

Phenotypic and dynamical transitions in model genetic networks I.

Emergence of patterns and genotype-phenotype relationships

I. Salazar-Ciudad,^{a,b,*} S. A. Newman,^c and R. V. Solé^b

^aComplex Systems Research Group, Department of Physics, (FEN) Universitat Politècnica de Catalunya, Campus Nord, Modul B5 08034 Barcelona, Spain; ^bDepartament de Genètica, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028, Barcelona, Spain; and ^cDepartment of Cell Biology and Anatomy, Basic Science Building, New York Medical College, Valhalla, NY 10595, USA

*Author for correspondence (email: isaac@complex.upc.es)

SUMMARY Genotype–phenotype interactions during the evolution of form in multicellular organisms is a complex problem but one that can be aided by computational approaches. We present here a framework within which developmental patterns and their underlying genetic networks can be simulated. Gene networks were chosen to reflect realistic regulatory circuits, including positive and negative feedback control, and the exchange of a subset of gene products between cells, or within a syncytium. Some of these networks generate stable spatial patterns of a subset of their molecular constituents, and can be assigned to categories (e.g., “emergent” or “hierarchic”) based on the topology of molecular circuitry. These categories roughly correspond to what has been discussed in the literature as “self-organizing” and “programmed” processes of development. The capability of such

networks to form patterns of repeating stripes was studied in network ensembles in which parameters of gene-gene interaction were caused to vary in a manner analogous to genetic mutation. The evolution under mutational change of individual representative networks of each category was also simulated. We have found that patterns with few stripes (≤ 3) are most likely to originate in the form of a hierarchic network, whereas those with greater numbers of stripes (≥ 4) originate most readily as emergent networks. However, regardless of how many stripes it contains, once a pattern is established, there appears to be an evolutionary tendency for emergent mechanisms to be replaced by hierarchic mechanisms. These results have potential significance for the understanding of genotype-phenotype relationships in the evolution of metazoan form.

INTRODUCTION

Over the course of hundreds of millions of years, evolution has produced both phenotypic complexity and the developmental mechanisms by which such complexity is generated. Evolutionary theory states that individuals in populations exhibit heritable phenotypic variations that affect their chance of being perpetuated in the next generation. The wealth of available information on variation at the DNA level has permitted the application of empirically confirmed theories of population genetics to molecular evolution. But whereas molecular biology can identify genetic contributions to phenotypic variation, most phenotypic characteristics in metazoan organisms are generated during development, not by individual genes acting alone but by networks of interacting gene products distributed in a complex pattern over space and time. The phenotypic changes that a given mutation can generate in an organism thus depend on the developmental mechanisms present in that organism. The relationship between genotype and phenotype is therefore complex and nonlinear, precluding a direct extrapolation from evolution at the genetic level to the evolution of, for example, morphology.

These considerations imply that in order to understand the evolution of form, understanding the mechanisms of development is required. Much earlier work has been devoted to studying the range of variation that may arise from alteration of specific developmental processes. Empirical studies are available on morphological variations in natural populations (Alberch 1980; Jernvall 2000; Shubin et al. 1995; Wright 1912), as well as studies comparing such variations, or those found between closely related species, with those induced by mutations or by experimental manipulation of development (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 coherently empirical data on the mechanisms that act in specific developmental processes and to predict the morphological variations found within those processes (Oster et al. 1988; Ho 1990; Goodwin 1994). Further, the relevance of such variational properties in constraining morphological evolution has also been discussed (Alberch 1982; Goodwin 1994).

These studies have mainly been concerned with the morphological variation produced when a parameter or aspect of a defined developmental process is altered, but have not ad-

dressed the question of how the developmental processes themselves can be generated or changed. The relevance of such an approach to the evolutionary process is therefore confined to the interval of time in which the nature of the developmental processes being considered remain unmodified by evolution, although it has been well recognized that forms and other phenotypic characters may persist while their underlying generative mechanisms evolve (Wagner 1989; Wagner and Misof 1993; Müller and Wagner 1996; Müller and Newman 1999).

Few previous studies directly address how developmental processes in and of themselves may change. However, there is now a great deal of information available on the genes and gene networks used by different organisms in the generation of homologous or analogous structures. Well-studied examples include anteroposterior and dorsoventral patterning of the insect body (Lipshitz 1991), gastrulation in vertebrates and invertebrates (Leptin 1999; Tam and Behringer 1997), formation of invertebrate and vertebrate appendages (Ng et al. 1999; Panganiban et al. 1997), and segmentation (Davis and Patel 1999). It can be concluded from such work that most developmental processes in present-day organisms are complicated and highly integrated. But it is far from certain that ancient organisms used the same complex mechanisms as their modern counterparts to produce homologous morphological structures (Newman 1994; Newman and Müller 2000). The fact that the developmental systems used to generate very similar structures can differ even between closely related species (Wagner and Misof 1993; Müller and Wagner 1996; Felix and Sternberg 1997; Müller and Newman 1999), and that patterns and forms can be generated by various independent mechanisms acting at roughly the same time (Wang and Sternberg 1999; Felix 1999; Tautz 1992), suggest that originating mechanisms often differ from evolutionarily derived ones.

The present study uses realistic model genetic networks, acting in the context of a multicellular system, to explore such questions as (1) which categories of pattern-forming mechanism are most likely to arise in the course of evolution, (2) which are most closely tied to the generation of certain types of morphological outcomes, and (3) which are most readily transformed into other categories of mechanism by gene mutation. Of course, which molecular mechanism is responsible for a given developmental outcome can only be answered definitively by direct experimentation. But the strategy adopted here is highly suitable for integrating such empirical data into evolutionary scenarios that can be simulated and computationally tested.

Our results indicate that certain variational properties of developmental mechanisms can provide insight into the central problems of developmental evolutionary biology, which we have outlined here. The properties we have analyzed are characteristics of model genetic networks that accurately re-

flect molecular features of well-studied developmental systems (see the accompanying article on segment formation in *Drosophila melanogaster* for a detailed example). By varying these model network properties in ways analogous to those by which natural genetic networks are altered by mutation, we have determined the following: the likelihood that specific types of mechanisms may arise; the characteristics of the phenotypes that can be generated by such mechanisms (e.g., the type, extent, and graduality of the phenotypic variation resulting from genotypic variation); and the degree of relatedness between genotype and phenotype. As we will show, these characteristics are mainly related to the internal logic of such mechanisms, and are thus biologically relevant despite their necessary simplifications.

We have focused our study on patterns that can arise from cells interacting through diffusible paracrine molecules. Two main classes of developmental mechanism have been suggested to employ such interactions: reaction-diffusion processes (Meinhardt 1982), in which a small number of reciprocally interacting molecules can produce patterns triggered by small deviations of a homogeneous initial state, and more hierarchic mechanisms, in which a generally larger number of gene products are organized into largely unidirectional pathways that generate pattern from an initially heterogeneous state. Each of these classes of mechanism has been found to underlie the formation of actual morphological patterns (see Jiang et al. 1999; Miura and Shiota 2000a, 2000b for reaction-diffusion processes, and the accompanying article for hierarchic mechanisms). These mechanisms can generate many of the same patterns, and indeed reaction-diffusion mechanisms have been shown to be capable of producing realistic patterns (Lacalli et al. 1988; Hunding et al. 1990) in a system in which hierarchic mechanisms have been experimentally demonstrated. Our results suggest that these two mechanisms are more often found in different evolutionary and developmental contexts. However, rather than confining our analysis a priori to these two classes of mechanism, we have addressed the question of whether they are the only classes that are likely to arise in the course of evolution and whether they appear in different evolutionary moments or contexts.

We have used two complementary approaches: In the first approach, we simulate an evolutionary process in which a population of networks is generated by mutation of a parental network and undergoes selection on the basis of particular patterns they generate. By this means we show that, in fact, types of networks may replace one other during evolution. In addition, this kind of simulation provides a characterization of the range of mechanisms that can produce patterns in the course of evolution. In the second approach, we take representative examples of each of the network types found and study their structural and variational properties in order to infer the reasons for the observed genotype-phenotype relationships and evolutionary substitutions.

Our general conclusion is that differences between distinct classes of developmental mechanism can be so extensive that probabilistic inferences about their relative roles in stages of evolution and development can be made in spite of the historical contingencies affecting all evolutionary processes.

THE MODEL

The model considers N_c cells arranged in a line (in which case it is assumed that all paracrine factors are secreted into the extracellular space), or N_c nuclei arranged in a line in a syncytium (e.g., along the anteroposterior axis in an insect embryo). Because these are formally identical, we will mainly refer to the syncytial case below. Each cell, having the same genome, has the same set of N_g genes and the same relationships between them. Genes in these networks interact according to a set of simple rules that simulate the most typical mechanisms by which real genes regulate one another (see Fig. 1). In fact, a similar model has been used to successfully reproduce segmentation gene patterns in the *Drosophila* embryo (Reinitz and Sharp 1995).

Gene products interact by binding (or by other potentially activating changes such as phosphorylation, proteolytic

cleavage, and so forth) to other gene products or by binding to *cis*-regulatory sequences on gene promoters. N_p of the genes are paracrine factors that can diffuse. The model assumes that the change in the activity, or chance of transcription induced by interaction, follows a saturating Hill function (a class of function widely used for molecular binding processes: see Cornish-Bowden 1979). However, the network topologies and properties also hold for most monotonically increasing functions that saturate. A more detailed characterization of the model can be found in Salazar-Ciudad et al. (2000). Enhancers and protein binding sites are characterized by a value W_{jk} that weights both the affinity of the transcriptional factor for the enhancer and the intensity of the response produced by the binding.

These networks are modeled by a dynamic system obeying the following set of equations:

Non-paracrine gene products

$$\frac{\partial g_{ij}}{\partial t} = \frac{\Phi(h_{ij})}{k_M + \Phi(h_{ij})} - \mu_j g_{ij}$$

Where $h_{ij} = \sum_{k=1}^{N_g} W_{jk} g_{jk}$ (1)

and $i = 1, \dots, N_c$ and $j = N_p + 1, \dots, N_g$

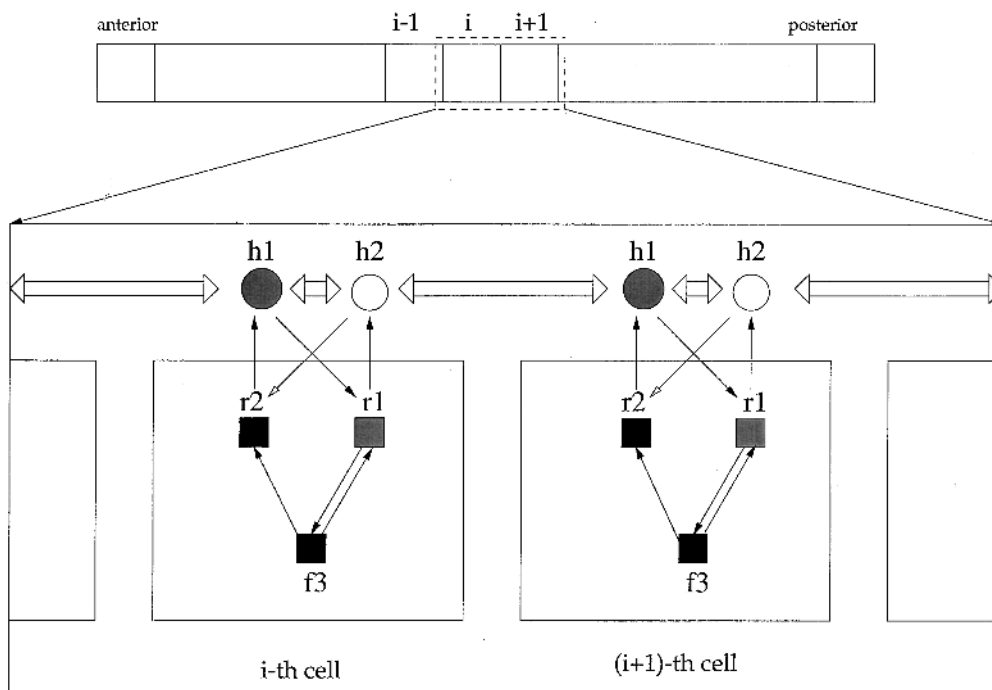


Fig. 1. The kinds of interactions included in the model are shown. The boxes are genes acting inside cells whereas circles are diffusible paracrine factors. Abbreviations: *h* designates a hormone or paracrine factor, *r* a receptor, and *f* a transcriptional factor. Arrows with black arrowheads indicate positive interactions and arrows with white arrowheads indicate inhibitory interactions. Thick horizontal arrows indicate diffusion.

Paracrine gene products

$$\frac{\partial g_{il}}{\partial t} = \frac{\Phi(h_{il})}{k_M + \Phi(h_{il})} - \mu_l g_{il} + D_l \nabla^2 g_{il}$$

$$\text{Where } h_{il} = \sum_{k=1}^{N_s} W_{lk} g_{lk} \quad (2)$$

and $i = 1, \dots, N_c$ and $l = 1, \dots, N_p$

The symbol Φ is the Heaviside function, which is introduced to ensure that inhibiting interactions do not lead to negative concentration values, $\Phi(x) = x \forall x > 0$ and $\Phi(x) = 0$ otherwise. The symbol μ is a factor related to the intrinsic rate of degradation affecting all gene products, and k_M , the binding rate constant. D_l is the diffusion coefficient for the gene product g_l . Zero-flux boundary conditions are used in all cases. At $t = 0$ all gene product levels have zero value except an arbitrarily chosen gene, which is the same in each network, in the cell at the central position (located at $(N_c/2) + 1$). We note that although this initial condition is not a gradient (as in the syncytial *Drosophila* embryo), a gradient appears shortly after $t = 0$ in many systems. The systems used in these simulations had 25 cells or nuclei. Other possibilities were considered in Salazar-Ciudad et al. (2000). Although the model is highly simplified, it embodies a set of rules of interaction that are typical for gene products involved in intercellular communication.

Whereas most real gene products have different binding rate constants and degradation constants, we have found that using different k_M and μ for different gene products does not change the basic network characteristics. We consider that the line of nuclei exhibits pattern if different nuclei express the same gene at different levels; then we call this gene the pattern gene. Of course, there can be more than one pattern gene.

To define two patterns as different we use the following rule: For each nucleus i , we compare the value of the pattern gene in such nucleus with that of the rest of nuclei in the array. We define a variable $\phi(i)$, which we call the state of nucleus i of syncytium j , that is equal to the number of nuclei in the syncytium j that have a value of the pattern gene equal or lower than that in nucleus i . Two patterns are considered to be equivalent if nuclei in analogous positions have the same values of ϕ (that is $\phi_1(i) = \phi_2(i) \forall i$). For example, in a three-nuclei system with the pattern gene taking the values $g_{11} = 0.1$, $g_{11} = 0.9$, and $g_{11} = 0.1$, the pattern will be characterized by $\phi(1) = 2$, $\phi(2) = 3$ and $\phi(3) = 2$.

We have performed two kinds of analysis using this model.

Artificial evolution protocol

In this approach, we consider a population of 100 one-dimensional syncytia that begin with the same genome (i.e., equal W_{jk} values). In each subsequent generation, we estimate the fitness of each individual by the degree of similarity or distance between the pattern they produce and an imposed op-

timal pattern consisting of a variable number of equally spaced stripes. This distance is measured as:

$$d_{jk} = \sum_{i=1}^{N_c} \|\phi_j(i) - \phi_k(i)\| \quad (3)$$

These simple stripe patterns have been found in organisms such as *Drosophila*, where such stripes (of *even-skipped*, *fushi tarazu*, *hairy*, and other pair-rule genes products) provide a prepattern for body segmentation. In the accompanying article, we provide an evolutionary developmental interpretation of our results.

While many genes in an individual can exhibit pattern, we choose the same gene in each individual and generation. In each generation, the 50 individuals with the lowest fitness were eliminated and substituted by copies of the 50 most-fit individuals. We have also tested more realistic modes of selection, in which the number of descendants of an individual in the next generation is proportional to its fitness divided by the sum of the fitnesses of the other individuals (Fisher 1930).

The way in which the genome is implemented allowed us to introduce types of mutations analogous to those found in DNA. These included point mutations (changes in a W_{jk} value), duplications, recombinations (i.e., interchange of various W_{jk} values between genes), and the acquisition of new interactions (i.e., a W_{jk} that was equal to zero acquires a small positive or negative value). Each evolution simulation started at generation one with a population of identical networks. These consist of a transcription factor activating itself as well as a diffusible gene product. This initial network produces a simple one-stripe pattern. In the beginning, 10 genes exist in each individual, although only these two are connected.

The types of networks that produced patterns during different phases of evolution, and the different patterns generated, were analyzed. In a previous paper (Salazar-Ciudad et al. 2000), we identified the two types of network that in such a model can produce pattern. These are ‘‘hierarchical’’ and ‘‘emergent.’’ The generic topological properties characteristic of each of them was also identified.

In each generation, we first identified which part of the whole individual network was responsible for the pattern formed. We did this by eliminating connections and determining if the pattern formed by such a mutated network was identical to the original. After this, we analyzed the topological properties of the sub-network responsible for the pattern to see whether it could be assigned to one of the two types. In a previous paper (Salazar-Ciudad et al. 2000), the topological characteristics of the different classes of networks and the methodology to identify them were presented. Briefly, in emergent networks at least two paracrine gene products are required. At least one of them directly or indirectly increases the levels of expression of both paracrine

products, whereas the other directly or indirectly inhibits the levels of expression of the first paracrine gene product. In hierarchic networks, there are no direct or indirect reciprocal effects between paracrine gene products.

In another set of experiments, we selected for the phenotypic complexity of the patterns produced. The complexity was estimated by using a measure previously presented (Salazar-Ciudad et al. 2000):

$$I = \sum_{i=1}^{N_c-1} \sum_{k=1}^{N_c-1} \sum_{l=1}^{N_c-1} [P[\phi(i) = k, \phi(i+1) = l]] \log P[\phi(i) = k, \phi(i+1) = l] \tag{4}$$

$$+ \sum_{i=1}^{N_c-1} \sum_{k=1}^{N_c-1} \sum_{l=1}^{N_c-1} [P[\phi(i) = k, \phi(i+1) = l]] \log P[\phi(i) = k, \phi(i-1) = l].$$

where $P[\phi(i) = k, \phi(i+1) = l]$ is the probability that a cell takes state k while its neighbor takes state l . This is termed the “spatial join information,” (Salazar-Ciudad et al. 2000) and it precisely indicates the relative entropy of the arrangement between cells with different states.

Static modular approach

In order to more clearly understand the evolutionary transitions between networks that took place in artificial evolution, we studied the variational properties of a large number of the networks that appeared during evolution. Representative examples of each type of network are shown in Fig. 2. To study the variational properties of such networks, we simulated a large number of them (1,000,000) with the same topology but different strengths (W_{ij}) of the interactions between gene products in the network. By this method, we obtained an estimation of some characteristics of the morphospace that each mechanism can explore. The morphospace of our system is a space with N_c dimensions where each dimension corre-

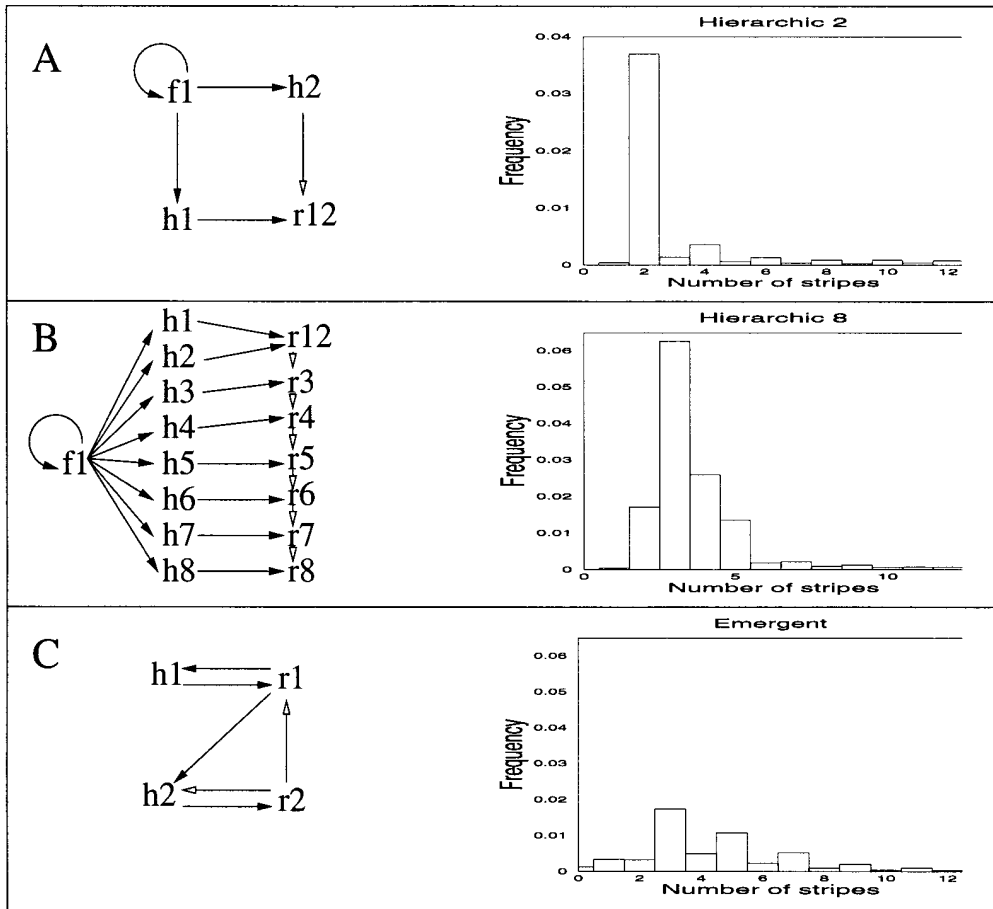


Fig. 2. Three network topologies for which the variational properties were studied. *h*, *r* and *f*, and arrows are as in Fig. 1. Hierarchic 2 and hierarchic 8 refer to hierarchic network topologies with two paracrine factors and eight receptors, respectively. The graphs show the relative frequency of patterns with indicated numbers of stripes that can be attained when specific W_{jk} values are assigned to each of the network topologies.

sponds to the state of a different cell. Thus each possible pattern occupies a unique point in this space.

RESULTS

Likelihood of origination of network types at the molecular level

When selecting for a specific number of stripes (we have performed 100 different simulations for each number of stripes), we found that when the optimal pattern is first attained it is produced by hierarchic networks if it has less than four stripes (Fig. 3). For more than three stripes, most often emergent networks were the ones that produced the optimal pattern (Fig. 3).

When selecting for complexity of the pattern, most often the more complex patterns were produced by emergent networks (Fig. 4). For both types of selective regime, the first patterns to emerge were almost always produced by simple hierarchic networks. These patterns were themselves simple, consisting of one or two stripes. In all the simulations performed only hierarchic and emergent networks were found, in agreement with previous results (Salazar-Ciudad et al. 2000).

Type and amount of morphological variation

In Figure 2 we have plotted the frequency with which patterns with different numbers of stripes are generated by the networks analyzed. A close look at the data in this figure shows that the emergent networks are typically simpler than the hierarchic ones. In fact, the same emergent network may generate any number of stripes. Indeed, we have found that

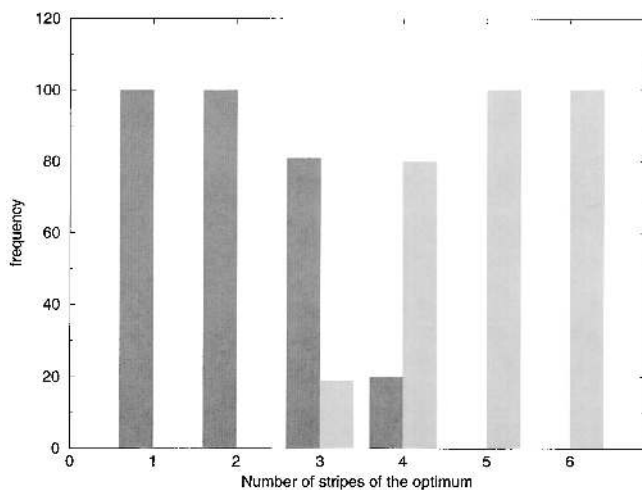


Fig. 3. The number of optimal patterns attained in the artificial evolution experiments by hierarchic networks (black) and by emergent networks (gray). The results shown are for optimal patterns with the indicated numbers of stripes.

simple emergent networks produce more patterns than a simple hierarchic network (results not shown). The range of phenotypic variation with hierarchic networks, however, was largely restricted to patterns with a limited number of stripes.

Probability of breeding true and the relationship between phenotype and genotype

A major issue in the evolution of pattern and form is the capacity of organisms to “breed true”—that is, to produce offspring that are phenotypically like their parents. To address this question, we have randomly taken 100,000 network examples for each mechanism (that is, network topologies with specific W_{jk} values), and we have performed small mutations on these W_{jk} values (of a maximum value of ± 0.33 over the maximum value that a W_{jk} can attain [± 1]), in only one W_{jk} each time. We then simulated the pattern that each mutated network produces. For each of such patterns, we measured its distance in morphospace (d) to the “wild type” pattern produced by the original network.

In Figure 5 we have plotted the relative frequency of all observed distances between the “wild type”-mutated pair patterns in the 100,000 different networks (30 mutations were performed for each network). It is clear that hierarchic networks exhibit shorter distances. In hierarchic networks, moreover, 12% in hierarchic 2 and 18% in hierarchic 8 exhibit the same pattern as the “wild type.” In contrast, this proportion is much smaller in emergent networks. This implies that hierarchic networks more often produce the same patterns when slightly mutated, that is, they breed true more often. The shorter distances between patterns formed from hierarchic networks implies that hierarchic networks have a closer relationship between phenotype and genotype. We note that such changes in W_{jk} values do not necessarily uniquely relate to mutations. Environmental effects—such as local or global changes in temperature, physiological stress, and so forth—that affect the activity of a gene or its product during the formation of a pattern can have the same effect as a mutation (such phenomena are termed “phenocopies”) (Goldschmidt 1938). Interestingly, when only transient environmental perturbations are considered, the patterns produced by hierarchic networks have been found to be more sensitive (Salazar-Ciudad et al. 2000).

Capacity for fine-tuning

Although we have just shown that closely related hierarchic networks produce more similar patterns than closely related emergent networks, these results do not imply that the patterns produced by hierarchic networks are actually closely related in an absolute sense. An important unanswered question is which kind of network can produce more finely tuned patterns—that is, for which category are patterns closer to one another in morphospace? We note that simply because

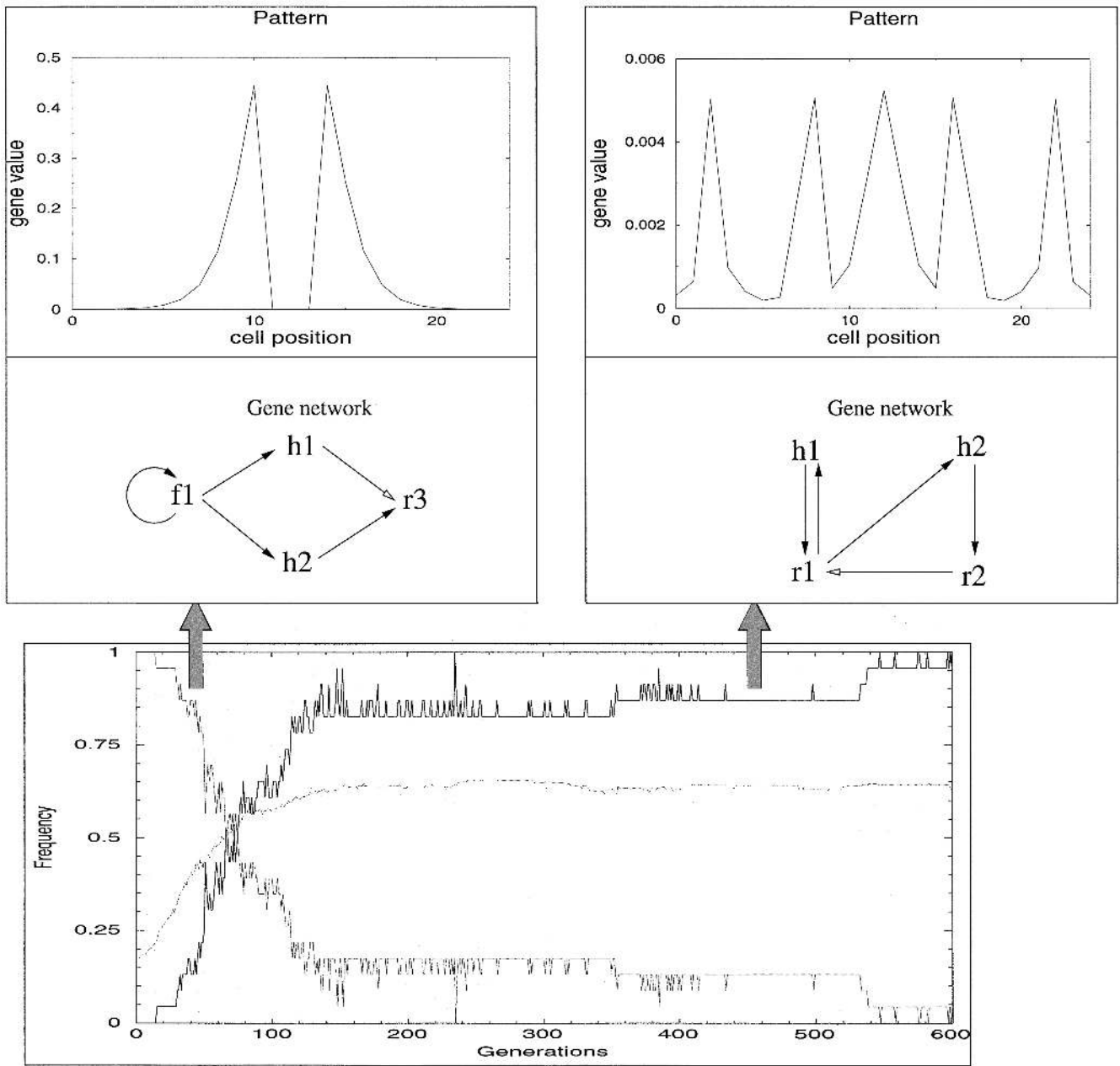


Fig. 4. Relative frequency of hierarchic (continuous line) and emergent (dashed line) networks that appeared over time when selecting for pattern complexity. Data were collected from 500 independent evolution experiments. The dotted line is the pattern complexity of the fittest network averaged over 500 simulations for each generation. For each type of network, a diagram is shown representing a typical network topology. *h*, *r* and *f*, and arrows are as in Fig. 1.

emergent networks have a more varied relationship between genotype and phenotype, this is no guarantee that the range of patterns they produce are more widely distributed in morphospace than patterns produced by hierarchic networks.

We measured the distances between the patterns generated from each type of network irrespective of the degree of similarity of the networks. In Figure 6, we have plotted the

frequencies of such distances for 100,000 different networks of each topology. Clearly, patterns in the hierarchic case are more similar to one another. This implies that hierarchic networks can produce more finely tuned pattern variations than emergent networks. This fact, along with the results shown in Figure 2, suggests that although a single emergent network allows the exploration of more morphospace than a sin-

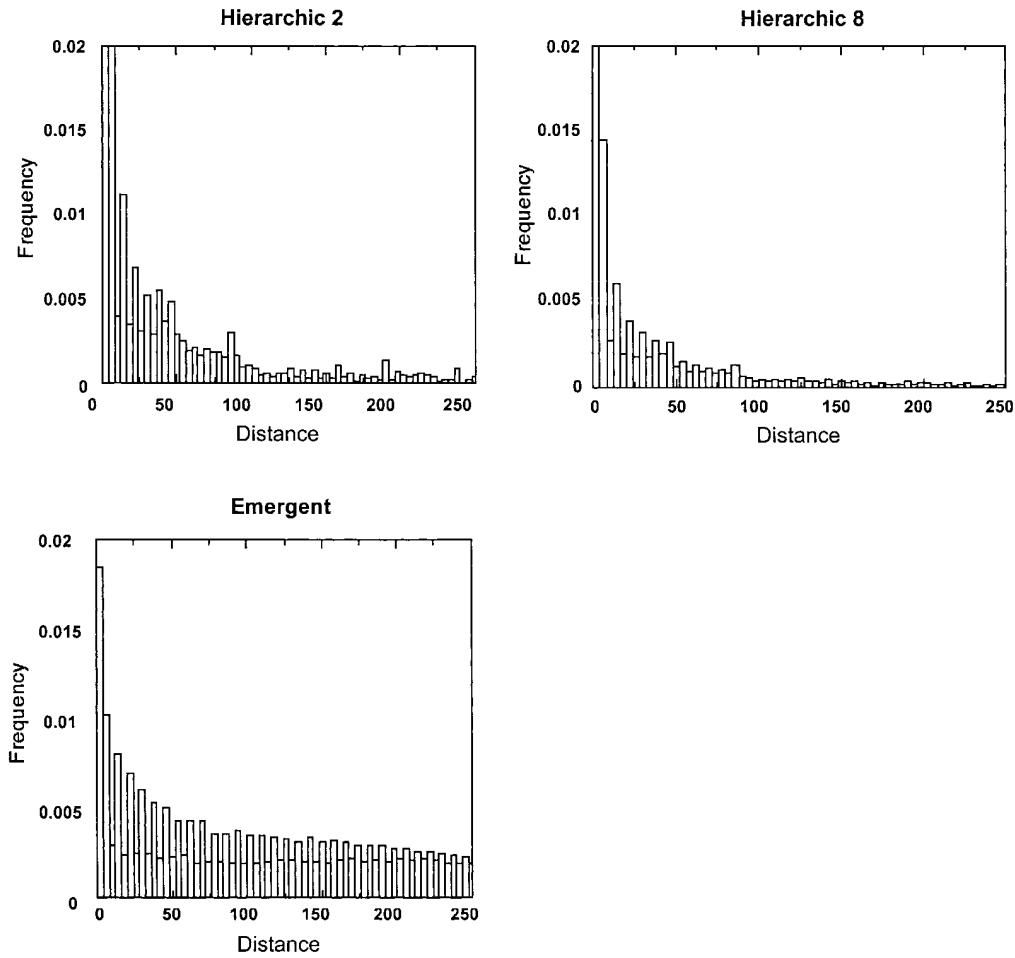


Fig. 5. Estimation of the relationship between genotype and phenotype in the three network topologies shown in Fig. 2. The *x* axis represents the distance between a network and a mutated version. The *y* axis represents the relative frequency of such distances between the 100,000 networks analyzed and 30 randomly mutated versions of each.

gle hierarchic network, the whole set of possible hierarchic networks can explore a larger volume of morphospace than the full set of possible emergent networks; further, each portion of morphospace is more intensively explored by a specific set of hierarchic network topologies.

DISCUSSION

The generation of morphological novelty has been recognized as an important evolutionary problem that is difficult to treat within present neo-Darwinian theory (Goodwin 1994; Gould 1977; Gould and Lewontin 1979). Thus, while there are known cases in which an innovation can appear because of variation in a developmental parameter of relatively well-characterized developmental mechanism (Alberch and Gale 1983; Müller 1990; Nijhout 1991; Streicher and Müller

1992; Belousov 1998; Landini and Iannaccone 2000), such findings do not account for the mechanism by which a morphological structure was originally generated in the course of evolution. In many cases, it seems probable that an already existing mechanism (for example, a network capable of pattern formation) can be recruited in a new developmental context to generate innovation. In fact, in present-day organisms the same networks and genes can be used in different places in the same organism (Martinez-Arias 1998; Bray 1998), or in different species (Wray 1999; von Dassow and Munro 1999; Huang 1998). In such cases, however, the question of how and why this network first appeared remains.

Our results suggest that there are a limited number of genetic mechanisms by which patterns can be attained, in agreement with previous work (Salazar-Ciudad et al. 2000). In fact, only reaction-diffusion and hierarchic mechanisms are involved in the formation of pattern in our model genetic networks, which embody basic interactions typical of devel-

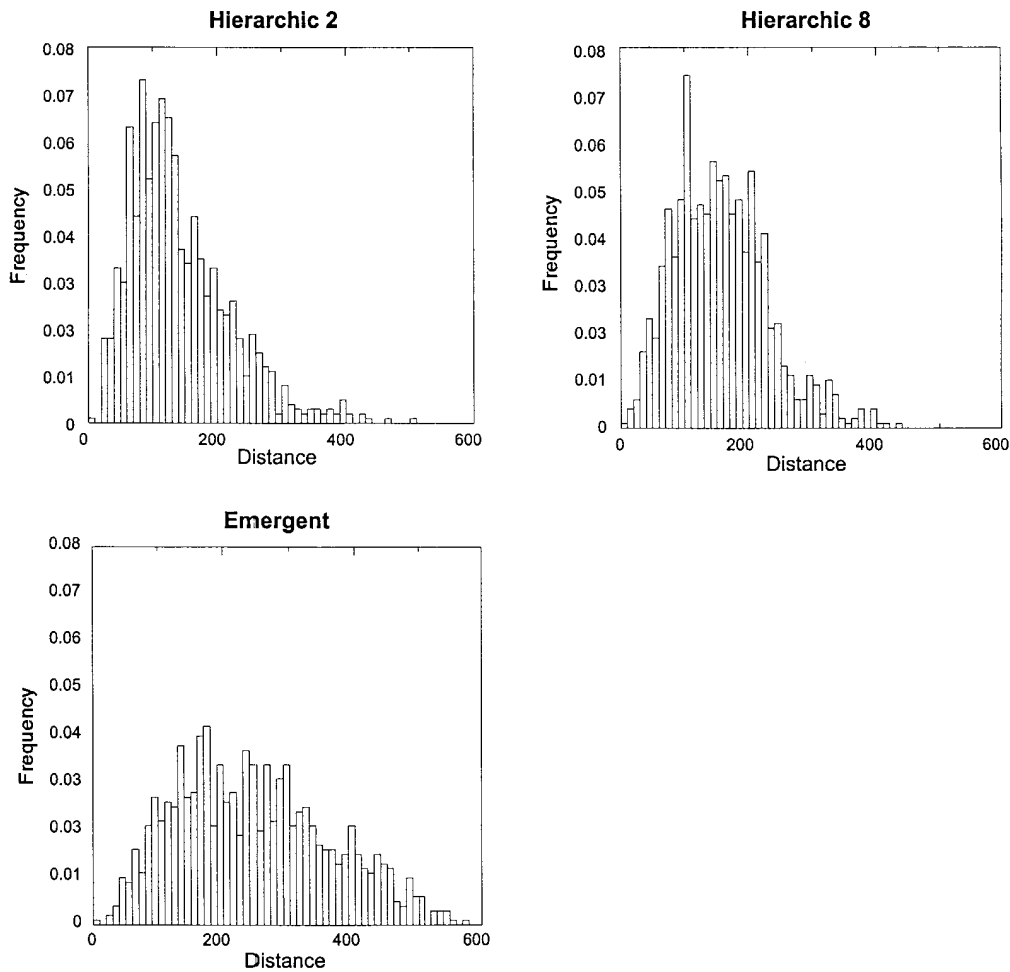


Fig. 6. Degree of fine-tuning allowed by the network topologies shown in Fig. 2. The x axis represents the distances between the patterns found when specific random W_{jk} values were assigned to such network topologies. The y axis represents the relative frequency of such distances.

opmental regulatory mechanisms. These findings imply that many problems in developmental evolutionary biology can be approached by studying the variational properties of such networks as well as other properties related to their likelihood of arising.

From these results, we can suggest reasons why emergent networks are the earliest arising mechanisms that produce complex patterns. This may be due to the fact that the emergent networks required to generate a large number of stripes are simpler at the molecular level than the hierarchic networks required for forming the same patterns (Fig. 2). Thus, emergent networks can produce increasingly complex patterns with a lower complexity at the molecular level. Hierarchic patterns are evolutionarily quicker to produce simple patterns. In fact, the hierarchic networks required to produce simple patterns are simpler (have fewer genes) than any emergent network. In contrast, the hierarchic networks required to produce patterns of a complexity similar to that

achieved by emergent networks have many more genes than the emergent networks (Fig 2). Although hierarchic networks can arguably be combined to form more complex patterns, the required changes at the genetic level ensures that such evolutionary pathways will require many steps (Fig. 2).

We expect that when complex patterns first appear, their underlying genetic networks will be of the emergent type. However, our results suggest that once certain patterns have emerged, there can be many selective pressures favoring substitution of emergent networks by hierarchic networks capable of producing the same patterns. As we discuss in the accompanying article, this may have happened in *Drosophila melanogaster*. This substitution cannot be produced suddenly, because a hierarchic network capable of producing the same pattern as an emergent network requires many genes and connections between them. However, any intermediate step in such a transition may be adaptive in itself. The reasons for this are multiple. First, having two indepen-

dent mechanisms producing the same pattern is adaptive by virtue of buffering against epigenetic noise and environmental perturbation (Newman 1993, 1994; Nowak et al. 1997; Wilkins 1997). Second, once a pattern becomes established, hierarchic mechanisms will produce offspring that more often exhibit the same pattern. Third, with hierarchical networks, a greater degree of fine-tuning of an established pattern to a slightly different pattern with potentially higher adaptive value can be obtained. This capacity for fine-tuning the patterns implies, for example, that hierarchic networks can be utilized to regulate independently the expression of each stripe in a stripe pattern like the ones leading to *Drosophila* segmentation. Finally, hierarchic networks can adapt more rapidly (so would prevail over a potentially emergent competitor) to small changes in the optimal pattern as they exhibit a closer relationship between genotype and phenotype.

In fact, our results suggest that emergent mechanisms may be involved in the generation of many forms and patterns, especially the first time they appear in the course of evolution. However, we would also expect a progressive trend for such networks to be replaced, or at least reinforced, by a hierarchic network producing the same pattern. It may be that in certain cases such transition is not possible, or it has not yet occurred because the time required is too long. For example, reaction-diffusion mechanisms have been suggested in the formation of fruiting bodies of slime molds. In this case, the substitution of an emergent network by a hierarchic network may be precluded because the hierarchic network capable of generating the same pattern would require many additional paracrine factors. In other cases, the variational properties that emergent mechanisms allow may themselves be adaptive. It has been suggested, for instance, that the principal selective pressures affecting butterfly wing coat patterns are coarse grained and mainly related to mimetic and other perceptive effects (Nijhout 1991). In such cases, emergent networks allow a large spectrum of phenotypic variation with the same genotype. In fact, reaction diffusion mechanisms have been implicated in the formation of eye spot patterns in the butterfly (Nijhout 1991), in the coat patterns of many vertebrates (Kondo and Asai 1995; Asai et al. 1999), in feather patterns in birds (Jiang et al. 1999), and in mollusk shells (Meinhardt 1982). It is plausible that the wide range of modifications seen in the vertebrate limb skeleton, all organized around a common format (a phenomenon that Darwin took note of), is a reflection of the generation of this structure, in part, by a reaction-diffusion system (Newman and Frisch 1979; Miura and Shioya 2000a; Miura and Shioya 2000b).

In general, at late stages of development, emergent networks may be more frequent, both because greater morphological variation can be tolerated in peripheral structures, and because some late-appearing structures may be neomorphic (e.g., the digits) and have not been subjected to the same

degree of selection as earlier appearing structures. In contrast, in patterns affecting many subsequent processes in development (the overall body plan, for example), the replacement of emergent networks is more likely (see accompanying article). This may be the case, for example, in developmental processes near the so-called phylotypic stage (Slack et al. 1993; Raff 1996) where few variations are presumed to be possible.

Acknowledgments

This work was supported by grants from the Generalitat de Catalunya (ISC; FI/98-00342), the Comisión Interministerial de Ciencia y Tecnología, CICYT, (RVS; PB97-0693), and the National Science Foundation (SAN; IBN-9603838 and IBN-0083653).

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, Berlin, 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.
- Asai, R., Taguchi, E., Kume, Y., Saito, M., and Kondo, S. 1999. Zebrafish leopard gene as a component of the putative reaction-diffusion system. *Mech. Dev.* 89: 87–92.
- Bray, S. 1998. Notch signaling in *Drosophila*: three ways to use a pathway. *Semin. Cell. Dev. Biol.* 9: 591–597.
- Belousov, L. V. 1998. *The Dynamic Architecture of a Developing Organism: An Interdisciplinary Approach to the Development of Organisms*. Kluwer Academic Publishers, Utrecht.
- Cornish-Bowden, A., 1979. *Fundamentals of Enzyme Kinetics*. Butterworths, London.
- Davis, G. K., and Patel, N. H. 1999. The origin and evolution of segmentation. *Trends Cell Biol.* 9: M68–72.
- Felix, M. A., and Sternberg, P. W. 1997. Two nested gonadal inductions of the vulva in nematodes. *Development* 124: 253–259.
- Felix, M. A. 1999. Evolution of developmental mechanisms in nematodes. *J. Exp. Zool.* 285: 3–18.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Goldschmidt, R. 1938. *Physiological Genetics*. McGraw-Hill, New York.
- Goodwin, B. C. 1994. *How The Leopard Changed Its Spots*. Weidenfeld and Nicolson, London.
- Gould, S. J., and Lewontin, R. C. 1979. The spandrels of San Marco and the Panglossian paradigm. *Proc. Roy. Soc. London B* 205: 581–598.
- Gould, S. J. 1977. *Ontogeny and Phylogeny*. Belknap Press, Cambridge, MA.
- Ho, M. W. 1990. An exercise in rational taxonomy. *J. Theor. Biol.* 147: 43–57.
- Hunding, A., Kauffman, S. A., and Goodwin, B. C. 1990. *Drosophila* segmentation: supercomputer simulation of prepatter hierarchy. *J. Theor. Biol.* 145: 369–384.
- Huang, F. 1998. Syntagms in development and evolution. *Int. J. Dev. Biol.* 42: 487–494.
- Jernvall, J. 2000. Linking development with generation of novelty in mammalian teeth. *Proc. Nat. Acad. Sci. USA* 97: 2641–2645.
- Jiang, T., Jung, H., Widelitz, R. B., and Chuong, C. 1999. Self-organization of periodic patterns by dissociated feather mesenchymal cells and the regulation of size, number, and spacing of primordia. *Development* 126: 4997–5009.
- Kondo, S., and Asai, R. 1995. A reaction-diffusion wave on the skin of the marine angelfish *Pomacanthus*. *Nature* 376: 765–768.
- Lacalli, T. C., Wilkinson, D. A., and Harrison, L. G. 1988. Theoretical aspects of stripe formation in relation to *Drosophila* segmentation. *Development* 104: 105–113.

- Landini, G., and Iannaccone, P. M. 2000. Modeling of mosaic patterns in chimeric liver and adrenal cortex: algorithmic organogenesis? *FASEB J.* 14: 823–827.
- Lipshitz, M. 1999. Gastrulation in *Drosophila*: the logic and the cellular mechanisms. *EMBO J.* 18: 3187–3192.
- Lipshitz, H. D. 1991. Axis specification in the *Drosophila* embryo. *Curr. Opin. Cell Biol.* 3: 966–975.
- Martinez-Arias, A. 1998. Interactions between wingless and notch during the assignment of cell fates in *Drosophila*. *Int. J. Dev. Biol.* 42: 325–333.
- Meinhardt, H. 1982. *Models of Biological Pattern Formation*. Academic Press, New York.
- Miura, T., and Shiota, K. 2000a. Extracellular matrix environment influences chondrogenic pattern formation in limb bud micromass culture: experimental verification of theoretical models. *Anat. Rec.* 258: 100–107.
- Miura, T., and Shiota, K. 2000b. TGF β 2 acts as an “activator” molecule in reaction-diffusion model and is involved in cell sorting phenomenon in mouse limb micromass culture. *Dev. Dyn.* 217: 241–249.
- Müller, G. B. 1990. Developmental mechanisms at the origin of morphological novelty: A side-effect hypothesis. In M. Nitecki (ed.), *Evolutionary Innovations*. University of Chicago Press, Chicago, pp. 99–130.
- Müller, G. B., and Wagner, G. P. 1996. Homology, *Hox* genes, and developmental integration. *Amer. Zool.* 36: 4–13.
- Müller, G. B., and Newman, S. A. 1999. Generation, integration, autonomy: three steps in the evolution of homology. *Novartis Found. Symp.* 222: 65–73.
- Newman, S. A. 1993. Is segmentation generic? *BioEssays* 15: 277–283.
- Newman, S. A. 1994. Generic physical mechanisms of tissue morphogenesis: A common basis for development and evolution. *J. Evol. Biol.* 7: 467–488.
- Newman, S. A., and Frisch, H. L. 1979. Dynamics of skeletal pattern formation in developing chick limb. *Science* 205: 662–668.
- Newman, S. A., and Müller, G. B. 2000. Epigenetic mechanisms of character origination. In G. P. Wagner (ed.), *The Character Concept in Evolutionary Biology*. Academic Press, San Diego.
- Ng, J. K., Tamura, K., Buscher, D., and Izpisua-Belmonte, J. C. 1999. Molecular and cellular basis of pattern formation during vertebrate limb development. *Curr. Top. Dev. Biol.* 41: 37–66.
- Nijhout, H. F. 1991. *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press, Washington, DC.
- Nowak, M. A., Boerlijst, M. C., Cooke, J., and Smith, J. M. 1997. Evolution of genetic redundancy. *Nature* 388: 167–171.
- Panganiban, G., et al. 1997. The origin and evolution of animal appendages. *Proc. Nat. Acad. Sci. USA*, 94: 5162–5166.
- Raff, R. 1996. *The Shape of Life: Genes Development and the Evolution of Animal Form*. University of Chicago Press, Chicago.
- Reinitz, J., and Sharp, D. H. 1995. Mechanism of eve stripe formation. *Mech. Dev.* 49: 133–158.
- Salazar-Ciudad, I., Garcia Fernandez, J., and Solé, R. V. 2000. Gene network capable of pattern formation: from induction to reaction-diffusion. *J. Theor. Biol.* 205: 587–603.
- 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.
- 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.
- Slack, J. M., Holland, P. W., and Graham, C. F. 1993. The zootype and the phylotypic stage. *Nature*. 361: 490–492.
- 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.
- Tam, P. P., and Behringer, R. R. 1997. Mouse gastrulation: the formation of a mammalian body plan. *Mech. Dev.* 68: 3–25.
- 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.
- von Dassow, G., and Munro, E. 1999. Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo. *J. Exp. Zool.* 285(4): 307–325.
- Wagner, G. P. 1989. The biological homology concept. *Annu. Rev. Ecol. Syst.* 20: 51–69.
- Wang, M., and Sternberg, P. W. 1999. Competence and commitment of *Caenorhabditis elegans* vulval precursor cells. *Dev. Biol.* 212: 12–24.
- 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.
- Wilkins, A. S. 1997. Canalization: a molecular genetic perspective. *BioEssays* 19: 257–262.
- Wray, G. A. 1999. Evolutionary dissociations between homologous genes and homologous structures. *Novartis Found. Symp.* 222: 189–203.
- Wright, S. 1912. Notes on the anatomy of trematode *Microphallus opacus*. *Trans. Amer. Soc.* 31: 167–175.