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TOPICAL REVIEW

Bioattractors: dynamical systems theory and the evolution of regulatory processes

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Abstract In this paper, we illustrate how dynamical systems theory can provide a unifying conceptual framework for evolution of biological regulatory systems. Our argument is that the genotype–phenotype map can be characterized by the phase portrait of the underlying regulatory process. The features of this portrait – such as attractors with associated basins and their bifurcations – define the regulatory and evolutionary potential of a system. We show how the geometric analysis of phase space connects Waddington's epigenetic landscape to recent computational approaches for the study of robustness and evolvability in network evolution. We discuss how the geometry of phase space determines the probability of possible phenotypic transitions. Finally, we demonstrate how the active, self-organizing role of the environment in phenotypic evolution can be understood in terms of dynamical systems concepts. This approach yields mechanistic explanations that go beyond insights based on the simulation of evolving regulatory networks alone. Its predictions can now be tested by studying specific, experimentally tractable regulatory systems using the tools of modern systems biology. A systematic exploration of such systems will enable us to understand better the nature and origin of the phenotypic variability, which provides the substrate for evolution by natural selection.

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Introduction

The evolution of complex traits depends on the dynamics of underlying metabolic, physiological and developmental regulatory processes. These *generative processes* form a non-linear map from genotype to phenotype (Alberch, 1991; Wagner & Altenberg, 1996; Pigliucci, 2010; Félix, 2012). (Terms in italics are defined and explained in the glossary.) The *genotype–phenotype map* determines which phenotypes can be realized by a specific genome embedded in its organismic and environmental context. In other words, the genotype–phenotype map defines the theoretically possible distribution of *phenotypic variability* produced by a given set of genotypes (Wagner & Altenberg, 1996). The actual, observable *phenotypic variation* in real world populations results from evolutionary processes such as natural selection and neutral drift acting on this a priori distribution (see, for example, Wagner & Altenberg, 1996; Salazar-Ciudad & Marín-Riera, 2013). While we

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have a solid understanding of how these population-level processes produce evolutionary change given an observed range of phenotypic traits, we know very little about how the distribution of theoretically possible phenotypes originates in the first place through the process of *ontogeny*.

The origin and nature of phenotypic variability poses a central problem for evolutionary theory. A better, more mechanistic and more detailed, understanding of the genotype–phenotype map is essential for us to address the question why organisms can evolve in the first place (for a range of perspectives on this topic, see Dawkins, 1989; Alberch, 1991; Wagner & Altenberg, 1996; Pigliucci, 2010; Wagner, 2011). For instance, we still do not know why there are beneficial mutations at all – why do some random perturbations improve fitness, rather than break the system – and why such adaptive mutations occur at frequencies sufficient for selection to act upon. Most engineered systems do *not* improve this way, as they are already designed for minimal redundancy and optimal performance. Biological systems are different, but we do not yet know why that is.

What is missing is a theory – or at least a more systematic, quantitative and integrative understanding – of ontogeny, including metabolism, physiology and development. How do complex traits originate? How do they react to genetic or environmental perturbations? How does the internal wiring of the underlying generative processes, their *regulatory organization*, influence and bias the direction of evolutionary change? What kind of phenotypic transitions are likely to occur? Besides, what kind of trait is impossible to evolve, even in principle? Generally, we do not have any satisfying answers – based on mechanistic, causative explanations – to any of these questions.

In this paper, we argue that the theory of dynamical systems can provide such answers. We show how dynamical systems theory allows us to connect important conceptual frameworks, such as Waddington's epigenetic landscape and the theory of genotype networks, to provide a more unified approach to the study of regulatory evolution. Considering the evolution of dynamical systems allows us to classify general types of evolutionary transitions, and provides insights into the mechanisms underlying robustness and evolvability of generative regulatory processes. Finally, we illustrate how dynamical systems theory can be used to understand the role of phenotypic plasticity and self-organization in the evolution of complex phenotypes.

From Waddington's landscape to genotype networks

One famous attempt to address the problem of regulatory evolution was made by Conrad Hal Waddington who aimed at reuniting genetics, evolution and embryology (Waddington, 1957, 1975; Gilbert, 2000; Slack, 2002). To this end, Waddington proposed the concept of the *epigenetic landscape* to explain how regulatory organization affects the evolution of generative processes (Fig. 1, top left, upper panel) (Waddington, 1939, 1940, 1957). In this landscape, the current *state* of a biological system is represented by a ball. Over time, the ball rolls down valleys within the landscape that represent *robust* or *canalized* physiological and developmental trajectories. Waddington called these trajectories '*chreods*'. Branch points in these valleys or chreods indicate ontogenetic decisions, e.g. differentiation into one cell type or another. Genes exert their effects by altering the landscape. Waddington represented this genetic influence by pegs attached to the underside of the surface via guy ropes (Fig. 1, top left, lower panel). Simply put, development occurs on a given topography, while evolution changes this topography over time. Both processes are therefore connected and constrained by the structure of the landscape.

Waddington admonished his readers on several occasions that his landscape is intended to apply at a metaphorical level only (see, for example, Waddington, 1957). It is not supposed to be mechanistically rigorous. This point has been criticized by several authors (Gilbert, 2000; Slack, 2002) as it limits the applicability of the concept in an empirical setting. It is difficult to measure a metaphor, after all.

A rather different approach to understand the influence of the genotype–phenotype map on evolutionary dynamics is based on the simulation of large sets (called *ensembles*) of discrete gene regulatory networks (reviewed in Kauffman, 1993, 2004; Wagner, 2005, 2011). In most of these studies, genes are represented as simple on/off switches. Such networks, it is argued, capture the essence of the genotype–phenotype map. The 'genotype' of a network is defined as the set of its regulatory interactions: an interaction between two genes can be activating, repressing or absent. Given some initial state, every genotype will produce a particular output state, or 'phenotype', consisting of a particular combination of genes that are switched either on or off. It turns out that, in general, many different genotypes produce the same phenotype (Kauffman, 1993; Borenstein & Krakauer, 2008; Munteanu & Solé, 2008). Such genotypes form an 'invariant set of genotypes', or simply, a '*genotype set*' (Wagner, 2011).

Genotypes are linked to each other through mutations, forming a network of networks (or 'meta-network'; Fig. 1, bottom). Neighbours in this meta-network are connected through a mutational change – addition, removal or sign reversal – in just one regulatory interaction. Genotype sets are usually highly connected, forming what are called '*neutral*' or '*genotype networks*' (Maynard Smith, 1970; Schuster *et al.* 1994; Fontana, 2002; Gavrilets, 2004; Wagner, 2005, 2008, 2011; Ciliberti *et al.* 2007*a*,*b*; Draghi

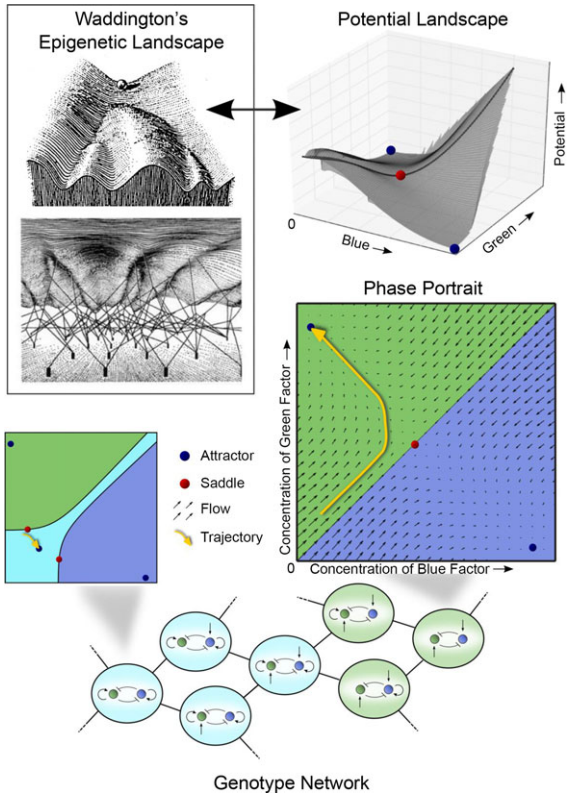

Figure 1. From Waddington's landscape to genotype networks
Top left: the upper panel shows Waddington's most famous illustration of his epigenetic landscape. Developmental trajectories are represented by a ball rolling down the valleys of the landscape. Branch points represent developmental decisions. The lower panel illustrates the influence of the genes, which are drawn as pegs connected to the underside of the landscape by guy ropes. Genes can alter the shape of the landscape by pulling on these ropes. Both illustrations are from Waddington (1957), *Strategy of the Genes*. At the bottom of the figure we illustrate the concept of a genotype network. Variants of a toggle switch model are represented by network diagrams, where green/blue nodes represent regulatory genes, while arrows indicate activating and T bars repressing regulatory interactions. A genotype network is a (meta-)network of such networks, which are connected by mutational steps (addition, removal or sign reversal of a regulatory interaction). Two genotype networks are shown: one for regulatory networks converging to a state where low levels of the blue and green factors coexist (cyan background), the other for networks converging to a blue off, green on state (green background). Middle panels show two examples of phase portraits corresponding to specific network structures or genotypes in the bottom panel. Axes of these portraits correspond to regulator concentrations (as indicated in the panel on the right). Black arrows represent the flow of the system. Blue circles are attractors, red circles saddle points. Basins of attraction are indicated by blue, green and cyan background respectively. Basin borders correspond to separatrices (black lines). An example trajectory is shown in yellow for both phase portraits. Top right: this panel shows the potential landscape derived from the phase portrait on the right. Attractors, saddles and separatrices are shown as in the phase portraits. The slope of the potential surface is determined by the flow of the system. Attractors lie in local troughs, valleys correspond to unstable manifolds that connect the saddle to the two attractors. The potential surface is a mathematically explicit formulation of Waddington's landscape metaphor. See text for details.

et al. 2010). This means that mutations can change the interactions within a network without changing the resulting output phenotype. Genotype networks are often large, spanning a considerable fraction of the space of all possible genotypes.

As an example to illustrate the concept, we consider mutational variants of a genetic toggle switch network (see Jaeger *et al.* 2012, and references therein), corresponding to *regulatory structures* such as those displayed at the bottom of Fig. 1. The basic version of this network consists of two regulatory factors (indicated in blue and green) that repress each other while exhibiting constitutive external activation and/or auto-activation. Connectors between network variants represent single mutational changes in regulatory structure. Two examples of genotype networks are shown: cyan background colour indicates networks that produce an output state, or phenotype, with coexisting low levels of the blue and the green gene product; green background indicates an alternative phenotype with high green and low blue (Fig. 1, bottom).

Genotype networks help us understand a number of systems-level properties of evolving regulatory processes. Their large size and high connectivity, for example, explain the mutational robustness of regulatory systems, and show that considerable cryptic variability can accumulate in a population as it drifts along mutational paths within the network (Wagner, 2005, 2008, 2011; Ciliberti *et al.* 2007*a*,*b*). Similarly, genotype networks provide an explanation for the capacity of regulatory networks to reach novel phenotypes – a concept called '*evolvability*', or sometimes more precisely '*innovability*' (Dawkins, 1989; Müller & Wagner, 1991; Wagner & Altenberg, 1996; Fontana, 2002; Müller & Newman, 2005; Wagner, 2005, 2008, 2011; Ciliberti *et al.* 2007*a*,*b*; Pigliucci, 2008; Draghi *et al.* 2010; Müller, 2010). As a population drifts across a genotype network, it reaches novel regions of genotype space and brings it in contact with alternative genotype networks implementing different kinds of phenotypes.

Simulation-based studies of genotype networks have provided many important and interesting new insights into the origin and evolution of phenotypic traits. However, most of this research is based on numerically sampling and simulating large numbers of regulatory networks, and the insights derived from it are therefore of a statistical and correlative nature. This limits the explanatory power of the approach, as it provides no mechanistic or causative explanations for the existence, size and geometry of genotype networks. In other words, these studies show that such networks exist and how they influence robustness and evolvability, but they do not provide us with answers to the deeper questions of *why* genotype networks exist in the first place, and *why* they have the particular extent and shape observed in ensemble simulations.

The evolution of dynamical systems

We can address the problem of the existence and geometry of genotype networks by treating regulatory processes as evolving dynamical systems (Goodwin, 1982; Oster & Alberch, 1982; Webster & Goodwin, 1996; François & Siggia, 2012; Jaeger & Crombach, 2012; Jaeger *et al.* 2012; Jaeger & Sharpe, 2014). Dynamical systems theory (Hirsch *et al.* 2004) provides us with powerful conceptual tools to understand the underlying causal mechanisms that produce genotype networks. The geometric analysis of *state* or *phase space* is particularly relevant in this context (for a highly accessible introduction, see Strogatz, 2000). Phase space is an abstract space. Its axes represent the values of the *state variables* of the system. In our toggle switch example, these are the concentrations of the blue and green regulatory factors that constitute the network.

As we have seen above, each variant of the network can be considered a particular genotype of the system (Fig. 1, bottom). At the level of our argument, we assume that each genotype (or network structure) is in turn associated with a particular geometry of phase space – a specific *phase portrait* (for justifications of this simplification, see Cotterell & Sharpe, 2010; Wagner, 2011). The phase portrait links genotype to phenotype, and thus represents the characteristics of the genotype–phenotype map as shown in Fig. 2.

Figure 1, middle panels, shows two specific examples of phase portraits for the toggle switch model. The system may begin at any combination of initial regulator concentrations, that is, at any point of phase space. This defines the *initial state* or *condition* of the system. Given a specific initial condition, the equations of the system determine a dynamic *trajectory* (yellow and magenta arrows in Figs 1 and 2) by describing the rate of change in system state over time. In other words, a trajectory reflects the temporal progression of the system, equivalent to the rolling ball in Waddington's epigenetic landscape. The totality of possible trajectories in phase space, from any arbitrary initial condition, constitutes the *flow* of the system (black arrows in the phase portrait on the right in Fig. 1).

Trajectories converge towards subregions of phase space called *attractors*. These are the stable steady states of the system. Attractors can be specific points (blue circles in Figs 1 and 2) or more complex features of phase space (limit cycles and strange attractors) (Strogatz, 2000; Hirsch *et al.* 2004). All but the simplest systems have multiple attractors, so their phase portrait encodes multiple, qualitatively distinct, dynamic behaviours. This defines the *dynamical repertoire* of a *multi-stable* system. It describes the developmental potential of a regulatory process: the different phenotypic outputs it can produce given different initial conditions. Our example phase portraits show the toggle switch network in its tristable (left) or bistable (right) regime, with three and two alternative point attractors. The two bistable attractors correspond to steady states with high blue and low green, or low blue and high green, respectively. In the tristable regime, there is an additional attractor at which low levels of blue and green factor coexist.

Each attractor has an associated region of phase space – called its *basin of attraction* – that contains the set of trajectories that converge towards it (different background colours in the phase portraits in Figs 1 and 2). The attractor that is reached by the system is determined by the basin of attraction to which the initial condition belongs. The boundaries between basins are called *separatrices*. Trajectories that lie on separatrices do not converge to any of the attractors of the system, but rather to an unstable steady state, called a *saddle point* (red circles in Fig. 1). A saddle point is unstable, as any trajectory leaving it in

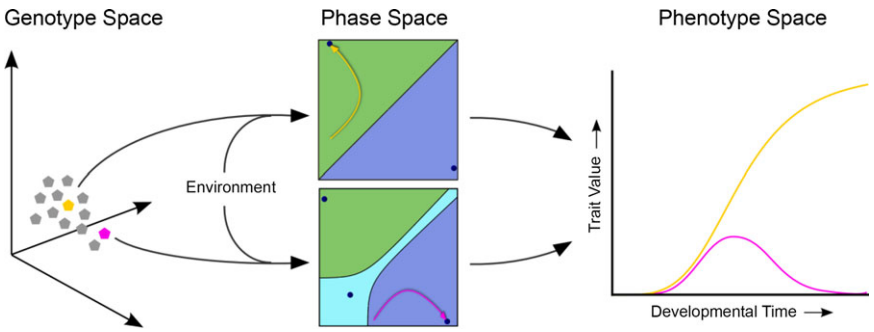

Figure 2. Phase portraits characterize the genotype–phenotype map
Genotype space is shown on the left, example phase portraits of the system in the middle, and the resulting trajectories in phenotype space on the right. A population of individuals with variation in initial conditions (due to the environment) and systems parameters (due to genetic variation) is represented by pentagons. Given a specific genotype and specific environmental conditions, the system will follow a particular trajectory to produce a particular phenotypic outcome. Examples of such trajectories are shown in yellow and magenta in the middle and right-hand panels). The geometry of phase space determines which phenotypic outcomes can be reached. See text for details.

a direction other than the separatrix will diverge to one of the attractors. Those trajectories that connect saddle points to attractors are called *unstable manifolds*.

For systems with one or two state variables, we can visualize the phase portrait in the form of a *potential surface* or *landscape* (Fig. 1, top right) (Strogatz, 2000). In this representation, the slope of the landscape corresponds to the local rate of change in state variables, as defined by the equations of the system. Attractors correspond to local troughs, and saddle points resemble mountain passes with their associated separatrix coming in along the ridgeline. Unstable manifolds form chreods that follow the valleys connecting saddles to attractors. The similarities between this representation of a phase portrait and the epigenetic landscape are obvious. Basically, the potential landscape of a dynamical system is a mathematically rigorous implementation of Waddington's famous metaphor (Huang, 2009, 2012; Bhattacharya *et al.* 2011; Wang *et al.* 2011; Ferrell, 2012; Furusawa & Kaneko, 2012; Verd *et al.* 2014).

Figure 1 summarizes how dynamical systems theory connects genotype networks to the epigenetic landscape. Genotype networks can be seen as regions of parameter space within which a particular local topography of the landscape is conserved. Inside such regions, the system converges to equivalent output states in a way that is qualitatively similar between different genotypes. In this sense, dynamical systems theory provides a unifying conceptual framework for the evolution of biological regulatory systems. This general proposition has been made previously (Goodwin, 1982; Oster & Alberch, 1982; Alberch, 1991; Webster & Goodwin, 1996). In the following sections, we go further by showing how dynamical systems concepts can be applied to specific problems of regulatory evolution.

Types of evolutionary transitions

The pioneering theoretical work of René Thom (1976, 1989) aimed at providing a mathematically rigorous foundation for Waddington's conceptual framework. Thom proved analytically that, for a precisely defined class of dynamical systems, there are only a limited number of possible types of phenotypic (or morphogenetic) transitions when parameters change. He called these transitions elementary *catastrophes*, and argued (quite differently from what we say here) that they correspond to specific types of valleys or chreods in Waddington's landscape. The main problem with Thom's work on catastrophe theory is that many real world regulatory networks do not conform to the specific class of systems for which his proofs are valid. Still, Thom's analysis raises the interesting possibility that a general classification of phenotypic transitions may be possible (see also Goodwin, 1982; Webster & Goodwin, 1996; Jaeger & Sharpe, 2014).

To take a first step towards such a classification, we have established in Jaeger *et al.* (2012) that there are only four distinct modes by which phase space can change during evolution (apart from a change in the dimensionality of phase space itself, which we will not consider further here) (Fig. 3). Only the first mode conforms to Darwin's concept of gradualism in evolution. Let us illustrate this with a specific example based on the toggle switch model: when the constitutive activating inputs to both regulatory genes are varied, the position of the system's attractors shift position in phase space, while their associated basins and the separatrix between them remains unchanged (Fig. 3, top panel). Thus, if we start at the same initial state, the system will remain in the same basin of attraction, while the exact concentration values of the steady state – the phenotypic output of the system – will change smoothly and continuously as parameter values are altered. Only concentration levels, but not the basic on/off switch behaviour of the system, are modified. This results in gradual 'phenotypic' change.

In the other three scenarios, however, threshold effects occur, and the change in phenotype is qualitative rather than quantitative. The resulting transition is drastic and discrete. In the first of these discontinuous situations, we alter the initial condition of the system (Fig. 3, left-hand panel). This reflects, among other possibilities, a mutation with a maternal effect. As long as we remain in the same basin of attraction, no phenotypic change occurs: the system is robust. As soon as the initial conditions cross a separatrix, however, an instantaneous switch to an alternative attractor will happen.

In the second scenario, the separatrix shifts its position (Fig. 3, right-hand panel). This can result from a change in the relative strength of the mutually repressive interactions between the two regulator genes. The system becomes asymmetric, and will converge to an alternative attractor as soon as its initial conditions are 'overtaken' by the moving separatrix. The resulting change in phenotype is again drastic and discrete, similar to the previous scenario.

Finally, parameter changes can create or annihilate attractor states. Such events are called *bifurcations* (Strogatz, 2000; Hirsch *et al.* 2004; Kuznetsov, 2004). In Fig. 3 (bottom panel), we show a bifurcation from the bistable to the tristable regime of the toggle switch model. It occurs due to the introduction of auto-activation for both regulatory genes. Above a certain strength, auto-activating inputs will allow a novel steady state to come into existence, at which both regulatory factors co-occur at low concentration levels (cyan attractor basin in Fig. 3). If the initial condition of the system comes to lie in the new basin, the system will converge to this intermediate attractor. Again, the transition is drastic and discrete, contradicting the gradualist view of small and smooth phenotypic change.

As far as we can see, these four modes of change are not limited to low-dimensional systems such as the toggle switch. Any transitions due to change in phase space geometry must involve the basic elements considered in our simple analysis. Therefore, we can draw a number of general conclusions about the evolution of regulatory networks.

First, our analysis shows that many changes in parameters (or initial conditions) do not change the qualitative behaviour of the system. This feature of biological processes is called *structural stability*. It provides an explanation for the robustness of regulatory networks towards mutational changes (Thom, 1976), and may contribute to the punctuated dynamics of evolution, where long periods of stasis alternate with relatively quick and drastic shifts in phenotypes (Jaeger *et al.* 2012).

Second, our analysis suggests that many changes in regulatory interactions *must* lead to discontinuous changes in the phenotype of the system. No smooth transition occurs, for example, between the two alternative steady states of the toggle switch in the three non-gradual scenarios described above. Similarly, bifurcations can lead to a sudden, discontinuous switch of the system to a novel steady state, or an equally drastic change away from an annihilated attractor state. This necessarily discrete – as opposed to gradual and smooth – nature of many phenotypic transitions is an important phenomenon to consider when trying to understand the difference between macro- and micro-evolutionary dynamics (Jaeger *et al.* 2012) and the origin of novel phenotypes (see below).

Finally, the geometry of phase space – separatrices, saddles and attractors with their basins, together with the bifurcations that occur when parameters are changed – strongly constrains the probability of transitions that can occur during regulatory evolution (see also, Goodwin, 1982; Oster & Alberch, 1982; Alberch, 1991; Webster

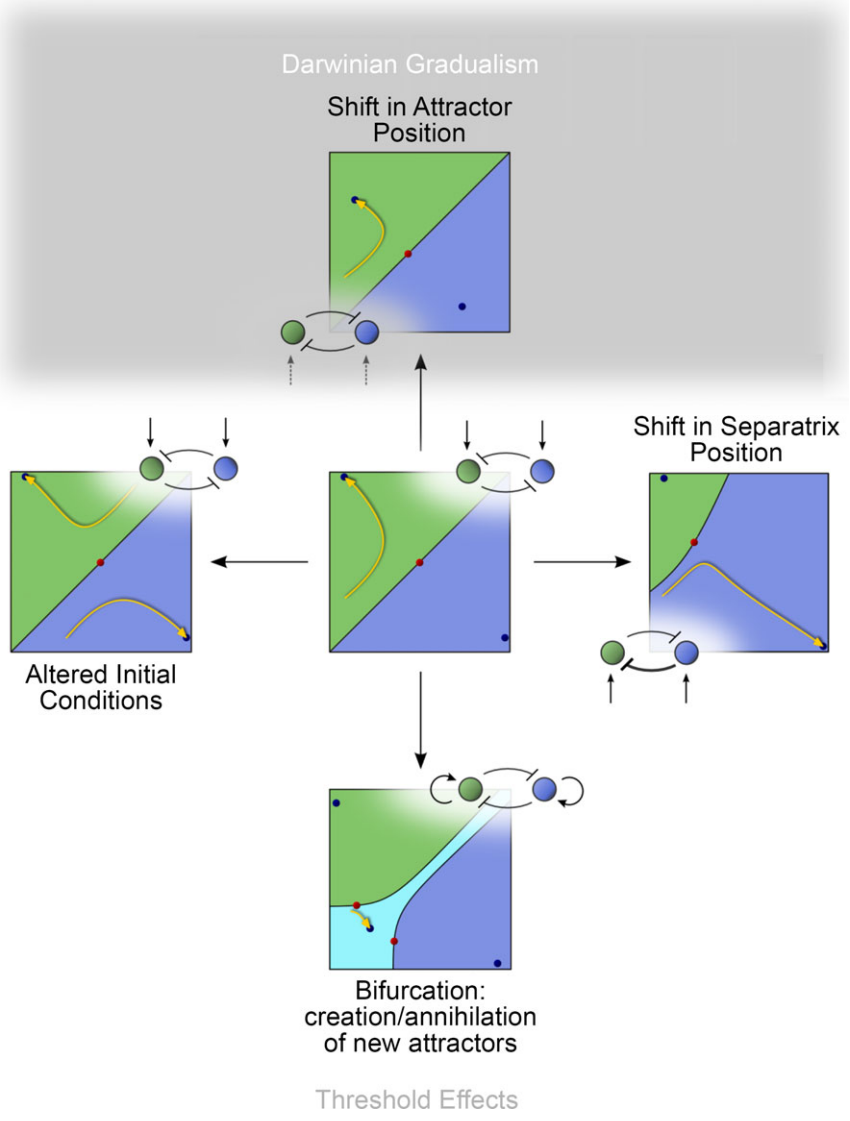

Figure 3. The four types of change in phase space geometry during evolution
Regulatory structures and phase portraits of toggle switch models are shown as in Fig. 1. The original network is shown in the middle. It converges to the green on, blue off attractor at the top left corner of the phase portrait. The four peripheral panels indicate the four possible ways in which phase space can affect evolutionary change. Top, shift in attractor position has occurred due to weakened activating inputs to the two regulators (dashed arrows in network diagram). The system converges to a different state with lower concentrations of the green factor, while its basic on/off behaviour remains unaffected. This corresponds to Darwin's concept of gradual evolution (indicated by the grey background colour). All three other types of change lead to discrete threshold effects. Left, a change in initial condition can either be buffered (as long as it remains in the same basin of attraction) or lead to a sudden and drastic switch to the alternative (blue) attractor state. Right, introducing an asymmetry in repressive strength between the two regulator genes causes the separatrix to shift. As soon as it crosses the position of the initial condition, the system will converge to the alternative (blue) attractor state. Bottom, introduction of autoactivation can lead to the creation of a third attractor state (shown in cyan) by a bifurcation event. If the initial condition comes to lie within the newly created attractor basin, the system will converge to the novel state. Again, this transition is abrupt and discrete. See text for details.

& Goodwin, 1996; Jaeger *et al.* 2012; Jaeger & Sharpe, 2014). In fact, some transitions simply cannot occur (we will revisit this point in the next section). Their probability is zero, as the required attractor states do not coexist in the same region of phase and parameter (or genotype) space. Understanding such constraints for specific evolving regulatory processes would enable us to make probabilistic, local predictions concerning the future direction of evolutionary change in biological systems. In other words, it could turn evolutionary biology from a purely historical into a more predictive branch of science.

Robustness, innovation and evolvability

So far, we have only briefly touched upon topics such as robustness, innovation and evolvability. Dynamical systems theory reveals its full explanatory power concerning these phenomena when combined with the concepts of Waddington's epigenetic landscape and Wagner's notion of genotype networks (see Fig. 1).

For the purpose of our argument, we will use a very specific, somewhat coarse-grained, definition of a genotype network: it is a mutationally connected set of genotypes for which a specific initial condition lies within the basin of attraction associated with a particular phenotype. This concept is illustrated in Fig. 4: the regulatory network associated with the phase space in *A* lies within the blue genotype network, as the trajectory originating from its given initial condition converges to the blue attractor. Likewise, phase spaces in *B*–*D* belong to the green genotype network, while the trajectory in *E* falls into the intermediate attractor basin shown in cyan. It is important to note that in our present context we do not consider smooth, quantitative changes in the attractor position as phenotypic transitions. Thus, the genotypes corresponding to phase portraits in Fig. 4*B* and *C* belong to the same genotype network although the exact concentration levels defining their respective attractor states differ between the two.

Ensemble simulations have shown that the robustness of a phenotype depends on the size and connectivity of its genotype network (Wagner, 2005, 2008, 2011; Ciliberti *et al.* 2007*a*,*b*). In our conceptual framework, genotype networks are associated with specific basins of attraction (Fig. 4). Therefore, the explanation of why genotype networks exist and how they lead to robustness is straightforward in dynamical systems terms. The probability of observing a given phenotype in an evolving population, and its robustness against perturbations, depend directly on the size, geometry and structural

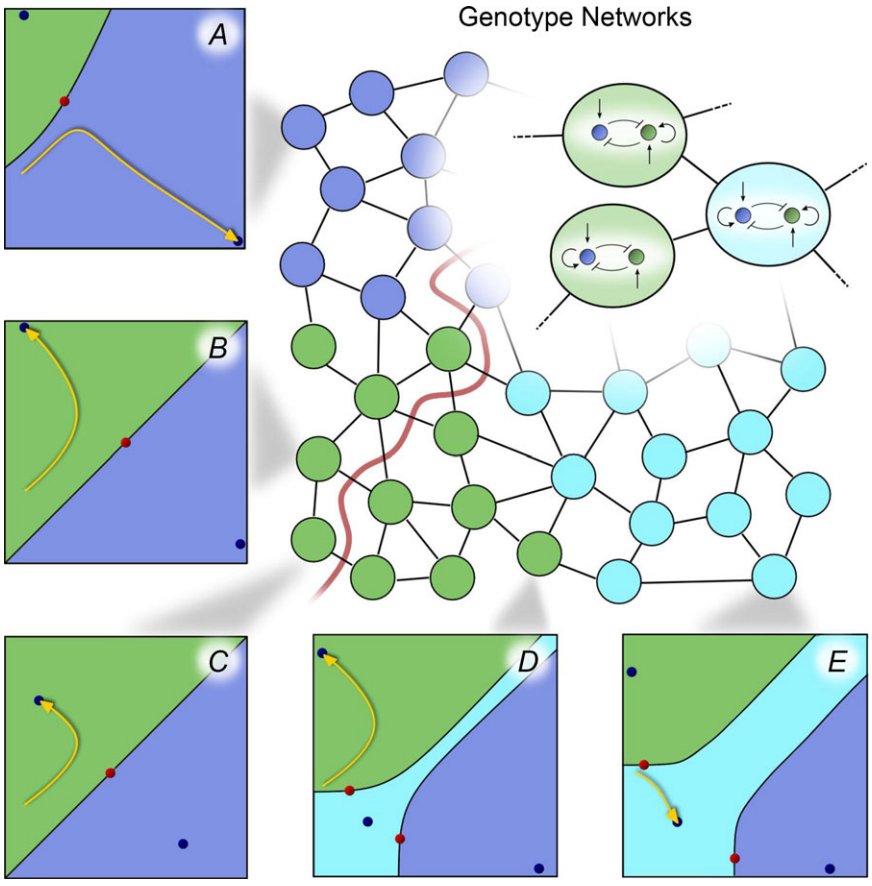

Figure 4. Robustness, innovation and evolvability
This figure illustrates how features of phase space can explain the presence and geometry of large genotype networks, which determine the robustness and evolvability of a regulatory system. It shows three genotype networks, one for each of the tristable attractor states (blue, green and cyan network nodes). Connectors between nodes represent mutational steps. The inset on the top right shows that each node corresponds to a specific genotype or regulatory structure (as in Fig. 1). *A*–*E*, phase portraits for selected nodes (axes and layout as in Fig. 1). Individual trajectories given a specific initial condition are shown as yellow arrows. Boundaries of genotype networks correspond to the initial condition crossing a separatrix, thus causing the system to converge to an alternative steady state. The curved red line in the genotype network diagram represents the bifurcation boundary where the cyan attractor state is created/annihilated. Note that it does not coincide with any genotype network boundary. This allows the system to cross a bifurcation boundary by neutral drift, thereby increasing its innovation potential by increasing the number of alternative genotype networks it can encounter in phase space. See text for details.

stability of the associated attractor basin in phase and parameter (or genotype) space. Basins that cover a large fraction of phase space across different genotypes will probably be encountered by selection or other evolutionary processes (see, for example, the green or blue basins in Fig. 4, which are present and cover large areas of phase space in all panels shown). Furthermore, a large percentage of changes in initial conditions or parameter values will retain the system in such a robust dynamical regime (see, for example, Fig. 4*B–D*). This is particularly true if the relevant initial condition for a system is located well away from any separatrices that bound the basin of attraction. Note that in this case, the system will also be buffered against stochastic fluctuations.

Thus, genotype networks conveying robustness correspond to large basins of attraction, which are spread across phase space, and persist across large regions of the parameter space of the underlying dynamical system. Translated into Waddington's terms, this means that robustness and canalization depend on deep valleys in the epigenetic landscape with a large associated 'drainage basin' that persists across a large range of genetic and environmental perturbations. Alternatively, as Thom put it and we have already mentioned above, robust morphogenesis crucially depends on the structural stability of the underlying dynamics (Thom, 1976). This is a fundamental mechanistic insight, which can now be tested empirically using the methods and tools of modern systems biology. In particular, we need to investigate whether large and stable basins of attraction are a rare exception, or whether they are a common phenomenon in biological regulatory processes. This is still an open question. A dedicated research programme to empirically sample, simulate and analyse phase spaces of experimentally tractable systems will be required to address it in a rigorous and systematic manner (Jaeger & Crombach, 2012; Jaeger *et al.* 2012; Jaeger & Sharpe, 2014).

Shifting our focus back to conceptual issues, we note that a number of additional systems-level properties of evolving networks can be fitted into our dynamical systems framework. For instance, the notion of a genotype network resolves the apparent contradiction between mutational robustness and the capacity of a regulatory system to innovate, that is, to produce novel phenotypes under suitable selective pressures (Müller & Wagner, 1991; Müller & Newman, 2005; Wagner, 2005, 2008, 2011; Ciliberti *et al.* 2007*a*,*b*; Draghi *et al.* 2010; Müller, 2010). The basic idea is the following: genotype networks allow (more or less) neutral systems drift as rewiring the network through mutations does not necessarily change the phenotypes they produce. In this way, a population of regulatory networks can explore large regions of genotype space. The wider the range of exploration, the higher the number of alternative genotype networks that occur in the population's mutational neighbourhood – the region of genotype space that can be reached within one mutational step. When the population encounters a change in environmental conditions that leads to selection for an alternative phenotype, it is more probable to have that particular phenotype mutationally available than if the population were confined to a small, localized region of genotype space. If any mutated individual in the population comes to lie within this new genotype network, its offspring can outcompete their peers due to the increased fitness associated with the 'invention' of the novel, more adapted, phenotype.

How does this translate to dynamical systems terms? As we have seen, large genotype networks correspond to large basins of attraction that persist across a wide range of parameter changes. If we study the geometry of phase portraits, we find that such structurally stable basins tend to be in contact with a greater number of alternative dynamical regimes. For example, Fig. 4*D* shows a phase portrait where the green and blue basins of attraction do not connect. It is impossible to transition from the green to the blue steady state without crossing the intermediate cyan basin. If we assume that altered environmental conditions favour the blue phenotype, but lead to a negative impact on fitness for the cyan state, then the green-to-blue transition becomes highly improbable. This evolutionary roadblock can be circumvented if the population spreads across the green genotype network. Through this neutral drift process, it will eventually cross the bifurcation boundary (indicated by a red line in Fig. 4) where the cyan state is annihilated. Beyond this boundary, the system will reach configurations such as those shown in Fig. 4*B* and *C*, where the green and blue basins do touch, and direct transitions now become possible. Therefore, transition probabilities between phenotypes are determined by the geometrical arrangement of attractor basins and their contacts through phase and parameter space. This provides a mechanistic explanation for another important concept: the idea that there are *regulatory* (or *developmental) constraints* or *biases* on the evolution of phenotypic traits (see, for example, Alberch, 1982, 1991; Maynard Smith *et al.* 1985; Wagner, 1988; Richardson & Chipman, 2003; Arthur, 2004; Salazar-Ciudad, 2006).

From the above example, it is evident that the boundaries of genotype networks correspond to the initial condition crossing a separatrix between two alternative basins of attractors. This differs from the traditional view that phenotypic or indeed any critical transitions are always directly caused by bifurcations (e.g. Thom, 1976; Scheffer, 2009). Figure 4 shows that the bifurcation creating the cyan state occurs well within the green and blue genotype networks. We will revisit this point when discussing the role of phenotypic plasticity below. In our current example, it means that, although the phase portraits in Fig. 4*C* and *D* differ by a bifurcation, they both

belong to the green genotype network, while Fig. 4*D* and *E* fall into different genotype networks despite exhibiting an equivalent number and arrangement of attractor basins.

Thus, we can summarize this section as follows. The existence of genotype networks can be explained by the presence of large, structurally stable basins of attraction in the underlying dynamical system. These correspond to deep, stable valleys in Waddington's landscape. The extent and geometrical arrangement of these attractor basins in phase space determines the evolutionary potential of the system – its robustness and evolvability, and its developmental constraints. Only large, structurally stable, basins are probably encountered by evolutionary processes. They determine the distribution of phenotypic variability, the substrate on which selection can act.

The role of the environment

We have seen above that the phase portrait of a system captures the essential features of the genotype–phenotype map (see Fig. 2): a developmental trajectory is specified by a combination of initial conditions and the parameters (which we called the 'genotype') of the system. The phase portrait provides a natural framework for understanding the role of environmental cues in the evolution of regulatory networks. In our course-grained framework, we can model the influence of such environmental factors through changes in the initial conditions of the system (while noting that, in general, such factors may also change the parameters of the system).

So far, we have considered the connection between phase space geometry and genotype networks for the simplified case of a precisely specified initial condition. This produces a unique developmental trajectory resulting in a *default* or *target phenotype* (yellow arrows in Fig. 4) (Nijhout, 2003; Fusco & Minelli, 2010; Espinosa-Soto *et al.* 2011; Wagner, 2011). It reflects the regulatory dynamics of a single instance of the network, that is, an individual organism, in the absence of environmental perturbations. To render our conceptual model a bit more realistic, we will extend our considerations to populations. We assume that the environment has a slightly different impact on the initial conditions of each individual in the population. Therefore, instead of a single default trajectory, we get a cloud of initial states as represented in yellow in the phase portraits of Fig. 5.

As most biological regulatory systems are multi-stable, environmental variability has the potential to provide access to multiple phenotypes for a given genotype. This results in *phenotypic plasticity*- the dependence of the phenotype on external conditions (reviewed in Schlichting & Pigliucci, 1998; West-Eberhard, 2003; Gilbert & Epel, 2009). Phenotypic plasticity can manifest itself in several ways. It can lead to a smooth shift in attractor position, and hence a smooth *reaction norm* of a phenotype (Schlichting & Pigliucci, 1998). Here, we will focus on a more drastic case where environmental changes result in a qualitatively distinct *alternative phenotype* because initial conditions come to lie in different basins of attraction (see, for example, phase portraits in Fig. 5*B*, *C* and *E*). This phenomenon is called *polyphenism*, and there is increasing evidence that it is much more common in nature than previously thought (see, for example, Emlen, 2000; Nijhout, 2003; Fusco & Minelli, 2010; Simpson *et al.* 2011).

Phenotypic plasticity and polyphenism in particular, have interesting consequences for the evolutionary dynamics of phenotypic transitions. To illustrate this, we adopt a modified version of the representation introduced by Espinosa-Soto and Wagner (2011) depicting each node of a genotype network with a pie chart. The sectors of each pie chart indicate the relative size of the attractor basins in the associated phase portrait. Let us consider Fig. 5*C* in a bit more detail. This phase portrait exhibits two equal-sized blue and green basins of attraction, which correspond to a pie chart that is half blue, half green. Note that the cloud of initial conditions lies mainly in the green basin, but does extend a little across the separatrix into the blue. Depending on the initial conditions, this phase portrait can fall either into the green or the blue genotype network. In this way, polyphenism – reflected by multi-stability of the system – causes different genotype networks to overlap (where pie charts show multiple sectors). The boundaries between them are no longer uniquely defined, but depend explicitly on the environment.

Polyphenism can facilitate phenotypic transitions through a process called *genetic assimilation,* another one of Waddington's pioneering concepts (Waddington, 1953, 1957, 1961, 1975; for recent reviews, see also West-Eberhard, 2003; Crispo, 2007). This type of assimilation occurs when an originally environmentally triggered phenotype becomes internally induced through genetic signals during evolution. It can easily be implemented in our framework. At the outset, a change in environmental conditions shifts the position of the cloud of initial conditions in the phase portrait (compare Fig. 5*C* and *D*, shift indicated by grey arrow). This can bring the population close to a separatrix such that some or all individuals fall into an alternative basin of attraction (Fig. 5*D*, cyan basin). Subsequently, selection will favour increased penetrance of the new, adaptive phenotype. Such an increase can be achieved, for instance, by genetic changes enlarging the size of the alternative basin (compare the cyan regions in Fig. 5*D* and *E*). Finally, the original environmental trigger is removed (grey arrow in Fig. 5*E*), yet the system remains in the basin of the novel, alternative phenotype (cyan basin, Fig. 5*E*).

Simulation-based studies have revealed the exact conditions that need to be met for genetic assimilation to occur (Espinosa-Soto *et al.* 2011; Wagner, 2011).

First, the system needs to be able to encounter environmentally induced alternative phenotypes while still drifting along the genotype network of the original default phenotype. Second, the penetrance of the adaptive alternative phenotype should have a tendency to increase as we approach the boundary of the original genotype network. Third, genotypes that produce a given alternative phenotype must be mutationally connected to other genotypes producing this alternative phenotype. Finally, increasing penetrance of an alternative phenotype should facilitate its transition to become the new default.

These four conditions for genetic assimilation can be explained by the following features of phase and parameter space. First, we have seen in the previous section (and Fig. 4) that bifurcation boundaries can occur well beyond the borders of a genotype network. We have further argued that multi-stability leads to phenotypic plasticity and overlapping genotype networks if we let the environment affect the initial conditions (see Fig. 5). Taken together, this shows that multi-stability is necessary and provides a mechanistic explanation for the first condition.

Second, as we move towards the boundary of the genotype network for the default phenotype, we tend to see a decrease in the size of its associated basin of attraction, and a consequent increase in basin size for the alternative phenotypes (see pie charts in Fig. 5).

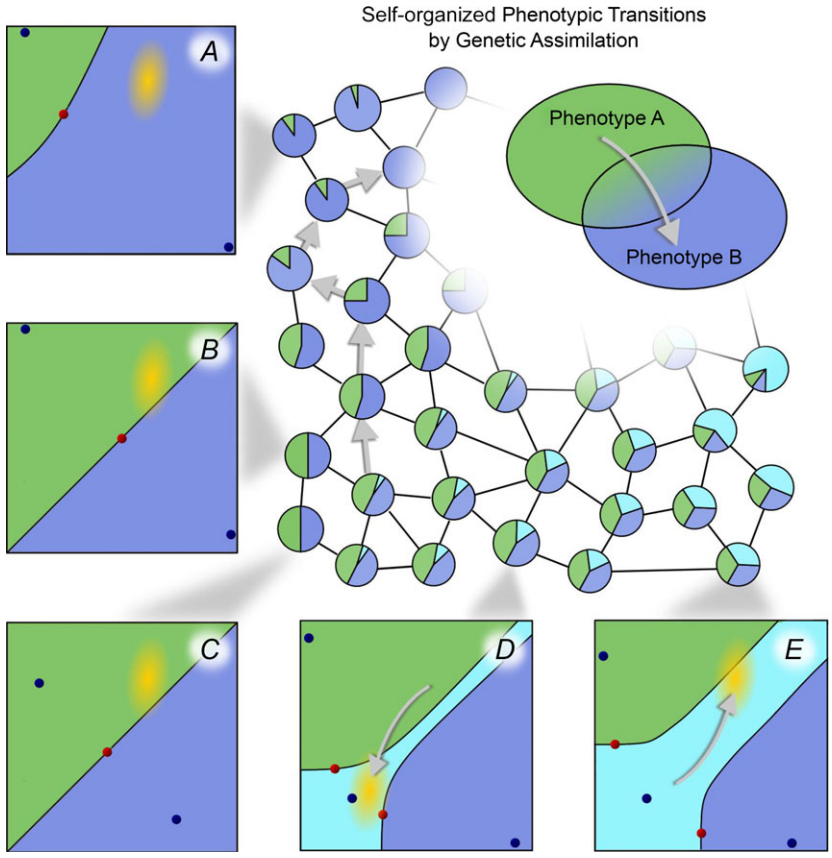

Figure 5. The role of the environment: genetic assimilation and self-organization
How polyphenism can lead to self-organized evolutionary convergence toward a novel adaptive phenotype through genetic assimilation. The basic layout is the same as Fig. 4. Nodes in genotype networks are now displayed as pie charts reflecting the relative size of the basins of attraction representing each phenotype in the phase portraits (*A*–*E*, blue, green and cyan). Multi-stable nodes (which have more than one coloured sector in their pie charts) can belong to different genotype networks depending on initial conditions. Genetic assimilation is modelled using a population of genotypes indicated by yellow clouds in phase portraits *A*–*E*. An environmental perturbation triggers the population to express an alternative phenotype (cyan; shift of initial conditions indicated by the grey arrow in *D*). Selection then causes stabilization of this phenotype by enlargement of its basin (transition from *D* to *E*). The novel phenotype is now expressed even in the absence of the environmental trigger (reverse shift of initial conditions represented by the grey arrow in *E*). Self-organization of phenotypic transitions is shown as follows: grey arrows between pie charts represent fastest evolutionary path from the green to the blue genotype network. This is summarized in the inset on the top right: circles indicate green and blue genotype networks respectively. Maximally increasing penetrance along the mutational path (grey arrow) leads to self-organized transition (evolutionary funnelling) of the population to the alternative (blue) state. See text for details.

This expansion involves movement of separatrices, which 'overtake' the cloud of initial conditions explaining the increasing recruitment of initial conditions into the novel basin, and thus the increasing penetrance of the alternative phenotype (see, for example, the blue attractor in Fig. 5*A* and *B*).

Third, in the absence of bifurcation events, which are generally quite rare, phase space geometry tends to change subtly and smoothly, as system parameters are varied. This continuity explains why genotypes producing the same set of alternative phenotypes tend to be connected. Their connection is based on the geometrical similarity of the underlying phase spaces (see, for example, Fig. 4*B* and *C*).

The final point is the most interesting. Selection for an advantageous phenotype will act to increase its penetrance (see above). Because of the continuous nature of phase space change between genotypes in the absence of bifurcation, this mechanism will 'push' the evolving population in the direction of the largest increase in basin size for the alternative attractor (indicated by grey arrows between pie charts in Fig. 5). In Waddington's terms, it will guide the population in the direction of the deepest valley with the largest drainage basin. This gives us an intuitive geometrical explanation of why genetic assimilation can accelerate phenotypic change. The topography of the landscape and the way it reacts to genetic changes induces an element of *self-organization* into the process (Kauffman, 1993; Camazine *et al.* 2003). Evolution is still a random process, but a random process biased towards the novel advantageous phenotype by the underlying geometry of phase and parameter space.

Conclusions: cursed by complexity?

In this paper, we have introduced some conceptual tools from dynamical systems theory, and have illustrated how they provide explanations for a number of systems-level phenomena relevant to the evolution of biological regulatory processes (see Figs 3, 4, 5). These concepts constitute a unifying framework for regulatory evolution that connects genotype networks to Waddington's epigenetic landscape (see Fig. 1). We argue that the geometrical analysis of phase and parameter space provides a deeper level of understanding than insights based on the numerical study of network ensembles, as it yields mechanistic and causative rather than statistical and correlative explanations.

One common objection to this approach is to criticize its limited practicality. It has been argued that potential problems – such as the high and dynamically changing dimensionality of complex regulatory systems – pose an insurmountable challenge for phase space analysis. We disagree with this point of view, for a number of reasons.

Of course, it is true that many biological processes will not be amenable to this level of scrutiny. However, selected model systems already are or will become so in the near future. Such an approach based on case studies in carefully chosen model systems is very common throughout biology. Phase space analysis has been successfully used to analyse the function and developmental potential of complex patterning systems in embryos of *Drosophila melanogaster* (Manu *et al.* 2009*a*,*b*; Vakulenko *et al.* 2009) and *Caenorhabditis elegans* (Corson & Siggia, 2012). This provides a proof-of-principle for the feasibility of the approach.

A number of additional considerations indicate that our suggested approach is practical and worth pursuing. First, we have shown (in Fig. 3) that there are only a limited number of possible phase space transitions (Jaeger *et al.* 2012). Furthermore, the relevant features of phase space and their bifurcations are often low dimensional (see, for example, Manu *et al.* 2009*b*; Corson & Siggia, 2012). Or if they are not, there are methods that allow the reduction of the dimensionality of the system, either by clustering or eliminating individual factors (reviewed in Hecker *et al.* 2009), by identifying and studying subsystems of attractors (Irons & Monk, 2007), or by more abstract mathematical approaches to dimensionality reduction (Radulescu *et al.* 2008; Vakulenko *et al.* 2009). And finally, by choosing a pragmatic bottom-up, rather than systematic top-down approach to phase space analysis, we can understand the regulatory and evolutionary potential of specific, experimentally tractable biological systems, in which we can rigorously test our model predictions (Jaeger & Crombach, 2012; Jaeger & Sharpe, 2014).

Based on this last point, we need to emphasize that our conceptual framework does not provide a general theory from which we can derive all possible regulatory behaviours. Instead, we propose a more empirical research programme to explore the space of possible phase space geometries from specific, real world instances. Phase space analysis could be used in the near future to gain new insights into models of evolving regulatory processes such as cell cycle regulation, microbial metabolism and physiology, stem cell differentiation, or aspects of development such as axis formation and segmentation in arthropods, vertebrate limb development and somitogenesis, and pattern formation in plant roots, stems and leaves. Only by examining a large number of such systems will we be able to derive general insights into potential regularities or rules that govern biological regulatory dynamics. Knowledge of such regularities would, in fact, amount to some sort of an empirical theory of physiology and development (Jaeger & Sharpe, 2014). Such a theory will bring our understanding of evolutionary dynamics to an entirely new level, and may lead one day to a locally predictive theory of phenotypic evolution in real world populations.

Box. Glossary

alternative phenotype	A phenotype – produced by a *multi-stable* regulatory system – which shows a lower penetrance than the *default phenotype.*
attractor	A point or higher-dimensional bounded subregion of phase space towards which trajectories converge from all directions over time.
basin of attraction	The subregion of *phase space* containing all *trajectories* that converge to a specific *attractor.*
bifurcation	An abrupt and drastic change in the *dynamical repertoire* of a dynamical system caused by smooth variation in parameter values. Usually involves the creation or annihilation of an *attractor* and its associated *basin*, or a change in the type of a steady state (e.g. from *attractor* to *saddle point*).
canalization	Characterizes the buffered nature of ontogenetic *trajectories* with respect to genetic or environmental perturbations. Is caused by *structural stability* of the underlying *attractors* and their *bifurcations.*
catastrophe	An abrupt and drastic change in the behaviour of a dynamical system, usually due to an underlying *bifurcation* event. Nowadays, more often called a 'critical transition'.
chreod	A robust or canalized ontogenetic pathway. Corresponds to the bottom of a valley in the *epigenetic* or *potential landscape.*
default phenotype	The phenotype produced by a regulatory system given a specific *initial condition* in the absence of any environmental variability or perturbation. Also: the phenotype produced with highest *penetrance* by a *phenotypically plastic* system under variable environmental conditions.
developmental constraint/bias	See *regulatory constraint/bias.*
dynamical repertoire	The set of outputs (dynamical behaviours or phenotypes) a system can produce. Defined by the *phase portrait* (*attractors* and their *basins*, *saddles* and *separatrices*) of the system.
(network) ensemble	A large set/class of networks with given characteristics.
epigenetic landscape	A metaphor proposed by C.H. Waddington to characterize the dynamical constraints and *robustness* or *canalization* affecting the production of phenotypic outcomes, and the evolution of ontogenetic *trajectories*.
evolvability	The capacity of an evolving regulatory system to produce adaptive phenotypes. Also called *innovability* by some authors taking the assumption that adaptive phenotypes can be considered evolutionary novelties or innovations.
flow	The set of all trajectories of a dynamical system starting from any possible *initial condition*. Defines the *phase portrait* and, hence, the *dynamical repertoire* of the system.
generative process	A metabolic, physiological and/or developmental regulatory process that produces some sort of phenotype. The *genotype–phenotype map* is formed by the combination of such processes.
genetic assimilation	The replacement of an environmental trigger for an ontogenetic *trajectory* by an internal, genetic one.
genotype network	A set of genotypes that, given identical initial conditions, produce the same *(default) phenotype*, and that are directly connected to each other via mutational steps, i.e. a change (addition, subtraction or sign reversal) in a single regulatory interaction.
genotype–phenotype map	A map (in the mathematical sense of the word) that connects genotypes to the phenotypes they produce via *generative processes*. This map is degenerate due to *phenotypic plasticity* (one genotype, many phenotypes) and *robustness* or *canalization* (many genotypes, one phenotype) of biological systems.
genotype set	A set of different genotypes producing the same *(default) phenotype*.
initial state/condition	Is comprised of the initial values of all *state variables* of the dynamical system.
innovability	The propensity of a system to produce novel adaptive phenotypes (innovation).
neutral network	See *genotype network*.
multi-stability	The characteristic of a system that can produce more than one (phenotypic) output. Implies the presence of more than one *attractor* in the *phase portrait*.
ontogeny	We use this term in a very broad sense here – true to its literal meaning, 'the generation of being' – to include not only developmental but also metabolic and physiological processes involved in the production of phenotypes.
parameter	See *system parameters.*
penetrance	The percentage of individuals in a population that show a given phenotype.
phase portrait	Characterizes the *phase space* of a specific dynamical system, e.g. a gene regulatory network with a given *regulatory structure* and a defined set of *parameter* values. Consists of an arrangement of *attractors* and their *basins*, with *separatrices* (and associated *saddle points*) that form the boundaries between them. Defines the *dynamical repertoire* of the system.

continued

Box. Continued

phase space	An abstract, usually multidimensional, space whose axes are defined by the *state variables* of the system. Contains features such as *attractors* and their associated *basins*, *saddle points* and *separatrices.*
phenotypic plasticity	The ability of a specific regulatory system, or *genotype–phenotype map,* to produce a range of phenotypes under varying environmental conditions.
phenotypic variability	Theoretical (a priori) distribution of phenotypes a given regulatory system, or *genotype–phenotype map*, can produce. Provides the probability of observing a possible phenotype before considering the effects of natural selection and genetic drift.
phenotypic variation	Observed (a posteriori) distribution of phenotypes in a population, based on the combined effect of a specific a priori distribution (*phenotypic variability*), and evolutionary processes such as natural selection and genetic drift.
polyphenism	A type of *phenotypic plasticity* where the resulting distribution of phenotypes is not continuous (see *reaction norm*), but discrete, producing a *default*, and one or several *alternative phenotypes*.
potential surface/landscape	Visualization of the *phase portrait* where the *flow* of the system is represented by the slope of the potential, i.e. fast change implies a steep slope and vice versa. Can be seen as a mathematically rigorous implementation of the *epigenetic landscape.*
reaction norm	A continuously varying distribution of phenotypes produced by a given regulatory system across a range of environmental conditions (as opposed to the discrete distribution of phenotypes in *polyphenism*).
regulatory constraint/bias	Describes a limitation or bias in the direction or rate of possible evolutionary change, imposed by the *regulatory organization* of a system.
regulatory organization	Corresponds to the wiring of a *generative process*: defines how the components constituting the process interact dynamically to produce a phenotypic outcome. Includes metabolic, physiological and gene regulation. We use this term interchangeably with *regulatory structure.*
regulatory structure	See *regulatory organization.*
robustness	See *canalization*.
saddle point	A point or higher-dimensional bounded subregion of phase space located on a *separatrix* or boundary between two *basins of attraction.* Saddle points are unstable: trajectories converge towards them from the direction of the *separatrix* only, while they diverge in all other directions.
self-organization	Describes a structure or dynamic pattern that arises due to internal regulatory interactions in a system, rather than an external forcing or drive.
separatrix	The boundary between two *basins of attraction*. Can contain *saddle points.*
state (of a system)	Is comprised of the values of all *state variables* of the dynamical system at a given time.
state space	See *phase space*.
state variable	Represents the current value of a specific component of a dynamical system that changes over a short timescale (e.g. the concentration of a regulatory factor).
structural stability	Characterizes the persistence of a specific *attractor* and its associated *basin* over a large range of changing *parameter* values. Underlies the *robustness* and *canalization* of biological regulatory processes.
system parameters	A set of numerical values that determine system properties, such as the sign and strength of regulatory interactions, or rates of production, decay, or diffusion for systems components. Parameters do not change over time, or if they do, they change more slowly than the *state variables* of the system.
target phenotype	See *default phenotype.*
trajectory	Describes the changing state of a biological system over time. Corresponds to the path of change through phase space that a dynamical system will take given a specific *initial state* or *condition.*
unstable manifold	A *trajectory* diverging from a *saddle point* towards an *attractor.* Concentrates bundles of trajectories in the subregion of *phase space* around it before they reach the attractor. Corresponds to a *chreod* (the bottom of a valley) in the *epigenetic* or *potential landscape.*

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Additional information

Competing interests

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