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A new necessary condition on interaction graphs for multistationarity

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Abstract

We consider a dynamical system, described by a system of ordinary differential equations, and the associated interaction graphs, which are defined using the matrix of signs of the Jacobian matrix. After stating a few conjectures about the role of circuits in these graphs, we prove two new results relating them to the dynamic behaviour of the system: a sufficient condition for qualitative unstability, and a necessary condition for the existence of several stationary states. These results are illustrated by examples of regulatory modules in two variables, such as those occurring in biological networks.
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1. Introduction

In this paper we study the qualitative properties of a dynamical system, described by a system of ordinary differential equations. For that purpose, we consider the interaction graphs attached to the system. These are defined using the signs of the entries of the Jacobian matrix. Several conjectures have been stated and/or proven in the past which relate circuits in these graphs to the behaviour of the corresponding dynamical system. We add here two new results. One is about the stability of a stationary state and the other is a new criterion for the existence of several stationary states.

Our interest in these results comes from biology: multistationarity in cellular networks can be viewed as a rationale for decision making and differentiation. This is explained in the next section, where we discuss how our problem originated from biological considerations. Then, after introducing some definitions, we formulate four conjectures relating circuits to the qualitative behaviour of a dynamical system. We discuss an example illustrating these conjectures and we summarize what is known about them.

The third section contains the statement of our main results. Theorem 1 gives a sufficient condition for a stationary point to be (strongly) unstable, and Theorem 2 gives a necessary condition for the existence of several nondegenerate stationary points. These theorems are proved in the Appendix. The case of two variables is discussed in detail.

In the fourth section we show with examples that the hypotheses of Theorem 2 cannot be weakened. Finally, we discuss in Section 5 how our results can be applied to a positive feedback system similar to those encountered in developmental Biology.

2. Some conjectures in nonlinear dynamics

2.1. Biological background

Epigenetic differences are those differences that are transmissible from cell to cell generation in the absence of any genetic difference. It has become clear for some time (Briggs and King, 1952; Wilmut et al., 1997) that with few exceptions all the cells of an organism contain all the genes of that organism. Thus, cell differentiation is essentially an epigenetic process.

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In a short but historical note, Delbrück (1949) mentioned (in other words) that epigenetic differences, including those involved in cell differentiation, can be understood in terms of a more general process, namely multistationarity. Multistationarity takes place when a system displays multiple steady states.

This urged us to identify the formal requirements of multistationarity and other nontrivial behaviour. We soon realized that the common point between all the biological systems that display multistationarity (e.g. Novick and Weiner, 1957; Cohn and Horibata, 1959; Monod and Jacob, 1961; Eisen et al., 1967; Kauffman, 1973) is the presence of a positive circuit in their underlying logic. In fact all these examples imply a switch from a "rest" state to an alternative latent state and thus point to the existence of a cellular memory based on a positive circuit. It was subsequently concluded (Thomas, 1981) that the presence of a positive circuit is not simply a convenient way to realize multistationarity, but is in fact a necessary condition for its occurrence. This conjecture was submitted to formal proofs in Plahte et al. (1995), Snoussi (1998), Gouzé (1998), Cinquin and Demongeot (2002) and Soulé (2003).

Of special relevance for the mechanisms of cell differentiation is the fact that a gene whose expression is under direct or indirect positive control of its own product can be switched on lastingly by a transient signal. This explains that during cell differentiation a gene can be switched on by the product of another gene and remains on after the disappearance of this product. On the other hand, in order to account for various cell types in terms of multiple steady states, one has to account for many steady states. As discussed in Kaufman and Thomas (1987) and Thomas and Richelle (1988), many regulatory interactions (and their composition) are sigmoid (or stepwise) in shape and a positive circuit thus usually results in no more than three steady states, two of which can be stable. However, m positive circuits can generate up to 3^m steady states, 2^m of which can be stable. Thus, eight genes under positive auto-control might suffice to generate $2^8 = 256$ cell types. More generally, *many* steady states (and thus many potential cell types) can be generated by *several* positive circuits.

As briefly mentioned above, positive circuits are involved in cellular memory. For more recent examples, see, e.g. Acar et al. (2005) and Sha et al. (2003). Moreover, one can reason that whenever a set of neurons are connected into a positive circuit, this set will usually persist in a rest state, but any signal that can move it away from this state will lead it to the alternative complementary state of the circuit (Demongeot et al., 2000; Tonnelier et al., 1999). In other words, such a simple network can evocate a latent state that is kept as a memory. In the immune system as well, differentiation and memory can be understood in terms of positive circuits between the various types of cells involved (see Kaufman et al., 1985; Kaufman and Thomas, 1987; Segel, 1998; Yates et al., 2004).

Negative circuits also play a fundamental role in biology. That homeostasis (already recognized by Claude Bernard as *elasticity*) operates, with or without oscillations, like a thermostat or a Watt regulator, has been understood for many years, and many authors (e.g. Szekely, 1965) have suggested an implication of what we now call *negative circuits* in these processes. A further step consisted of conjecturing that negative circuits are not only involved in homeostasis and periodicity, but are in fact a necessary condition of this type of behaviour (Thomas, 1981).

Although all biological processes are complex and involve many variables, essential qualitative features of these processes can usually be understood in terms of a small number of crucial variables. This view is strongly supported by the observation that extremely complex behaviour can arise from simple combinations of positive and negative circuits. In particular, we will deal with small modules (or "regulons") whose consideration can be of interest in various domains. For example, it has become clear recently that such complex behaviours as deterministic chaos can take place in the presence of one positive and one negative circuit, and even in the presence of a single circuit, provided this circuit can be positive or negative depending on the values of relevant variables (Thomas, 1999).

2.2. Definitions

As explained in the previous section, the biological examples led us to propose several mathematical conjectures relating the behaviour of a dynamical system to the topology of its interaction graph. To state them precisely, we first need to introduce some terminology.

Given a positive integer n, we consider a differentiable map $F : \mathbb{R}^n \to \mathbb{R}^n$, i.e. a collection $F = (f_1, \ldots, f_n)$ of n differentiable maps $f_i(x_1, \ldots, x_n)$, $1 \leqslant i \leqslant n$ (see 3.1 below). We are interested in the dynamical system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = F(x), \tag{1}$$

where $x = (x_1(t), \ldots, x_n(t))$ is a trajectory in the n-dimensional Euclidean space.

The *interaction graph* $G(x)$ of F at the point $x \in \mathbb{R}^n$ is the finite oriented graph with $\{1, \ldots, n\}$ as set of vertices and such that there is a positive (resp. negative) arrow from j to i if and only if the partial derivative $(\partial f_i/\partial x_j)(x)$ is positive (resp. negative). Each edge in $G(x)$ is thus both oriented and endowed with a sign. The variable x is viewed as the *phase space location* of the graph $G(x)$.

A *circuit* in the graph $G(x)$ is a sequence of distinct vertices $i_1, i_2, \ldots, i_k$ such that there is an edge from i_α to $i_{\alpha+1}$, $1 \leqslant \alpha \leqslant k-1$, and from i_k to i_1.

The *sign of a circuit* is the product of the signs of its edges.

A circuit is thus determined by a set of nonzero coefficients in the *Jacobian matrix* $J(x) = ((\partial f_i/\partial x_j)(x))$ whose rows and columns are in cyclic permutation. Its sign is the sign of the product of these coefficients.

Several circuits are called *disjoint* when they do not share any vertex.

A *nucleus* is a union of one or more disjoint circuits which involves all the vertices of $G(x)$ (this was called an "Hamiltonian hooping" in Soulé, 2003).

The *sign of a nucleus* is $(-1)^{p+1}$, where p is the number of positive circuits in the nucleus (this last definition is due to Eisenfeld and DeLisi, 1985).

A nucleus (or a circuit) is called *variable* when at least one of its edges display more than one sign according to the location in phase space.

A nucleus (or a circuit) is called *ambiguous* when its sign varies with the location in phase space.

2.3. The conjectures

Here are four conjectures that can be made about the behaviour of a dynamical system as above:

Conjecture 1 (*Thomas*). *The presence of a positive circuit (somewhere in phase space) is a necessary condition for multistationarity.*

Conjecture 2 (*Kaufman*). *Multistationarity requires either the presence of a variable nucleus or else the presence of two nuclei of opposite signs.*

Conjecture 3 (*Thomas*). *The presence of a negative circuit of length at least two (somewhere in phase space) is a necessary condition for stable periodicity.*

Conjecture 4 (*Thomas*). *A chaotic dynamics requires both a positive and a negative circuit.*

Remarks. In Conjectures 1 and 2 above, multistationarity means that there exist $a \neq b$ in $\mathbb{R}^n$ such that $F(a) = F(b) = 0$ when $\det J(a) \neq 0$ and $\det J(b) \neq 0$ (nondegenerate stationary points). Conjectures 1 and 3 have been formulated by Thomas (1981) and Conjecture 2 by Kaufman (Thomas and Kaufman, 2001) in slightly different forms.

In Conjecture 4 (Thomas, 1999), the idea is that a positive circuit is required to allow for multistationarity—at least in a subsystem, and a negative circuit (of length at least two) is required to allow for permanent periodicity. Notice that this double requirement can be achieved by a single circuit if it is ambiguous.

2.4. Proven results

Conjecture 1 was proved by Soulé, 2003 (see Plahte et al., 1995; Snoussi, 1998; Gouzé, 1998; Cinquin and Demongeot, 2002, for previous partial results).[1]

We shall prove below Conjecture 2 under some mild condition on F (Theorem 2), and we shall disprove it for a very special choice of F (see Section 4.2).

Conjecture 3 was proven by Snoussi (1998) and Gouzé (1998) under additional assumptions, including the fact that the graph $G(x)$ does not depend on x. We prove in Theorem 1 that the presence of a negative circuit in $G(x)$ is a necessary condition for a steady state x to be stable (see also Plahte et al., 1995). Note finally that the presence of an attractor implies that there exists a state x such that $G(x)$ contains a negative circuit of length one (see Appendix A.1)

The paper Toni et al. (1999), discusses Conjecture 4.

2.5. An example

Let us illustrate the conjectures with the extremely simple system:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \pm x^3 \pm y,$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \pm x \pm y,$$

which comprises three circuits: a loop on x, a loop on y and a circuit between x and y. The nonlinearity x^3 was chosen because its derivative is positive everywhere, and consequently none of the circuits can be ambiguous. For each choice of signs it is easy to determine the number, location and nature of the steady state(s) and to check the consistency of these results with the conjectures. For each case the steady states and trajectories are shown in Fig. 1.

Let us first consider system (A):

$$\mathrm{d}x/\mathrm{d}t = -x^3 + y,$$

$$\mathrm{d}y/\mathrm{d}t = -x - y,$$

for which the Jacobian matrix has signs: $\left(\begin{smallmatrix} - & + \\ - & - \end{smallmatrix} \right)$. All three circuits are negative. In the absence of any positive circuit, the system has only one steady state, as implied by the proof of Conjecture 1. This unique steady state is stable—a stable focus (Fig. 1A), as implied by the well-known criterion of qualitative stability (Eisenfeld and DeLisi, 1985, Section 3.1).

In systems (B) and (C) with matrices of signs $\left(\begin{smallmatrix} + & + \\ + & + \end{smallmatrix} \right)$ and $\left(\begin{smallmatrix} + & - \\ - & + \end{smallmatrix} \right)$, respectively, all the circuits are positive. Not surprisingly, these systems display multistationarity; there are three steady states, consistent with Conjecture 1. Since both circuits of length one are positive, they have no attractor and in particular no stable steady state. The conventional methods indeed show that the external steady states are unstable nodes and the internal one is a saddle point (Fig. 1B and C). As a matter of fact, this result can be obtained without any calculation, by considering that in system (B), for each of $x \ll 0$, $x = 0$ and $x \gg 0$, there is a nucleus whose representative product is dominant in absolute value. These "leading" nuclei are, respectively:

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[1] In Craciun and Feinberg, (2006a,b) and Craciun et al. (2006), chemical reaction networks satisfying the law of mass action are studied. A necessary condition is given for such a network to lead to multiple positive steady states. This condition is expressed in terms of a bipartite "species-reaction graph", which is different from the interaction graphs considered in this paper, and it is unrelated to the conditions given above.
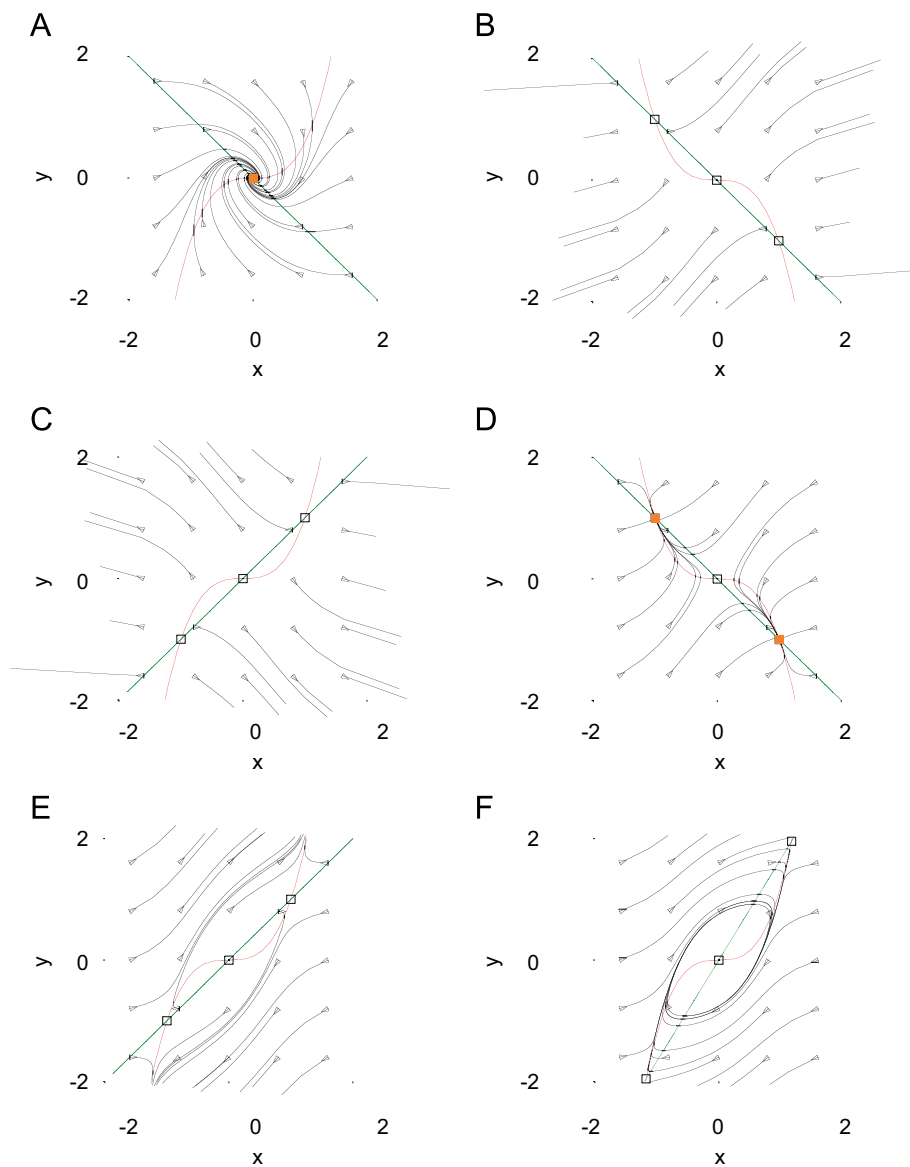
Fig. 1. Phase portraits for the different sign patterns of the example in Section 2.5. Stable steady states are indicated by filled squares, unstable ones by open squares.

$\begin{pmatrix} + & \\ & + \end{pmatrix}$, $\begin{pmatrix} & + \\ + & \end{pmatrix}$ and $\begin{pmatrix} + & \\ & + \end{pmatrix}$, and the corresponding steady states are, respectively, an unstable node, a saddle point, and an unstable node again.

Let us now reexamine these systems in terms of Conjecture 2. In system (A) all three circuits are negative and both nuclei are negative. The fact that the steady state is unique is consistent not only with Conjecture 1 (there is no positive circuit), but also with Conjecture 2 (the two nuclei have the same sign). In contrast, systems (B) and (C) comprise no negative circuit but they have two nuclei of opposite signs: the union of two positive circuits, which is a negative nucleus, and a positive 2-circuit. These systems have multiple steady states, consistent with both Conjectures 1 and 2. If, starting from system (B) where all the elements of the Jacobian matrix are positive, one changes the sign of one element of the Jacobian matrix, the two

nuclei have now the same sign, and the system has a single steady state, in agreement with Conjecture 2. If instead one changes the signs of two elements of the Jacobian matrix, the two nuclei have opposite signs and multistationarity is kept.

Note that when all four elements of the Jacobian matrix are negative: $\begin{pmatrix} - & - \\ - & - \end{pmatrix}$ (system (D)), the 2-circuit is positive. In this system, there are thus both positive and negative circuits, and there are both positive and negative nuclei. Not surprisingly, this system has multiple steady states, two of which are stable (Fig. 1D).

Of particular interest is system (E) with signs $\begin{pmatrix} - & + \\ - & + \end{pmatrix}$. In this case, there is one positive and two negative circuits, and the two nuclei have opposite signs. There are three steady states, consistent with Conjectures 1 and 2. Like in systems (B) and (C), all three steady states are unstable (Fig. 1E).

However, in contrast with systems (B) and (C), in which there are no attractors, the following system (F):

$$dx/dt = -x^3 + y,$$

$$dy/dt = -x + 0.6y,$$

which has the same sign pattern as (E), happens to be such that the intermediate unstable steady state is surrounded by a limit cycle (Fig. 1F), consistent with Conjecture 3 and with the presence of a negative circuit of length one.

One additional purpose of this section is to draw the attention on a possible confusion between the number of steady states (multistationarity) and the number of attractors. In the absence of any negative circuit one can well have multistationarity, but, as follows from the discussion in Section 2.4, there should not be any attractor.

3. Statement of the main results

3.1. More definitions

As in Section 2.2, we consider a map

$$F : \Omega = \mathbb{R}^n \to \mathbb{R}^n,$$

which is *differentiable*, i.e. such that, for each $i, j \in \{1, \ldots, n\}$ and any $a \in \Omega$, the ith component f_i of F has a partial derivative $(\partial f_i / \partial x_j)(a)$ at the point a and

$$f_i(x) = f_i(a) + \sum_{j=1}^n \frac{\partial f_i}{\partial x_j}(a)(x_j - a_j) + o(\|x - a\|),$$

where $\|x - a\|$ is the norm of $x - a$ and o is the Landau o-symbol.

For any $a \in \Omega$, the *Jacobian* of F at a is the n by n real matrix

$$J(a) = J(F)(a) = \left(\frac{\partial f_i}{\partial x_j}(a) \right).$$

For any $a \in \Omega$ we let

$$G(a) = G(J(a))$$

be the interaction graph of F at the point a. In other words, there is a positive (resp. negative) edge e with $o(e) = j$ and $t(e) = i$ in $G(a)$ if and only if $(\partial f_i / \partial x_j)(a)$ is positive (resp. negative).

Given any permutation $\sigma \in \Sigma_n$ and a point $a \in \Omega$ such that $\prod_{i=1}^n (\partial f_i / \partial x_{\sigma(i)})(a)$ does not vanish, we can define as follows a nucleus $C(\sigma)$ in $G(a)$. There is a unique decomposition

$$\{1, \ldots, n\} = I_1 \sqcup I_2 \sqcup \ldots \sqcup I_k$$

of $\{1, \ldots, n\}$ into a disjoint union of nonempty subsets such that the restriction σ_α of σ to I_α is a *cyclic permutation* for all $\alpha = 1, \ldots, k$. Let $C(\sigma_\alpha)$ be the circuit of G with edges $(i, \sigma_\alpha(i))$, $i \in I_\alpha$. We denote by $C(\sigma)$ the nucleus of G which is the disjoint union of the circuits $C(\sigma_\alpha)$, $\alpha = 1, \ldots, k$.

We say that G has a *variable* nucleus if there exists two points $a \neq b$ in $\mathbb{R}^n$ and a permutation $\sigma \in \Sigma_n$ such that both

$G(a)$ and $G(b)$ contain a nucleus $C(\sigma)$, but there exists an edge in $C(\sigma)$ with opposite signs in $G(a)$ and $G(b)$. In other words, $\prod_{i=1}^n (\partial f_i / \partial x_{\sigma(i)})(a)$ and $\prod_{i=1}^n (\partial f_i / \partial x_{\sigma(i)})(b)$ are nonzero, and there exists $i \in \{1, \ldots, n\}$ such that $(\partial f_i / \partial x_{\sigma(i)})(a)$ and $(\partial f_i / \partial x_{\sigma(i)})(b)$ have opposite signs.

3.2. The statements

Given F as above, we let $\mathscr{F}$ be the finite set of functions from $\mathbb{R}^n$ to $\mathbb{R}$ of the form $\pm \prod_{i \in I} (\partial f_i / \partial x_{\tau(i)})$, where $I \subset \{1, \ldots, n\}$ is any subset and $\tau : I \to \{1, \ldots, n\}$ is any injective map. We shall consider the following condition:

(C) Given two functions f and g in $\mathscr{F}$ such that f is not identically zero and g is strictly positive somewhere in $\mathbb{R}^n$, there exists $x \in \mathbb{R}^n$ such that $f(x) \neq 0$ and $g(x) > 0$.

Condition (C) is very often fulfilled. This is the case for instance when all the components f_i of F are polynomials or quotients of polynomials or, more generally, when they are real analytic. Indeed, in that case, the zero-locus of f has codimension one, therefore its complement meets the open set where g is positive.

To get an example of two functions f and g in one variable which satisfy the hypothesis of (C) and not the conclusion, let $f(x) = x^2$ when $x < 0$, $f(x) = 0$ when $x \geqslant 0$, $g(x) = 0$ when $x \leqslant 0$, and $g(x) = x^2$ when $x > 0$. Both f and g are differentiable on the real line and positive somewhere, but there does not exist any $x \in \mathbb{R}$ such that $f(x) \neq 0$ and $g(x) > 0$. See Section 4.2 below for a similar construction.

A point $a \in \mathbb{R}^n$ is a *nondegenerate zero* of F (i.e. a *stationary point*) when $F(a) = 0$ and $\det J(a) \neq 0$. Such a point is called *strongly unstable* when the matrix $J(a)$ has a positive eigenvalue.

Our main results are the following :

Theorem 1. *Assume that a is a nondegenerate zero of F and that the graph $G(a)$ contains only positive circuits. Then a is strongly unstable.*

Theorem 2. *Assume that F has two nondegenerate zeroes and that condition (C) is satisfied. Then:*

(a) *either there is $a \in \Omega = \mathbb{R}^n$ such that $G(a)$ has two nuclei with opposite signs;*
(b) *or G has a variable nucleus.*

Theorems 1 and 2 are proved in Appendix A.2 and A.3, respectively.

Remarks. (i) Let $\Omega = \prod_{i=1}^n]a_i, b_i[$ be a product of open real intervals, with $a_i \geqslant -\infty$ and $b_i \leqslant +\infty$, and let $F : \Omega \to \mathbb{R}^n$ be a differentiable function. Theorem 2 remains valid in that situation (with $a \in \Omega$ in case (a)), see below Appendix A.4.

(ii) Assume $G(a) = G$ does not depend on $a \in \Omega$. Then condition (C) is satisfied and (b) cannot happen, therefore Theorem 2 states that if F has two nondegenerate zeroes
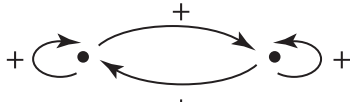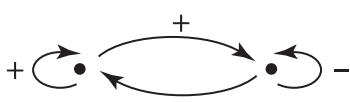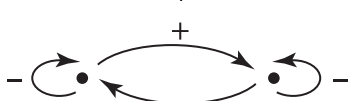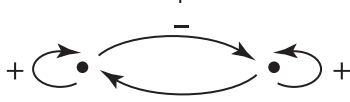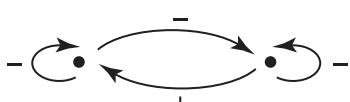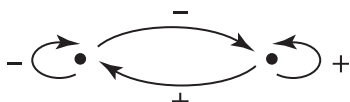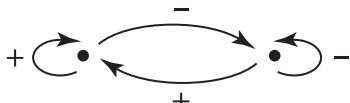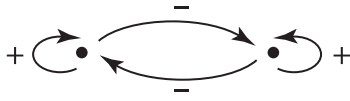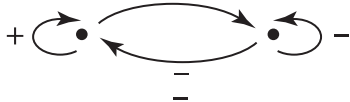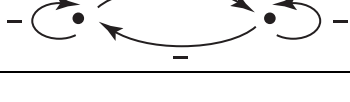
then G has two nuclei with opposite signs. See also Soulé (2003, Theorem 4).

3.3. The case $n = 2$:

Let us specialize further to the case where $n = 2$, assuming that $G(a) = G$ does not depend on $a \in \Omega$, and that G has four arrows. Up to symmetry, there are 10 possible such graphs, which are listed in Table 1 below. In this table we indicate what is known about multistationarity and qualitative stability for the different types of graphs. For instance, a graph of type one contains both a positive and a negative nucleus, therefore it may have several stationary states. These are unstable because the trace of the Jacobian matrix is positive (or by the criterion of Eisenfeld and DeLisi, 1985, Theorem 3.5 which gives, in any dimension, a sufficient condition for qualitative unstability). Both nuclei in the graph of type two are positive, therefore, by Theorem 2, there can be at most one stationary state, which will be unstable by Eisenfeld and DeLisi (1985, Theorem 3.5), i.e. because the determinant of the Jacobian matrix is negative. The nuclei in the graph of type three have opposite signs, hence it may have several stationary states. Nothing can be said a priori on the stability of these states. The case of type four is similar to type two. Both nuclei of the graph of type five are negative,

Table 1
Regulons with two variables

Type	Interaction graph	Number of steady states	Stability
1		Possibly > 1	Unstable
2		⩽ 1	Unstable
3		Possibly > 1	Unknown a priori
4		⩽ 1	Unstable
5		⩽ 1	Stable
6		Possibly > 1	Unknown a priori
7		Possibly > 1	Unknown a priori
8		Possibly > 1	Unstable
9		⩽ 1	Unstable
10		Possibly > 1	Unknown a priori

therefore, by Theorem 2 (or by Conjecture 1), this module contains at most one stationary state. This state is stable because the trace of the Jacobian matrix is negative and its determinant is positive (or by the criterion for qualitative stability of May, 1974; Quirk and Ruppert, 1965, Eisenfeld and DeLisi, 1985, Section 3.1). The other types in Table 1 can be discussed by similar arguments.

4. Additional mathematical comments

In this section we show that stronger versions of Theorem 2 do not hold.

4.1. The graph G can be variable without being ambiguous

When $n = 2$, it was shown in Soulé (2003, Theorem 2), that the statement (b) in Theorem 2 can be replaced by the stronger assertion that G has an *ambiguity*: there is $a, b \in \mathbb{R}^n$ and a positive circuit in $G(a)$ which is negative in $G(b)$.

This is not always the case when $n \geqslant 3$. In case (b) of Theorem 2 the nucleus $C(\sigma)$ may keep the same sign in $G(a)$ for all $a \in \mathbb{R}^n$ (although some of its edges will change sign). For instance, $C(\sigma)$ may be negative. Then, by Theorem 1 in Soulé (2003), there is also $a \in \mathbb{R}^n$ and a positive circuit C in $G(a)$, different from $C(\sigma)$.

Here is an example. Let x, y, z be the coordinates of $\mathbb{R}^3$ and $F = (f_1, f_2, f_3) : \Omega \to \mathbb{R}^3$ be the map defined by

$$f_1(x, y, z) = xy^2 + (2x - 1)z + x - \tfrac{5}{4},$$

$$f_2(x, y, z) = -x^2 y - y + 2,$$

$$f_3(x, y, z) = -x^2 + x + 2,$$

where Ω is the product of open intervals

$$-1 - \varepsilon < x < 2 + \varepsilon, \quad \tfrac{2}{5} - \varepsilon < y < 1 + \varepsilon, \quad -\tfrac{14}{25} - \varepsilon < z < -\tfrac{38}{75} + \varepsilon,$$
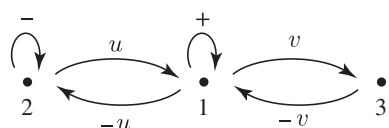
and $\varepsilon > 0$ is a very small constant. The map F has two zeroes in Ω:

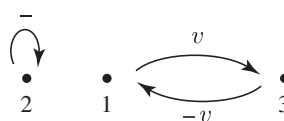$$F\left(-1, 1, -\tfrac{14}{25}\right) = F\left(2, \tfrac{2}{5}, -\tfrac{38}{75}\right) = 0.$$

Its Jacobian matrix is

$$J(F) = \begin{pmatrix} y^2 + 2z + 1 & 2xy & 2x - 1 \\ -2xy & -x^2 - 1 & 0 \\ -2x + 1 & 0 & 0 \end{pmatrix}.$$

Therefore the zeroes of F are nondegenerate. Since ε is small, $y^2 + 2z + 1$ is positive in Ω. When $a = (x, y, z) \in \Omega$, the graph $G(a)$ is the following:

where u is the sign of $2xy$ and v is the sign of $2x - 1$. The only nucleus of $G(a)$ is

which is negative. It is variable but G is not ambiguous. As implied by Conjecture 1 (proved in Soulé, 2003), G contains a positive circuit, namely

Note that this circuit is not contained in the previous nucleus.

By a change of variables as in Appendix A.4, we get a map $\mathbb{R}^3 \to \mathbb{R}^3$ with the same iteration graph as F above.

4.2. Conjecture 2 is not true in full generality

Theorem 2 and Conjecture 2 are not valid for an arbitrary differentiable map $F : \mathbb{R}^n \to \mathbb{R}^n$, without assuming (C).

For example, let $n = 2$ and consider the map $F : \mathbb{R}^2 \to \mathbb{R}^2$ defined by the formula

$$F(x, y) = (\psi(x), y\varphi(x)),$$

where

$$\psi(x) = \begin{cases} (x - 1)^2 - 1 & \text{when } x \geqslant 0, \\ 1 - (x + 1)^2 & \text{when } x \leqslant 0, \end{cases}$$

and

$$\varphi(x) = \begin{cases} (x - 1)^2 & \text{when } x \geqslant 1, \\ 0 & \text{when } -1 \leqslant x \leqslant 1, \\ (x + 1)^2 & \text{when } x \leqslant -1. \end{cases}$$

This map is differentiable (as can be seen from the definition in 3.1). Its Jacobian matrix is

$$JF = \begin{pmatrix} \psi'(x) & 0 \\ y\varphi'(x) & \varphi(x) \end{pmatrix}.$$

Note that the function $-\psi'(x)$ is strictly positive when $-1 < x < 1$ and $\varphi(x)$ is not identically zero, but $-\psi'(x)\varphi(x)$ is nowhere positive. Therefore (C) does not hold. The interaction graph $G(x, y)$ is
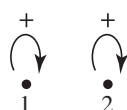
when $x < -1$ or $x > 1$,

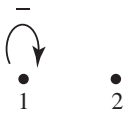when $-1 < x < 1$, and

when $x = \pm 1$. Therefore neither (a) nor (b) in Theorem 2 is true. On the other hand, F has two nondegenerate zeroes (which lie in different domains of definition of F) :

$$F(2,0) = F(-2,0) = 0.$$

Therefore Conjecture 2 does not hold for F.

5. A regulatory module

When a dynamical system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = F(x)$$

as (1) is used to model a biological network, each coordinate $x_i(t)$, $1 \leq i \leq n$, of x is usually positive or zero and represents the concentration at time t of a given item (for instance a protein, resp. a cell type) which, in addition to interacting with itself and other constituents of the system, undergoes a spontaneous decay, due to degradation or dilution (via cell growth for proteins, resp. cell death for cell types). This means that each component $f_i(x)$ of $F(x)$ can be written

$$f_i(x) = g_i(x) - \gamma_i x_i, \qquad (2)$$

where γ_i, the degradation rate, is a positive constant. In most realistic models, there exists $x \in \mathbb{R}^n$ such that

$$\frac{\partial f_i}{\partial x_i}(x) = \frac{\partial g_i}{\partial x_i}(x) - \gamma_i < 0$$

for all $i = 1, \ldots, n$. In that case, the interaction graph $G(x)$ contains a negative loop e_i at each of its vertices $i = 1, \ldots, n$. As explained in Appendix A.3 below, under condition (C), Theorem 2 is then a direct consequence of Conjecture 1 (proved in Soulé, 2003). The idea of the argument is the following: either the diagonal terms in the Jacobian are everywhere negative, in which case the presence somewhere of a positive circuit ensures the existence of two nuclei of opposite signs; or the diagonal terms change signs, in which case there is a variable nucleus.

On the other hand, we may consider a product of open intervals $\Omega = \prod_{i=1}^{n}]a_i, b_i[$ such that each function $(\partial g_i / \partial x_i)(x) - \gamma_i$, $i = 1, \ldots, n$, and every nucleus has a fixed sign in Ω. We can then apply Theorem 2 to the restriction of F to Ω and get interesting information about the possibility of several stationary points in Ω.
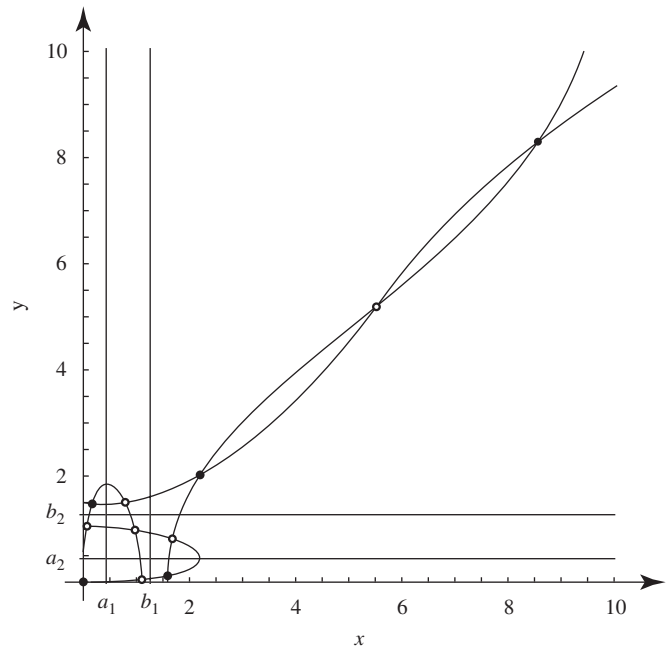

Fig. 2. Nullclines corresponding to the regulatory module of Section 5. The intersections of these nullclines provide eleven steady states. Stable steady states are indicated by black circles, unstable ones by open circles. The vertical and horizontal lines partition phase space into nine domains in which the nucleus formed by the diagonal terms of the Jacobian matrix has a fixed sign. For $a_1 < x < b_1$ (respectively, $a_2 < y < b_2$) autoactivation is stronger than degradation.

To illustrate this point, we have chosen a rather simple two-variable system, with sigmoid interactions, described by the set of equations

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{1.95x^3}{1 + x^3} + \frac{9y^3}{6^3 + y^3} - x,$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \frac{10x^3}{7^3 + x^3} + \frac{1.95y^3}{1 + y^3} - y$$

and corresponding Jacobian matrix

$$\begin{pmatrix} \dfrac{5.85x^2}{(1+x^3)^2} - 1 & \dfrac{27 \times 6^3 y^2}{(6^3 + y^3)^2} \\ \dfrac{30 \times 7^3 x^2}{(7^3 + x^3)^2} & \dfrac{5.85y^2}{(1+y^3)^2} - 1 \end{pmatrix}.$$

This "regulon" (see also Snoussi and Thomas, 1993) comprises two nuclei: a 2-circuit, which is positive everywhere in the positive quadrant, and an ambiguous nucleus formed by the union of two one-element circuits. The necessary condition for multistationarity of Theorem 2(b), i.e. the existence of a variable nucleus, is thus fulfilled in the whole region defined by $x \geq 0$ and $y \geq 0$ and, for our choice of parameter values, the system has 11 steady states[2] five of which are stable: a steady state where both x and y are

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[2]A system with two isolated positive circuits (with sigmoid interactions) can have up to nine steady states. Additional interactions usually reduce the number of steady states, except, however, when these additional interactions create an additional positive circuit.

Table 2
Partition of phase space according to the sign of the ambiguous nucleus

type 3	type 2	type 3
type 2	type 1	type 2
type 3	type 2	type 3
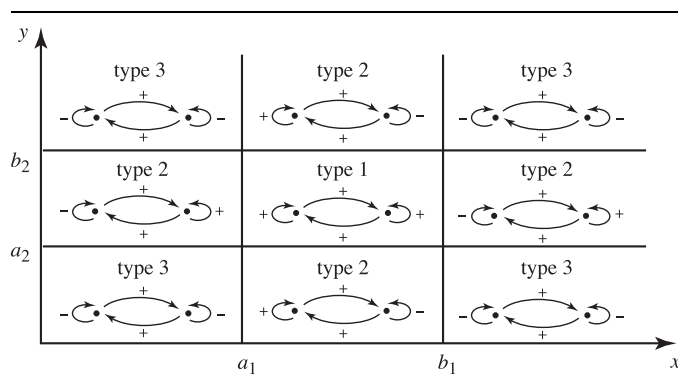
In each domain the graph of interactions has a given type, described in Table 1.

absent; two steady states where either x or y is present; a steady state where x and y are both present at medium level and a steady state where x and y are present at high level. Which of these stable steady states will eventually be reached depends on the duration of a transient signal that switches on the positive circuits.

When phase space is partitioned into domains according to the sign of the ambiguous nucleus, it is seen in Fig. 2 that the only domain that contains more than one, and in fact three, steady states[3] does fulfil condition (a) of Theorem 2, i.e. the presence of two nuclei with opposite sign. More precisely, in the present case phase space is partitioned into nine domains (Table 2) where the graph is either of type 1, type 2 or type 3 of Table 1. It can be seen that the condition of Theorem 2(a) is fulfilled in the domains of types 1 and 3, but not in the domains of type 2. In the present case, of the five domains of types 1 or 3, only one contains more than one steady state. Note that in addition to the information concerning the number of steady states in each region, Theorem 1 and the discussion in Section 3.3 provide information about the stability of these steady states as given in Table 1.

6. Conclusion

Several authors have noticed that cellular networks often contain interaction motives of a simple type as, for instance, the regulatory modules describing the cross-regulation of genes. It is thus of interest to determine those qualitative properties of the behaviour of these modules which remain valid independently of the value of the parameters. We proved two new results in that direction: a sufficient condition for unstability and a necessary condition for multistationarity. The latter result indicates

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[3]This partition into domains according to whether or not Kaufman's conditions for multistationarity are fulfilled should not be confused with the partition into domains in terms of the signs of the eigenvalues, as described in Thomas and Kaufman (2005).

that, in order to have several steady states, the number of variables is not the most relevant factor, but rather the existence of enough circuits in the system, and especially appropriate combinations of positive and negative circuits, as shown in the example discussed in Section 5.

Appendix A

A.1. Attractors and negative loops

Assume the dynamical system (1) has an attractor, i.e. a compact subset $A \subset \mathbb{R}^n$, invariant under the flow, which attracts a fundamental family of open neighbourhoods $U_m, m \geqslant 0$. Let $div(F)(x) = tr(J(x))$ be the divergence of F, and $grad(F)(x)$ its gradient. The Stokes formula says that the integral of $div(F)(x)$ over an open set U_m is equal to the integral on the boundary ∂U_m of the scalar product of $grad(F)(x)$ with the normal unit vector pointing outwards. Since this scalar product is negative, its integral over ∂U_m is negative and we conclude that there exists a point $x \in U_m$ such that $div(F)(x) < 0$. Consequently, the graph $G(x)$ contains a negative circuit of length one.

A.2. Proof of Theorem 1

Following Eisenfeld and DeLisi (1985), let us define the sign of a disjoint union of circuits in $G(a)$ to be $(-1)^{p+1}$ where p is the number of positive circuits. Eisenfeld and DeLisi (1985, Theorem 3.5) states that a is strongly unstable if there exists an integer k such that any disjoint union of circuits of total length k in $G(a)$ is positive.

Now assume that all circuits in $G(a)$ are positive. Let k be the largest integer such that $G(a)$ does not contain any circuit of length less than k. Then any disjoint union $C_1 \coprod C_2 \coprod \cdots \coprod C_n$ of circuits in $G(a)$ of total length k consists of a single circuit ($n = 1$). As a consequence, any disjoint union of circuits of total length k is positive. Therefore a is strongly unstable.

A.3. Proof of Theorem 2

We first make the following remark. Let $F = (f_i)$ be as in Theorem 2 and fix $i \in \{1, \ldots, n\}$. We denote by $F' : \mathbb{R}^n \to \mathbb{R}^n$ the map obtained from F by replacing f_i by $-f_i$. Clearly F' satisfies the hypotheses of Theorem 2. The graph $G'(a)$ of F' is obtained by changing the sign of every edge with endpoint i, leaving unchanged the other edges. It follows

that G satisfies (a) or (b) if and only if G' does. So, Theorem 2 is true for F iff it is true for F'.

Similarly, let $\sigma \in \Sigma_n$ be any permutation and $F' = (f_{\sigma(i)})$. The function F' satisfies the hypotheses of Theorem 2. Its interaction graph G' was described in Soulé (2003, 1.1 and 4.4). In particular, to any nucleus in G is attached a nucleus in G', the sign of which gets multiplied by the signature of σ (Soulé, 2003, Lemma 3). Therefore G' satisfies (a) or (b) if and only if G does. This means again that Theorem 2 is true for F iff it is true for F'.

Now, if F is as in Theorem 2, the determinant of its Jacobian matrix does not vanish everywhere. So there is $\sigma \in \Sigma_n$ and $a \in \mathbb{R}^n$ such that

$$\prod_{i=1}^{n} \frac{\partial f_i}{\partial x_{\sigma(i)}}(a) \neq 0.$$

If we let $f'_j = f_{\sigma^{-1}(j)}$ for every $j \in \{1, \ldots, n\}$ we get

$$\prod_{i=1}^{n} \frac{\partial f'_i}{\partial x_i}(a) \neq 0.$$

By changing the signs of some of the functions f'_i, we can assume that

$$\frac{\partial f'_i}{\partial x_i}(a) < 0 \quad \text{for every } i \in \{1, \ldots, n\}.$$

According to the previous remarks, Theorem 2 holds for $F = (f_i)$ iff it holds for $F' = (f'_i)$ (note that the class $\mathscr{F}$ is the same for F and F'). So we only need to prove Theorem 2 under the assumption that there exists $a \in \mathbb{R}^n$ such that

$$\frac{\partial f_i}{\partial x_i}(a) < 0 \quad \text{for every } i \in \{1, \ldots, n\}. \tag{3}$$

Condition (3) means that, for every vertex i, there is a negative edge from i to itself in $G(a)$. On the other hand, by Soulé (2003, Theorem 1) (Thomas' conjecture), there exists $c \in \mathbb{R}^n$ such that $G(c)$ contains a positive circuit. In other words, there is a subset $I \subset \{1, \ldots, n\}$ and a permutation σ of I such that $g(c) > 0$, where

$$g(x) = \prod_{i \in I} \frac{\partial f_i}{\partial x_{\sigma(i)}}(x).$$

Let

$$f(x) = \prod_{i=1}^{n} \frac{\partial f_i}{\partial x_i}(x).$$

We know that $f(a) \neq 0$ and $g(c) > 0$. Since both f and g are in $\mathscr{F}$, condition (C) tells us that we can find $d \in \mathbb{R}^n$ with $f(d) \neq 0$ and $g(d) > 0$. In other words, $G(d)$ contains a positive circuit C and, for every vertex i, $G(d)$ contains an edge e_i from i to itself (which can be negative or positive).

Assume first that there exists i such that e_i is positive. Then, the signs of e_i in $G(a)$ and $G(d)$ are opposite and the nucleus $\coprod_{i=1}^{n} e_i$ of G is variable. Therefore, (b) holds true.

Assume, on the contrary, that every edge e_i is negative in $G(d)$. The nucleus $\coprod_{i=1}^{n} e_i$ is then negative. Since the nucleus $C \coprod \coprod_{i \notin I}^{n} e_i$ is positive, we conclude that $G(d)$ has two

nuclei with opposite signs. Therefore, (a) is satisfied. This ends the proof of Theorem 2.

A.4. A remark about Theorem 2

Let $\Omega = \prod_{i=1}^{n}]a_i, b_i[$ be a product of open real intervals, with $a_i \geqslant -\infty$ and $b_i \leqslant +\infty$, and let $F : \Omega \to \mathbb{R}^n$ be a differentiable function. Theorem 2 and its proof remain valid in that situation. In fact Theorem 2 for $\mathbb{R}^n$ or for an arbitrary domain Ω are equivalent statements.

Indeed, for every $i \in \{1, \ldots, n\}$, we can choose a differentiable increasing isomorphism:

$$u_i :]a_i, b_i[\to \mathbb{R}.$$

For instance, when a_i and b_i are finite, after some affine transformation we can assume that $a_i = -1$ and $b_i = 1$. The map from $]-1, 1[$ to $\mathbb{R}$ sending x to $x/(\sqrt{1-x^2})$ is such an isomorphism.

Let then $h_i = f_i \circ u_i^{-1} : \mathbb{R} \to \mathbb{R}$ and $H = (h_i) : \mathbb{R}^n \to \mathbb{R}^n$. If $y_i = u_i(x_i)$ and $\beta_i = u_i(\alpha_i)$, Leibnitz' formula gives

$$\frac{\partial f_i}{\partial x_j}(\alpha) = \frac{\partial h_i}{\partial y_j}(\beta) \times u'_j(\alpha),$$

where the derivative $u'_j(\alpha)$ is strictly positive. Therefore, the interaction graph of F at α is the same as the interaction graph of H at β. Hence, Theorem 2 for F and H are equivalent.

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