

Adaptive walks in a gene network model of morphogenesis: insights into the Cambrian explosion

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ABSTRACT The emergence of complex patterns of organization close to the Cambrian boundary is known to have happened over a (geologically) short period of time. It involved the rapid diversification of body plans and stands as one of the major transitions in evolution. How it took place is a controversial issue. Here we explore this problem by considering a simple model of pattern formation in multicellular organisms. By modeling gene network-based morphogenesis and its evolution through adaptive walks, we explore the question of how combinatorial explosions might have been actually involved in the Cambrian event. Here we show that a small amount of genetic complexity including both gene regulation and cell-cell signaling allows one to generate an extraordinary repertoire of stable spatial patterns of gene expression compatible with observed anteroposterior patterns in early development of metazoans. The consequences for the understanding of the tempo and mode of the Cambrian event are outlined.

KEY WORDS: *evo devo*, pattern formation, gene networks, morphogenesis, landscape

Introduction

Morphological complexity in metazoa experienced an extraordinary leap close to the Cambrian boundary (around 550 Myrs ago). Such event has been labeled the *Cambrian explosion*. When this radiation began and how rapidly it unfolded, is still the subject of active research (Morris, 1998; Raff, 1994; Valentine *et al.*, 1999; Solé *et al.*, 2000). The early origins of the last common ancestor and the structure of its bodyplan are controversial issues (Erwin and Davidson, 2002). The Cambrian event established essentially all the major animal body plans and hence all the major phyla which would exist thereafter. A body plan can be described anatomically but also in terms of the spatiotemporal pattern of expression of some key genes (Arthur, 1997). In this context, the origins of evolutionary novelty emerges as an integrative field involving gene regulation, development and paleobiology (Arthur, 2002; Carroll, 2001). As Shubin and Marshall pointed out, untangling the web of ecological, developmental and genetic interactions is a difficult task and a key question is which changes occur first (Shubin and Marshall, 2000).

A possible interpretation of the uniqueness, tempo and mode of the Cambrian event in terms of generic features of complex evolving systems was suggested by Kauffman in terms of adaptive walks on rugged fitness landscapes (Kauffman, 1993, 1989). In a

nutshell, the idea is that, given the fundamental constraints imposed by early developmental dynamics, early exploration of the universe of possible body plans took place quickly (once some underlying requirements were met) but then slowed down as the repertoire of possible bodyplans was filled out (Kauffman, 1989; Raff, 1994). Starting from some initial condition where low-fit multicellular organisms were present, a rapid exploration of the landscape was allowed to occur. This initial exploration led to an increase of diversity of improved alternative morphologies thereby establishing phyla. As the rate of finding fitter mutants that alter early developmental processes (which define body plans) slowed down, lower taxonomic groups became established.

The argument is completed by the assumption that mutants affecting early development have more profound effects than those affecting late development. In this scenario, by the Permian extinction (200 Myr later), when an estimated 95% of species (82% of genera, see (Erwin, 1998)) went extinct, early developmental pathways would be expected to have become largely frozen in after the Cambrian event and no new phyla would be reachable. Fitter variants altering basic body plans would be very hard to find but not variants affecting late development. This would allow the radiation of new families and lower taxa. Fitness landscapes, first introduced by Wright in 1932, allow to describe changes in the fitness of a

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given species through time in a well-defined fashion (Palmer, 1991; Kauffman, 1993; Rowe, 1994; Stadler, 2002). In an idealized situation, we can imagine the landscape as a surface. Here the tops and bottoms of the landscape would identify good and bad combinations of traits, respectively. This picture allows one to visualize the evolution of species as a hill climbing on the fitness surface.

A more precise definition is provided by describing each individual (or species) is defined as a set of N binary variables $S_i \in \{0, 1\}$ defining a set of characteristic traits (Kauffman and Levin, 1987; Kauffman, 1993). The total number of combinations is 2^N and each occupies a node of a N -hypercube Γ_N . In Fig. 1 we show an example of this fitness landscape for $N=3$. Each string has a well-defined fitness value, which can be represented by means of a N -dimensional function $\phi = \phi(S_1, \dots, S_N)$. If the number of epistatic interactions K is zero, i. e. if different traits do not influence each other, then a smooth landscape is obtained, with a single peak. But if different traits interact, then the landscape becomes rugged and multiple local fitness peaks are allowed to occur. The simplest evolution on a fitness cube occurs by means single, one-bit steps. In other words, a given species can perform a random *adaptive walk* from a given node towards one of its N nearest neighbors if this leads to an increase in fitness (alternatively, a neutral change can also occur and thus random drift is also allowed). A direct consequence of this assumption is that once a local peak is reached, no further changes are allowed.

There is a universal feature of adaptation on statistically correlated landscapes (Kauffman and Levin, 1987) which can be appropriately used to test these ideas. This can be formulated as follows: if S is the cumulative number of assumed improvements (new body plans) originated and T is the cumulative number of tries, then S will grow with T in a logarithmic fashion. Graphically, this means that S grows rapidly at the beginning and then slows down. The cumulative number of tries can be approximated by the cumulative number of lower-level

entities (e. g. genera) viewed as successive experiments in the generation of higher-level entities. The analysis of the cumulative increase of phylum originations against cumulative genera seems to confirm this prediction (Eble, 1998).

A further step in exploring the possible scenarios for an explosion of patterns in the evolution of development should consider the developmental program in an explicit way. One important factor not addressed by previous theoretical studies deals with the spectrum of possible spatial patterns of gene expression that can be generated from a given number of genes. This is a relevant question since combinatorial explosions can occur once complexity thresholds (in number of genes or their interactions) are reached. In previous studies, it has been shown that some particular types of combinations of gene-gene interactions involving cell-cell communication allow to easily generate a number of spatial patterns including stripes, gradients or spots (Salazar et al., 2000, 2001; Solé et al., 2002). These studies were performed on randomly wired networks and revealed a large fraction of spatial patterns that could be generated and their nature (Solé et al., 2002). One question immediately emerges: what are the minimal complexity requirements in terms of number of pattern-forming genes- in order to reach a high diversity of phenotypes?

One possible approach to the problem is to consider an artificial evolution model where real organisms and their development are replaced by digital organisms (Wilke and Adami, 2002) with simplified developmental programs. Such an approximation is becoming more common in evolutionary studies. The success of some of these models is extraordinary in providing insight into the evolution of complex biological systems. As pointed out by Gould, such a range of success is a consequence of universal laws of change that are common to all complex systems (Gould, 2003). In this context, we can conjecture that digital developmental programs might also share generic properties with real, early development.

Previous theoretical studies have shown that the fact of randomly wiring gene networks within a tissue context allows a high diversity of patterns and that spatial patterns are easily obtained (Solé et al., 2002). But no theoretical study has addressed the question of the potential diversity of (stable) patterns that can be obtained by tuning the number of genes involved in creating them, provided that some simple initial signal (an activated gene) is present at the beginning of development. Here we will explore this question by means of a simplified model of early development in which the number of different cell types is optimized. In this context, we specifically ask the following questions:

1. What are the consequences of searching for stable patterns of increasing complexity, being complexity measured in terms of the number of cell types?
2. What are the minimal requirements in terms of gene regulation complexity in order to be able to reach a wide spectrum of spatial patterns?
3. What type of spatial patterns are obtained?
4. How does the evolution of these patterns take place in terms of the underlying fitness landscape?
5. Can a combinatorial explosion partially explain the tempo and mode of the Cambrian event?

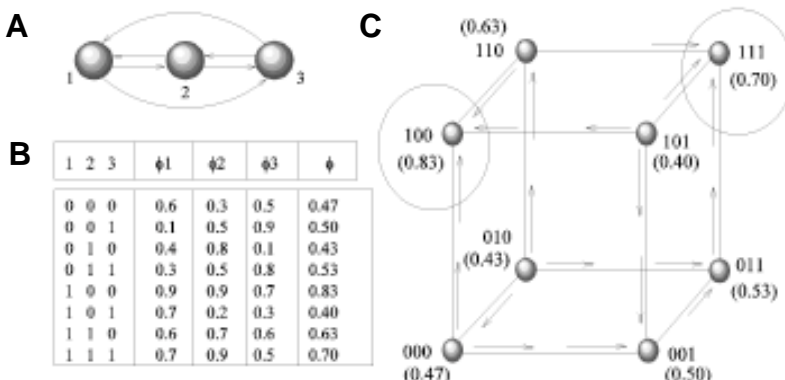


Fig. 1. A simple fitness landscape with $N=3$ traits and $K=2$ epistatic interactions. Here the three traits involved interact with the other two (A). The possible states of the system are given by the vertices of the fitness cube (this time a $N=3$ -dimensional cube Γ_3). The resulting average fitness, $\phi = \sum \phi_i / N$ is obtained from a fitness table (B) where the contribution of each trait ϕ_i (under the presence of the other two) is generated as a random number between zero and one. The table provides the local maxima. Adaptive walks (indicated as arrows) take place to nearest sites with higher fitness. In this example, adaptive walks end in two possible local peaks (C).

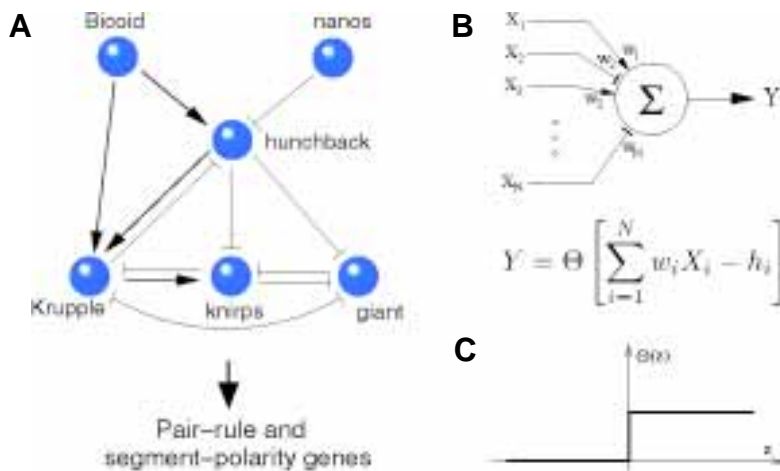


Fig. 2. (A) Gene interactions in early development. These are a subset of gene regulatory interactions that take place in *Drosophila* development. Arrows indicate different types of positive and negative interactions among different genes. In **(B)** the simplified, discrete threshold model considered here is shown. Each gene is treated as a binary (Boolean) variable with only two states: active (1) or inactive (0). It integrates the input signals which are weighted through a matrix of links W (either positive or negative). The output Y is obtained by using a threshold function, such as the one indicated in **(C)**.

As will be shown below, the model approach provides non-trivial, tentative answers to the previous questions.

Gene network model

A complete model of the gene activity pattern even at early stages of development would require a detailed description of the different levels of gene regulation and signaling (Arnone and Davidson, 1997). Such a description should take into account the continuous nature of mRNA and protein levels as well as other considerations related to the nature and distribution of cell signaling molecules and the stochastic nature of their dynamics. In this context, abstract models only taking into account a small amount of key features often capture the essential ingredients of gene regulation dynamics (Hasty *et al.*, 2001; Solé and Pastor-Satorras, 2002).

As a matter of fact, it has been recently shown that discrete, ON-OFF models of early development can actually provide complete enough information in order to reproduce the key traits of a developmental pattern (Bodnar, 1997; Mendoza *et al.*, 1999; Sanchez and Thieffry, 2001; Albert and Othmer, 2003). As discussed in (Albert and Othmer, 2003) within the context of the expression pattern of segment polarity genes in *Drosophila*, the observed patterns are determined by the topology of the network and the type of regulatory interactions between components. This is consistent with recent computer models indicating that a robust segment polarity module exists and that it is rather insensitive to variation in kinetic parameters (von Dassow *et al.*, 2000) (see also (Meir *et al.*, 2002; Gibson, 2002)). In other words, a complete description of both wild type patterns and various mutants is successfully reached by using simple Boolean networks. Here we take the same approach.

Several choices can be made in dealing with the structure of the wiring scheme to be used in the regulation network. One

possibility is to restrict ourselves to a hierarchical cascade of interactions inspired in the topology of interactions of *Drosophila*. But it is known that even gap genes are not completely hierarchical (Gehring, 1998). Some of them may act to switch on others (Fig. 2A). Actually, this property characterizes other gene groups as well, reminding us that we are actually dealing with a complex network instead of a linear chain of steps (Arthur, 1997).

The model explored here is a gene threshold system, described by a set of N genes per cell, interacting through a one-dimensional domain involving C cells, as shown in Fig. 3. The set of genes involved represent those required to build the basic structure and are thus bauplan genes (as defined in (Tautz and Schmid, 1998)). Here gene states will be Boolean: genes are either active (1) or inactive (0). Two types of elements will be considered: G genes and H hormones, with $N = G + H$. Genes interact within the cell, whose state at a given time t will be indicated as $g_i^j(t)$, with $i = 1, \dots, G$ as the gene number and $j = 1, \dots, C$ as the cell number (ordered from anterior to posterior). Generically labeled *microhormones* (Jackson *et al.*, 1986) involve some implicit local mediators communicating neighboring cells. These hormones can receive inputs from any of the first G units, but they can only make output to genes in other cells. The state of these H hormones will be accordingly indicated as $h_j^i(t)$.

Two matrices will be required in order to define the whole spectrum of links between different elements. These two matrices will be indicated by $A = (A_{ik})$ and $B = (B_{ik})$, defining interactions among the G genes and between genes and hormones, respectively.

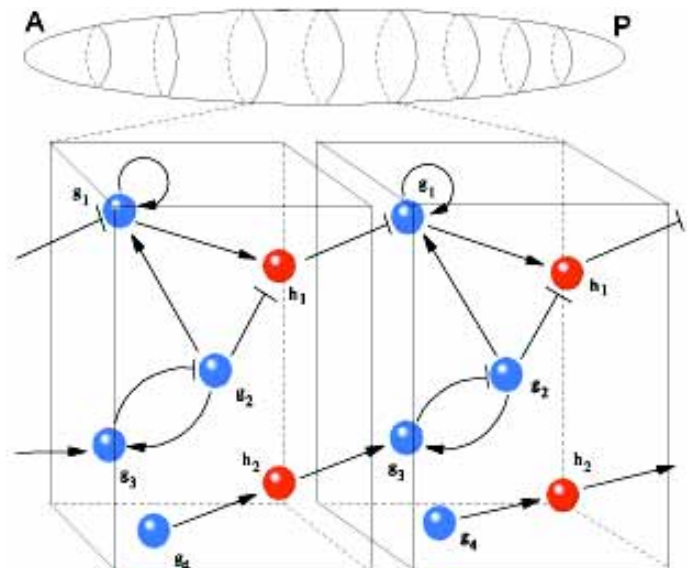


Fig. 3. Modeling morphogenesis using simple gene network models. Here the simulated organism is a one-dimensional array of cells. Each cell contains the same set of N genes, G of which (indicated by blue balls) only interact inside the cell whereas H others (red balls) act as microhormones, exchanging information with neighboring cells.

The basic set of equations of our gene network model are:

$$g_i^j(t+1) = \Theta \left[G_i^j(t) + \mathcal{H}_i^j(t) \right] \quad (1)$$

$$h_i^j(t+1) = \Theta \left[G_i^j(t) \right] \quad (2)$$

with

$$G_i^j(t) = \sum_{k=1}^G A_{ik} g_i^k(t)$$

$$\mathcal{H}_i^j(t) = \sum_{k=1}^G B_{ik} \delta \left(h_k^{j+1}(t), h_k^{j-1}(t) \right)$$

where $\delta(x,y)$ is an “OR” function (that is, $\delta(x,y) = 1$ if either $x = 1$ or $y = 1$ and zero if $x = y = 0$). The function $\Theta(x)$ is a threshold function (that is, $\Theta(x) = 1$ if $x > 0$ and $\Theta(x) = 0$ otherwise) and is shown in Fig. 2C.

Therefore, genes are influenced either by genes or hormones and hormones are influenced just by genes and the influences G_i^j and \mathcal{H}_i^j add up to determine if genes will be active or inactive by the comparison to a threshold (in this case 0), as shown in Fig. 2B. The OR function, or $\delta(x,y)$, is used here as a substitute for the diffusion of microhormone to the neighbours, since a microhormone will be seen as active in cell j if either of its neighbours is active.

For cells at the boundaries we have the special terms:

$$\mathcal{H}_i^1(t) = \sum_{k=1}^G B_{ik} h_k^2(t)$$

$$\mathcal{H}_i^C(t) = \sum_{k=1}^G B_{ik} h_k^{C-1}(t)$$

indicating that cells at the poles just have one neighbour.

Finally, we need to define an initial condition. The simplest choice that can be defined involves the activation of a single gene at the anterior ($j = 1$) pole, as well as the symmetric case in which the signal is present at the two poles ($j = 1$ and $j = C$). Specifically, we set $g_j^i(0) = h_j^i(0) = 0$ for all $j = 1, \dots, N_C$ except $g_1^1(0) = 1$ in the single pole case and an additional $g_1^{N_C}(0) = 1$ in the symmetric case. These choices correspond to maternal signals confined to the embryo’s extremes. Such initial change will propagate to the rest of the tissue provided that the network is sensitive to it.

As an example of the dynamics, the temporal evolution of one sample organism with one-pole maternal signal is shown in Fig. 4.

Adaptive walks

Using the above mentioned gene network model of pattern formation, we now explore how pattern complexity emerges through a simple evolutionary algorithm where a population of \mathcal{P} organisms evolve through adaptive walks. Such type of algorithm has been successfully used in different contexts (see for example (Niklas, 1994, 1997)).

In order to define the evolution algorithm, we need to first define a fitness function. Here we restrict ourselves to measuring the number of different cell types. The number of cell types is a good measure of complexity which is known to increase through metazoan evolution (Valentine *et al.*, 1994; Carroll, 2001). Increases in cell type number provide a high potential for further

evolution of anatomical and functional complexity, essentially through division of labor and the formation of specialized tissues (Maynard-Smith and Szathmáry, 1995). It is clear that the diversity of organisms involving a small number of cell types is fairly limited and thus a first step towards the evolution of complex organisms requires an expansion of their diversity of cell types. Actually, as discussed by Erwin and Davidson, regulatory processes underlying cell-type specification are very old and display conserved plesiomorphic features (Erwin and Davidson, 2002). Morphogenetic processes would have evolved independently and added at later stages.

We consider, then, the number of cell types n_{ct} as our complexity measure and formulate the following question. What are the consequences for the diversity of spatial patterns that can be generated if we increase the number of cell types?

Starting from a homogeneous population of \mathcal{P} identical organisms, we perform adaptive walks on the fitness landscape. At each generation in the algorithm, we sequentially choose each

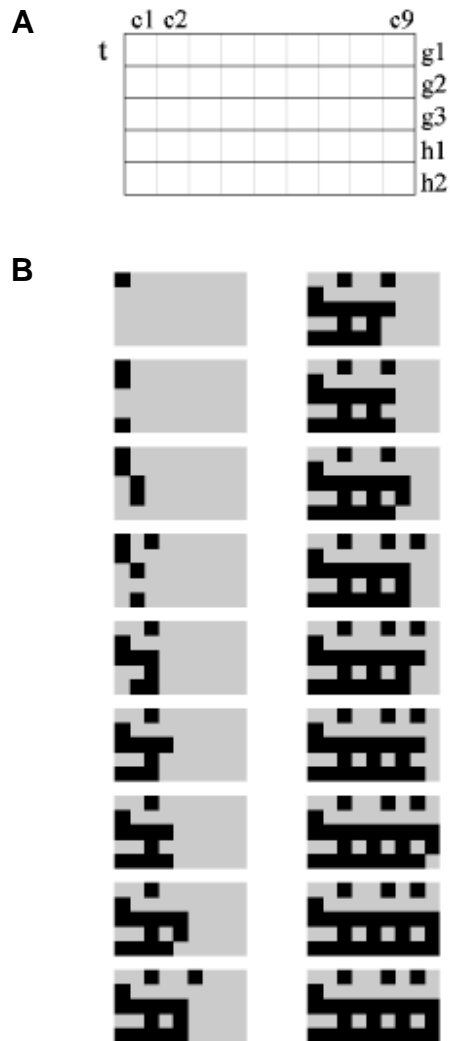


Fig. 4. The temporal construction of a pattern in a $G = 3$ gene and $H = 2$ hormone network. (A) Scheme of representation. (B) The 20 steps necessary to reach a stable pattern. Genes in black are active and genes in gray are inactive.

organism and introduce a single change in its gene network. Three types of changes can occur, with probability 1/3, all of them affecting the dependencies between genes:

- addition of new links,
- removal of previously present links, and
- randomization of links' weights.

In this way the complexity of the network can be tuned independently for each organism.

After the change, the developmental pattern generated by the organism is simulated. The number of different cell types n_{ct}^t is computed using this new pattern and it is compared with the previous one, n_{ct}^{t-1} . If $n_{ct}^t > n_{ct}^{t-1}$ the change is accepted and the new organism replaces the old one. Otherwise the change is rejected and the old organism is kept. The fact that we keep the new organism if $n_{ct}^t = n_{ct}^{t-1}$ (the exact developmental pattern might be different), introduces a certain amount of neutrality that may reduce the probability that an organism gets trapped because it cannot find any changes that give it a higher number of cell types. As an additional requirement, the stability of the final pattern is enforced by rejecting those organisms which do not reach a stable state in l iterations. This is a strong constrain, since puts a hard limit on the attainable patterns, but it also seems sensible, for developmental processes are very robust and only display transient oscillations.

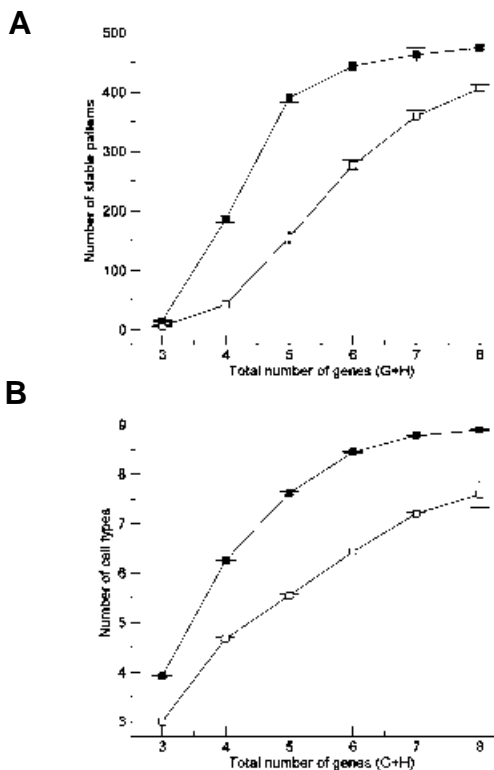


Fig. 5. (A) The number of stable spatial patterns obtained for different combinations of genes and microhormones from the adaptive walk algorithm. Here two sets of numerical experiments were performed, involving one and two hormones and varying numbers of genes. In **(B)** the corresponding numbers of cell types are shown for the two cases. Note the saturation towards the maximum number $n_{ct} = C$ as the total number of genes increases. For each point, 5 simulations of 15,000 generations were performed with $C = 9$, $P = 500$ and $l = 100$.

The size of the space of possible patterns is rather high, since the number of combinations of the activation of genes in each cell gives rise to a potential number of patterns of

$$|\Gamma| = 2^{(H+G)C}.$$

Actually, the tissue context exponentially expands the allowed space Γ_c of cell types reachable by isolated cells, which has a size $|\Gamma_c| = 2^{(H+G)}$ (i.e. we have $|\Gamma| = |\Gamma_c|^C$). Even for a small number of cells and a small amount of genes, the resulting space is hyperastronomically large. For example, if we take $G + H = 4$ (which is one particularly interesting case, as shown below) we have $|\Gamma| = 2^{60}$ (approximately 10^{17}).

In order to explore the potential repertoire of structures that can be generated, we will focus in the set of stable spatial patterns Γ_g associated to each single gene (independently of others), from the set of possible spatial patterns, with $|\Gamma_g| = 2^C$. This is equivalent to consider the raw capacity to generate specific patterns without regarding any gene as more important than any other. Therefore, a population of organisms at any given generation has a corresponding set of patterns, \mathcal{P} , the ones taken from all the patterns that the genes in each organism generate individually in the developmental process. Any pattern in this list occurs in some specific genes in some organisms, across the different cells and with a different frequency, which we also measure.

Results

As an overall trend, simulations proceed in the direction of increasing the number of patterns $|\mathcal{P}|$, as the average number of cell types grows generation by generation. Given that every organism in the population performs its own adaptive walk, one would expect the behavior of the average of the population would reflect some of the structure of the landscape. For instance, should there be any special local peaks in which an organism could get trapped, one would find many organisms there. As we will see, this is not the case.

A graph of $|\mathcal{P}|$ and the number of cell types achieved after 15000 generations for different numbers of genes and hormones is shown in Fig. 5. As the number of genes increases, the number of patterns discovered increases, with a tendency to saturate at high G . Since the total number of possible patterns is, for $C = 9$, $|\Gamma_g| = 2^9 = 512$, it is clear that the population almost finds all the possible patterns for high G . This is even more evident if we look at the number of cell types, which reaches a value of almost 9, the maximum. From this curves it is also clear the simple change from one to two microhormones makes the system much less constrained and therefore more patterns are found.

As mentioned earlier, given a population, many organisms generate some patterns more than once. In fact, the distribution $F(r)$ of patterns generated has an interesting structure, as shown in Fig. 6. Here patterns are sorted out by their rank r (i. e. by their order from the most to the least frequent). Some patterns, especially those with a small number of active cells at the anterior pole, are very frequent. Some others, on the contrary, are very scarce. The distribution follows approximately the form of a power law, i.e. $F(r) \sim r^{-\alpha}$, with α close to one.

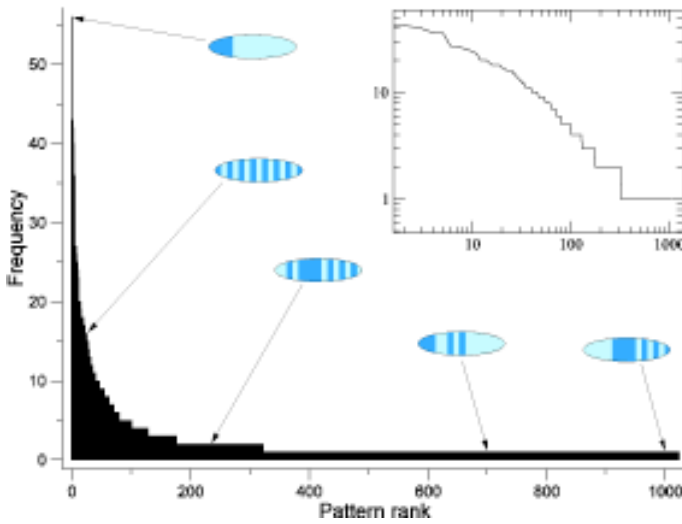


Fig. 6. Frequency-rank distribution of complex patterns obtained from the adaptive walk evolutionary algorithm, for 15,000 generations, here with $G = 3, H = 2$ and $P = 500$ (a log-log plot is shown in the inset). A power decay is obtained, indicating a majority of patterns present in the final population, but also a long tail of less common patterns. Some examples are indicated. Most common patterns involve activation close to the anterior pole, but more complex patterns, such as stripes, appear to be rather frequent. As we move towards the tail of the distribution, more rich, asymmetric patterns are observed.

The frequent patterns are, roughly, those that start the repeating process that generates a wave across the organism but remain active only near the anterior pole. Since less combinations exist for that type of patterns, more organisms will generate the same ones. The rare patterns seem to be those that are the result of the construction process and may vary considerably in its exact

values, since many more different combinations exist. In Fig. 4, the pattern generated by g_2 would be an example of a “starter” and thus more frequent and the g_1 would be an example of a possible rare one.

Given a fixed G and H , two other parameters affect the number of patterns one obtains. On the one hand, the maximum number of iterations to attain a stable pattern, I and, on the other, the number of organisms in the population, P . If I is smaller, less patterns are discovered since those patterns that may stabilize in a higher number of iterations are filtered out. If P is larger, the landscape is explored more extensively and more patterns are discovered. Two figures demonstrate this dependence: Fig. 7 shows two different runs with different population sizes all other parameters being equal, showing how the bigger population attains a bigger number of patterns; Fig. 8 shows the exact dependency.

The most surprising results, however, involve the repertoire of spatial patterns for small-sized gene networks. The basic set of patterns obtained is shown in Fig. 9 for $C = 15$ cells, where the results from the two types of initial activations (one pole at left, two poles at right) are shown. As we can see, the repertoire is fairly limited, even if we use $G = 3$ genes and $H = 1$ hormones. Most patterns are rather homogeneous, displaying a small gradient or single stripes, although some patterns with wider stripes are also observable, thus indicating that the richness of dynamical patterns transiently generated by coupled genes can stably propagate even with only one hormone.

The situation, however, dramatically changes once we cross a complexity threshold. If $H = 2, G = 2$, a very large number of patterns becomes available. In Fig. 10 the resulting stable patterns are shown under the same simulation conditions. Now we can see that the number of different patterns, $|P_1| = 239$ and $|P_2| = 154$ for one and two maternal signals, respectively, is comparable to the number of organisms involved. The diversity is also remarkable: many different orderings in the stripe and gap distributions are obtained, suggesting that a very high universe of combinations is compatible with a high diversity of cell types.

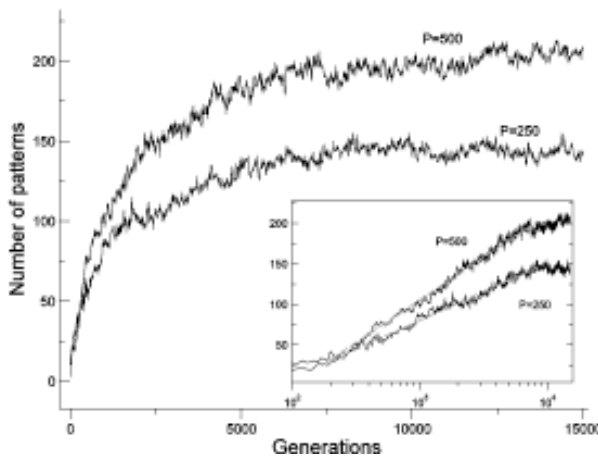


Fig. 7 (Left). Time evolution of the number of stable patterns (P) for two different population sizes. The other parameters are $C = 15, G = 2, H = 2$. The inset displays the same evolution in log-linear scale. A straight line in such a plot indicates a logarithmic increase in the number of patterns, consistent with a rugged landscape.

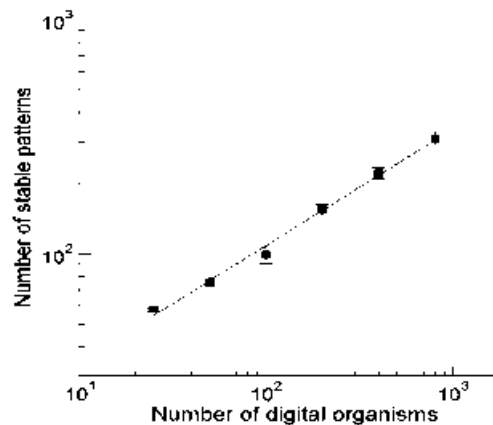


Fig. 8 (Right). The number of stable patterns (P) for different population sizes. The other parameters are $C = 15, G = 2, H = 2$ and 15,000 generations. The dashed line shows a curve that fits the data with a power function, i.e. $f(x) = \alpha x^\beta$ with an exponent of $\beta = 0.498 \pm 0.02$.

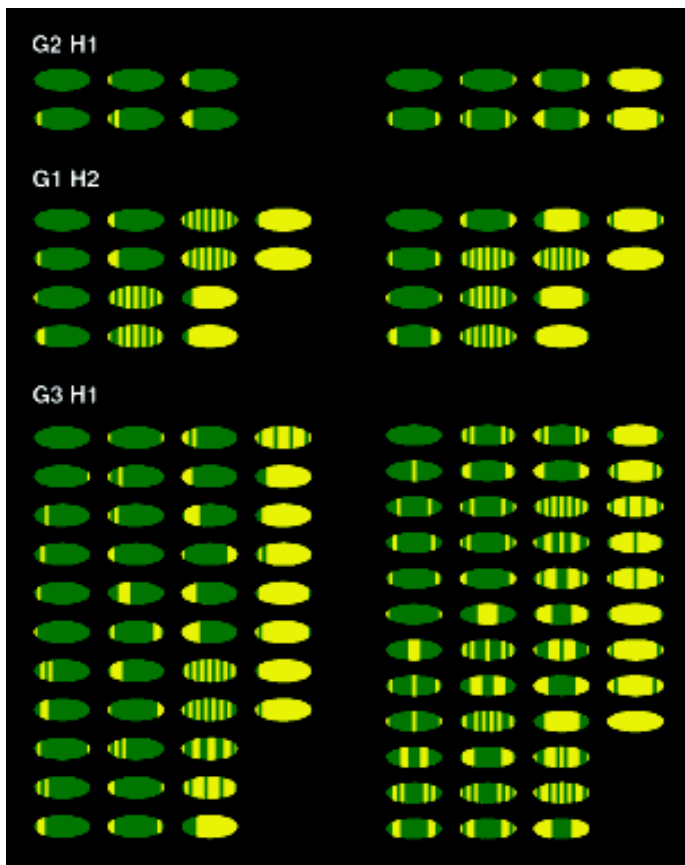


Fig. 9 (Left). Taxonomy of patterns created from a system with different numbers of genes and microhormones at the subcritical regime. The left side of this figure shows the single pole case, whereas the right shows the symmetric one. A small amount of possible patterns is obtained, indicating that the possible repertoire of structures is fairly limited. Once two hormones are involved, it gets possible to obtain stable stripes, but only the most regular combinations are allowed. The experiment involved a population of $P = 500$ individuals for 15,000 generations.

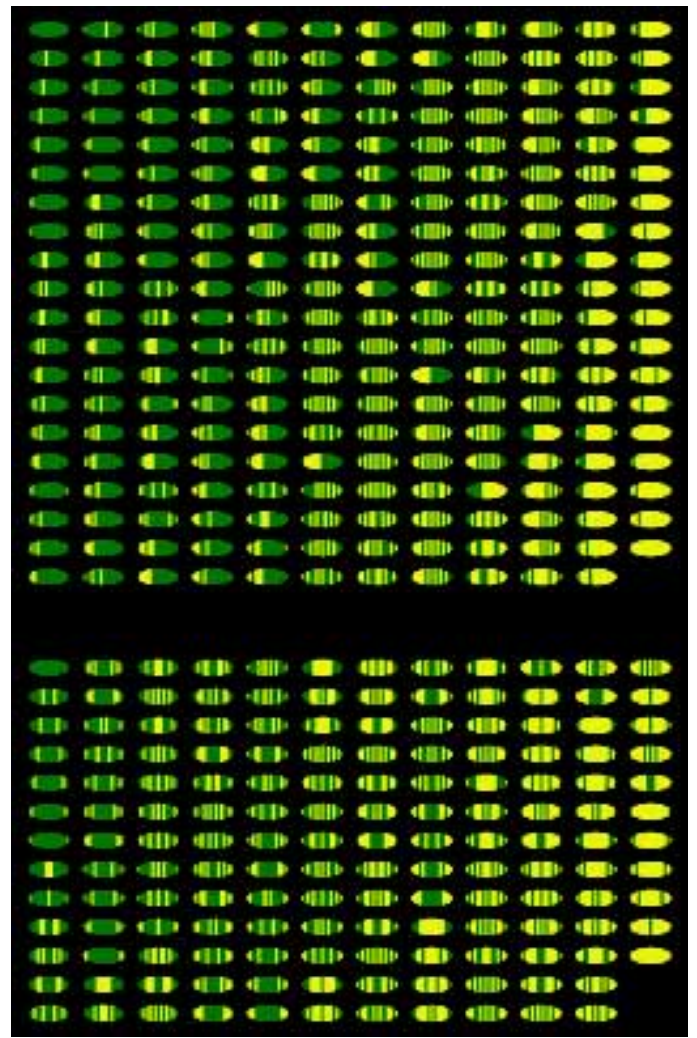


Fig. 10 (Right). As in Fig. 9, but now using $G = 2$ and $H = 2$. A much higher diversity of patterns is generated, revealing a combinatorial explosion of spatial motifs. The combination of complex patterns of gene interactions together with a combination of two microhormones leads to a great diversity of patterns of gene expression. The upper part shows the patterns for a single-pole maternal signal, whereas the lower part show the ones for the symmetric case.

Discussion

Any simple model of pattern formation does necessarily introduce shortcuts that limit the range of conclusions that can be safely reached. Our model does not include a large number of relevant features, from cell division to biologically realistic regulatory mechanisms. But again, as stressed in previous sections, some key, large scale features of complex systems do not depend on the specific details involved at lower levels (Solé and Goodwin, 2001). This is fairly well exemplified by Boolean models of *Drosophila* development.

Our results can be summarized as follows (providing a tentative list of answers to the list of questions presented in the first section):

1. Using the number of cell types as a complexity measure, our model indicates that the whole spectrum of spatial patterns is potentially reachable, provided that the population of digital organisms is large enough. Maximal diversity of cell types is positively correlated with the diversity of spatial patterns generated. This suggests that increasing cellular diversity is consistent with highly flexible pattern-forming mechanisms.
2. If a single hormone is involved, complex patterns can be obtained by increasing the network complexity. A much more rapid increase is obtained by using two hormones. Actually, the combination of two genes + two hormones leads to a combinatorial explosion of spatial patterns. As a consequence, our results indicate that, provided the number of organisms is large enough, any pattern seems to be available.
3. The spectrum of spatial patterns of gene expression is dominated by gap-like structures, stripes and all kind of combinations between them. It is remarkable that all classes of spatial structures are easily identified as matching the spatial distribution of maternal, gap and pair-rule gene expression patterns. Some genes (as it occurs in real development) are only transiently activated and thus appear in the end as absent.

4. The population climbs the underlying landscape in such a way that the rate of finding local peaks increases in a logarithmic fashion. This implies that a rapid diversification is followed by a further slowdown, in agreement with a rugged fitness landscape.
5. The jump in pattern diversity experienced at $H = G = 2$ indicates that thresholds in network complexity, even at small-gene numbers exist and can lead to combinatorial explosions. Such explosions would open a whole spectrum of available structures. Reaching such a threshold might require the formation of a minimal regulatory network and might also require other prerequisites dealing with body size, cellular interactions and tissue specialization. However, once in place, the whole universe of patterns can be made suddenly available.

The previous results support the idea that the Cambrian event (and perhaps other rapid diversification events) might result from changes in the pattern of gene regulation. Of course the model does not consider ecological or other factors that might have played a key role. Instead, we concentrate in the study of the generative potential of such a simple model and in the universe of possible spatial patterns that can be generated. As a result, we obtain a surprising jump once a threshold of genetic complexity is reached. Although the whole repertoire of patterns might need some exploratory effort to be reached, most patterns are easily found and once a large fraction of them has been obtained, further innovation occurs at a slower pace.

The main message of this paper is that rapid diversification events in terms of generation of evolutionary novelty in developmental processes can take place through combinatorial explosions. Although it can be argued that the model is too simple, the exploration of continuous counterparts of these results give very similar outcomes (particularly the thresholds of network complexity). Additionally, the continuous models allow to increase the repertoire of patterns and the same applies to discrete models using Boolean (instead of threshold) functions. In other words, the choices made here actually limit the diversity of patterns that can be generated.

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