JOURNAL OF EXPERIMENTAL ZOOLOGY (MOL DEV EVOL) 304B:619–631 (2005)

# Graduality and Innovation in the Evolution of Complex Phenotypes: Insights from Development

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*ABSTRACT* The neo-Darwinian paradigm benefits from the assumption that phenotypic variation is gradual and that phenotype and genotype have a relatively simple relationship. These assumptions are historically inherited from the times of the neo-Darwinian synthesis and, consequently, do not include present understanding about development. In this study, understanding about the dynamics of pattern formation is used to explore to that extent phenotypic variation can be expected to be gradual and simply related to molecular variation. Variation in simple phenotypes seems to fit neo-Darwinian assumptions but variation in complex phenotypes does not. Instead, variation in complex phenotypes would have a tendency to relatively less gradual evolution, even at microevolutionary time scales, that would make phylogenetic reconstructions more difficult. In addition, they will have a tendency to exhibit specific trends in innovation rates over group radiations with early accelerations and late decelerations. This work also explores further consequences of these results in our understanding of phenotypic evolution. *J. Exp. Zool. (Mol. Dev. Evol.) 304B:619–631, 2005*. 

Evolution has produced, over hundreds of millions of years, the diversity of complex phenotypes observed in metazoans and the developmental mechanisms by which such complexity is generated in each generation. The classical neo-Darwinian paradigm can explain the dynamics of replacement between variant phenotypes based on selective pressures and population genetic structure but not which variant phenotypes can appear due to mutation. Thus the structure of the phenotype cannot be explained except for the short time intervals in which selective pressures and possible heritable phenotypic variants are known. To understand which phenotypic variation is possible, some understanding about the developmental processes producing it is required. The relevance of this problem has been undermined by the assumption that, for most traits, there is enough heritable gradual variation for selection to be the main determinant of the direction and rate of evolution. This view is based on the assumption of a simple relationship between genotype and phenotype, and thus proposes that development itself is not critical for the understanding of evolution (Fisher, '30; Dobzhansky, '37; Mayr, '63). Although most evolutionary changes in phenotype are due to changes in DNA, the relationship between molecular and phenotypic variation is far from simple. In fact, this relationship depends on the ways in which development functions. It is currently understood that development proceeds by complex spatio-temporal patterns of interaction between many genes and between those genes and the spatio-temporally dynamic epigenetic context of the embryo phenotype (Newman and Müller, 2000; Gilbert, 2003; Salazar-Ciudad et al., 2003). The graduality of variation has long been intensively discussed in relationship to phenotypic evolution and the extrapolation of microevolutionary dynamics into macroevolution (Provine, '71; Charlesworth et al., '82). Gradualistic views have had very influential proponents (Fisher, '30; Haldane, '32; Dobzhansky, '37; Mayr, '63) but also many opponents (Goldschmidt, '40; Gould and Elredge, '77; Alberch, '82). Perhaps because in the neo-Darwinian view the genotype and phenotype are assumed to have a rather simple relationship, there is often a non-separation between the

Grant sponsor: Marie Curie fellowship; Grant number: HPMF-CT-2002-01720; Grant sponsor: Academy of Finland grants; Grant number: SA-1207547 and SA-1208582.
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Received 5 November 2004; Accepted 2 May 2005
Published online 19 July 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.b.21058.

possibility that relevant evolutionary changes are gradual and that variation occurring in populations before the action of selection is gradual. Fisher's work (e.g., Fisher, '30) suggested, on theoretical grounds, that only small gradual variation does play a significant role in evolution. This may have produced a relative neglect of non-gradual variation by many evolutionary biologists. However, even before the neo-Darwinian synthesis there were extensive studies on meristic variation (e.g., Bateson, 1894) and meristic variation is now attracting renewed interest (Weiss et al., '98; Ahn and Gibson, '99; Arthur et al., '99; Arthur and Kettle, 2001; Pilbeam, 2004). Furthermore, variation in the morphology of organs itself rather than in their number has also been described by many authors as essentially non-gradual or as not totally gradual (Bateson, 1894; Gregory, '34; Sawin and Edmonds, '49; Manville, '59; James, '60; Haitlinger, '62; Puèek, '62; Berry, '63; Yablokov, '66; Alberch, '82, '83; Wolsan, '89; Szuma, 2002). It is also noteworthy that quantitative and population genetic studies do not necessarily consider variation as gradual and simply related to the genotype (e.g., Orr, 2003; Shapiro et al., 2004).

The discussion about the magnitude of evolutionary change overshadows the qualitative problem of which kind of variation is possible. Discussions about the magnitude of evolutionary change cannot be naturally separated from the question about how phenotype changes because the measure of change is highly dependent on the complex multivariate structure of phenotypes (Eble, '99).

The problem of evolutionary innovation illustrates well the qualitative nature of phenotypic variation. In fact, we suggest that innovation is often used in a rough way to describe those qualitative changes that poorly fit into the more gradual univariate views of neo-Darwinism. Morphological variation and evolutionary change are often studied in a number of arbitrarily defined traits. But nature may be unaware of those arbitrary choices made by researchers. There is, in principle, a spectrum of possibilities between innovation, as change in the characteristics that define a trait, and variation in a trait. What may be considered innovation and what gradual variation in a trait depends on the criteria used to define a trait. Without an understanding of the developmental basis of phenotypic variation, there may not be a non-arbitrary method to define traits. Then the distinction between innovation and other kinds of changes may not reflect a real phenomenon but our incapacity to understand the variation that is possible from development.

Evolutionarily relevant phenotypic variation has been suggested to be qualitatively and quantitatively different for organisms of different phenotypic complexity (Vermeij, '73, '74) and using different developmental mechanisms (Alberch, '82; Wagner and Misof, '93; Goodwin, '94; Salazar-Ciudad et al., 2000, 2001a, b, 2003; Salazar-Ciudad and Jernvall, 2004). These differences have been suggested to dramatically affect the evolutionary process (Alberch, '82; Wake et al., '83; Newman and Müller, 2000; Salazar-Ciudad et al., 2001a, 2003) and allow to make evolutionary predictions about phenotypic and developmental evolution that are simply not possible from the neo-Darwinian paradigm (Salazar-Ciudad et al., 2001a; Salazar-Ciudad and Jernvall, 2004).

Mammalian tooth morphogenesis is a developmental system whose understanding has dramatically increased during the last decade due to the use of modern molecular techniques (Jernvall and Thesleff, 2000). The basic cellular and genetic interactions that lead to tooth morphology are known to the extent that, in contrast to most other developmental systems, mechanistic hypothesis about pattern formation and morphogenesis have been produced and tested with a significant degree of accuracy. This has been done by the construction of a mathematical model that, by implementing these basic cellular and genetic interactions, gives rise to teeth morphologies closely resembling known species (Salazar-Ciudad and Jernvall, 2002). This amount of integrated experimental and theoretical work offers a quite explicit and accurate way to relate phenotype and genotype. The genotype is represented in the model by the parameter values and the topology of the gene network implemented. The model includes an implementation of the interaction between gene products and how these affect proliferation (which can be regarded as how they regulate mitogenic genes). The model does not implement the genotype at the level of sequences but at the level of the biochemical binding constants between gene products and DNA regulatory regions or between gene products through protein–protein binding sites. These constants depend on the structure of these proteins and in the sequence of DNA binding sites. Thus our model implements only the more basic level at which function exists. Not all the genes involved in tooth development are known. The gene network included in the model is just a

minimal network of interactions between known gene products that is able to produce teeth. Mutations in genes other than those included in the model can affect evolutionary dynamics. Our model does not include all the relevant genotype but some caricature of a part of the genotype that is responsible for the generation of the phenotype during development. In spite of this limitation, our model may be useful to understand the relationship between phenotype and genotype because in contrast to more detailed genetic approaches it includes realistic developmental dynamics.

The phenotype is simply the result of the model, a three-dimensional tooth morphology composed of epithelial and mesenchymal cells. Development is the model or mechanistic hypothesis itself, the wiring of gene interactions and its effect on mitosis. The model, as being based on the presumably realistic implementation of experimentally found interactions, provides relatively accurate predictions of tooth morphology and patterns of gene expression in different mammalian species (Salazar-Ciudad and Jernvall, 2004). It is important to note that the model does not include any prefixed information about the position of cusps or gene expression; three-dimensional morphology and patterns of gene expression appear from the dynamics of the model. In other words, the model, by implementing what we know about the basic genetic and cellular interactions in tooth development, predicts the morphology of teeth. In this article we use this model to explore the basic underlying dynamics of the relationship between phenotype and genotype.

It has been suggested that the developmental mechanism responsible for tooth morphogenesis is of morphodynamic type (Salazar-Ciudad and Jernvall, 2002). In morphodynamic mechanisms, pattern formation occurs because of reciprocal signaling between groups of cells that are simultaneously changing their proliferative and adhesive properties (in response to the molecular signals they receive). In morphostatic mechanisms, on the contrary, pattern formation takes place through a first phase in which cells interchange molecular signals and a second phase in which cells undergo proliferation and adhesion changes in response to the signals received. It has been suggested that many vertebrate organs use morphiodynamic mechanisms (Salazar-Ciudad et al., 2003). It has also been suggested that all possible morphodynamic mechanisms may exhibit similar relationships between phenotype and genotype (Salazar-Ciudad and Jernvall, 2004). To the extent that this is true, the conclusions of our study may be of general utility not only for teeth but also for other organs in which signaling and proliferative and adhesive changes occur simultaneously.

In this study, we specifically explore how well graduality and the simple genotype–phenotype relationship fit the variation produced by the model. In previous studies, we have evaluated different kinds of developmental mechanisms (Salazar-Ciudad et al., 2000, 2001a; Salazar-Ciudad and Jernvall, 2003). Here, the focus is on the variation produced from different types of phenotypes. In other words, the aim is to see if some of the variation produced by a morphodynamic mechanism fits the neo-Darwinian ''gradualistic'' assumptions and from which types of phenotypes this variation can be produced. As far as the model grasps the basic dynamics of tooth development, these comparative predictions can be expected to be relatively insensitive to the unavoidable simplifications of mathematical models and thus still be useful for comparisons of the evolution of different animal groups.

We focus on questions related to the graduality of variation and its relationship with genetic variation. These questions include the distribution of the magnitudes of phenotypic changes obtainable by mutation and the comparative relevance of the qualities of such variation. This can have important consequences in the evaluation of the relative mode and tempo of evolution of species and on the causes of phyletic trends. Among these, special attention will be paid to the patterns of change in disparity among species in the radiation of a group over time, the disparity among close species in a phylogeny and the frequent ultimate decreases in phenotypic innovation rates over time (Foote, '91, '92, '95, '97; Briggs et al., '92; Erwin, '94; Wagner, '95; Jernvall et al., '96; Labandeira and Eble, 2004). Moreover, our analysis provides a null model to evaluate the likelihood of morphological transformations that can provide a better way to evaluate both the intensity of selection and the probabilities of character transformations for phylogenetic reconstructions. More in general our study aims to exemplify how development-based understanding of variation can transform our explanations and approaches to phenotypic evolution.

## METHODS

The model includes four cell behaviors: cells can secrete signaling molecules, receive signaling

molecules (and change their behaviors in consequence), divide and differentiate. It also includes a network of gene products that regulates these behaviors and interactions between them. The epithelial growth rate is a constant ($R_e$) intrinsic to the cells, minus the activator concentration. 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 non-dividing knot cells. These knot cells also secrete inhibitor at a rate equal to the local activator concentration. This inhibitor counteracts activator secretion on receiving cells and enhances growth of the mesenchyme. Experimental data suggest that the activator may include bone morphogenetic protein and inhibitor may include fibroblast growth factor and/or sonic hedgehog. As a result of these 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.

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 the non-dental mesenchyme lies (where the activator and inhibitor can diffuse out of the system). The rate of activator secretion in non-knot epithelial cells is

$$\frac{\partial \mathrm{A}}{\partial t} = \frac{k_1[\mathrm{A}]}{k_2[\mathrm{I}] + 1} + k_3 + D_\mathrm{A}\nabla^2[\mathrm{A}], \qquad (1)$$

where $D_\mathrm{A}\nabla^2[\mathrm{A}]$ is the diffusion term and $D_\mathrm{A}$ 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

$$\frac{\partial \mathrm{I}}{\partial t} = [\mathrm{A}] + D_\mathrm{I}\nabla^2[\mathrm{I}], \qquad (2)$$

where $D_\mathrm{I}\nabla^2[\mathrm{I}]$ is the diffusion term and $D_\mathrm{I}$ is the diffusion coefficient of the inhibitor. Epithelial growth is implemented by making epithelia increase their 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 downgrowth of valleys along with the retention of the crown base. Epithelial growth rate is $R_e$–[A] and at least zero. 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 the 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 (for example, all the posterior columns next to column $i$). This reads

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

where $j$ can be any of the four directions (anterior, posterior, buccal and 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

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

where $D_j = S_j/(S_\mathrm{p}+S_\mathrm{a}+S_\mathrm{b}+S_\mathrm{l})$ for $j$ [p,a,b,l].

$R_j(i)$ is the rate of growth of column $i$ in direction $j$. $[\mathrm{I}]_{ik}$ is the concentration of the inhibitor in cell $k$ in column $i$ and $R_\mathrm{m}$ is the rate constant of mesenchymal growth. $S_j$ is the inverse of the resistance and $(S_\mathrm{p}+S_\mathrm{a}+S_\mathrm{b}+S_\mathrm{l})$ 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

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

Models were programmed in Xbasic (http://www.maxreason.com/software/xbasic/share.html) and are available with the code from the authors (http: //www.biocenter.helsinki.fi/bi/craniofacial/PNAS02Program/Program.htm).

### *Analysis of morphospace*

A large ensemble of teeth was produced by giving random values to the parameters in between the ranges. The parameter ranges were: for $k_1$ it was between 0 and 6; for $k_2$ it was between 0 and 1,000; 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 0.007; for $R_m$ it was between 0 and 0.009; for $B_a$ it was between 0 and 0.004; for $B_p$ it was between 0 and 0.004; for $B_l$ it was between 0 and 0.004; for $B_c$ it was between 0 and 0.004. The ranges were chosen because outside these ranges the model does not produce teeth. Each tooth was simulated until an arbitrary time (iteration 24,000). For each tooth produced (we call this the wild-type tooth) we made 1,000 mutants (100 per each parameter) and run them until iteration 24,000. The value changes from the wild type were regularly spaced between 50∗range∗0.001 and −50∗range∗0.001. Range represents the value ranges for the random values of the parameters of the wild-type teeth. All teeth were scaled and then for each tooth we measured the phenotypic complexity and the phenotypic distance to the wild type.

The obtained teeth were scaled in order to allow the comparison between teeth of different sizes. The original teeth were enclosed in an epithelium with a maximum of $25 \times 25$ cells. For each cell the larger dimension in the bucco-lingual or antero-posterior axis was identified. This distance was made equal to 25 and the rest of the teeth were scaled proportionally. The height was also scaled in such a way that the lower cell had a height of zero and the higher cell a height of 30. Scaling was performed to compare tooth morphology irrespective of tooth size. The phenotypic information or complexity of a tooth can be measured in any surface that can be characterized as a matrix with values representing heights. 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, in fact, we will use the proportion between the surface and volume ($S/V$) as a measure of complexity. This measure also indicates how difficult it is to guess the height of a point if the height of its neighbor is known.

A second morphological measure, the phenotypic distance or distance, among two teeth is the sum of the differences of height among homologous points within the grid space (all teeth are included, after scaling, in an equally large grid $25 \times 25$ after being scaled) divided by the sum of the volume of the two compared teeth.

A qualitative coarse characterization of morphology has been applied to all teeth. This classification has been previously applied to characterize patterns of teeth morphological disparity over time in the fossil record (Jernvall et al., '96). In brief, teeth are classified into crown types characterized by four numbers: the number of cusps in the buccal side, the number of cusps in the lingual side and the number of lophs in the anterio-posterior and bucco-lingual directions.

This study explores the structure of the morphospace and its relationship to the parameter space that we use as a proxy for the genospace. The morphospace is an abstract space in which all possible morphologies are ordered while the genospace is the same but for the ensemble of possible genetic sequences. Essentially what we do is to explore a hypersphere around a large number of points in parameter space and see how this hypervolume maps into the morphospace. This allows us to study the variational properties of the developmental mechanism that is supposed to produce teeth. By variational properties (Salazar-Ciudad et al., 2003) of a developmental mechanism, we mean the ensemble of phenotypes produced by environmental changes and mutations that alter the parameter values of the mechanism without altering its topology (i.e., without altering which genes affect each other and how they affect cell behaviors).

The model considers, essentially, haploid organisms because the focus of our research is on the

variational properties of development. Diploidy and other characteristics of the genetics of transmission can later affect that some of the variation possible from development are not expressed.

## RESULTS

Figure 1 shows a density plot of the complexity of wild types (ancestral shape) against its neighbors (descendants). The colors in the graphic indicate the relative density of teeth per each interval (box) of wild type and mutant complexity (at 0.01 intervals). The relative density is obtained by dividing by the number of teeth in each wild-type interval. The red squares represent the averaged mutant complexity for each interval of wild-type complexities. The figure indicates that complex teeth have typically complex teeth as neighbors.

Figure 2 shows the phenotypic distance between each type of teeth and their neighbors. Phenotypic distances are averaged over all the teeth that have the same genetic distance (here the genotypic distance is counted as the order of the mutant (from −50 to 50)) to the wild type and the wild

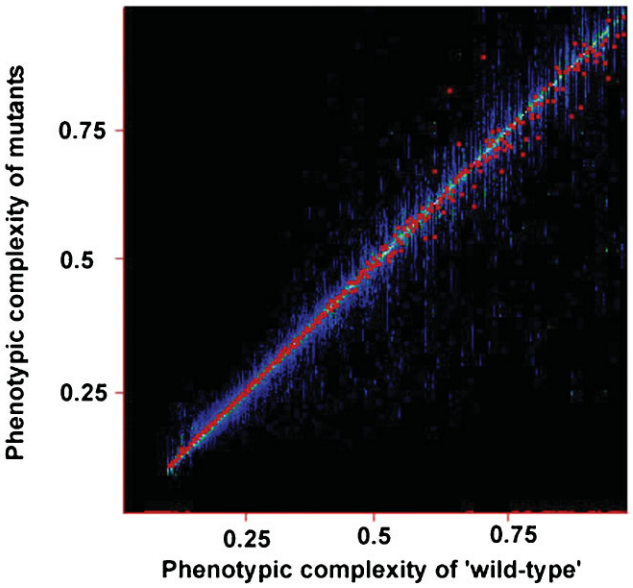


Fig. 1. Density plot of the complexity of wild types against its neighbors. The colors in the graphic indicate the density of teeth per each interval (box) of wild type and mutant complexity. These intervals are of 0.01 in complexity. This density is divided by the number of teeth in each wild-type interval. The red squares represent the averaged mutant complexity for each interval of wild-type complexities. $r^2 = 0.961$, $r$ Spearman rank order test, $P < 0.001$.

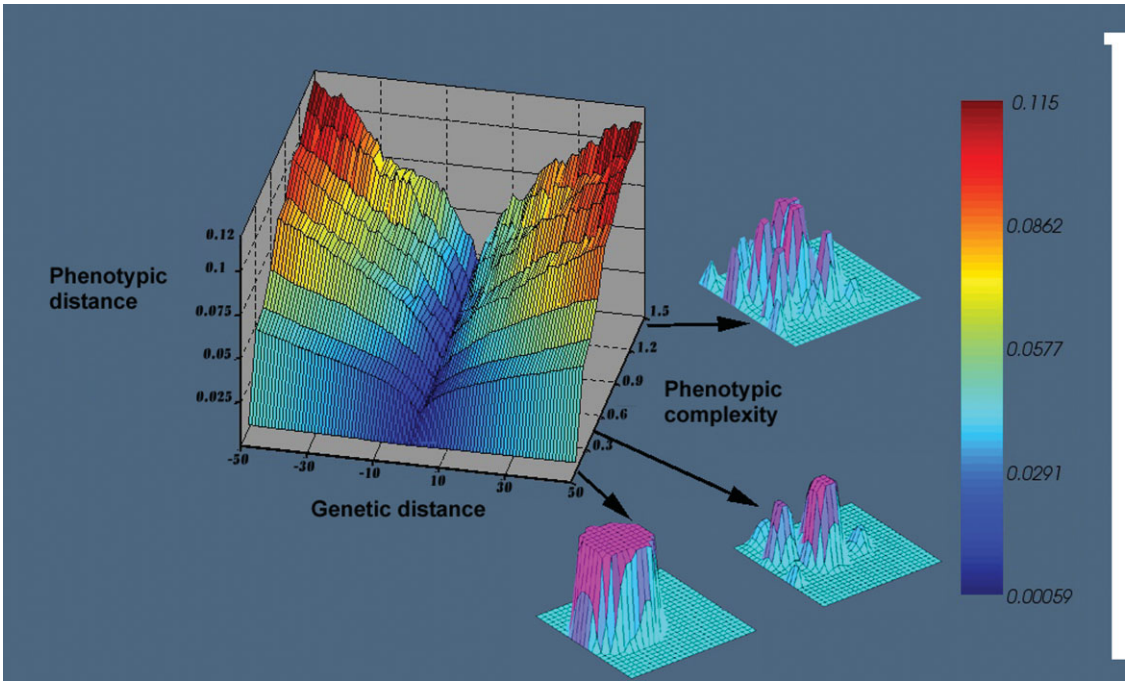


Fig. 2. Phenotypic distance between each type teeth and their neighbors. Phenotypic distances are averaged over all the teeth that have the same genetic distance (here the genotypic distance is counted as the order of the mutant (from −50 to 50)) to the wild type and the wild types between intervals of complexity of 0.15. The small plots are simply example teeth produced by the model and showing the pointed complexity.

types between intervals of complexity of 0.15. The small plots are simply examples of teeth produced by the model showing specific complexity values. It is clear from the figure that simple wild-type teeth have neighbors that are more similar to them. The disparity among neighbors is larger for complex teeth. It is important to note that this relationship does not appear by the nature of the measure itself. Distance and complexity are independent measures. This result indicates that the hypervolume in parameter space around each tooth that has a phenotypically similar tooth is, on average, much smaller for complex teeth than for simple teeth. Essentially, the figure shows that the variation produced from complex teeth is much less gradual than that produced from simple teeth.

This does not preclude that there is still an equally simple relationship between genotype and phenotype in complex and simple teeth. It would be possible, for example, that in both kinds of teeth the phenotypic distance increases linearly with genetic distance. In fact, Fig. 2, because it is made of averages, does not indicate how the relationship between genetic and phenotypic distance is per each wild-type tooth. To explore this possibility, the ruggedness of the map between genotypic and phenotypic distances has been measured for each wild type. The ruggedness is measured for each wild type by comparing the genetic and phenotypic distance between it and its mutants. The sum of the differences in distances to the wild type between consecutive neighbors is divided by the sum of all the distance to the wild type. This is equivalent to ploting, per each wild type, the genetic and phenotypic distances and calculating the ratio between the perimeter of the contour made by joining all the points of the map and the area enclosed between the contour and the axes. As before, the genetic distance is counted as the order of the mutant (from $-50$ to 50). For each wild type, this measure is divided by the value that this ruggedness measure will have if the contour is a straight line (with the maximum phenotypic distance observed for mutants with maximum genetic distance to the wild type. This measure has the advantage that reflects the complexity or ruggedness of the relationship between phenotype and genotype without assuming how this relationship is (linear, quadratic, etc.). The normalization allows the comparison with the linear case (which would have a value close to 1). This measure is just a two-dimensional analog for our complexity measure but applied to the map between the genotype and the phenotype.

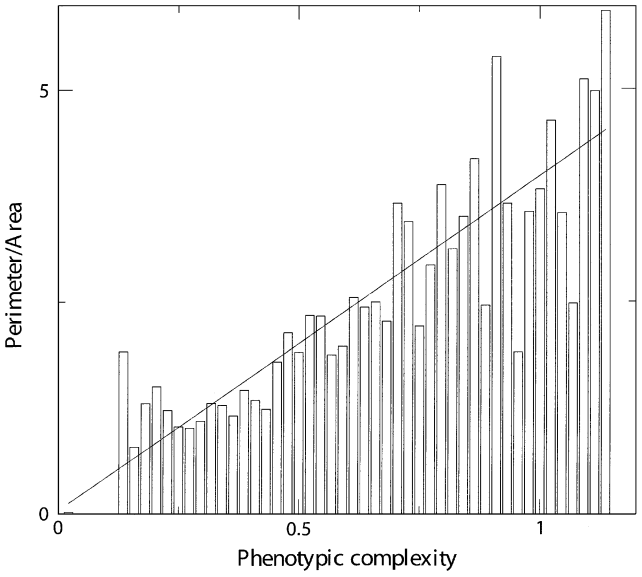


Fig. 3. Ruggedness of the relationship between genetic and phenotypic distance against the phenotypic complexity of wild-type teeth. The values are averaged for all the wild-type teeth in each 0.03 complexity interval because otherwise there are too many points. $r^2 = 0.792$, $r$ Spearman rank order test, $P < 0.001$.

Figure 3 shows the value of this measure averaged for complexity intervals. The figure clearly shows that the ruggedness of the relationship between genotype and phenotype increases with phenotype complexity and is only linear for very simple teeth.

To have a more qualitative and understandable evaluation of the significance of this difference in phenotypic distances, the differences in crown type between wild type and mutants have been measured. The crown-type-based phenotypic distance between two teeth is the sum of the difference between the four numbers characterizing a tooth crown type. A distance of one, for example, means that one tooth has one cusp or loph more than the other. This is a considerably large distance that in functional terms can have dramatic consequences (Jernvall et al., '96). In Fig. 4, the average crown-type distance between wild type and mutants for wild-type teeth in complexity intervals of 0.03 is plotted against the complexity of the wild type. The figure shows that the likelihood of crown-type changes increases with the complexity of the wild type.

A qualitative analysis of the variation produced indicates that simple and complex teeth vary in quite different ways. In all teeth, at least one of the taller cusps forms in the center of the grid. This is because of the dynamics of the model, but it seems to be the case also for real teeth (Butler,

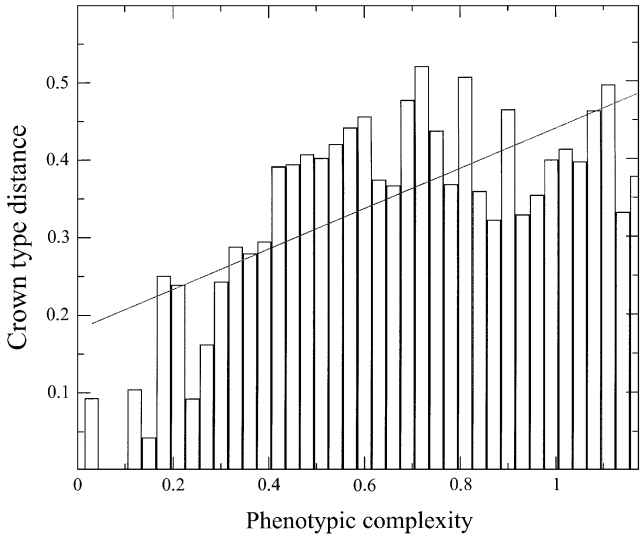


Fig. 4. Crown-type distance between wild type and mutants averaged for all wild-type teeth in complexity intervals of 0.03 plotted against the complexity of the wild type. $r^2 = 0.476$, $r$ Spearman rank order test, $P < 0.001$.

'56; Jernvall, 2000). In complex teeth there can be several cusps of roughly the same height. In simple teeth instead, there are few cusps and there is normally one that is much taller than the rest. Short cusps vary more in position, height and shape. The taller a cusp is, the more unlikely it changes its shape, height and, especially, position. In simple teeth, variation consists essentially in variation in the size and sharpness of the higher cusp. In complex teeth instead, variation can give rise to teeth that visually look quite different from the wild type. This can be, for example, changes in the number and relative positions of high cusps that alter the whole morphology of the tooth. These observations are totally consistent with the larger disparity existing among complex teeth in the model.

## DISCUSSION

This study shows that the kind of variation that can be produced by a developmental mechanism can vary substantially for different types of teeth. In summary, in the model complex teeth produce, comparatively, less gradual variation with a complex relationship between genotype and phenotype. Variation consistent with the neo-Darwinian assumptions is only found for very simple teeth (essentially one cusped teeth). This variation is due to the differences in parameter values but it can also be related, to some extent, to aspects of the phenotype produced. In other words, the degree of ''gradual'' variation produced by mutating a parameter value is strongly dependent on the complexity of the tooth that a set of parameter values produce in the first place. This is an effect of the phenotype itself because there is no clustering among the hypervolumes of the parameter space that can produce complex teeth (data not shown) and, as explained, the network topology is the same in all the simulations. This effect of the phenotype has already been suggested to be common among morphodynamic mechanisms when they produce complex phenotypes (Salazar-Ciudad et al., 2003). This work shows these suggestions in the dynamics of simulated morphodynamics mechanisms. This phenomenon can be explained by taking into account that in morphodynamic mechanisms the phenotype at intermediate stages in development can be considered as a causal factor directing development. This is because signaling and morphogenetic mechanisms are taking place simultaneously and in an interdependent way that needs to be considered to understand the dynamics of morphogenesis. These morphological changes are due to changes in cell behavior like adhesion, mitosis, apoptosis, etc., that are affected by the signals received (see Salazar-Ciudad et al. (2003) and Salazar-Ciudad and Jernvall (2004) for a more detailed discussion about these mechanisms). Thus, in a sense, the morphology at every time instant is affecting how signaling and form changes are taking place. This view seems to be close to the role that Waddington suggested for the epigenotype in shaping the phenotype (Waddington, '56). Complex teeth are, normally, more complex in intermediate stages than simpler teeth, resulting in a more complex interdependence between signaling and morphogenesis. In developing complex teeth, any small change can have large effects on subsequent development because the phenotype itself is not gradual (the teeth themselves are more rugged).

The variational properties that simple teeth produce by the morphodynamic model are very similar to those found by the morphostatic model (Salazar-Ciudad and Jernvall, 2004). This is not surprising because the dynamics of morphodynamic mechanisms are affected by the intermediate stages phenotype while morphostatic dynamics are not. For morphodynamic mechanisms, the more complex the intermediate phenotype, the more development dynamics depend on it. In a sense, morphostatic mechanisms, where inductions precede morphogenetic changes, cannot use

the spatial information existing in the intermediate phenotype.

The prediction from our model can be tested against natural variation by relatively simple measurements of it. There are, however, two problems that preclude our predictions to be tested against existing studies on tooth variation. First the prediction concerns variation produced by development and thus should be tested against all the variation produced by development. This discards natural populations in which selection may have acted. A possibility would be to study only variation in newborn individuals because, apart from wear, tooth shape does not change after eruption. Another possibility would be to study industrialized human or laboratory mammal populations in which selection can be presumed to be weaker on tooth phenotype. Similarly, in certain groups of mammals, such as in seals, the functional demands of tooth shape may have been relaxed due to the lack of occlusion (Jernvall, 2000). Studies like that exist in systems other than teeth, mainly in *Mus musculus* mandible (Klingenberg and Leamy, 2001; Klingenberg et al., 2003) and *Drosophila melanogaster* wings (Pezzoli et al., '97; Gilchrist et al., 2000; Klingenberg and Zaklan, 2000), but do not address the questions of this article although their data may provide valuable information in this sense. To our knowledge, no study has directly compared the variation on complex and simple phenotypes produced from the same developmental mechanisms. However, some studies in *Martes* show that the number of discrete variants (morphotypes) is larger for molars than for premolars (Wolsan, '89). Molars in *Martes* are more complex than premolars and the number of discrete variants is the equivalent to the probability of producing disparate variation (otherwise the variant would not be classified in a different morphotype than the ''wild type''). This pattern of variation fits our results. More in general, we expect that non-gradual changes like variation in the number and position of cusps (especially the small ones) are larger in molars than in premolars, canines or incisors because they are more complex.

Another issue is the way in which morphology is measured. In this study, morphology consists in a grid of equally spaced (in the *xy* plane) points with height values. It is thus a multidimensional measure that keeps many of the proportions of the teeth. In contrast, many studies summarize morphology in one or few arbitrarily defined landmarks. If the number of landmarks is small, these studies could not be used to test our hypothesis. Both types of measurements may give rise to totally different conclusions even when dealing with identical morphologies. Studies reporting that phenotypic variation is gradual in populations and evolution are normally using this kind of low-dimensionality measures (Fisher, '30; Wright, '78; Falconer, '96; Brakefield, 2003). It is quite significant that the only teeth that produce gradual variation in our model are simple one-cusped teeth. For this kind of teeth, univariate measures can grasp morphology accurately enough. On the contrary, non-gradual variation is found for complex teeth (that have multiple cusps) whose morphology would be missed unless multivariate measures are used. Views supporting graduality may be influenced by simplistic measures of morphology or focuses on simple morphologies. Multivariate studies on complex morphologies may not be so common because of its higher difficulty and because the assumption of a relatively simple relationship between phenotype and genotype may suggest that univariate measures of simple or complex phenotypes can be extrapolated to understand complex phenotypes. On the other hand, univariate studies can support the assumption of a simple relationship between phenotype and genotype. It is significant in this sense that in spite of the long tradition of the study of morphological variation, the methods to analyze multivariate morphology are relatively recent and still in development (Bookstein, '82; Polly, 2001; Klingenberg, 2002).

Our study also suggests that development may produce different likelihoods for character transitions used in evolutionary taxonomy. Particularly for complex phenotypes, phylogenetic reconstructions giving equal likelihoods to all character transitions may produce inaccurate results. For complex phenotypes, considerably large character state transitions may be likely (although in each case development determines few possible ones), as it has already been found in mouse that have altered levels of a single signaling molecule (Kangas et al., 2004). This may make phylogenetic reconstructions based on complex teeth less reliable, while at the same time complex teeth are also more likely to have more easily identifiable characters. It is also possible that in mammals with complex teeth, developmental mechanisms, such as morphostatic, have evolved to temper the variation.

Our simulations implement only one developmental mechanism and consequently do not

provide any useful information about how developmental mechanisms or genetic architecture may evolve. This paper studies the phenotypic variation produced by small mutations that do not affect the topology of the genetic networks involved in tooth morphogenesis. Other studies have addressed the problem of the evolution of developmental mechanisms (Salazar-Ciudad et al., 2001a, b; Salazar-Ciudad and Jernvall, 2004).

The above results have implications on how complex and simple phenotype evolve. Evolution from complex phenotypes can be expected to be punctuated. But here, in contrast to the punctuated equilibrium hypothesis (Gould and Elredge, '77), the punctuation could be observable at a microevolutionary scale. If selection is conservative, long periods of stasis are expectable. When evolutionary change would happen, it would be due to relatively (compared to simple teeth) large phenotypic changes. In other words, since small phenotypic changes are relatively unlikely, changes will occur only when environment changes in a way that does not negatively select these comparatively larger variants. Under very permissive selective regimes, like for example when new unoccupied habitat becomes available, complex phenotypes would easily give rise to an explosion in disparity that would be difficult to phylogenetically reconstruct based on morphology.

Simple phenotypes can evolve in a much more gradual way. Since they exhibit a simpler relationship between phenotype and genotype, they can adapt faster and in a finer way to selective pressures that filter for largely divergent phenotypes. For small time periods, rates of evolution would often be larger than in complex teeth. However, since the probabilities of phenotype reversal are much higher, and the likelihood of substantial innovation very low, for large time intervals the evolutionary rates would be disproportionately small. Under very permissive selective regimes, lineages with simple phenotypes would be less likely to produce sudden increases of disparity and thus may occupy a smaller proportion of available ecospace compared to complex phenotypes (essentially because they can occupy only a smaller proportion of morphospace).

Obviously, simple phenotype organs can evolve into complex ones and vice versa. In fact, radiations starting with species with relatively simple phenotypes in an organ and progressively exhibiting species with more complex organ phenotypes are not rare. Of course, this increase in complexity does not need to be due to complexity per se. Our study suggests some important predictions on those kinds of radiations. If complexity increases over time in some lineages, then those lineages can be seen as evolving in an available morphospace that is changing in form (see Fig. 5). How this form is in each moment and how it relates to the genospace affects the evolution of such lineages. It can be expected that in lineages increasing in complexity, and in the radiations they participate, there is at some point an acceleration in the production of disparity. This is due to the more disparate variation that appears from complex phenotypes produced by morphodynamic mechanisms. This possibility is inherent to the developmental mechanisms used but its realization may need to be allowed, of course, by existing selective pressures. At some point, complex phenotypes may become too complex to produce variation that is not too disparate to be adaptive, thus producing a deceleration or stopping of innovation rates, in a sense creating a ''complexity trap''. By too disparate we mean that most variants appearing in a population would be too often too different from the wild type to produce adaptive changes. This would lead to stasis and lineage pruning by extinction. Here replacement between morphologically similar species may be more common and innovation rare. In these radiations selection may

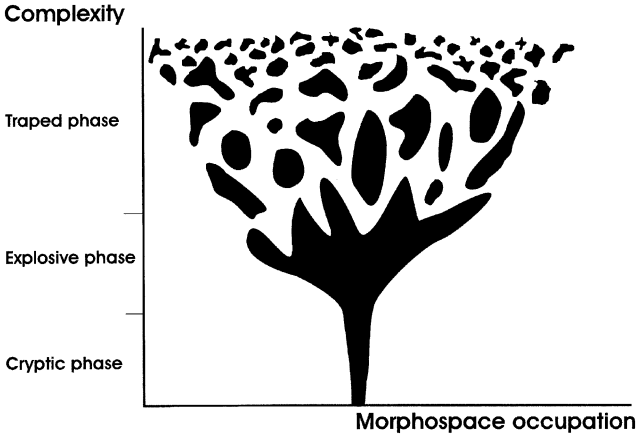


Fig. 5. Schema representing how the phenotypic disparity changes through time in those radiations in which complexity increases. There are three temporal phases as indicated in the text. A first ''cryptic'' phase where complexity is low and morphological evolutionary rates lineal. There is also an explosive phase where complexity is large enough to allow an acceleration of the rate of production of phenotypic disparity. Finally, when complexity is large enough, most variants are too different from the wild types and the morphospace is occupied in a less gradual way (it is occupied in small discontinuous islands).

have determined at which time, and after which complexities, acceleration and deceleration took place. These predictions are consistent with, and can explain, some general trends in the disparity over time of some fossil groups. Thus it has been found (Foote, '91, '92, '95, '97; Briggs et al., '92; Erwin, '94; Wagner, '95; Jernvall et al., '96; Labandeira and Eble, 2004) that disparity increases in a relatively sudden way some time after the origin of some groups. Also, as expected from our work, the amount of disparity remains constant or decreases after this acceleration period. These relationships lead to expect a specific relationship between patterns of disparity and species diversity. Lineages can split at any point in the beginning but its identification as morphologically distinct species (and consequently its identification in the fossil record) may be more likely and massive during the acceleration phase of the radiation. Those same studies (Foote, '91, '92, '95, '97; Briggs et al., '92; Erwin, '94; Wagner, '95; Jernvall et al., '96; Labandeira and Eble, 2004) have found that, indeed, maximum speciation takes place during, or slightly after, this accelerated disparity evolution. Major taxa within the group also appear during this period.

The stasis expectable in complex phenotypes can be described as a morphospace trapping or a complexity trap because the morphospace for complex teeth is partitioned in very small islands in which morphologies are similar. Close islands have rather different morphologies and thus it is unlikely that populations can jump between them and not be maladapted. Similar phenomena have been observed in simulations of the relationship between genotype and phenotype in RNA molecules (Fontana and Schuster, '98; Stadler et al., 2001).

It has been suggested that under some selective regimes, morphostatic mechanisms may replace morphodynamic mechanisms in the production of a phenotype because they allow a simpler relationship between phenotype and genotype and, consequently, a faster adaptation (Salazar-Ciudad et al., 2003; Salazar-Ciudad and Jernvall, 2004). There are other reasons by which complex phenotypes may often exhibit stasis. These are not due to the developmental dynamics by which phenotypes are produced, but to the number of interactions at the genetic (Kauffman, '93; Duboule and Wilkins, '98), physiological or anatomical level (Whyte, '65; Wake, '91; Schwenk, '95) necessary for complex systems to operate (Solé et al., '99). This entrenchment, or internal selection, is very strong in morphostatic mechanisms producing complex phenotypes because they require much more gene interaction. This suggests that whichever developmental mechanism is used, at some point, phenotypes become too complex to further evolve. Our suggestion would be that at some point the only possibility for further change would be to simplify the phenotype (on which new or already existing developmental mechanisms would be able to produce more gradual variation) or produce new unrelated developmental mechanisms acting on existing phenotypes. Interestingly, the origins of some major groups have been suggested to implicate some dramatic simplifications of the phenotype (Garstang, '28; de Beer, '58).

Our study suggests that complex and simple phenotypes evolve in different ways. Microevolution and macroevolution may be understood under the same light but without the mentioned neo-Darwinian assumptions, at least for complex phenotypes. In fact, possible disagreement between population genetics studies and morphology-based macroevolutionary studies about the tempo and mode of evolution (and about the magnitude and nature of evolutionary change) may be due to focuses on qualitatively different kinds of phenotypes rather than to the existence of different rules at different scales. This may not preclude the existence of different processes at macroevolutionary and microevolutionary time scales but may provide a unified explanation for both scales.

## ACKNOWLEDGMENTS

This work was supported by a Marie Curie fellowship (HPMF-CT-2002-01720) to I.S.-C. and Academy of Finland grants (SA-1207547) to J.J. and (SA-1208582) I.S.C.

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