Positive circuits and maximal number of fixed points in discrete dynamical systems

Adrien Richard

Laboratoire I3S, UMR 6070 CNRS & Université de Nice-Sophia Antipolis, 2000 route des Lucioles, 06903 Sophia Antipolis, France.
Email: richard@i3s.unice.fr

Abstract

We consider a product X of n finite intervals of integers, a map F from X to itself, the asynchronous state transition graph $\Gamma(F)$ on X that Thomas proposed as a model for the dynamics of a network of n genes, and the interaction graph $G(F)$ that describes the topology of the system in terms of positive and negative interactions between its n components. Then, we establish an upper bound on the number of fixed points for F, and more generally on the number of attractors in $\Gamma(F)$, which only depends on X and on the topology of the positive circuits of $G(F)$. This result generalizes the following discrete version of Thomas' conjecture recently proved by Richard and Comet: If $G(F)$ has no positive circuit, then $\Gamma(F)$ has a unique attractor. This result also generalizes a result on the maximal number of fixed points in Boolean networks obtained by Aracena, Demongeot and Goles. The interest of this work in the context of gene network modeling is briefly discussed.

Key words: Discrete dynamical system, Discrete Jacobian matrix, Interaction graph, Positive circuit, Fixed point, Gene network.

1 Introduction

We are interested in the number of fixed points, and more generally in the number of attractors, in discrete dynamical systems used to model genetic regulatory networks. These networks are often symbolically described by biologists in terms of *interaction graphs*. These are directed graphs where vertices correspond to genes and where edges are labeled with a sign: a positive (resp. negative) edge from j to i means that the protein encoded by gene j activates (resp. represses) the synthesis of the protein encoded by gene i. These graphs are then used as a basis to generate *dynamical models* describing the temporal evolution of the concentration of the encoded proteins; see [1] for a literature

review. Unfortunately, these models require informations on the strength of the interactions that are most often unavailable. One is thus faced with the following difficult problem: *Which dynamical properties of a gene network can be inferred from its interaction graph (in the absence of information on the strength of the interactions)?*

The biologist René Thomas stated a well-known conjecture providing a partial answer to this question [2]: *The presence of a positive circuit in the interaction graph is a necessary condition for the presence of multiple stable states* (a circuit is positive if it contains an even number of negative edges). At this stage, it is worth noting that the number of stable states is a key feature of gene network dynamics: according to an idea of Delbrück [3], the presence of multiple stable states is one possible mechanism for biological differentiation.

Thomas' conjecture has been proven in differential frameworks [4–9], and, more recently, in discrete frameworks [10,11] in which the concentration level of each protein is assumed to evolve inside a finite interval of integers, $\{0, 1\}$ in the Boolean case. Such discrete frameworks are increasingly used to model gene networks because reliable experimental data are mostly qualitative and the sigmoidal shape of genetic regulations leads to a natural discretization of concentrations [12–16]. Furthermore, discrete descriptions allow the use of powerful computational tools; see for instance [17,18].

In this paper, we establish, in the general discrete framework proposed in [10], an upper bound on the number of attractors that the dynamics of a network contain, according to the set of states of the network and the topology of the positive circuits of its interaction graph. This result generalizes, and is proved with, the discrete version of Thomas' conjecture established by Richard and Comet [10]. This result also generalizes a result, obtained by Aracena, Demongeot and Goles [19,20], on the maximal number of fixed points in a particular class of Boolean networks.

The paper is organized as follows. In Section 2, we consider a product X of n finite intervals of integers, a map F from X to itself, and the directed graph $\Gamma(F)$ on X, called *asynchronous state transition graph* of F, that Thomas proposed as a model for the dynamics of a network of n genes. The fixed points of F then correspond to the *stable states* of the system and are seen as particular *attractors* of $\Gamma(F)$. In Section 3, we associate with F *local interaction graphs* based on the *discrete Jacobian matrix* of F, and we define the *global interaction graph* $G(F)$ of the system as the union of all the local interaction graphs. In Section 4, we state and prove the main result (Theorem 2), and we successively derive from it: an upper bound on the number of attractors in $\Gamma(F)$ that only depends on X and on the positive circuits of the local interaction graphs associated with F (Corollary 1); and a less accurate upper bound that only depends on X and on the positive circuits of $G(F)$ (Corollary 2).

Final comments are provided in Section 5. These concern the influence of connections between positive circuits and the interest of the established bounds in the context of *Thomas' logical method* [14,21–23] which is one of the most commonly used discrete modeling methods for gene networks.

2 Asynchronous state transition graph and attractors

Let $X = \prod_{i=1}^{n} X_i$ be a product of n finite intervals of integers, each of cardinality strictly greater than 1, and consider a map F from X to itself,

$$x = (x_1, \ldots, x_n) \in X \;\mapsto\; F(x) = (f_1(x), \ldots, f_n(x)) \in X.$$

In the following definition, we attach to F a directed graph on X, called *asynchronous state transition graph* of F. According to Thomas [14,21–23], this state transition graph can be seen as a model for the dynamics of a network of n genes: the set of vertices X is the set of possible states for the network (each interval X_i corresponds to the set of possible concentration levels for the protein encoded by gene i), and each path corresponds to a possible evolution of the system. Asynchronous state transition graphs can also be seen as discretizations of piecewise-linear differential systems; see for instance [13–15].

Definition 1 *We call* asynchronous state transition graph *of F, and we denote by $\Gamma(F)$, the directed graph whose set of vertices is X and that contains an edge from x to y if there exists $i \in \{1, \ldots, n\}$ such that*

$$y = x + e_i \text{ and } x_i < f_i(x) \qquad \text{or} \qquad y = x - e_i \text{ and } x_i > f_i(x),$$

where e_i is the n-tuple whose components are all 0 except the ith, which is 1.

[$f_i(x)$ can be seen as the level toward which the concentration x_i of the protein encoded by gene i evolves at state x: there exists a transition starting from x that allows the ith component to increase (resp. decrease) if and only if $x_i < f_i(x)$ (resp. $x_i > f_i(x)$).]

The fixed points of F have no successor in $\Gamma(F)$ and naturally correspond to the *stable states* of the system. In the next definition, we introduce a notion of *attractor* which extends, in a natural way, the notion of stable state.

Definition 2 *A* trap domain *of $\Gamma(F)$ is a non-empty subset $A \subseteq X$ such that, for all edges (x, y) of $\Gamma(F)$, if $x \in A$ then $y \in A$. An* attractor *of $\Gamma(F)$ is a smallest trap domain with respect to the inclusion relation.*

In other words, the attractors of $\Gamma(F)$ are the smallest subsets of states that the system cannot leave. They extend the notion of stable state in the sense

that x is a fixed point of F if and only if $\{x\}$ is an attractor of $\Gamma(F)$. Note also that there always exists at least one attractor (since X is a trap domain). Other basic observations follow: (i) from each state, there is a path that leads to an attractor (this is why one can say that attractors perform, in a weak sense, an attraction); (ii) attractors are strongly connected components; and (iii) attractors are mutually disjointed.

3 Discrete Jacobian matrix, interaction graph and positive circuit

In this section, we define the interaction graph of the network whose dynamics are described by $\Gamma(F)$. We proceed as in [10] by first introducing a discrete Jacobian matrix for F based on a notion of discrete directional derivative (the notion of discrete Jacobian matrix was first considered by Robert [24–26] in the *Boolean case*, i.e. when X is the n-dimensional hypercube $\{0,1\}^n$).

Let X' be the set of couples (x, v) such that $x \in X$, $v \in \{-1, 1\}^n$ and $x+v \in X$.

Definition 3 *For all $(x, v) \in X'$, we call* Jacobian matrix *of F evaluated at x along the directional vector v the $n \times n$ matrix $F'(x, v) = (f'_{ij}(x, v))$ defined by*

$$f'_{ij}(x, v) = \frac{f_i(x + v_j e_j) - f_i(x)}{v_j} \qquad (i, j = 1, \ldots, n).$$

[If v_j is positive (resp. negative), then $f'_{ij}(x, v)$ may be seen as the right (resp. left) partial derivative of f_i with respect to the jth variable evaluated at x. In both cases, $f'_{ij}(x, v)$ is a natural discrete analogue of $(\partial f_i/\partial x_j)(x)$.]

An *interaction graph* is here a directed graph whose set of vertices is $\{1, \ldots, n\}$ and where each edge is provided with a sign. More formally, each edge is characterized by a triple (j, s, i) where j (resp. i) is the initial (resp. final) vertex and where $s \in \{-1, 1\}$ is the sign of the edge. Let $\mathcal{G}$ and $\mathcal{G}'$ be interaction graphs with edge sets E and E', respectively. $\mathcal{G}$ is a *subgraph* of $\mathcal{G}'$ if $E \subseteq E'$. We denote by $\mathcal{G} \cup \mathcal{G}'$ the interaction graph whose set of edges is $E \cup E'$.

Definition 4 *We call* interaction graph of F evaluated at $(x, v) \in X'$, *and we denoted by $\mathscr{G}_F(x, v)$, the interaction graph that contains a positive (resp. negative) edge from j to i if $f'_{ij}(x, v)$ is positive (resp. negative).*

[To illustrate this definition, assume that $f'_{ij}(x, v)$ is positive and that $v_j = 1$. Then, $f_i(x) < f_i(x+e_j)$, and therefore we can say that, at state x, an increase of x_j induces an increase of f_i, that is, an increase in the level toward which the ith component of the system evolves. In other words, j acts as an activator of i, and we have a positive edge from j to i in $\mathscr{G}_F(x, v)$.]

In the following definition, we characterize a subgraph $G_F(x,v)$ of $\mathscr{G}_F(x,v)$ whose interest is twofold. Firstly, this subgraph will allow us to obtain stronger results (Remark 4). Secondly, this subgraph only depends on $\Gamma(F)$ [10], and thanks to this property, we will be able to: (i) define without possible ambiguity the interaction graph of the network whose dynamics are described by $\Gamma(F)$ (Definition 6 and Remark 2), and (ii) interpret the obtained results in the context of Thomas' logical method (Sections 5.2 and 5.3). We refer the reader to [10] for an illustration of the definition of $G_F(x,v)$ and further comments.

Definition 5 *We call* interaction graph of F evaluated at $(x,v) \in X'$ with thresholds*, and we denote by $G_F(x,v)$, the interaction graph that contains a positive (resp. negative) edge from j to i if $f'_{ij}(x,v)$ is positive (resp. negative) and if $f_i(x)$ and $f_i(x+v_je_j)$ are on both sides of the threshold $t = x_i + v_i/2$.*

Here, we say that a and b are *on both sides* of c if $a < c < b$ or $b < c < a$.

Remark 1 In the Boolean case, we have $G_F(x,v) = \mathscr{G}_F(x,v)$, but in the general discrete case, $G_F(x,v)$ is often a strict subgraph of $\mathscr{G}_F(x,v)$ since the additional condition "on both sides of the threshold" is rather strong.

As in [8,10,11], we define the global interaction graph of the system as the union of all the local interaction graphs.

Definition 6 *We call* global interaction graph *of F, and we denote by $G(F)$, the interaction graph defined by $G(F) = \bigcup_{(x,v)\in X'} G_F(x,v)$.*

Remark 2 Since $G_F(x,v)$ only depends on $\Gamma(F)$, the global interaction graph $G(F)$ only depends on $\Gamma(F)$ and can be seen as the interaction graph of the network whose dynamics are described by $\Gamma(F)$. The following basic property highlights the fact that $G(F)$ only depends on $\Gamma(F)$: $G(F)$ has a positive (resp. negative) edge from j to i if and only if, for all maps $H : X \to X$ such that $\Gamma(H) = \Gamma(F)$, there exists $(x,v) \in X'$ such that $h_{ij}(x,v)$ is positive (resp. negative).

We now recall the notions of positive circuit and of positive feedback vertex set.

Definition 7 *A* positive circuit *in an interaction graph $\mathcal{G}$ is a non-empty sequence of edges, say $(j_1,s_1,i_1),(j_2,s_2,i_2),\ldots,(j_r,s_r,i_r)$, such that: $i_k = j_{k+1}$ for $1 \le k < r$ (the sequence is a path); $i_r = j_1$ (the path is a circuit); the vertices j_k are mutually distinct (the circuit is elementary); and the product of the signs s_k is positive (even number of negative edges).*

Definition 8 **[19]** *A* positive feedback vertex set *of an interaction graph $\mathcal{G}$ is a subset $I \subseteq \{1,\ldots,n\}$ such that each positive circuit of $\mathcal{G}$ has a vertex in I.*

Note that: (i) the vertex set of $\mathcal{G}$ is always a positive feedback vertex set of $\mathcal{G}$;

(ii) the empty set is a positive feedback vertex set of $\mathcal{G}$ if and only if $\mathcal{G}$ has no positive circuit; and (iii) if $\mathcal{G}$ is a subgraph of $\mathcal{G}'$ then all the positive feedback vertex sets of $\mathcal{G}'$ are positive feedback vertex sets of $\mathcal{G}$.

A last definition is needed to state the main result.

Definition 9 *Let $\mathcal{G}$ be a map defined on X' and whose images are interaction graphs. For each $i \in \{1, \ldots, n\}$, we denote by $T_i(\mathcal{G})$ the set of real numbers t such that i belongs to a positive circuit of $\mathcal{G}(x, v)$ for at least one $(x, v) \in X'$ such that $t = x_i + v_i/2$.*

Observe that $|T_i(\mathcal{G})|$ is always strictly less than $|X_i|$.

4 Positive circuits and attractors

As before, let $X = \prod_{i=1}^{n} X_i$ be a product of n finite intervals of integers, each of cardinality strictly greater than 1, and let F be a map from X to itself.

We are interested in the relationships between the local interaction graphs $G_F(x, v)$ and the number of attractors in $\Gamma(F)$. The following discrete version of Thomas' conjecture gives such a relation.

Theorem 1 **[10]** *If $G_F(x, v)$ has no positive circuit for all $(x, v) \in X'$, then $\Gamma(F)$ has a unique attractor.*

The next theorem extends the previous one by providing, without any condition on the local interaction graphs $G_F(x, v)$, an upper bound on the number of attractors in $\Gamma(F)$ that only depends on the map G_F (defined on X').

Theorem 2 *If I is a positive feedback vertex set of $G_F(x, v)$ for all $(x, v) \in X'$, then the number of attractors in $\Gamma(F)$ is at most*

$$\prod_{i \in I} \Big(|T_i(G_F)| + 1 \Big)$$

(with the usual convention that this product equals 1 if I is empty).

Proof —We reason by induction on I. Suppose I to be, for every $(x, v) \in X'$, a positive feedback vertex set of $G_F(x, v)$.

Base case. If $I = \emptyset$, this means that there is no $(x, v) \in X'$ such that $G_F(x, v)$ has a positive circuit. So, following Theorem 1, $\Gamma(F)$ has at most one attractor and the theorem holds.

Induction step. Suppose that $I \neq \emptyset$. The induction hypothesis is the following:

Induction hypothesis: Let $\tilde{F}$ be a map from X to itself. If $\tilde{I}$ is a positive feedback vertex set of $G_{\tilde{F}}(x,v)$ for all $(x,v) \in X'$, and if $\tilde{I}$ is *strictly included* in I, then $\Gamma(\tilde{F})$ has at most $\prod_{i\in\tilde{I}}(|T_i(G_{\tilde{F}})|+1)$ attractors.

Without loss of generality, suppose that $1 \in I$. Let $\mathbb{P}$ be the partition of X_1 whose elements Y are the maximal intervals of X_1 (with respect to the inclusion relation) verifying

$$\forall t \in T_1(G_F), \qquad t < \min(Y) \quad \text{or} \quad \max(Y) < t. \tag{1}$$

Note that, by definition,

$$|\mathbb{P}| = |T_1(G_F)| + 1. \tag{2}$$

Let Y be any interval of $\mathbb{P}$, and consider the map $\tilde{F} = (\tilde{f}_1, \ldots, \tilde{f}_n) : X \to X$ defined by $\tilde{f}_i = f_i$ for $i > 1$ and by

$$\forall x \in X, \qquad \tilde{f}_1(x) = \begin{cases} \min(Y) & \text{if } f_1(x) < \min(Y) \\ f_1(x) & \text{if } f_1(x) \in Y \\ \max(Y) & \text{if } f_1(x) > \max(Y). \end{cases}$$

Then, for all $x, y \in X$,

$$\tilde{f}_i(x) < \tilde{f}_i(y) \quad \Rightarrow \quad f_i(x) \le \tilde{f}_i(x) < \tilde{f}_i(y) \le f_i(y) \qquad (i = 1, \ldots, n). \tag{3}$$

Indeed, this is obvious for $i > 1$, and for $i = 1$ it is sufficient to note that:

$$\tilde{f}_1(x) < \tilde{f}_1(y) \;\Rightarrow\; \tilde{f}_1(x) < \max(Y) \;\Rightarrow\; f_1(x) \le \tilde{f}_1(x);$$

$$\tilde{f}_1(x) < \tilde{f}_1(y) \;\Rightarrow\; \min(Y) < \tilde{f}_1(y) \;\Rightarrow\; \tilde{f}_1(y) \le f_1(y).$$

Now, we prove that, for all $(x,v) \in X'$,

$$G_{\tilde{F}}(x,v) \text{ is a subgraph of } G_F(x,v). \tag{4}$$

Let $(x,v) \in X'$ and suppose (j,s,i) to be an edge of $G_{\tilde{F}}(x,v)$. According to (3), $\tilde{f}'_{ij}(x,v)$ and $f'_{ij}(x,v)$ have the same sign (here s), and $f_i(x)$ and $f_i(x+v_je_j)$ are on both sides of $x_i+v_i/2$ since $\tilde{f}_i(x)$ and $\tilde{f}_i(x+v_je_j)$ are. In other words, (j,s,i) is an edge of $G_F(x,v)$. So (4) is proved and, as an immediate consequence,

$$T_i(G_{\tilde{F}}) \subseteq T_i(G_F) \qquad (i = 1, \ldots, n). \tag{5}$$

Then, for all $(x,v) \in X'$, we have the following:

$$G_{\tilde{F}}(x,v) \text{ has no positive circuit involving vertex 1.} \tag{6}$$

Indeed, suppose, by contradiction, that vertex 1 belongs to a positive circuit of $G_{\tilde{F}}(x,v)$. Let j be the predecessor of 1 in this circuit, and let $t = x_1 + v_1/2$. By definition, $t \in T_1(G_{\tilde{F}})$, and from (5) it follows that $t \in T_1(G_F)$. We then deduce, from (1) and the fact that the images of $\tilde{f}_1$ are in Y, that $\tilde{f}_1(x)$ and $\tilde{f}_1(x + v_j e_j)$ are not on both sides of t. In other words, there is no edge from j to 1 in $G_{\tilde{F}}(x,v)$, a contradiction.

Let $\tilde{\mathbb{A}}$ be the set of attractors of $\Gamma(\tilde{F})$, and let

$$\tilde{I} = I \setminus \{1\}. \tag{7}$$

Let (x,v) be any element of X'. Since I is a positive feedback vertex set of $G_F(x,v)$, and since $G_{\tilde{F}}(x,v)$ is a subgraph of $G_F(x,v)$, I is also a positive feedback vertex set of $G_{\tilde{F}}(x,v)$. We then deduce from (6) that $\tilde{I}$ is a positive feedback vertex set of $G_{\tilde{F}}(x,v)$. Since this holds for all $(x,v) \in X'$, and since $\tilde{I}$ is strictly included in I, by induction hypothesis, we have

$$|\tilde{\mathbb{A}}| \leq \prod_{i \in \tilde{I}} \Big(|T_i(G_{\tilde{F}})| + 1 \Big),$$

and from (5) we obtain:

$$|\tilde{\mathbb{A}}| \leq \prod_{i \in \tilde{I}} \Big(|T_i(G_F)| + 1 \Big). \tag{8}$$

Now, let $\mathbb{A}$ be the set of attractors of $\Gamma(F)$, and let $\mathbb{A}_Y$ be the set of $A \in \mathbb{A}$ containing a point x such that $x_1 \in Y$. We claim that:

$$\forall A \in \mathbb{A}_Y, \text{ there exists } \tilde{A} \in \tilde{\mathbb{A}} \text{ such that } \tilde{A} \subseteq A. \tag{9}$$

So let $A \in \mathbb{A}_Y$, and consider the set $\bar{A}$ of $x \in A$ such that $x_1 \in Y$. We prove that $\bar{A}$ is a trap domain of $\Gamma(\tilde{F})$. Suppose (x,y) to be an edge of $\Gamma(\tilde{F})$ such that $x \in \bar{A}$. By definition, there exists an index i such that $y = x + e_i$ and $x_i < \tilde{f}_i(x)$, or $y = x - e_i$ and $x_i > \tilde{f}_i(x)$. We consider two cases:

1) Case $i > 1$. Then, $y_1 = x_1 \in Y$. Moreover, $\tilde{f}_i(x) = f_i(x)$ so (x,y) is an edge of $\Gamma(F)$. Hence $y \in A$ (since $x \in A$), and we deduce that $y \in \bar{A}$.

2) Case $i = 1$. Suppose that $x_1 < \tilde{f}_1(x)$ (the proof is similar if $x_1 > \tilde{f}_1(x)$). Then, $x_1 < y_1 \leq \tilde{f}_1(x)$, and since x_1 and $\tilde{f}_1(x)$ are in Y, we have $y_1 \in Y$. Moreover, $\min(Y) \leq x_1 < \tilde{f}_1(x)$ so $x_1 < \tilde{f}_1(x) \leq f_1(x)$. Thus, (x,y) is an edge of $\Gamma(F)$. Hence $y \in A$ (since $x \in A$), and we deduce that $y \in \bar{A}$.

Since $y \in \bar{A}$ in both cases, $\bar{A}$ is trap domain of $\Gamma(\tilde{F})$. Thus, there exists at least one attractor $\tilde{A} \in \tilde{\mathbb{A}}$ such that $\tilde{A} \subseteq \bar{A}$, and (9) holds, since $\bar{A} \subseteq A$.

Following (9), there exists a map $H : \mathbb{A}_Y \to \tilde{\mathbb{A}}$ such that $H(A) \subseteq A$ for all $A \in \mathbb{A}_Y$. Since the attractors of $\Gamma(F)$ are mutually disjointed, the elements of $\mathbb{A}_Y$ are mutually disjointed, and we deduce that the images of H are also mutually disjointed. Consequently, H is an injection. So $|\mathbb{A}_Y| \leq |\tilde{\mathbb{A}}|$ and we deduce from (8) that

$$|\mathbb{A}_Y| \leq \prod_{i \in \tilde{I}} \Big(|T_i(G_F)| + 1 \Big).$$

Since this inequality holds for all $Y \in \mathbb{P}$, and since $\mathbb{A} = \bigcup_{Y \in \mathbb{P}} \mathbb{A}_Y$, we have:

$$|\mathbb{A}| \leq \sum_{Y \in \mathbb{P}} |\mathbb{A}_Y| \leq \sum_{Y \in \mathbb{P}} \Big(\prod_{i \in \tilde{I}} \Big(|T_i(G_F)| + 1 \Big) \Big) = |\mathbb{P}| \prod_{i \in \tilde{I}} \Big(|T_i(G_F)| + 1 \Big).$$

Using (2) and (7), we obtain:

$$|\mathbb{A}| \leq \Big(|T_1(G_F)| + 1 \Big) \prod_{i \in \tilde{I}} \Big(|T_i(G_F)| + 1 \Big) = \prod_{i \in I} \Big(|T_i(G_F)| + 1 \Big).$$

□

From the bound of Theorem 2 (which only depends on G_F) we now state: a less accurate bound, which only depends on X and on the images of G_F, and which still generalizes Theorem 1 (Corollary 1); a bound, again less accurate, which only depends on X and $G(F)$ (Corollary 2).

Corollary 1 *If I is a positive feedback vertex set of $G_F(x, v)$ for every $(x, v) \in X'$, then $\Gamma(F)$ has at most $\prod_{i \in I} |X_i|$ attractors.*

Proof —Straightforward from Theorem 2 and the fact that $|T_i(G_F)| < |X_i|$. □

Corollary 2 *If I is a positive feedback vertex set of $G(F)$, then $\Gamma(F)$ has at most $\prod_{i \in I} |X_i|$ attractors.*

Proof —Straightforward from Corollary 1 and the fact that each local interaction graph $G_F(x, v)$ is a subgraph of $G(F)$. □

Remark 3 Since the number of fixed points for F is less than or equal to the number of attractors in $\Gamma(F)$, we have the following: *If I is a positive feedback vertex set of $G(F)$, then F has at most $\prod_{i \in I} |X_i|$ fixed points*. This property has been proved by Aracena, Demongeot and Goles [19,20] in the Boolean case and under the rather strong hypothesis that $G(F)$ does not contain both a positive and a negative edge from one vertex to another (i.e. the entries of the Jacobian matrix of F are either everywhere ≥ 0 or everywhere ≤ 0).

Remark 4 Since $G_F(x, v)$ is a subgraph of $\mathscr{G}_F(x, v)$, Theorem 2 and Corollary 1 remain true but become less strong when stated with $\mathscr{G}_F$ instead of G_F (and for the same reasons, Corollary 2 remains true but becomes less strong when stated with $\mathscr{G}(F) = \bigcup_{(x,v) \in X'} \mathscr{G}_F(x, v)$ instead of $G(F)$). To illustrate

this, suppose that $X = \{0, 1, 2, \ldots, b\}^n$ and that $f_i(x) = \min(x_i + 1, b)$ for $i = 1, \ldots, n$. Then, $f'_{ii}(x, v) = 1$ if $x_i < b$ and $x_i + v_i < b$, and $f'_{ii}(x, v) = 0$ otherwise. In other words, $\mathscr{G}_F(x, v)$ has a positive edge from i to itself, and hence a positive circuit of length 1 on i, whenever x_i and $x_i + v_i$ are $< b$. We deduce that $|T_i(\mathscr{G}_F)| = b - 1$, and that there is no strict subset of $\{1, \ldots, n\}$ that has the property to be, for all $(x, v) \in X'$, a positive feedback vertex set of $\mathscr{G}_F(x, v)$. Consequently, the smallest upper bound given by Theorem 2 when stated with $\mathscr{G}_F$ is b^n. Now, it is easy to see that $G_F(x, v)$ has no edge for every $(x, v) \in X'$. So the smallest upper bound given by Theorem 2 is only 1.

5 Comments

5.1 Influence of connections between positive circuits

Corollary 2 is sufficient to highlight the fact that: *"A high level of connection between positive circuits leads to a small number of attractors"*. Suppose, for the sake of simplicity, that all the intervals X_i are of cardinality q, and let r be the smallest number of vertices that a positive feedback vertex set of $G(F)$ can contain. Then, the smallest upper bound on the number of attractors in $\Gamma(F)$ given by Corollary 2 is q^r, and the more the positive circuits of $G(F)$ are connected, the smaller r is. Indeed, let us say that a vertex *represents* a circuit when it belongs to this circuit. Then, r is the smallest number of vertices allowing the representation of each positive circuit. Therefore, the more the positive circuits are connected, the more it is possible to choose vertices representing a number of positive circuits, and the smaller r is. For instance, r is always $\leq$ the number p of positive circuits that $G(F)$ contains, but $r < p$ whenever $G(F)$ has connected positive circuits, and in the extremal case where all the positive circuits of $G(F)$ share a same vertex, we have $r = 1$.

5.2 Thomas' logical method

In practice, the dynamics of a gene network are often modeled from its interaction graph $\mathcal{G}$, typically by using *Thomas' logical method* [14,22,23]. Basically, Thomas associates with $\mathcal{G}$ a finite state space X and describes the behavior of the interactions of $\mathcal{G}$ by *logical parameters*. Then, he deduces from the value of these parameters a map F from X to itself whose asynchronous state transition graph describes possible dynamics for the network; see [17] for a formal presentation.

This modeling method is coherent with our notion of interaction graph in the

sense that, for all parameters values, the resulting map F has the property to be such that $G(F)$ is a subgraph of $\mathcal{G}$ [27]. So, thanks to Corollary 2, we can say, in the total absence of information on the value of the parameters, that following Thomas' logical method, the number of attractors in the dynamics of the network is at most

$$\mu(\mathcal{G}, X) = \min_{I \in \mathcal{I}(\mathcal{G})} \prod_{i \in I} |X_i|,$$

where $\mathcal{I}(\mathcal{G})$ is the set of positive feedback vertex sets of $\mathcal{G}$. This result is of practical interest since the value of the parameters is most often unknown and difficult to estimate, and since the number of attractors is an important feature of the dynamics of the network. For instance, if the network is known to control a differentiation process into k cell types, one often considers that the dynamics of the network have to contain at least k attractors. The bound $\mu(\mathcal{G}, X)$ can then be used to check whether the data of $\mathcal{G}$ and X are consistent with the presence of k attractors (there is inconsistency whenever $\mu(\mathcal{G}, X) < k$).

5.3 Feedback circuit functionality

Finally, Theorem 2 is related to one of the main concepts raised by Thomas' logical method: the concept of *feedback circuit functionality* [15,22,23,28]. Roughly speaking, it has been observed that some inequality constraints on the logical parameters describing the behavior of the interactions of a positive (resp. negative) circuit of $\mathcal{G}$ often lead to dynamics that contain several attractors (resp. that describe oscillations). For that reason, when these constraints are satisfied, the corresponding circuit is said to be functional. Even if this notion is not well understood and often informally stated, it is often used in practice to establish the value of the logical parameters; see for instance [29–34].

A natural formalization of the notion of functional circuit, also proposed in [27,35], is the following: given a map F from X to itself whose interaction graph $G(F)$ is a subgraph of $\mathcal{G}$, a circuit C of $\mathcal{G}$ is *functional* at $(x, v) \in X'$ if C is a circuit of $G_F(x, v)$. It is then easy to see that the upper bound on the number of attractors given by Theorem 2 depends only on the localization (inside X') and on the connections of the functional positive circuits of the system. To our knowledge, this is one of the first mathematical results relating the functional circuits of the system to its *global* dynamical properties (for relations between functional circuits and *local* dynamical properties, see the recent paper [35]).

Acknowledgement

I wish to thank Christophe Soulé for his valuable suggestions that led to significant improvements in the preliminary version of this work.

A Example

This appendix illustrates Theorem 2 for a very simple class of Boolean networks for which the computation of the bound of Theorem 2 is straightforward, in the sense that it can be computed by regarding only the global interaction graph of the network.

First observe that, in the Boolean case, Theorem 2 and Corollary 1 are equivalent: they have the same conditions, and if I is a smallest subset of $\{1, \ldots, n\}$ verifying these conditions, then $|T_i(G_F)| + 1 = |X_i| = 2$ for all $i \in I$, so that the conclusions are identical.

Now, let $\mathcal{G}$ be an interaction graph that does not have both a positive and a negative edge from one vertex to another. For each vertex i, let $\mathcal{G}_i^+$ (resp. $\mathcal{G}_i^-$) be the set of positive (resp. negative) predecessors of i in $\mathcal{G}$, i.e. the set of vertices j such that $\mathcal{G}$ has a positive (resp. negative) edge from j to i. Let $X = \{0,1\}^n$, and let F be the map from X to itself defined from $\mathcal{G}$ by

$$f_i(x) = \min\Big(\{x_j \,|\, j \in \mathcal{G}_i^+\} \cup \{1 - x_j \,|\, j \in \mathcal{G}_i^-\} \cup \{1\}\Big) \qquad (i = 1, \ldots, n).$$

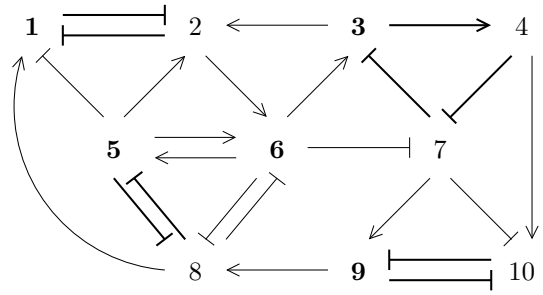
So $f_i(x) = 1$ if and only if, at state x, all the positive predecessors j of i are present ($x_j = 1$) and all the negative predecessors j of i are absent ($x_j = 0$). Observe that $G(F) = \mathcal{G}$.

Let C be a circuit of $\mathcal{G}$. We say that a vertex j of $\mathcal{G}$ is a *bad vertex* for C if $\mathcal{G}$ has a positive edge e and a negative edge e', both starting from j, such that e and e' do not belong to C and such that the final vertices of e and e' are distinct vertices of C. It is easy to see that C is a circuit of $G_F(x, v)$ for at least one $(x, v) \in X'$ if and only if C has no bad vertex. In this context, Corollary 1 can thus be reformulated as follows:

If I is a set of vertices such that each positive circuit of $\mathcal{G}$ without a bad vertex has a node in I, then $\Gamma(F)$ has at most $2^{|I|}$ attractors. (A.1)

For instance, suppose that $\mathcal{G}$ is as follows (arrows $\rightarrow$ correspond to positive

edges, and T-end arrows $\dashv$ correspond to negative edges):



Then, $\mathcal{G}$ has 46 positive circuits (and no negative circuit), and a positive feedback vertex sets of $\mathcal{G}$ of minimal cardinality is, for instance, $\{1, 3, 5, 6, 9\}$. So the smallest upper bound on the number of attractors in $\Gamma(F)$ given by Corollary 2 is $2^5 = 32$. Now, in order to use A.1, consider the positive circuits of $\mathcal{G}$ that do not involve vertex 6. There are four such circuits, and each of these has a bad vertex (given in brackets):

$$1 \dashv 2 \dashv 1 \qquad (5)$$

$$3 \rightarrow 4 \dashv 7 \dashv 3 \qquad (6)$$

$$5 \dashv 8 \dashv 5 \qquad (6)$$

$$9 \dashv 10 \dashv 9 \qquad (7)$$

We deduce that each positive circuit of $\mathcal{G}$ without a bad vertex has a node in $\{6\}$. So according to the reformulation A.1 of Corollary 1, $\Gamma(F)$ has at most $2^1 = 2$ attractors. This bound is reached since $(1, 0, 0, 0, 0, 0, 1, 1, 1, 0)$ and $(0, 1, 1, 1, 1, 1, 0, 0, 0, 1)$ are fixed points of F.

References

[1] H. de Jong, Modeling and simulation of genetic regulatory systems: a literature review, *Journal of Computational Biology*, 9 (2002) 67-103.

[2] 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.

[3] O. Delbrück, Discussion, in *Unités Biologiques Douées de Continuités Génétiques*, Volume 33, Edition CNRS, Lyon, 1949.

[4] E. Plathe, T. Mestl, S.W. Omholt, Feedback loops, stability and multistationarity in dynamical systems, *Journal of Biological Systems*, 3 (1995) 569-577.

[5] J. L. Gouzé, Positive and negative circuits in dynamical systems, *Journal of Biological Systems*, 6 (1998) 11-15.

[6] E.H. Snoussi, Necessary conditions for multistationarity and stable periodicity, *Journal of Biological Systems*, 6 (1998) 3-9.

[7] O. Cinquin, J. Demongeot, Positive and negative feedback: striking a balance between necessary antagonists, *Journal of Theoretical Biology*, 216 (2002) 229-241.

[8] C. Soulé, Graphical requirements for multistationarity, *ComPlexUs*, 1 (2003) 123-133.

[9] C. Soulé, Mathematical approaches to differentiation and gene regulation, *C.R. Paris Biologies*, 329 (2006) 13-20.

[10] A. Richard, J.-P. Comet, Necessary conditions for multistationarity in discrete dynamical systems, *Discrete Applied Mathematics*, 155 (2007) 2403-2413.

[11] E. Remy, P. Ruet, D. Thieffry, Graphics requirement for multistability and attractive cycles in a boolean dynamical framework, *Advances in Applied Mathematics*, 41 (2008) 335-350.

[12] L. Glass, S.A. Kauffman, The logical analysis of continuous non linear biochemical control networks, *Journal of Theoretical Biology*, 39 (1973) 103-129.

[13] E.H. Snoussi, Qualitative dynamics of a piecewise-linear differential equations : a discrete mapping approach, *Dynamics and Stability of Systems*, 4 (1989) 189-207.

[14] R. Thomas, R. d'Ari, *Biological Feedback*, CRC Press, 1990.

[15] E.H. Snoussi, R. Thomas, Logical identification of all steady states: the concept of feedback loop caracteristic states, *Bulletin of Mathematical Biology*, 55 (1993) 973-991.

[16] H. de Jong, J.-L. Gouzé, C. Hernandez, M. Page, S. Tewfik, J. Geiselmann, Qualitative simulation of genetic regulatory networks using piecewise-linear models, *Bulletin of Mathematical Biology*, 66 (2004) 301-340.

[17] G. Bernot, J.-P. Comet, A. Richard, J. Guespin, Application of formal methods to biological regulatory networks: extending Thomas' asynchronous logical approach with temporal logic, *Journal of Theoretical Biology*, 229 (2004) 339-347.

[18] A.G. Gonzales, A. Naldi, L. Sánchez, D. Thieffry and C. Chaouiya, GINsim: a software suite for the qualitative modelling, simulation and analysis of regulatory networks, *Biosystems*, 84 (2006) 91-100.

[19] J. Aracena, J. Demongeot, E. Goles, Positive and negative circuits in discrete neural networks, *IEEE Transactions of Neural Networks*, 15 (2004) 77-83.

[20] J. Aracena, Maximum number of fixed points in regulatory boolean networks, *Bulletin of Mathematical Biology*, 70 (2008) 1398-1409.

[21] R. Thomas, Boolean formalization of genetic control circuits, *Journal of Theoretical Biology*, 42 (1973) 563-585.

[22] R. Thomas, Regulatory Networks Seen as Asynchronous Automata: A logical Description, *Journal of Theoretical Biology*, 153 (1991) 1-23.

[23] R. Thomas, M. Kaufman, Multistationarity, the basis of cell differentiation and memory. I. & II., *Chaos*, 11 (2001) 170-195.

[24] F. Robert, Dérivée Discrète et Comportement Local d'Une Itération Discrète, Linear Algebra Appl. 52/53 (1983) 547–589.

[25] F. Robert, Discrete iterations: a metric study, in: Series in Computational Mathematics, Vol. 6, Springer-Verlag, Berlin-Heidelber-New York, 1986.

[26] F. Robert, Les systèmes dynamiques discrets, in: Mathématiques et Applications, Vol. 19, Springer-Verlag, Berlin-Heidelber-New York, 1995.

[27] A. Richard, *Modèle formel pour les réseaux de régulation génétique et influence des circuits de rétroaction*, Ph.D. Thesis, University of Evry Val d'Essonne, France, 2006.

[28] R. Thomas, D. Thieffry, M. Kaufman, Dynamical behaviour of biological regulatory networks - I Biological role of feedback loops an practical use of the concept of the loop-characteristic state, *Bulletin of Mathematical Biology*, 57 (1995) 247-276.

[29] D. Thieffry, R. Thomas, Dynamical behaviour of biological regulatory networks - II Immunity control in bacteriophage lambda, *Bulletin of Mathematical Biology*, 57 (1995) 277-297.

[30] E. Muraille, D. Thieffry, O. Leo, M. Kaufman, Toxicity and neuroendocrine regulation of the immune response: a model analysis, *Journal of Theoretical Biology*, 183 (1996) 285-305.

[31] L. Sánchez, J. van Helden, D. Thieffry, Establishement of the dorso-ventral pattern during embryonic development of drosophila melanogaster: a logical analysis, *Journal of Theoretical Biology*, 189 (1997) 377-389.

[32] L. Mendoza, D. Thieffry, E.R. Alvarez-Buylla, Genetic control of flower morphogenesis in Arabidopsis thaliana: a logical analysis, *Bioinformatics*, 15 (1999) 593-606.

[33] L. Sánchez, D. Thieffry, A logical analysis of the Drosophila gap-gene system, *Journal of Theoretical Biology*, 211 (2001) 115-141.

[34] L. Sánchez, D. Thieffry, Segmenting the fly embryo: a logical analysis of the *pair-rule* cross-regulatory module, *Journal of Theoretical Biology*, 224 (2003) 517-537.

[35] E. Remy, P. Ruet, From elementary signed circuits to the dynamics of Boolean regulatory networks, *Bioinformatics*, 24 (2008) 220-226.