the border of the tooth a new cell is added to extend the perimeter. All the new cells appearing are considered epithelial if they are in contact with the space apical to the epithelium. Lateral growth is biased by increasing the lateral force on cells in the perimeters of the tooth. There is a bias in the posterior (B_p), anterior (B_a), buccal (B_b), and lingual (B_l) direction. For cells in the border j thus reads as follows:

$$R_j(i) = D_j R_m \sum_{k=0}^{k=m(i)} [I]_{ik} + B_j \text{ for } j\,[\mathrm{p,a,b,l}] \quad (5)$$

Models were programmed in Xbasic (www.maxreason.com/software/xbasic/share.html) and is available with the code from the authors (http://biocenter.helsinki.fi/bi/craniofacial/Jernvall.htm).

Morphostatic model

In the morphodynamic model, pattern formation takes place in two phases. In the first phase, the epithelium and the mesenchyme grow at the same rate (so no bending of the epithelium is produced) and only by lateral apposition of epithelial and mesenchymal cells in the borders due to the aforementioned biases (bias that is multiplied by R_m). Thus, growth is not affected by the activator or inhibitor. During this phase, the teeth are made of a flat epithelium and an underlying mesenchyme. Cells secrete and receive activator and inhibitor, and they differentiate following the same rules and kinetics as those in the morphodynamic model. After this signaling phase, the tooth consists of a flat epithelium with some cells being knot cells and a spatially heterogeneous distribution of activator and inhibitor concentrations. In the morphogenetic phase, the epithelium starts to bend in such a way that the depth that each cell reaches is equal to a constant value minus the concentration of the inhibitor at such point multiplied by R_e.

Morphostatic and morphodynamic comparison

Current knowledge of teeth morphogenesis clearly points to the use (at least in mouse and vole; Jernvall and Thesleff 2000; Salazar-Ciudad and Jernvall 2002) of a morphodynamic mechanisms. This is because the epithelium is sending and receiving signals while it is proliferating and changing its form. It is important to note that the number of parameters and their significance is almost the same for both models (except for R_e, which has a slightly different effect; see above). The gene network used is the same in both models, with the only difference that in the morphostatic model inhibitor does not affect mesenchymal growth and that activator affects epithelial growth only after patterning has taken place. Thus, at a genetic level the differences between the two models are very small, but the difference between the two types of mechanisms is essentially structural. In other words, the difference between the models lies in how interactions are organized rather than the interactions themselves. In both morphodynamic and morphostatic models, tooth formation is stopped at the same arbitrary time.

Analysis of morphospace

To compare the variational properties of these two models, we generated a large ensemble of teeth (100,000 for each model) by giving random values to the parameters of the models. The parameter ranges were as follows: for k_1 it was between 0 and 3; for k_2 it was between 0 and 200; for k_3 it was 0.0001; for D_A it was between 0 and 1; for D_I it was between 0 and 1; for R_e it was between 0 and 0.001; for R_m it was between 0 and 0.001; for B_a it was between 0 and 0.001; for B_p it was between 0 and 0.001; for B_l it was between 0 and 0.001; for B_e it was between 0 and 0.001. Each tooth was simulated until iteration 12,000. The obtained forms were scaled to allow the comparison between teeth of different sizes. The original teeth were enclosed in an epithelium with 30×30 cells. For each cell the larger dimension in the bucco–lingual or anteroposterior axis was identified. This distance was made equal to 40, and the rest of the teeth were scaled proportionally (thus, 40×40 teeth were obtained). The height was also scaled in such a way that the lower cell had a height of zero and the higher a height of 30.

Two measures were used to analyze the morphology of teeth. The first measure is the phenotypic information or complexity of a tooth. In any surface that can be characterized as a matrix with values representing heights, a measure of complexity can be calculated. This measure reflects height differences among near points in the surface of a tooth. A very rugged surface can be seen as a complex surface, and we use ruggedness as a measure of complexity. We are interested in a measure that indicates how difficult it is to guess the height of a point if we know the height of its neighbor. We calculated the difference in heights among a point ($h(i,j)$) and all its neighbors at several arbitrary distances or ranges (r).

$$\begin{aligned} d(i,j,r) = \sum_{k=0}^{k=r} \sum_{l=0}^{l=r-k} & (\|h(i,j) - h(i+k,j+l)\| \\ & + \|h(i,j) - h(i-k,j-l)\|.) \\ & + \sum_{k=0}^{k=r} \sum_{l=0}^{l=r-k} (\|h(i,j) - h(i-k,j+l)\| \\ & + \|h(i,j) - h(i+k,j-l)\|.) \end{aligned} \quad (6)$$

This distance $d(i,j,r)$ is calculated for all the points and then averaged over all the pairs of differences calculated.

A second morphological measure, the phenotypic distance or distance, among two teeth is the sum of the difference of height among homologous points within the grid space (all teeth are included in an equally large grid 40×40).

We performed another set of simulations in which, as previously, random values were given to the parameters. For each tooth produced by the same parameter values (we call this the wild-type tooth), we made 100 mutants (10 for each parameter) differing only in small random values: for k_1 it was ± 0.0006; for k_2 it was ± 0.04; for k_3 it was 0; for D_A it was ± 0.0002; for D_I it was ± 0.0002; for R_e it was ± 0.0000002; for R_m it was ± 0.0000002; for B_a it was ± 0.0000002; for B_p it was ± 0.0000002; for B_l it was ± 0.0000002; for B_b it was ± 0.0000002. For each set the distance between the mutant teeth and the wild-type tooth was measured. This procedure was performed for 1000 different wild-type teeth.

RESULTS

Tooth disparity

Every tooth produced by the models has a unique location in the morphospace of 6000 dimension (one per each of the 40×40 grid positions). Each position takes height values between 0 and 30. We will call morphostatic morphospace to the part of the morphospace where the teeth produced by the morphostatic are positioned. The morphodynamic morphospace will then be the part of the morphospace with the teeth produced by the morphodynamic model.

In a first analysis we compared the phenotypic distances among those teeth produced by the morphostatic and morphodynamic mechanisms in the first set of simulations. First we asked whether the teeth produced by morphodynamic mechanisms are, on average, more different among them than those produced by morphostatic mechanisms. In Fig. 2 we plotted the distances between a tooth and its nearest neighbors, averaged for all the teeth produced by each mechanism. We plotted the same measure for the first, second, third, and fourth closest (morphologically most similar) neighbors. In all cases, the teeth produced by the morphodynamic mechanisms are more different from each other. Because only closest neighbor distances are measured, it cannot be detected whether some teeth are more clustered than others. For example, neighboring morphostatic teeth could be very similar, but all these teeth may be confined to a small part of the morphospace (not observable by looking only at the nearest neighbors). To explore this possibility, we also measured the volume occupied by the produced teeth in each morphospace for different coarser grain–scaled grid point heights. The coarse scale was necessary because our 100,000 tooth sample is too small for directly comparing volumes in a significant way. Each grid point can have a continuous value between 0 and 30, and even if we round these heights to take discrete values between 0 and 30, we still have a morphospace with 31^{1600} different tooth positions. Therefore, to estimate the occupied morphospace, we scaled the 40×40 grid to a 10×10 grid. In most cases after this transformation, the main features of the teeth were still

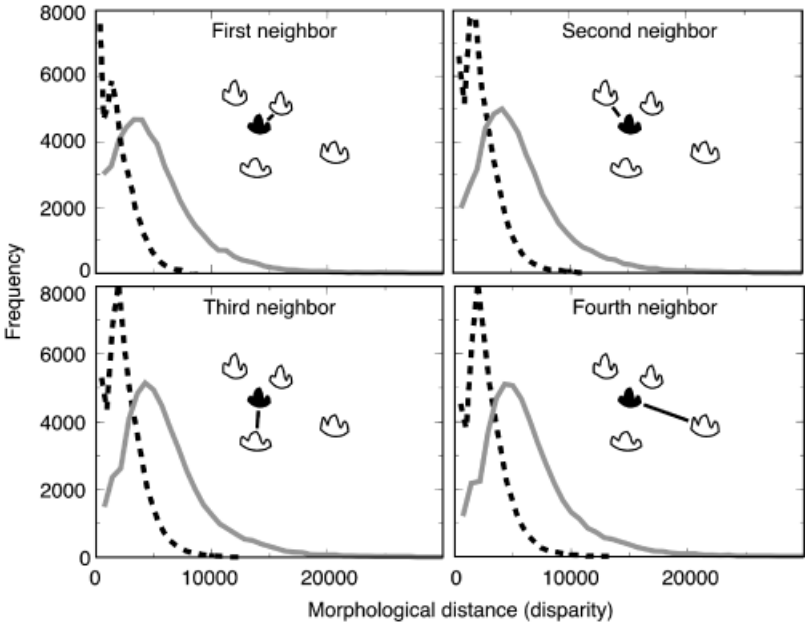

Fig. 2. Frequency distributions of distances of the four most similar teeth in morphodynamic (gray) and morphostatic (hatched black) teeth. The distances are averages of all shape to shape distances. Note how the peak frequency of morphological distances (disparity) is among the longer distances in morphodynamic teeth.

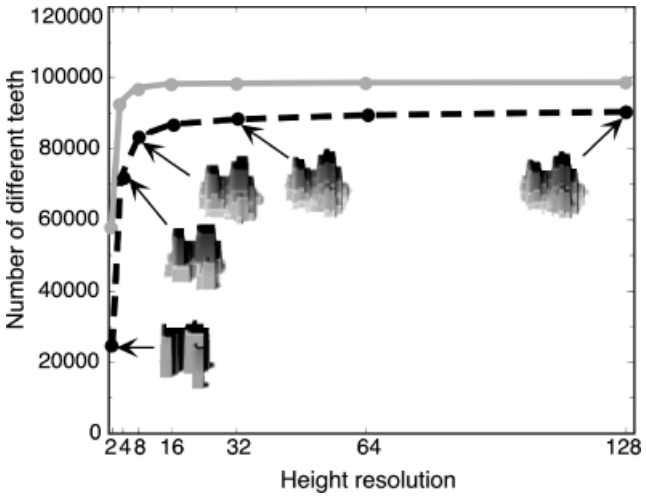

Fig. 3. Number of morphologically different teeth and the effect of rounding the resolution of cusp heights. Note how there is a higher number of morphodynamic teeth (gray line) than morphostatic teeth (hatched black line) even when only two height values are used and only basic gross morphologies remain.

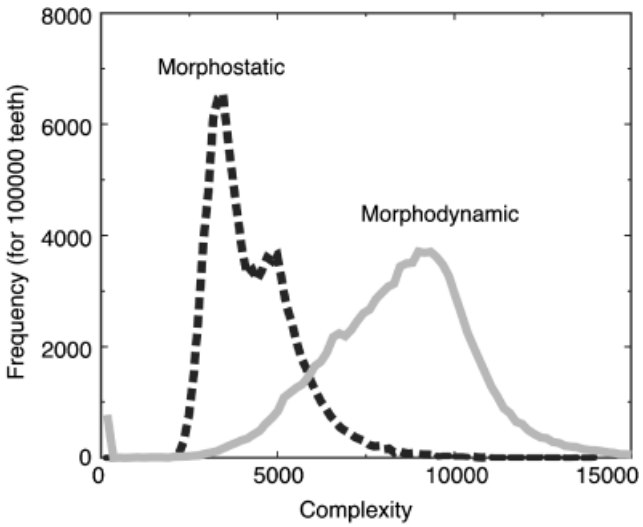

Fig. 4. Frequency distribution of tooth complexity (measured as surface ruggedness). The figure is for distances between 1 and 4. Other neighboring distances show similar results.

recognizable. Then we made the height values discrete by dividing the height coordinate into different number of intervals. The number of intervals used were 128, 64, 32, 16, 8, 4, and 2. This way all grid point height took values between 1 and 128, 1 and 64, 1 and 32, 1 and 16, 1 and 8, 1 and 4, and 1 and 2. Even in the case of heights taking values between 1 and 2, there were still 2^{100} different possible teeth. This transformation is equivalent to partitioning the morphospace into hyperboxes of different sizes. From Fig. 3 we can observe that the number of different teeth, and hence the volume of the morphodynamic morphospace, is always larger than the number of morphostatic teeth. Because this pattern is consistent over the whole range of rounding of the morphospace, the morphodynamic teeth can be interpreted to be more widespread over the whole morphospace.

Tooth complexity

For each of the 40×40 teeth produced, we measured the form complexity at different ranges (see above). In Fig. 4 we plot the relative frequencies of teeth of different complexity for morphodynamic and morphostatic mechanisms. The complexity of morphodynamic teeth was more than twice that of morphostatic teeth. Furthermore, morphostatic teeth had a narrower range of variation.

Relationship between phenotype and genotype

The distances between each wild-type tooth and its mutationally related neighbors were calculated. This analysis thus tests how different morphologies result from small changes in the model parameters. In Fig. 5 the relative frequency of tooth–tooth distances is plotted. Our results indicate that small parameter changes in the morphostatic mechanism (and by extension small genetic changes affecting the parameters) produce teeth that are morphologically more similar to each other than that obtained by changing the parameters of morphodynamic mechanism (Fig. 5). In other words, morphostatic mechanisms exhibit a simpler relationship between genotype and phenotype than morphodynamic mechanisms.

DISCUSSION

Variational properties and mechanism dynamics

The different variational properties of morphodynamic and morphostatic mechanisms can be understood by taking into account what we know about the functioning of each of the two mechanisms. As we discussed, the parameters of the two models are very similar. However, the consequences of parameter changes (this is essentially mutation) are quite different in the two models. Essentially, the parameters involved in the kinetics of signaling (k_1, k_2, k_3, D_A, and D_I) and the growth parameters (R_e and R_m) have very different effects in morphostatic mechanisms. The contrary is true for morphodynamic mechanisms because growth and signaling are causally interdependent.

In morphostatic mechanisms, changes in the kinetic parameters affect essentially the heights of the cusps and their relative spacing. Growth parameters only affect the height of the cusps and whole size of the tooth. R_m can affect the number of cusps because it affects the size of the whole tooth.

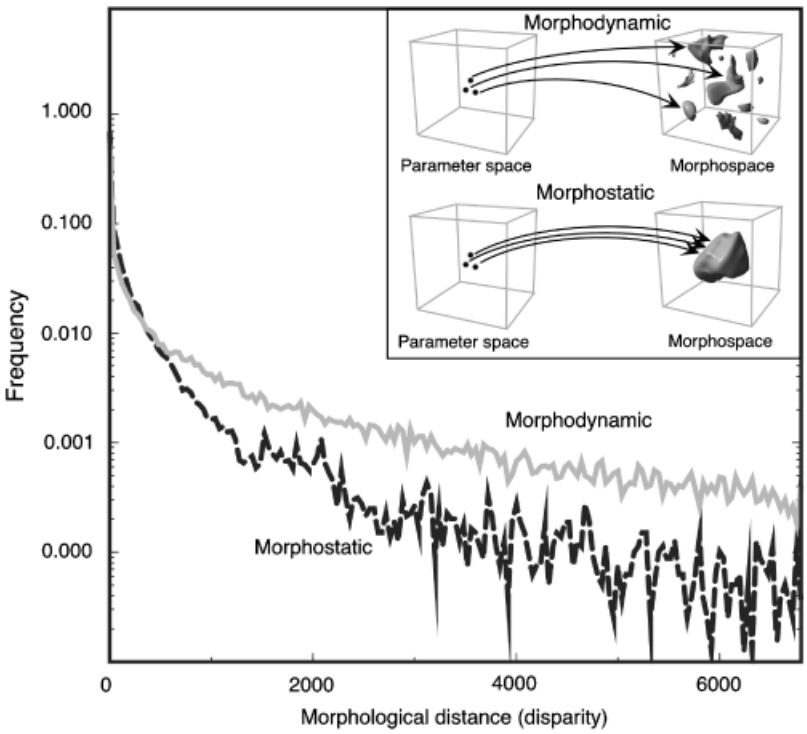

Fig. 5. Small random changes (mutations) in the parameters produce frequently more similar morphostatic teeth than morphodynamic teeth. This suggests that the relationship between genotype and phenotype (parameter space and morphospace) is more complex in morphodynamic teeth (inset).

In contrast, in the morphodynamic model, changes in the parameters more often include changes in the whole tooth form. Changes in different parameters give rise to phenotypic changes that are not qualitatively different. These results are a consequence of molecular interactions taking place on a complex morphological space that is changing because of growth. The concentration of molecules on a part of the tooth does not only depend on the presence of other signaling molecules. Growth parameters affect the effective volume and form in which these molecules are diffusing and, consequently, the effective concentration that arrives to epithelial cells. For example, in the morphostatic mechanism, changes in R_e would change the height of the cusps formed in the tooth, but the positions and forms of the rest of the cusps of the tooth that will form would not be affected. In the morphodynamic mechanism, instead, different values of R_e would change the positions and forms of the knots and cusps that would form later. Comparatively low values of R_e would make the forming cusps blunt, and thus more mesenchymal space would be present for the dilution of the activator and the inhibitor. This change in signal concentrations allows new knots to form closer to already existing knots. Thus, the position of new knots is affected by R_e in the morphodynamic mechanisms but not in the morphostatic mechanisms. The forms of the new knots would also be affected by R_e in the morphodynamic mechanism because new knots will form in the epithelium in between the existing knots and the tooth borders. This space has a form that depends on the relative distribution of existing knots and their size in relationship to the borders.

Kinetic parameters also affect form because activator and inhibitor directly affect growth. There is a strong interdependence between the parameter effects. This interdependence is not due to any direct genetic interaction but rather is epigenetic: Parameters "interact" through their effects on the developing phenotype. Essentially, the form at each stage (i.e., intermediate phenotype) has a causal role in later development. Thus, activator and inhibitor spatial distributions are both a cause and a consequence of form.

Changes in R_e in morphostatic mechanisms result in teeth that differ in the height of their cusps. Those teeth resemble each other more than those produced by morphodynamic mechanisms with the same parameter values. In the teeth produced using the morphodynamic mechanisms, not only the height but also the relative position and number of cusps changes for different values of R_e. In general, parameter changes in morphodynamic mechanisms produce change in the whole pattern of the tooth. In morphostatic mechanisms, different parameter changes produce different effects (e.g., the heights or the relative spacing of the cusps) but do not alter

the whole pattern so dramatically. This difference explains why the morphodynamic mechanism can produce variation with more disparity and why it exhibits a more complex relationship between genotype and phenotype (Figs. 5 and 6).

Another implication of the simulations is that morphostatic mechanisms can produce tooth forms that very similar to each other. We have found, by purposely searching for close neighbors of randomly chosen tooth, that in morphodynamic mechanisms it is often not possible to produce the intermediate form between two possible forms. The morphospace can be seen as being large but with a complex form with many holes and islands (Fig. 6). The morphostatic morphospace occupies less space but occupies it more uniformly and densely (Fig. 6). The existence of these holes in the morphodynamic morphospace can be regarded as true developmental constraints (sensu Alberch 1982) because the intermediate phenotype dependence biases further development to specific paths. It should be noted that the genetic information in both mechanisms is the same and that almost the same number of different teeth can be produced by each mechanism. Those teeth produced by the morphodynamic mechanisms are simply more different/disparate and more complex. This additional complexity and disparity does not require additional genetic information (in comparison with morphostatic mechanisms) and is due to the causal role that the intermediate phenotype has on development dynamics. Thus, it can be said that the epigenetic information existing in the intermediate phenotype can be used in morphodynamic mechanisms.

The results just described hold for the models presented and probably for teeth. Our suggestion is that they also hold for morphodynamic and morphostatic mechanisms in general. The different variational properties of these two types of mechanisms are due to their different use of the spatial information produced in the intermediate phenotype. Morphodynamic mechanisms can use this information for their functioning, whereas morphostatic mechanisms cannot. This difference suggests that the association between variational properties and the type of mechanism is more generally not restricted to the model used or to its implementation. It should be noted that the morphodynamic–morphostatic distinction is the result of how morphogenetic and inductive mechanisms are combined. Exactly the same morphogenetic and inductive mechanisms can be combined to produce a morphodynamic or a morphostatic mechanism. The differences at the genetic level can be subtle and by themselves would not provide clear clues to understand functioning. These genetic differences can consist, for example, of delays in the activations of some genes that would delay the activation of morphogenetic mechanisms.

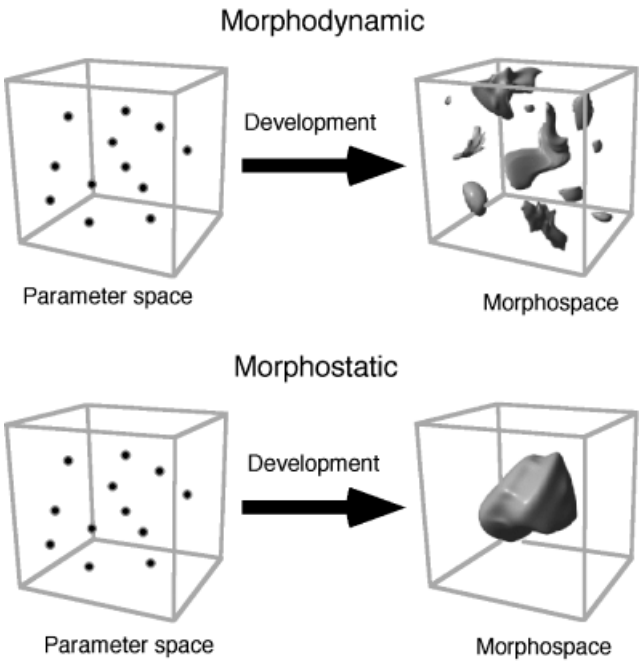

Fig. 6. This figure exemplifies that the relationship between genotype and phenotype (parameter space and morphospace) is more complex in morphodynamic than in morphostatic teeth. The possible occupied morphodynamic morphospace is also more discontinuous, with some intermediate forms having low probability to evolve.

Predictions

Developmental origin of morphological innovations

One of the central problems in current evolutionary theory is its inability to satisfactorily explain which type of phenotypic variation is possible and likely due to mutation at the molecular level. This problem is intimately related to the innovation problem in evolutionary biology. However, different definitions of evolutionary innovations exist (Simpson 1953; Mayr 1960; Liem 1974; Wiley 1981; Müller and Wagner 1991) because of different views about how phenotypic variation appears or because of a focus on more ecological or developmental questions. For our purposes, an evolutionary innovation is simply a new pattern. It can be that a previously existing pattern is replaced by a new one (e.g., a skin coat pattern made of colored spots is replaced by a skin coat pattern made of colored stripes) or that a new pattern appears where nothing existed before (e.g., an epithelial extremity appears in a flat epithelium that did not have any extremity in the ancestors). We call the first possibility an *evolutionary transition of a pattern* and the second one an *evolutionary addition of a pattern*.

At the level of developmental mechanisms, we distinguish two main types of changes. *W mutations* are genetic changes that affect quantitative aspects of the mechanisms. This type

of genetic changes affect the structure of the gene products involved in the mechanism, thereby affecting the functioning of the mechanism. Examples might include changes in the binding affinities of a transcriptional factor for its binding site in the promoter region of gene that affects proliferation rate or changes in the diffusivity of a signaling molecule. These are the types of mutations we have been simulating. Molecular changes can also affect the topology of a developmental mechanism; in other words, which gene product interactions are taking place. Those changes can also affect which cellular behaviors are implicated in a developmental mechanism. This way new developmental mechanisms are produced. These two types of changes in developmental mechanisms can also be considered innovations (but just innovations at the mechanism level), but they do not necessarily produce morphological innovations.

As we have seen from Figs. 2, 3, and 5, morphodynamic mechanisms produce more diverse phenotypic variation than morphostatic mechanisms. The distinction between these two pattern-forming mechanisms is based on the relative timing of action between morphogenetic and inductive mechanisms and does not imply any difference in the genetic complexity. In principle, the likelihood that genetic mutations recruit inductive and morphogenetic mechanisms to make a morphodynamic or morphostatic mechanism is equal. As such, morphodynamic mechanisms produce more phenotypic patterns for the same amount of genetic variation. In other words, for a pattern of a given complexity, the morphodynamic mechanisms capable of producing it would be genetically more simple, on average, than the morphostatic mechanisms capable of producing them. In addition, many patterns simply cannot be produced by morphostatic mechanisms. This suggests that, in general, morphodynamic developmental mechanisms would be more often implicated in the generation of new evolutionary patterns. However, which kind of mechanism would be implicated in the origination, in evolution, of a new pattern from an ancestral one depends on selective pressures. Several selective scenarios are possible.

In some cases, ancestral patterns are under strong selection, and only a limited number of other patterns are more adaptive in the environment. If these more adaptive patterns are very similar to the existing patterns, then one would expect that evolutionary transition of the pattern is more likely to be produced by morphostatic mechanisms. This expectation results from the observation that morphostatic mechanisms produce more gradual variation. Furthermore, they can produce it faster because of a closer relationship between genotype and phenotype. On the contrary, if the more adaptive patterns are not very similar to the ancestral pattern, then morphodynamic mechanisms would be more likely involved in the generation of innovation. The same situation holds true in the case where the intermediate patterns that a morphostatic mechanism would be able to produce are maladaptive.

In a second evolutionary scenario, the ancestral pattern is under light selection or no selection, and thus many different patterns are allowed in a environment. In this case, one would expect that morphodynamic mechanisms are more often involved in the generation of innovation because they have the capability to produce more variation. Consequently, over time, the likelihood that at least one adaptive pattern is found would be higher for those evolutionary lineages with morphodynamic mechanisms. These consideration suggests that the proportion of individuals whose ancestors used a morphodynamic mechanism would increase over time (although developmental mechanisms are rarely selected per se).

In the case of pattern evolutionary additions we also expect morphodynamic mechanisms to be more commonly recruited. This kind of innovation, however, might be expected to be either too deleterious or neutral the first time they appear. In the first case, they would be selectively eliminated, and in the second case, they fit to the second evolutionary scenario described above. Indeed, it has been suggested that many innovations remain for large periods as vestigial-like nonselected or weakly selected patterns (Bock 1959).

Dynamics of evolutionary replacement among mechanisms

Once a new pattern is produced it is likely that the mechanisms used to produce it change, over time, from morphodynamic to morphostatic mechanism. The likelihood of this evolutionary replacement depends on the kind of selective pressures acting on a pattern, but in many cases it is likely that once a well-fit pattern is attained, most variation in this pattern would be maladaptive. Morphostatic mechanisms produce smaller variations and can produce it more quickly because of the simpler relationship between phenotype and genotype. This allows them to fine tune the adaptive patterns. This evolutionary replacement from morphodynamic to morphostatic mechanisms may be very slow, because many patterns that can be produced by morphodynamic mechanisms may be unlikely or impossible for morphostatic mechanisms.

To have several independent mechanisms producing the same pattern buffers its development in a larger range of environmental and genetic backgrounds(Nowak et al. 1997). This buffering is probably more often the case when the mechanisms implicated function in very different ways, such as in the case of morphodynamic and morphostatic mechanisms. Thus, in many cases replacement would be incomplete.

From these considerations, it follows that the developmental mechanisms that presently produce a pattern in an organism are not necessarily the ones that produced it for the first time in the evolution of the lineage of this organism.

Indeed, Weiss and Fullerton (2000) suggested that changes in developmental mechanisms through time, which they call phenogenetic drift, may make it "more difficult to understand evolution at gene level than at the trait level."

The present study directly predicts which kind of variation is expected to be found in patterns using morphodynamic mechanisms and morphostatic mechanisms. In this sense, recent morphological studies in human twins specifically suggest that the kind of variation and relationship between phenotype and genotype observed in teeth is consistent with a morphodynamic mechanisms (Townsend et al. 2003).

Temporal structure of development

Developmental mechanisms acting in late development cannot affect the functioning of developmental mechanisms acting earlier in development. Then, in general, changes in the late-acting developmental mechanisms are less often deleterious than changes in early-acting developmental mechanisms. This suggests that phenotypic innovation, and thus morphodynamic mechanisms, would be comparatively more frequent in late development. In fact, in late development the intermediate phenotype is often more complex and thus more chances for innovation exist for morphodynamic mechanisms. But over time, as we argued, we expect that morphodynamic mechanisms would be replaced by morphostatic mechanisms. In early development more time has been elapsed since the recruitment of the original mechanisms, and then it is more likely than this original mechanism becomes replaced. Then, early stages of development can be expected to be more often morphostatic. In addition, the comparatively large phenotypic variation produced by morphodynamic mechanisms would be too disruptive for early development.

Disparity, phylogeny, and tempo and mode of evolution

The use of a particular type of developmental mechanism by a lineage conditions its further evolution. Lineages using morphodynamic mechanisms in the development of a morphological structure would be expected to exhibit larger disparity among their branches than lineages using morphostatic mechanisms for the same amount of time since last common ancestor in each group of lineages. Of course, whereas it would also depend on the history of selective pressures, here we focus on developmental effects. Our approach adds an explanatory factor in understanding morphological evolution. In general, we predict that because variation that morphodynamic mechanisms allow is comparatively larger, adaptive change would take place less often but would be larger. Lineages using morphostatic mechanisms in the development of a morphological structure would change the pattern of this structure very often but very gradually (Fig. 7). Those changes, however, may not give rise, even gradually, to any large pattern change but are quite restricted in their possibilities for variation. This creates the effect that in comparing the amount of morphological change in a lineage at time intervals of different length, different trends would be found. Lineages using morphostatic mechanisms in the development of a structure would be expected to exhibit high rates of morphological change when considering short time intervals but relatively slow rates if measured over long time intervals. This phenomenon is the result of the frequent small changes characteristic of morphostatic mechanisms.

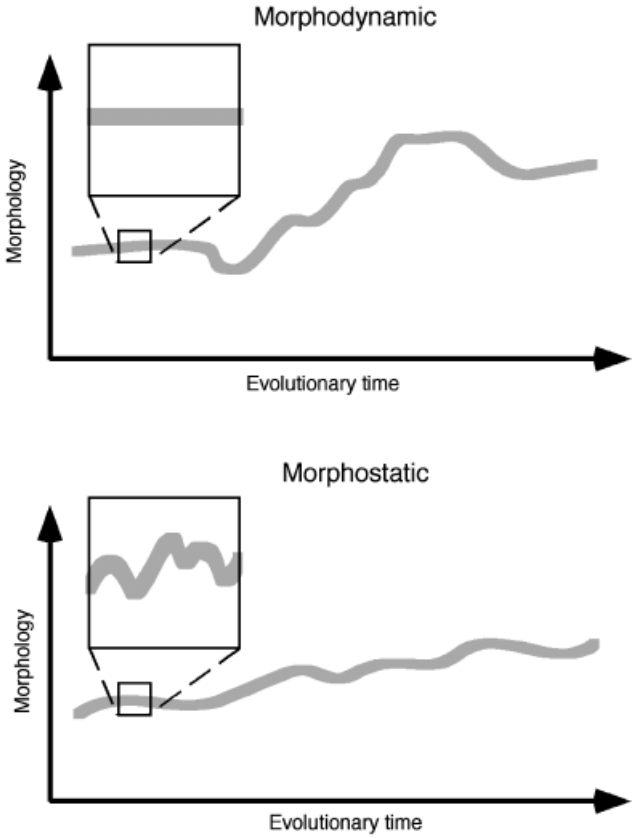

Fig. 7. Hypothetical evolution of a lineage with morphodynamic and morphostatic developmental mechanisms. Everything else being equal, morphodynamic lineage can be expected to show more stasis with larger jumps in morphology when there is an evolutionary change. Morphostatic lineages can be expected to show more continuous and gradual changes but lack larger morphological jumps. The rates of evolution will be different in morphostatic and morphodynamic lineages for different time scales.

For morphodynamic mechanisms, the contrary would be found. Stasis would be found for short intervals, but if measured over long time intervals, high rates would be found (Fig. 7). In a paleontological time scale, these changes may even seem saltational. Indeed, Weiss and Fullerton (2000) suggested that changes in developmental mechanisms through time, which they call phenogenetic drift, may make it "more

difficult to understand evolution at gene level than at the trait level." In fact, this perspective gives a possible developmental explanation for punctuated equilibrium (Gould and Eldredge 1977). It also suggests that both punctuated equilibrium and gradualism are possible and that they may be part of a more general phenomena that does not only apply to paleobiological time scales (Wake et al. 1983). From our perspective, which kind of macroevolutionary trends will be found in a lineage depends on the developmental mechanisms it uses (and on selective pressures; e.g., see Sheldon 1996). A corollary of this hypothesis is that lineages with complex phenotypes can be expected to use morphodynamic mechanisms more often and, consequently, more often exhibit punctuated equilibrium.

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REFERENCES

Alberch, P. 1980. Ontogenesis and morphological diversification. *Amer. Zool.* 20: 653–667.

Alberch, P. 1982. Developmental constraints in evolutionary processes. In J. T. Bonner (ed.). *Evolution and Development*. Dahlem Konferenzen. Springer-Verlag, Heidelberg, pp. 313–332.

Alberch, P., and Gale, E. A. 1983. Size dependence during the development of the amphibian foot. Colchicine-induced digital loss and reduction. *J. Embryol. Exp. Morphol.* 76: 177–197.

Bard, J. B. L. 1990. *Morphogenesis: the Cellular and Molecular Processes of Developmental Anatomy*. Cambridge University Press, Cambridge.

Bock, W. J. 1959. Preadaptation and multiple evolutionary pathways. *Evolution* 13: 194–211.

Felix, M. A. 1999. Evolution of developmental mechanisms in nematodes. *J. Exp. Zool.* 285: 3–18.

Felix, M. A., and Sternberg, P. W. 1997. Two nested gonadal inductions of the vulva in nematodes. *Development* 124: 253–259.

Goodwin, B. C. 1994. *How the Leopard Changed Its Spots*. Weidenfeld and Nicolson, London.

Gould, S. J., and Eldredge, N. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. *Paleobiology* 3: 115–151.

Ho, M. W. 1990. An exercise in rational taxonomy. *J. Theor. Biol.* 147: 43–57.

Jernvall, J. 2000. Linking development with generation of novelty in mammalian teeth. *Proc. Natl. Acad. Sci. USA* 97: 2641–2645.

Jernvall, J., and Thesleff, I. 2000. Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mech. Dev.* 92: 19–29.

Liem, K. F. 1974. Evolutionary strategies and morphological innovations: chichlid pharyngeal jaws. *Syst. Zool.* 20: 425–441.

Mayr, E. 1960. The emergence of evolutionary novelties. In S. Tax (ed.). *Evolution after Darwin*. University of Chicago Press, Chicago, pp. 349–380.

Müller, G. B., and Wagner, G. P. 1991. Novelty in evolution: restructuring the concept. *Annu. Rev. Ecol. Syst.* 22: 229–256.

Newman, S. A., and Müller, G. B. 2000. Epigenetic mechanisms of character origination. *J. Exp. Zool.* 288: 304–317.

Nijhout, H. F. 1991. *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institute Press, Washington, DC.

Nowak, M. A., Boerlijst, M. C., Cooke, J., and Smith, J. M. 1997. Evolution of genetic redundancy. *Nature* 388: 167–171.

Oster, G. F., Shubin, N., Murray, J. D., and Alberch, P. 1988. Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny. *Evolution* 42: 862–884.

Polly, P. D. 2001. On morphological clocks and paleophylogeography: towards a timescale for Sorex hybrid zones. *Genetica* 112–113: 339–357.

Salazar-Ciudad, I., Garcia-Fernandez, J., and Solé, R. V. 2000. Gene networks capable of pattern formation: from induction to reaction-diffusion. *J. Theor. Biol.* 205: 587–603.

Salazar-Ciudad, I., and Jernvall, J. 2002. A gene network model accounting for development and evolution of mammalian teeth. *Proc. Natl. Acad. Sci. USA* 99: 8116–8120.

Salazar-Ciudad, I., Jernvall, J., and Newman, S. A. 2003. Mechanisms of pattern formation in development and evolution. *Development* 130: 2027–2037.

Salazar-Ciudad, I., Newman, S. A., and Solé, R. V. 2001a. Phenotypic and dynamical transitions in model genetic networks. I. Emergence of patterns and genotype-phenotype relationships. *Evol. Dev.* 3: 84–94.

Salazar-Ciudad, I., Solé, R. V., and Newman, S. A. 2001b. Phenotypic and dynamical transitions in model genetic networks II Application to the evolution of segmentation mechanisms. *Evol. Dev.* 3: 95–103.

Sheldon, P. R. 1996. Plus ça change: a model for stasis and evolution in different environments. *Paleogeography, Paleoclimatology and Paleoecology* 127: 209–227.

Shubin, N., Wake, D. B., and Crawford, A. J. 1995. Morphological variation in the limbs of Taricha granulosa (Caudata: Salamandridae): evolutionary and phylogenetic implications. *Evolution* 49: 874–884.

Simpson, G. G. 1953. *The Major Features of Evolution*. Columbia University Press, New York.

Streicher, J., and Müller, G. B. 1992. Natural and experimental reduction of the avian fibula: developmental thresholds and evolutionary constraint. *J. Morphol.* 214: 269–285.

Tautz, D. 1992. Redundancies, development and the flow of information. *Bioessays* 14: 263–266.

True, J. R., Edwards, K. A., Yamamoto, D., and Carroll, S. B. 1999. Drosophila wing melanin patterns form by vein-dependent elaboration of enzymatic prepatterns. *Curr. Biol.* 9: 1382–1391.

Townsend, G., Richards, L., and Hughes, T. 2003. Molar intercuspal dimensions: genetic input to phenotypic variation. *J. Dent. Res.* 82: 350–355.

Wagner, G. P., and Misof, B. Y. 1993. How can a character be developmentally constrained despite variation in developmental pathways. *J. Evol. Biol.* 6: 449–455.

Wake, D. B., Roth, G., and Wake, M. H. 1983. On the problem of stasis in organismal evolution. *J. Theor. Biol.* 101: 211–224.

Wang, M., and Sternberg, P. W. 1999. Competence and commitment of *Caenorhabditis elegans* vulval precursor cells. *Dev. Biol.* 212: 12–24.

Weiss, K. M., and Fullerton, S. M. 2000. Phenogenetic drift and the evolution of genotype phenotype relationships. *Theor. Pop. Biol.* 57: 187–195.

Wiley, E. O. 1981. *Phylogenetics*. Wiley, New York.

Wolpert, L. 1969. Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* 25: 1–47.

Wolpert, L. 1989. Positional information revisited. *Development* 107: 3–12.

Wright, S. 1912. Notes on the anatomy of trematode *Microphallus opacus*. *Trans. Am. Soc.* 31: 167–175.