Robustness as an Evolutionary Principle

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We suggest to simulate evolution of complex organisms constrained by the sole requirement of robustness in their expression patterns. This scenario is illustrated by evolving discrete logical networks with epigenetic properties. Evidence for dynamical features in the evolved networks is found that can be related to biological observables.

I. INTRODUCTION

A common concept in evolution is fitness and fitness landscapes [1], and often evolution is viewed as hill climbing, possibly with jumps between fitness maxima [2,3]. However, fitness landscapes implicitly assume that fitness is varying over a well-defined metric in genomic space. This would be the case if single point mutations were a driving force. However, significant genome rearrangements are observed already in the rather brief real-time evolution experiments of \textit{Escherichia coli} cultures of Papadopoulos et al. [4]. Genomic rearrangements short-circuit the simple metric generated by one point mutations, usually underlying the intuition of evolution on landscapes. As a consequence the combinatorial distance for moving from a genome A to a genome B may easily be different from the distance of the opposite move, simplest exemplified by deletions and insertions. Thus, although fitness landscapes have a meaning for the small scale adjustments associated to fine-tuning of binding constants, it is an unjustified concept for evolutionary changes on the scale of speciation events.

Abandoning fitness landscapes we here instead discuss the possibility that evolution progresses through a process where genotypes and phenotypes subsequently set the frame at which the other may change. Of particular relevance for this view of evolution is the fact that one often observes different phenotypes for the same genotype. This viewpoint is in part supported by cell differentiation within one organism, in part supported with epigenetics and the large class of organisms which undergo metamorphosis and thus exist in several phenotypes for the same genotype. Recently, it has also been proposed that genotype-phenotype ambiguity [5] is governing speciation events.

A class of systems that exhibits epigenetics is represented by the logical networks, where nodes in the network take values on or off, as function of the output of specified other nodes. This has been suggested to model the regulatory gene circuits [6,7] where specific genes may or may not be expressed as function of other genes. In terms of these models it is natural to define genotypes in form of the topology and rules of the nodes in the network. The phenotypes are similarly associated to the dynamical expression patterns of the network.

To define the rules under which phenotypes and genotypes set the frame for each other’s development, a model for evolution should fulfill the requirement of robustness. Robustness is defined as the ability to function in face of substantial change in components [8,9]. Robustness is an important ingredient in simple molecular networks and probably also an important feature of gene regulation on both, small and large scale. In terms of logical networks, robustness is implemented by constraining subsequent networks to have similar expression patterns.

This article is organized as follows: First we discuss dynamics on logical networks and numerically review the basic properties of attractors of random threshold networks and Boolean networks. Then we propose a minimal evolution model and investigate its statistical and structural implications for the evolved networks. Finally, biological implications, and possible experimental approaches to the dynamics of real genetic networks are discussed.

II. DYNAMICS ON LOGICAL NETWORKS

Let us first discuss two prototype networks that exhibit epigenetics, Boolean networks [10] and threshold networks [11]. These are both networks of logical functions and share similar dynamical properties. We here briefly describe their definition and dynamical features. In both networks each node is taking one of two discrete values, ±1, that at each timestep is a discrete function of the value of some fixed set of other nodes specified by a wiring diagram. If we denote the links that provide input to node $i$ by $\{w_{ij}\}$, with $w_{ij} = \pm 1$ also, then for the threshold network case the updating rule is additive

$$\sigma_i = 1 \text{ if } \sum_{j \in \{w_i\}} w_{ij} \sigma_j \geq 0 \quad (1)$$

$$\sigma_i = -1 \text{ if } \sum_{j \in \{w_i\}} w_{ij} \sigma_j < 0 \quad (2)$$

In the Boolean network case the updating is a general Boolean function of the input variable

$$\sigma_i = B(\sigma_j’s \text{ which provide input to } i). \quad (3)$$

Thus, the threshold networks form a hugely restricted set of the Boolean networks. Boolean networks include all
nonlinear combinations of input nodes, including functions as, for example, the “exclusive or”.

The basic property of logical networks is a dynamics of the state vector \( \{ \sigma_i \} \) characterized by transients that lead to subsequent attractors. The attractor length depends on the topology of the network. Below a critical connectivity \( K_c \approx 2 \) the network decouples into many disconnected regions, i.e., the corresponding genome expression would become modular, with essentially independent gene activity. Above \( K_c \) any local damage will initiate an avalanche of activity that may propagate throughout most of the system. For any \( K \) above \( K_c \) the attractor period diverges exponentially with respect to system size \( N \) and in some interval above \( K_c \) the period length in fact also increases nearly exponentially with connectivity \( K \) \[16\].

III. STRUCTURAL EVOLUTION OF NETWORKS

Dynamics may occur on networks as defined by the rule above, but at least as important is the dynamics of network topology. In terms of network topology an evolution means a change in the wiring \( \{ w_{ij} \} \rightarrow \{ w_{ij}' \} \) that takes place on a much slower timescale than the \( \{ \sigma_j \} \) updating. The evolution of such networks represents the extended degree of genetic network engineering that seems to be needed to account for the large differences in the structure of species genomes \[14\], given the slow and steady speed of single protein evolution \[17\].

We have in an earlier publication proposed to evolve Boolean networks with the sole constraint of continuity in expression pattern \[2\]. Here we simplify this model by simple damage spreading testing:

The model evolves a new single network from an old network by accepting rewiring mutations with a rate determined by expression overlap.

This is a minimal constraint scenario with no outside fitness imposed. Further the model tends to select for networks which have high overlap with neighbor mutant networks, thus securing robustness.

Now let us formulate an operational version of the evolution in terms of threshold networks as these have comparable structural and statistical features to the Boolean ones \[13\]. Consider a threshold network with \( N \) nodes.

To each of these let us assign a logical variable \( \sigma_i \), \(-1 \) or \(+1 \). The states \( \{ \sigma_i \} \) of the \( N \) nodes are simultaneously updated according to (1) where the links \( w_{ij} \) are specified by a matrix. The entry value of the connectivity matrix \( w_{ij} \) may take values \(-1 \) and \(+1 \) in case of a link between \( i \) and \( j \), and the value 0 if \( i \) is not connected to \( j \).

The system that is evolved is the set of couplings \( w_{ij} \) in a single network. One evolutionary time step of the network is:

1) Create a daughter network by a) adding, b) removing, or c) adding and removing a weight in the coupling matrix \( w_{ij} \) at random, each option occurring with probability \( p = 1/3 \). This means turning a \( w_{ij} = 0 \) to a randomly chosen \( \pm 1 \) or vice versa.

2) Select a random input state \( \{ \sigma_i \} \). Iterate simultaneously both the mother and the daughter system from this state until they either have reached and completed the same attractor cycle, or until a time where \( \{ \sigma_i \} \) differs between the two networks. In case their dynamics is identical then replace the mother with the daughter network. In case their dynamics differs, keep the mother network.

Thus, the dynamics looks for mutations which are phenotypically silent, i.e., these are neutrally inherited under at least some external condition. Notice that adding a link involves selecting a new \( w_{ij} \), thus changing the rule on the same timescale as the network connectivity. Iterating these steps represents an evolution which proceeds by checking overlap in expression pattern between networks. If there are many states \( \{ \sigma_i \} \) that give the same expression of the two networks, then transitions between them are fast. On the other hand, if there are only very few states \( \{ \sigma_i \} \) which result in the same expression for the two networks, then the transition rate from one network to the other is small. If this is true for all its neighbors then the evolutionary process will be hugely slowed down.

In Fig. 1 the connectivity change with time for a threshold network of size \( N = 32 \) is shown.

![FIG. 1. Long time evolution for the connectivity of of a threshold network with N=32 nodes. Connectivities are constrained to be below K = 8. One observes long periods of stasis interrupted by sudden changes, reminiscent of punctuated equilibrium.](image-url)

Time is counted as number of attempted mutations, and one observes that especially for high connectivity the system may stay long time at a particular network before an allowed mutation leads to punctuations of the stasis. The overall distribution of waiting times is \( \sim 1/t^{2\pm0.2} \).

One feature of the evolution is the structure of the
evolved networks, which can be quantified by the average length of attractors for the generated networks. This is shown in Fig. 2, where they are compared with attractor lengths for random networks at the same connectivity.

![Fig. 2. Average length of periodic attractors for evolved and random networks. Also the periods of the unsuccessful mutations in the presence of newly chosen random initial conditions are shown, demonstrating that selection of networks is indeed operating in structure space and the specific input configuration in the event of selection does not play a major role.](image)

One observes that the evolved networks have much shorter attractors than the random ones, thus our evolution scenario favors simplicity of expression.

To examine further the expression behavior of the networks let us consider the size of frozen components as introduced by Kauffman for Boolean networks. A frozen component is the set of nodes connected to a given attractor that does not change at any time when you iterate along the attractor, i.e., a frozen component represents genes which are anesthesized under a given attractor/initial conditions. In Fig. 3 one sees that the frozen component for the evolved network typically involves half the system, and thus is much larger than the typical frozen component associated to attractors of randomly generated threshold networks. Also we test frozen components for random one mutant neighbors of the selected ones, and find that these networks also have huge frozen components.

![Fig. 3. Average size of frozen components as a function of connectivity for evolved and random networks. The frozen component is the set of all nodes that do not switch during the attractor. One observes that the robustness constraint in evolution favors a larger frozen component.](image)

In Fig. 4 this quantity is shown for random networks as well as evolved networks. One observes that the active part of the evolved networks exhibits a much simpler expression pattern than that of a random network of comparable connectivity.

![Fig. 4. Average number of flips per node in the non-frozen part of the network, as a function of connectivity for evolved and random networks. The evolved networks show a reduced activity in the non-frozen nodes resulting in simple expression patterns as compared to those of random networks of same connectivities. Notice that the number counts off-on and on-off transitions of the nodes as separate events.](image)

Overall, requiring robustness as an evolution criterion has observable consequences for both, the temporal evolution pattern, and for confining possible genetic network architectures to the ones with simple expression patterns.
IV. DISCUSSION

Some quantitative testing of the minimal evolution scenario is possible on the macro-evolutionary scale. Here the intermittent evolution of the networks bears resemblance to the punctuated equilibrium observed for species in the fossil record [22]. Quantitatively the $1/f$ power spectra and $1/t^2$ stability distribution for single networks, that one finds for this model as well as for the earlier version [21], compares well with the similar scalings observed for the statistics of birth and death of individual species in the evolutionary record [18,19]. Obviously the here ignored features related to co-evolution prevent us from discussing co-extinctions [18]. In fact the analogy can even be fine-grained into a sum of characteristic lifetimes, each associated to a given structural feature of the networks [21]. A similar decomposition is known from the fossil record [22], where groups of related species display Poisson distributed lifetimes and therefore similar evolutionary stability.

A validation on the microlevel based on statistical properties of genetic regulatory circuits has to be based either on properties of genetic networks [3] or on evolution and mutation experiments of fast lived organisms as E. coli [4]. A key number is the estimated average connectivity $K$ of 2 → 3 in the E. coli genome [23]. Information on the overall organization of these genetic networks is obtained from gene knock out experiments. A quantitative support for a connected genome can be deduced from Elena and Lenski’s [2] experiments on double mutants, which demonstrated that about 30-60% of these (dependent on interpretation) change their fitness in a cooperative manner. In terms of our networks, we accordingly should expect a coupled genetic expression for about half of the of pairs of genes. Although our evolved networks can give such correlations for the connectivity estimate of 2-3 given by [23], the uncertainty is still so large that random networks also are in accordance with data. Further one should keep in mind that the E. coli genome is large and not well represented by threshold dynamics of all nodes, and also that only between 45 and 178 of the E. coli’s 4290 genes are likely to mediate regulatory functions [23]. Thus, most of the detected gene-gene correlations presumably involve genes which are not even regulatory, but instead metabolic and their effect on each other more indirect than in the case of the regulatory ones. Presumably one would obtain stronger elements of both coupling and correlation if one specialized on regulatory genes. Thus one may wish for experiments where one and two point mutations are performed in regulatory genes only. A more direct test of our hypothesis of damage control as a selection criterion may be obtained from careful analysis of the evolution of gene regulation in evolving E. coli cultures.

Another interesting observation is the simplicity of biological expression patterns. For example as observed in yeast many genes are only active one or two times during the expression cycle [20], thus switching from off to on or on to off occurs for each gene in this system only a few times during expression. For random dynamical networks of comparable size one would expect a much higher activity. Thus surprisingly simple expression patterns are observed in biological gene regulatory circuits. This compares well with our model observation where simplicity of expression patterns emerges as a result of the evolutionary constraint.

V. SUMMARY

In this article we have proposed a computer simulation of evolution operating on logical networks. The scenario mimics an evolution of gene regulatory circuits that is governed by the requirement of robustness only. The resulting dynamics evolves networks which have very large frozen components and short attractors. Thus they evolve to an ordered structure that counteracts the increasing chaos when networks become densely connected. The evolved architecture is characterized by simplicity of expression pattern and increased robustness to permanent mutational fluctuations in the network architecture – features that are also seen in real molecular networks.

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