

## Neutral Mutations and Punctuated Equilibrium in Evolving Genetic Networks

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Boolean networks may be viewed as idealizations of biological genetic networks, where each node is represented by an on-off switch which is a function of the binary output from some other nodes. We evolve connectivity in a single Boolean network, and demonstrate how the sole requirement of sequential matching of attractors may open for an evolution that exhibits punctuated equilibrium. [S0031-9007(98)06567-3]

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Evolution of life is presumably a random process with selection [1]. It has been discussed whether this process can be viewed as some hill climbing process [2], or whether evolution mostly happens as a random walk where changes do not influence the phenotype, and thus are neutral [3]. Originally, the case of evolution as adaptation in an externally imposed fitness landscape has been proposed by Wright [2], and later formed the basis for models of punctuated equilibrium by Newman [4] and Lande [5]. The case for neutral evolution has been presented by Kimura [3], and is experimentally supported on the microlevel by the observation that there are many functionally identical variants of most of the important macromolecules of life.

The observation of punctuated equilibrium in the fossil record, recently discussed by Gould and Eldredge [6], may be taken as an indication that evolution of a species consists of exaptations of jumping from one hilltop to another nearby in some fitness landscape. Naturally such jumps will be rare, separated by large time intervals where species are located at a fitness peak, and the resulting evolutionary pattern will show punctuations as indeed seen in the fossil record. This picture of single species evolution in a given fixed landscape has been modeled explicitly by Newman [4] and Lande [5].

However, neutral evolution also may show punctuations as, for example, might be visualized by finding the exit in a labyrinth or from finding a golf hole by means of a random walk in a flat landscape. The picture here is that genetic changes always take place, but that the phenotypic changes only rarely occur. This has recently been demonstrated by modeling the evolution of RNA secondary structure by Schuster and co-workers [7]. For these molecules, mutating a single nucleotide often does not induce any changes in their secondary structure, and the mutation is considered neutral. Occasionally, however, one mutation can lead to a complete readjustment of the structure, usually accompanied by a major change in its functionality.

In any case, as demonstrated by Bak and Sneppen [8], punctuated equilibrium on the organism level might be

connected to the episodic punctuations observed on the ecosystem level. The crucial element of such an extrapolation is that the environment of each species depends on species which are ecological neighbors, thereby allowing punctuations to propagate across the ecosystem.

In the present paper we propose to evolve a single genetic network, ideally representing a single species. The evolution is driven by a noisy environment. The evolutionary step consists of random mutations combined with selection of mutants preserving the phenotype with respect to a given environment. Thus, the only requirement in this minimalistic model is continuity in phenotype. Other changes in genotype are allowed, creating a path of neutral mutations. We will discuss how this requirement of continuity in evolution may constrain and guide the evolution of an individual species in the face of a constantly changing environment.

Our fundamental constituents are the genes of the organism, and the evolution we consider is on the genetic network level. Although genetic networks consist of biochemical switches [9], it has been proposed that the on-off nature of these switches can be well approximated by Boolean functions [10–12], eventually with asynchronous updating [13]. We here consider networks of random Boolean functions, idealized by synchronized updating. The functionality we test for is attractors of these networks [11]. Boolean networks are known to exhibit a rich dynamical behavior, including fixed points, periodic attractors, and long transients. Further, the number of attractors, their length, and the length of the transients strongly depend on the connectivity number [14]. In this paper we do not address any question about the time scale of these attractors. Instead we consider a longer evolutionary time scale connected to the change in geometry of the networks under mutation.

We implement continuity in evolution by testing for reaching a given attractor on subsequent steps, but allowing changes that modify attractors that are not tested from the actual initial condition. In subsequent steps, the initial condition (modeling the environment) assumes new random values which subsequently allow previous neutral

mutations to surface in the phenotype. The philosophy of this subdivision between initial state and function is that while the Boolean functions are manifested by various DNA binding proteins, the initial state of the system is set by the chemical composition of the environment. This environment changes due to conditions beyond the control of the Boolean gene regulatory circuit.

Consider a genetic network with  $N$  genes. Each of these genes can be assigned a Boolean variable  $\sigma_i = 0$  or 1. For each of the  $N$  genes we define an updating matrix in the form of a lookup table which determines its output for each of the possible  $2^N$  input states from the  $N$  genes in the system. This Boolean updating matrix is assigned random values, all rules are *a priori* equally possible. The matrix is effectively quenched on evolutionary time scales. Finally, we define which gene is actively connected to which, by a matrix  $w_{ij}$  that defines the input to gene number  $i$  from gene number  $j$  as  $w_{ij}\sigma_j$ . The entry value of the connectivity matrix  $w_{ij}$  can take values 0, if  $i$  is not connected to  $j$ , and 1, if  $i$  is connected to  $j$ . Typically only a fraction of the connectivity matrix entries is in use, and the average number of connected inputs per gene is called the connectivity  $K$ . It varies between 0 and  $N$ , meaning that  $K$  may include self-couplings. Thus  $K = 0$  means that all is fixed to the output state specified by input  $(0, \dots, 0)$  to all genes.

The system we evolve is the set of couplings  $w_{ij}$  in a single Boolean network. The simulation starts with a low but finite connectivity, here an initial average connectivity of  $K = 1$  per site. One evolutionary time step of the network is

(1) Select a random input state to the network  $\{\sigma_i\}$ . Iterate the system, called the mother, from this state until a final attractor is determined.

(2) 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  $p = 1/3$ . Iterate the daughter system from the same initial state as that selected for the mother and test whether it reaches the same attractor as the mother system did. In case it does, then replace mother with daughter network and go to step 3. In case another attractor is reached, keep mother network and go to step 3.

(3) Then, finally, one random bit of the total  $N \times 2^N$  lookup table entries is flipped to another value. This allows for a convenient self-averaging of the system, and in fact represents a very slow change.

Iterating these steps makes an evolutionary algorithm that represents the sole requirement of continuity in evolution and how this may proceed under an environment that fluctuates. No selective pressure is applied. Step 3 rarely affects the active part of the network because  $K$  typically remains low compared to  $N$ , and thus  $2^K \ll 2^N$ . It is included in order to obtain a self-averaging of the system, that otherwise tends to have small effective statistics even for the small  $N$  we can simulate.

In Fig. 1a we show how the connectivity ( $K$ ) of this system evolves with time in a network of size  $N = 16$ . One observes that the typical  $K$  of the network is confined to lower values than of random networks. This is further quantified in Fig. 2 where the distributions of average connectivities are displayed in the statistically stationary state. Notice that there are two distributions: one counting the frequency of connectivities for all new “species,” and one counting the time averaged distribution. These two distributions diverge strongly for high  $K$ , because the few species with high  $K$  have very long lifetimes, i.e.,

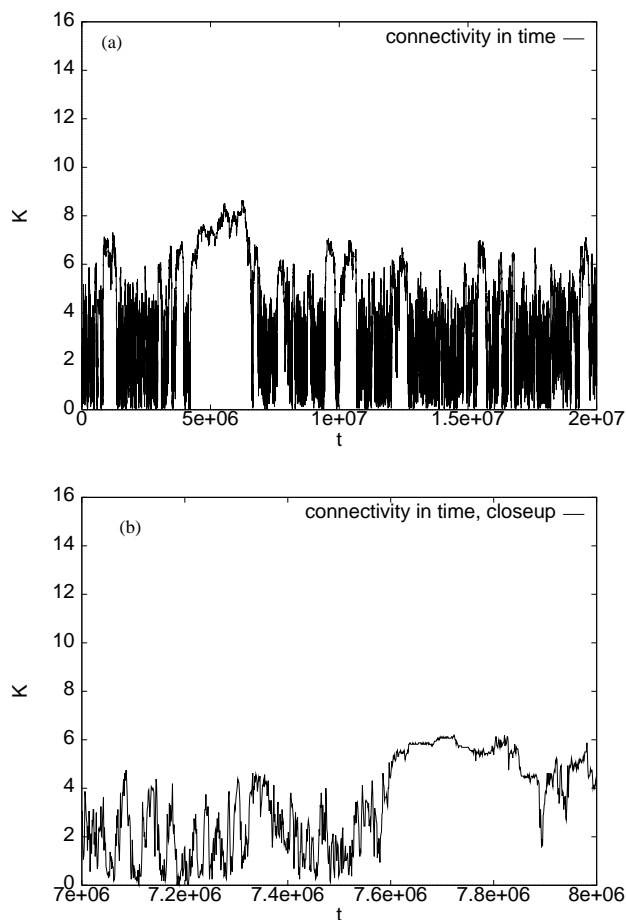


FIG. 1. Evolution of the Boolean network connectivity with time (a) and close-up on a part of the connectivity evolution (b). Note that periods of approximate stasis and sudden punctuations appear on both time scales. A single network of size  $N = 16$  has been simulated starting with an initial average connectivity of  $K = 1$  active inputs per node. The connectivity matrix as well as the Boolean updating matrix were chosen completely randomly, with all possible Boolean rules allowed. Apart from the slow adjustment of Boolean rules under step 3 in the model, the system thus evolves in a quenched “landscape” of Boolean rules. The connectivity  $K$  shown is directly measured from the connectivity matrix of the network. The effective connectivity [15], defined as  $K$  minus the number of connected inputs that do not contribute due to specific Boolean updating matrix entries, has somewhat lower values but shows similar overall behavior.

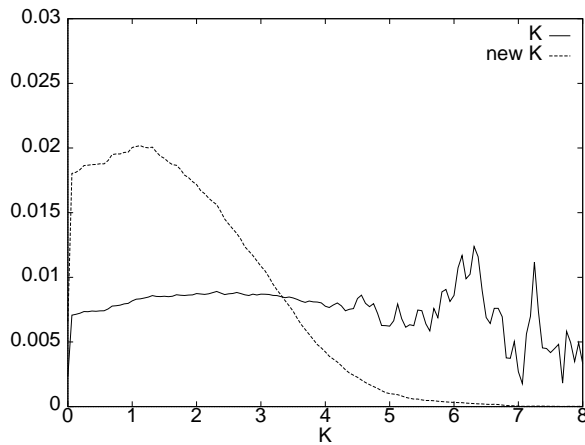


FIG. 2. Distributions of connectivity  $K$  in the statistically stationary state, obtained from the same simulation as in Fig. 1. The frequency of connectivities of the new “species” is shown (“new  $K$ ”), as well as the time averaged distribution of connectivities of all mutated “species.” Note that for higher values of  $K$ , a mutant network is less likely to match the phenotype of its parent.

for high  $K$  it is difficult to find mutations which do not change the activity pattern of the networks. In our case, the activity pattern consists of the transient and the final periodic attractor following the given initial state. The time scale of these patterns becomes large for networks with high  $K$ , making it more difficult to keep the exact dynamic pattern under the mutation of a weight. In popular terms, an increased complexity of the network makes further evolution difficult. One may speculate that this is the reason for real genetic networks to keep their connectivity low: It will be easier to evolve by increasing the number of genes  $N$  at a fairly low connectivity level (the present model, however, does not consider variable  $N$ ).

In Fig. 1a we further see that marked punctuations occur, where long periods of nearly fixed average connectivity sometimes are interrupted by a sudden change in connectivity. This interplay between long waiting times and short times for actual changes is in fact observed in the fossil record. The phenomenon has been coined “punctuated equilibrium” by Gould and Eldredge [6]. As also seen from Fig. 1b, the periods of stasis show a similar structure on shorter time scales as they do on longer time scales. This is explored further in Fig. 3a where we show this distribution averaged over the simulation. Approximately the distribution of stasis times is  $\propto 1/t^2$ . Periods of stasis at high values of  $K$  can become long, which in practice calls for very long equilibration times.

In Fig. 3b we decompose the stasis time distribution into times obtained for different values of the average connectivity. Again we observe that higher  $K$  typically shows longer stasis times. Remarkably, when looking at the statistics of a small interval of low  $K$  values, we observe exponentially distributed stasis times. The power

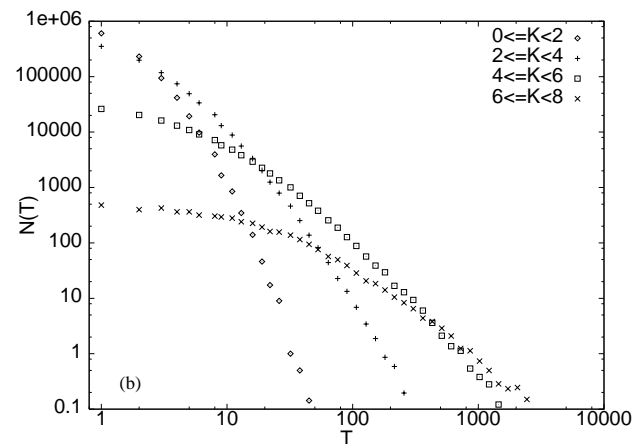
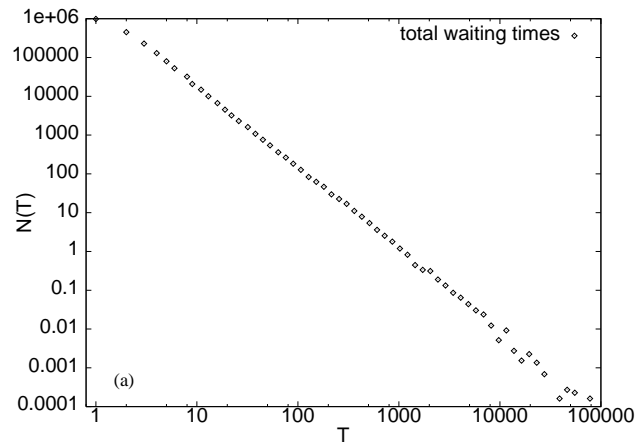


FIG. 3. Stasis time distribution in the neutral evolution of networks (a) and the decomposition into stasis time distributions for different intervals of the average connectivity (b). The simulation is the same as in Fig. 1. While the average over all networks approximately follows a power law, the distributions for networks with restricted  $K$  do not. One observes that large connectivity typically implies a lower degree of evolvability.

law behavior then comes about by averaging over the range of all  $K$  values.

In order to test for the robustness of our model we tried other mutation rules (again without any evolutionary pressure, i.e., symmetric in adding and removing weights). In one variant a daughter network was created by (a) adding or (b) removing a weight in the coupling matrix at random, with  $p = 1/2$  each, thus allowing for  $K$ -changing mutations only. In another variant a daughter network was created by independently adding a random weight with  $p = 1/2$  and removing a random weight with  $p = 1/2$ . We also tested a scenario where we demanded complete match between attractors of mother and daughter for two different initial configurations. Also, we considered this case with demanding only partial overlap between mother and daughter, i.e., match in at least one of the two tested environments, only. Finally, we tested networks with weights  $w_{ij} \in \{-1, 0, 1\}$  such that the signal transmitted from an inactive node can differ from the

value of a disconnected input node. In all cases our results were robust.

Let us briefly discuss the meaning of the stasis times and punctuations observed here. According to the definition of our model, we quantify the waiting time in terms of the number of times mutant networks are exposed to new environments before a neutral mutation occurs that fulfills continuity. Thus they are not to be confused with the “neutral evolution” introduced by Kimura [3] which leads to waiting times consisting of a number of neutral mutations. The genetic networks are formally defining a species and the length of the waiting times indicates the “genetic flexibility” of a species.

Associating the interconnectedness of the networks with the genetic flexibility of real organisms, one may attempt to understand a puzzling decomposition of lifetimes of species in the fossil record. First, it was noted by Van Valen [16] that each group of closely related species has exponentially distributed lifetimes. Second, an analysis of the overall distribution of genera lifetimes, tabulated by Raup and Sepkoski [17], showed that this is rather distributed as  $\propto 1/t^2$  [18] for genera lifetimes exceeding  $10 \times 10^6$  yr. It is tempting to speculate that groups of closely related species are associated to the same genetic flexibility, and thus evolve, and eventually get extinct, with a frequency given by this genetic flexibility. This would explain the exponential distribution of Van Valen’s. Averaging over all genetic flexibilities is then an average over different characteristic lifetimes, and our simplified evolution scenario demonstrates how such an averaging can give an overall  $1/t^2$  distribution.

The obtained  $1/t^2$  scaling may be an inherent part of our neutral evolution scenario [19]. In comparison, for evolution on fitness landscapes one typically obtains a distribution  $\propto 1/t$  corresponding to a sampling of waiting times for passing over barriers [18,20], although a  $1/t^2$  distribution can be obtained by supplementing a hill climbing concept with the assumption that extinction of a given species is determined by evolutionary rates of older species [21]. Abandoning fitness landscapes, the ecological network model of Refs. [22,23] instead determines the fate of a species through an ecological connectivity matrix. When this evolving network of species is assigned a connectivity that is comparable to (eco)system size, it shows a  $1/t^2$  distribution of genera lifetimes. In contrast to these macroevolutionary models, our study of Boolean networks considers only one species, with a comparison to species extinction data that is based on an extrapolation of the obtained evolutionary clock.

In conclusion, we have studied evolution of Boolean networks in the absence of any competition. This simplification allowed us to discuss how the requirement of evolving robust networks in itself may lead to an evolution which exhibits punctuated equilibrium.

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