Chapter 8
Evolution In Silico: From Network Structure to Bifurcation Theory

Paul François

Abstract I describe an evolutionary procedure in silico that creates small gene networks performing basic tasks. I use it to evolve a wide range of models for very different biological functions: multistability, adaptive networks and entire developmental programmes like somitogenesis and *Hox* gene pattern. In silico evolution finds both known and original network designs, and can be used to make predictions on biological behaviours. This computation illustrates how complex traits can evolve in an incremental way, and suggests that dynamical systems theory could be used to get new insights towards a predictive evolutionary theory.

1 Introduction: Can We Make Evolution Predictive?

Evolution is a retrospective theory: phylogenies are reconstructed based on comparison between current species, at all biological level (from DNA to morphologies and even behaviours). It is assumed that evolutionary divergences from a common ancestor are due either to genetic drift or to environmental constraints imposing some selective pressures. In both cases, since both mutations and environmental changes are largely random processes, the results of biological evolution itself are often considered as purely contingent [33]. As a consequence, it seems *a priori* impossible to use evolution in a predictive way: for instance, we typically do not use evolutionary principles to answer specific biological questions such as the amino-acid sequence of a protein which evolved to perform some biochemical activities or the structure of gene networks performing some known biological functions.

P. François (✉)
McGill University, Ernest Rutherford Physics Building, 3600 rue University, H3A 2T8 Montreal, QC, Canada
e-mail: paulf@physics.mcgill.ca

O.S. Soyer (ed.), *Evolutionary Systems Biology*, Advances in Experimental Medicine and Biology 751, DOI 10.1007/978-1-4614-3567-9_8,

However, despite strong evolutionary contingencies, history of life also shows that evolution kept finding very similar solutions to the same evolutionary problems. This phenomenon is called convergent evolution. There are multiple examples at all scales of the living world, from convergent evolution of biochemical pathways (such as independent evolution of the C-4 photosynthesis [59]) to evolution of organs (the classical example of the camera eye [50]) and even convergent evolution of social structures (such as eusociality in insects and mole-rats [8]). These examples are especially interesting because if there is some reproducibility in nature, this means that there are some laws to discover to explain it.

It seems, however, very difficult—at least for now—to build a general theory based on known biological examples of convergent evolution for at least two reasons. The first one is the lack of mathematical tools to describe the genotype to phenotype mapping and therefore to model its evolution. The second one is the fitness problem: we do not really have a good quantitative way to describe generic selective pressures which could explain convergent evolution. If we could solve both difficulties, one could in principle predict and derive biological structures and dynamics from evolutionary theory.

Fruitful ideas have been recently developed in systems biology. Systems biology descriptions rely on the modelling and the simulation of so-called networks, loosely defined as ensembles of biological objects (such as genes, RNAs, proteins, etc.) interacting with each other. One of the advantage of this approach is that it considerably simplifies the problem of connecting network structure and kinetics to biological behaviour, while being complex enough to have access to a wide range of realistic dynamics. Therefore, it is tantalizing to use systems biology modelling to study evolutionary theory in a more predictive way [23, 28, 64].

In this chapter, I describe and illustrate a generic method to study *in silico* evolution of such networks. The goal of this approach is to ask an "inverse question": given a biologically plausible function, what are the possible networks that could evolve to perform it? The genotype to phenotype mapping is not trivial but can be easily simulated, and I will describe on specific examples how to solve the fitness problem introduced above. Study of computational evolution and its result gives considerable insights into the network dynamics and the evolutionary process itself, especially the problem of convergent evolution. It also connects in a surprising way evolution to dynamical systems—in particular bifurcation theory—which suggests some potentially fruitful tracks to follow for future research.

This chapter is organized as follows: Sect. 2 summarizes the general approach used. Then, I review results of evolutionary computations, firstly starting with the description of evolution of alternative biochemical reactions for classical functions in single cell systems biology in Sect. 3, and secondly describing how computational evolution can be used to evolve complex developmental programmes in Sect. 4.

2 Evolutionary Algorithm

2.1 Network Definition

A standard approach is followed where a biological network is defined as an ensemble of interacting genes and proteins. Dynamics of genes and proteins are modeled in single cells using the "rate equation paradigm" [56]:

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = f_i(\mathbf{x}), i = 1, \ldots, N. \tag{8.1}$$

$\mathbf{x}$ is a vector of variables modelled within an individual cell, e.g. if we model only protein concentration evolution, $x_i, i = 1, \ldots, N$ corresponds to the concentration of proteins considered in the system. Functions f_i are non-linear functions accounting for the biological interactions between genes and proteins. The goal of the evolutionary algorithm is essentially to randomly modify both these functions f_i and the number of variables N to select for a prescribed dynamics encoded via a fitness function as described in Sect. 2.2. Some simpler formalism can be used to model gene networks dynamics; however, ODE-based formalism gives rise to both richer and more realistic dynamics than discrete ones and was therefore preferred.

The precise mathematical forms of f_i used in our algorithm depend on the specific problem considered, its known biological property, and level of details we want to model. Two different kinds of simulations are described in this chapter:

- Evolution of new biochemical motifs, where the goal is to figure out, given a simple list of possible interactions, what kind of networks spontaneously evolve to perform a given biological function (bistable switch, adaptation).
- Evolution of network organization associated with a developmental programme, where the goal is to study evolutionary emergence of new dynamics and structures driving emergence of a complex pattern (somitogenesis, *Hox* pattern).

In the first category of evolutionary simulations, we model explicitly different types of genetic interaction. This level of details corresponds to a situation where we actually want to explore the influence on evolution of the presence of specific standard non-linear processes such as protein–protein interactions or phosphorylations. Binding of proteins onto DNA promoters [23] and/or of subsequent biochemical post-transcriptional interactions [25] are also modelled using standard mass-action laws.

Table 8.1 recapitulates these interactions with typical associated equations used. Roman letters correspond to variables simulated (fractional gene activity and protein concentrations), and Greek letters correspond to parameters randomly chosen and are allowed to evolve. In our simplified description, we condensate the two biological steps of transcription and translation into one single rate. Transcriptional interactions in this chapter thus refer to regulations of production rate of a protein by a transcription factor.

Table 8.1 Possible biochemical interactions. Each term should be added to the corresponding rate equations to account for several interactions

Example of interaction	Equations	Parameters
Regulation of A transcription by a protein B	$\frac{\mathrm{d}a}{\mathrm{d}t} = \theta a : B - \gamma aB$	a: "naked" promoter for gene a
	$\frac{\mathrm{d}a:B}{\mathrm{d}t} = \gamma aB - \theta a : B$	$a : B$: protein B bound to a promoter
	$\frac{\mathrm{d}A}{\mathrm{d}t} = \rho_A a + \rho'_A a : B$	γ kinetic constant for binding of B to a promoter
		θ kinetic constant for unbinding
		ρ_A production rate of A with no regulation
		ρ'_A production rate of A when B is bound to A promoter
Degradation of protein A	$\frac{\mathrm{d}A}{\mathrm{d}t} = -\delta_A A$	δ_A: degradation rate of A
Dimerization: A and B form a dimer C	$\frac{\mathrm{d}A}{\mathrm{d}t} = -\gamma AB + \delta C$	γ: association constant
	$\frac{\mathrm{d}B}{\mathrm{d}t} = -\gamma AB + \delta C$	δ: dimer disassociation
	$\frac{\mathrm{d}C}{\mathrm{d}t} = \gamma AB - \delta C$	
Phosphorylation: kinase K phosphorylates protein A into protein A^*	$\frac{\mathrm{d}A}{\mathrm{d}t} = -\rho K \frac{(A/\alpha_0)^\eta}{1+(A/\alpha_0)^\eta} + \delta A^*$	ρ: phosphorylation rate
	$\frac{\mathrm{d}A^*}{\mathrm{d}t} = \rho K \frac{(A/\alpha_0)^\eta}{1+(A/\alpha_0)^\eta} - \delta A^*$	Phosphorylation cascade is modelled via a Hill function with threshold
		α_0 and coefficient η
		δ: dephosphorylation rate

To explore the evolution of bigger combinatorial transcriptional networks (like in development), our description is simplified. A formalism inspired by transcription network [56] is used as a coarse-grained description of interactions between different genes and proteins (another reason for this specific choice being most developmental networks heavily rely on transcriptional interactions [14]). Regulation of a protein B is modelled as a combination of Hill functions. Assuming that transcription factors A_1 and A_2 activate expression of gene B and that repressor R represses it, equation for B would then be

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \rho_B \max\left(\frac{A_1^{\eta_1}}{A_1^{\eta_1} + \alpha_{1*}^{\eta_1}}, \frac{A_2^{\eta_2}}{A_2^{\eta_2} + \alpha_{2*}^{\eta_2}}\right) \times \frac{\rho_*^{\eta_3}}{R^{\eta_3} + \rho_*^{\eta_3}} - \delta_B B, \tag{8.2}$$

where α_{i*} and ρ_* are threshold concentrations in Hill functions and η_i are Hill coefficients accounting for cooperativity. It is also possible to add a delay in transcription for some problems (see, e.g. in [27]). As in the previous case, parameters corresponding to Greek letters are chosen and allowed to evolve

randomly. Equation (8.2) expresses that we assume an "OR" combinatorial between activators (i.e. one single activator is enough to activate transcription) while repressors act multiplicatively. Of course, gene regulation is vastly more complex than these simplified models, but they are generic enough so that we can capture realistic changing trends of response with variation of activators and repressors.

2.2 Evolutionary Dynamics

Genetic networks are evolved by repeated rounds of selection, growth and mutation.

Typically 100 networks are followed in parallel. At each step of the algorithm, equations corresponding to the networks are integrated, and a fitness or scoring function is computed (see below). Networks are then ranked according to this fitness; the best half is retained, then each network kept is copied and mutated.[1] By analogy with minimization of energy in physics, practically, our algorithm actually minimizes the fitness function, this is of course mathematically equivalent to flipping a sign and maximizing minus the fitness. Details are given below for specific examples on which function is minimized or maximized.

Mutations are of two types: mutations changing the kinetic constants within the networks or mutations changing the topology of the network, i.e. adding/removing new proteins or interactions among the one listed above. At each generation, a given mutation has a predetermined probability to happen. Mutations changing kinetic parameters in the network are assumed to have a higher probability of occurrence than mutations changing topologies of network, and within the latter, probability of removing existing interactions are higher than probability of adding new interactions. This choice of probability corresponds to a biologically realistic limit for which the most probable evolutionary event is to modify existing interactions, the second most probable event being to delete interactions and creating new ones is the least probable. Results of evolution described in this chapter are essentially largely independent from the precise choice of mutation rates as long as simulations are run in this limit.

After the mutation step, the entire process is iterated. A generation is one iteration of this selection/growth/mutation process and corresponds to many generations in a real organism since we are only concerned with mutations in the one network under study. This procedure favours the evolution with time network topologies and parameters satisfying the fitness function. An overview of the algorithm is provided in Fig. 8.1.

[1] Schemes based on a more probabilistic selection process have been tested and never significantly altered the outcomes of evolution described: the main reason is that in all cases described in this chapter, evolution happens in a very incremental way so that evolutionary innovations spread rapidly in the population.

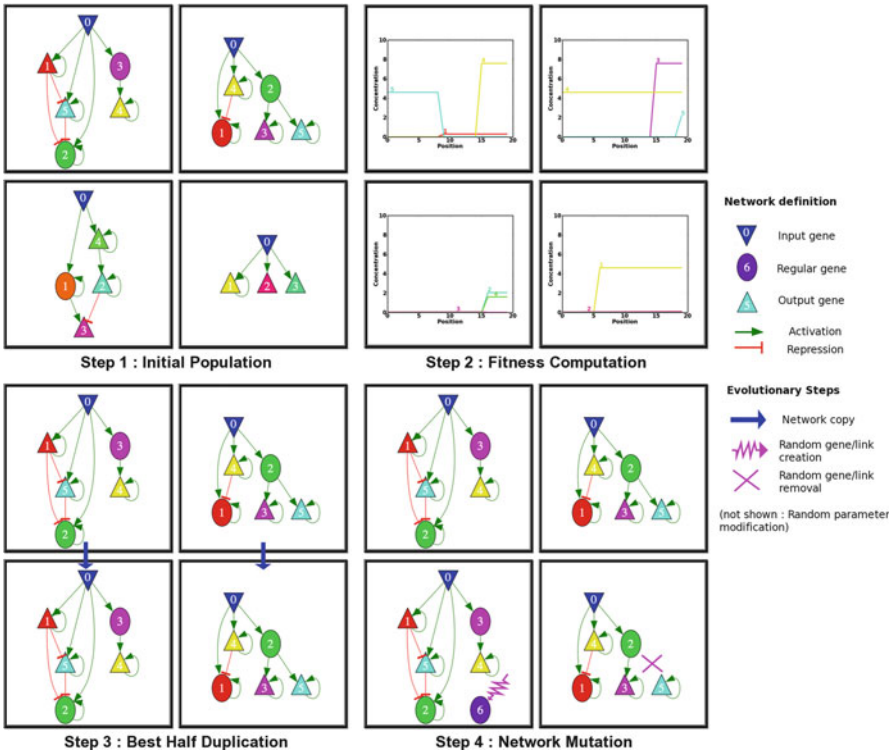

Fig. 8.1 *Overview of evolutionary algorithm*. Differential equations for each network are integrated, prescribing dynamics of input gene if needed (step 1). The fitness function is computed for each network on output genes (step 2). The best half of the networks is retained (selection), copied (growth, step 3), and randomly mutated (mutation, step 4). Mutations change parameters (kinetics) or the network itself, as exemplified here. In this chapter, we will follow the following conventions for network sketches: genes/proteins are represented as *big circles*, output genes on which fitness is computed by *triangles*, input genes with imposed dynamics by *inverted triangles*. Transcriptional activation is represented by *green arrows*, repression by *t-headed red arrows*, and other interactions (e.g. protein–protein interactions, PPI) by *blue arrows*. Figure reproduced from [26]

One characteristic of evolutionary simulations is the phenomenon of code bloat: core working networks are often embedded into bigger ones due to past evolutionary history but without any functional roles. To identify the most parsimonious subnetwork accounting for a function, we use a pruning evolutionary procedure: once a working network topology has been identified, we run our evolutionary simulations in a mode were nodes are randomly pruned and only networks keeping a constant fitness are selected. All networks displayed in this chapter represent such core networks.

2.3 Fitness

Our goal is to select for networks performing specific biological functions. It can be quite challenging to make reasonable assumptions about what we want to select for and on the mathematical way to define it, and classical evolutionary works from Wright have stressed the influence of the fitness landscape on evolutionary dynamics [74]. It is obvious that the topography of this fitness function over the space of possible networks could greatly change the results of evolution and its chance of convergence. For instance, if this topography is analogous to a funnel leading to a unique minimum, evolution is guided towards it and convergence towards this evolutionary solution should be rather systematic when simulating evolutions several times. The opposite case consists in a golf-course evolutionary landscape, with local minima but without any cue leading towards the optimal solution: evolution in that case reduces to a random walk in space of networks.

Our definition of fitness is guided by the fact that pathways and networks do not evolve independently, are commonly shared by many species but with slightly different dynamics and are reused in many contexts.

All of this suggests to use a rather smooth fitness landscape, and our fitness functions are therefore defined in a very coarse-grained way. Then, evolution occurs incrementally, following Darwin's intuition for the evolution of complex structures as he exemplified for eye evolution [13] and as theoretically studied by Nilsson and Pelger [50]. More details on the fitnesses chosen are given for specific examples in this chapter.

3 Single-Cell Dynamics: Alternative Biochemistry for Complex Dynamics

Many theoretical works on functions of gene networks motifs focus on purely transcriptional networks [62]. Transcriptional interactions can, however, be embedded in many other non-transcriptional interactions, which can surely play important roles in network dynamics. In this section, we use our evolutionary approach to look for alternative network motifs performing classical biochemical functions with the help of post-transcriptional interactions.

3.1 Bistable Networks

Bistability is a paradigmatic network function, widely considered in systems and synthetic biology [7, 21, 29]. Bistability has been shown in many contexts to lock important cellular decisions such as epigenetic switches [6, 53]. We first study the evolution of a bistable gene network [23]. Two genes and associated proteins A and

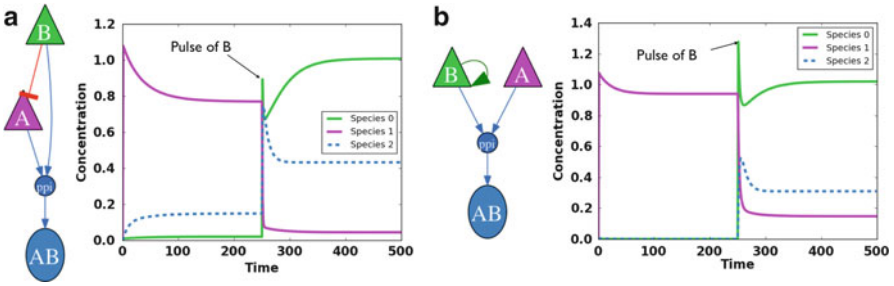

Fig. 8.2 *Example of evolved bistable networks.* Each panel displays network topology on the *left* and dynamics of it on the *right*. Simulated pulse of protein B is indicated. Fitness is computed on genes A and B (*triangles*); "PPI" indicates a protein–protein interaction (titration). (**a**) Mixed-feedback loop bistable motif and (**b**) Positive feedback loop motif

B are considered. The goal is to evolve networks with two possible stable steady states: one state with high A concentration and low B concentration and one state with high B concentration and low A concentration.

Networks are initialized close to the first desired steady state with A high and B low. Then the dynamics is allowed to relax from time 0 to time T. A pulse of B is induced, aiming at switching the steady state. The network is then followed from T to $2T$. The score or fitness of the network is computed in the following way: from 0 to T, integral $\int \left((A(t)-A_1)^2+(B(t)-B_1)^2\right) \mathrm{d}t$ is computed and from T to $2T$ $\int \left((A(t)-A_2)^2+(B(t)-B_2)^2\right) \mathrm{d}t$, where A_i, B_i the desired concentration for steady state i. Minimization of these two integrals ensures that the system spends as much time as possible close to each of this state (by analogy with energy minimization in physics, lower fitness is better here).

We first checked that, when post-transcriptional interactions are forbidden within the algorithm, networks spontaneously evolve towards classical toggle switch scheme with mutual repression [23]. Then, we ran the evolutionary algorithm, allowing for post-transcriptional interactions between proteins.

Figure 8.2 illustrates two typical networks found with this procedure, with their respective dynamics. Interestingly, these networks are quite different from classical "toggle switches" based on mutual repression [29]. In Fig. 8.2a, protein B represses protein A, and then A and B titrate each other to form a dimer AB. So, if B concentration is high, it represses protein A, which defines the first steady state. On the contrary, if protein A is high, it titrates protein B and therefore prevents it from repressing A production, locking the second steady state. Figure 8.2b is a network without even a single transcriptional repression. If B is high, it activates itself and titrates protein A, maintaining it at a low concentration. If A is high, it titrates protein B and prevents it from activating itself, stabilizing the system in a high A low B concentration.

These networks are therefore clearly not symmetric between A and B, the two proteins implicated in these switches. One common feature of these networks is that

protein A and B titrate each other. Indeed, molecular titration induces strong non-linearity [5]. Because of this post-transcriptional non-linearity, these networks are bistable even without cooperativity in transcriptional regulation as shown in [24].

Interestingly, the two motifs of Fig. 8.2 are quite common in actual biological networks. Network from panel a is called a "mixed-feedback loop" (MFL) network, has been shown to be statistically overrepresented in gene network structure as compared to randomized ones [75] and is implicated in many decision processes in yeast [24]. Bistability of this system has been experimentally shown for two important epigenetic switches in bacteria: for the lac operon first, where allolactose induced by permease production binds to the lac repressor which represses permease [38, 51], and for the daughter cell separation in *Bacillus subtilis*, where SinR represses transcription of SlrR and titrates it [6]. This motif has also been suggested to be at the core of microRNA switches [63]. Network from panel b is at the basis of known bistable/excitable system such as the development of competence in *B. subtilis*, where MecA binds to ComK, preventing its autoactivation [65, 69].

3.2 Adaptation: A Tale of Two Fitnesses

Biochemical adaptation is ubiquitous in biological signalling, from vertebrate photoreceptors [45, 49] to bacterial chemotaxis [4]. In adaptive networks, a jump between two constant input levels (e.g. a ligand, light intensity) drives a transient output pulse between two nearly identical constant states. If the output steady state is independent from the input, adaptation is qualified as "perfect".

Figure 8.3a illustrates a "typical" input/output relationships for a generic genetic network. Assuming a step of input occurs between two levels I_1 and I_2, we define $\Delta O_{\mathrm{ss}} = |O(I_1) - O(I_2)|$ the difference between steady state concentrations of the output. Perfect adaptation is $\Delta O_{\mathrm{ss}} = 0$. Of course, there are many networks for which this condition is realized, including networks where the output concentration is simply independent from the input level. To select for adaptation, we are therefore interested in another quantity, ΔO_{max}, which is the maximum absolute deviation of the output concentration after a change of input level $\Delta O_{\mathrm{max}} = \max_{t>0} |O(t) - O(I_1)|$.

Computational evolution of adaptation therefore is a problem of simultaneous optimizations of two fitnesses: we aim at both minimizing ΔO_{ss} and maximizing ΔO_{max}. However, in our evolutionary setup, we have to define a single fitness function, so we ran several evolutionary simulations combining these two quantities in different ways [25], e.g.

$$f_a = \Delta O_{\mathrm{ss}} - \Delta O_{\mathrm{max}}, \tag{8.3}$$

$$f_b = \Delta O_{\mathrm{ss}} + \varepsilon / \Delta O_{\mathrm{max}}, \tag{8.4}$$

$$f_c = (\Delta O_{\mathrm{ss}} + \varepsilon) / \Delta O_{\mathrm{max}}, \tag{8.5}$$

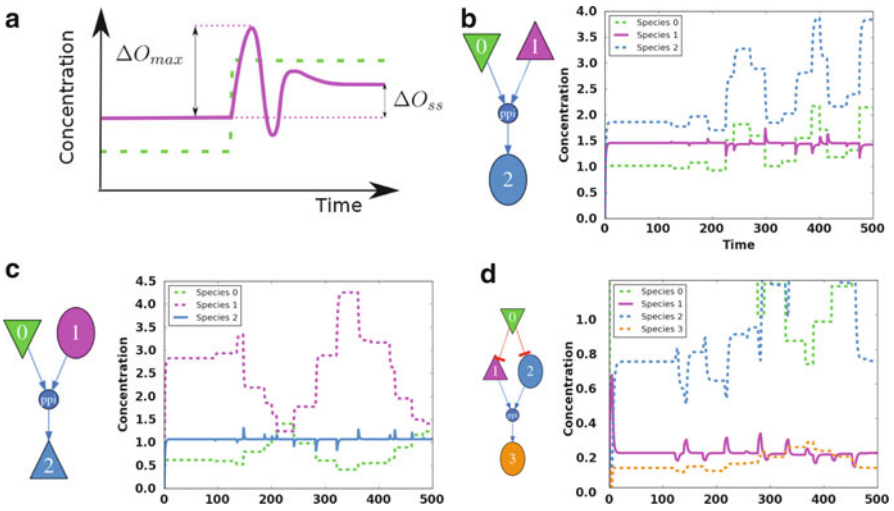

Fig. 8.3 *Evolution of adaptive networks.* (**a**) Illustration of input and output relationships for a general network after a step in input. $\Delta O_{\max}$ and ΔO_{ss} are represented. The (external) input of the network is displayed in *dashed green* while the output is in *purple*. Reproduced from [25] (**b**) First type of network evolved from [25]. Input 0 converts constitutively expressed output 1 into a species 2. Adaptation is realized if species 2 has a zero degradation rate. (**c**) Second type of network evolved. Input 0 converts constitutively expressed gene 1 into output 2. Adaptation is realized if species 1 has a zero degradation rate. (**d**) Feed-forward network. Input gene 0 regulates transcriptions of ouptut gene 1 and gene 2 in a similar way. Protein–protein interaction between 1 and 2 creates an incoherent feed-forward loop explaining adaptation

where ε is a small fixed number, small in comparison with typical values of O. Again by analogy with statistical physics, we have defined the fitness as a cost function to be minimized.

Evolutionary simulations were run using successive steps of log-normally distributed inputs, and averaging the quantities ΔO_{ss} and $\Delta O_{\max}$ over each plateau for fitness computation. Surprisingly, the solutions found by the evolutionary algorithm were largely independent from the specific fitness function actually chosen, as long as it maximized $\Delta O_{\max}$ and minimized ΔO_{ss}.

Two types of networks spontaneously evolve:

- Buffered output-type network (Fig. 8.3b).
- Ligand receptor-type network (Fig. 8.3c).

Again, these networks heavily rely on protein–protein interactions to perform adaptive functions. Since, as previously, protein–protein interactions impose strong and specific non-linearities, we also forced the input of the network to act purely transcriptionally in a new set of simulations. We then found a third type of feedforward network (Fig. 8.3d). Interestingly, an analogous typology of networks, feedback and feed-forward based, was also observed in a later work focused on the enumeration of purely transcriptional networks [43].

So, why do these topologies evolve? Equations of these networks are simple enough so that we can actually study the evolutionary pathways leading to them analytically [25]. For instance, equations for a generalized network such as the one in Fig. 8.3c are

$$\dot{P}_1 = \rho - \gamma P_1 P_0 - \delta_1 P_1, \tag{8.6}$$

$$\dot{P}_2 = \gamma P_1 P_0 - \delta_2 P_2, \tag{8.7}$$

where P_i indicates the protein indexed by i (P_0 being the input). As can be seen, apart from transcription and degradation, there is only one interaction in this network, the formation of a complex between P_1 and the input P_0 which gives protein P_2. This interaction appears very quickly during any evolutionary simulation and is immediately selected and mutated since, interestingly, it can be shown that this interaction and subsequent parameter optimization *simultaneously* increases $\Delta O_{\max}$ and decreases $\Delta O_{\rm ss}$ without trade-off between these two quantities [25]. Computational evolution eventually converges to $\delta_1 = 0$ so that this network clearly is adaptive if we take P_2 as the output O [3, 11, 25]. In that case, while P_2 comes back to the same steady state value, P_1 essentially buffers variations of Input P_0.

Two features of this specific example are generically observed:

- First, evolution is quick and easy: only very few interactions are implicated, and subsequent parameter optimization simply follows the fitness gradient.
- Second, absence of trade-off between the two sub-fitnesses is the general rule for networks similar to those displayed in Fig. 8.3b, c,[2] and therefore, evolution of these motifs is independent of the precise shape of the fitness function: computational evolution essentially selects for the pathways optimizing the two sub-fitnesses at the same time.

Taken together, these arguments explain why these network topologies evolve: they are simply the ones easy to evolve along a smooth fitness gradient. Details of the simulations such as the precise fitnesses from (8.3) to (8.5) are irrelevant. Details of the interaction between input and output for networks in Fig. 8.3 b, c are actually irrelevant too: for instance, if in our evolutionary simulations we assume rather that the input acts rather as a kinase, the same motifs are selected with a non-linear term of the form $P_0 \frac{P_1^\nu}{P_1^\nu + P_*^\nu}$ instead of $P_1 P_0$ in (8.6)–(8.7). Selection of network motifs such as the one presented in Fig. 8.3b, c is therefore generic.

Finally, it is worth mentioning that the maximum responses of these networks are easy to compute analytically, for instance, for network on Fig. 8.3c, we have

$$\Delta O_{\max} \simeq O_{\rm ss} \Delta I / I_1 .$$

[2] On the contrary, there is a trade-off between these two fitnesses for network of Fig. 8.3d, this is the main reason why it does not spontaneously appear without imposing extra evolutionary constraints.

Such proportionality between response and ratio of input actually is ubiquitous in adaptive networks and is called "Weber's law" [22], which evolved spontaneously here. This dependency purely comes from the multiplicative term P_1P_0 in (8.6) and (8.7). Similar logarithmic dependencies to Weber's law would be expected for any adaptive variable where the buffer variable scales inversely with the input concentration and evolutionary computation reproduces this simple design principle.

4 Evolving a Developmental Programme: Vertebrate Antero-posterior Patterning

In the previous section, we considered evolution of small networks performing specific biochemical functions. *In silico* evolution can also be used to study emergence of transcriptional networks controlling development [26–28, 70]. In this chapter, we are especially interested in two important morphogenetic events during vertebrate embryogenesis: segmentation and *Hox* genes pattern, reviewed below.

4.1 Modelling Embryos and Input Dynamics

Some specific assumptions need to be made to generalize the approach and apply it to embryonic development.

An embryo is modelled as a linear array of cells (typically 100), sharing the same genetic networks. Here, our goal is to see if a minimum number of hypothesis gives rise to reasonable networks. We therefore rather make a conservative hypothesis where dynamics is cell-autonomous and purely controlled by some prescribed inputs. Other related modelling works have included a role for diffusion in antero-posterior patterning [10, 39, 47, 60, 61, 71], but precise role of diffusion in actual embryos is still unclear. For instance, Jaeger and co-worker found that their model was essentially not depending on the presence of diffusion [39], which is consistent with our hypothesis. It is also important to mention that, contrary to fly embryos where expression of gap genes first occurs in a syncytium, vertebrate embryos are cellularized [73]. There is also evidence that many dynamical wave-like expressions such as the anterior spreading of *Hox* genes are unaffected by a glass barrier and therefore are mostly cell-autonomous [30].

So in our model, the only difference between cells is encoded in different time evolution of an input variable. Dynamics of this input models in a simplified way the existence of a growth zone from head to tail, characteristic of early development of vertebrates and of many arthropods [55]. We assume a morphogen profile translating down a line of cells, from left to right corresponding to the anterior-posterior direction (see Fig. 8.4b). High morphogen corresponds to the extending tail bud. As a consequence, cells differ only in the amount of time spent in the growth zone.

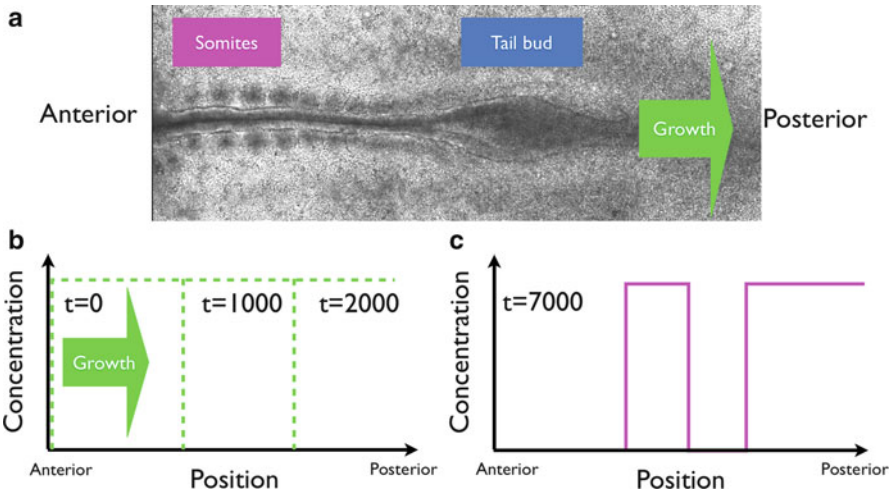

Fig. 8.4 *Modelling embryonic growth.* (**a**) Bright field image of the posterior part of a chick embryo. Anterior is on the *left*, posterior on the *right*; tail bud extends from anterior to posterior and lays somites in the anterior. (**b**) Imposed input dynamics during evolutionary computation. A step of input slides from anterior to posterior to model tail bud extension. (**c**) Fitness computation for somitogenesis. A reporter protein is monitored at steady state, and then number of transitions between low and high values are simply counted. The profile shown here therefore has fitness 3

4.2 Evolving Somitogenesis

The body plan of many bilaterians is made of metameric structures called segments [55]. In vertebrates, segmentation is called somitogenesis (see [15] for a review), and somites are precursors of future vertebrae. Sequential clustering of cells into somites occurs from head to tail as the antero-posterior axis elongates (Fig. 8.4a).

Mechanism underlying somites formation has been well studied in recent years. Somites are defined through the interaction of a molecular "clock" with a wavefront of morphogens (as predicted in 1976 by Cooke and Zeeman [9]), following tail bud regression from anterior to posterior [54]. Many oscillating genes have been identified in different vertebrate species, in three different molecular pathways (Notch, FGF, Wnt) [16]. Morphogenetic movements, elongation and segmentation clock are controlled by morphogen gradients expressed in the tail bud, such as FGF8 and Wnt3a [2,18]. As cells get more and more anterior, the segmentation clock slows down and finally interacts with a "determination front" (assumed to be controlled by morphogens) to define segments [19]. The precise mechanism converting a temporal oscillation into a spatial one implicates specific genes such as Mesp2/Meso2, whose expression delineates the future segment before localizing in the rostral part of the somite [48, 52, 66]. Eventually, rostral and caudal parts of each somites express specific genes, creating stripes of genetic expression [58] qualitatively very similar to what is observed for segmentation genes in fly [73]. However, from a dynamical

systems standpoint, the precise mechanism giving rise to this striped pattern is still unclear, and in this section, we show how a predictive somitogenesis model can be obtained through computational evolution [27].

4.2.1 Fitness

The goal is to evolve a spatial periodic pattern, qualitatively similar to what is observed during vertebrate segmentation [54]. There are a priori many ways to evolve such a pattern: for instance, one could try to select for a spatial profile at steady state considered as "ideal". Our choice of fitness rather relies on the idea that evolution should be incremental and that a specific pattern does not have much sense per se, what matters is having a robust mechanism creating such pattern. We therefore consider the steady state profile of a single segmentation gene (subsequently called "gene" 1). We then simply count the number of steps up and down of this gene 1 at steady state as we go from anterior to posterior as explained on Fig. 8.4c. This integer is then used as the fitness.

4.2.2 Evolution

Figure 8.5, panels a–d, illustrates a typical evolutionary path followed by evolution with this fitness function.

The first step of evolution gives rise to a bistable system based on gene 1 (Fig. 8.5a). This is done here through the selection of a positive feedback loop of gene 1 on itself. A multistable system is a very natural way to build a memory of past history, and evolving a bistable network defines at least one zone of low concentration of 1 and one zone of high concentration of 1 (which, following our definition, has fitness 1). The bifurcation diagram illustrates that for high concentration of input, only the high state exists, while for low concentration of input, the network can exist in both a low and high state. Low state appears through a saddle-node bifurcation.

Then, once a bistable system has evolved, evolution selects for regulators upstream of it. Very often in evolution, a repressor of gene 1 evolves (gene 2), expressed with a slower timescale (Fig. 8.5b). The bistable system, after being first turned on, is consequently turned off after some time. This defines now three different zones in the embryo and has fitness 2 (i.e. one single stripe). Network associated to this stripe module is generically feed-forward, as observed in an analogous simulation [28]. In terms of bifurcation, for high input concentration, the repressor is fully expressed and essentially squashes the expression of the high state.

Once a stripe sub-module has evolved, it is easy for evolution to multiply it. Evolution selects for a sequence of repressors acting in succession on the stripe module. Such cascade relieves and reactivates repression at different times and creates new alternations of stripes. One such step is illustrated on (Fig. 8.5c). Gene

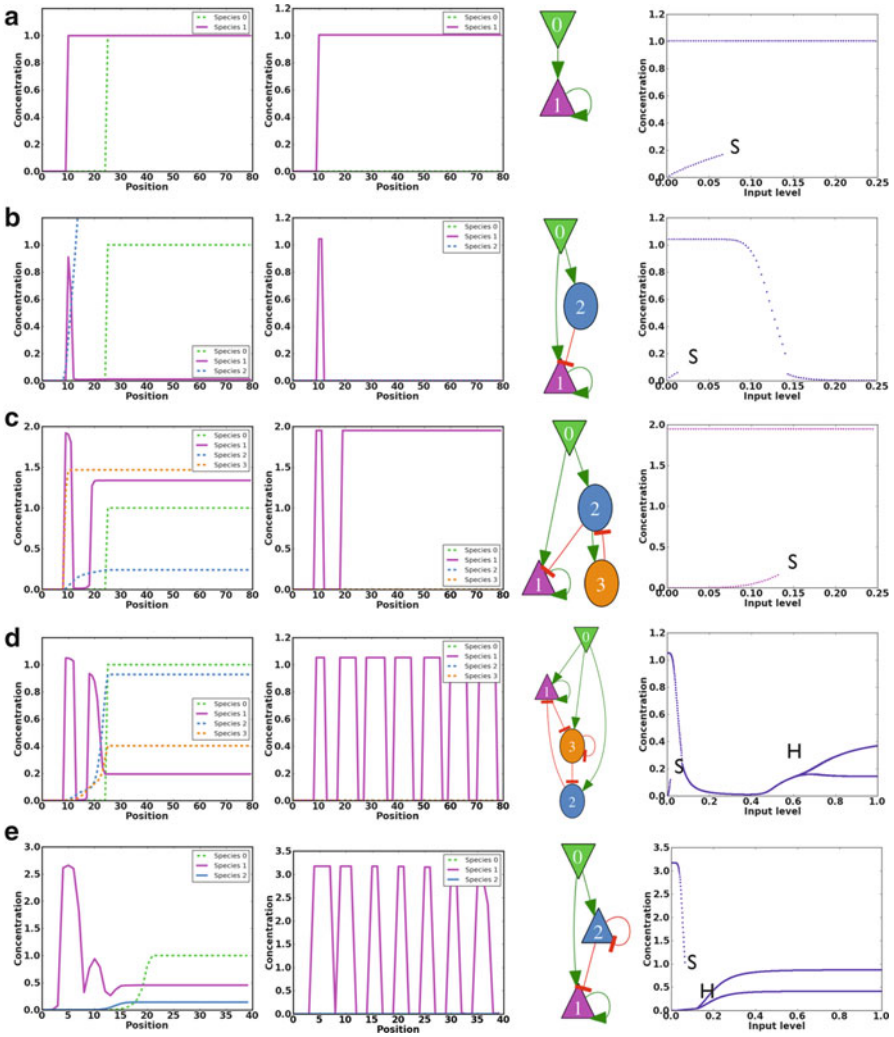

Fig. 8.5 *Example of in silico evolution of somitogenesis*. (**a**)–(**d**): four consecutive evolutionary steps selected in an example of evolutionary simulations. (**a**)–(**d**) represent generation 7, 140, 360, and 490. From *left* to *right*: behaviour of the network early in time, final pattern, and network topology. *Last column* represents the stable orbits of the concentration of output gene 1 for fixed constant values of input gene 0. *S* denotes the position of a saddle-node bifurcation, and *H* the position of a Hopf bifurcation leading to oscillations. Panel (**e**): another network selected for a different input shape and allowing transcriptional delays in the dynamics so that negative feedback with delays creates an oscillating gene 2 (see [27])

3 is activated by gene 2 and represses it, the effect is that after gene 2 is turned on and represses gene 1, it is later turned off by gene 3 which derepresses gene 1 and creates a new zone of high 1 activity. The network has now fitness 3.

Finally, once a cascade of repressors starts evolving, it becomes very easy for computational evolution to create a potentially infinite alternation of repression and activation of the stripe. Evolution closes a negative feedback loop in this sequence of repressors, which rapidly evolves into a clock, with faster and faster period. In Fig. 8.5d, this is done through evolution of the classical repressilator [21] between genes 1, 2 and 3. A Hopf bifurcation appears at high input concentration accounting for the oscillation, while the system is still bistable at low input concentration.

So computational evolution incrementally selects for a clock and wavefront mechanism by simply imposing selection for stripe formation. It is worth noticing again that some natural properties of segmentation evolve without imposing them explicitly, namely, both the existence of a clock mechanism and the equal size of stripes (after a transitory start).

When evolution of somitogenesis is reproduced from one simulation to the other, we systematically see this very stereotypical sequence of evolutionary events: bistability, then stripe module, and then eventually, oscillation upstream of this stripe module. Any of this step can be implemented by different network types. For instance, if we allow for delays in transcription, a simple negative feedback loop with delay can give rise to a clock and spontaneously evolves (Fig. 8.5e and our original paper [27]). So there is no systematic convergence on one network topology; rather what is generic and conserved is the dynamical properties of these steps, i.e. the nature of the bifurcations. Bifurcation diagrams in Fig. 8.5, panels d and e, are qualitatively identical: when decreasing morphogen concentration, one goes from an oscillating system to a bistable system. Furthermore, if we consider the stable orbits from panels a to d, we clearly see that each evolutionary step corresponds very simply to the emergence of new dynamics and bifurcations, which is done incrementally and, roughly, from anterior to posterior. Panel a corresponds to the evolution of bistability when no input is present, the state of the system at high concentration of input in panels b and c corresponds respectively, to the low and high branch of the cycle evolving through a Hopf bifurcation in panel d. Each of this new dynamics corresponds to the selection of a very clear sub-module: e.g. bistability corresponds to the selection of a simple auto-regulating positive feedback loop and the clock behaviour corresponds to the selection of a negative feedback loop oscillators upstream of it. Modularity of these evolved networks therefore is a consequence of incremental evolution of successive bifurcations.

Despite its simplicity, our evolved model of somitogenesis suggests specific interactions that may be checked experimentally. In particular, the interplay between clock, wavefront and genes fixing the pattern is still rather unclear experimentally; we predict that the stripe pattern observed in vertebrate somitogenesis is the expression of a bistable subnetwork, defining rostro-caudal polarity within somites. Possibly, another similar layer of upstream bistability might also define somites vs. non-somites as suggested by Godbeter and co-workers [32]. Future time-lapse experiments [2], correlating gene expression pattern to cell fates, will be necessary to confirm or falsify the idea that a downstream bistable network discretizes the phase of the segmentation clock.

4.3 Evolving Hox Genes Patterning

Hox patterns develop contemporaneously [1, 34] with somitogenesis. *Hox* genes are "master control genes" defining the identity of the different parts of the embryo, notably the segments along the antero-posterior axis [40]. *Hox* genes are extremely conserved, and it is very clear that Urbilateria, the ancestor of all bilaterians, indeed had *Hox* genes [31, 57], while there is still debate to know if it was segmented. In both insects and vertebrates, *Hox* genes are organized as cluster on chromosomes, and, very surprisingly, the order of the anterior expression boundaries along the AP axis matches the $3'$ to $5'$ arrangement on the *Hox* cluster [17].

In vertebrates, *Hox* pattern is dynamic, e.g. in Xenopus, there are particularly clear data showing that *Hox* genes are expressed in a $3'$ to $5'$ temporal progression in the non-organizer mesoderm and acquire a fixed position when the cells converge into the organizer and then extend to create the AP axis [20, 72]. This correlation between temporal expression of *Hox* genes with their position on the *Hox* cluster is called "temporal colinearity" [17, 37].

Another property of *Hox* pattern is called the posterior prevalence or dominance rule [46]. Schematically, posterior *Hox* genes impose their fates on anterior ones. Loss of function mutations on *Hox* genes results in a characteristic homeotic transformation: e.g. in fly, a parasegment adopts the fate of the *Hox* gene immediately anterior to it. Gain of function results in a posterior homeotic transformation when performed anteriorly and has no phenotype when it is done posteriorly.

In this section, evolution of *Hox* gene pattern with properties similar to actual networks is presented.

4.3.1 Fitness

It is a priori difficult to define a reasonable fitness function, incremental and generic to evolve pattern similar to *Hox* genes.

We first make a selector gene approximation: we assume that cellular identity is defined by a subset of master control genes, corresponding to the *Hox* genes. This is of course an oversimplification of the real situation where some *Hox* overlap can happen; however, the number of functional *Hox* combinations is a linear function of the number of genes, not an exponential one (e.g. see Fig. 2 in [35]), which is consistent with the idea that very few genes control *Hox* identity of a given cell. Therefore, our fitness favours:

- *Diversity of genetic expression*: A selective pressure imposing as many domains as possible in a given embryo.
- *A unique cell fate at any given position*: Only one selector gene should be expressed in any given cell.

Our computation of fitness is inspired by the notion of entropy in physics. The analogy is the following: imagine a system has N possible states available to it.

If it spends equal time in each state, the entropy is maximum and equal $\log(N)$ while it is 0 if it stays in only one fate. So, if we define occupancy of a state as the probability of observing one given fate in the whole embryo, we define an entropy term $\mathscr{H}(diversity)$ that will be 0 if only one selector gene is expressed in the embryo and maximum equal to $\log(N)$ if N selector genes are expressed in the same number of cells.

However, unicity of cell fate means only one master control gene is expressed locally. Using our entropic analogy, this means that a *conditional* entropy, $\mathscr{H}(diversity|position)$ is minimum: in any given cell, we want to have a precise determination of cellular fate. Precise mathematical definitions of these two functionals are rather technical and provided in [26].

Like the adaptation case above, we therefore require the optimization of these two a priori contradictory constraints: maximizing entropy at the embryo level $\mathscr{H}(diversity)$ while minimizing $\mathscr{H}(diversity|position)$ locally. We then make the following assumption on their trade-off: we assume that if a selector gene is duplicated while keeping its domain of expression, this event is evolutionary neutral. With our definitions, it can be shown analytically [26] that a working combination is indeed the difference between these two fitnesses $fitness = \mathscr{H}(diversity|position) - \mathscr{H}(diversity)$ which corresponds to the mathematical notion of mutual information between *Hox* identity of a cell and its position in the embryo (for a review of mutual information in gene network, see [68]). Intuitively, optimizing this function simply means that from its *Hox* identity, a cell can determine its position, and vice versa, which clearly makes sense biologically.

Practically, this functional is a generic and incremental way to compute the logarithm of the number of domains of equal sizes expressing different *Hox* genes. Figure 8.6 illustrates this fitness on several examples: embryos with two domains of equal size have fitness $\log 2$, those with three domains of equal size $\log 3$ and intermediate situations are given. We therefore aim at maximizing this fitness to increase the number of domains of equal size. We used this fitness function to realize evolution of gene network under control of both static morphogen and sliding growth zone [26], in this chapter, we focus on the latter case.

4.3.2 *Hox* Gene Pattern Evolution

Networks are evolved using the mutual information fitness function under control of a sliding growth zone like in the somitogenesis case [26]. In this section, results are derived with the two extra following assumptions:

- For this problem only, gene duplications are allowed. This makes particular sense in this context since all *Hox* genes are paralogs.
- To accelerate evolution, evolution is initialized with the small network shown on the left of Fig. 8.7, panel a. This feed-forward network is common in developmental biology and is a generic network found by evolutionary simulations limited to two domains of *Hox* gene expression (data not shown) or for stripe

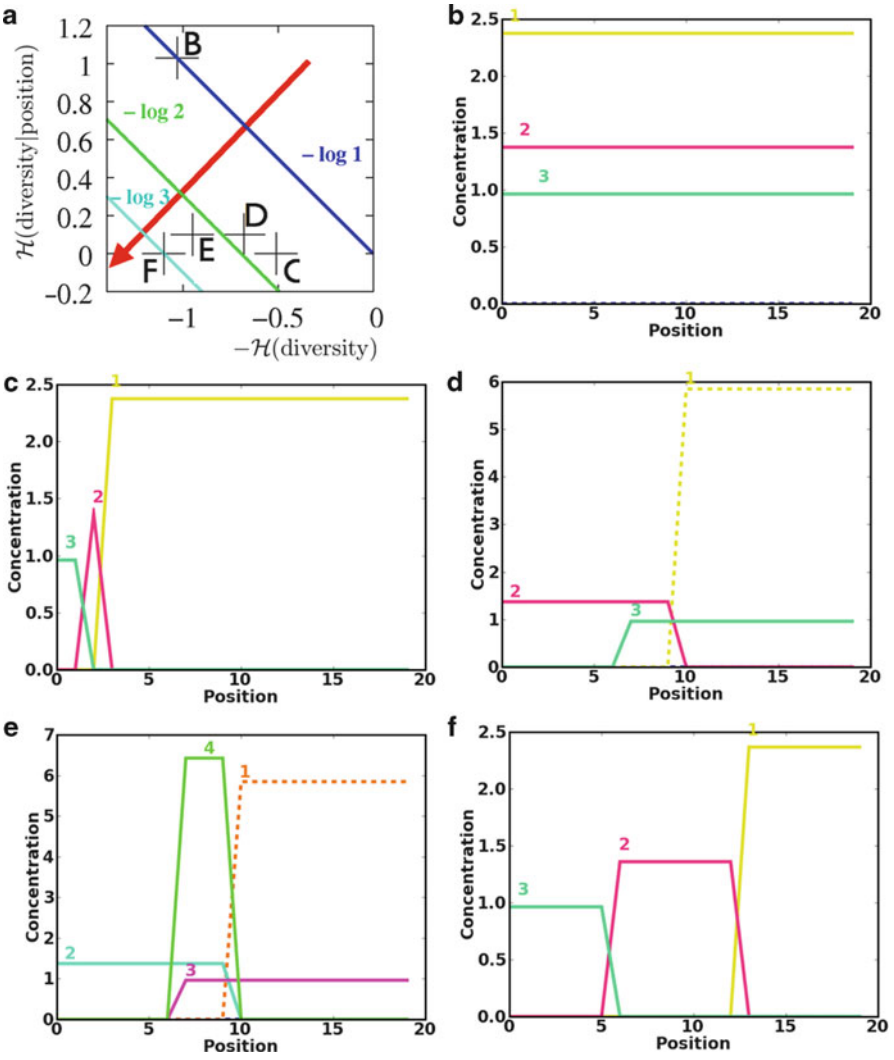

Fig. 8.6 *Examples of fitness.* Fitness diagram and gene expression profiles as a function of anterior-posterior (AP) position from cell 1 to 20 illustrate properties of the fitness. Only the output genes (*solid lines*) enter the fitness; other network genes are represented by *dashed lines*. (**a**) The two components of fitness are plotted with *diagonal coloured lines* showing contours of constant total fitness (better fitness in *red*). (**b**)–(**f**) mark the fitness of subsequent panels. (**b**) For three genes ubiquitously expressed, both $\mathscr{H}(diversity)$ and conditional entropy $\mathscr{H}(diversity|position)$ are high and equal, so the actual fitness is zero. (**c**) Each cell expresses a single gene resulting in zero conditional entropy, but gene 1 occupies most of the embryo, lowering the diversity, giving a fitness of $\log 1.64$. (**d**) Fitness is defined when outputs overlap, but neither the diversity nor the conditional entropy is optimal, and the fitness is $\log 1.78 > \log 2$. (**e**) The network in (**d**) can be improved by the addition of a new output 4 that accounts for the overlap of genes 2 and 3, giving a fitness of $\log 2.33 < \log 2$ (**f**) Optimal configuration for three outputs; diversity is high ($\log 3$) and conditional entropy is zero, so the actual fitness is $\log 3$. Reproduced from [26]

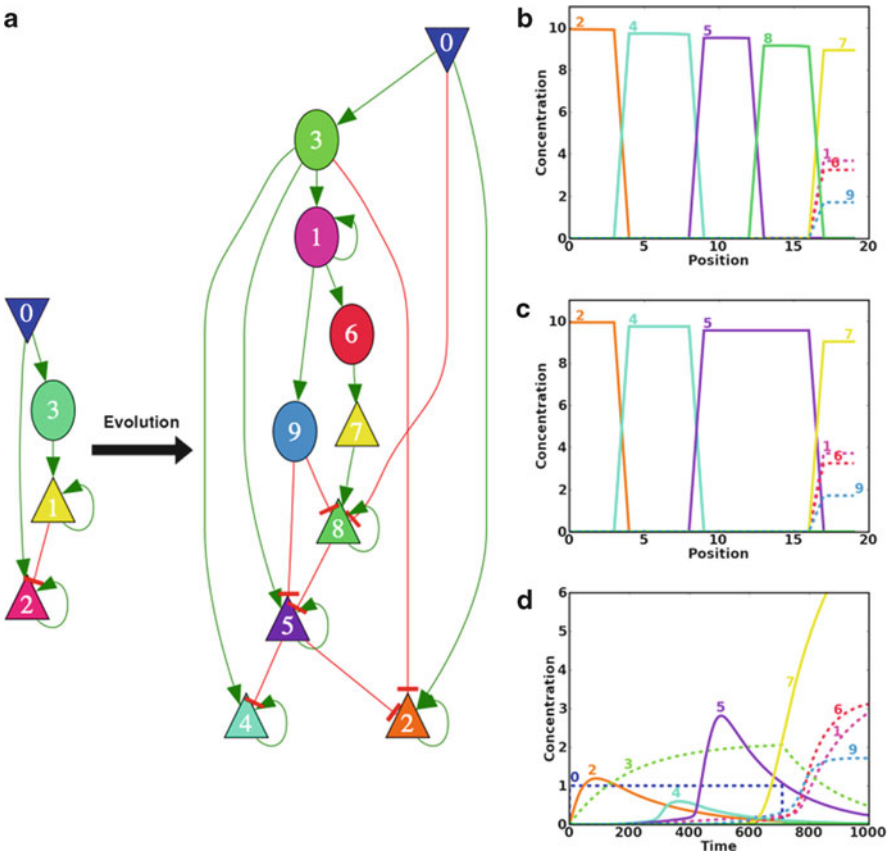

Fig. 8.7 *Example of in silico evolution of Hox gene pattern* (**a**) Initial network topology and the evolved network topology after 5,000 generations. (**b**) Steady-state profile for the evolved network: fitness, log 4.95, following convention of Fig. 8.4, anterior/posterior corresponds to *left/right direction*. (**c**) Change in expression domains when a single gene 8 is forced to zero. Gene 5 directly anterior to 8 extends posteriorly, until it is repressed by the next posterior gene. (**d**) Gene expression as a function of time in the posterior-most cell follows the AP order in (**b**), with the exception of the one realizator (gene 8) that is repressed by the input

definition [36]. We generalize slightly by adding a slow timescale in the form of an intermediate protein 3 which is activated by the input to delay activation of gene 1 by the morphogen.

A typical subsequent evolution of this small network is shown in Fig. 8.7a, along with the expression profile it generates.

We found that the initial topological bias and the presence of timer 3 facilitate evolution of simple networks with many states that exhibit many properties similar to actual *Hox* genes networks:

- *Posterior prevalence*: Repressive links are indicated in Fig. 8.7a, and as can be seen on Fig. 8.7b, anterior genes are only repressed by posterior ones. One consequence is that if one output gene is artificially down-regulated, the gene just anterior to it spreads more posteriorly (Fig. 8.7c).
- *Temporal colinearity*: Genes are expressed in the posterior-most cell in the same order as they are expressed along the AP axis as can be seen on Fig. 8.7d.

Study of network dynamics reveals that these properties are direct consequence of the structure selected. Timer 3 is recruited during evolution to play the role of a dynamical morphogen: as it accumulates with time, it successively reaches different threshold of concentration, activating different output genes in the cascade, which explains temporal colinearity. The posterior prevalence is coming from a more subtle effect: sharp boundaries imposed by the fitness function are dynamically more easily realized by repression between contiguous regions. Genes are expressed temporally from anterior to posterior because of the combination of temporal colinearity and of the growth of the embryo. So what happens during evolution is that later expressed genes (i.e. posterior genes) are simply selected to repress earlier ones (i.e. anterior genes). Once again, evolution simply follows the fitness gradient, and the resulting network reproduces many properties of real networks without any specific selective pressure for those.

There is a last interesting aspect in the network displayed in Fig. 8.7: if we simulate an embryo where gene 3 is static in time but graded along the AP axis (e.g. similarly to *caudal* in fly), the same network topology gives rise to a qualitatively similar pattern of genetic expression where the orders of the output along the AP axis is conserved (see [26] for more details). This led us to suggest that the interconversion from short germ insects (which have patterning tied to growth just like vertebrates) to long germ insects (which pattern with an embryo of fixed size under control of maternal morphogens gradient like fly) might have occurred via the interconversion of a timer gene like gene 3 into a more classical morphogen like *caudal* [26].

5 Conclusion

In silico evolution is very powerful at recapitulating models, structure, and dynamics found in actual networks, from specific biochemical structures for bistable switches to general properties of developmental programmes. It also gives ways to test the usual adaptive assumption that a given network structure evolves in response to a specific selective pressure.

One of the first lesson of this approach is to show that important well-known properties of some networks might actually come out spontaneously without explicit selection. For instance, Weber's law comes naturally from evolution of adaptation as well as a segmentation clock from stripes evolution or posterior dominance for *Hox*-like gene pattern. One can actually speculate that if we had selected explicitly

for those properties, we would have impinged evolution. For instance, in the somite case, if we had selected for a pre-specified pattern, it would have been unlikely that evolution would have found the same topologies and dynamical organizations in such an easy incremental way.

Fitness were designed in most cases to favour incremental evolution. This creates a smooth evolutionary landscape where fast evolution is possible. A biological analogy would be a selection at the global phylum level: e.g. for somitogenesis, we select for segments evolution, not for a predetermined segment number. This makes perfect sense biologically: species specific features (e.g. the precise segment number in one species) can be highly variable. They are presumably the consequence of higher order contingent environmental constraints which we cannot predict at this stage. This is at a higher phylogenetic level that generic results can be obtained and predicted.

The second lesson is that there is no very strong correlation between network function and network structure. As shown in this chapter, different networks can have the exact same function. Conversely, the same network structure can adopt very different functions: for instance, feed-forward loop motifs such as the one evolved in Fig. 8.3c can behave either adaptively, either as a detector of persistence as proposed by Alon and co-workers [44] (data not shown).

It is therefore legitimate to ask if the gene network level is the right one to study evolution. Recent network comparisons between different species suggest that indeed there might be a huge evolutionary flexibility for the same biological function. For instance, somitogenesis clearly is implemented in different ways in different organisms [41], and there are big divergences in stress networks between different yeasts [67]. Some authors even suggested that specific gene network structures we observe are nothing more than manifestation of population genetics, with very little functional significance [42].

However, coming back to our question of evolutionary convergence and predictive evolution, one should also stress that even if networks are divergent between species, their global function can be highly conserved. Somitogenesis is a paradigmatic example where the clock-based mechanism, predicted theoretically more than 30 years ago, is qualitatively identical in all known vertebrate organisms [15]. This is therefore close to this level of global dynamics that generic properties exist and that predictions can be made.

As shown in this chapter on the somite example, evolutionary convergence happens in the form of parallel evolution of new dynamics (such as bifurcations), implemented in different simulations in different ways. Our proposal is that evolved models are somewhat “minimal” in the sense that they are very good at capturing, with a small number of parameters, these sequences of bifurcations. “Genes” in our networks might not necessary correspond strictly to actual genes, but rather variables accounting for global state of the cellular dynamical systems (e.g. fate control), such as microRNAs or even possibly epigenetic states at the chromatin level. Fine details in network topology might just be random. Rather, flows in the phase space and global network organization explaining them can be recovered and predicted.

One can finally ask why use *in silico* evolution and not simple network enumeration, as proposed in other related works [10, 43]. One obvious reason is that, for any complex function, it becomes exponentially costly to explore all possible networks when increasing network size, while computational evolution has no problem to incrementally generate networks with many nodes (such as the one in Fig. 8.7). It also makes more sense from a biological standpoint to use evolutionary computation rather than enumeration, which would rapidly lead to a paradox similar to the famous Levinthal's paradox for protein folding [12]. Levinthal noticed that a typical protein could not explore its entire space of folding configuration in a reasonable time, which strongly suggested the existence of a kinetic folding funnel. Similarly, biological evolution cannot explore all possible network configurations for a given functions and has therefore no reason to find a global optimum. Rather, we suggest that evolution follows fitness funnels, converging towards easy to evolve dynamics in an incremental way. One challenge in the future will be to develop the right formalism to study this problem, at the intersection between classical network and dynamical system theories.

Acknowledgements I would like to thank Eric Siggia, Vincent Hakim, Olivier Pourquié, Bertrand Benazeraf, Alexander Aulehla, Ali Brivanlou, Nicolas Buchler, Alin Vonica, Aryeh Warmflash, and Francis Corson for, useful discussions. Tail bud picture of Fig. 8.4 was taken during a short stay in the Pourquié lab in Stowers Institute, Kansas City, and I further thank Olivier Pourquié for inviting and hosting me numerous times in his lab.

References

1. Aulehla A, Pourquié O (2010) Signaling gradients during paraxial mesoderm development. Cold Spring Harbor Perspect Biol 2(2):1–17
2. Aulehla A, Wiegraebe W, Baubet V, Wahl MB, Deng C, Taketo M, Lewandoski M, Pourquie O (2008) A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. Nat Cell Biol 10(2):186–193
3. Behar M, Hao N, Dohlman HG, Elston TC (2007) Mathematical and computational analysis of adaptation via feedback inhibition in signal transduction pathways. Biophys J 93(3):806–821
4. Block SM, Segall JE, Berg HC (1983) Adaptation kinetics in bacterial chemotaxis. J Bacteriol 154(1):312–323
5. Buchler NE, Louis M (2008) Molecular titration and ultrasensitivity in regulatory networks. J Mol Biol 384(5):1106–1119
6. Chai Y, Norman T, Kolter R, Losick R (2010) An epigenetic switch governing daughter cell separation in Bacillus subtilis. Gene Dev 24(8):754–765
7. Cherry JL, Adler FR (2000) How to make a biological switch. J Theor Biol 203(2):117–133
8. Conway Morris S (2004) Life's solution: inevitable humans in a lonely universe. Cambridge University Press, Cambridge
9. Cooke J, Zeeman EC (1976) A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. J Theor Biol 58(2):455–476
10. Cotterell J, Sharpe J (2010) An atlas of gene regulatory networks reveals multiple three-gene mechanisms for interpreting morphogen gradients. Mol Syst Biol 6:425
11. Csikász-Nagy A, Soyer OS (2008) Adaptive dynamics with a single two-state protein. J R Soc Interface 5(Suppl 1):S41–S47

12. Cyrus L (1969) How to fold graciously. In: Proceedings of a Mossbauer spectroscopy in biological systems, Meeting held at Allerton House, Monticello, IL, pp 22–24
13. Darwin CR (1861) On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life. John Murray, London
14. Davidson EH, Erwin DH (2006) Gene regulatory networks and the evolution of animal body plans. Science 311(5762):796–800
15. Dequeant ML, Pourquie O (2008) Segmental patterning of the vertebrate embryonic axis. Nat Rev Genet 9(5):370–382
16. Dequeant ML, Glynn E, Gaudenz K, Wahl M, Chen J, Mushegian A, Pourquie O (2006) A complex oscillating network of signaling genes underlies the mouse segmentation clock. Science 314(5805):1595–1598
17. Duboule D, Morata G (1994) Colinearity and functional hierarchy among genes of the homeotic complexes. Trends Genet 10(10):358–364
18. Dubrulle J, Pourquie O (2004) fgf8 mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. Nature 427(6973):419–422
19. Dubrulle J, McGrew MJ, Pourquie O (2001) FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. Cell 106(2):219–232
20. Durston AJ, Jansen HJ, Wacker SA (2010) Review: time-space translation regulates trunk axial patterning in the early vertebrate embryo. Genomics 95(5):250–255
21. Elowitz MB, Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. Nature 403(6767):335–338
22. Fechner GT (1966) Elements of psychophysics. Holt, Rinehart and Winston, New York
23. Francois P, Hakim V (2004) Design of genetic networks with specified functions by evolution in silico. Proc Natl Acad Sci USA 101(2):580–585
24. Francois P, Hakim V (2005) Core genetic module: the mixed feedback loop. Phys Rev E Stat Nonlinear Soft Matter Phys 72(3 Pt 1):031908
25. Francois P, Siggia ED (2008) A case study of evolutionary computation of biochemical adaptation. Phys Biol 5(2):26009
26. François P, Siggia ED (2010) Predicting embryonic patterning using mutual entropy fitness and in silico evolution. Development 137(14):2385–2395
27. Francois P, Hakim V, Siggia ED (2007) Deriving structure from evolution: metazoan segmentation. Mol Syst Biol 3:9
28. Fujimoto K, Ishihara S, Kaneko K (2008) Network evolution of body plans. PLoS ONE 3(7):e2772
29. Gardner TS, Cantor CR, Collins JJ (2000) Construction of a genetic toggle switch in Escherichia coli. Nature 403(6767):339–342
30. Gaunt SJ, Strachan L (1994) Forward spreading in the establishment of a vertebrate Hox expression boundary: the expression domain separates into anterior and posterior zones, and the spread occurs across implanted glass barriers. Dev Dynam 199(3):229–240
31. Gehring WJ, Kloter U, Suga H (2009) Evolution of the Hox gene complex from an evolutionary ground state. Curr Top Dev Biol 88:35–61
32. Goldbeter A, Gonze D, Pourquie O (2007) Sharp developmental thresholds defined through bistability by antagonistic gradients of retinoic acid and FGF signaling. Dev Dynam 236(6):1495–1508
33. Gould SJ (1989) Wonderful life. W. W. Norton, New York
34. Iimura T, Pourquie O (2006) Collinear activation of Hoxb genes during gastrulation is linked to mesoderm cell ingression. Nature 442(7102):568–571
35. Iimura T, Pourquie O (2007) Hox genes in time and space during vertebrate body formation. Dev Growth Differ 49(4):265–275
36. Ishihara S, Fujimoto K, Shibata T (2005) Cross talking of network motifs in gene regulation that generates temporal pulses and spatial stripes. Gene Cell 10(11):1025–1038

37. Izpisúa-Belmonte JC, Falkenstein H, Dollé P, Renucci A, Duboule D (1991) Murine genes related to the Drosophila AbdB homeotic genes are sequentially expressed during development of the posterior part of the body. EMBO J 10(8):2279–2289
38. Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol 3(3):318–356
39. Jaeger J, Surkova S, Blagov M, Janssens H, Kosman D, Kozlov KN, Manu, Myasnikova E, Vanario-Alonso CE, Samsonova M, Sharp DH, Reinitz J (2004) Dynamic control of positional information in the early Drosophila embryo. Nature 430(6997):368–371
40. Kmita M, Duboule D (2003) Organizing axes in time and space; 25 years of colinear tinkering. Science 301(5631):331–333
41. Krol AJ, Roellig D, Dequeant ML, Tassy O, Glynn E, Hattem G, Mushegian A, Oates AC, Pourquie O (2011) Evolutionary plasticity of segmentation clock networks. Development 138(13):2783–2792
42. Lynch M (2007) The evolution of genetic networks by non-adaptive processes. Nat Rev Genet 8(10):803–813
43. Ma W, Trusina A, El-Samad H, Lim WA, Tang C (2009) Defining network topologies that can achieve biochemical adaptation. Cell 138(4):760–773
44. Mangan S, Alon U (2003) Structure and function of the feed-forward loop network motif. Proc Natl Acad Sci USA 100(21):11980–11985
45. Matthews HR, Murphy RL, Fain GL, Lamb TD (1988) Photoreceptor light adaptation is mediated by cytoplasmic calcium concentration. Nature 334(6177):67–69
46. McGinnis W, Krumlauf R (1992) Homeobox genes and axial patterning. Cell 68(2):283–302
47. Meinhardt H (2008) Models of biological pattern formation: from elementary steps to the organization of embryonic axes. Curr Top Dev Biol 81:1–63
48. Morimoto M, Takahashi Y, Endo M, Saga Y (2005) The Mesp2 transcription factor establishes segmental borders by suppressing Notch activity. Nature 435(7040):354–359
49. Nakatani K, Yau KW (1988) Calcium and light adaptation in retinal rods and cones. Nature 334(6177):69–71
50. Nilsson DE, Pelger S (1994) A pessimistic estimate of the time required for an eye to evolve. Proc Biol Sci 256(1345):53–58
51. Novick A, Wiener M (1957) Enzyme induction as an all-or-none phenomenon. Proc Natl Acad Sci USA 43(7):553
52. Oginuma M, Takahashi Y, Kitajima S, Kiso M, Kanno J, Kimura A, Saga Y (2010) The oscillation of Notch activation, but not its boundary, is required for somite border formation and rostral-caudal patterning within a somite. Development 137(9):1515–1522
53. Ozbudak EM, Thattai M, Lim HN, Shraiman BI, van Oudenaarden A (2004) Multistability in the lactose utilization network of Escherichia coli. Nature 427(6976):737–740
54. Palmeirim I, Henrique D, Ish-Horowicz D, Pourquie O (1997) Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. Cell 91(5):639–648
55. Peel AD, Chipman AD, Akam M (2005) Arthropod segmentation: beyond the Drosophila paradigm. Nat Rev Genet 6(12):905–916
56. Phillips R, Kondev J, Theriot J (2008) Physical biology of the cell, 1st edn. Garland Science, New York
57. Ryan JF, Mazza ME, Pang K, Matus DQ, Baxevanis AD, Martindale MQ, Finnerty JR, Fay J (2007) Pre-bilaterian origins of the Hox cluster and the Hox code: evidence from the sea anemone, Nematostella vectensis. PLoS ONE 2(1):e153
58. Saga Y, Takeda H (2001) The making of the somite: molecular events in vertebrate segmentation. Nat Rev Genet 2(11):835–845
59. Sage R (2004) The evolution of C4 photosynthesis. New Phytologist 161:341–370
60. Salazar-Ciudad I, Newman SA, Solé RV (2001) Phenotypic and dynamical transitions in model genetic networks, I. Emergence of patterns and genotype-phenotype relationships. Evol Dev 3(2):84–94

61. Salazar-Ciudad I, Solé RV, Newman SA (2001) Phenotypic and dynamical transitions in model genetic networks, II. Application to the evolution of segmentation mechanisms. Evol Dev 3(2):95–103
62. Shen-Orr SS, Milo R, Mangan S, Alon U (2002) Network motifs in the transcriptional regulation network of Escherichia coli. Nat Genet 31(1):64–68
63. Shimoni Y, Friedlander G, Hetzroni G, Niv G, Altuvia S, Biham O, Margalit H (2007) Regulation of gene expression by small non-coding RNAs: a quantitative view. Mol Syst Biol 3:138
64. Soyer OS, Pfeiffer T, Bonhoeffer S (2006) Simulating the evolution of signal transduction pathways. J Theor Biol 241(2):223–232
65. Süel GM, Garcia-Ojalvo J, Liberman LM, Elowitz MB (2006) An excitable gene regulatory circuit induces transient cellular differentiation. Nat Cell Biol 440(7083):545–550
66. Takahashi Y, Koizumi K, Takagi A, Kitajima S, Inoue T, Koseki H, Saga Y (2000) Mesp2 initiates somite segmentation through the Notch signalling pathway. Nat Genet 25(4):390–396
67. Tirosh I, Wong KH, Barkai N, Struhl K (2011) Extensive divergence of yeast stress responses through transitions between induced and constitutive activation. Proc Natl Acad Sci USA 108(40):16693–16698
68. Tkačik G, Walczak AM (2011) Information transmission in genetic regulatory networks: a review. J Phys Condens Matter 23(15):153102
69. Turgay K, Hamoen LW, Venema G, Dubnau D (1997) Biochemical characterization of a molecular switch involving the heat shock protein ClpC, which controls the activity of ComK, the competence transcription factor of Bacillus subtilis. Gene Dev 11(1):119–128
70. ten Tusscher KH, Hogeweg P (2011) Evolution of networks for body plan patterning; interplay of modularity, robustness and evolvability. PLoS Comput Biol 7(10):e1002208
71. Vakulenko S, Manu, Reinitz J, Radulescu O (2009) Size regulation in the segmentation of Drosophila: interacting interfaces between localized domains of gene expression ensure robust spatial patterning. Phys Rev Lett 103(16):168102
72. Wacker SA, Jansen HJ, McNulty CL, Houtzager E, Durston AJ (2004) Timed interactions between the Hox expressing non-organiser mesoderm and the Spemann organiser generate positional information during vertebrate gastrulation. Dev Biol 268(1):207–219
73. Wolpert L (2006) Principles of development. Oxford University Press, Oxford
74. Wright S (1932) The roles of mutation, inbreeding, crossbreeding and selection in evolution. In: Proceedings of the VI international congress of genetics, vol 1, pp 356–366
75. Yeger-Lotem E, Sattath S, Kashtan N, Itzkovitz S, Milo R, Pinter RY, Alon U, Margalit H (2004) Network motifs in integrated cellular networks of transcription-regulation and protein-protein interaction. Proc Natl Acad Sci USA 101(16):5934–5939