

From wiring to function and back: a case study in feed-forward networks

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Biological and technological systems process information by means of cascades of signals. Be they interacting genes, spiking neurons, or electronic transistors, signals travel across these systems, producing, for each set of external conditions, an appropriate response. In technology, circuits that perform specific, complex tasks are designed by humans. In biology, however, design has to be ruled out, confronting us with the question of how these systems could have arisen by accumulation of small changes. The key factor is the genotype-phenotype map, which modulates the adaptation process profoundly. Although a detailed study of RNA secondary structures has helped illuminate the structure of this map for combinatorial molecules, until now it wasn't known whether this ideas had a more general applicability. Here we show that some important features of the genotype-phenotype map of simple feed-forward circuits follow a similar pattern: they have a large degree of neutrality, by which a circuit can be completely rewired keeping its input-output function intact, and there is a relatively small neighbourhood of a given circuit containing almost all phenotypes. However, some key differences arise, the most important being that across the connected webs of neutral neighbours circuits have vastly different degrees of evolvability.

Keywords: feed-forward networks, Boolean networks, neutrality, evolvability

I. INTRODUCTION

Many biological systems perform computations by internally processing external stimuli. Some have information processing capabilities that rival those of computers. Signaling transduction, metabolic pathways, gene regulatory networks, immune responses and neural assemblies are examples of such form of processing (1), which is carried out by different kinds of networks. All of them perform some class of *computation* (2; 3), an essential ingredient of adaptation, whose evolutionary dynamics is largely unknown.

The evolution of multicellular life is pervaded by the computational nature of biological networks. They benefit from extensive cross-talk among different parts and are able to buffer mutational change and/or generate a wide repertoire of responses. When compared with artificial designs, such as electronic circuits (4) it is possible to identify common traits: both of them are definable in terms of an input-output structure with well-defined functional meaning. But their evolutionary rules strongly diverge. As early pointed by F. Jacob (5; 6) it is tinkering –not design– what shapes biological structures. Tinkering implies re-use and local, instead of top-down, planned decisions. Yet in spite of its apparent limitations, it is obviously successful (7) but not well understood. This is due to a lack of knowledge of the mapping between structure (genotype) and function (phenotype). With the exception of studies at the molecular level, (8; 9) little is known about the nature of such mapping.

In order to uncover such mapping and its consequences for network evolution, we have explored the class of so called *feed forward networks* (FFN). They all involve the presence of a set units acting as receptors and a downstream cascade of signaling events ending up in a set of output units. Such systems are simple (and yet very general) models of biological networks, from intracellular signaling (10) to layered cortical maps (11). Consistently with previous work on RNA folding (8), we observe that: (a) *neutral networks* percolate the entire

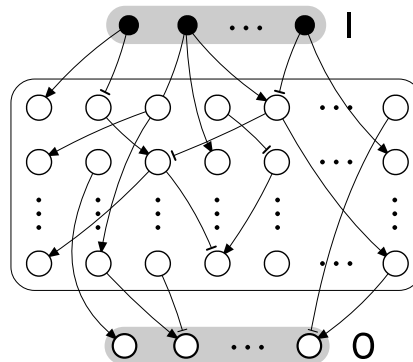


FIG. 1 Topology of the model of FFN used. It consists of inputs (I units), hidden units ($H \times M$ units), and outputs (O units). Units can connect strictly to the layers above, thus avoiding cycles, except for the outputs, which cannot connect directly to the inputs.

genotype space: there are always single-mutation neighbours of a given wiring that have the same input-output function, to the point of enabling us to go arbitrarily far in genotype space; and (b) it is not necessary to search all of the genotype space to find a given phenotype, since all phenotypes are present around a relatively small neighbourhood of a given sequence, compared to the size of the whole space. A third piece of information suggests an even more interesting picture: (c) the search neighbourhood becomes much smaller if we start at specifically chosen genotypes which have been optimized for mutant diversity, suggesting large differences in evolvability.

II. FEED-FORWARD BOOLEAN NETWORKS

Network structure and function. The model used is a very simple feed-forward structure. The network has I inputs, O outputs, and a $H \times M$ block of hidden units, as figure 1 shows. Units in the hidden block can connect only to the layers above

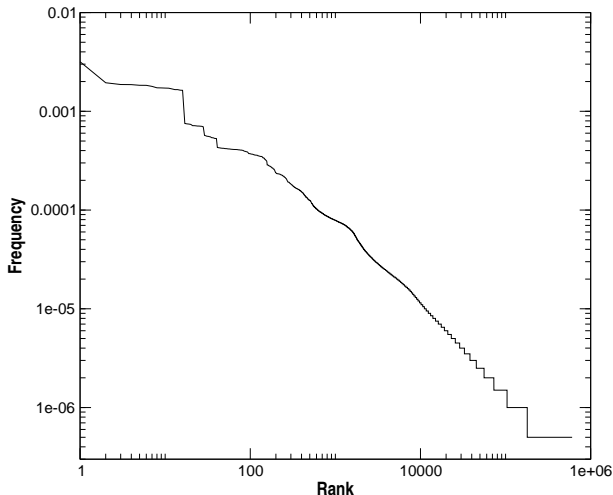


FIG. 2 Rank-frequency distribution of functions. A network with $I = 3, O = 4, H = 8, M = 3$ and average connectivity $\langle k \rangle = 3$ was chosen and a sample of 2×10^6 was taken. The distribution follows a general form of Zipf's law, i.e. $P(r) = a(b + r)^{-\alpha}$ with $\alpha \approx 0.75$.

them (thus avoiding cycles and cyclic behavior), including inputs, and the outputs can connect to the hidden units but not directly to the inputs. In addition, the number E of connections is fixed.

The units s_i of the network have a Boolean nature (i.e. $s_i \in \{0, 1\}$), and perform a simple integer threshold function of the inputs, that is,

$$s_i(t+1) = \Theta \left(\sum_{j=1}^N w_{ij} s_j(t) \right). \quad (1)$$

The Θ function is defined as: $\Theta(x) = 0$ for $x \leq 0$, and $\Theta(x) = 1$ for $x > 0$ (thus the XOR function is not possible with only one unit). The weights w_{ij} are drawn from the set $\{+1, -1, 0\}$ representing positive, negative, or absent regulation, respectively. When an input is presented, the output can be computed propagating the inputs in a non-dynamical way just as if all units changed at once.

By this definition, the input layer of the circuit models external states (or the result of sensing external states) being presented to the network, and the bottom layer models the output, representing needed response. The network, therefore, ‘‘computes’’ the appropriate set of responses for each external state. This feed-forward topology is widely used in artificial neural networks, although in most cases units connect to the preceding layer only, and generally weights are floating point numbers (12). This distinction is what enables us to more appropriately define a genotype and a phenotype. Additionally, the model presented has an asymmetrical treatment of activation since to produce activity in one unit, its preceding units have to be also active (an all-zero input always produces an all-zero output).

Wiring-function mapping. Given this structure, we can easily define a genotype and a phenotype. The *genotype*, W_i ,

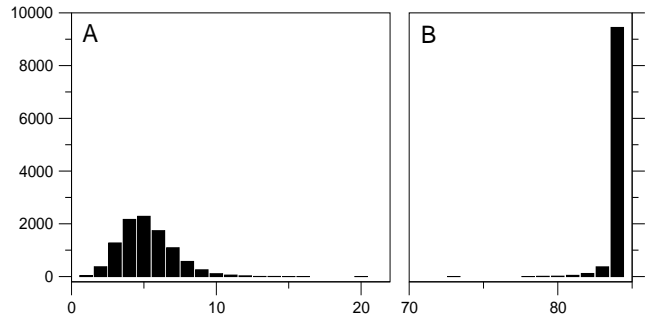


FIG. 3 Probability densities of genotype distance d_G in two different experiments. (A) A target wiring is chosen at random, and an approach in genotype space to this target is tried by 10^4 different wirings. For each trial wiring, a neutral path is performed in which neighbours have to maintain function and at the same time be closer to the target (average final distance to the target: 5.18 ± 1.82). (B) A similar neutral path is tried. In this case, a random genotype is chosen and a trail copy is mutated, step by step pushing it away in genotype space, while again conserving function (94.4% of genotypes were completely rewired while still maintaining phenotype).

is defined as the ordered string of all weights w_{ij} . To compute the phenotype we first calculate all the input-output pairs, with all possible different inputs from $I_1 = \{0, 0, 0, \dots, 1\}$ to $I_{2^N-1} = \{1, 1, \dots, 1\}$ (with the exception of $I_0 = \{0, 0, \dots, 0\}$ which, by definition, yields an all-zero output). The entire list of outputs fully describes the Boolean function Φ_i , or *phenotype*.

Two sets, \mathbf{W} and Φ , describe the universe of possible wirings and functions, i.e. the sets of all possible genotypes and phenotypes. The genotype-phenotype map between wiring and function is then defined as

$$\Omega : \mathbf{W} \longrightarrow \Phi. \quad (2)$$

For each genotype $W_i \in \mathbf{W}$, we have a phenotype $\Phi_i \equiv \Omega(W_i) \in \Phi$. Evolution and adaptation occurs through changes in wiring eventually leading to changes in function. How adaptation proceeds largely depends on the nature of the mapping Ω (13).

In order to characterize Ω , a metric or topological measure is needed (ref). Given the discrete nature of both spaces, phenotypic and genotypic distances can be defined, respectively, as

$$d_P(\Phi_a, \Phi_b \in \Phi) = \sum_k |\Phi_a^k - \Phi_b^k|, \quad (3)$$

$$d_G(W_a, W_b \in \mathbf{W}) = \frac{1}{2} \sum_k |W_a^k - W_b^k|. \quad (4)$$

Phenotype distance is, therefore, equivalent to the Hamming distance of a bit string, and genotype distance is similar, measuring the number of different connections (that is, either displaced or with reversed sign, which contribute 2 to the sum, hence the $1/2$ factor).

Throughout the work, we have used small networks, usually with $I \in \{3, 4\}$, $O \in \{4, 5\}$, $H = \{7, \dots, 11\}$, and $M \in \{3, 4\}$, with an average connectivity of $\langle k \rangle \approx 3.0$ which

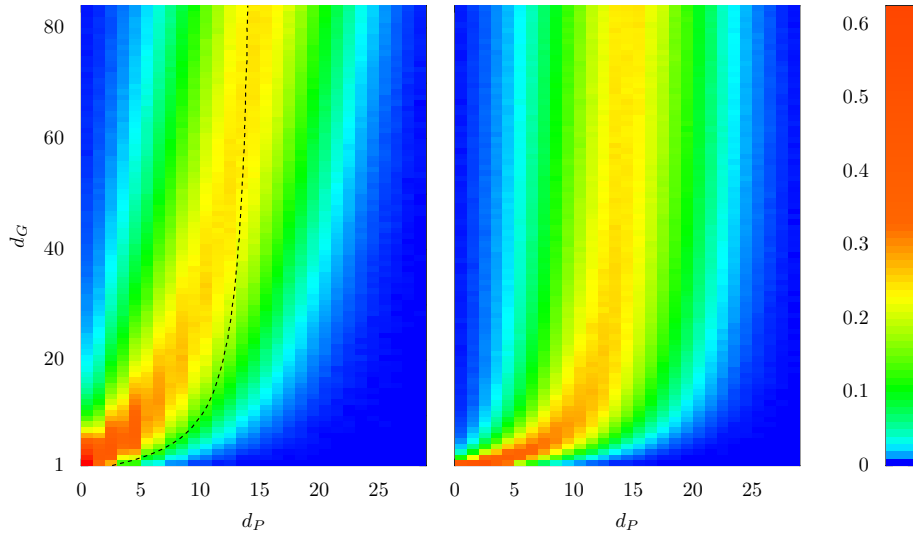


FIG. 4 The genotype-map structure as seen from a group of genotypes in two different numerical experiments $((I, O, H, M, \langle k \rangle) = (3, 4, 8, 3, 3.0))$. **Left.** From 10^3 random wirings, the histogram of phenotypic distances of 10 other wirings at progressively higher distances starting at them. **Right.** The same histogram starting at the 80 selected genotypes with increased mutational diversity. The average phenotypic distance of the right part is plotted as a dashed black line in the left part for comparison.

allowed us to more exhaustively explore genotype and phenotype spaces.

Network wiring changes. Mutation is implemented as the simplest random procedure that alters the wiring of the network: an existing edge is chosen at random and it is removed, and a new edge is chosen also at random and it is added, with a negative weight with probability $1/3$ and a positive weight with probability $2/3$. The bias in the weights tries to compensate for the fact that a balanced network is less active overall.

III. RESULTS

Frequencies of shapes. The frequencies of different functions were obtained using a sample of 2×10^6 random wirings and computing the input-output table by the rules given. The rank-plot of this data is shown in figure 2, evidencing a general form of power law. Thus, there are some frequent functions and many rare ones. The most frequent is the all-zero outputs function: there is a certain probability that no activation path exists between inputs and outputs, although overall, the probability is rather low (3.1×10^{-3}). The small plateau following this first value at the low rank zone corresponds to those functions with an equal output for all different inputs. Also, within groups of 10 or more genotypes with the same phenotype, we calculated the average genotypic distance. In all cases we obtained the same distance (within 1%) as that of a random sample, confirming that phenotypes have genotypes distributed uniformly over genotype space.

Neutral paths. Two experiments were performed to check for the existence of neutral networks (i.e., regions in \mathbf{W} consisting of neighboring genotypes with the same phenotype), both involving neutral paths with monotonously decreasing (and increasing) distance from a reference sequence. In the

first (fig. 3A), a target wiring W is chosen at random, and a second random wiring is chosen as trial genotype, T . Next, if a random neighbour T' of T conserves the phenotype and has a smaller $d_G(W, T')$, it is accepted as the new T . The process is repeated 10^4 times. The final $d_G(W, T)$ is an upper bound of the minimum distance of the two phenotypes Φ^W and Φ^T . It is remarkable that this distance is on average 5 (out of 84).

In the second experiment, a random wiring W is chosen, and a copy of it is taken as trial, T . At each step, if a random neighbour T' of T has the same phenotype as W and $d_G(W, T')$ is larger, it is accepted as the new T . The process is repeated 10^4 times. The final $d_G(W, T)$ correlates with the size in genotype space of the neutral networks. In this experiment, 94.4% of the genotypes could be completely rewired (maximum genotype distance of 84) while keeping the phenotype (the smaller distance being 73). Neutral networks, therefore, percolate through genotype space.

Map Structure. To understand how the map Ω is seen from the viewpoint of an average genotype W , we evaluated the probability that another genotype W' at distance $d_G(W, W')$ had a phenotype at a certain distance d_P (the Structure Density Surface, or SDS (8)). This probability was evaluated by producing progressively distant mutants from a given starting wiring, and evaluating the distance d_P of their respective functions. We took 10^3 reference wirings, and for each one, we chose 10 different wirings at all values of d_G and computed d_P . Figure 4 (left) shows the resulting two-dimensional histogram. As d_G increases, we have a picture of how the average phenotypic distance behaves. For values of d_G between 1 and 20, there is correlation with the phenotype (a few changed wires usually produce a few changes in function), but after that, the distance to phenotypes is progressively similar to the random case, i.e. we can expect to find an almost random phenotype if we change 20 or more of a given network's

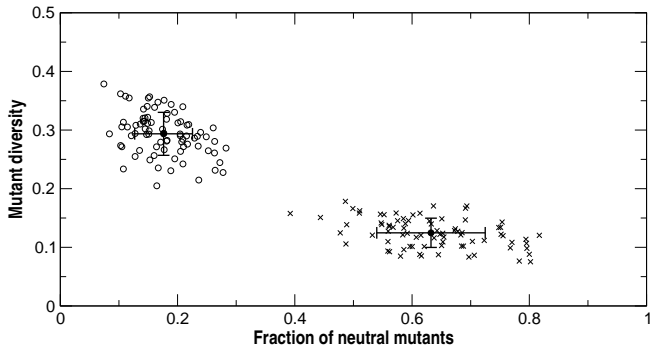


FIG. 5 A set of 80 random genotypes (crosses) was optimized for higher mutant diversity, conserving their phenotype (circles). A hill-climbing process was performed with each one so that either the fraction of neutral mutants (μ) decreased, or the diversity of the different mutants (δ) increased. The initial average values of (0.63, 0.12), indicating very robust networks, were changed into (0.17, 0.29), suggesting the big differences in the sensitivity to mutations of genotypes within the same neutral network.

links (a 25% of the total).

Together with the covering of genotype space by the average phenotype, these results suggest the presence of a neighbourhood (a high-dimensional ball) around a particular FFN whose wirings include all common functions, in consonance with the RNA case (8). However, there are some differences. Firstly, a few changes in an RNA sequence mostly result in a changed shape: even in the case of only one mutation, an RNA molecule can have a drastically different structure (up to a 66% change in phenotype distance). This is in contrast to FFNs, which in general are more robust for a small number of mutations (fig. 4, left). Secondly, the radius of the high-dimensional ball around which a genotype can find all common functions is somewhat smaller in the RNA case (around 15%). These differences lead us to think whether a special group of FFNs could be more sensitive to mutations, and therefore deeply alter the perception of genotype space in the same experiment with them as starting points.

Mutational Sensitivity. To test this hypothesis, we searched FFNs with a higher average sensitivity. Starting at a random genotype W , we measured its mutant diversity with two parameters: μ (satisfying $0 < \mu < 1$), indicating the fraction of mutants with a different phenotype (i.e., non-neutral), and δ (satisfying $0 < \delta < 1$), measuring the fraction of unique phenotypes within the non-neutral group (or diversity). A pair (μ, δ) with values (0.85, 0.1) describes a robust FFN with an 85% of neutral mutants, in which non-neutral phenotypes (the remaining 15%) are repeated 10 times on average.

We chose a group of 80 random FFNs and with each one, we performed a hill climbing process, successively choosing mutants with either lower μ or higher δ , but conserving phenotype. The size of the mutant samples was 2×10^3 . The starting and ending sets are shown in figure 5. It is immediately clear that the differences in mutation sensitivity are enormous. This differences suggest that within a neutral network, special FFNs could serve as gateways, giving populations access to a very high number of different phenotypes from the same spot.

This is confirmed by the structure of the genotype-phenotype map (fig. 4, right) as viewed from the sensitive group of FFNs. The average phenotype distance is plotted as a dashed line on the left, for comparison. The distance separating this group from a random genotype is halved, indicating a much smaller search space for this special FFNs.

Dynamical transitions. The structure of the mapping is further made clear by studying evolutionary dynamics. Following previous approaches (14) we did some optimization experiments in which a population of FFNs evolves towards a target function. We choose a very unfrequent target phenotype Φ_T (the phenotype with the highest average output-pair entropy in a sample of 10^5), and a group of 10^3 FFNs chosen at random serves as initial population. At each iteration, a new population results from fitness-proportionate reproduction, with the fitness of a genotype W_i being $F_i = e^{-d_P(\Phi_i, \Phi_T)}$. Every reproduced FFN has a probability $p = 0.3$ of being mutated.

An example of the dynamics displayed by this kind of process is shown in figure 6. The average distance to the target decreases with time, showing punctuated events in which fitter genotypes spread rapidly within the population. Between these transitions, a stable regime characterized by an increase in genetic diversity takes place. A sample of the average d_G of FFNs in the population shows increasing values, which drop abruptly whenever a fitter genotype takes over. This is the typical result that should be expected from the random drift of a population within a genotype space in the presence of neutrality (14; 15).

IV. DISCUSSION

In FFNs, neutrality is a consequence of the numerous connections in a specific network that can be added or removed without directly affecting its functionality (3). Therefore, it is a robust result that is insensitive to the parameters I , O , M or H , but depends on the existence of a threshold at each unit, as the consistent results we have observed suggest. The exception is the rank-frequency distribution, which already for the values of $I = 6$ and $O = 6$ turns out to be too large to sample with enough significance, and therefore, is different if sampled with the same density. In addition, we are aware of some caveats of our approach.

First, the parameters affect other aspects of the FFNs, such as their ability to compute a randomly chosen phenotype. As in the case of neural networks, the number of hidden layers (H here) and their size (M here), affects the complexity of the computations available to the system, or its capacity (16), and, in the case of FFNs, to the attainable phenotypes (which also depends on the number of links E). In this sense, many dynamics experiments such as the one shown in figure 6 failed when performed with a target phenotype chosen at random (and never with a phenotype computed from a random *genotype*). In general, we do not know what is the precise dependence between an increase in M or H and the diversity of phenotypes, but we expect to find an increasing coverage of phenotype space as H and M increase.

Second, and concerning explanatory power, the all-or-none,

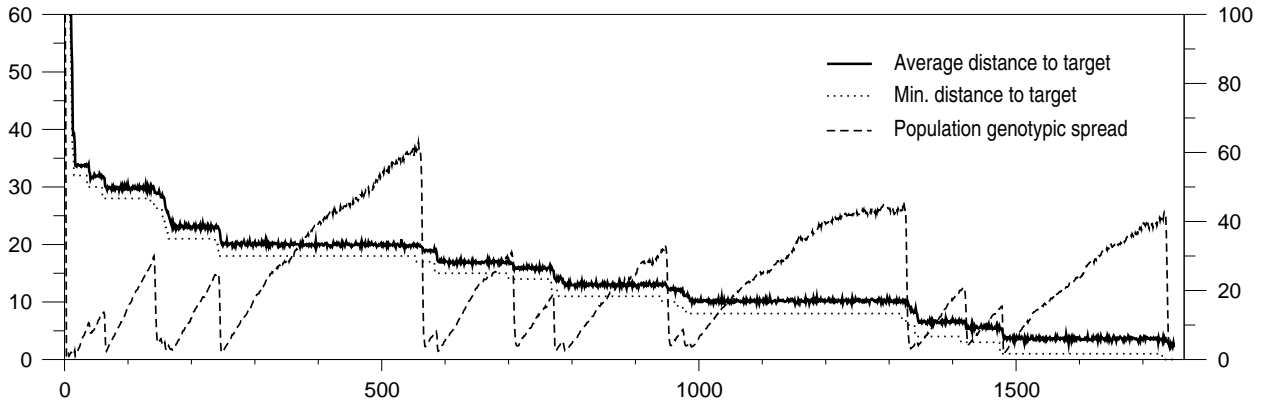


FIG. 6 An example of the evolutionary optimization towards a specially chosen phenotype (see text). The parameters of the FFNs are $I = 6$, $O = 5$, $H = 10$, $M = 5$, $L = 3.0$. The initial population consists of 10^3 random FFNs. The population finds the target in 1749 generations, and the dynamics shows punctuated events in which population diversity falls abruptly. The vertical axis on the left shows distance to the target, or d_P . On the right, it shows genotypic distance, or d_G . Genotypic spread is the average d_G between 10^3 random pairs of genotypes within the population.

binary nature of the model dynamics used here is certainly an oversimplification. However, it is consistent with the switch-like behavior of proteins within signaling cascades (10; 17), and some studies show how a Boolean treatment of gene networks is very successful (18). In our study, a Boolean idealization was a necessity since the proper definition of a phenotype and genotype requires discretization, and we feel is also pertinent to the issues treated. A similar argument applies to the feed-forward nature of the networks studied, since real networks are in general recurrent but the methodological issues involved in modeling them make the problem much more difficult.

And third, there is no agreed measure of evolutionary adaptability, or evolvability. Although its definition is mostly clear (19), it leaves room for interpretation and proposed measures are inevitably defined in terms of the models presented, and our sensitivity measure (μ, δ) is no exception. Nevertheless, sensitivity to mutation is informative about the plasticity of a given genotype, in relation with the second point of the evolvability definition: “to reduce the number of mutations needed to produce phenotypically novel traits” (19). Since neutrality is assumed, the first point, “to reduce the potential lethality of mutations”, is fulfilled (other authors have already studied evolvability as affected by neutrality (20), but in the sense of “the ability of random variations to sometimes produce improvement”).

Despite these limitations, and as already discussed in (8), the presence of neutrality in the genotype-phenotype map has immediate consequences for an evolutionary process, and our results extend those of RNA and combinatorial molecules to a new domain and provide a good example of a more general applicability of these ideas. In the case of biology, living systems have evolved mechanisms of computation able to optimize their chances of survival. As a consequence, convergence towards networked structures able to integrate and process external inputs into reliable outputs has been widespread. Our results suggest that evolving such functional networks is not as difficult as it may seem, complementing the strictly

topological results in the field of network biology (21). Current research is actually aimed at building synthetic molecular interaction networks (22; 23). Not surprisingly, special interest is being focused on the possibility of exploiting the computational potential of such networks. As other authors have suggested before (7; 24), reprogramming of control pathways can be produced by slight changes coming from protein domain recombination, and our results confirm this picture.

On the other hand, many attempts have been made at the evolutionary design of digital (and analog) circuits, using genetic representations. In this context, some authors have already pointed out the importance of neutrality in artificial circuit evolution (25) and genetic programming (26). However, most of this work has been focused on the production of small electronic circuits and their application to other domains is not straightforward. The results presented here could also contribute to this field. Although there is a consensus about the need for a so called complex systems engineering (27), success is still modest.

A very informative result in these lines is the remarkable difference in sensitivity among the genotypes in a neutral network, suggesting that these networks have finer structure inside. This opens up interesting new questions, and in particular it cleanly visualizes the already mentioned, and somewhat controversial issue of evolvability (19; 28), about which precise models are scarce. If special FFNs have a broader spectrum of mutants, they could more readily access new functions, and hence adapt more rapidly. If this is translated in some way to the domain of circuit evolution, adaptation would be greatly speeded up, maybe allowing us to design larger circuits. This sensitivity is in contrast with the natural tendency of populations to drift towards the more connected parts of a neutral network, which are occupied by the more robust phenotypes (29; 30). It remains to be seen if such sensitivity is found in biological networks, how it could be maintained in the course of an artificial adaptation experiment, and, perhaps more importantly, what is the underlying structure that supports it.

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