

Necessary conditions for multistationarity in discrete dynamical systems

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Abstract

R. Thomas conjectured, twenty years ago, that the presence of a positive circuit in the interaction graph of a dynamical system is a necessary condition for the presence of several stable states. Recently, E. Remy *et al.* stated and proved the conjecture for Boolean dynamical systems. Using a similar approach, we generalize the result to discrete dynamical systems, and by focusing on the asynchronous dynamics that R. Thomas used in the course of his analysis of genetic networks, we obtain a more general variant of the R. Thomas' conjecture. In this way, we get a necessary condition for genetic networks to lead to differentiation.

Key words: Discrete dynamical system, Discrete Jacobian matrix, Interaction graph, Positive circuit, Multistationarity.

1 Introduction

The biologist René Thomas proposed, in the course of his analysis on the qualitative behaviors of genetic regulatory networks, a conjecture on dynamical systems. When studying genetic regulatory networks, biologists rarely obtain qualitative results. The main issues of their investigations are often represented by finite directed graphs where each edge is labelled by a sign. The vertices correspond to the genes of the network and a positive (resp. negative) edge from a gene i to a gene j means that the protein encoded by i activates (resp. represses) the synthesis of the protein encoded by j . The so called interaction graphs are then used as basis to design dynamical models, using either a differential or a discrete framework (one can refer to [1,2] for an

overview of the different approaches). In both cases, to each gene is associated a numerical value, called expression level, which describes the concentration of the corresponding encoded protein. The temporal evolution of these values define the dynamics of the system. Unfortunately, the dynamics generally depends on a great number of kinetic parameters which are most often unknown and difficult to measure. Given an interaction graph, a lot of possible dynamics have then to be considered. In this context, it can be very useful to extract general properties on the possible behaviors of a system according to its interaction graph.

At the beginning of the 80s, R. Thomas conjectured that *the presence of a positive circuit in the interaction graph (i.e. a circuit containing an even number of inhibitions) is a necessary condition for the presence of several stable states in the dynamics* [3]. From a biological point of view, multistationarity is an important dynamical property since it is related to epigenetic differences, included those involved in cell differentiation [3–5] (*e.g.* immune response, hematopoiesis).

This conjecture has been proved by several authors in the differential framework during the last decade [6–11], the more general proof having been done by C. Soulé in [10]. Recently, E. Remy *et al.* proved the conjecture in the Boolean framework [12,13], *i.e.* when each expression level can be either 0 (gene not expressed) or 1 (gene expressed). To prove the R. Thomas’ conjecture in such a discrete framework is of great interest because discrete approaches are increasingly used in biology: available experimental data are mostly qualitative and regulatory relationships are generally highly non-linear. However, the Boolean idealization is frequently too caricatural to give realistic models of biological systems. That led R. Thomas and coworkers to develop the so called general logical analysis in which genes can have more than two possible expression levels [2]. In this framework, E. Remy *et al.* [14] have recently prove a weak version of the R. Thomas’ conjecture.

In this paper, we prove a strong version of the R. Thomas’ conjecture in a general discrete framework (which includes the generalized logical analysis) with an approach similar to the one used by C. Soulé [10] (in the differential framework) and E. Remy *et al* [12] (in the Boolean one).

Section 2 presents our discrete framework. The set of states of a system involving n genes is assumed to be a product X of n finite intervals of integers, and the dynamics of the system is represented by a binary relation on X defined from a map $f : X \rightarrow X$. More precisely, the dynamics defined from f is the asynchronous dynamics that R. Thomas uses in his generalized logical analysis. Section 3 shows how to deduce from these dynamics the interaction graph $G(f)$ of the system. We first introduce a new Jacobian matrix for the discrete map f . Then, for any $x, y \in X$, we define a local interaction graph $G(x, y)$

from the discrete Jacobian matrix of f evaluated at state x with variations in the direction of y . This allows us to define the global interaction graph $G(f)$ as the superposition of all the local interaction graphs. In Section 4 the main result of the paper is presented. It gives a sufficient condition for the presence of a shortest path between two given states in the asynchronous dynamics. In Section 5, using the main result, we state and prove the R. Thomas' conjecture in our discrete framework: we show that if a map $f : X \rightarrow X$ has two fixed points x and y , then there exists $z \in X$ such that $G(z, y)$ has a positive circuit (which is, by definition, also present in the global interaction graph $G(f)$). More generally, we prove that the presence of a positive circuit in a local interaction graph is required for the presence of several attractors in the asynchronous dynamics. Section 6 is devoted to conclusion.

2 Asynchronous dynamics and attractors

We are interested in the evolution of a biological system involving n genes, denoted from 1 to n . The set of possible expression levels of each gene $i \in \{1, \dots, n\}$ is assumed to be a finite interval of integers X_i of cardinality greater than or equals to 2. A *state* of the system is an element $x = (x_1, \dots, x_n)$ of $X = X_1 \times \dots \times X_n$, where x_i is the expression level of gene i . A set of the form of X is called a *n-finite set of states* (in the Boolean case, $X = \{0, 1\}^n$).

The dynamics of the system is represented by a binary relation on X , defined from a map $f : X \rightarrow X$, $f(x) = (f_1(x), \dots, f_n(x))$, and an updating rule. For each $x \in X$, $f_i(x)$ is the value to which x_i , the expression level of gene i , tends when the system is in state x , and is called the *target level of i at state x* . If x is not a fixed point ($x \neq f(x)$), then at least one gene i such that $x_i \neq f_i(x)$ has to change its expression level in direction of its target level $f_i(x)$. The updating rule precises the expression levels that evolve, and how they evolve toward their respective target level.

The more classical updating rule leads to the synchronous dynamics $\{(x, f(x)) \mid x \in X\}$, where all expression levels x_i are simultaneously updated to $f_i(x)$ in one step. In this article, we focus on another dynamics, the asynchronous dynamics proposed by R. Thomas for modeling the qualitative behavior of genetic networks [2,5]. To define this dynamics, we will use the following notations:

- (1) For all $x \in X$, $i \in \{1, \dots, n\}$ and $\varepsilon \in \{-1, +1\}$, $x^{i \triangleleft \varepsilon}$ denotes the n -tuple obtained by adding ε to the i th component of x :

$$x^{i \triangleleft \varepsilon} = (x_1, \dots, x_i + \varepsilon, \dots, x_n).$$

- (2) For all $x, y \in X$, $I(x, y)$ denotes the set of $i \in \{1, \dots, n\}$ such that $x_i \neq y_i$.

(3) For all $a \in \mathbb{Z}$, we set $sign(a) = 0$ if $a = 0$ and $sign(a) = a/|a|$ otherwise.

We are now in position to define the *asynchronous dynamics of f* , denoted AD_f , by:

$$AD_f = \{(x, x^{i \triangleleft \varepsilon}) \mid x \in X, i \in I(x, f(x)) \text{ and } \varepsilon = sign(f_i(x) - x_i)\}.$$

In this dynamics, at each step, only one gene $i \in I(x, f(x))$ changes its expression level from x_i to $x_i + sign(f_i(x) - x_i)$. From a biological point of view, the asynchronous dynamics is clearly more realistic than the synchronous one [2,5]. Moreover, it has been shown that the asynchronous dynamics extracts the main qualitative behaviors of piecewise linear differential systems [15,16].

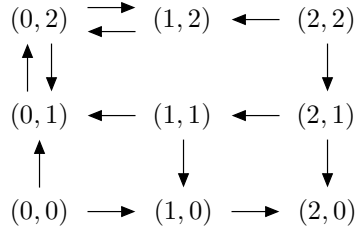
We are particularly interested in the paths and in the trap domains of the asynchronous dynamics. A *path of AD_f of length $k \in \mathbb{N}$* is a sequence of states (x^0, \dots, x^r) such that if $r > 0$ then $(x^k, x^{k+1}) \in AD_f$ for $k = 0, \dots, r - 1$. A *trap domain of AD_f* is a non-empty subset A of X such that, $\forall (x, y) \in AD_f, x \in A \Rightarrow y \in A$. In other words, a trap domain is a set of states that we cannot leave in the asynchronous dynamics. A trap domain A is a *smallest trap domain*, or an *attractor*, if there is no trap domain strictly included in A . Remark that the fixed points of f are smallest trap domains, and that the smallest trap domains are strongly connected components of the oriented graph (X, AD_f) .

Example 1 $n = 2, X = \{0, 1, 2\} \times \{0, 1, 2\}$ and f is given by the table:

| x | $f(x)$ | $sign(f_1(x) - x_1)$ | $sign(f_2(x) - x_2)$ |
|--------|--------|----------------------|----------------------|
| (0, 0) | (2, 1) | +1 | +1 |
| (0, 1) | (0, 2) | 0 | +1 |
| (0, 2) | (2, 0) | +1 | -1 |
| (1, 0) | (2, 0) | +1 | 0 |
| (1, 1) | (0, 0) | -1 | -1 |
| (1, 2) | (0, 2) | -1 | 0 |
| (2, 0) | (2, 0) | 0 | 0 |
| (2, 1) | (1, 0) | -1 | -1 |
| (2, 2) | (0, 1) | -1 | -1 |

In the following figure, an arrow from state x to state y means that (x, y)

belongs to the asynchronous dynamics of f :



The asynchronous dynamics contains 11 trap domains. It is easy to see that the smallest trap domains are $A = \{(0, 1), (0, 2), (1, 2)\}$ and $B = \{(2, 0)\}$. $B \cup \{1, 0\}$, $A \cup B$, and $A \cup B \cup \{(1, 1), (2, 1)\}$ are other examples of trap domains.

3 Discrete Jacobian matrices and interaction graphs

In the differential framework, C. Soulé [10] associates to each state an interaction graph, which is defined from the Jacobian matrix at this state. More precisely, the state of the system obeys a differential equation $\dot{x} = f(x)$, where $f : \mathbb{R}^n \rightarrow \mathbb{R}^n$, and at state x , the interaction graph $G(x)$ contains a positive (resp. negative) interaction from j to i if and only if $(\partial f_i / \partial x_j)(x) > 0$ (resp. $(\partial f_i / \partial x_j)(x) < 0$). The global interaction graph is then defined as the superposition of all local interaction graphs: it contains a positive (resp. negative) interaction from j to i if and only if there exists $x \in \mathbb{R}^n$ such that $(\partial f_i / \partial x_j)(x) > 0$ (resp. $(\partial f_i / \partial x_j)(x) < 0$).

In the Boolean framework, *i.e.* when f is a map from $\{0, 1\}^n$ to itself, E. Remy *et al.* [12] propose analogous definitions using the discrete Jacobian matrix defined by F. Robert [17]. Here, we extend these definitions to our general discrete case.

3.1 Discrete Jacobian matrices

In this section, we introduce two Jacobian matrices, the first one being a pedagogical step in the definition of the second one.

Given a n -finite set of states X , for each $x \in X$, we define the set of *variation vectors* $V(x)$ by:

$$V(x) = \{\varepsilon \mid \varepsilon \in \{-1, +1\}^n \text{ and } x + \varepsilon \in X\}.$$

Definition 1 Let X be a n -finite set of states and let $f : X \rightarrow X$. For all $x \in X$ and $\varepsilon \in V(x)$, we call usual Jacobian matrix evaluated at state x with the variation vector ε , and we denote by $df(x, \varepsilon) = (df_{ij}(x, \varepsilon))$, the $n \times n$ matrix with (i, j) -entry:

$$df_{ij}(x, \varepsilon) = \varepsilon_j \cdot \text{sign}(f_i(x^{j \leftarrow \varepsilon_j}) - f_i(x)).$$

So, if ε_j is positive (resp. negative), then $df_{ij}(x, \varepsilon) = +1$ if and only if an increase (resp. a decrease) of x_j induces an increase (resp. a decrease) of f_i . In this case, we said that there is a positive interaction from j to i . Inversely, $df_{ij}(x, \varepsilon) = -1$ if and only if an increase (resp. a decrease) of x_j induces a decrease (resp. an increase) of f_i , and in this case, we said that there is a negative interaction from j to i . However, such interactions may be not “visible” in the asynchronous dynamics, as shown in Figure 1.

In order to detect only the interactions “visible” in the asynchronous dynamics, we slightly modify the usual Jacobian matrix in the following way:

Definition 2 Let X be a n -finite set of states and let $f : X \rightarrow X$. For all $x \in X$ and $\varepsilon \in V(x)$, we call non usual Jacobian matrix evaluated at state x with the variation vector ε , and we denote by $\partial f(x, \varepsilon) = (\partial f_{ij}(x, \varepsilon))$, the $n \times n$ matrix with (i, j) -entry:

$$\partial f_{ij}(x, \varepsilon) = \begin{cases} df_{ij}(x, \varepsilon) & \text{if } f_i(x) \text{ and } f_i(x^{j \leftarrow \varepsilon_j}) \text{ are on both sides of } x_i + \varepsilon_i/2, \\ 0 & \text{otherwise.} \end{cases}$$

(Integers a and b are on both sides of a real number c if $a < c < b$ or $b < c < a$.)

The condition “on both sides of $x_i + \varepsilon_i/2$ ” is illustrated in Figure 2.

Remark 1

- (1) If, instead of the non usual Jacobian matrix we use the usual Jacobian matrix, then all the results incoming remains valid but become less strong (cf. Remarks 4 and 5).
- (2) It is straightforward to show that the non usual Jacobian matrix of f only depends on the asynchronous dynamics of f (if $\text{AD}_f = \text{AD}_g$ then $\partial f = \partial g$).
- (3) In the Boolean case, the non usual Jacobian matrix is identical to the usual Jacobian matrix ($df = \partial f$). Moreover, $V(x)$ is a singleton $\{\varepsilon\}$, and the (i, j) -entry of the Robert’s Jacobian matrix is then $|\partial f_{ij}(x, \varepsilon)|$ [17].

3.2 Local interaction graphs

We now introduce our notion of local interaction graph based on the non usual Jacobian matrix. Intuitively, we associate to each couple of states (x, y) an interaction graph $G(x, y)$ which gives a graphical representation of the interactions between genes of $I(x, y)$ which are detected by the non usual Jacobian matrix evaluated at state x when we use variations in direction of y :

Definition 3 *Let X be a n -finite set of states. Given $f : X \rightarrow X$, for all $x, y \in X$, the local interaction graph evaluated at state x with variations in direction of y , denoted $G(x, y)$, is the oriented graph whose set of nodes is $\{1, \dots, n\}$ and whose set of arcs is:*

$$\{(j, \alpha, i) \mid i, j \in I(x, y) \text{ and } \alpha = \partial f_{ij}(x, \varepsilon) \neq 0\};$$

ε being any variation vector of $V(x)$ such that $\varepsilon_i = \text{sign}(y_i - x_i)$ for all $i \in I(x, y)$.

In this definition, we use a second state y instead of a variation vector ε in order to simplify the formulation of the properties that we prove in the following sections.

Remark 2 *In the Boolean case, $V(x)$ is a singleton $\{\varepsilon\}$, and the interaction graph that E. Remy et al. [12] associate to x is the interaction graph $G(x, x+\varepsilon)$ (which is simply denoted $G(x)$). Thus, our notion of local interaction graph generalizes the one of E. Remy.*

Remark 3 *Let us denote by $\tilde{G}(x, y)$ the interaction graph defined as $G(x, y)$ with the usual Jacobian matrix instead of the non usual one. Since for all $x \in X$ and $\varepsilon \in V(x)$ we have*

$$\partial f_{ij}(x, \varepsilon) \neq 0 \Rightarrow \partial f_{ij}(x, \varepsilon) = df_{ij}(x, \varepsilon) \quad (i, j = 1, \dots, n),$$

any arc of $G(x, y)$ is an arc of $\tilde{G}(x, y)$ i.e. $G(x, y)$ is a subgraph of $\tilde{G}(x, y)$.

As in [12], we define the *global interaction graph* $G(f)$ of a map $f : X \rightarrow X$ as the superposition of all local interaction graphs: $G(f)$ has an interaction from node j to node i labelled by a positive (resp. negative) sign if and only if there exists $x, y \in X$ such that $G(x, y)$ contains such an interaction. Note that $G(f)$ can have both a positive and a negative interaction from one gene to another one. In such a case, the sign of the interaction depends on the state of the system, *i.e.* is context-sensitive, as it is often observed in biology.

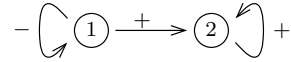
A *circuit of length* $r \in \mathbb{N}^*$ in an interaction graph G is a sequence $(i_1, \alpha_1, \dots, i_r, \alpha_r)$ such that i_1, \dots, i_r are distinct nodes of G , such that (i_r, α_r, i_1) is an arc of

G , and such that if $k > 1$ then (i_k, α_k, i_{k+1}) is an arc of G for $k = 1, \dots, r-1$. The *sign of a circuit* $(i_1, \alpha_1, \dots, i_r, \alpha_r)$ is $\prod_{k=1}^r \alpha_k$. In other words, a circuit is positive if it contains an even number of inhibitions, and negative otherwise.

Example 2 Consider the map f from $X = \{0, 1, 2\} \times \{0, 1, 2\}$ to itself defined in the first example. We have:

$$\partial f((1, 2), (-, -)) = \begin{vmatrix} - & + \\ + & 0 \end{vmatrix} \quad (+1 \text{ and } -1 \text{ are simply denoted } + \text{ and } -).$$

Thus, the local interaction graph $G((1, 2), (0, 0))$ (which is equal to $G((1, 2), (0, 1))$) is:



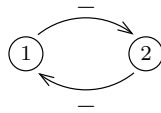
It contains a positive circuit and a negative circuit. We deduce easily from $G((1, 2), (0, 0))$ that the local interaction graph $G((1, 2), (1, 0))$ (which is equal to $G((1, 2), (1, 1))$) is:



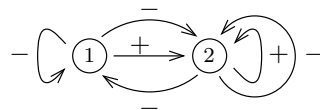
We have:

$$\partial f((0, 1), (+, -)) = \begin{vmatrix} 0 & - \\ - & 0 \end{vmatrix}$$

Thus, the local interaction graph $G((0, 1), (1, 0))$ (which is equal to $G((0, 1), (2, 0))$) is:



It corresponds to a positive circuit of length 2. The global interaction graph $G(f)$ is:



It contains two positive circuits and three negative circuits.

4 The shortest path theorem

Let X be a n -finite set of states and let f be a map from X to itself. A path from a state x to a state y in the asynchronous dynamics of f is necessarily of length greater than or equal to $d(x, y) = \sum_{i=1}^n |x_i - y_i|$, *i.e.* to the *Manhattan*

distance between x and y . In the following, we call *shortest path* from x to y any path of length $d(x, y)$, and we give a sufficient condition for the presence of a shortest path between two given states in the asynchronous dynamics of f .

For each $x, y \in X$, define $\pi(x, y)$ to be the smallest hyperrectangular region of X containing both x and y :

$$\pi(x, y) = \prod_{i=1}^n \{\min(x_i, y_i), \dots, \max(x_i, y_i)\}.$$

Obviously, all the states of a shortest path from x to y belong to $\pi(x, y)$. This lead us to focus on the dynamics described inside $\pi(x, y)$ and to introduce the notion of y -stability: state x is said y -stable if

$$\forall i \in I(x, y), \quad f_i(x) \leq x_i < y_i \quad \text{or} \quad y_i < x_i \leq f_i(x).$$

Equivalently, x is y -stable if for all $z \in X$ such that $(x, z) \in \text{AD}_f$ we have $z \notin \pi(x, y)$, *i.e.* if, from x , it is not possible to evolve inside $\pi(x, y)$.

Lemma 1 *Let X be a n -finite set of states, let $f : X \rightarrow X$ and let $x, y \in X$. If x is y -stable then $G(x, y)$ has no negative circuit.*

Proof — Let ε be a variation vector of $V(x)$ such that $\varepsilon_i = \text{sign}(y_i - x_i)$ for all $i \in I(x, y)$, and suppose that (j, α, i) is an arc of $G(x, y)$. Since $i \in I(x, y)$ and since x is y -stable, we have:

$$\begin{cases} f_i(x) \leq x_i < y_i & \text{if } \varepsilon_i = +1 \\ y_i < x_i \leq f_i(x) & \text{if } \varepsilon_i = -1. \end{cases} \quad (1)$$

By definition, $\alpha = \partial f_{ij}(x, \varepsilon) \neq 0$, so $f_i(x)$ and $f_i(x^{j \triangleleft \varepsilon_j})$ are on both sides of $x_i + \varepsilon_i/2$. Thus, from (1), we deduce that:

$$\begin{cases} f_i(x) \leq x_i < f_i(x^{j \triangleleft \varepsilon_j}) & \text{if } \varepsilon_i = +1 \\ f_i(x^{j \triangleleft \varepsilon_j}) < x_i \leq f_i(x) & \text{if } \varepsilon_i = -1. \end{cases}$$

Consequently $\text{sign}(f_i(x^{j \triangleleft \varepsilon_j}) - f_i(x)) = \varepsilon_i$, thus $\alpha = \partial f_{ij}(x, \varepsilon) = \varepsilon_j \cdot \varepsilon_i$. Now, suppose that $G(x, y)$ has a circuit $C = (i_1, \alpha_1, \dots, i_r, \alpha_r)$. Using the previous reasoning, we have:

$$\begin{aligned} \alpha_1 &= \varepsilon_{i_1} \cdot \varepsilon_{i_2} \\ \alpha_2 &= \varepsilon_{i_2} \cdot \varepsilon_{i_3} \\ &\vdots \\ \alpha_{r-1} &= \varepsilon_{i_{r-1}} \cdot \varepsilon_{i_r} \\ \alpha_r &= \varepsilon_{i_r} \cdot \varepsilon_{i_1} \end{aligned}$$

Thus:

$$\prod_{i=1}^r \alpha_i = (\varepsilon_{i_1} \cdot \varepsilon_{i_1}) \cdot (\varepsilon_{i_2} \cdot \varepsilon_{i_2}) \cdot \dots \cdot (\varepsilon_{i_r} \cdot \varepsilon_{i_r}) = +1.$$

Consequently, C is positive. Therefore, $G(x, y)$ has no negative circuit. \square

Lemma 2 *Let X be a n -finite set of states, let $f : X \rightarrow X$, and let x and y be two distinct states of X . If x is y -stable and if $G(x, y)$ has no positive circuit, then there exists a state $z \in \pi(x, y)$ which is y -stable and such that $(z, x) \in \text{AD}_f$.*

Proof — Let ε be a variation vector of $V(x)$ such that $\varepsilon_i = \text{sign}(y_i - x_i)$ for all $i \in I(x, y)$. Since x is y -stable and since $G(x, y)$ has no positive circuit, following Lemma 1, $G(x, y)$ has no circuit. Thus, there exists a node belonging to $I(x, y)$ without successor in $G(x, y)$. Let j be such a node and let $z = x^{j \triangleleft \varepsilon_j}$. Obviously, $z \in \pi(x, y)$. Let us prove that $(z, x) \in \text{AD}_f$. Since x is y -stable, we have

$$\begin{cases} f_j(x) \leq x_j < z_j \leq y_j & \text{if } \varepsilon_j = +1 \\ y_j \leq z_j < x_j \leq f_j(x) & \text{if } \varepsilon_j = -1. \end{cases} \quad (2)$$

Then, since there is no interaction from j to itself in $G(x, y)$, we have $\partial f_{jj}(x, \varepsilon) = 0$. Consequently, $f_j(x)$ and $f_j(x^{j \triangleleft \varepsilon_j}) = f_j(z)$ are not on both sides of $x_j + \varepsilon_j/2$. So, we deduce from (2) that:

$$\begin{cases} f_j(z) \leq x_j < z_j \leq y_j & \text{if } \varepsilon_j = +1 \\ y_j \leq z_j < x_j \leq f_j(z) & \text{if } \varepsilon_j = -1 \end{cases} \quad (3)$$

Consequently, $\text{sign}(f_j(z) - z_j) = -\varepsilon_j$. Thus $(z, z^{j \triangleleft (-\varepsilon_j)}) \in \text{AD}_f$ and since

$$z^{j \triangleleft (-\varepsilon_j)} = (x^{j \triangleleft \varepsilon_j})^{j \triangleleft (-\varepsilon_j)} = x,$$

we have $(z, x) \in \text{AD}_f$. Let us finally prove that z is y -stable. Since x is y -stable, we have

$$\forall i \in I(x, y) \setminus \{j\}, \quad \begin{cases} f_i(x) \leq x_i = z_i < y_i & \text{if } \varepsilon_i = +1 \\ y_i < z_i = x_i \leq f_i(x) & \text{if } \varepsilon_i = -1 \end{cases}$$

and since j has no successor in $G(x, y)$, we have $\partial f_{ij}(x, \varepsilon) = 0$ for all $i \in I(x, y)$. As previously, we deduce that

$$\forall i \in I(x, y) \setminus \{j\}, \quad \begin{cases} f_i(z) \leq x_i = z_i < y_i & \text{if } \varepsilon_i = +1 \\ y_i < x_i = z_i \leq f_i(z) & \text{if } \varepsilon_i = -1. \end{cases}$$

Because $I(z, y) \subseteq I(x, y)$, we deduce from these inequalities and from (3) that z is y -stable. \square

The main result of this paper follows.

Theorem 1 (Shortest path theorem) *Let X be a n -finite set of states, let $f : X \rightarrow X$ and let $x, y \in X$. If x is y -stable and if, for all $z \in \pi(x, y)$, $G(z, y)$ has no positive circuit, then there is a shortest path from y to x in AD_f .*

Proof — We prove the theorem by induction on the Manhattan distance $d(x, y) = \sum_{i=1}^n |x_i - y_i|$ between x and y . If $d(x, y) = 0$ then $x = y$ and there is nothing to prove. So, suppose that $d(x, y) > 0$, and suppose that the theorem holds for all $x', y' \in X$ such that $d(x', y') < d(x, y)$. Under the conditions of the theorem, x is y -stable and $G(x, y)$ has no positive circuit. Thus, following Lemma 2, there exists a state $z \in \pi(x, y)$ which is y -stable and such that $(z, x) \in \text{AD}_f$. Because $d(x, z) = 1$ and $z \in \pi(x, y)$, we have $d(z, y) = d(x, y) - 1$. Moreover, $\pi(z, y)$ is a strict subset of $\pi(x, y)$. Thus, for all $z' \in \pi(z, y)$, $G(z', y)$ has no positive circuit, and following the induction hypothesis that there exists a shortest path from y to z in AD_f . Since $(z, x) \in \text{AD}_f$, we deduce that there exists a shortest path from y to x , of the form (y, \dots, z, x) , in the asynchronous dynamics of f . \square

Remark 4 *If we use the usual Jacobian matrix instead the non usual one, the theorem remains valid, but becomes less strong. Indeed, following Remark 3, if, for all $z \in \pi(x, y)$, $\tilde{G}(z, y)$ has no positive circuit then, for all $z \in \pi(x, y)$, $G(z, y)$ has no positive circuit.*

An illustration of the proof of the shortest path theorem is given in Figure 3.

5 Necessary conditions for multistationarity

From the shortest path theorem, we can easily prove that the presence of a positive circuit in a local interaction graph is a necessary condition for the coexistence of distinct attractors and, more generally, for the coexistence of disjointed trap domains.

Corollary 1 (Discrete version of the R. Thomas' conjecture) *Let X be a n -finite set of states, and let $f : X \rightarrow X$. Suppose that A and B are two disjointed trap domains of AD_f . Let (x, y) be a couple of $A \times B$ such that $\pi(x', y') \not\subset \pi(x, y)$ for all $(x', y') \in A \times B$. Then, there exists $z \in \pi(x, y)$ such that $G(z, y)$ has a positive circuit.*

Proof — We first prove that under the conditions of the theorem, x is y -stable. For a contradiction, suppose that it is not the case. Then, there exists $z \in \pi(x, y)$ such that $(x, z) \in \text{AD}_f$. Thus $\pi(z, y)$ is strictly included in $\pi(x, y)$, and since x belong to the trap domain A , we have $z \in A$, a contradiction.

Consequently, x is y -stable. So, if we suppose that $G(z, y)$ has no positive circuit for all $z \in \pi(x, y)$, by the shortest path theorem, there exists a path from y to x . Since y belongs to the trap domain B , we deduce that $x \in B$, a contradiction. Consequently, there exists $z \in \pi(x, y)$ such that $G(z, y)$ has a positive circuit. \square

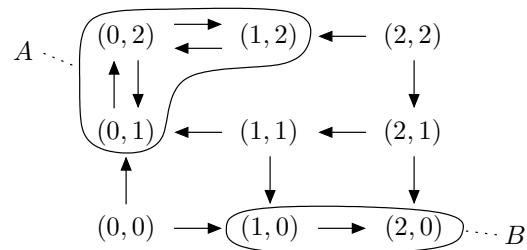
Remark 5 *Again, if we use the usual Jacobian matrix instead the non usual one, the result remains valid, but becomes less strong. Indeed, following Remark 3, if $G(z, y)$ has a positive circuit then $\tilde{G}(z, y)$ has this circuit.*

Corollary 1 gives an information on the localization of the positive circuit required for the coexistence of the two disjointed trap domains A and B (it belongs to an interaction graph evaluated in a state which is “between” x and y), and on the genes involved in this circuit (they belong to $I(x, y)$). However, the presence of a positive circuit in a local interaction graph is not sufficient for the presence of disjointed trap domains as shown in Figure 4.

Note also that Corollary 1 is a generalization of the R. Thomas’ conjecture. Indeed, since a fixed point is an attractor: *if x and y are two distinct fixed points of f then there is $z \in \pi(x, y)$ such that $G(z, y)$ has a positive circuit.* Actually, multistationarity requires the presence of a positive circuit in a local interaction graph. This necessary condition for multistationarity is stronger than the one initially proposed by R. Thomas since the presence of a positive circuit in a local interaction graph of f implies its presence in the global interaction graph $G(f)$.

Finally, note that if $X = \{0, 1\}^n$ and if the trap domains A and B are reduced to fixed points, the Boolean version of the R. Thomas’ conjecture given by E. Remy *et al.* [12] is recovered (we have thus an independent proof of the Boolean version of the R. Thomas’ conjecture).

Example 3 *Consider again the map f from $\{0, 1, 2\} \times \{0, 1, 2\}$ to itself defined in Example 1. $A = \{(0, 1), (0, 2), (1, 2)\}$ and $B = \{(0, 1), (0, 2)\}$ are two disjointed trap domains of the asynchronous dynamics of f :*



Let $x = (1, 2) \in A$ and $y = (1, 0) \in B$. For all $(x', y') \in A \times B$, $\pi(x', y')$ is not a strict subset of $\pi(x, y)$. Thus, following Corollary 1, there exists $z \in \pi(x, y)$

such that $G(z, y)$ has a positive circuit. Indeed, if we take $z = x$, as shown in Example 2, the local interaction graph $G(z, y)$ has a positive circuit of length 1. Now, let $x = (0, 1) \in A$ and $y = (1, 0) \in B$. Again, for all $(x', y') \in A \times B$, $\pi(x', y')$ is not a strict subset of $\pi(x, y)$. Thus, there exists $z \in \pi(x, y)$ such that $G(z, y)$ has a positive circuit. Indeed, if we take $z = x$, as shown in Example 2, the local interaction graph $G(z, y)$ has a positive circuit of length 2.

6 Conclusion

By introducing a non usual discrete Jacobian matrix for maps f from a product of n finite intervals of integers to itself, we prove the shortest path theorem giving a sufficient condition for the presence of a shortest path between two given states in the asynchronous dynamics of f . From this theorem, we proof a strong discrete version of the first R. Thomas conjecture generalizing the boolean version the conjecture proved by E. Remy *et al.* [12]. In [3], R. Thomas proposed a second conjecture stating that negative circuits are necessary for the presence of stable cycles (a particular kind of attractor) in the asynchronous dynamics. This conjecture, proved in the Boolean case [12], is also important since, in biology, stable cycles are associated to homeostasis or sustained oscillatory behaviors (*e.g.* cell cycle, circadian rhythms). The Jacobian matrix that we have introduced could also be useful to state and prove, in our general discrete framework, the second conjecture of R. Thomas as well as other rules giving general properties on the possible behaviors of a system according to its interaction graph.

References

- [1] H. de Jong. Modeling and simulation of genetic regulatory systems: a literature review. *Journal of Computational Biology*, 9(1):67-103, 2002.
- [2] R. Thomas and R. d'Ari. *Biological Feedback*. CRC Press, 1990.
- [3] R. Thomas. On the relation between the logical structure of systems and their ability to generate multiple steady states and sustained oscillations. In *Series in Synergetics*, volume 9, pages 180-193. Springer, 1981.
- [4] R. Thomas. Laws for the dynamics of regulatory networks. *Int. J. Dev. Biol.*, 42(3):479-85, 1998.
- [5] R. Thomas and M. Kaufman. Multistationarity, the basis of cell differentiation and memory. I. & II. *Chaos*, 11:170-195, 2001.

- [6] E. Plathe, T. Mestl and S.W. Omholt. Feedback loops, stability and multistationarity in dynamical systems. *Journal of Biological Systems*, 3:569-577, 1995.
- [7] J.L. Gouzé. Positive and negative circuits in dynamical systems. *Journal of Biological Systems*, 6:11-15, 1998.
- [8] E.H. Snoussi. Necessary conditions for multistationarity and stable periodicity. *Journal of Biological Systems*, 6:3-9, 1998.
- [9] O. Cinquin and J. Demongeot. Positive and negative feedback: striking a balance between necessary antagonists. *J. Theor. Biol.*, 216(2):229-241, 2002.
- [10] C. Soulé. Graphical requirements for multistationarity. *ComplexUs*, 1:123-133, 2003.
- [11] C. Soulé. Mathematical approaches to differentiation and gene regulation. *C.R. Paris Biologies*, 329:13-20, 2006.
- [12] E. Remy, P. Ruet and D. Thieffry. Graphics Requirement for Multistability and Attractive Cycles in a Boolean Dynamical Framework. Technical Report, IML, 2005.
- [13] E. Remy and P. Ruet. On differentiation and homeostatic behaviours of Boolean dynamical systems. In *Transactions on Computational Systems Biology VII*, volume 4230 of LNCS, pages 153-162, Springer, 2006.
- [14] E. Remy, P. Ruet and D. Thieffry. Positive or negative regulatory circuit inference from multilevel dynamics. In *Positive Systems: Theory and Applications*, volume 341 of LNCIS, pages 263-270, Springer, 2006.
- [15] E.H. Snoussi. Qualitative dynamics of a piecewise-linear differential equations : a discrete mapping approach. *Dynamics and stability of Systems*, 4:189-207, 1989.
- [16] E.H. Snoussi and R. Thomas. Logical identification of all steady states : the concept of feedback loop characteristic states. *Bull. Math. Biol.*, 55(5):973-991, 1993.
- [17] F. Robert. *Les systèmes dynamiques discrets*, volume 19 of *Mathématiques et Applications*. Springer, 1995.

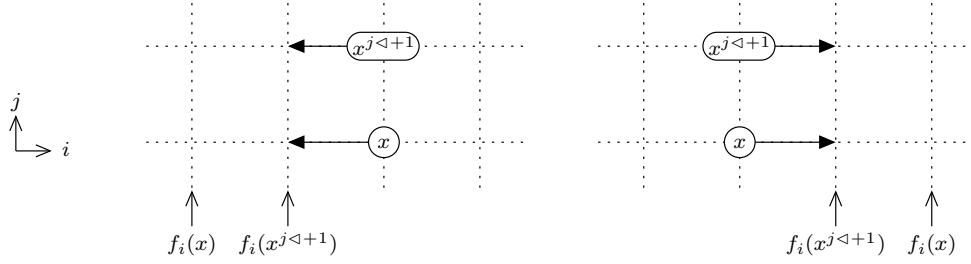


Fig. 1. The variation ε_j is positive. On the left, an increase of x_j induces an increase of f_i : we have $df_{ij}(x, \varepsilon) = +1$. However, this interaction from j to i is not “visible” in the asynchronous dynamics: before and after the variation of x_j , the level of i is greater than its target level and tends to decrease. On the right, an increase of x_j induces a decrease of f_i (we have $df_{ij}(x, \varepsilon) = -1$), and this interaction from j to i is also not “visible” in the asynchronous dynamics.

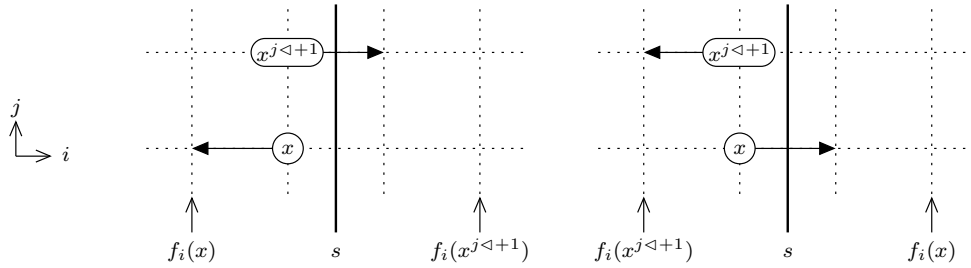
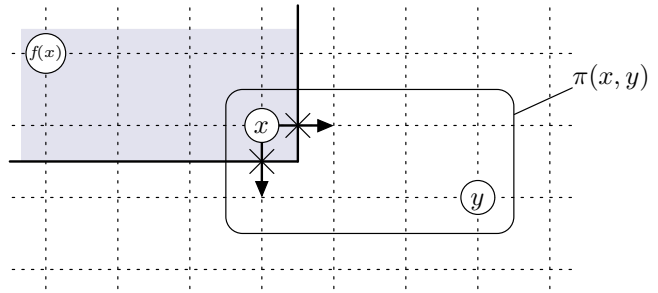
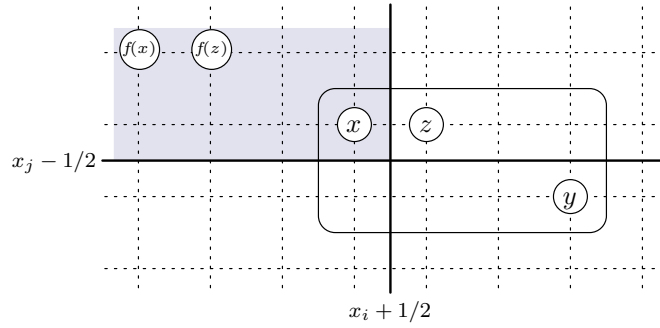


Fig. 2. The variations ε_i and ε_j are positive. The threshold $s = x_i + \varepsilon_i/2$ to consider is thus between x_i and $x_i + 1$. On the left, an increase of x_j induces an increase of f_i allowing f_i to cross the threshold s : we have $\partial f_{ij}(x, \varepsilon) = +1$. This interaction from j to i is “visible” in the asynchronous dynamics: before the variation of x_j , the level of i tends to decrease whereas after the variation of x_j , the level of i tends to increase. On the right, an increase of x_j induces a decrease of f_i allowing f_i to cross the threshold s (we have $\partial f_{ij}(x, \varepsilon) = -1$), and this interaction from j to i is also “visible” in the asynchronous dynamics.

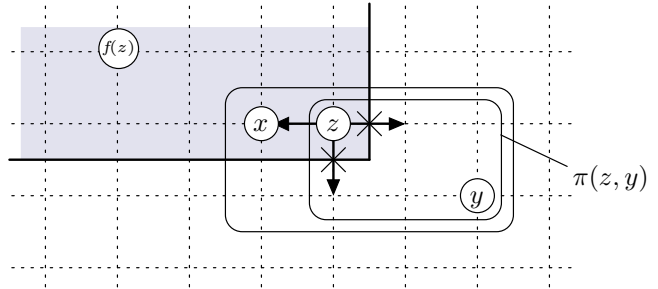
1) Under the conditions of the theorem, x is y -stable and $G(z, y)$ has no positive circuit for all $z \in \pi(x, y)$. In particular, $G(x, y)$ has no positive circuit. Thus, following Lemma 1, $G(x, y)$ has no circuit.



2) Consequently, $G(x, y)$ has a node (belonging to $I(x, y)$) without successor. Suppose that i is such a node (horizontal axis). Then, at state x , a variation of the level of i in direction of y does not allow $f_i(x)$ (resp. $f_j(x)$) to cross the threshold $x_i + 1/2$ (resp. $x_j - 1/2$). Thus, $f(z) = f(x^{i \triangleleft + 1})$ belongs to the grey zone.



3) Therefore, there is a transition from z to x in the asynchronous dynamics ((z, x) belongs to AD_f), and z is y -stable.



4) Since $\pi(z, y)$ is a strict subset of $\pi(x, y)$, $G(z', y)$ has no positive circuit for all $z' \in \pi(z, y)$. Thus, by induction hypothesis there is a shortest path from y to z . Since there is a transition from z to x , we deduce that there is a shortest path from y to x .

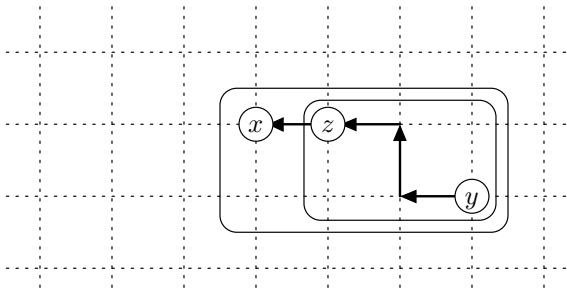


Fig. 3. Illustration of the proof of the shortest path theorem.

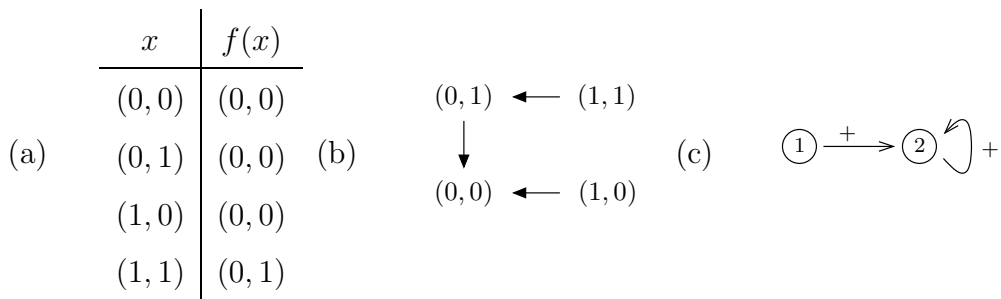


Fig. 4. (a) A map $f : \{0,1\}^2 \rightarrow \{0,1\}^2$. (b) The corresponding asynchronous dynamics AD_f whose trap domains are: $\{(0,0)\}$, $\{(0,0), (0,1)\}$, $\{(0,0), (1,0)\}$, $\{(0,0), (1,0), (1,1)\}$ and $\{(0,0), (0,1), (1,0), (1,1)\}$. (c) The local interaction graph $G((1,1), (0,0))$ contains a positive circuit, but there is no disjointed trap domains.