Criticality in gene networks

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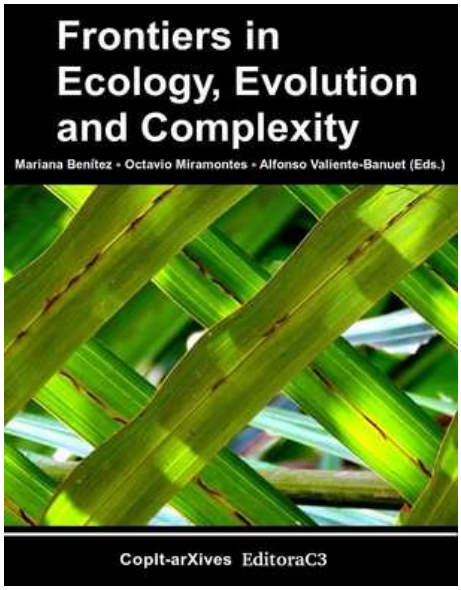

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1 Abstract

Along evolution, living systems have prevailed in different and constantly changing environments, each one demanding a distinctive set of phenotypic traits in order to survive. Over their lifetime, most organisms need to cope with a huge spectrum of perturbations ranging from external temperature and pressure changes to inherent disruptions such as genetic mutations. Life in these circumstances have forced organisms to be phenotypically robust, in the sense that their phenotypes have to maintain functionality under many conditions. At the same time, living organisms must be flexible enough as to develop new phenotypes in order to keep up with new environmental challenges. At the heart of the problem of how organisms reach this equilibrium between phenotypic robustness and phenotypic innovation, we find the concept of dynamical criticality. For it is at criticality, namely at the brink of a phase transition between ordered and chaotic dynamics, where phenotypic robustness and innovation can coexist. Here we present a theoretical framework for the evolution of genetic regulatory networks that provides a very likely explanation of how criticality emerges in evolution. Under this framework, we evolve populations of networks subjected to mutations and demand the fulfilment of two selection criteria that are common to the evolution of all living organisms: (i) at each evolutionary step the already acquired phenotypic traits must be conserved, and (ii) in the long term new phenotypic traits must emerge. (We will refer to "phenotypic traits" as the dynamical attractors of the network). Surprisingly, these two selection criteria are enough to rapidly produce populations of networks operating at criticality. Additionally, by demanding a non-trivial information content in the phenotypic traits of the network, we obtain topologies similar to the ones observed in real organisms, characterized by the presence of global regulators or "hubs" (i.e. nodes that regulate the expression of a great number of other genes). This last point is a clear example where restrictions imposed on the dynamical properties of the network can shape its topological structure.

2 Resumen

Durante su desarrollo, los organismos vivos tienen que contender con una gran variedad de perturbaciones que van desde cambios de temperatura y humedad en el medio ambiente, hasta alteraciones permanentes en su metabolismo y material genético. Por lo tanto, a lo largo de la evolución han tenido que generar dos características importantes para su supervivencia. Por un lado, el fenotipo de los organismos vivos tiene que ser lo suficientemente robusto para seguir funcionando adecuadamente en presencia de perturbaciones. Por otro lado, dicho fenotipo tiene que ser lo suficientemente flexible para eventualmente generar nuevas características que le permitan al organismo contender con nuevos retos ambientales. Existe evidencia teórica y experimental de que este balance entre robustez e innovación fenotípicas se logra al "borde del caos", es decir, cuando las redes genéticas de los organismos vivos operan en el punto crítico de una transición de fase entre dinámicas ordenadas y dinámicas caóticas. Sin embargo, aún no se sabe cómo es que a lo largo de la evolución se generaron redes genéticas operando con dinámicas críticas. En este capítulo presentamos un modelo evolutivo de redes genéticas que se basa en un principio muy sencillo pero fundamental de la evolución: La emergencia de nuevos fenotipos, necesarios para adaptarse a nuevos entornos, ocurre sin destruir las características fenotípicas que ya se habían adquirido antes. Esto se puede resumir coloquialmente diciendo: "la mosca no perdió las patas cuando le salieron las alas". Veremos como este principio es el responsable de generar redes operando con dinámicas críticas.

3 Introduction

One of the most challenging problems in Systems Biology is to understand the relationship of the structure and dynamics of the genome with the collection of phenotypes of the organism. There are two different approaches to this problem. One of them is to understand how gene expression patterns derive in distinct phenotypes [1–3]. This approach is of particular interest because it may lead to important therapeutic applications. For instance, one would like to predict the existence or absence of certain diseases (e.g. cancer or diabetes) from a particular set of gene expression patterns [4, 5]. On the other hand, we could also tackle this question from an evolutionary point of view, where one would aim to understand how adaptive constrains on the phenotypes influence the structure and dynamics of an underlying genetic network. It is clear that both the structure and dynamics of the genome have been crafted through evolution to determine the phenotypic traits of the organism. Indeed, experimental studies have shown that modifying the topology of a regulatory network directly alters its dynamics, which in turn affects the phenotypic traits of the organism. As an example of the above, in Ref. [6] it is shown that gene expression patterns resembling logic gates (AND, OR, NOR, etc.) arise by synthetically shuffling the regulatory architecture of a bacterial promoter. Depending on the logic gate coded in a particular promoter, the bacterium can codify (or not) a fluorescent

protein. Analogously, constraining the dynamics of a system during its evolution results in the appearance of important topological properties. For example, in Ref. [7] Parotti and his co-workers showed that imposing some stability constraints on the dynamics of a growing network, results in the emergence of complex topological properties, such as the ubiquitous scale-free topology.

However, understanding how the genomes acquired their observed structural and dynamical properties, and how such properties are intertwined to determine the organism's phenotypic traits, is not a trivial matter. The main limitations lie in understanding how evolution has molded phenotypes, as we do not know the exact series of environmental changes that each species underwent throughout its evolutionary history. Additionally, we know neither the particular constraints that each environment imposes, nor the effect that such restrictions would have in the genome's structure and dynamics. Nonetheless, despite the particularities of the evolutionary history of each species, there are two common characteristics in the evolution of every organism:

(a) evolution occurs in changing environments, and

(b) the new phenotypic traits, needed to cope with new environmental challenges, emerge on top of the already existing ones.

Property (a) states the widely accepted fact that evolution towards different (and perhaps more complex) forms of life is necessary, only because the environment changes and confronts organisms with new survival challenges. If the environment, as perceived by the organism, stayed always the same, evolution would be unnecessary. This does not rule out the existence of diversity, as even in the absence of selective pressure genetic drift is known to occur. Property (b) states that when new phenotypic traits are developed, previous ones do not disappear. Instead they are most likely conserved or slightly transformed. Thus, changing environments represent the driving force that generates new phenotypic traits across evolution.

So, the fact that organisms develop and survive in changing environments has two important consequences: *phenotypic robustness* and *phenotypic innovation* [8–10]. This essentially means that living systems must be able to maintain certain functionality in the face of perturbations, imposed by the changing environments, and at the same time be able to transform their phenotypes, and consequently their gene expression patterns, when the new environmental challenges become so demanding that the emergence of new functionalities is required. In this context, phenotypic robustness is a measure of how resilient the organism's phenotypes are when faced with a wide variety of perturbations [11, 12]. Highly robust systems would be those that preserve their phenotypes and functionality under perturbations; while lesser robust systems would lose functionality and drastically transform their phenotypes, even in the presence of small perturbations. We will discuss later how a system can gain or lose phenotypes.

Very importantly, two broad types of perturbations must be differentiated. First, we have transient perturbations, which range from environmental noise or brief chemical exposures, to even some epigenetic modifications. These perturbations affect the patterns of gene expression and the corresponding phenotypes only in the short-term. Second, there are much more stable changes such as point mutations, DNA recombinations, gene duplications, deletions etc. These perturbations are of particular interest, since they may affect the evolutionary path of a living organism, and its descendants, in an almost permanent manner. Although it is true that most of the genetic mutations are either neutral or unfavorable, eventually a set of those changes could derive in a new and fitter phenotype. This is exactly what phenotypic innovation refers to. Therefore, phenotypic innovation will be defined as the capability of an organism to generate new phenotypes, in order to successfully adapt to new conditions.

Later on we will give a precise definition of phenotypic robustness and innovation in terms of the dynamical properties of genetic network models. Here, suffices it to mention that evolving in changing environments will have repercussions in the ability of the genetic network to innovate and be robust; properties which in turn are dictated by the topology and dynamics of the network. In fact, previous work in this direction shows that changing environments, each one demanding new abilities or posing new constraints, can significantly speed up evolution [13]. Interestingly, the highest speedup was found in environments that changed gradually and therefore shared some requirements with the previous ones. Such environments changed the network topology through the spontaneous modularization of the system [14].

The ability of living systems to generate new phenotypes while preserving the previous ones is called *evolvability* [15–18]. This term can be summarized in colloquial terms by saying that "the fly got the wings without losing the legs". In other words, at the core of evolvability is the fact that, in the presence of new environmental challenges, new phenotypes emerge on top of the already existing ones. This requirement demands a delicate balance between forces of opposite nature: phenotypic robustness, in which organisms do not respond to perturbations (transient or permanent); and phenotypic innovation, which entails the generation of new phenotypes as a response to permanent mutations.

In physical systems, a very similar balance between robustness and responsiveness is often attained close to a critical point, namely at the brink of a phase transition, between ordered and chaotic dynamics [19]. Systems operating in the ordered regime are impervious to change, as they are able to gradually vanish every perturbation. On the contrary, chaotic systems are extremely sensitive to perturbations and their behavior is often unpredictable, with small initial perturbations propagating rapidly throughout the entire system. The delicate balance between robustness and sensitivity to perturbations is achieved close to criticality, where perturbations neither disappear nor propagate indefinitely, but typically remain confined to a small subset of elements. Because of this particularity of critical systems, it is natural to ask whether the juxtaposition in living systems of phenotypic robustness and phenotypic innovation could also be understood in

terms of critical dynamics.

In 1969 Stuart Kauffman empirically found a phase transition between ordered and chaotic dynamics, with a non-trivial critical point, in simple genetic regulatory network models [20]. His studies led him to propose that real genetic networks should operate at (or close to) criticality, as it is exactly at this point where the system exhibits the aforementioned balance [21]. Since then, his idea has been known as the *life at the edge of chaos hypothesis*. Nonetheless, in that pioneering work, Kauffman analyzed the network response exclusively under *transient perturbations*. Despite this analysis is useful to describe the behavior of an organism under perturbations that occur during its life time, evolution also requires living systems to be phenotypically evolvable under genetic mutations, which occur at much longer time scales and across generations.

Unfortunately, the life at the edge of chaos hypothesis, as insightful and appealing as it is, was formulated on not very solid grounds. This is because for many years, the implications of the dynamical phase in which the network operates, to the evolution of such network under permanent mutations, were unclear. Here we discuss a model of network evolution, and show that when genetic mutations are properly considered, Kauffman's hypothesis still holds. We will see that the two properties (a) and (b) mentioned above, although simple, have profound implications in the evolution of genetic networks. These two properties constitute the main ingredients that generate critical dynamics, and consequently, phenotypic robustness and innovation. As a prototype model for genetic regulatory networks we will use the Boolean network model proposed by Kauffman [20], as there is now plenty of evidence showing that it effectively captures the essential aspects of the gene regulatory process [22–27]. So, in the next section we will describe the Kauffman model of gene regulation and its three dynamical phases: ordered, critical and chaotic.

4 Boolean networks and criticality

In the Boolean approach proposed by Kauffman, the dynamical state of the genetic network is encoded in a set of N boolean variables, $\sigma_1, \sigma_2, \ldots, \sigma_N$, each representing the state of expression of a given gene. Thus, $\sigma_n(t) = 1$ or $\sigma_n(t) = 0$ according to whether the n^{th} gene is expressed or not at time t, respectively. The state of expression σ_n of the n^{th} gene changes in time and is determined by the state of expression of its regulators according to the equation

$$\sigma_n(t+1) = F_n(\sigma_1^n(t), \sigma_2^n(t), \ldots, \sigma_{k_n}^n(t)) \tag{1}$$

where $\{\sigma_1^n, \sigma_2^n, \ldots, \sigma_{k_n}^n\}$ are the k_n regulators of σ_n and $F_n(\cdot)$ is a Boolean function of k_n arguments that is constructed according to the activatory or inhibitory nature of the regulations. For networks of real organisms, the regulators of each gene and the corresponding Boolean functions are constructed with base on the biological knowledge of the system. Nowadays there is solid evidence showing that the Boolean approach is able to reproduce the gene expression patterns observed experimentally in several organisms.

Since we are not interested in a particular network of any specific organism, in the initial population we use random networks in which the k_n regulators of a given gene σ_n are chosen randomly from anywhere in the system. The Boolean functions are also assigned randomly in a way such that for each of the 2^{k_n} configurations of the k_n regulators, the Boolean function evaluates to 1 with probability p and to 0 with probability $1-p$. This is just the standard Kauffman model whose dynamical properties have been extensively studied. In particular, it is known that this simple model exhibits a continuous phase transition between ordered and chaotic dynamics [28–31]. In the ordered phase, any perturbation in the initial condition eventually disappears whereas in the chaotic phase any such perturbation propagates to a large fraction of the network. The parameter that determines in which dynamical phase the network operates is the so called *network sensitivity* S defined as

$$S = 2p(1-p)K, \tag{2}$$

where K is the average number of regulators per gene. If $S < 1$ the network will be in the ordered phase, and if $S > 1$ it will be in the chaotic phase. The critical phase is attained for $S = 1$, where the dynamics are not extremely sensitive to perturbations in the initial conditions (as in the chaotic phase), but the perturbations will not always disappear (as in the ordered phase). Fig. 1 illustrates the dynamical behavior of the network in the three different phases.

Because the network has a finite number of genes, there is also a finite number $\Omega = 2^N$ of possible dynamical states, ranging from $000\ldots0$ where all the genes are inactive, to the state $111\ldots1$ where all the genes are active, including all the intermediate states. This does not mean that, starting from a given initial condition, the network will necessarily explore all the $\Omega = 2^N$ possible states. In fact, before the network can go through all the possible states, it gets trapped in a *dynamical attractor*. Since the dynamics given by Eq. (1) are deterministic, starting out from one initial state, the network will go through a series of transients until a previously visited state is reached. At this point the network enters into a periodic pattern of expression that repeats itself over and over again. These periodic patterns are exactly the dynamical attractors mentioned above. Usually, several attractors may exist for the same network. All the states that converge to the same attractor constitute its particular basin of attraction. Networks operating in the ordered phase typically have a small number of attractors, whereas networks in the chaotic phase have a really large number of them [29]. Thus, the dynamical rule given in Eq. (1) partitions the state space into disjoint sets consisting of the attractors and their corresponding basins of attraction. The set of all the attractors (and their basins of attraction) is known as the *attractor landscape* of the network. The biological relevance of the dynamical attractors was first pointed out by Kauffman, who formulated the hypothesis that the attractors correspond to the stable patterns of expression of the genetic network, which in turn correspond to the different cell types or, more accurately, to the different functional states of the organism (its phenotypic traits). This hypothesis has been firmly demonstrated for

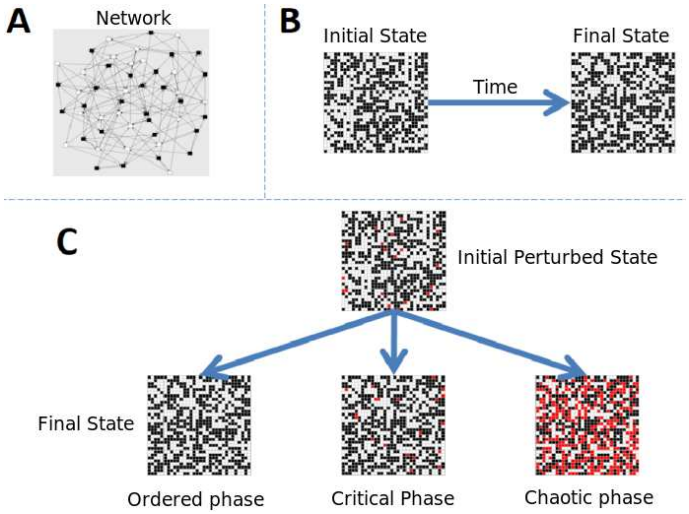

Figure 1: Network dynamics in the three different phases. (A) Graphic representation of the network state at a given time point. The color of the nodes represent their activity: white if the node is active and black if it is not active. (B) The N genes of the network have been placed on a square lattice just for visualization purposes. Starting with one initial state (on the left) the system develops in time until a stable state is reached (on the right). (C) The initial state is perturbed so that a few genes (less than 1%) are forced to change its activity. The perturbed genes are represented in red. If the network were operating in the ordered regime, the initial perturbation would disappear after some time and the network would reach the same stable state as without the perturbation. By contrast, in the chaotic regime the initial perturbation amplifies and propagates to a very large portion of the network, which ends up in a completely different state. In the critical phase, typically what happens is that the perturbation neither disappears nor propagates to the entire network, but remains confined to a small subset of genes.

several cases [22, 32, 33].

5 Waddington Epigenetic landscape

The fact that a given network has multiple attractors solves an important problem posed by Conrad H. Waddington with respect to the Modern Evolutionary Synthesis (MES), where it is proposed that a genotype (a given set of genes) corresponds to exactly one phenotype. In the 1950s Waddington noted that MES could not explain multicellularity, as this theory assumes that genetic mutation is the only source of phenotypic variation. But then, how could cell differentiation occur without any inheritable genetic mutation? Waddington proposed that throughout the development of an organism, intrinsic restrictions, imposed by gene expression and shaped by evolution, would occur. This idea led

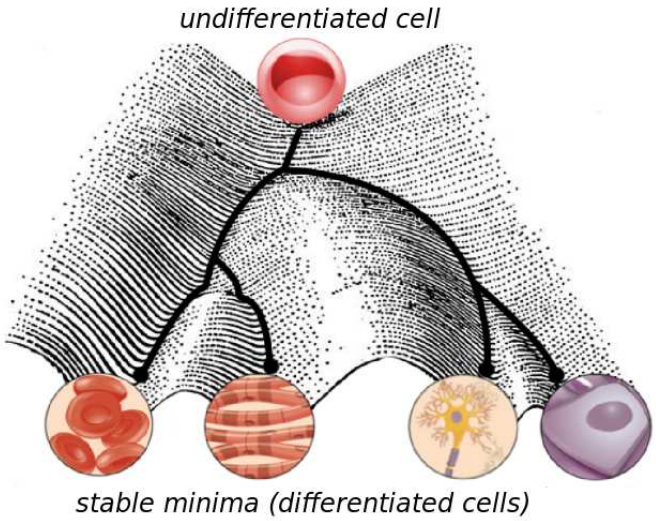

Figure 2: Waddington epigenetic landscape. In this metaphor, the undifferentiated embryonic cell is imagined like a ball that can roll down the hill on a surface that represents all possible states of expression in the genome. The stable minima in this surface would correspond to the stable phenotypes of the organism. The different bifurcation points represent alternative differentiation pathways.

him to formulate the concept of the *epigenetic landscape* as a metaphor of such restrictions. Waddington's epigenetic landscape consists of an hypothetical surface, with crests and valleys, over which a ball rolls down from the highest point of the surface, and ends into any of the possible lower minima (see Fig. 2). Along the way, there are ramification points where the ball can take different paths that lead to different minima. In this metaphor, the ball represents an embryonic undifferentiated cell whereas the surface represent all the possible states of expression of the genome. Thus, the undifferentiated cell "rolls down the hill" searching for the stable expression minima. In each ramification point of the development, the embryonic cell could take one path or another, depending on the presence of certain inductors, homeotic genes or even stochastic fluctuations. This mechanism was interpreted by Waddington as the effect of the environment over gene expression. The stable minima of the surface would then correspond to the stable phenotypes which the cell can get to.

Waddington's epigenetic landscape was considered for many years as a metaphor that could not be proved experimentally. However, after Kauffman's work, it was clear that the attractor landscape of a genetic network represents the formal materialization of Waddington's metaphor. The dynamical attractors correspond to the stable minima of Waddington's surface (stable phenotypes), whereas the basins of attraction correspond to the grooves and furrows (developmental pathways) that lead to these minima. This

correspondence was first proved experimentally by S. Huang et al. and subsequently corroborated by other research groups [32]. Thus, the attractor landscape resolves the question of how the same genotype can give rise to a variety of different phenotypes.

6 Criticality of the attractor landscape

It is important to stress that the definition of the ordered, critical and chaotic phases given before, is closely related to the dynamical response of the network to transient perturbations (see Fig. 1). However, there is a much more profound manifestation of these dynamical phases, in relation to the way in which the attractor landscape changes when the network is permanently mutated [34]. Indeed, since the attractor landscape is determined by the network topology and the Boolean functions, one would expect that changing either of these properties consequently modifies the attractor landscape. One can also expect that the magnitude of this change will depend on the dynamical phase in which the network operates. In our group we have investigated the relationship between the dynamical regime of the network and the evolvability of its attractor landscape. To do this, we first formulate an operational definition of phenotypic robustness and phenotypic innovation as follows:

- A network is phenotypically robust, under a given mutation, if its dynamical attractors do not change as a result that mutation.
- A network is phenotypically innovative, under a given mutation, if new attractors appear as a result of that mutation.
- A network is evolvable under a given mutation if it is both phenotypically robust and innovative. In other words, if all the attractors it had before the mutation are conserved and also new attractors appear.

According to the previous definitions, it is also important to define the mutations under which a network is going to be considered evolvable. We have implemented a particular type of mutation that is the main cause of genome growth and evolution: gene duplication followed by divergence [34]. We start from a Boolean network with N genes, $\{\sigma_1, \sigma_2, \ldots, \sigma_N\}$, which we will call the *original network*, and randomly chose one of its genes for duplication. Let σ_i be the randomly chosen gene. We duplicate this gene and form a new network with $N+1$ genes, in which $\sigma_i = \sigma_{N+1}$. This means that immediately after the duplication event σ_{N+1} has the exact same regulators (inputs), the same regulated genes (outputs), and the same Boolean functions as σ_i. Afterwards, we mutate some of these properties in the duplicated gene σ_{N+1}, making it different from the parent gene σ_i. This process, called genetic divergence, is known to occur very rapidly after the duplication event [35]. We will call the network resulting from this duplication-divergence event the *mutated network*. As a result of the duplication-divergence event, the attractors

of the mutated network may change, or they may even disappear whereas new attractors may appear. Robustness will be then quantified as the fraction of attractors of the original network that are conserved in the mutated network after the duplication-divergence event. If all the original attractors are conserved, then the network has robustness $R = 1$, whereas if none of the attractors are conserved, the network has no robustness: $R = 0$.

Fig. 3 shows the probability $P(R)$ that a random Boolean network has robustness R at each of the three different phases; ordered ($S = 0.5$), critical ($S = 1$) and chaotic ($S = 1.5$ and $S = 2$). Clearly, the network robustness decreases as the dynamics transit from the ordered to the chaotic regime. This can be observed as the probability $P(1)$ that the network conserves all of its original attractors rapidly decreases, whereas the probability $P(0)$ to conserve none of them increases. These results show that networks operating in the ordered regime are very robust, as with high probability their attractors do not change under mutations. But precisely because of this, ordered networks cannot evolve since their attractor landscape is "frozen", which makes them incapable of generating new attractors. On the contrary, chaotic networks are very innovative. In such networks, there is a very high probability that the attractor landscape completely changes after the duplication-divergence event. These chaotic network are innovative but they are not robust, so they cannot evolve either. Critical networks are peculiar in the sense that they are robust and innovative at the same time. This can be observed in Fig. 4, which shows the probability $P_e(S)$ that after a gene duplication-divergence event, a network with sensitivity S conserves all of its attractors and generates at least a new one. Note that this probability is maximum for critical networks ($S = 1$).

Thus, from the theoretical point of view, criticality is a desirable property that confers the phenotypic robustness and innovation the network needs to evolve. Two important questions arise from this conclusion: Are the genetic networks of real organisms critical? And if so, how did criticality emerge throughout evolution? The first question was answered affirmatively by several groups, who reported experimental evidence showing that the networks of real organisms exhibit dynamics compatible with criticality [36–40]. As for the second question, our group has investigated the evolutionary mechanisms that generate critical dynamics. In particular, we arre interested in knowing whether ordered or chaotic networks can evolve towards criticality, or if critical networks need to be born being critical. In the next section we present a simple evolutionary model, rooted on biological grounds, that gives a general answer to these questions.

7 Evolution towards criticality

In the previous section we assumed that the network is already operating in a given dynamical regime (ordered, critical or chaotic) and then proceed to determine the effect of mutations on the evolvability of the attractor landscape. The main result was that, under a gene duplication-divergence event, critical networks exhibited the highest evolvability,

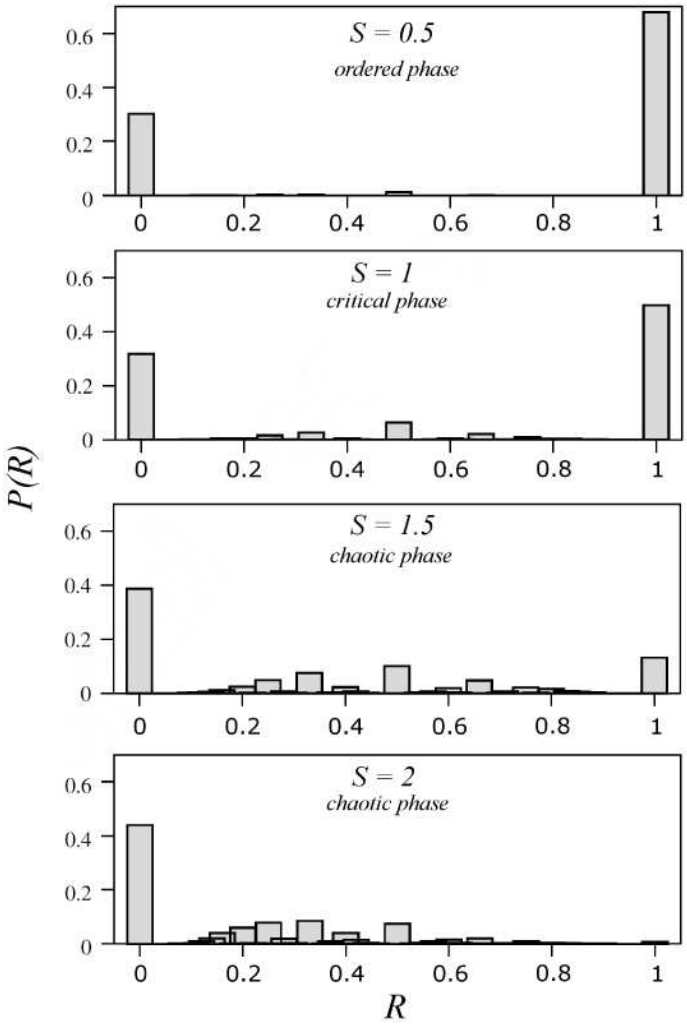

Figure 3: Probability $P(R)$ for the network to have phenotypic robustness R under gene duplication-divergence events. The different graphs correspond to networks operating in the three different dynamical phases: ordered ($S = 0.5$), critical ($S = 1$), chaotic ($S = 1.5$) and super chaotic ($S = 2$). Note that as the dynamical regime passes from ordered to chaotic, the network becomes less robust, as the probability $P(1)$ for the network to conserve all of its original attractors decreases and the probability $P(0)$ to conserve none of its attractors increases.

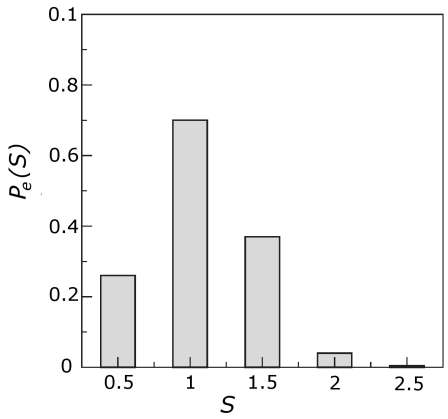

Figure 4: Probability $P_e(S)$ for a network with sensitivity S to be evolvable, namely, to conserve all its attractors and generate at least a new one, after a gene duplication-divergence event. Note that this probability is maximum for critical networks.

as they conserved all the original attractors (phenotypic robustness) and were able to generate new ones (phenotypic innovation). In this section we will proceed in the opposite direction, starting with random networks operating in arbitrary dynamical phases and evolving them through mutations and gene duplication-divergence events. It is throughout this process that we will demand evolvability. This means that only the networks that conserve their already acquired phenotypes (attractors) and also generate new ones, will be the ones selected to survive and continue further trough the simulation. Our goal is to determine if the requirement of evolvability across the evolutionary process will favor a particular dynamical regime.

We start with a population of $M_0 = 1000$ random Boolean networks (all different), each with $N = 20$ genes. At this point, all the genes have exactly K regulators and the Boolean functions have a bias of $p = 0.5$. Hence, the sensitivity of the networks in the initial population is entirely determined by the network connectivity K as $S_0 = 2p(1-p)K = K/2$. Through evolution, we mutate the networks in the population by adding or removing connections between the genes, changing the Boolean functions that regulate the expression of the genes, and adding new genes to the network. Although for each network we perform these mutations randomly, the way in which we implement them is deeply rooted on the biological phenomenology of genome growth and evolution. More specifically, we assume that each gene is composed of two parts, a regulatory region and a coding region, and that mutations can occur in any of these two parts with equal probability. Mutations in the regulatory region consist in the addition or deletion of binding sites to DNA, which in turn change the way in which the gene is regulated. In Ref. [41] the mutagenic algorithm is described in detail. Here we briefly mention that mutations in

the regulatory region of a given gene σ_n will cause the loss or gain of regulators, as well as changes in its Boolean function. On the other hand, mutations in the coding region of σ_n change how this gene regulates its targets, which translates into the gain or loss of targets, as well as modifications of the Boolean functions of such gained or lost targets. Finally, the network growth is implemented through gene duplication followed by divergence up to a maximum size $N = 100$.

In each generation and for each network in the population, there is a probability μ for each gene to be mutated in either its regulatory or coding region. After the mutations, we check whether or not the mutated networks conserve the same attractors they had before the mutations, and eliminate from the population those networks which do not conserve *all* their attractors. Thus, only the phenotypically robust networks can go through the next generation. We will call this selection process the *phenotypic robustness criterion* (PRC). The elimination of the networks that do not satisfy this criterion reduces the population size to a new value, and therefore we have to replicate each of the surviving networks to restore the population to its original size. This replication is carried out with a certain bias (or fitness) α per network that will be discussed later.

Every two thousands generations all the networks in the population simultaneously undergo a duplication-divergence event, after which the only networks that survive and pass to the next generation will be the ones that in addition to fulfilling the PRC, also generate at least one new attractor. Thus, every two thousands generations we are demanding evolvability. Therefore, we will call this selection process the *phenotypic evolvability criterion* (PEC). Under this criterion we eliminate from the population all the networks which do not satisfy the PRC or do not generate new attractors (even if some of these latter networks do fulfill the PRC).

There are two important points to be considered when new attractors emerge. First, every time a new attractor is found, it is added to the set of attractors that must be conserved to fulfill the PRC. We will call this growing set of attractors, which will be under selective pressure, the *phenotypic attractors*. Each network has its own set of phenotypic attractors. Second, the genes in the phenotypic attractors must do something. More precisely, networks whose phenotypic attractors have all the genes in the same state (active or inactive) will have a low fitness and consequently a lower replication rate. We define the average genetic expression variability of the network as $\alpha = 1 - |\psi_1 - \psi_0|$, where ψ_0 and ψ_1 are the average fractions of 0's and 1's in all the states of all the attractors of the network (clearly, $\psi_0 + \psi_1 = 1$). Thus, $\alpha \approx 0$ if almost all the genes in the attractors are in only one state (either 0 or 1), whereas $\alpha \approx 1$ if more or less half of the genes in the attractors are in the state 1 and the other half in the state 0. In each generation, we replicate each surviving network in a quantity proportional to its average genetic activity α, which introduces competition in the replication of the surviving networks, being more favored the ones with an average genetic variability close to $\alpha = 1$.

Fig. 5A shows the evolution of the average network sensitivity $\langle S \rangle$, where the average is taken over all the networks in the population. The different curves depicted in Fig. 5A

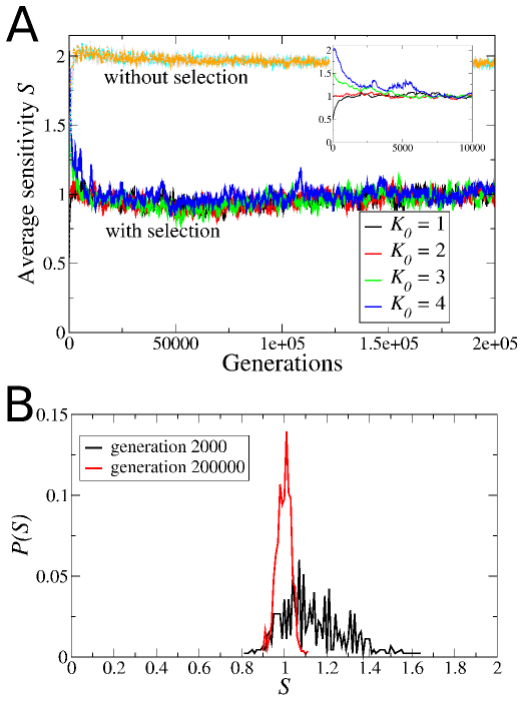

Figure 5: Evolution towards criticality. (A) Evolution of the average network sensitivity for four different populations, each initially composed of networks in one of the three dynamical regimes: ordered ($K = 1$, $S = 0.5$, black), critical ($K = 2$, $S = 1$, red), and chaotic ($K = 3$, $S = 1.5$, green; $K = 4$, $S = 2$, blue). Under the Darwinian selection given by the PRC and PEC, all the populations become critical ($\langle S \rangle \to 1$) in less than 5000 generations (see inset) regardless of their initial dynamical regime. The control curves (in light gray) were obtained evolving populations without selection, and show that the mutagenic method alone drives the networks into the chaotic regime ($\langle S \rangle \to 2$). Therefore, in our simulations evolution towards criticality is not an artifact of the mutagenic algorithm. (B) Distribution of sensitivities at two different generations for the population that started with $K = 3$ (chaotic networks). Early in the simulation, at generation $g = 2 \times 10^3$, $P(S)$ is quite broad (black line), reflecting a great diversity of networks. However, through evolution, all the surviving networks become critical and the distribution $P(S)$ narrows down (red line). The distribution shown here at generation $g = 2 \times 10^5$ has $\langle S \rangle = 0.998 \pm 0.035$.

correspond to four different populations that started with networks in the ordered, critical, and chaotic regimes. The curves in light gray that converge to $\langle S \rangle = 2$ show the effect of the mutagenic algorithm only, as they correspond to populations evolving with mutation but without selection (all the networks survive in each generation). Clearly, the mutagenic algorithm alone produces chaotic networks. Contrary to this, when the evolution takes place with selection, i.e. implementing the PRC and PEC, the sensitivity of the

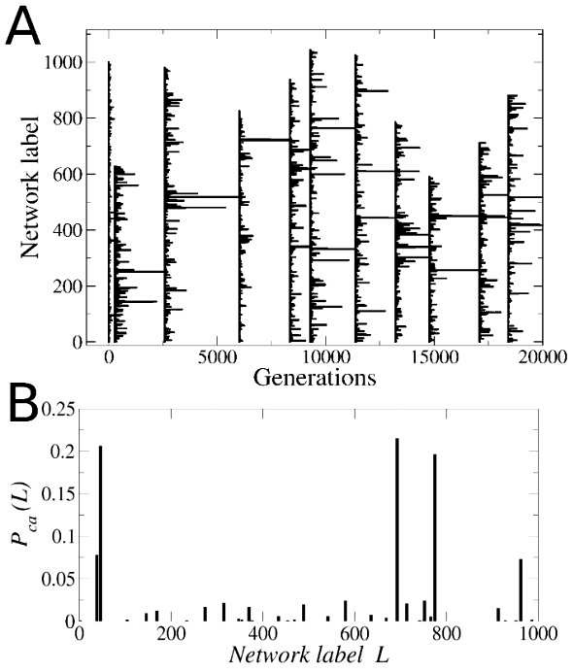

Figure 6: Evolutionary bottlenecks. (A) This plot shows the evolution of the lineages (network labels) across generations. Each horizontal line indicates the survival time of a particular lineage. The vertical lines indicate the fixation events in which all the networks in the population are relabelled again after only one lineage was left in the entire population. (B) Probability $P_{ca}(L)$ that a network with label L in the original population becomes the common ancestor (this is the lineage that goes through the first bottleneck, giving rise to the first fixation event). Note that only very few networks (less than 4%) in the original population can become common ancestors. Among this 4%, only 5 networks are selected in about 80% of the realizations.

networks in all the populations converge, on average, to the critical value $\langle S \rangle \approx 1$. This demonstrates that the Darwinian selection given by the PRC and PEC indeed makes the networks evolve towards criticality. Furthermore, Fig. 5B shows the distribution of sensitivities $P(S)$ in one of the populations that started with chaotic networks ($S = 1.5$), and for two distinct generation times: Very early in the simulation, at generation $g = 2 \times 10^3$ (black curve); and at the end of the simulation, at generation $g = 2 \times 10^5$ (red curve). It is clear that at the beginning of the evolutionary process a great diversity of networks is present, which is reflected in the broad distribution $P(S)$. Nonetheless, throughout evolution, the networks become critical and the final distribution of sensitivities $P(S)$ becomes very narrow, with mean $\langle S \rangle = 0.998$ and standard deviation $\Delta S = 0.035$. This shows that each network in the population is converging towards criticality.

As we mentioned before, the networks that do not satisfy the PRC in each generation,

or the PEC after the gene duplication events every two thousands generations, are removed from the population. In order to determine how restrictive these selection criteria are, it is important to measure the survival times of the networks in the population. To do this, at generation $g = 0$ we label all the networks in the population with an integer ranging from 1 to 1000. Throughout generations, each network conserves its original label. Furthermore, when one network is replicated into several copies, the "daughter" networks acquire the same label from the "mother". Therefore, the labels are inherited from mother to daughters, which makes it possible to identify different "lineages" through the evolutionary process. Each network in the initial population gives rise to a different lineage and therefore, at the beginning of the process there are 1000 different lineages. However, since the networks that fail the selection criteria are removed from the population, some lineages might disappear. If at generation g only one lineage is left in the entire population, we relabel the networks in that particular lineage from 1 to M_g, being M_g the number of networks in the population. This can be considered as the "fixation" of that lineage in the population. (Note that the existence of only one lineage in the population does not mean that there is only one network. Rather, it means that all the M_g networks have the same label, and therefore, all of them share a common ancestor.) Fig. 6A shows the evolution of lineages throughout generations. The vertical lines show the fixation events, and the horizontal lines the survival time of a particular lineage. It is clear that the majority of lineages disappear from the population very quickly, and only very few lineages survive for long times. These results indicate that evolution towards criticality via the PRC and PEC confronts the population against a series of selective filters (bottlenecks) which only very few networks are able to go through.

A very important consequence of these bottlenecks is that the final population comes entirely from only one common ancestor. This rises the question of how reproducible is obtaining the same common ancestor in different realizations of the evolutionary process. In other words, if we perform one million different simulations, always starting with the same initial population of networks but with a different history of mutations and duplications in each realization, how many times the same network in the original population would be selected as the common ancestor? Since the networks in the original population were constructed randomly, one might expect that all of them have the same probability of making it through the bottlenecks imposed by the PRC and PEC. If this were the case, the probability $P_{ca}(L)$ that the initial network with label L becomes the common ancestor would be the same for all values of L. Nonetheless, Fig. 6B shows that this is not the case, as only very few networks are selected as common ancestors.

Another remarkable result is the topological structure of the networks in the final population. We start the simulation with homogeneous random networks for which all the nodes have the same number of inputs K and a number of outputs drawn from a Poisson distribution. However, at the end of the simulation the networks have global regulators (hubs), namely, nodes with a great number of output connections as it is illustrated in Fig. 7A. This topological structure is known to occur in the genetic networks of real or-

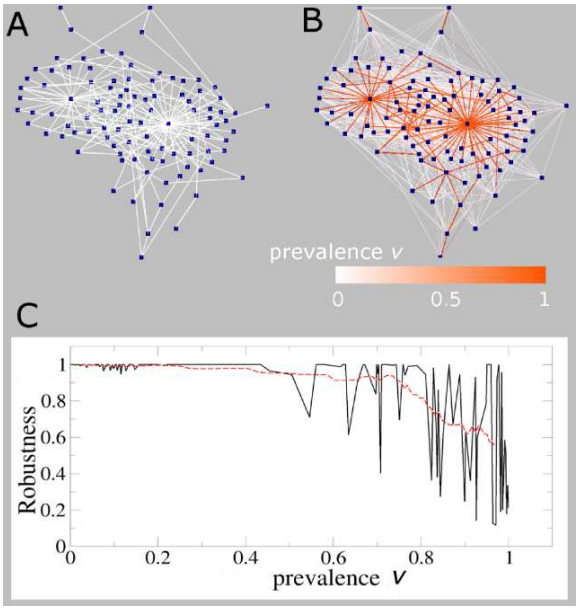

Figure 7: (A) Structure of a randomly chosen network in the final population. Note the existence of highly connected nodes (global regulators or "hubs"). (B) Diagram showing the superposition of all the networks in the final population. The color of a given link indicates its prevalence in the population, which is the fraction of final networks in which that link occurred. (C) Robustness of the network when a link with prevalence v is removed. The black curve corresponds to one randomly chosen network and the red dashed line is the average over the population. Note that on average, the robustness of the network decreases as the prevalence of the removed link increases.

ganisms, such a *E. coli*, *S. cerevisiae*, *S. pombe* and *B. subtilis* [34, 36, 42]. The existence of global regulators in the final networks was a very unexpected result for two reasons. First, the topological structure of the network was never considered in the selection mechanism. Second, and more importantly, global regulators introduce strong correlations in the network dynamics, and it is not obvious that these correlations can survive to the selection pressure imposed by the PRC and PEC. Interestingly, when the α-fitness criterion is not enforced, i.e. when we allow the possibility for all the genes in the attractors to be "frozen" in the same state (either 0 or 1), the networks never develop hubs. This strongly suggests that the existence or absence of global regulators is related to the information content of the attractor landscape but further investigation is necessary in this matter.

Fig. 7A shows a representative network of the final population. It is important to mention that the final networks, although similar, are not identical even though they all have the same set of phenotypic attractors. Fig. 7B shows a superposition of all the networks in the final population and the color code indicates the *prevalence of the links* in such networks. This prevalence is measured as $v_{ij} = m_{ij}/M_f$, where m_{ij} is the number of net-

works in the final population in which the nodes σ_i and σ_j are connected, and M_f is the total number of final networks. It is clear from Fig. 7B that the most prevalent links are the ones connecting the global regulators. This suggests that these hubs play an important role in the evolvability of the attractor landscape. Indeed, Fig. 7C shows the robustness of the network (the number of attractors that are conserved) when we remove links with different prevalence. It is clear that on average, the robustness greatly decreases when we remove the most prevalent links.

8 Discussion

Phenotypic robustness and innovation are two central properties common to all living organisms. These two properties are closely related to the dynamical regime in which the underlying genetic network operates. This is because networks that are dynamically critical are also robust and innovative not only under transient changes in the environment, but also under permanent mutations either in the topological structure of the network or in its regulatory interactions (the Boolean functions). Therefore, evolution towards criticality stems out as a fundamental process that can help us understand how living organisms are robust and at the same time have the ability to generate adaptable diversity. In this work we have shown that dynamical criticality can indeed emerge by means of a simple and biologically meaningful Darwinian selection process, that imposes two main constraints on the attractor landscape. First, the networks must conserve the attractors they have acquired through evolution and second, networks that generate new attractors as a consequence of mutations, are preferred over the networks that do not generate new attractors. In this sense, the balance between conservation and innovation of the attractor landscape plays an important role in the selection process. We should note that innovation of phenotypes occurs in two distinct ways. On the one hand, the emergence of new attractors can be considered as the generation of new phenotypes. On the other hand, the addition of new genes to the network also adds new information to the already existing attractors (the attractor states grow). In either case, for this information to be useful, the new genes must have some activity that changes from one attractor to another. Therefore, a third selection constraint comes up naturally, and consists in that the the genes in the attractors should not be "frozen". This important biological constraint is not fundamental for the evolution towards criticality, as the populations become critical even without the fulfillment of the α-fitness criterion. But it is essential for the existence of global regulators in the final networks, which suggests a strong relationship between the network structure and the information content of the attractor landscape.

It is also important to mention that in our simulations the attractor conservation and innovation criteria are not as stringent as one may think. The reason is that, due to computer limitations, the attractor landscape can be known in full only for small networks. Thus, we completely determine the attractor landscape for all the networks in the popula-

tion only for the first generation where the networks are small. After that, in order to find new attractors we just sampled a small fraction of the state space (we sampled about 10^4 states for each network). Clearly, we can apply the PRC and the PEC only to the attractors that are found by means of this under sampling (the set of phenotypic attractors). However, there can be "hidden" attractors that do not come out through this under sampling process. It is quite remarkable that even when many attractors may not be taken into account, the PRC and the PEC make the population evolve towards criticality. The under sampling in our numerical simulations has a biological counterpart, which is that for an organism like *E. coli*, with $N \approx 300$ regulatory genes, it is very unlikely that all the 2^{300} possible configurations could be explored throughout evolution in order to reach all the possible existing phenotypes.

Even though there is a great genotypic and phenotypic diversity in the initial population (because all the networks are structurally different and have different attractor landscapes), throughout generations the population passes through a series of selective filters which decrease this diversity by eliminating from the population the majority of lineages. At the end of the simulation all the networks have the same set of phenotypes (the same set of phenotypic attractors), but slightly different genotypes (different topological structures). Additionally, as we have mentioned before, the existence of highly connected nodes in the final networks seems to be a consequence of restrictions imposed on the information content of the dynamical attractors. Thus, our results are consistent with the idea that restrictions on the dynamics of the network can play an important role in shaping its topology, as it has been suggested for other types of networks [43, 44].

In conclusion, although dynamical criticality is not a necessary condition in the functioning of living organisms, it can be a consequence of evolution. For it naturally emerges from the very same forces that allow living organisms to evolve in changing environments: phenotypic robustness and phenotypic innovation.

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