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Propagation of genetic variation in gene regulatory networks☆

Erik Plahte [a], Arne B. Gjuvsland [a,*], Stig W. Omholt [b,c]

[a] Centre for Integrative Genetics (CIGENE), Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, P.O. Box 5003, N - 1432 Ås, Norway
[b] Centre for Integrative Genetics (CIGENE), Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N - 1432 Ås, Norway
[c] NTNU Norwegian University of Science and Technology, Department of Mathematical Sciences, N - 7491 Trondheim, Norway

HIGHLIGHTS

- We show that a diploid gene can be modelled as one entity.
- Propagation functions describe how genetic variation propagates through the network.
- Their derivatives can be approximated by observable quantities—and are related to the feedback structure of the system.
- The observable allele interaction value is related to the dominant feedback loop.

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ABSTRACT

A future quantitative genetics theory should link genetic variation to phenotypic variation in a causally cohesive way based on how genes actually work and interact. We provide a theoretical framework for predicting and understanding the manifestation of genetic variation in haploid and diploid regulatory networks with arbitrary feedback structures and intra-locus and inter-locus functional dependencies. Using results from network and graph theory, we define propagation functions describing how genetic variation in a locus is propagated through the network, and show how their derivatives are related to the network's feedback structure. Similarly, feedback functions describe the effect of genotypic variation of a locus on itself, either directly or mediated by the network. A simple sign rule relates the sign of the derivative of the feedback function of any locus to the feedback loops involving that particular locus. We show that the sign of the phenotypically manifested interaction between alleles at a diploid locus is equal to the sign of the dominant feedback loop involving that particular locus, in accordance with recent results for a single locus system. Our results provide tools by which one can use observable equilibrium concentrations of gene products to disclose structural properties of the network architecture. Our work is a step towards a theory capable of explaining the pleiotropy and epistasis features of genetic variation in complex regulatory networks as functions of regulatory anatomy and functional location of the genetic variation.

1. Introduction

Understanding the *genotype to phenotype map* is essential for a whole range of problems in evolutionary biology, production biology and biomedicine. As gene regulatory networks are the main mediating agents for setting up this map, a theory that can tell us how genetic variation is phenotypically manifested in gene regulatory networks as a function of regulatory anatomy may prove most helpful. Such a theory will be an important contribution to a future quantitative genetics theory linking genes, phenotypes and population level genetic phenomena in causal models based on how genes actually work and interact. More specifically, by being able to describe how the effects of genetic variation propagate in a network one will be able to predict how genetic variation in a gene affects network pathways and processes. In this way one may be able to tie genetic variation in gene networks to a whole range of biological processes that generate high-level phenotypic features. Moreover, at the generic level such a theory can be used in a systematic way to reveal recurrent patterns of how variation is propagated in specific types of regulatory anatomies.

We assume that the network is composed of a set of interacting nodes or loci. Each locus can in principle be regarded as a module by being a functional unit or subsystem of molecular processes whose working may be unknown, but which includes the whole transcriptional and translational machinery that produces the output of the locus [1,2]. The phenotypes of a network are the

* Corresponding author. Tel.: +47 64965292.
E-mail addresses: erik.plahte@umb.no (E. Plahte), arne.gjuvsland@umb.no (A.B. Gjuvsland), stig.omholt@umb.no (S.W. Omholt).

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stable equilibrium values of the gene products of all the loci in the network. Each locus is susceptible to genetic variation, and we assume that the genetic variation affects the promoter region of a given gene, but that there is no variation in the coding region of the gene. Many experimental results justify the relevance of this assumption. There are examples of noncoding mutations affecting production rates [3], mRNA processing rates [4,5], the shape of the cis-regulatory input function [6–8], and mRNA decay rates [9–11]. In a recent study of adaptive evolution in threespine sticklebacks, Jones et al. found that in 41% of the genes allelic variation was regulatory, in 42% it was probably regulatory, and in only 17% it was coding [12].

To fully understand the functional properties of a diploid gene it is desirable to model its two alleles as separate quantities. This was first done by Omholt et al. [13] to show how the phenomena of genetic dominance, overdominance, additivity, and epistasis could be seen as generic features of simple diploid gene regulatory networks. This model framework was later used to introduce the so-called *allele interaction* concept [14]. In the present paper, we develop these ideas further by proposing a way by which a diploid gene modelled in this fashion can be represented as a single entity and described by a single ODE for its gene product.

Based on these premises we provide a new vocabulary for analysing how genetic variation is manifested in a wide class of haploid and diploid gene regulatory networks possessing negative and positive feedback loops. We introduce terms to describe how a change in equilibrium value at one locus affects the equilibrium values of all other loci, how to identify the causal chains of loci conveying a genetic signal from one locus to another, and how genetic variation at a particular locus affects the equilibrium value phenotype of the locus itself. In [14] we investigated the relationships between single locus gene action concepts and regulatory network anatomy in small networks. Here we extend the analysis to gene regulatory networks with arbitrary number of loci and complex feedback structures. This extension is highly relevant for understanding *epistasis* and *pleiotropy* in genotype–phenotype maps. Epistasis refers to situations where the effect of a genetic substitution at one locus depends on the genotype at another locus. Pleiotropy describes situations where one gene influences several phenotypes rather than a single one. Since epistasis and pleiotropy are inherent to biological networks, a system-level understanding of these phenomena is needed [15,16].

By this work we contribute to the long and strong tradition originating with the works of René Thomas on relating generic systemic properties to the web of feedback loops [17,18], while at the same time elucidating the link between genetics and systems dynamics. Our results provide further support to the view that nonlinear system dynamics will make up a major part of the core of the mathematical foundation of a future quantitative genetics theory [19,20].

2. Propagation of genetic variation: features shared by haploid and diploid networks

At this stage we are not concerned with the inner workings of each gene due to genetic variation, but assume that the output rate of a locus is a given function of the concentration levels of its regulators, which we assume are one or several gene outputs. Thus in the first part of the paper we deal with characteristics of propagation of genetic variation that are shared by both haploid and diploid networks.

We combine results from linear algebra and graph theory (see e.g. [21]) with gene network ideas to describe how genetic variation in one locus propagates to the other loci in the system in terms of the equilibrium values of the state variables. We introduce the term *propagation function* to describe how a change in equilibrium value of one node affects the equilibrium values of all other nodes, the term *propagation chain* to describe a chain of actions conveying a genetic signal from one node in the network to another, and finally, the term *feedback function* to describe how genetic variation at any particular locus affects the equilibrium value of the locus product itself.

A brief explanation of our notation is found in Appendix A.

2.1. Basic rate equations

We assume the network $\mathcal{N}$ is composed of a set of n loci X_i, $i \in N = \{1, 2, \ldots, n\}$, where $n \geq 2$. The non-negative variable z_i represents the possibly time dependent concentration or amount of the output of X_i and acts as input to other loci in the network or contributes of the network's net output. The dynamics of $\mathcal{N}$ is described by a set of autonomous rate equations E_i for z_i, $i \in N$,

$$\dot{z}_i = f_i(z, a_i) = r_i(z, a_i) - \gamma_i z_i, \tag{1}$$

where $z \in R^n_+$ is the n-component vector with non-negative components z_i, $r_i(z, a_i)$ is differentiable with respect to z in a certain open and convex domain W, and $\gamma_i > 0$ is the relative degradation rate of z_i. The quantity $a = \{a_i\}$, $i \in N$, represents a set of parameters defining the system's genotype, the subset a_i defining the genotype of X_i and comprising quantities like maximum production rate, activation thresholds, affinities of activators and inhibitors, mRNA to protein conversion rate, etc. In many modelling approaches of this type, r_i is a Boolean or Boolean-like functional of sigmoidal functions or piecewise constant functions; see [22] for a review of modelling approaches for gene networks. It should be noted that there could be long and complicated chains of effects incorporated into $r_i(z, a_i)$ [23].

We assume that for each combination of genotypes of the loci X_i in $\mathcal{N}$, the system composed of Eqs. (1) has a single hyperbolic, asymptotically stable and differentiable point-like solution x in W. We show in Section 2.2 that under reasonable assumptions an equilibrium x always exists. If $\mathcal{N}$ has no positive loops, x is unique [24,25]. To avoid having to discuss possible problems related to multistationarity, we invoke the additional assumption that the equilibrium of the system is unique within the domain of phase space of interest even if there are positive loops in the system.

2.2. Propagation functions

A shift in the equilibrium value of some x_k due to a change in parameters specific for X_k will propagate through the network and lead to shifts in other equilibrium values. The propagation follows the network connections, which can be read out from the Jacobian J of Eq. (1) in the stable state x. To the network $\mathcal{N}$ corresponding to Eq. (1) we associate a signed digraph $\mathcal{G}$. To each node or locus X_i is associated a vertex X_i in $\mathcal{G}$. Let $X_j \to X_i$ indicate a direct effect from X_j to X_i if $J_{ij} = \partial r_i(z, a)/\partial z_j \neq 0$ in $z = x$. The effect of X_j on X_i is positive (negative) if the rate of change $\dot{z}_i$ increases (decreases) when z_j increases. For this direct effect there is a corresponding directed arc in $\mathcal{G}$ from X_j to X_i with a sign equal to the sign of J_{ij} associated to it. The sequence of direct effects $X_k \to X_j \to \cdots \to X_l$ is called *a chain from* X_k to X_l if each node in the chain occurs only once [26]. This chain corresponds to a simple path in $\mathcal{G}$ from X_k to X_l. We will use the term *propagation chain*.

The following proposition shows that for each pair $k, l \in N$, where $l \neq k$, there exists a propagation function p_{lk} which determines how the perturbed value of x_l due to a genetic variation in X_k is given in terms of x_k.

Proposition 1. *Let $k \in N$ be given, let $L = N \setminus \{k\}$, and consider the set of equilibrium conditions*

$$f_l(x_L, x_k, a_l) = r_l(x_L, x_k, a_l) - \gamma_l x_l = 0, \quad l \in L, \tag{2}$$

where all $x_i \geq 0$, and all r_l satisfy $r_l(x_L, x_k, a_l) > 0$ for $x_l = 0$. For any x_k the system of equations $f_L(x_L, x_k, a_L) = 0$ has at least one set of solutions $x_l = p_{lk}(x_k, a^{(k)})$, where $a^{(k)}$ is the set of parameters not occurring in the rate equation of X_k.

The proposition follows directly from Theorem 4.9 in [27]. Because the equation $f_k(x_L, x_k, a_k) = 0$ is not included in the system of equations $f_L(x_L, x_k, a_L) = 0$, the solution x_L is independent of the X_k-specific parameters a_k. This fact is important because it implies that the effect on x_l of any genetic variation of X_k is given by a fixed propagation function p_{lk}.

From this follows the usefulness of the propagation functions. A genotypic variation (mutation) in a gene may lead to new equilibrium values of the gene products in the network. One way of addressing this would be to try to parametrise the genotypic variation, and then model the dependence of the equilibrium values on the relevant parameters. The propagation functions offer a simpler solution because they require no knowledge of how the mutated gene could be modelled. They only relate the observable equilibrium concentrations. There is no need to take account of what is the cause of the genetic variation of X_k, how this manifests itself in a shift of parameter values in a_k, or how this parameter value shift might influence p_{lk}. All that matters are the shifted values of x_k and x_l. For a given k the set of all the functions p_{lk} contain all information about how the genetic change in X_k becomes manifested in the network against a fixed genetic background (the genotypes of all the other genes).

In the following we explore the properties of the propagation functions and show how they are related to the structure and interactions in the network. In the following we will try and derive the propagation functions from network properties, and also use what can be learned about propagation functions from observed equilibrium values to obtain information about causal chains in the network.

For a given k the functions p_{lk} are in principle observable by varying the genotype of X_k while keeping the other loci fixed and recording the shifted equilibrium values. Of course, solving p_{lk} for a given model is in general prohibited due to the nonlinearities in the system. However, finding the derivative of p_{lk} is a linear problem. In the following we relate the derivative $p'_{lk}(x_k, a^{(k)}) = q_{lk}(x_k, a^{(k)})$ to the values of the elements of the Jacobian J of Eqs. (1) for a given k and any $l \neq k$. Let $L = N \setminus \{l, k\}$ and $j \in L$. All the equilibrium conditions E_L define x_j as a function of x_k, i.e. $x_L = p_{Lk}(x_k)$. Then, when the expression in Eq. (6) below for $\mathrm{d}x_l/\mathrm{d}x_k$ exists,

$$\gamma_l x_l = r_l(x_l, x_k, p_{Lk}(x_k)) \tag{3}$$

defines x_l as a function of x_k around the steady state. Differentiating Eq. (3) with respect to x_k gives

$$\sum_{j \neq k} J_{lj} q_{jk} = -J_{lk}. \tag{4}$$

Let $Q^{(k)}$ be the column vector with components q_{ik} and $v^{(k)}$ the column vector with elements $\partial f_i/\partial x_k$, both with $i = k$ excluded. Then

$$J^{(kk)} Q^{(k)} = v^{(k)}. \tag{5}$$

Using Cramer's rule and interchanging columns in the numerator finally leads to

$$\frac{\mathrm{d}x_l}{\mathrm{d}x_k} = q_{lk}(x_k, a^{(k)}) = (-1)^{k+l} \frac{D^{(kl)}}{D^{(kk)}}. \tag{6}$$

Note that the right hand side is in fact independent of $f_k(x, a_k)$ because row number k in J is deleted in both determinants. This confirms Proposition 1. However, despite this, genotype variation in X_k will shift the equilibrium values and indirectly affect the values of the matrix elements of J. Furthermore, it follows from the implicit function theorem (see e.g. [28]) that if $D^{(kk)} \neq 0$ in x, then there is a unique differentiable mapping $p_{lk} : x_k \mapsto x_l$ in a neighbourhood of x whose derivative can be given as above.

Eq. (6) shows that the propagation of genetic variation in locus X_k is intimately linked to the feedback loop structure of the network in the stable state. While the left hand side of Eq. (6) can be approximated by finite differences of observable equilibrium values after a perturbation of X_k, its right hand side depends on the feedback structure of the network, which is not directly accessible. In the following section we introduce the propagation chain concept and show how it is linked to J, and how it discloses the biological implications of Eq. (6). First, however, we show that Eq. (6) sheds some light on the conditions for the validity of the chain rule for functions defined implicitly by a set of equations.

From an imprudent application of the chain rule to $x_m = p_{ml}(x_l)$ and $x_l = p_{lk}(x_k)$ one might be tempted to conclude that $x_m = p_{ml} \circ p_{lk}(x_k)$ and

$$p'_{ml} p'_{lk} = p'_{mk}, \tag{7}$$

where $k \in N$, $m \in N$ and $k \neq m$. This, however, is not generally true. In Appendix C we prove and comment on the following result:

Proposition 2. *Assume the variables have been renumbered such that $k = 1$ and $1 < l < m < n$, and define the sets $L = \{1 : l\}$, $M = \{(l+1) : n\}$, $Q = \{1 : (l-1)\}$, $R = \{l : n\}$, where $\{i : j\} = \{i, i+1, \ldots, j\}$ for $i < j$ and $\{i : i\} = \{i\}$. In terms of partitioned matrices*

$$J = \begin{pmatrix} J_{LQ} & J_{LR} \\ J_{MQ} & J_{MR} \end{pmatrix}. \tag{8}$$

If $J_{MQ} = 0$, the chain rule Eq. (7) is fulfilled.

The opposite conclusion is not true, however, as there may be nonzero elements in $J_{MQ} = 0$ that do not enter into feedback loops without jeopardising the rule.

Because the numbering of the nodes is arbitrary and immaterial, this result can be interpreted as follows. If all chains of effects from X_k to X_m pass through X_l, then the chain rule Eq. (7) is fulfilled, even if there are return chains from X_m to X_k so that both nodes are members of a feedback loop. However, if there exists a chain from X_k to X_m that does not pass through X_l, the chain rule may be violated. Apart from this, the network structure is immaterial.

As a simple illustration we consider the three-gene system

$$\begin{aligned} x_1 &= r_1(x_3), \\ x_2 &= r_2(x_1), \\ x_3 &= r_3(x_1, x_2), \end{aligned} \tag{9}$$

in which all $\gamma_i = 1$. The two chains $X_1 \to X_2 \to X_3$ and $X_1 \to X_3$, constitute a feedforward loop from X_1 to X_3, X_2 playing the role of the intermediate element X_l in Eq. (7). Then

$$\begin{aligned} \frac{\mathrm{d}x_2}{\mathrm{d}x_1} &= q_{21} = \frac{\mathrm{d}r_2}{\mathrm{d}x_1}, \\ \frac{\mathrm{d}x_3}{\mathrm{d}x_2} &= q_{32} = \frac{\frac{\partial r_3}{\partial x_2}}{1 - \frac{\partial r_3}{\partial x_1}\frac{\partial r_1}{\partial x_3}}, \\ \frac{\mathrm{d}x_3}{\mathrm{d}x_1} &= q_{31} = \frac{\partial r_3}{\partial x_1} + \frac{\partial r_3}{\partial x_2} q_{21}. \end{aligned} \tag{10}$$

Obviously, $q_{31} \neq q_{32}\, q_{21}$ if r_3 depends explicitly on x_1, in which case there is a chain from X_1 to X_3 not passing through X_2. On the other hand, the arc $X_3 \to X_1$ causes no problem.

2.3. Propagation chains and feedback loops

We start this section with a few standard definitions and clarifications.

- A *circuit* is a set of elements in the Jacobian J whose circuit product (the product of all the elements in the circuit) contributes to $\det(J)$ or one of its principal subdeterminants. An element in a circuit represents either an action from one node to another or to itself (a regulatory element), or a degradation term. Thus, a circuit with i elements involves i nodes. *The signed circuit product* of a circuit equals its circuit product times a signature factor defined in Appendix B. *A full circuit* is a circuit with n elements. *The length of a circuit* equals the number of elements in the circuit. *The sign of a circuit* equals the sign of its circuit product.
- If there is a circuit among a subset of nodes and another circuit among another disjoint subset of nodes, the two circuits are *subcircuits* in *a composite circuit*. The circuit product of a composite circuit can always be factorised as a product of two or more subcircuit products. The sign of the composite circuit equals the product of the signs of all the subcircuits.
- *A proper circuit* is a circuit that is not composite. Its circuit product cannot be factorised into subcircuit products.
- *A feedback loop* or just a loop is a circuit that only comprises regulatory elements. A feedback loop comprises one or more closed chains of actions or effects (closed paths) in the network in which any node in the chains occurs just once.
- *An autoregulatory loop* is a loop with one member, arising from a node whose product acts on its own dose-response function.

For example, if

$$J = \begin{pmatrix} -\gamma_1 & 0 & 0 \\ 0 & -\gamma_2 & c_{23} \\ 0 & c_{32} & -\gamma_3 \end{pmatrix}, \tag{11}$$

there is just one regulatory loop in the network ($X_2 \leftrightarrows X_3$), but several (composite) circuits, for instance $-\gamma_1 c_{23} c_{32}$. The degradation terms in Eq. (1) ensure that all nodes are members of one or more circuits which could be purely regulatory loops or a mixture of regulatory effects and degradation terms. A circuit product is therefore always either a loop product or equal to a loop product times one or more factors $-\gamma_j$. Accordingly, in the mathematical sense there exists at least one (proper or composite) circuit $\mathcal{L}_N$ comprising all nodes, even in cases where there is no full (regulatory) loop. In Appendix B we recall a few useful facts about subdeterminants and circuits.

Let $U = \{u_1, u_2, \ldots, u_\rho\}$ be a subset of N with k as its first element and l as its last, and let $\rho = |U|$ be the number of elements in U. Then $\mathcal{C}_U$ is *a propagation chain* $X_{u_1} \to X_{u_2} \to X_{u_3} \to \cdots \to X_{u_\rho}$ if the product

$$C_U = J_{u_\rho u_{\rho-1}} J_{u_{\rho-1} u_{\rho-2}} \cdots J_{u_2 u_1} \tag{12}$$

is nonzero. If $\mathcal{C}_U$ is made to close on itself by appending the action $X_{u_\rho} \to X_{u_1}$, it becomes the loop $\mathcal{L}_U$ with loop product $P_U = J_{u_1 u_\rho} C_U$.

Next we show that if some $q_{lk}(x_k, a^{(k)}) \neq 0$, there must be a chain propagating the effect of a shift in x_k from X_k to X_l. (The opposite is not true, as the contributions from two or more chains might accidentally cancel.) Combining Eq. (6) with known formulae for the expansion of determinants in terms of minors [27], we can express q_{lk} as

$$q_{lk}(x_k, a^{(k)}) = \frac{1}{D^{(kk)}} \sum_U (-1)^{\rho-1} D_{VV} C_U, \tag{13}$$

where U is any chain set with $U_1 = k$ and $u_\rho = l$, $V = N \setminus U$, $C_U = C_U(J)$ is the chain product of U, and the sum runs over all such U. Keep in mind that Eq. (6) presupposes $k \neq l$. Combining Eqs. (6) and (13) we see that $D^{(kl)}$ is a weighted sum of the chain product in J of all chains leading from X_k to X_l. If no such chain exists for given k and l, then $q_{lk}(x_k, a^{(k)}) = 0$, as expected.

For a given gene regulatory network model, Eq. (6), or alternatively Eq. (13), allows us to obtain analytical expressions predicting how variation in a gene X_k affects the equilibrium concentrations of all other genes in the network. In those cases where $q_{lk}(x_k, a^{(k)})$ equals zero, the genetic variation in X_k does not become manifested in the output of node X_l even though the equilibrium concentration of x_k is changed. If the variation becomes manifested in the output of more than one locus, the introduced polymorphism is pleiotropic. Since $q_{lk}(x_k, a^{(k)})$ depends explicitly on all chains leading from X_k to X_l, a change in genotype at one or more loci involved can potentially modify the effect on x_l of a shift in x_k, leading to epistasis. This implies that the epistasis and pleiotropy features of all loci can be cartographed in a systematic way. This information can be used to validate a particular model against experimental measurements of $q_{lk}(x_k, a^{(k)})$ as well as to identify generic characteristics of how variation is manifested as a function of regulatory anatomy.

2.4. The regulatory feedback effect on x_k of genetic variation in X_k

The formula (13) for q_{lk} is only valid for $k \neq l$. We now want to define a function which can be used to determine the effect of genotypic variation in X_k on x_k itself. It is obvious from Eq. (1) that even in an isolated node X_k without autoregulation, a change of genotype manifested as a change of a_k will in general lead to a shifted value of x_k. We will call this *an unmediated effect*. In addition there may be contributions from *mediated effects* due to the feedback loops involving X_k, including an autoregulatory loop. For example, in a system with the Jacobian in Eq. (11), a change of genotype in X_2 will lead to a shift in x_2 for two reasons: a change of the dose-response function r_2, and because of the loop $X_2 \leftrightarrows X_3$. The resultant of both effects determines how x_k responds to genetic variation in X_k.

We let X_L be the set of nodes apart from X_k itself that act directly on X_k, and X_M the remaining set of nodes, such that $\{k\} \cup L \cup M = N$. The stationarity condition for node X_k is

$$\gamma_k x_k = r_k(x_k, x_L, a_k). \tag{14}$$

According to Proposition 1 we can in principle find $x_l = p_{lk}(x_k, a^{(k)})$ for all $l \in L \cup M$, i.e. all $l \neq k$. Inserting this into Eq. (14) gives

$$\gamma_k x_k = r_k(x_k, p_{Lk}(x_k, a^{(k)}), a_k). \tag{15}$$

We define *the feedback function* ϕ_k for X_k by

$$\phi_k(x_k, a) = r_k(x_k, p_{Lk}(x_k, a^{(k)}), a_k), \tag{16}$$

or just $\phi_k(x_k) = r_k(x_k, p_{Lk}(x_k))$, and express the stationarity condition for X_k as

$$x_k = \frac{1}{\gamma_k} \phi_k(x_k, a). \tag{17}$$

For a given genotype, expressed as given a value of the parameter set a, the value of x_k can be found as the (by assumption stable and unique) solution of this equation. If $\psi_k(x_k, a) = \phi_k'(x_k, a) \equiv 0$, where the prime denotes the derivative with respect to x_k, then X_k is not involved in any regulatory feedback loop. However, if $\psi_k(x_k, a) = \phi_k'(x_k, a) \neq 0$, there is an effective feedback of X_k on itself, mediated by one or more loops. Therefore the feedback function ϕ_k describes and quantifies the feedback effects of changes in the equilibrium value of X_k on itself.

The derivative of ϕ_k can be expressed in terms of the Jacobi matrix elements. Differentiating Eq. (16) with respect to x_k, using

$x_l = p_{lk}(x_k, a^{(k)})$, Eq. (6) and that $\partial r_k/\partial x_m = 0$ for all $m \in M$ defined just before Eq. (14), we find

$$\psi_k(x_k, a) = \frac{\partial r_k}{\partial x_k} + \sum_{l\in L} \frac{\partial r_k}{\partial x_l} q_{lk} = \gamma_k + \frac{D}{D^{(kk)}}. \tag{18}$$

Let F_k be the sum of the signed circuit products (defined in Appendix B) of all full circuits in J in which there is a real regulation of X_k, but not necessarily of the other nodes. (We do not consider the linear degradation as a regulation. For example, in J defined in Eq. (11), there are two full circuits with circuit products $-\gamma_1\gamma_2\gamma_3$ and $-\gamma_1 c_{23}c_{32}$, respectively, but only the latter includes a real regulation of X_2 (by X_3) and would contribute to F_2. Neither contributes to F_1.)

As an illustration we consider the system

$$\begin{aligned} \gamma_1 x_1 &= r_1(x_1, x_2, x_3), \\ \gamma_2 x_2 &= r_2(x_1), \\ \gamma_3 x_3 &= r_3(x_2), \end{aligned} \tag{19}$$

with the two loops $X_1 \leftrightarrows X_2$ and $X_1 \to X_2 \to X_3 \to X_1$. With $k = 1$ we readily find

$$\psi_1 = \gamma_1 + \frac{1}{\gamma_2\gamma_3}(\gamma_2\gamma_3 J_{11} + \gamma_3 J_{12}J_{21} + J_{13}J_{32}J_{31}). \tag{20}$$

The expression in the parenthesis is F_1. Its second term comes with a positive sign because the minus sign for $-\gamma_3$ is cancelled by the negative signature factor of the loop $X_1 \leftrightarrows X_2$. Here as always, F_k is independent of γ_k, but not of the other degradation rates.

We also note that $D^{(kk)}$ is the sum of the signed circuit product of all circuits (proper and composite) of length $n-1$ which do not involve X_k. Expanding D along row k gives

$$D = \frac{\partial r_k}{\partial x_k} D^{(kk)} - \gamma_k D^{(kk)} + \sum_{j\neq k} \frac{\partial r_k}{\partial x_j} D^{(kj)}. \tag{21}$$

According to the lemma in Appendix B a determinant can be expanded as a sum of its signed circuit products. The first term in Eq. (21) is the sum of all full, composite circuit products with an autoregulatory subcircuit in X_k. Each term in the last sum is the determinant of a matrix K^{kj} obtained by setting all elements in row k and column j except J_{kj} equal to zero. Then $\det(K^{kj})$ is the sum of all circuit products in J which involve the element J_{kj}, i.e. in which X_k contributes with an active regulation. This gives $D = F_k - \gamma_k D^{(kk)}$, and if $D^{(kk)} \neq 0$,

$$\psi_k(x_k, a) = \frac{F_k}{D^{(kk)}} = \gamma_k \frac{F_k}{F_k - D}. \tag{22}$$

By this we have obtained a formula that relates the gain of the feedback function of a locus X_k to the circuit products of the full circuits in which X_k is regulated. This circuit would be either a full loop or a set of subloops, one of them involving X_k, and a number of degradation terms. It provides an analytic basis for the intuition that a high gain is obtained if the loops that X_k enters into are much stronger than the rest, i.e. if $|F_k| \gg |D^{(kk)}|$. Note that because x is hyperbolic by assumption, $D \neq 0$, thus $\psi_k(x_k, a) \neq \gamma_k$.

If $\psi_k(x_k, a) = 0$, then $F_k = 0$, which means that there is no effective regulation of X_k or the effects of the regulating loops happen to cancel. Then assume $\psi_k(x_k, a) \neq 0$. Solving Eq. (22) with respect to D and using that $(-1)^n D > 0$ (see Appendix B) leads to

$$(-1)^n F_k \Omega_k(x_k, a) > 0, \tag{23}$$

where

$$\Omega_k(x_k, a) = \frac{\psi_k(x_k, a) - \gamma_k}{\psi_k(x_k, a)}. \tag{24}$$

Assume there exists a full circuit composed of a proper loop $\mathcal{L}$ involving X_k and a perhaps number of degradation terms. Let P be the loop product of $\mathcal{L}$. As is illustrated in Eq. (20), the sign of this circuit product is equal to $\mathrm{sign}(P)$ independently of the number of degradation terms, because the negative signs of the degradation terms are compensated by the signature factor (see Lemma 2 in Appendix B) of the full loop. If $\mathrm{sign}(F_k) = \mathrm{sign}(P)$, we call $\mathcal{L}$ *a sign-dominant loop* of X_k. The signature factor of $\mathcal{L}$ is $(-1)^{n-1}$ because it has n members. The sign of its contribution to F_k is therefore $(-1)^{n-1}\mathrm{sign}(P)$, yielding the result

$$P\Omega_k(x_k, a) < 0, \tag{25}$$

which will be used to prove Proposition 5. From this follows readily

Proposition 3. *If $P > 0$, then $0 < \psi_k(x_k, a) < \gamma_k$, and if $P < 0$, then $\psi_k(x_k, a) < 0$ or $\psi_k(x_k, a) > \gamma_k$, and vice versa.*

Thus, a positive sign-dominant proper loop implies a feedback function with positive slope bounded by the degradation rate, while a negative sign-dominant loop implies either negative slope or a large positive slope of ϕ_k. If $\mathcal{L}$ is a composite loop, Eq. (25) is replaced by

$$(-1)^{n+\varepsilon_{\mathcal{L}}} P\Omega_k(x_k, a) > 0, \tag{26}$$

where $(-1)^{\varepsilon_{\mathcal{L}}}$ is the signature of the loop. If $F_k \neq 0$, there is always at least one sign-dominant loop for X_k.

To compute the values of x_k for a slight change of genotype in X_k, the shift in a_k must also be taken into account. Let $x_k = x_k(a)$ be the solution of Eq. (17), and let $b \in a_k$ be a single parameter. Differentiating Eq. (17) and introducing Jacobi elements as in the derivation of Eq. (18) we find

$$\frac{\partial x_k}{\partial b} = -\frac{D^{(kk)}}{D}\frac{\partial r_k}{\partial b} = -\frac{D^{(kk)}}{D}\frac{\partial \phi_k}{\partial b} = \frac{1}{\gamma_k - \psi_k(x_k, a)}\frac{\partial \phi_k}{\partial b}. \tag{27}$$

This formula emphasises the importance of the feedback function as a source of information about the phenotypic effects of genotype changes.

We are now ready to use these results to analyse diploid networks.

3. Allele interaction in networks with diploid loci

The rest of the paper deals with models of diploid systems, that is, systems in which chromosomes come in pairs with one variant of each gene, called an *allele*, on each of the two chromosomes. Thus, each gene is composed of two alleles, each allele being regulated more or less independently of the other, and the product of the gene is some combination of the product of each of the two alleles. If the two alleles are identical, the gene is called *homozygotic*, if they are different, the gene is *heterozygotic*, and if one of the alleles has been knocked out, it is *hemizygotic*.

Since the dawn of genetics, additive and dominant gene actions in diploids have been defined by comparing heterozygote and homozygote phenotypes without reference to, or model of, the functional dependency between the two alleles composing each genotype. However, from [14] as well as the present paper it is clear that it is precisely the interaction between the two alleles that gives rise to nonadditive gene action. Consequently, the genetics concepts of additive and dominant gene actions cannot explain basic phenomena in genetics theory from regulatory biology. Exploiting the additivity and nonadditivity properties of the two alleles, Gjuvsland et al. [14] showed that by means of the new concept of *allele interaction*, gene regulatory systems with one or two loci can be linked to single locus genetic theory.

We first present ways of modelling a network of genes involving diploid loci in an efficient way, and then introduce the concept of allele interaction. Finally, we study how the sign of the allele interaction is related to the feedback structure of the network. When studying allele interaction, we contrast different genotypes at a single focal locus without specifying the genotype of the rest of the loci.

3.1. Allele-specific diploid gene regulatory network models

As our objective is to relate the changes in genotypic value (i.e. the phenotype) due to allelic variation of a locus X_i to a potential interaction between its two alleles, we need to model the function and regulation of the two alleles as two distinct entities. A biallelic node X_i with two alleles sitting on each of the two chromosomes, splits into two subnodes X_i^1 and X_i^2 with z_i^1 and z_i^2 representing the concentration of gene product from each of the two chromosomes, respectively.

As stated in the introduction we assume that the outputs of the two alleles are functionally equivalent in the sense that they regulate other genes in the same fashion, genetic differences manifesting themselves only in the regulation of the two alleles, not in qualitative differences in their output. This implies that the nodes in the network are regulated by the total gene product $z_i = z_i^1 + z_i^2$, not by its two constituents separately. If this assumption should not hold for a gene X_i, the dose-response function of a downstream gene could depend on z_i^1 and z_i^2 separately. In such cases the simplifications described below would not be justified, and one would have to model the two alleles of this gene by two separate equations.

We let superscripts α_i and β_i denote the alleles in X_i^1 and X_i^2, respectively, $\alpha_i \in \{1, 2\}$, $\beta_i \in \{1, 2\}$. If the genotype of X_i is biallelic with alleles α_i, β_i, we model its rate equations by

$$\begin{aligned} \dot{z}_i^1 &= f_i^{\alpha_i}(z) = r_i^{\alpha_i}(z) - \gamma_i^{\alpha_i} z_i^1, \\ \dot{z}_i^2 &= f_i^{\beta_i}(z) = r_i^{\beta_i}(z) - \gamma_i^{\beta_i} z_i^2, \end{aligned} \tag{28}$$

where $z_i = z_i^1 + z_i^2$. For simplicity we suppress the parameters a_i from the arguments of the dose-response functions in the following. If $(\alpha_i, \beta_i) = (1, 1)$ or $(\alpha_i, \beta_i) = (2, 2)$, the equations describe a homozygous locus X_i, while $(\alpha_i, \beta_i) = (1, 2)$ describes the heterozygote. This model for a diploid node was first proposed by Omholt et al. [13].

If X_i is a homozygous locus, $\alpha_i = \beta_i$, and simple addition of the two equations gives

$$\dot{z}_i = 2r_i^{\alpha_i}(z) - \gamma_i^{\alpha_i} z_i. \tag{29}$$

In the hemizygous genotypes where one allele has been knocked out and the remaining copy is of genotype α_i, Eqs. (28) are reduced to

$$\dot{z}_i^1 = f_i^{\alpha_i}(z) = r_i^{\alpha_i}(z) - \gamma_i^{\alpha_i} z_i^1, \tag{30}$$

and $z_i = z_i^1$. For each polymorphic locus we may therefore consider five different genotypes: the biallelic genotypes 11, 12, and 22, and the mono-allelic genotypes 1 and 2.

In the following we consider a network in which X_n is polymorphic while the genotypes of the remaining loci are unspecified but fixed. We first describe it by *the extended system* S_E defined by the rate equations

$$S_E = \begin{cases} \dot{z}_i = r_i(z) - \gamma_i z_i, & i = 1, \ldots, n-1, \\ \dot{z}_n^1 = r_n^{\alpha_n}(z) - \gamma_n^{\alpha_n} z_n^1, \\ \dot{z}_n^2 = r_n^{\beta_n}(z) - \gamma_n^{\beta_n} z_n^2, \end{cases} \tag{31}$$

where $z_n = z_n^1 + z_n^2$ and $z = [z_1, \ldots, z_n]$. For simplicity we drop the subscript n to α_n and β_n in the following. As above, we denote the presupposed asymptotically stable state of Eq. (31) by $x = [x_1, \ldots, x_n]$.

3.2. Aggregating diploid loci

Contrary to Eq. (28), common ways of modelling gene regulatory networks describe a gene by a single equation for the total output of the gene, even when the gene is diploid. In the present section we investigate whether these two contrasting modelling schemes can be unified into a common modelling approach.

By exploiting the assumption that the diploid node X_n only acts on the other nodes by its total output z_n, we want to convert the extended model into a new model expressed in terms of the total product of a locus, while still keeping track of the properties of each of the two alleles. In other words, we want to construct a system S_A obtained by merging X_n^1 and X_n^2 into one *aggregated node* X_n with a single rate equation for z_n. We will call this conversion an *aggregation*. The rationale for this operation is that an aggregated model facilitates considerably the theoretical analysis of allele interaction in high-dimensional systems.

In fact, almost all gene regulatory models occurring in the literature are aggregated in the sense that they describe each gene by just one variable representing the amount or concentration of the gene's output. This is so even if the gene is diploid, and even in cases where several of the genes probably have allelic variation in the coding region as well, and perhaps produce qualitatively different outputs. Gene transcription and translation are very complicated processes which are only very crudely modelled by the kind of equations studied in the present paper. Even if the two allele products act in the same way such that only their total concentration matters as regulatory agents, there may be different degradation rates operating at the mRNA stage, during the translation process or later. If $\gamma_n^\alpha = \gamma_n^\beta = \gamma_n$, then obviously $\dot{z}_n = r_n^\alpha(z, a_n) + r_n^\beta(z, a_n) - \gamma_n z_n$. However, when $\gamma_n^\alpha \neq \gamma_n^\beta$, it is impossible to combine the two last equations in Eqs. (31) into one rate equation for z_n. The crucial problem in these cases is to perform the aggregation in such a way that the aggregated model reproduces the properties of the original extended model.

A natural solution would be to assume that the total dose-response function of the gene is the sum of the dose-response functions for each of the two alleles, and that the relative degradation rate of the total gene product $z_n = z_n^1 + z_n^2$ is an average of the two allelic degradation rates γ_n^1 and γ_n^2. We will call such a model an aggregated model S_A of S_E:

$$S_A = \begin{cases} \dot{y}_i = r_i(y) - \gamma_i y_i, & i = 1, \ldots, n-1, \\ \dot{y}_n = r_n^\alpha(y) + r_n^\beta(y) - \gamma_n^{\alpha\beta} y_n, \end{cases} \tag{32}$$

where

$$\gamma_n^{\alpha\beta} = \frac{\gamma_n^\alpha x_n^{\alpha|\circ} + \gamma_n^\beta x_n^{\beta|\circ}}{x_n^{\alpha|\circ} + x_n^{\beta|\circ}}. \tag{33}$$

Let $z(t, z^0)$ and $y(t, y^0)$ be the solutions of S_E and S_A, respectively, satisfying $z(0, z^0) = z^0$ and $y(0, y^0) = y^0$. It is easy to see that if $z^* = [z_1^*, \ldots, z_n^*]$ is a steady point of S_E, then $y^* = z^*$ is a steady point of S_A. It is not obvious that if z^* is an asymptotically stable point of S_E, then $y^* = z^*$ is an asymptotically stable point of S_A, and if z^* is hyperbolic, then y^* is hyperbolic. However, we show in Appendix D that this is in fact the case. We also show by extensive numeric simulations that in the majority of cases the temporal behaviours of $z(t, z^0)$ and $y(t, z^0)$ are approximately equal and qualitatively similar for a range of actual parameter values and realistic common initial values $y^0 = z^0 \neq z^*$. This being the case, we call S_A *a well-founded aggregation of* S_E.

These results strongly suggest that the idea of aggregating a diploid model in this way makes sense. If S_E has several biallelic nodes, we use this aggregate procedure of S_A on each node. If each aggregation is well-founded, we finally arrive at a *well-founded,*

fully aggregated system S_{FA}. Its diploid loci X_i of genotype $\alpha_i\beta_i$ are described by

$$\dot{y}_i = r_i^{\alpha_i}(y) + r_i^{\beta_i}(y) - \gamma_i^{\alpha_i\beta_i} y_i, \tag{34}$$

where $\gamma_i^{\alpha_i\beta_i}$ is given by Eq. (33) with n replaced by i. Haploid nodes X_j are described by

$$\dot{y}_j = r_i(y) - \gamma_j y_j. \tag{35}$$

If all nodes are diploid and admit the above aggregation process, the dimensionality of the model has been reduced from $2n$ to n, leading to the fully aggregated model S_{FA}. In the next section we use S_{FA} to investigate the consequences of knockout behaviour and different allele combinations in genotype–phenotype maps (GP maps).

3.3. The allele interaction concept

The concept of allele interaction for a polymorphic locus X for some specific phenotypic trait in a regulatory network was defined by Gjuvsland et al. [14]. Recall that $x_i^{1|\circ}$ and $x_i^{2|\circ}$ are the hemizygote genotypic values of a locus X_i when only allele 1, respectively allele 2 is present, and x_i^{11}, x_i^{12} and x_i^{22} are the biallelic homozygote and heterozygote genotypic values. The *heterozygote allele interaction value* Δ_i^{12} of X_i is defined as

$$\Delta_i^{12} = x_i^{12} - (x_i^{1|\circ} + x_i^{2|\circ}). \tag{36}$$

We define the two homozygote allele interaction values Δ_i^{11} and Δ_i^{22} in the same way, in general

$$\Delta_i^{\alpha\beta} = x_i^{\alpha\beta} - (x_i^{\alpha|\circ} + x_i^{\beta|\circ}), \tag{37}$$

where $\alpha, \beta \in \{1, 2\}$. An allele interaction is said to be negative if $\Delta_i^{\alpha\beta} < 0$ and positive if $\Delta_i^{\alpha\beta} > 0$.

Mendelian dominance is expressed by the dominance value

$$d_i = x_i^{12} - \frac{x_i^{11} + x_i^{22}}{2}. \tag{38}$$

The name "dominance value" stems from the fact that if $d_i \neq 0$, then one of the alleles contributes more to (dominates) the equilibrium value, shifting the heterozygous value away from the midpoint between the two homozygous equilibrium values. Allele interaction is closely related to d_i because $d_i = \Delta_i^{12} - (\Delta_i^{11} + \Delta_i^{22})/2$. Gjuvsland et al. [14] showed that if an isolated node X is under negative autoregulation, then its three allele interaction values are negative, while if the autoregulation is positive, they are positive. Building upon the theoretical machinery developed above, we show in the following that these results can be generalised to higher dimensional gene regulatory networks with more complex feedback structures. In this way we are able to build new theory relating gene action concepts and regulatory network anatomy to quantitative genetics.

If the two alleles of X_i were completely independent, one would expect $\Delta_i^{12} = 0$, as in this case the total output of the gene would be just the sum of the outputs from the two alleles. A nonzero value would therefore indicate some kind of one-way or mutual action between the alleles. In a single-locus model a nonzero allele interaction value could be a consequence of the feedback between the two alleles. Non-feedback mechanisms, such as transvection [29] in which one allele has an effect on the other (but not the other way round), could also lead to nonzero allele interaction [14].

To search for systemic causes of nonzero allele interaction values we examine the two-locus systems in Fig. 1. Their rate equations are given in Appendix E. The node X_1 is diploid and splits

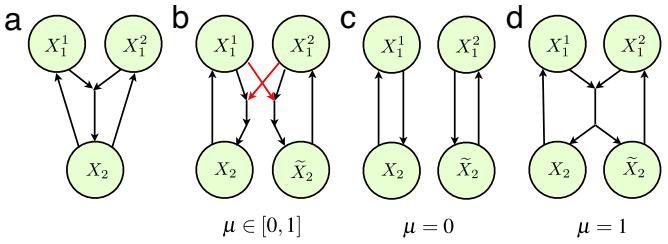

Fig. 1. a. The two-locus system analysed in Section 3.3 to investigate the source of nonzero allele interaction values. The nodes X_1^1 and X_1^2 are the two alleles of the locus X_1. Black arrows indicate direct effects. b. The interaction diagram of the artificial μ-system. The node $\widetilde{X}_2$ is a genetically identical copy of X_2. The two red, crossing arrows indicate actions whose strengths depend on μ. c. If $\mu = 0$, the actions are zero. The μ-system splits in two independent subsystems, each of them equivalent to the original system i a with one allele knocked out. d. If $\mu = 1$, the strengths of the actions are the same as in the system i a. In this case the μ-system is equivalent to the original system in a without any allele knockout. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in two subnodes X_1^1 and X_1^2 of type $\alpha_1 = 1$ and $\beta_1 = 2$, respectively. Taking the total equilibrium concentration of X_1 as the system's phenotype, we want to investigate the allele interaction value Δ_1^{12} of the system in Fig. 1a. We then have to compare the equilibrium value y_1^{12} with $y_1^{1|\circ} + y_1^{2|\circ}$, the phenotype values when only one allele is present. Let the node $\widetilde{X}_2$ be a copy of X_2. Then $y_1^{1|\circ}$ and $y_1^{2|\circ}$ are the phenotype values of the two systems in Fig. 1c.

To ease the comparison between these two systems we introduce the artificial *μ-system* in Fig. 1b. The two red, crossing arcs represent actions whose strengths (expressed by the magnitudes of the corresponding Jacobian elements) are proportional to a parameter μ which can be varied in [0, 1]. The equilibrium values of X_1^1 and X_1^2 in the μ-system are $y_1^{1|2}(\mu)$ and $y_1^{2|1}(\mu)$, and the corresponding allele interaction value is $\Delta_1^{12}(\mu)$.

If $\mu = 0$, the μ-system simplifies to the two independent subsystems in Fig. 1c. The one to the left (right) is the allele knockout system with only X_1^1 (X_1^2) left because $\widetilde{X}_2$ and X_2 are presumed identical. The phenotypic value of the whole system is $y_1^{12}(0) = x_1^{1|\circ} + x_1^{2|\circ}$. Therefore $\Delta_1^{12}(0) = y_1^{12}(0) - y_1^{1|\circ}(0) - y_1^{2|\circ}(0) = x_1^{1|\circ} + x_1^{2|\circ} - x_1^{1|\circ} - x_1^{2|\circ} = 0$.

If $\mu = 1$, the μ-system is represented by Fig. 1d. From its rate equations it follows that the equilibrium conditions of this system are the same as for Fig. 1a, leading to equal allele interaction values. Therefore the μ-system interpolates continuously between the diploid system in Fig. 1a and the allele knockout system in Fig. 1c.

Differentiating the equilibrium conditions of the μ-system, we find that $\mathrm{d}\Delta_1^{12}/\mathrm{d}\mu|_{\mu=0}$ is in general nonzero. Details are given in Appendix E. Because $\Delta_1^{12}(0) = 0$, this implies that even for infinitesimally small μ the μ-system has a nonzero allele interaction value. One might think that this nonzero value is caused by the feedback loop $X_1^1 \to \widetilde{X}_2 \to X_1^2 \to X_2 \to X_1^1$. However, there is still a nonzero allele interaction value if the arc from X_1^2 to X_2 is removed. In this case there is no mutual interaction between the alleles, only an indirect action from X_1^1 to X_1^2, but no chain from X_1^2 to X_1^1. We conclude that a nonzero allele interaction value could be caused by feedback among the two alleles, but that a one-way action is sufficient.

3.4. Allele interaction, feedback functions and feedback loops

The allele interaction values can be computed from directly observable quantities. In this section, we show how they can be related to properties of the network. Using finite differences and

the mean value theorem, the derivatives of the dose-response functions can be estimated in terms of the *single allele effects*

$$\delta_l^{\alpha_k\beta_k\backslash\alpha_k} = x_l^{\alpha_k\beta_k} - x_l^{\beta_k|\circ},$$
$$\delta_l^{\alpha_k\beta_k\backslash\beta_k} = x_l^{\alpha_k\beta_k} - x_l^{\alpha_k|\circ} \tag{39}$$

which quantify the effect on any locus X_l of activating the second allele in the initially hemizygous locus X_k. Then

$$p'_{lk}(c_l^{\alpha_k\beta_k}) = q_{lk}(c_l^{\alpha_k\beta_k}) = \frac{\delta_l^{\alpha_k\beta_k\backslash\beta_k}}{\delta_k^{\alpha_k\beta_k\backslash\beta_k}}, \tag{40}$$

where $c_l^{\alpha_k\beta_k} \in (x_k^{\alpha_k|\circ}, x_k^{\alpha_k\beta_k})$. We use the subscript l in $c_l^{\alpha_k\beta_k}$ because its value clearly depends on l.

Because the function p_{lk} is independent of the allelic composition of X_k, we can get four independent estimates of q_{lk} by combining X_k^{11} with X_k^1, X_k^{22} with X_k^2, and X_k^{12} with X_k^1 and with X_k^2. Note however that they will refer to different and unknown arguments, so that all together they will provide an estimate of the average value of q_{lk} in the interval between the minimum and maximum of the five genotypic values $x_k^{1|\circ}, x_k^{2|\circ}, x_k^{11}, x_k^{12}$ and x_k^{22}.

If a model for a given network exists, we can use Eq. (40) to estimate how the single allele effect propagates through the network as a consequence of polymorphism in X_k and the network connectivities, and use this to test the model. Conversely, measurements of the single allele effects from a polymorphic locus give information about the network connections [30].

Again dropping the subscript k from α_k and β_k, we denote in the following the feedback functions of X_k by ϕ_k^α, ϕ_k^β and $\phi_k^{\alpha\beta}$, and similarly for F_k, etc. According to Eq. (17) the stationarity conditions for the allele combinations $\alpha|\circ$, $\beta|\circ$ and $\alpha\beta$ are

$$\gamma_k^\alpha x_k^{\alpha|\circ} = \phi_k^\alpha(x_k^{\alpha|\circ}, a),$$
$$\gamma_k^\beta x_k^{\beta|\circ} = \phi_k^\beta(x_k^{\beta|\circ}, a), \tag{41}$$
$$\gamma_k^{\alpha\beta} x_k^{\alpha\beta} = \phi_k^\alpha(x_k^{\alpha\beta}, a) + \phi_k^\beta(x_k^{\alpha\beta}, a) = \phi_k^{\alpha\beta}(x_k^{\alpha\beta}, a),$$

where $\gamma_k^{\alpha\beta}$ is computed in accordance with Eq. (33). The last equation follows because, as is evident from Proposition 1, to derive p_{Lk} we do not use the stationarity condition for X_k, and all the other stationarity conditions are invariant under polymorphism of X_k and do not have superscripts α and β. The allele interaction value $\Delta_k^{\alpha\beta} = x_k^{\alpha\beta} - x_k^{\alpha|\circ} - x_k^{\beta|\circ}$ is then given in terms of the solutions of the three Eqs. (41). The following proposition relates $\Delta_k^{\alpha\beta}$ to the derivatives ψ_k^α and ψ_k^β of the feedback functions ϕ_k^α and ϕ_k^β.

Proposition 4. *For any biallelic locus X_k, $k \in N$, there exist numbers $c_k^{\alpha\beta} \in (x_k^{\alpha|\circ}, x_k^{\alpha\beta})$ and $c_k^{\beta\alpha} \in (x_k^{\beta|\circ}, x_k^{\alpha\beta})$ such that*

$$\Delta_k^{\alpha\beta} = \frac{\psi_k^\alpha(c_k^{\alpha\beta}, a)x_k^{\beta|\circ} + \psi_k^\beta(c_k^{\beta\alpha}, a)x_k^{\alpha|\circ}}{\gamma_k^{\alpha\beta} - \psi_k^\alpha(c_k^{\alpha\beta}, a) - \psi_k^\beta(c_k^{\beta\alpha}, a)}. \tag{42}$$

Proof. From Eqs. (17) and (41) it follows that

$$\Delta_k^{\alpha\beta} = \frac{1}{\gamma_k^{\alpha\beta}}\left(\phi_k^\alpha(x_k^{\alpha\beta}, a) + \phi_k^\beta(x_k^{\alpha\beta}, a)\right) - x_k^{\alpha|\circ} - x_k^{\beta|\circ}.$$

Inserting $x_k^{\alpha\beta} = x_k^{\alpha|\circ} + (\Delta_k^{\alpha\beta} + x_k^{\beta|\circ})$ into $\phi_k^\alpha(x_k^{\alpha\beta}, a)$ and $x_k^{\alpha\beta} = x_k^{\beta|\circ} + (\Delta_k^{\alpha\beta} + x_k^{\alpha|\circ})$ into $\phi_k^\beta(x_k^{\alpha\beta}, a)$ and using the mean value theorem on both functions lead after some elementary algebra and repeated use of Eqs. (33) and (17) to Eq. (42). □

By combining Eq. (42) with Eq. (23) and using the following lemma, we are able to relate the sign of $\Delta_k^{\alpha\beta}$ to properties of the feedback loops of the system.

Lemma 1. *Let E be an open subset of R_+, let $\phi_1 : E \to R_+$ and $\phi_2 : E \to R_+$ be two positive, strictly monotonic and differentiable functions. Define $\phi_{12}(x) = \phi_1(x) + \phi_2(x)$, and assume that $\gamma_i x = \phi_i(x)$, $i = 1, 2$, have unique solutions x_1, x_2 in E, where $\gamma_i > 0$ and $x_1 \le x_2$ by convention. Define*

$$\gamma = \frac{\gamma_1 x_1 + \gamma_2 x_2}{x_1 + x_2}, \tag{43}$$

and assume the solution x_{12} of $\gamma x = \phi_{12}(x)$ is also in E. Define $\Delta_{12} = x_{12} - x_1 - x_2$ and $\delta_1 = x_{12} - x_2$, $\delta_2 = x_{12} - x_1$.

1. *If $\phi_1'(x) < 0$ and $\phi_2'(x) < 0$ for all $x \in E$, then $\Delta_{12} < 0$ and $\delta_2 > 0$.*
2. *If $0 < \phi_1'(x) < \gamma_1$, $0 < \phi_2'(x) < \gamma_2$ for all $x \in E$, then $\Delta_{12} > 0$ and $\delta_1 > 0$, $\delta_2 > 0$.*
3. *If $\phi_1'(x) > \gamma_1$, $\phi_2'(x) > \gamma_2$ for all $x \in E$, then $\Delta_{12} < 0$ and $\delta_1 < 0$.*

The proof is in Appendix F.

Recall the definition $\Omega_i(x) = (\phi_i'(x) - \gamma_i)/\phi_1'(x)$ in Eq. (24). Assume Ω_1 and Ω_2 have the same sign. It follows from Lemma 1 that if $\Omega_i < 0$ for $i = 1, 2$, then $\Delta_{12} > 0$, and if $\Omega_i > 0$ for $i = 1, 2$, then $\Delta_{12} < 0$. Combining this with Eqs. (23)–(26) we readily arrive at

Proposition 5. *If F_k^α and F_k^β, $\alpha \ne \beta$, have the same sign S_k, then $(-1)^n S_k \Delta_k^{\alpha\beta} < 0$. If P is the loop product of a sign-dominant, proper loop for X_k, then $P\Delta_k^{\alpha\beta} > 0$. If the loop is sign-dominant but composite and has signature factor $(-1)^\varepsilon$, then $(-1)^{n+\varepsilon-1} P\Delta_k^{\alpha\beta} > 0$.*

The allele interaction values $\Delta_k^{\alpha\beta}$ are directly observable by subjecting each X_k to allele knockout and recording the unperturbed and perturbed equilibrium values of x_k. If this is done for all X_k, a set of exact sign conditions on the loop structure of the system is obtained. This may be particularly useful for homozygous systems, because then $F_k^\alpha = F_k^\beta$ and there will be no problem with the sign S_k.

4. Discussion and conclusions

Combining graph theory and linear algebra results with mathematical models of gene regulatory networks, we have introduced relevant concepts and provided analytical insights on how genetic variation is propagated in gene networks. We hope that our results may contribute to a future theory on the pleiotropy and epistasis features of genetic variation in haploid and diploid gene networks as a function of regulatory architecture and functional location of genetic variation.

We have also shown that the modelling framework for diploid gene networks developed by Omholt et al. [13] in which a diploid node is described by two rate equations, can be transformed – in our language: aggregated – into a standard type model in which each locus, haploid or diploid, is described by just one rate equation.

The time-dependent solutions of the aggregated models are qualitatively equivalent to the corresponding model by the modelling framework of Omholt et al., and the equilibrium solutions of the former are stable when the solutions of the latter are. Qualitative equivalence is here to be taken in an informal sense, meaning that the graphs of the solution curves look similar, and that the curves are relatively close to each other in a sense given in Appendix D.

The variables of the aggregated model are the observable total gene product of each locus. The model depends explicitly on the genotypes of the two alleles of the diploid loci. This property facilitates investigations on how the genotypic value of a diploid locus (i.e. its phenotype) depends on its genotype. It further

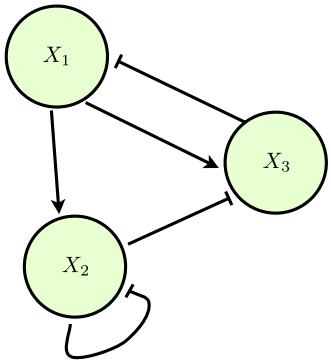

Fig. 2. A system with an incoherent feedforward motif (from X_1 to X_3) and three feedback loops. An arrow denotes positive action, a crossbar negative action. The two full loops $X_1 \to X_2 \to X_3 \to X_1$ and $(X_1 \to X_3 \to X_1)\,(X_2 \to X_2)$ are incoherent: their contributions to each F_i have opposite signs.

reduces the size of the model from perhaps $2n$ down to n. This reduction also makes it much easier to read out the connection and the feedback loops between the loci in "everyday" language in which we talk about a gene as one entity despite the fact that it is composed by two more or less independent alleles. To the best of our knowledge this provides for the first time a rationale for modelling diploid gene regulatory networks with one node for each locus even though the locus may be polymorphic and show intra-locus interaction effects.

Finally, we have shown that for a wide range of network architectures the sign of the allele interaction is independent of the shape of the rate functions and parameter values, and does not change with mutations in the other nodes or under external noise. More specifically, Proposition 5 confirms and generalises the result in [14] for an isolated gene. It shows the close connection between the sign of the allele interaction for a polymorphic locus X_k and the feedback loops it is involved in. Its main importance is that recording the equilibrium values x_k for a hemizygotic and either a homo- or heterozygotic locus X_k gives information about the network interactions and feedback loops involving X_k. These genotypes are within experimental reach for several organisms, and the machinery developed above can be tested in several settings. Hemizygous collections are already available for yeast [31]. Of course there may be networks for which the actual genotypes lead to more complex sign relations so that the above results would not be valid. Irrespective of whether the sign relations are valid or not, if these three allele interaction values $\Delta_k^{\alpha\alpha}$, $\Delta_k^{\alpha\beta}$ and $\Delta_k^{\beta\beta}$ have equal signs s_k, a tentative hypothesis is that X_k has one or more sign-dominant loop with sign s_k.

Gjuvsland et al. [14] showed that in systems with one or two loci, a biallelic locus can display up to 18 qualitatively different allele interaction sign patterns (triplets of $+$, $-$ and 0 representing the signs of Δ^{11}, Δ^{12} and Δ^{22}). In a single locus system with autoregulation only a subset of 7 of these could be realised with monotonic dose-response functions. With non-monotonic dose-response functions, however, 16 sign patterns could be generated. They also showed analytically that for each allele combination, the allele interaction value and the sign of the autoregulatory loop were equal (their Supporting Information, Result 1). For the autoregulatory system of an isolated node X_1, the sign of F_1 is just the sign of the autoregulatory loop, which equals the sign of the derivative of the dose-response function. Therefore, a non-monotonic dose-response function implies that F_1 – and the allele interaction value – may take both signs, depending on parameter values.

Consider then a multi-locus system with monotonous dose-response functions (Fig. 2). The two full loops $X_1 \to X_2 \to X_3 \to X_1$ and $(X_1 \to X_3 \to X_1)(X_2 \to X_2)$ are incoherent (their contributions to F_1 have opposite signs) because $F_1 = J_{13}J_{32}J_{21} - J_{13}J_{31}J_{22}$ and $J_{13}J_{32}J_{21} > 0, J_{13}J_{31}J_{22} > 0$. Depending on parameter values either the one or the other may determine the sign of F_1 and give opposite signs to $\Delta_1^{\alpha\beta}$. In this multi-node network, varying sign of $\Delta_1^{\alpha\beta}$ can be obtained with monotonic dose-response functions, while this could only be obtained with non-monotonic dose-response functions in the single node autoregulatory system. Based on this we conjecture that with monotonic dose-response functions a much wider range of allele interaction sign motifs can be obtained in multi-gene systems than for autoregulated genes.

Our results provide a theoretical basis for two kinds of experimental tests of network models: (i) checking the sign of the allele interaction for any node by allele knockout in the same node and (ii) checking the effect of allele knockout in one node on the equilibrium values of other nodes. In both cases the checking can be made independently on either of the homozygotes and on the heterozygote. This gives three possible combinations for each polymorphic locus. If the allelic composition of each of these loci can be selected or imposed experimentally and independently for each locus, the number of different test can in principle be very large. The formalism developed above may be combined with systematic measurement of the effects of allele knockouts and their effects on the other nodes in the network to deduce the connectivity of networks for which no model so far exists. This approach would be very similar to the approach suggested by Kholodenko et al. [30].

We have deliberately refrained from dealing with networks with multiple stable states. Surely, multistationarity is a generic characteristic of nonlinear dynamic systems, but is not a relevant issue in a large number of biological systems. Nor have we allowed genetic variation affecting the coding part of a gene. For such genes aggregation is generally not possible, as the two allele products may have different effects on other genes. It would not make sense to sum the two product concentrations, and the two alleles would simply have to be modelled by separate rate equations. The model framework we have used for the theory development is of course very simple both in terms of the relationship between the gene product expression level and the production rate from downstream loci and the neglect of more complex regulatory anatomies involving for example noncoding RNA (see e.g. [32–35]). Including more biological realism along these lines would make it more complicated to develop the theory, but might at the same time disclose deeper insight into the propagation of genetic variation in real networks. Our formalism can easily also account for other network agents than gene loci, and can be used to study e.g. regulatory structures involving gene networks, metabolic networks and protein signalling networks. We anticipate that such an endeavour will yield new insight into the manifestation of genetic variation in nonlinear biological systems.

Acknowledgements

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Appendix A. Notation

In this appendix, we explain our notation for subsets of vectors and matrices, and for equilibrium values for different genotypes (allelic compositions) of diploid genes.

Let U and V be subsets of $N = \{1, 2, \ldots, n\}$. We use the notation $z_U = \{z_k\}_{k\in U}$, $X_U = \{X_k\}_{k\in U}$, etc. In matrix equations

z_U denotes the corresponding column vector. The $n \times n$ Jacobian matrix of Eq. (1) in the stable point x is denoted by J, and J_{UV} is the matrix obtained from J by selecting the rows U and the columns V (without interchanging rows or columns). We use the notation $X^{(\hat{U})}$ to denote the set of nodes not in X_U, etc., and denote the corresponding set of variables by $z^{(U)}$. Similarly, $x^{(k)}$ is the set of all x_i except x_k or the vector obtained by removing x_k from x. Let $i \in U$ and $j \in V$. The matrix $J^{(ij)}_{UV}$ is obtained from J by selecting the rows U and the columns V in J and deleting row i and column j in J. The superscript $(i\circ)$ indicates that only row i and no column is deleted, and $(\circ j)$ that only column j and no row is deleted. We also define $D = \det(J)$, $D^{(ij)}_{UV} = \det(J^{(ij)}_{UV})$ if $|U| = |V|$, and $D^{(ij)} = \det(J^{(ij)})$. It goes without saying that if there is no superscript, no row or column is deleted, and if there is no subscript, all rows and columns are included. Similarly, if $L \subset N$, $p_{Lk}(x_k, a^{(k)}) = \{p_{lk}(x_k, a^{(k)}) \mid l \in L\}$.

The genotype of a diploid gene X_i is denoted $g_i = \alpha_i\beta_i$, where α_i and β_i take the values 1 or 2, indicating two different alleles. All equilibrium values depend on the total genotype $g = [g_1 \cdots g_n]$ of the system, but we do not complicate formulae by stating this explicitly. Instead, we let $x_i^{\alpha_i\beta_i}$ denote the equilibrium value of X_i when its genotype is $\alpha_i\beta_i$. Thus, x_i^{11}, x_i^{12} and x_i^{22} are the stable equilibrium values of X_i when both alleles are present and both are of type 1, of types 1 and 2, and both of type 2, respectively.

The stable equilibrium value for X_i when one of the alleles has been knocked out is $x_i^{1|\circ}$ and $x_i^{2|\circ}$, where $\circ$ indicates a nil value, i.e. that the allele is absent. Finally, $x_i^{1|1}$ and $x_i^{1|2}$ represent the equilibrium value of the output from a subnode of X_i with allele of type 1 when the other allele is of type 1 or 2, respectively. For example, $x_i^{11} = x_i^{1|1} + x_i^{1|1} = 2x_i^{1|1}$, $x_i^{12} = x_i^{1|2} + x_i^{2|1}$, and $x_i^{22} = x_i^{2|2} + x_i^{2|2} = 2x_i^{2|2}$. Note however that while e.g. z_i^1 is the (time dependent) output of X_i^1 whatever its actual genotype, $x_i^{1|\circ}$ is the equilibrium concentration of the gene product of X_i when the copy of the gene on one chromosome is knocked out and the one present is allele $\alpha_i = 1$.

Appendix B. Circuits and loops

In this appendix, we recall some useful facts related to the circuit structure of a real $n \times n$ matrix A.

Lemma 2 ([24]). *Let $k \in N$ be given, let U be any subset of N with k elements, and let $\pi(U)$ be the set of permutations of U, including the identity permutation. Let $V \in \pi(U)$ and define the circuit product*

$$P(U, V) = A_{U_1V_1}A_{U_2V_2}\cdots A_{U_kV_k} \tag{B.1}$$

and

$$S_U = \sum_{V\in\pi(V)} (-1)^{\varepsilon(U,V)}P(U, V), \tag{B.2}$$

where $\varepsilon(U, V)$ is the number of subcircuit products in the circuit product $P(U, V)$ with an even number of factors, and

$$s_k = \sum_U S_U, \tag{B.3}$$

where the sum runs over all U for which $|U| = k$. Then $S_U = D_{UU}$, and the characteristic polynomial of A is

$$p_n(\lambda) = \lambda^n - s_1\lambda^{n-1} + s_2\lambda^{n-2} + \cdots + (-1)^n s_n. \tag{B.4}$$

In particular, the trace $T = \mathrm{tr}(A) = s_1$ and the determinant $D = \det(A) = s_n$. Of course, $s_n = S_N$. We call $(-1)^{\varepsilon(U,V)}$ the *signature factor* and $(-1)^{\varepsilon(U,V)}P(U, V)$ *the signed circuit product* of the circuit corresponding to the circuit product $P(U, V)$. To express signs we use the sign function defined by $\mathrm{sign}(x) = -1$ if $x < 0$, $\mathrm{sign}(0) = 0$, $\mathrm{sign}(x) = +1$ if $x > 0$.

A square matrix for which all eigenvalues have a negative real part, will be called *a stable matrix*. The following result should be well-known.

Lemma 3 ([36, vol. 2, p. 220]). *If the real $n \times n$ matrix A is stable, then*

$$(-1)^j s_j > 0, \quad \text{all } j \in N. \tag{B.5}$$

Appendix C. Proof of Proposition 2

Proof. When $F_{MQ} = 0$, the four determinants $D^{(1l)}$, $D^{(lm)}$, $D^{(1m)}$ and $D^{(ll)}$ are all block triangular, and can be expressed as

$$\begin{aligned} D^{(1l)} &= D^{(1l)}_{LQ}D_{MM}, \\ D^{(lm)} &= D_{QQ}D^{(\circ m)}_{MR}, \\ D^{(1m)} &= D^{1l}_{LQ}D^{(\circ m)}_{MR}, \\ D^{(ll)} &= D_{QQ}D_{MM}. \end{aligned} \tag{C.1}$$

The notation for subscripts and superscripts was defined in Appendix A. From Eqs. (C.1) follows trivially that

$$D^{(1l)}D^{(lm)} = D^{(1m)}D^{(ll)} \tag{C.2}$$

which is equivalent to the chain rule due to Eq. (6). □

Appendix D. Justification of aggregated models

In this appendix, we justify the claim in Section 3.2 that the aggregated model S_A is a well-founded aggregation of S_E.

The simple model for transcription regulation developed by Bintu et al. [37,38] and Buchler et al. [39] is based upon setting the transcription rate proportional to the binding probability of transcription factors and polymerase to the gene's binding site. They used traditional Boltzmann statistics to derive formulae for the binding probabilities. Extending this analysis to biallelic genes remains to be done. Unfortunately, a physico-chemical analysis of transcription soon gets very complicated [40,41], but the following simple argument lends some justification to the assumption that the production rate of the biallelic gene is just the sum of the two monoallelic production rates. Assume the number of transcription factor molecules is much larger than the number of binding sites of the gene, and that the effect of non-specific binding sites for the transcription factors can be disregarded. Then the number of transcription factor molecules available for binding to one chromosome is not appreciably reduced if a small fraction of them are bound to the other chromosome. If the probability of binding to the one chromosome is independent of what happens at the other chromosome, the total probability that transcription factors will bind to the gene and initiate transcription is just the sum of the two single-allele probabilities, and the total transcription rate is the sum of the two single-allele transcription rates.

The following proposition shows that S_A possesses the same asymptotic stability properties as S_E.

Proposition 6. *Let $z^* = [z_1^*, \ldots, z_n^*]$ be an asymptotically stable point for S_E. If the Jacobian J of S_A is diagonalisable in z^*, then z^* is an asymptotically stable point for S_A.*

Proof. If $\gamma_n^1 = \gamma_n^2 = \gamma_n$, the equations for z_n^1 and z_n^2 of S_E can be added, leading to the equations for S_A.

We then assume $\gamma_n^1 \neq \gamma_n^2$. Let $z(t, z^0)$, where $z_n = z_n^1 + z_n^2$, be a solution of S_E as given by Eqs. (31) such that $\lim_{t\to\infty} z(t, y^0) = z^*$,

and define $u(t) = z(t, y^0) - z^*$. The definition of S_A ensures that z^* is a stationary point for both systems.

Because z^* is an asymptotically stable state for S_E, for any $\varepsilon > 0$ there exists a $T > 0$ such that $\|u(t)\| < \varepsilon$ for $t > T$. By choosing y^0 sufficiently close to z^* we can ensure that $\|u\| < \varepsilon$ for all positive t.

We proceed by investigating the rate equations for the n-component vector $u(t)$.

$$\begin{aligned}\dot{u}_i &= r_i(z^* + u) - \gamma_i z_i = r_i(z^* + u) - r_i(z^*) - \gamma_i u_i,\\ \dot{u}_n &= r_n(z^* + u) - \gamma_n^1 z_n^1 - \gamma_n^2 z_n^2 \\ &= r_n(z^* + u) - r_n(z^*) - \gamma_n^1 u_n^1 - \gamma_n^2 u_n^2,\end{aligned} \tag{D.1}$$

where $i = 1, \ldots, n-1$ and $r_n = r_n^1 + r_n^2$. After a little algebra the equation for $\dot{u}_n$ can be written as

$$\dot{u}_n = r_n(z^* + u) - r_n(z^*) - \gamma_n u_n + e_n(u), \tag{D.2}$$

where γ_n is defined in Eq. (33), and

$$e_n(u) = \frac{1}{z_n^*}\left(\gamma_n^1 z_n^{*2} - \gamma_n^2 z_n^{*1}\right)\left(u_n^2 - u_n^1\right). \tag{D.3}$$

The mean value theorem for a mapping $r : R^n \to R^n$ is [28].

Theorem 1. *Suppose $r : W \to R^n$ is differentiable on the open set $W \subset R^n$, and that the line segment joining z^* and z lies in W. Then there exist numbers α_i, $0 < \alpha_i \leq 1$, and vectors $w^i = (1 - \alpha_i)z^* + \alpha_i z$, $i = 1, \ldots, n$, such that*

$$r_i(z) - r_i(z^*) = Dr_i(w)(z - z^*), \quad i = 1, \ldots, n, \tag{D.4}$$

where $D = [\partial/\partial z_1, \ldots, \partial/\partial z_n]$, and r_i, z and z^* are column vectors.

Note that w^i lies on the line segment between z^* and z.

Let $J(z)$ be the Jacobian of S_A, defined by $J_{ij}(z) = \partial f_i(z)/\partial z_j$. Applying the mean value theorem to Eq. (D.2) we get

$$\dot{u} = H(u, z^*, a)u + e(u), \tag{D.5}$$

where $H(u, z^*, a)$ is obtained as follows: Let $v^i = (1 - \alpha_i)z^* + \alpha_i(z^* + u) = z^* + \alpha_i u$, where $0 < \alpha_i < 1$, and define $a = [\alpha_1, \ldots, \alpha_n]$. Then $H(u, z^*, a)$ is the matrix obtained by evaluating the elements of $J(z)$ in row number i in the point v^i, $i = 1, \ldots, n$. Obviously, $H(u, z^*, a) \to J(z^*) = J^*$ when $t \to \infty$ and $u(t) \to 0$.

We write $H(u, z^*, a) = J^* + E(u, a) = PD^*P^{-1} + E(u, a)$, where D^* is the diagonal eigenvalue matrix and P the eigenvector matrix for J^*. Then $E(u, a) \to 0$ when $t \to \infty$. Considering Eq. (D.5) as an inhomogeneous ODE for $u(t)$ and introducing $v(t) = P^{-1}u(t)$, its solution is

$$v(t) = e^{D^*t}v^0 + \int_0^t e^{D^*(t-\tau)}\left(E(Pv(\tau), a) + e(Pv(\tau))\right)d\tau. \tag{D.6}$$

With $w(t) = E(Pv(\tau), a) + e(Pv(\tau))$ we write this simpler as

$$\begin{aligned}v(t) &= e^{D^*t}v^0 + \int_0^t e^{D^*(t-\tau)}w(\tau)d\tau\\ &= e^{D^*t}v^0 + \int_0^t e^{D^*(t-\tau)}d\tau\ \bar{w}(t)\\ &= e^{D^*t}v^0 + (D^*)^{-1}(I - e^{D^*t})\bar{w}(t),\end{aligned} \tag{D.7}$$

where $\bar{w}(t)$, which is the vector of mean values of the components of $w(t)$, is bounded by the minimum and maximum of $w(t)$ in $[0, t]$ because the remaining integrand is positive for each component of $v(t)$.

Let $\{v^{0j}\}$, $j = 1, \ldots, n$ be a set of linearly independent vectors, and $v^j(t)$ the corresponding solutions given by Eq. (D.7). Because $\bar{w}(t) \to 0$ when $t \to \infty$, the set of $v^j(t)$ is also linearly independent for sufficiently large t. Letting V^0, $V(t)$ and $\bar{W}(t)$ be the matrices with v^{0j}, $v^j(t)$ and $\bar{w}^j(t)$ as columns, respectively, we get

$$V(t) = e^{D^*t}V^0 + (D^*)^{-1}\left(I - e^{D^*t}\right)\bar{W}(t), \tag{D.8}$$

leading to

$$e^{D^*t} = \left(V(t) - (D^*)^{-1}\bar{W}(t)\right)\left(V^0 - (D^*)^{-1}\bar{W}(t)\right)^{-1}. \tag{D.9}$$

The last factor in Eq. (D.9) is well-defined for sufficiently large t and approaches $(V^0)^{-1}$ when $t \to \infty$ because $\bar{W}(t)$ approaches the zero matrix. It follows that $\|e^{D^*t}\| \to 0$ when $t \to \infty$. Let μ be the spectral abscissa of D^*. For all $t \geq 0$, $\exp(\mu t) \leq \|\exp(D^*t)\|$ [42, Theorem 15.3]. This shows that $\exp(\mu t) \to 0$ when $t \to \infty$. Therefore $\mu < 0$, and z^* is an asymptotically stable and hyperbolic point for S_A. □

To compare the temporal behaviours of S_E and S_A we had to rely on numerical simulations. To justify the aggregation, the temporal behaviours of $z(t, z^0)$ and $y(t, z^0)$ should be approximately equal and qualitatively similar for a range of actual parameter values and realistic common initial values $z^0 \neq z^*$. Although very close similarity far from the common equilibrium point cannot be expected, at least the behaviours near the equilibrium should be quantitatively similar. We quantify the degree of similarity of the solution curves by the relative discrepancy

$$RelErr(y, z) = \left\{\frac{\int_0^\infty (y_i(t, z_0) - z_i(t, z_0))^2 dt}{\int_0^\infty (z_i(t, z_0) - z_i^*)^2 dt}\right\}_{i \in N}. \tag{D.10}$$

One advantage of this discrepancy measure is that both integrals converge exponentially if z^* is hyperbolic so that they can easily be computed numerically by integrating to a sufficiently large and finite T. As a measure of the similarity of solutions near the equilibrium we used

$$EigDiff(z^*; S_E, S_A) = \log\left(\sum_{j \in N} \frac{|\lambda_j - \Lambda_j|}{|\Lambda_j|} e^{\Re(\Lambda_j)}\right), \tag{D.11}$$

where $\{\Lambda_j\}$ and $\{\lambda_j\}$ are the sets of eigenvalues of the Jacobians of S_E and S_A, respectively. Because S_E has one additional eigenvalue, one of its eigenvalues has to be excluded from the sum in Eq. (D.11). We excluded the eigenvalue that minimises the sum. The purpose of the exponential factor is to simulate the fact that an eigenvalue contributes to the solution of the linearised equations around z^* by this factor. This similarity measure is justified if there is a corresponding similarity between the two sets of eigenvectors, because then for a given solution $z(t)$ it will be possible to construct a solution $y(t)$ which will be quantitatively similar to $z(t)$ close to the equilibrium.

Numeric simulations for a range of n-values show that in almost all cases the eigenvalues of S_A match the eigenvalues of S_E very closely (Fig. D.3). The scatterplots in the left column show that except in a few cases, $RelErr(y, z)$ and $EigDiff(z^*; S_E, S_A)$ are much smaller than 0, showing that the solution of S_A lies relatively close to the solution of S_E, and that the differences between the eigenvalues $\{\Lambda_j\}$ of S_E and $\{\lambda_j\}$ of S_A are much smaller in magnitude than the eigenvalues of the Jacobians of S_E.

Except in a small number of cases, the temporal behaviours of the solutions also match closely. Typically, at least when n is large, there are appreciable differences between the two solutions only for a few variables (Fig. D.4). Frequently this happens for variables that do not approach their final state monotonically, either because they oscillate towards the equilibrium or because

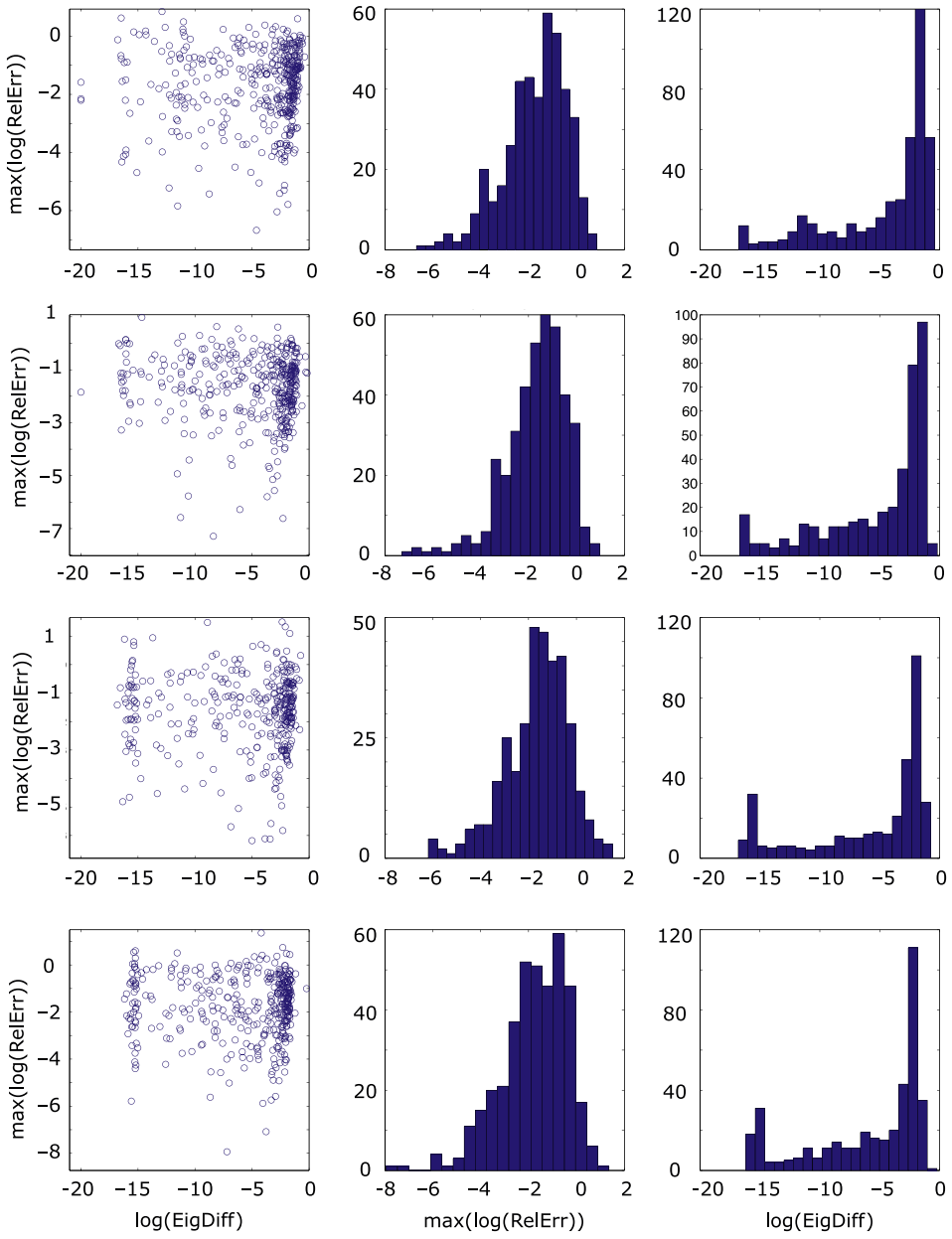

Fig. D.3. Comparisons of the temporal behaviour of the extended model S_E and the aggregate model S_A for varying parameter combinations. Top row: 3 aggregated nodes, 423 data points. Second row: 7 aggregated nodes, 394 data points. Third row: 14 aggregated nodes, 352 data points. Bottom row: 24 aggregated nodes, 392 data points. Left panels: scatterplot of $RelErr(y, z)$ vs. $EigDiff(z^*; S_E, S_A)$ for 423 parameter sets. Middle panels: the distribution of $RelErr(y, z)$. Right panels: the distribution of $EigDiff(z^*; S_E, S_A)$. Parameter values and details about the simulations are given in the text.

they approach a limit cycle. Also, there could be multistationarity in the systems such that the two solutions approach different final states.

Below follows a summary of the simulation details. The rate functions f_k, $k = 1, \ldots, n-1$, f_n^1 and f_n^2 were given by the function

$$f_i(z) = a_i B_j(Z_k, Z_l) - \gamma_i z_i, \tag{D.12}$$

where a_i and γ_i were scalars chosen at random from a uniform distribution over $(0, 1)$, and B_j is any of the 14 non-constant Boolean functions of two variables, chosen at random for each i, but equal for f_n^1 and f_n^2. The function

$$Z_k = \frac{z_k^{p_k} + h_k \theta_k^{p_k}}{z_k^{p_k} + \theta_k^{p_k}} \tag{D.13}$$

is the generalised Hill function derived from applying Boltzmann statistics to transcription regulation [43]. Note that here the superscripts are powers. The thresholds θ_k are chosen at random uniformly over $(0, 1)$, the steepness parameters p_k (equivalent to the Hill exponent) were picked from a uniform distribution of integers in $[1, 10]$, and the inverse fold changes h_k were also chosen at random from a uniform distribution over $(0, 1)$. The two inputs to the Boolean functions were chosen at random among the variables Z_i, but the same for r_n^1 and r_n^2. For each value of n we ran 500 simulations. For each parameter set, both solutions were started from the same randomly chosen initial point. The systems that did not converge to a stable point or in which the two systems approached different attractors, were disregarded. That left us with the number of cases mentioned in the caption of Fig. D.3.

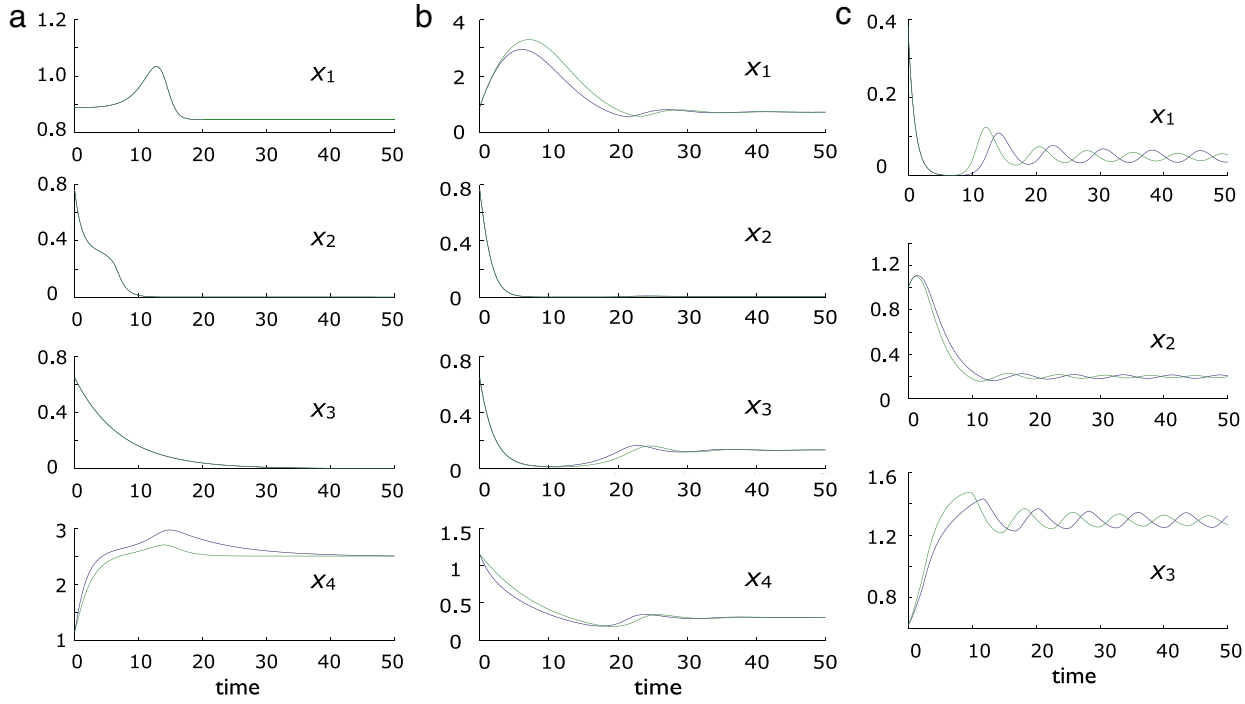

Fig. D.4. Selected examples of solution curves for extended systems (blue) and the corresponding aggregated systems (green). a: a typical case with $n = 4$ in which the two systems differ significantly in just one variable. b: a case with $n = 4$ of particularly bad similarity. In both cases, however, the two sets of curves are qualitatively similar, but the oscillations and dips are shifted in time. c: a system with $n = 3$ having a stable limit cycle for both systems. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Appendix E. The root of nonzero allele interaction values

The basic two-node system in Fig. 1a is in our standard notation given by the rate equations

$$\begin{aligned}\dot z_1^1 &= r_1^1(z_2) - \gamma_1^1 z_1^1,\\ \dot z_1^2 &= r_1^2(z_2) - \gamma_1^2 z_1^2,\\ \dot z_2 &= r_2(z_1) - \gamma_2 z_2,\end{aligned} \tag{E.1}$$

where $z_1 = z_1^1 + z_1^2$. For the μ-system in Fig. 1b the rate equations are

$$\begin{aligned}\dot z_1^1 &= r_1^1(z_2) - \gamma_1^1 z_1^1,\\ \dot z_1^2 &= r_1^2(\widetilde z_2) - \gamma_1^2 z_1^2,\\ \dot z_2 &= r_2(z_1^1 + \mu z_1^2) - \gamma_2 z_2,\\ \dot{\widetilde z}_2 &= r_2(\mu z_1^1 + z_1^2) - \gamma_2 \widetilde z_2,\end{aligned} \tag{E.2}$$

where $\mu \in [0, 1]$. By assumption the equilibrium conditions of Eq. (E.2) define unique stable equilibrium values $y_1^{12}(\mu) = y_1^{1|2}(\mu) + y_1^{2|1}(\mu)$, $y_2(\mu)$ and $\widetilde y_2(\mu)$. The allele interaction value is $\Delta_1^{12}(\mu) = y_1^{12}(\mu) - y_1^{1|\circ}(\mu) + y_1^{2|\circ}(\mu)$. Using implicit differentiation, doing some straightforward algebra and finally taking the limit $\mu \to 0$, we find

$$\lim_{\mu\to 0} \frac{\mathrm{d}\Delta_1^{12}(\mu)}{\mathrm{d}\mu} = \frac{u_1^2 u_2}{\gamma_1^2\gamma_2 - u_1^2 u_2} y_1^1 + \frac{u_1^1 u_2}{\gamma_1^1\gamma_2 - u_1^1 u_2} y_1^2, \tag{E.3}$$

where u_1^α and u_2 represent the derivatives of the corresponding dose-response functions with respect to their argument. Because $\Delta_1^{12}(0) = 0$ (see the main text), it follows that for arbitrarily small $\mu > 0$ the μ-system has a nonzero allele interaction value. We may conclude that in general, this is true also for $\mu = 1$, in which case the μ-system is equivalent to the basic system defined by Eqs. (E.1).

If the arrow from X_1^2 to X_2 in Fig. 1b is missing, the loop $X_1^1 \to \widetilde X_2 \to X_1^2 \to X_2 \to X_1^1$ is broken, and there is no longer a regulatory loop in the system. In this case the subsystem X_1^2, X_2 does not act on the two other nodes, and $\lim_{\mu\to 0} \mathrm{d}\Delta_1^{12}(\mu)/\mathrm{d}\mu$ no longer depends on y_1^2. Only the first term in Eq. (E.3) remains, and the conclusion is still valid.

Appendix F. Proof of Lemma 1

Proof. We adapt the numbering such that $x_1 \le x_2$. The intersections between the curves $y = \phi_i(x)$ and $y = \gamma_i x$, $i = 1, 2$, and $y = \phi_1(x) + \phi_2(x)$ and $y = \gamma x$ define the solutions x_1, x_2 and x_{12}, respectively. We consider three cases separately.

1. *The case* $\phi_i'(x) < 0$, $i = 1, 2$. Assume $x_{12} \ge x_1 + x_2$. Then

   $$\begin{aligned}\gamma x_{12} &= \phi_1(x_{12}) + \phi_2(x_{12}) \le \phi_1(x_1 + x_2) + \phi_2(x_1 + x_2)\\ &< \phi_1(x_1) + \phi_2(x_2) = \gamma_1 x_1 + \gamma_2 x_2 = \gamma(x_1 + x_2),\end{aligned}$$

   contradicting the assumption. Thus, $x_{12} < x_1 + x_2$.

   Then assume $x_{12} \le x_1$. This leads to

   $$\begin{aligned}\gamma x_{12} &\ge \phi_1(x_1) + \phi_2(x_1)\\ &> \phi_1(x_1) + \phi_2(x_2) = \gamma(x_1 + x_2) > \gamma x_{12},\end{aligned}$$

   which is impossible. Thus, $x_{12} > x_1$. In passing we note that it is not possible to draw a definite conclusion about which is the larger of γ_1 and γ_2.
2. *The case* $0 < \phi_i'(x) < \gamma_i$, $i = 1, 2$. In this case, too, existence of x_{12} has to be assumed. The three curves $y = \phi_1(x)$, $y = \phi_2(x)$, and $y = \phi_{12}(x)$ intersect the lines $y = \gamma_1 x$, $y = \gamma_2 x$ and $y = \gamma x$, respectively, from above as in case 1. Then $\phi_{12}(x_1 + x_2) > \phi_1(x_1) + \phi_2(x_2) = \gamma_1 x_1 + \gamma_2 x_2 = \gamma(x_1 + x_2)$. This implies that in $x = x_1 + x_2$, $\phi_{12}(x) > \gamma x$, from which it follows that in this point the curve $y = \phi_{12}(x)$ lies above the line $y = \gamma x$. Therefore the intersection x_{12} lies to the right of $x_1 + x_2$, i.e. $x_{12} > x_1 + x_2$.
3. *The case* $\phi_i'(x) > \gamma_i$, $i = 1, 2$. The existence of x_{12} is not ensured, but we have assumed it exists. In this case $\phi_{12}'(x) > \gamma_1 + \gamma_2 > \gamma$, and the three curves intersect the corresponding lines from

below. As $\phi_{12}(x) > \phi_i(x)$, it follows that $\phi_i(x_i) > \gamma_i x_i$. Assume $\gamma_1 < \gamma_2$. Thus, in both points x_1 and x_2, $\phi_{12}(x) > \gamma x$. This implies that $x_{12} < x_1$, $x_{12} < x_2$, thus $x_{12} < x_1 + x_2$. The case $\gamma_2 < \gamma_1$ goes likewise. □

References

[1] L.H. Hartwell, J.J. Hopfield, S. Leibler, A.W. Murray, From molecular to modular cell biology, Nature 402 (1999) C47–C52.
[2] D.A. Lauffenburger, Cell signaling pathways as control modules: complexity for simplicity? Proc. Natl. Acad. Sci. USA 97 (2000) 5031–5033.
[3] B. Hoogendoorn, S.L. Coleman, C.A. Guy, K. Smith, T. Bowen, P.R. Buckland, M.C. O'Donovan, Functional analysis of human promoter polymorphisms, Hum. Mol. Genet. 12 (2003) 2249–2254.
[4] N.H. Gehring, U. Frede, G. Neu-Yilik, P. Hundsdoerfer, B. Vetter, M.W. Hentze, A.E. Kulozik, Increased efficiency of mRNA 3′ end formation: a new genetic mechanism contributing to hereditary thrombophilia, Nat. Genet. 28 (2001) 389–392.
[5] J. Peng, E.L. Murray, D.R. Schoenberg, The poly(A)-limiting element enhances mRNA accumulation by increasing the efficiency of pre-mRNA 3′ processing, RNA 11 (2005) 958–965.
[6] R.L. Wang, A. Stec, J. Hey, L. Lukens, J. Doebley, The limits of selection during maize domestication, Nature 398 (1999) 236–239.
[7] N. Rosenfeld, J.W. Young, U. Alon, P.S. Swain, M.B. Elowitz, Gene regulation at the single-cell level, Science 307 (2005) 1962–1965.
[8] A.E. Mayo, Y. Setty, S. Shavit, A. Zaslaver, U. Alon, Plasticity of the *cis*-regulatory input function of a gene, PLoS Biol. 4 (2006) e45.
[9] J. Duan, M.A. Antezana, Mammalian mutation pressure, synonymous codon choice, and mRNA degradation, J. Mol. Evol. 57 (2003) 694–701.
[10] F. Capon, M.H. Allen, M. Ameen, A.D. Burden, D. Tillman, J.N. Barker, R.C. Trembath, A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups, Hum. Mol. Genet. 13 (2004) 2361–2368.
[11] J.V. Chamary, L. Hurst, Evidence for selection on synonymous mutations affecting stability of mRNA secondary structure in mammals, Genome Biol. 6 (2005) R75.
[12] F.C. Jones, M.G. Grabherr, Y.F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M.C. Zody, S. White, E. Birney, S. Searle, J. Schmutz, J. Grimwood, M.C. Dickson, R.M. Myers, C.T. Miller, B.R. Summers, A.K. Knecht, S.D. Brady, H. Zhang, A.A. Pollen, T. Howes, C. Amemiya, E.S. Lander, F. Di Palma, K. Lindblad-Toh, D.M. Kingsley, The genomic basis of adaptive evolution in threespine sticklebacks, Nature 484 (2012) 55–61.
[13] S.W. Omholt, E. Plahte, L. Øyehaug, K.F. Xiang, Gene regulatory networks generating the phenomena of additivity, dominance and epistasis, Genetics 155 (2000) 969–980.
[14] A.B. Gjuvsland, E. Plahte, T. Ådnøy, S.W. Omholt, Allele interaction—single locus genetics meets regulatory biology, PLoS One 5 (2010) e9379.
[15] G. Gibson, Epistasis and pleiotropy as natural properties of transcriptional regulation, Theor. Popul. Biol. 49 (1996) 58–89.
[16] A.L. Tyler, F.W. Asselbergs, S.M. Williams, J.H. Moore, Shadows of complexity: what biological networks reveal about epistasis and pleiotropy, Bioessays 31 (2009) 220–227.
[17] R. Thomas, R. D'Ari, Biological Feedback, CRC Press, Boca Raton, USA, 1990.
[18] R. Thomas, M. Kaufman, Multistationarity, the basis of cell differentiation and memory, II, logical analysis of regulatory networks in terms of feedback circuits, Chaos 11 (2001) 180–195.
[19] S.M. Welch, Z.S. Dong, J.L. Roe, S. Das, Flowering time control: gene network modelling and the link to quantitative genetics, Aust. J. Agric. Res. 56 (2005) 919–936.
[20] S.W. Omholt, From Bean-Bag Genetics to Feedback Genetics: Bridging the Gap Between Regulatory Biology and Classical Genetics, Landes Bioscience, Georgetown, Texas, USA, 2006.
[21] J.S. Maybee, D.D. Olesky, P. van den Driessche, G. Wiener, Matrices, digraphs and determinants, Technical Report, University of Colorado, 1987.
[22] H. de Jong, Modeling and simulation of genetic regulatory systems: a literature review, J. Comput. Biol. 9 (2002) 67–104.
[23] P. Brazhnik, A. de la Fuente, P. Mendes, Gene networks: how to put the function in genomics, Trends Biotechnol. 20 (2002) 467–472.
[24] E. Plahte, T. Mestl, S.W. Omholt, Feedback loops, stability and multistationarity in dynamical systems, JBS 3 (1995) 409–413.
[25] C. Soulé, Graphic reguirements for multistationarity, ComPlexUs 1 (2003) 123–133.
[26] J.S. Maybee, Principal minor determinant formulas, Technical Report CU-CS-033-73, University of Colorado, 1973.
[27] O. Radulescu, S. Lagarrigue, A. Siegel, P. Veber, M. Le Borgne, Topology and static response of interaction networks in molecular biology, J. R. Soc. Interface 3 (2006) 185–196.
[28] J.E. Marsden, M.J. Hoffman, Elementary Classical Analysis, second ed., W.H. Freeman, New York, 1993.
[29] I.W. Duncan, Transvection effects in *Drosophila*, Annu. Rev. Genet. 36 (2002) 521–556.
[30] B.N. Kholodenko, A. Kiyatkin, F.J. Bruggeman, E. Sontag, H.V. Westerhoff, J.B. Hoek, Untangling the wires: a strategy to trace functional interactions in signaling and gene networks, Proc. Natl. Acad. Sci. USA 99 (2002) 12841–12846.
[31] G. Giaever, A.M. Chu, L. Ni, C. Connelly, L. Riles, S. Veronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. Andre, A.P. Arkin, A. Astromoff, M. El Bakkoury, R. Bangham, R. Benito, S. Brachat, S. Campanaro, M. Curtiss, K. Davis, A. Deutschbauer, K.-D. Entian, P. Flaherty, F. Foury, D.J. Garfinkel, M. Gerstein, D. Gotte, U. Guldener, J.H. Hegemann, S. Hempel, Z. Herman, D.F. Jaramillo, D.E. Kelly, S.L. Kelly, P. Kotter, D. LaBonte, D.C. Lamb, N. Lan, H. Liang, H. Liao, L. Liu, C. Luo, M. Lussier, R. Mao, P. Menard, S.L. Ooi, J.L. Revuelta, C.J. Roberts, M. Rose, P. Ross-Macdonald, B. Scherens, G. Schimmack, B. Shafer, D.D. Shoemaker, S. Sookhai-Mahadeo, R.K. Storms, J.N. Strathern, G. Valle, M. Voet, G. Volckaert, C.-Y. Wang, T.R. Ward, J. Wilhelmy, E.A. Winzeler, Y. Yang, G. Yen, E. Youngman, K. Yu, H. Bussey, J.D. Boeke, M. Snyder, P. Philippsen, R.W. Davis, M. Johnston, Functional profiling of the *Saccharomyces cerevisiae* genome, Nature 418 (2002) 387–391.
[32] J.S. Mattick, RNA regulation: a new genetics? Nat. Rev. Genet. 5 (2004) 316–323.
[33] O. Hobert, Gene regulation by transcription factors and microRNAs, Science 319 (2008) 1785–1786.
[34] E.V. Makeyev, T. Maniatis, Multilevel regulation of gene expression by microRNAs, Science 319 (2008) 1789–1790.
[35] Y. Shimoni, G. Friedlander, G. Hetzroni, G. Niv, S. Altuvia, O. Biham, H. Margalit, Regulation of gene expression by small non-coding RNAs: a quantitative view, Mol. Syst. Biol. 3 (2007).
[36] F.R. Gantmacher, The Theory of Matrices, AMS Chelsea Publishing, Providence, Rhode Island, 2000.
[37] L. Bintu, N.E. Buchler, H.G. Garcia, U. Gerland, T. Hwa, J. Kondev, T. Kuhlman, R. Phillips, Transcriptional regulation by the numbers: applications, Curr. Opin. Genet. Dev. 15 (2005) 125–135.
[38] L. Bintu, N.E. Buchler, H.G. Garcia, U. Gerland, T. Hwa, J. Kondev, R. Phillips, Transcriptional regulation by the numbers: models, Curr. Opin. Genet. Dev. 15 (2005) 116–124.
[39] N.E. Buchler, U. Gerland, T. Hwa, On schemes of combinatorial transcription logic, Proc. Natl. Acad. Sci. USA 100 (2003) 5136–5141.
[40] A.M. Sengupta, M. Djordjevic, B.I. Shraiman, Specificity and robustness in transcription control networks, Proc. Natl. Acad. Sci. USA 99 (2002) 2072–2077.
[41] P.H. von Hippel, O.G. Berg, On the specificity of DNA-protein interactions, Proc. Natl. Acad. Sci. USA 83 (1986) 1608–1612.
[42] L.N. Trefethen, M. Embree, Spectra and Pseudospectra: The Behavior of Nonnormal Matrices and Operators, Princeton University Press, Princeton, NJ, 2005.
[43] N.E. Buchler, U. Gerland, T. Hwa, Nonlinear protein degradation and the function of genetic circuits, Proc. Natl. Acad. Sci. USA 102 (2005) 9559–9564.