



On the robustness of update schedules in Boolean networks

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ABSTRACT

Deterministic Boolean networks have been used as models of gene regulation and other biological networks. One key element in these models is the update schedule, which indicates the order in which states are to be updated. We study the robustness of the dynamical behavior of a Boolean network with respect to different update schedules (synchronous, block-sequential, sequential), which can provide modelers with a better understanding of the consequences of changes in this aspect of the model. For a given Boolean network, we define equivalence classes of update schedules with the same dynamical behavior, introducing a labeled graph which helps to understand the dependence of the dynamics with respect to the update, and to identify interactions whose timing may be crucial for the presence of a particular attractor of the system. Several other results on the robustness of update schedules and of dynamical cycles with respect to update schedules are presented. Finally, we prove that our equivalence classes generalize those found in sequential dynamical systems.

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1. Introduction

Robustness is a ubiquitously observed and necessary property of biological systems (Kitano, 2004), and is therefore a key aspect in the analysis of biological models, and in particular of regulatory networks. A dynamical property is said to be robust when it is not affected by small perturbations. When we talk about a real system, these perturbations model the noise which is intrinsic to reality, and apply to the states of the system. When we are dealing with a model instead, they can refer to changes in the state variables (in which case the word *stability* may be more appropriate) or to changes in the specification of the model itself.

The kinds of model in which we are interested are Boolean networks, first introduced by Kauffman as a mathematical tool to study the dynamics of gene regulatory networks (Kauffman, 1969, 1993). In this case a gene expression level is modeled by binary values, 0 or 1, indicating two transcriptional states, either active or inactive, respectively, and this level changes in time according to some local activation function which depends on the states of a set of nodes (genes). The joint effect of the local activation functions defines a global transition function; thus, the other element required in the description of the model is an update schedule which determines

when each node has to be updated, and hence, how the local functions combine into the global one (in other words, it must describe the relative timings of the regulatory activities).

Given those elements, there are mainly three kinds of perturbations for a BN: perturbations of the states of the nodes (the state variables), changes of the local activation functions, and modifications of the update schedule. The last two are changes to the model itself and therefore can cause variations in the dynamical trajectories. Importantly, they can change the set of attractors, which is of great interest when modeling genetic regulatory networks. The reason for this interest is twofold: on one hand, the attractors are usually identified with distinct types of cells defined by patterns of gene activity. In particular, the fixed points are often associated with phenomena such as cell proliferation and apoptosis, and the dynamical cycles with cellular cycle, division, etc. (Huang, 1999; Aracena et al., 2006). On the other hand, the attractors of the system are the most easily obtained information in the laboratory, and the most reliable one (being less prone to the noise that creates difficulty in time series analysis).

The first kind of robustness (which we prefer to call stability) has been widely studied, mostly from a statistical point of view, in random BNs (RBN), where the local activation functions are probabilistically chosen. The topic at large has been the relation between parameters and topological features describing the network, and the stability of its dynamics and the structure of its phase space. Unfortunately, there exist only a few analytical studies. Aldana and Cluzel (2003) show that RBNs with scale-free architecture, where

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a small set of nodes are highly connected and the rest poorly connected, are robust. Shmulevich et al. (2003) study the robustness of RBNs whose local functions belong to certain Post classes. The effect of function perturbation has been studied by Gershenson et al. (2006), but without emphasis on the attractors. Xiao and Dougherty (2007) study the impact of function perturbations on attractors in synchronous BNs.

Update schedules have been a slightly neglected topic, in particular when it comes to the robustness with respect to changes in them. An early introduction of strongly asynchronous timing in genetic models is due to (Thomas, 1973), through the use of time delays and the elimination of simultaneity. Since real time delays are difficult to determine (and are probably stochastic), the model is analyzed in probabilistic terms, or by studying the set of all admissible transitions; only when all time delays are fixed and known do we have a deterministic update schedule, which falls in case 5 of the list given below. For a long time the simpler synchronous update was the default choice for RBN researchers (Kauffman et al., 2003), in part because of the scarcity of actual models of real networks; when the need for asynchrony was recognized, it was usually introduced through stochasticity. Yet deterministic, non-synchronous update schedules have appeared in the literature (Mendoza and Alvarez-Buylla, 1998; Albert and Othmer, 2003), and are a necessary part of our understanding of Boolean network dynamics.

One reason for this slow introduction of asynchronous determinism into actual models is the difficulty of really knowing the order (if any) in which events take place in the cell; there may be spatial reasons determining the timings of regulatory activities, or the asynchrony may be needed to accommodate different speeds in reaction times. This makes an understanding of model robustness with respect to update schedules specially important: we need to know, for instance, what set of update schedules may produce a certain observed attractor.

Regarding perturbations of the update schedule, Chaves et al. (2005) study the effect of different asynchronous updates of the nodes on the dynamics of BNs for the *Drosophila melanogaster* segment polarity genes. Fauré et al. (2006) analyze the dynamics of a generic Boolean model for control of the mammalian cell cycle, considering different update schedules and their effects on the attractors. Willadsen and Wiles (2007) propose a method for quantifying robustness and dynamics in terms of state-space structures, for Boolean models of known genetic regulatory systems.

To the best of our knowledge, little analytical work has been done about this kind of perturbations. One of the exceptions, which (as will be seen in Section 5) comes close to the approach presented in this manuscript, is the study of a particular class of discrete dynamical system where the connection digraph is symmetric (or equivalently, an undirected graph). For this class of networks, the team of Mortveit and Reidys studied the set of update schedules preserving the whole dynamical behavior of the network (Mortveit and Reidys, 2001) and the set of attractors in a certain class of cellular automata (Hansson et al., 2005).

We focus our attention on the robustness of Boolean networks with respect to different deterministic update schedules. The choice of deterministic update schedules is given by the fact that information processing performed in the living cell has to be extremely robust and therefore requires a quasi-deterministic dynamics, what Schrödinger (1948) called a “clockwork mode”. Another reason for determinism is the need to model some periodical behaviors; when randomness is introduced, attractors become regions of the phase space, but are no longer exact dynamical cycles. Both stochastic and deterministic models are common in the biological literature, and a frequent strategy is to consider a deterministic dynamics and look at its robustness under small random perturbations. Here, we look at the robustness of deterministic update schedules within the space of deterministic models.

There exist different kinds of deterministic update schedules for a Boolean network, some of which are particular cases of another. The best known are:

1. Parallel or synchronous: every node is updated at the same time.
2. Sequential: in every time step, every node is updated in a defined sequence.
3. Block-sequential: the set of nodes is partitioned into blocks; nodes in a same block are updated in parallel, but blocks follow each other sequentially.
4. Asynchronous deterministic: in every time step only one node is updated following a defined sequence.
5. Asynchronous generalized: in every time step only one node is updated following a defined sequence, where every node can appear several times.

Here we consider the first three kinds, i.e., the classes where every node is updated once in every step. Nevertheless, note that the last two cases can be reduced to the third one through a simple network transformation (which duplicates nodes when necessary and considers the least common multiple of the periods). Thus, some results presented here may still be of interest for those models after an appropriate translation.

The structure of the paper is as follows. Section 2 introduces the basic definitions and notations. Section 3 studies the equivalence classes of update schedules, both through an easily checked equivalence condition introduced below, and through the equivalence of dynamical behavior; the connection between these two notions is discussed. Section 4 looks at the robustness of dynamical cycles of a network with respect to changes in its update schedule, seeing when cycles can be shared under different update regimes and how interactions whose timing is critical to a certain attractor can be identified. Section 5 deals with the relation of our results with those of Mortveit and Reidys. Finally, in Section 6 we discuss the insights that can be gained from the present results and the open problems of interest.

2. Definitions and Notation

A BN $N = (F, s)$ is defined by a finite set of variable states $x \in \{0, 1\}^n$, a global activation function $F : \{0, 1\}^n \rightarrow \{0, 1\}^n$, where $F(x) = (f_1(x), \dots, f_n(x))$ (f_i are called local activation functions), and an update schedule s .

An update schedule is defined by an update function that we denote $s : \{1, \dots, n\} \rightarrow \{1, \dots, n\}$, such that $s(\{1, \dots, n\}) = \{1, \dots, m\}$ for some $m \leq n$. A synchronous or parallel update is given by an update function s such that $\forall i \in \{1, \dots, n\}, s(i) = 1$. A sequential update corresponds to a permutation function over $\{1, \dots, n\}$. Others kinds of update functions can be considered as a block-sequential update.

The iteration of the BN with an update function s is given by:

$$x_i^{r+1} = f_i(x_1^r, \dots, x_n^r), \quad (1)$$

where $l_j = r$ if $s(i) \leq s(j)$ and $l_j = r + 1$ if $s(i) > s(j)$.

This is equivalent to applying a function $F^s : \{0, 1\}^n \rightarrow \{0, 1\}^n$ in a parallel way, with $F^s(x) = (f_1^s(x), \dots, f_n^s(x))$ defined by:

$$f_i^s(x) = f_i(g_{i,1}^s(x), \dots, g_{i,n}^s(x)),$$

where the function $g_{i,j}^s$ is defined by $g_{i,j}^s(x) = x_j$ if $s(i) \leq s(j)$ and $g_{i,j}^s(x) = f_j^s(x)$ if $s(i) > s(j)$. Thus, the function F^s corresponds to the dynamical behavior of the network $N = (F, s)$.

We say that two BNs $N_1 = (F_1, s_1)$ and $N_2 = (F_2, s_2)$ have the same dynamical behavior if $F_1^{s_1} = F_2^{s_2}$.

Since $\{0, 1\}^n$ is a finite set, we have two limit behaviors for the iteration of a network:

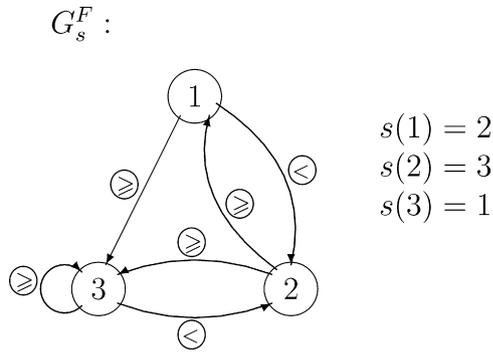


Fig. 1. Example of a labeled digraph.

- **Fixed point:** We define a fixed point as $x \in \{0, 1\}^n$ such that $F^s(x) = x$.
- **Cycle:** We define a cycle of length $p > 1$ as the sequence $[x^0, \dots, x^{p-1}, x^0]$ such that $x^j \in \{0, 1\}^n$, x^j are pairwise distinct and $F^s(x^j) = x^{j+1}$, for all $j = 0, \dots, p - 2$ and $F^s(x^{p-1}) = x^0$.

Fixed points and cycles are called *attractors* of the network.

We say that a node is *frozen* for a cycle if its state is constant on it (Greil et al., 2007; Kauffman, 1990).

The digraph associated to a BN $N = (F, s)$, called *connection digraph*, is the directed graph $G^F = (V, A)$, where $V = \{1 \dots, n\}$ and $(i, j) \in A$ if and only if f_j depends on x_i , i.e., if there exists $x \in \{0, 1\}^n$ such that

$$f_j(x_1, \dots, x_{i-1}, 0, x_{i+1}, \dots, x_n) \neq f_j(x_1, \dots, x_{i-1}, 1, x_{i+1}, \dots, x_n).$$

The node set of G^F is referred to as $V(G^F)$, its arc set as $A(G^F)$. An arc $(i, i) \in A(G^F)$ is called a *loop* of G^F .

A function $f : \{0, 1\}^n \rightarrow \{0, 1\}$ is *monotonic* on input i if for every $x \in \{0, 1\}^n$

$$f(x_1, \dots, x_{i-1}, 0, x_{i+1}, \dots, x_n) \leq f(x_1, \dots, x_{i-1}, 1, x_{i+1}, \dots, x_n).$$

A loop (i, i) is *monotonic* if f_i is monotonic on input i . A monotonic loop is the simplest network motif built out of a transcription factor activating its own transcription. Monotonic loops (also known as positive loops) are often found in gene regulatory networks (Kielbasa and Vingron, 2008).

We define the labeled digraph associated to a BN $N = (F, s)$ as: $G_s^F = (G^F, \text{lab}_s)$, where every arc has associated a label given by the function $A(G^F) \rightarrow \{\otimes, \odot\}$ defined as:

$$\text{lab}_s(i, j) = \begin{cases} \otimes & \text{if } s(i) \geq s(j) \\ \odot & \text{if } s(i) < s(j) \end{cases}$$

Example 1. See an example of a labeled digraph in Fig. 1. Note that the label on a loop will always be \otimes .

We define the following sequential schedules which correspond to elementary permutations, very useful in the sequel.

$$\pi_{i,j}(k) = \begin{cases} j & \text{if } k = i \\ i & \text{if } k = j \\ k & \text{otherwise.} \end{cases}$$

Also we denote by π_0 the schedule function such that $\pi_0(i) = i$. Observe that $\forall i, \pi_{i,i} = \pi_0$.

We denote $I(j) = \{i \in \{1, \dots, n\} / (i, j) \in A\}$. Thus, we can say $f_j(x) = f_j(x_i : i \in I(j))$.

3. Equivalent Update Schedules

Two different update schedules may induce the same or a different labeled graph. On the other hand, they may (in combination

with a given activation function) generate the same or a different dynamics of the system. The results in this section explore the connection between these two equivalence relations.

Theorem 1. Let $N_1 = (F, s_1)$ and $N_2 = (F, s_2)$ be two BNs which are different only in the update schedule. If $G_{s_1}^F = G_{s_2}^F$, then both dynamical behaviors are identical.

Proof. Without loss of generality, we suppose s_1 is such that, for all $i \in \{1, \dots, n\}$, $s_1(i + 1) \geq s_1(i)$ and $s_1(1) = 1$. Now, we prove by induction that $\forall j = 1, \dots, n, f_j^{s_1}(x) = f_j^{s_2}(x)$.

Basis. After the assumptions, if f_1 depends on x_i , then $s_1(i) \geq s_1(1)$, and by the condition $G_{s_1}^F = G_{s_2}^F, s_2(i) \geq s_2(1)$. Thus:

$$f_1^{s_1}(x) = f_1(g_{1,j}^{s_1}(x) : j \in I(1)) = f_1(x_j : j \in I(1)) = f_1(g_{1,j}^{s_2}(x) : j \in I(1)) = f_1^{s_2}(x).$$

Induction hypothesis. For all $j \leq k$

$$f_j^{s_1}(x) = f_j^{s_2}(x).$$

Case $k + 1$. By definition,

$$f_{k+1}^{s_1}(x) = f_{k+1}(g_{k+1,j}^{s_1}(x) : j \in I(k + 1)).$$

On the other hand, since $G_{s_1}^F = G_{s_2}^F$,

$$\forall j \in I(k + 1) : s_1(j) \geq s_1(k + 1) \Leftrightarrow s_2(j) \geq s_2(k + 1).$$

Thus, $\forall j \in I(k + 1)$ such that \otimes :

$$g_{k+1,j}^{s_1}(x) = x_j = g_{k+1,j}^{s_2}(x).$$

And $\forall j \in I(k + 1)$ such that \odot :

$$g_{k+1,j}^{s_1}(x) = f_j^{s_1}(x) \text{ and } g_{k+1,j}^{s_2}(x) = f_j^{s_2}(x).$$

Because of $s_1, \forall j \in I(k + 1), \otimes$ if and only if $j < k + 1$. Hence, according to the induction hypothesis: $f_j^{s_1}(x) = f_j^{s_2}(x)$ for all $j \in I(k + 1)$ such that \odot .

Therefore, for all $j \in I(k + 1), g_{k+1,j}^{s_1}(x) = g_{k+1,j}^{s_2}(x)$, and thus, $f_{k+1}^{s_1}(x) = f_{k+1}^{s_2}(x)$. \square

A corollary of this theorem is this result proved by Tchuente (1988):

Theorem 2. Let N be a BN such that $\forall i = 1, \dots, n, \forall j \in I(i), s(i) \leq s(j)$. Then the dynamics of N with the update schedule s and with the parallel update schedule are identical.

Proof. It is easy to see that a parallel update is equivalent to having a schedule function $s_p : V \rightarrow \{1, \dots, n\}$ such that $s_p(i) = 1, \forall i = 1, \dots, n$, and in this case $\text{lab}_{s_p}(i, j) = \otimes, \forall (i, j) \in A$ and if for all $i = 1, \dots, n, s(i) \leq s(j), \forall j \in I(i), \text{lab}_s(i, j) = \odot, \forall (i, j) \in A$, then $G_{s_p}^F = G_s^F$. \square

For a given global activation function F , we would like to classify the schedules that yield the same dynamical behavior. If we consider the relation between updates schedules s_1 and s_2 defined by having $G_{s_1}^F = G_{s_2}^F$, Theorem 1 shows that it yields a partition which is finer than the partition of identical dynamical behavior. In fact, the partition is strictly finer, since the converse of Theorem 1 turns out to be false in general.

Example 2. Fig. 2 shows two different labeled digraphs, without loops and strongly connected, associated to BNs $N_1 = (F, \pi_{0,0})$ and $N_2 = (F, \pi_{2,3})$ respectively, where $f_1(x) = x_2 \vee x_4, f_2(x) = x_4, f_3(x) = x_1 \vee x_2$ and $f_4(x) = x_3$. Both BNs have the same dynamical behavior, shown in Fig. 3.

However, in the following Proposition we show that, when two update schedules are dynamically equivalent but with different labeled graphs, then a small perturbation of the activation function can be found which preserves the connectivity and the labeled graphs, while causing a different dynamical behavior. Thus, while

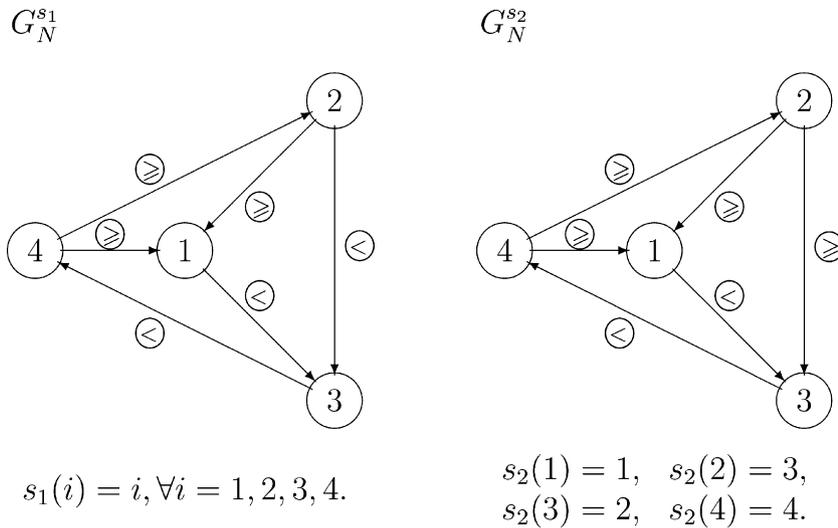


Fig. 2. BNs from Example 2, with two non-equivalent schedules with different labeled digraphs that have the same dynamical behavior. Their respective equivalence classes (giving the same labeled graphs) are {1234, 1123} and {1324, 1323, 1223}. The union of them gives the class of update schedules that yield this dynamical behavior.

Theorem 1 shows that the classes of update schedules with equal labeled graphs are the “atoms” that any particular global function F will group into classes with identical dynamical behavior, the next Proposition shows that this grouping will finely depend on the details of F .

Proposition 3. Let $N_1 = (F, s_1)$ and $N_2 = (F, s_2)$ be two BNs such that $G_{S_1}^F \neq G_{S_2}^F$ and their dynamical behavior are identical. Then there exists \tilde{F} , different from F in at most two local activation functions, such that $G^{\tilde{F}} = G^F$ and the dynamical behavior of $\tilde{N}_1 = (\tilde{F}, s_1)$ and $\tilde{N}_2 = (\tilde{F}, s_2)$ are different.

Proof. Without loss of generality, we suppose s_1 is such that, for all $i \in \{1, \dots, n\}$, $s_1(i+1) \geq s_1(i)$ and $s_1(1) = 1$. Let be:

$$i_* = \min\{i \in \{1, \dots, n\} : \exists j \in I(i), \text{lab}_{s_1}(j, i) \neq \text{lab}_{s_2}(j, i)\},$$

which is well defined, because by hypothesis $G_{S_1}^F \neq G_{S_2}^F$. Let us suppose that there exists $j_* \in I(i_*)$ and $x^* \in \{0, 1\}^n$ such that $\text{lab}_{s_1}(j_*, i_*) \neq \text{lab}_{s_2}(j_*, i_*)$ and $f_{j_*}^{s_1}(x^*) \neq x_{j_*}^*$.

Let $y^1, y^2 \in \{0, 1\}^n$ defined by $y_k^1 = x_k^*$ if $s_1(k) \geq s_1(i_*)$ and $y_k^1 = f_k^{s_1}(x^*)$, and $y_k^2 = x_k^*$ if $s_2(k) \geq s_2(i_*)$ and $y_k^2 = f_k^{s_2}(x^*)$. Hence, $f_{i_*}^{s_1}(x^*) = f_{i_*}(y^1)$ and $f_{i_*}^{s_2}(x^*) = f_{i_*}(y^2)$. Observe that $f_{j_*}^{s_1}(x^*) \neq x_{j_*}^*$ implies $y^1 \neq y^2$. Besides, for each $k \in I(i_*)$ we denote the vector $y^{2,k} \in \{0, 1\}^n$ such that $y_j^{2,k} = -y_j^2$ if $j = k$ and $y_j^{2,k} = y_j^2$ if $j \neq k$. Then, we define the function $\tilde{F} = (\tilde{f}_1, \dots, \tilde{f}_n)$ as:

$$\tilde{f}_i(x) = f_i(x) \quad \forall i \neq i_*$$

$$\tilde{f}_{i_*}(x) = \begin{cases} -f_{i_*}(y^2) & \text{if } x = y^2 \\ f_{i_*}(y^2) & \text{if } x = y^{2,k}, k \in I(i_*) \\ f_{i_*}(x) & \text{otherwise.} \end{cases}$$

Thus, $\tilde{F}_{i_*}^{s_1}(x^*) = \tilde{f}_{i_*}^{s_1}(x^*) = \tilde{f}_{i_*}(y^1) = f_{i_*}(y^1)$ and $\tilde{F}_{i_*}^{s_2}(x^*) = \tilde{f}_{i_*}^{s_2}(x^*) = \tilde{f}_{i_*}(y^2) = f_{i_*}(y^2)$. Since by hypothesis $f_{i_*}(y^1) \neq f_{i_*}(y^2)$, then $\tilde{F}_{i_*}^{s_1}(x^*) \neq \tilde{F}_{i_*}^{s_2}(x^*)$.

On the other hand, $\forall k \in I(i_*)$, $\tilde{f}_{i_*}^{s_1}(y^{2,k}) \neq \tilde{f}_{i_*}^{s_1}(y^2)$. Therefore, $G^{\tilde{F}} = G^{\tilde{F}}$.

If for all $x \in \{0, 1\}^n$ and for every $j \in I(i_*)$ such that $\text{lab}_{s_1}(j, i_*) \neq \text{lab}_{s_2}(j, i_*)$ verify $f_j^{s_1}(x) = x_j$, then we can define $\hat{f}_{j_*} = -f_{j_*}$ and $\hat{f}_i = f_i$, $\forall i \neq j_*$, where $j_* \in I(i_*)$ and $\text{lab}_{s_1}(j_*, i_*) \neq \text{lab}_{s_2}(j_*, i_*)$. Since

$$f_{j_*}(x_1, \dots, x_{k-1}, 0, x_{k+1}, \dots, x_n) \neq f_{j_*}(x_1, \dots, x_{k-1}, 1, x_{k+1}, \dots, x_n)$$

if and only if

$$\hat{f}_{j_*}(x_1, \dots, x_{k-1}, 0, x_{k+1}, \dots, x_n) \neq \hat{f}_{j_*}(x_1, \dots, x_{k-1}, 1, x_{k+1}, \dots, x_n),$$

then $G^{\tilde{F}} = G^F$. If $\hat{F}^{s_1} \neq \hat{F}^{s_2}$ we obtain the result of this proposition. Otherwise, we can apply the above reduction from $\hat{N} = (\hat{F}, s_1)$. \square

Example 2, continued. If we apply the previous Proposition to the example given in Fig 2, we obtain that $i_* = 3$ and $j_* = 2$. Thus, for $x^* = (0, 0, 0, 1)$: $y^1 = (1, 1)$, $y^2 = (1, 0)$, $\tilde{f}_3(y^1) = f_3(y^1) = y_1^1 \vee y_2^1 = 1 \vee 1 = 1$; $\tilde{f}_3(y^2) = f_3(y^2) = -(y_1^2 \vee y_2^2) = -(1 \vee 0) = 0$. Besides, $y^{2,1} = (0, 0)$ and $y^{2,2} = (1, 1)$. Hence, $\tilde{f}_3(y^{2,1}) = 1 = \tilde{f}_3(y^{2,2})$ and $\tilde{f}_3(x) = f_3(x)$, otherwise.

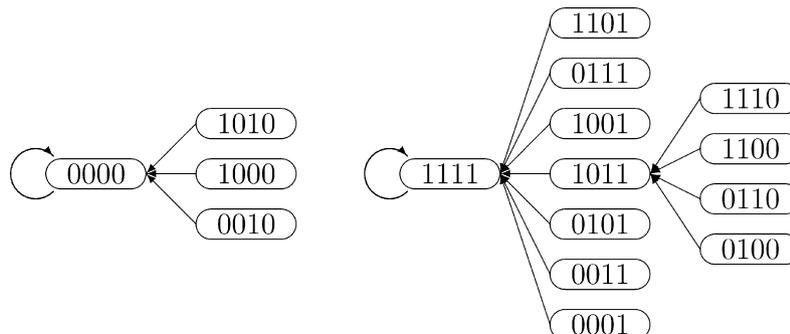


Fig. 3. Dynamical behavior of the networks N_1 and N_2 in Example 2.

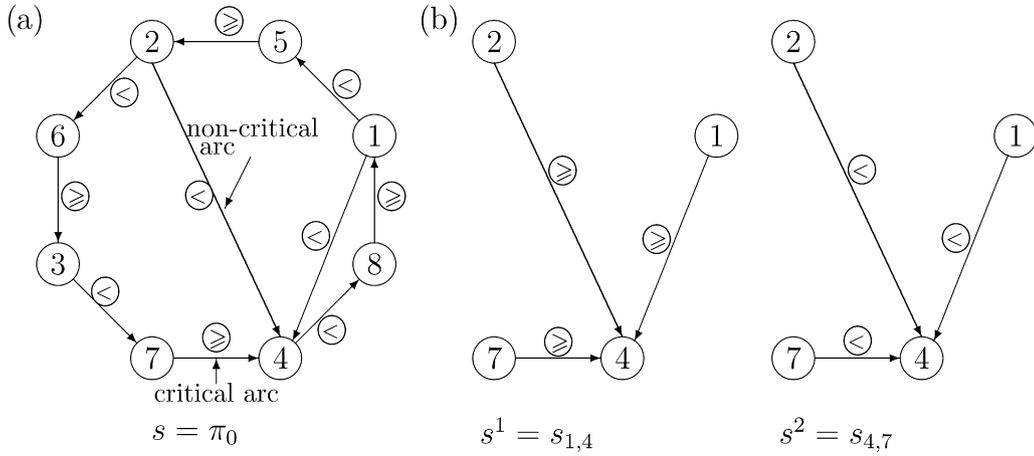


Fig. 4. (a) Critical and non-critical arcs for the cycle C_0 . (b) Application of conditions of Theorem 4 on the node $i = 4$.

4. Robustness of Attractors

In this section, we study different changes in the update schedule of a BN which keep or change its attractors. It is known that the set of fixed points of a discrete network does not change with respect to different update schedules. Therefore, the focus here is on the dynamical cycles.

Critical arcs: Given $N = (F, s)$ a BN and $[x^0, \dots, x^{p-1}, x^0]$ a dynamical cycle of length $p \geq 1$, we will say that the arc $(i, j) \in A(G^F)$ is critical for the cycle if either there exists $r \in \{0, \dots, p-2\}$ such that $F^{s_{ij}}(x^r) \neq x^{r+1}$ or $F^{s_{ij}}(x^{p-1}) \neq x^0$, where $s_{i,j}$ denote the update schedule defined by $s_{i,j} = s \circ \pi_{i,j}$ (in particular $s_{i,i} = s$). In other words, the arc (i, j) is critical for a dynamical cycle if the BN with update schedule $s_{i,j}$ does not preserve the cycle. Hence, a loop $(i, i) \in A(G^F)$ is never a critical arc.

Example 3. Fig. 4a shows an example of a BN $N = (F, s = \pi_0)$, where $F : \{0, 1\}^8 \rightarrow \{0, 1\}^8$ is defined by:

$$\begin{aligned} f_1(x) &= x_8 & f_2(x) &= x_5 & f_3(x) &= x_6 & f_4(x) &= (x_1 \wedge x_2) \vee x_7 \\ f_5(x) &= x_1 & f_6(x) &= x_2 & f_7(x) &= x_3 & f_8(x) &= x_4 \end{aligned}$$

N has a cycle

$$C_0 = [(10001000), (01000100), (00100010), (00010001), (10001000)].$$

For this cycle the arc $(2,4)$ is non-critical, since C_0 is also a cycle of the BN $N_1 = (F, s_{2,4})$. On the other hand, the arc $(7, 4)$ is a critical one, because the BN $N_2 = (F, s_{7,4})$ does not have the cycle C_0 as attractor.

Theorem 4. Let $N = (F, s)$ be a BN and $C = [x^0, \dots, x^{p-1}, x^0]$ a dynamical cycle of length $p > 1$. If i is a non-frozen node in C such that $\exists ! q, s(q) = \max\{s(j) : j \in I(i) \cup \{i\}\}$, then there exists a critical arc (j, i) for C .

Proof. Let $C = [x^0, \dots, x^{p-1}, x^0]$ a dynamical cycle of length $p > 1$ of $N = (F, s)$.

Let us now prove the result by contradiction. Let us suppose that $\exists q, r \in I(i) \cup \{i\}, \forall j \in I(i), s_{i,q}(j) \geq s_{i,q}(i)$ and $s_{i,r}(j) < s_{i,r}(i)$, and C is cycle of $N = (F, s_{i,q})$ and $N = (F, s_{i,r})$. Hence, $\forall l = 0, \dots, p-1$:

$$f_i^{s_{i,q}}(x_j^l : j \in I(i)) = f_i^{s_{i,q}}(x_j^l : j \in I(i)) = f_i^{s_{i,r}}(x_j^l : j \in I(i)).$$

But

$$f_i^{s_{i,q}}(x_j^l : j \in I(i)) = f_i(x_j^l : j \in I(i))$$

and

$$f_i^{s_{i,r}}(x_j^l : j \in I(i)) = f_i(x_j^{l+1} : j \in I(i)).$$

From here,

$$f_i(x_j^{l+1} : j \in I(i)) = f_i(x_j^l : j \in I(i)), \forall l = 0, \dots, p-1,$$

where $x^p \equiv x^0$. Thus, $f_i^s(x^l) = x_i^l = a, a \in \{0, 1\} \forall l = 1, \dots, p-1$, which is a contradiction with the hypothesis of x_i being non-constant. \square

Example 3, continued. An example of application of Theorem 4 is shown in Fig 4b.

Observe from the above proof that if $\forall j \in I(i), s(j) \geq s(i)$ and $\exists r \in I(i), s_{i,r}(j) < s_{i,r}(i)$ or $\forall j \in I(i), s(j) < s(i)$ and $\exists r \in I(i), s_{i,r}(j) \geq s_{i,r}(i)$, then (r, i) is a critical arc. Besides, the condition: $\exists r \in I(i) \cup \{i\}, \forall j \in I(i), s_{i,r}(j) < s_{i,r}(i)$ is not satisfied by a node j with a loop associated. However, it is easy to show that the result is also valid if a monotonic loop in the node is allowed. Thus, the following is a direct corollary of Theorem 4.

Corollary 5. Let $N = (F, s)$ and $N' = (F, s')$ be two BNs with different update schedules, and j a node without a loop or with a monotonic loop, such that $\otimes, \forall i \in I(j)$ and $\odot, \forall i \in I(j) \setminus \{j\}$. If C is a dynamical cycle for N and N' then j is a frozen node for C .

Observe from Theorem 4 and Corollary 5 that non-frozen nodes are important to the robustness of the cycle with respect to the change of the update schedule.

The following result allows us to define a new update schedule for a given BN such that the dynamical cycles are not kept.

Theorem 6. Let $N = (F, s)$ be a BN where the loops are monotonic. There exists an update schedule s' such that the dynamical cycles of $N' = (F, s')$ and $N = (F, s)$ are different.

Proof. Let $\{i_1, i_2, \dots, i_n\}$ be a labeling of the nodes of G^F so that $i_j \leq i_k \Leftrightarrow s(i_j) \leq s(i_k)$. We define s' by: $s'(i_j) = n + 1 - j, \forall j = 1, \dots, n$. Thus, $s'(i_1) > s'(i_2) > \dots > s'(i_n)$.

Given $[x^0, \dots, x^{p-1}, x^0]$ a dynamical cycle of length $p > 1$ of N , let i_* be the node of G^F such that $s'(i_*) = \max\{s'(i) : \exists l \in \{0, \dots, p-1\}, x_i^l \neq x_i^{l+1}\}$.

Suppose that $[x^0, \dots, x^{p-1}, x^0]$ is also a dynamical cycle of N' . Then, $\forall r = 0, \dots, p-1$:

$$f_{i_*}^s(x_j^r : j \in I(i_*)) = f_{i_*}^{s'}(x_j^r : j \in I(i_*)),$$

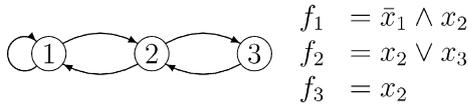


Fig. 5. This BN $N = (F, s)$ has a dynamical cycle $C = [(0, 1, 1), (1, 1, 1), (0, 1, 1)]$, which is invariant against any change in the update schedule s . Here, $(1, 1)$ is a non-monotonic loop of G^F .

with

$$f_{i_*}^s(x_j^r : j \in I(i)) = f_{i_*}(x_j^{r+1} : j \in I(i) \setminus I^*(i_*); a_j : j \in I^*(i_*)),$$

where $I^*(i_*) = \{j \in I(i) : x_j^r = a_j, a_j \in \{0, 1\}, \forall r = 0, \dots, p-1\}$.

Let us suppose that f_{i_*} does not depend on i_* , i.e. there does not exist the loop (i_*, i_*) in G^F . Then,

$$x_{i_*}^{r+1} = f_{i_*}^s(x_j^r : j \in I(i)) = f_{i_*}(x_j^r : j \in I(i) \setminus I^*(i_*); a_j : j \in I^*(i_*)),$$

Hence,

$$\begin{aligned} f_{i_*}(x_j^r : j \in I(i) \setminus I^*(i_*); a_j : j \in I^*(i_*)) \\ = f_{i_*}(x_j^{r+1} : j \in I(i) \setminus I^*(i_*); a_j : j \in I^*(i_*)). \end{aligned}$$

Therefore, x_{i_*} has constant value in the dynamical cycle, which is a contradiction with the definition of i_* .

Suppose now that f_{i_*} depends on i_* .

Observe that if there exists $l = 0, \dots, p-1$ such that $x_{i_*}^{l-1} = x_{i_*}^l$ then

$$\begin{aligned} x_{i_*}^l &= f_{i_*}^s(x_j^{l-1} : j \in I(i)) = f_{i_*}(x_j^l : j \in I(i) \setminus I^*(i_*); a_j : j \in I^*(i_*)) \\ x_{i_*}^{l+1} &= f_{i_*}^s(x_j^l : j \in I(i)) = f_{i_*}(x_j^l : j \in I(i) \setminus I^*(i_*); a_j : j \in I^*(i_*)) \end{aligned}$$

Thus $x_{i_*}^l = x_{i_*}^{l+1}$ and by induction we obtain that the i_* th component of the vectors in the cycle is constant, which contradicts again the definition of i_* .

Now, we suppose that f_{i_*} depends on x_{i_*} and $x_{i_*}^l \neq x_{i_*}^{l+1}, \forall l = 0, \dots, p-1$. Then, there exists $l = 0, \dots, p-1$, such that $x_{i_*}^{l-1} = 1, x_{i_*}^l = 0$ and $x_{i_*}^{l+1} = 1$:

$$\begin{aligned} x_{i_*}^l = 0 &= f_{i_*}(x_j^l : j \in I(i) \setminus \{i_*\}; x_{i_*}^l = 1) \\ x_{i_*}^{l+1} = 1 &= f_{i_*}(x_j^l : j \in I(i) \setminus \{i_*\}; x_{i_*}^l = 1) \end{aligned}$$

This is a contradiction with the monotonicity of f_{i_*} with respect to x_{i_*} . Therefore, $[x^0, \dots, x^{p-1}, x^0]$ is not a dynamical cycle of $N' = (F, s')$. \square

The hypothesis of monotonicity in the loops is essential in the previous theorem, which is noteworthy. The theorem says that if no negative loops are present, then all dynamical cycles can be destroyed by a change in the update schedule. If a negative loop does exist, then there *may* exist indestructible dynamical cycles. The simplest way to think about it is to imagine an isolated node with a negative loop: it will be a dynamical cycle by itself, and since loops are not affected by changes in the update schedule, it is an indestructible one. When the node is not isolated, this may still be true, but the conditions under which this will happen are not easily precised. Negative feedback loops have been associated with the existence of periodic behaviors (originally by Thomas, 1980; see also Thomas et al., 1995 and some recent work and references in Remy et al., 2008, Sontag et al., 2008, and specially Remy and Ruet, 2008). It is important here to bear in mind that we use the word “loop” in its restricted graph-theoretic sense (a node with a non-mediated influence on itself); thus negative *circuits* of greater length (often called loops too in the literature) are not excluded by the hypotheses. The relevance of negative loops in the previous proof is an indication of their importance not only for the existence,

but also for the robustness of periodic behavior: they are responsible for making dynamical cycles immune to changes in the update schedule.

Example 4. Fig. 5 shows a BN with a non-monotonic loop, for which a dynamical cycle exists that cannot be destroyed by any change in the update schedule.

Observe that if s is a synchronous update schedule in the proof of the previous theorem, then each sequential update schedule s' verifies that $s'(i_1) > s'(i_2) > \dots > s'(i_n)$ with i_1, \dots, i_n nodes of G^F . Thus, no sequential update schedule can share a dynamical cycle with the synchronous one (for a given BN without non-monotonic loops). This result was previously obtained by Goles and Salinas (2008).

5. Update Schedules in Symmetric Networks

One of the main studies of equivalent update schedules in discrete networks has been made in sequential dynamical systems (SDS) (Hansson et al., 2005; Mortveit and Reidys, 2001). These networks correspond to BNs with sequential schedules and where the connection digraph is symmetric, that is $(i, j) \in A(G^F) \Leftrightarrow (j, i) \in A(G^F)$. Besides, each node has also a loop.

While genetic regulatory networks are directed graphs, there exist some other biological networks where relations are symmetric (for instance, contact networks in epidemics, associative models of neural dynamics, and protein interaction networks—see Képès, 2007). In such cases it is worth considering this additional mathematical structure, which allows further theoretical results. One example of such a study, which deals with the topic of the present work, is Mortveit and Reidys (2001), who used the symmetric structure to provide an upper bound on the number of non-equivalent dynamics of SDS (something not yet available for the more general case considered here).

In the following, we work with BNs where the update schedule is sequential and G^F is a symmetric digraph.

Hansson et al. (2005); Mortveit and Reidys (2001) characterized the set of equivalent sequential schedules yielding a same dynamical behavior of a given SDSN $= (F, s_1)$, through the following relation: $s_1 \sim s_2$ if and only if:

$$\exists \{i_1, \dots, i_l\} \subseteq V(G^F), (s_1 = s_2 \circ \pi_{i_1, i_2} \circ \pi_{i_2, i_3} \circ \dots \circ \pi_{i_{l-1}, i_l})$$

and

$$\forall 1 \leq j < l, |s_1(i_j) - s_1(i_{j+1})| = 1 \wedge (i_j, i_l) \notin A(G^F) \wedge (i_l, i_j) \notin A(G^F)$$

They also estimated the number of schedules in each class.

In this section, we prove that the equivalence classes, determined by the relation ‘ \sim ’ defined above, coincide, in this particular family of BNs, with those defined in Section 3.

Theorem 7. Let $N_1 = (F, s_1)$ and $N_2 = (F, s_2)$ be two BNs with symmetric connection digraph and s_1, s_2 sequential update functions. Then, $s_1 \sim s_2$ if and only if $G_{s_1}^F = G_{s_2}^F$.

Proof. Sufficient Condition. It is easy to see that if $s_1 = s_2 \circ \pi_{i_1, i_2}$ with $|s_1(i_1) - s_1(i_2)| = 1$ and such that $(i_1, i_2) \notin A(G^F)$ and $(i_2, i_1) \notin A(G^F)$, then $G_{s_1}^F = G_{s_2}^F$. Thus, by using induction on the number of permutations needed to transform s_2 into s_1 , $G_{s_1}^F = G_{s_2}^F$.

Necessary Condition. We prove now that if $G_{s_1}^F = G_{s_2}^F$, then there exists a sequence of permutations $\pi_{i,j}$ such that the update schedule s_1 is the composition of these permutations with s_2 , that is $s_1 = s_2 \circ \pi_{i_1, i_2} \circ \pi_{i_2, i_3} \circ \dots \circ \pi_{i_{l-1}, i_l}$.

Without loss of generality, we suppose $s_1 = \pi_0$, and therefore for all $(i, j) \in A$ if $i \geq j$ then $s_1(i) \geq s_1(j)$, and if $i < j$ then $s_1(i) < s_1(j)$.

Now we proceed by induction on k to prove that $\forall k \in \{1, \dots, n\}$, $\exists i_1, \dots, i_k, \forall j \leq k, s_1(j) = j = s_2 \circ \pi_{i_1, i_2} \circ \dots \circ \pi_{i_{k-1}, i_k}(j)$ and $G_{s_2 \circ \pi_{i_1, i_2} \circ \dots \circ \pi_{i_{k-1}, i_k}}^F = G_{s_1}^F$.

Basis. Let us suppose that $s_2(1) = l \neq 1$, since $\forall j \in I(1), s_1(j) = j > 1$ and by hypothesis $G_{s_1}^F = G_{s_2}^F$, so that $s_2(j) > s_2(1) = l, \forall j \in I(1)$.

Therefore, $\forall j \in \{1, \dots, n\}$ such that $s_2(j) < l, (j, l) \notin A$ and $(l, j) \notin A$. Thus,

$$s_1(1) = 1 = s_2 \circ \pi_{i_1, i_2} \circ \dots \circ \pi_{i_{l-1}, i_l}(1)$$

where i_j is such that $s_2(i_j) = j, \forall j = 1, \dots, l$. In particular, $i_1 = 1$ and $i_l = l$. Besides, by Sufficient Condition result, $G_{s_2 \circ \pi_{i_1, i_2} \circ \dots \circ \pi_{i_{l-1}, i_l}}^F = G_s^F$.

Induction hypothesis. There exists a sequence of permutations such that, for all $j \leq k$:

$$s_1(j) = j = s'(j),$$

with $s' = s_2 \circ \pi_{i_1, i_2} \circ \dots \circ \pi_{i_{p-1}, i_p}$ and $G_{s'}^F = G_s^F$.

Case $k + 1$. If $s'(k + 1) = k + 1 = s_1(k + 1)$, then the result is direct.

Suppose that $s'(k + 1) = l \neq k + 1$. Thus, $l > k + 1$. Let $i_{k+1}, i_{k+2}, \dots, i_{l-1}$ be such that $s'(i_j) = j, \forall j = k + 1, \dots, l$. Hence, $i_j > k + 1, \forall j = k + 1, \dots, l - 1$ and $i_l = k + 1$.

Let us suppose that $(i_j, i_l) \in A$. Then, \odot . Since by hypothesis $G_{s'}^F = G_s^F, \odot$. This implies that $i_j < i_l = k + 1$ and hence $s'(i_j) = i_j < k + 1$, which contradicts $s'(i_j) = j \geq k + 1$.

Therefore, $(i_j, i_l) \notin A(G^F)$ and $(i_l, i_j) \notin A(G^F)$ for all $j = k + 1, \dots, l - 1$.

And by Sufficient Condition result, $G_s^F = G_{s'}^F = G_{s' \circ \pi_{i_{k+1}, i_{k+2}} \circ \dots \circ \pi_{i_{l-1}, i_l}}^F$ and

$$s_1(j) = s' \circ \pi_{i_{k+1}, i_{k+2}} \circ \dots \circ \pi_{i_{l-1}, i_l}(j), \forall j = 1, \dots, k + 1.$$

□

Note that the relation ' \sim ' defined above, as well as the proof of Theorem 7, are valid in any BN, not only for those with symmetric connection digraphs. Therefore, the results of Mortveit and Reidys (2001) relative to the equivalence relation ' \sim ' are also valid in another family of BNs different from SDSs. However, the problem of determining whether two update functions are equivalent is much more simple when the characterization given by their labeled digraphs is used.

6. Discussion

The use of non-trivial deterministic update schedules, though far from prevalent, has already appeared in models in the literature and as an option in modeling software (the last version of GINsim, for instance, makes them available through the use of *priority classes* Gonzalez et al. (2006); Fauré et al. (2006)). As more information flows in regarding reaction speeds and spatial constrains of the regulatory interactions, they are likely to continue surfacing, as they are one of the elements that define deterministic dynamical models (or even stochastic, since stochasticity, in the form of noise, needs a schedule of reference). Modelers of gene regulation or other biological networks must be aware of the role of this element, which may, for instance, explain the presence or absence of dynamical cycles in the observed dynamics of a system where the interactions are known but their timing is unclear. The results presented here are a first step toward understanding the flexibility or rigidity (in short, the robustness) of Boolean models with respect to their update schedules; a number of further questions of theoretical and practical interest remain open.

The labeling of graphs introduced here seems to be a useful way of looking at the relation between update schedules and dynamics. There is a close connection between the equivalence of labeled

graphs and the equivalence of dynamical behavior; the first implies the second (Theorem 1), and although the converse does not hold (Example 2), the cases where this happens are rare. Labeled graphs are not only easier to compare than dynamical behaviors, but also more tractable as combinatorial objects, if, for instance, we want to list the update schedules that preserve them. The number of update schedules for a given network is huge; the number of different induced label graphs, however, seems to be much smaller, and closer to the number of induced dynamical behaviors. There is work in progress towards a better understanding of the classes of labeled graphs that are possible for a given network (their sizes and numbers, and the way to enumerate them), and their relation to the classes of equivalent behaviors.

Another important aspect is the sensibility of dynamical cycles to changes in the update schedules, which was the topic of Section 4. Given a certain dynamical cycle, we would like to know the update schedules that preserve or destroy it, and the timings of interactions that are critical for its existence. A (partial) answer for the last question is given in Theorem 4, which gives sufficient conditions for the presence of critical arcs where a change in the update order would destroy the cycle. On the other hand, Theorem 6 gives a condition under which all the dynamical cycles of a network will be sensitive to changes in the update schedule. A complete characterization of indestructible dynamical cycles (besides the presence of non-monotonic loops, implied by the theorem) is an open problem, as is the characterization of all the update schedules which preserve a given dynamical cycle, or a set of them.

Section 5 shows that our results generalize those of Hansson et al. (2005) and Mortveit and Reidys (2001) for sequential dynamics systems. These particular systems are of no direct use in the modeling of gene regulatory networks, even if they may find a place when working with other biological networks where symmetry does appear. Yet, the connection is worth noticing, since it may open a possible source of insights or tools. Whether some techniques of their approach may be imported to the less algebraically tractable non-symmetric systems, or whether some results obtained in SDS may be used for giving bounds on quantities of interest in more general cases, remains to be seen.

There are several ways in which a better understanding of update schedules may be useful. Our results may shed light on some of the questions related to them, while other problems (some of them mentioned above and below) remain open. On one hand, the analytical study of the space of possible update schedules is a path for the study of deterministic asynchrony, and the question naturally arises of the connection between this perspective and that of stochastic noise. Do the sizes of equivalence classes of update schedules, for instance, correlate with the probability of observing some dynamics under noise? We do not know the answer, but the consideration of increasingly general deterministic updates and the understanding of their equivalence classes should bring us closer. A different area where the insights gained may be useful is in the engineering of artificial genetic systems (Andrianantoandro et al., 2006); there, deterministic asynchrony may be forced by using clock-like genetic circuitry, and the update schedules may become an element of design.

The most important current area of application, however, is certainly in the reconstruction and analysis of models of regulatory networks. Modelers must remember the importance of the updating scheme; as we show here, it is very relevant for the dynamical behavior of the model. It is an additional "degree of freedom" in the construction of the model, but one that must be used with care; any deterministic strict order of interactions must have a biological reason (even if presently unknown), and thus if it turns out to be required in order to suppress or cause a certain observed periodic behavior, that reason should be given (or researched as an hypothesis). How do we know whether an update schedule exists

that generates a certain periodic behavior? So far, the problem is open, as is the more general problem of having an efficient reconstruction algorithm for Boolean networks that considers the update schedule as one of the variables to be determined. In order to be useful, such an algorithm such be able to deal with a restricted set of legitimate update schedules (those which are deemed biologically reasonable). Without such an algorithm, the best option is just to use a synchronous reconstruction algorithm like REVEAL Liang et al. (1998), obtaining a network that generates the appropriate fixed points, and then look for changes in the labeled graph that may enforce in the model the dynamical cycles observed in the real system.

A final caveat (and line of future work) is due. In the language of this article, the global states of the network which are considered (and hence, those that are listed as states in a dynamical cycle) are those reached after a whole cycle of updating, i.e., when every node has been updated one time. In some biological systems, the observations may show an intermediate step (where, for instance, the first block of updates has taken place, but not the rest). If such intermediate observations are considered as part of the global dynamical behavior, then the results presented here do not hold in their current form, and need to be adapted. For instance: where without intermediate observations two update schedules are said to share no dynamical cycle, with these observations they would be able to share some dynamical cycles, of a very restricted kind; such restrictions on partial dynamics are another line of current work.

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