Multistate nested canalizing functions and their networks

Claus Kadelka [a,b,*], Yuan Li [c], Jack Kuipers [d], John O. Adeyeye [c],
Reinhard Laubenbacher [e,f]

[a] *Institute of Medical Virology, University of Zurich, 8006 Zurich, Switzerland*
[b] *Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, 8091 Zurich, Switzerland*
[c] *Department of Mathematics, Winston-Salem State University, NC 27110, USA*
[d] *D-BSSE, ETH Zurich, Mattenstrasse 26, 4058 Basel, Switzerland*
[e] *Center for Quantitative Medicine, University of Connecticut Health Center, Farmington, CT 06030, USA*
[f] *Jackson Laboratory for Genomic Medicine, Farmington, CT 06030, USA*

ARTICLE INFO

ABSTRACT

This paper provides a collection of mathematical and computational tools for the study of robustness in nonlinear gene regulatory networks, represented by time- and state-discrete dynamical systems taking on multiple states. The focus is on networks governed by nested canalizing functions (NCFs), first introduced in the Boolean context by S. Kauffman. After giving a general definition of NCFs we analyze the class of such functions. We derive a formula for the normalized average c-sensitivities of multistate NCFs, which enables the calculation of the Derrida plot, a popular measure of network stability. We also provide a unique canonical parametrized polynomial form of NCFs. This form has several consequences. We can easily generate NCFs for varying parameter choices, and derive a closed form formula for the number of such functions in a given number of variables, as well as an asymptotic formula. Finally, we compute the number of equivalence classes of NCFs under permutation of variables. Together, the results of the paper represent a useful mathematical framework for the study of NCFs and their dynamic networks.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Many biological networks, in particular gene regulatory networks, are inherently stochastic in nature [1,2] and questions regarding the robustness of such networks have received much attention [3–5]. The properties of molecular networks are frequently studied using time- and state-discrete models like Boolean and logical network models that have been used for this purpose since the 1970s [6,7]. Many of the more recently published discrete dynamical models include, however, variables that take on more than two states, needed to capture mechanisms that are not binary in nature; see, e.g., [8–11].

The impact of structural and topological network properties on the resulting dynamics is of particular interest when trying to understand the robustness of molecular networks, see, e.g., [12–14]. One popular measure of network robustness is the Derrida value of a network [15]. It assesses how perturbations, caused, for instance, by changes in the environmental conditions, propagate through the network.

---

* Corresponding author at: Institute of Medical Virology, University of Zurich, 8006 Zurich, Switzerland.
E-mail addresses: kadelka.claus@virology.uzh.ch (C. Kadelka), liyu@wssu.edu (Y. Li), jack.kuipers@bsse.ethz.ch (J. Kuipers), adeyeyej@wssu.edu
(J.O. Adeyeye), laubenbacher@uchc.edu (R. Laubenbacher).

Closely related to questions regarding robustness, Waddington had already in the 1940s developed the concept of canalization as a possible explanation of why the outcome of embryonal development leads to predictable phenotypes in the face of widely varying environmental conditions as well as frequent genetic mutations [16]. Different phenotypes can be thought of as "valleys" into which development is channeled by canalizing mechanisms, inferring protection from ubiquitous perturbations. Kauffman was the first to use a version of this concept in Boolean network modeling. He studied gene regulatory networks with canalizing Boolean functions [17], as well as the special subclass of so-called nested canalizing functions (NCFs) [18]. Since then, these functions and the dynamics of the networks they govern have been extensively studied [19–25]. The discovery of some invariants of a canalizing function enabled a further categorization of the set of all canalizing Boolean functions, see, e.g., [26–28]. Due to the increased need to model update functions with multistate variables, the concept of nested canalization has been generalized to functions taking values from any finite field [23,29]. A *canalizing* function possesses at least one input variable such that, if this variable takes on a certain "canalizing" value, then the output value is already determined, regardless of the values of the remaining input variables. If this variable takes on a non-canalizing value, and there is a second variable with this same property, and so on, then the function is *nested canalizing*.

The number of Boolean (nested) canalizing functions are known [20,30], and for the multistate case there exists at least a recursive formula for the number of NCFs [29]; the probability that a random Boolean function is canalizing decreases rapidly as the number of inputs increases (less than 0.5 for three inputs, and less than 0.01 for five inputs). Nested canalization is more restrictive and the probabilities are even lower (0.25 for three inputs, and less than 10^{-5} for five inputs). Interestingly, an analysis of published Boolean models of molecular networks revealed that all 139 investigated rules with at least three inputs are canalizing and even that 133 are nested canalizing [18,23,31]. (Unfortunately, a similar study has yet to be conducted for multistate models.) These findings clearly motivate the study of general multistate NCFs in the context of understanding the regulatory logic of gene networks.

In this paper, we introduce a collection of computational tools that can aid in the construction and analysis of multistate discrete dynamical systems, with a focus on nested canalization and robustness. Specifically, the novel contributions of the paper are as follows:

- Multistate, rather than Boolean, models of gene regulatory and signaling networks are increasingly used to model biological phenomena [8–11]. This suggests that theoretical studies of these, such as robustness, should be carried out in the multistate context. (We are not aware of any arguments that show that Boolean networks are sufficiently general for this purpose.) Therefore, we treat the multistate case and provide general results that can be used in that context.
- The Derrida values of a network can be expressed as a weighted sum of the normalized average *c*-sensitivities of the canalizing update functions [25]. We provide a formula for the normalized average *c*-sensitivities of multistate NCFs. This greatly simplifies the application of the Derrida plot for robustness analyses of multistate nested canalizing networks, which otherwise requires extensive simulations (difficult or infeasible for large networks).
- In [32], the authors showed that polynomial functions over a finite field serve as a rich mathematical framework for the modeling of discrete dynamical systems. Using this framework, we provide a canonical parametrized polynomial form for multistate NCFs. This canonical form can be used to easily generate such functions, a nontrivial task for functions with many variables; as many robustness investigations require a large number of randomly generated networks, this parametrization is particularly useful.
- Using the polynomial framework, we present a closed formula for the number of multistate NCFs in a given number of variables, in terms of the Sterling numbers of the second kind, and derive an explicit exponential generating function for them. From this generating function, we can derive a simple asymptotic approximation of the number of NCFs. This allows to carry out asymptotic studies, which we also provide. Knowing the proportion of NCFs among all functions provides information about the richness of this class of functions for the purpose of capturing a variety of regulatory mechanisms.
- We compute the number of equivalence classes of NCFs under permutation of variables. This question has received recent attention [33,34].

Together, these results provide a detailed characterization of multistate NCFs, including explicit formulas that tell us how prevalent these functions are, and that simplify the use of the common Derrida value metric for robustness analyses of dynamic networks constructed from these functions. We conclude the paper with a discussion of avenues for future work as well as problems and limitations in generalizing the concept of nested canalization.

2. The concept of canalization

This paper investigates multistate functions with inputs and outputs in finite sets. Functions appearing in models of gene regulatory networks typically take values in sets of different cardinality, as variables can take on different numbers of states; for instance in logical models, many variables are Boolean, while some take on 3 or more states [35]. This complicates the general mathematical framework for such functions and their networks. It is explained in detail in [32,36] that one can make the assumption that the functions we are considering take their inputs and produce their outputs in a finite field with a prime number of elements, rather than in a general finite set. This assumption does not limit the generality of the considered functions because the domain and range of any function can be enlarged, if needed, to a finite field with a prime

number of elements by adding "dummy" states to the domain. Thus, **throughout this paper, we will make the assumption that all functions are defined over a finite field $\mathbb{F} = \mathbb{F}_p$ with a prime number p of elements**. (Such a field can be thought of as the set of integers modulo p, with the usual addition and multiplication modulo p.) The Boolean case corresponds, of course, to $p = 2$. An important consequence of this assumption, used heavily here, is that any function $f : \mathbb{F} \times \cdots \times \mathbb{F} \longrightarrow \mathbb{F}$ can be represented by a polynomial function with coefficients in $\mathbb{F}$ [32].

In this section we review some concepts and definitions from [23,29] to introduce the computational concept of *canalization*.

Definition 2.1. A function $f(x_1, x_2, \ldots, x_n)$ is essential in the variable x_i if there exists $r, s \in \mathbb{F}$ and $(x_1, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n) \in \mathbb{F}^{n-1}$ such that

$$f(x_1, \ldots, x_{i-1}, r, x_{i+1}, \ldots, x_n) \neq f(x_1, \ldots, x_{i-1}, s, x_{i+1}, \ldots, x_n).$$

Definition 2.2. A function $f : \mathbb{F}^n \to \mathbb{F}$ is $\langle i : a : b \rangle$ canalizing if there exists a variable x_i, $a, b \in \mathbb{F}$ and a function $g(x_1, \ldots, x_n)$ such that

$$f(x_1, \ldots, x_n) = \begin{cases} b & \text{if } x_i = a \\ g \not\equiv b & \text{if } x_i \neq a, \end{cases}$$

in which case x_i is called a canalizing variable, the input a is the canalizing input, and the output value b when $x_i = a$ is the corresponding canalized output.

We now assume that $\mathbb{F} = \{0, 1, \ldots, p - 1\}$ is ordered, in the natural order $0 < 1 < \cdots < p - 1$. A proper subset S of $\mathbb{F}$ is called a segment if and only if $S = \{0, \ldots, j\}$ or $S^c = \mathbb{F} - S = \{0, \ldots, j\}$ for some $0 \leq j < p - 1$. Hence, a proper subset S is a segment if and only if S^c is a segment.

Definition 2.3. ([29]) Let $f : \mathbb{F}^n \to \mathbb{F}$ be a function in n variables, let $\sigma \in \mathcal{S}_n$ be a permutation of $\{1, 2, \ldots, n\}$ and let S_i be segments of $\mathbb{F}$, $i = 1, \ldots, n$. Then f is a *nested canalizing function (NCF)* in the variable order $x_{\sigma(1)}, \ldots, x_{\sigma(n)}$ with canalizing input sets $S_1, \ldots, S_n$ and canalized output values $b_1, \ldots, b_n, b_{n+1}$ with $b_n \neq b_{n+1}$ if it can be represented in the form

$$f(x_1, \ldots, x_n) = \begin{cases} b_1 & x_{\sigma(1)} \in S_1, \\ b_2 & x_{\sigma(1)} \notin S_1, x_{\sigma(2)} \in S_2, \\ b_3 & x_{\sigma(1)} \notin S_1, x_{\sigma(2)} \notin S_2, x_{\sigma(3)} \in S_3, \\ \vdots & \\ b_n & x_{\sigma(1)} \notin S_1, \ldots, x_{\sigma(n-1)} \notin S_{n-1}, x_{\sigma(n)} \in S_n, \\ b_{n+1} & x_{\sigma(1)} \notin S_1, \ldots, x_{\sigma(n-1)} \notin S_{n-1}, x_{\sigma(n)} \notin S_n. \end{cases}$$

In short, the function f is said to be nested canalizing if f is nested canalizing in some variable order with some canalizing input sets and some canalized output values.

Let $\mathbb{S} = (S_1, S_2, \ldots, S_n)$ and $\beta = (b_1, b_2, \ldots, b_{n+1})$ with $b_n \neq b_{n+1}$. We say that f is $\{\sigma : \mathbb{S} : \beta\}$ NCF if it is nested canalizing in the variable order $x_{\sigma(1)}, \ldots, x_{\sigma(n)}$, with canalizing input sets $\mathbb{S} = (S_1, \ldots, S_n)$ and canalized output values $\beta = (b_1, \ldots, b_{n+1})$.

Example 2.4. Let $\mathbb{F} = \{0, 1, 2\}$. The function $f : \mathbb{F}^2 \to \mathbb{F}$ given by

$$f(x_1, x_2) = \begin{cases} 2 & \text{if } x_1 \in \{1, 2\}, \\ 1 & \text{if } x_1 \notin \{1, 2\}, x_2 \in \{2\}, \\ 0 & \text{if } x_1 \notin \{1, 2\}, x_2 \notin \{2\} \end{cases}$$

is nested canalizing in the variable order x_1, x_2, with canalizing input sets $S_1 = \{1, 2\}$, $S_2 = \{2\}$, and canalized output values $b_1 = 2, b_2 = 1, b_3 = 0$. An NCF like f may for instance occur in the modeling of gene regulatory networks where the three states correspond to low (0), mediocre (1) and high (2) concentration. Here, f may describe the concentration of a target protein at a certain time point given the concentration levels of the two transcription factors of its encoding gene at the previous time point. x_1 represents a strong transcription factor, which even in mediocre concentration leads to high protein levels, while x_2 is a weaker transcription factor, which only at high concentration leads to mediocre protein levels.

Definition 2.3 immediately implies the following technical result, used subsequently.

Proposition 2.5. *A function f is $\{\sigma : \mathbb{S} : \beta\}$ NCF if and only if f is $\{\sigma : \mathbb{S}' : \beta'\}$ NCF, where $\mathbb{S}' = (S_1, S_2, \ldots, S_n{}^c)$ and $\beta' = (b_1, b_2, \ldots, b_{n-2}, b_{n+1}, b_n)$.*

Table 1
Truth table and c-sensitivities of the function f from Example 2.4.

x_1	x_2	$f(\mathbf{x})$	$S_1^f(\mathbf{x})$	$S_2^f(\mathbf{x})$
0	0	0	3	4
0	1	0	3	4
0	2	1	4	4
1	0	2	1	2
1	1	2	1	2
1	2	2	1	2
2	0	2	1	2
2	1	2	1	2
2	2	2	1	2

3. Normalized average c-sensitivities

Gene regulatory networks must be robust to small perturbations in order to cope with the ubiquitous changes in environmental conditions. The Derrida plot is a common technique to quantify the robustness of a discrete dynamical system. It describes how a network perturbation of a certain size propagates on average over time [15]. A system operates in the ordered regime if a small perturbation vanishes on average over time. Such networks typically possess many steady states and short limit cycles. A system in which a small perturbation typically amplifies over time is in the chaotic regime, often possessing long limit cycles. Lastly, if a small perturbation remains on average of similar size, the system operates close to the so-called critical threshold. Working at this "edge of chaos" seems essential for many biological systems; it provides robustness to withstand perturbations caused by environmental changes but also flexibility to allow adaptation [14,37].

In [25], the c-sensitivity of a function was introduced as a generalization of the popular concept of sensitivity. It measures how likely a simultaneous change in c inputs of a function leads to a change in the output, and can be directly adopted to the multistate case.

Definition 3.1. Any vector that differs at exactly c bits from a given vector $\mathbf{x}$ is called a c-Hamming neighbor of $\mathbf{x}$. Let $f : \mathbb{F}^n \to \mathbb{F}$. The c-sensitivity of f at $\mathbf{x}$ is defined as the number of c-Hamming neighbors of $\mathbf{x}$ at which the function value is different from its value at $\mathbf{x}$. That is,

$$S_c^f(\mathbf{x}) = \sum_{\substack{\mathbf{y} \in \mathbb{F}^n \\ d(\mathbf{x},\mathbf{y})=c}} \chi[f(\mathbf{x}) \neq f(\mathbf{y})],$$

where χ is an indicator function and $d(\mathbf{x},\mathbf{y}) = \left| \{i \in \{1, 2, \ldots, n\} : x_i \neq y_i\} \right|$ is the Hamming distance. Note that by definition, $S_0^f(\mathbf{x}) = 0$. Assuming a uniform distribution of $\mathbf{x}$,

$$S_c^f = \mathbb{E}[S_c^f(\mathbf{x})] = \frac{1}{p^n} \sum_{\mathbf{x} \in \mathbb{F}^n} \sum_{\substack{\mathbf{y} \in \mathbb{F}^n \\ d(\mathbf{x},\mathbf{y})=c}} \chi[f(\mathbf{x}) \neq f(\mathbf{y})]$$

is the average c-sensitivity of f. The range of S_c^f is $[0, \binom{n}{c}(p-1)^c]$. Let us therefore define the normalized average c-sensitivity of f as

$$s_c^f = \frac{S_c^f}{\binom{n}{c}(p-1)^c} \in [0, 1].$$

Example 3.2. Table 1 shows the truth table as well as the c-sensitivities of the function f, defined in Example 2.4. The average c-sensitivities of f are $S_1^f = \frac{16}{9}$ and $S_2^f = \frac{24}{9} = \frac{8}{3}$. The normalized average c-sensitivities of f are $s_1^f = \frac{4}{9}$ and $s_2^f = \frac{2}{3}$.

It was shown in [25] that the Derrida values of a network can be expressed as a weighted sum of the normalized average c-sensitivities of its canalizing update functions. Since the Hamming weight of two multistate vectors is still defined as the number of bits where two vectors differ, the Derrida values of a network governed by multistate functions are also defined like in the Boolean case [25]. The following theorem provides a formula for the normalized average c-sensitivities of multistate NCFs, which enables the calculation of the Derrida plot for any network governed by multistate NCFs – a task that otherwise requires extensive simulations, which is difficult or infeasible for large networks.

Theorem 3.3. *For $p \geq 2$, the expected normalized average c-sensitivity of a multistate NCF $f : \mathbb{F}_p^n \to \mathbb{F}_p$ is*

$$\mathbb{E}[s_c^f] = \frac{p+1}{3p}\left(\frac{c2^c}{n2^n}\left(\frac{p}{p-1} - {_2}F_1\left[1, n; n+1-c; \frac{1}{2}\right]\right)\left(\frac{p-2}{3p-3}\right)^{c-1}\right.$$
$$\left.+ \sum_{i=1}^{c}\frac{\binom{n-i}{c-i}}{\binom{n}{c}}{_2}F_1\left[i, c-n; i-n; \frac{1}{2}\right]\left(\frac{p-2}{3p-3}\right)^{i-1}\right),$$

with ${_2}F_1$ the hypergeometric function.

Proof. See Appendix. □

For $p = 2$, this formula reduces to the same simple equation as in [25, Corollary 3.4].

4. Characterization of nested canalizing functions

As mentioned in the introduction, it is important to better understand the class of multistate NCFs, in particular their density among all multistate functions. These questions have been largely addressed in the Boolean case, but little information is available in the more general case. The main results in this section include a canonical parametrized representation of NCFs as a particular form of polynomial function. This representation is then used to derive a closed formula for the number of multistate NCFs in a given number of variables, as well as an asymptotic formula as the number of variables grows. An important practical application of the polynomial form of NCFs is that it allows the easy generation of such functions by choosing a particular collection of parameters. This is very helpful in simulation studies involving large numbers of networks governed by NCFs. Finally, we derive a formula for the number of equivalence classes of NCFs under permutation of variables, a question that has received recent interest [33,34].

In the Boolean case, the extended monomial plays an important role in deriving a polynomial form of NCFs [26]. In the multistate case, the product of indicator functions, also used in [29], takes over this role.

Definition 4.1. Given a proper subset S of $\mathbb{F}$, the indicator function (of S^c) is defined as

$$Q_S(x) = \begin{cases} 0 & x \in S, \\ 1 & x \in S^c. \end{cases}$$

The following theorem gives an algebraic characterization of NCFs.

Theorem 4.2. For $n \geq 2$, the function $f(x_1, \ldots, x_n)$ is nested canalizing if and only if it can be uniquely written as

$$f(x_1, \ldots, x_n) = M_1\Big(M_2\Big(\cdots\big(M_{r-1}(B_{r+1}M_r + B_r) + B_{r-1}\big)\cdots\Big) + B_2\Big) + B_1, \tag{4.1}$$

where each M_i is a product of indicator functions of disjoint sets of variables. More precisely,

- $k_1 + \cdots + k_r = n$, and $k_i \geq 1$ for all $i = 1, \ldots, r$
- For all $i = 1, \ldots, r$, $M_i = \prod_{j \in A_i}(Q_{S_j}(x_j))$, where $A_i \subseteq \{1, \ldots, n\}$, $|A_i| = k_i$, $A_{i_1} \cap A_{i_2} = \emptyset$ if $i_1 \neq i_2$, $A_1 \dot\cup \ldots \dot\cup A_r = \{1, \ldots, n\}$, and $S_1, \ldots, S_n$ are segments of $\mathbb{F}$
- $B_1 \in \mathbb{F}$, $B_2, \ldots, B_{r+1} \in \mathbb{F} - \{0\}$
- if $k_r = 1$, then $B_{r+1} + B_r \neq 0$

Proof. First, let f be a function written as in Equation (4.1). Let $b_i := \sum_{j=1}^{i} B_j$. Then f is a $\{\sigma' : \mathbb{S}' : \beta'\}$ NCF, where

$$\sigma'(x_1, \ldots, x_n) = (x_{1_1}, \ldots, x_{1_{k_1}}, \ldots, x_{r_1}, \ldots, x_{r_{k_r}}),$$
$$\mathbb{S}' = (S_{1_1}, \ldots, S_{1_{k_1}}, \ldots, S_{r_1}, \ldots, S_{r_{k_r}}),$$
$$\beta' = (\underbrace{b_1, \ldots, b_1}_{k_1}, \underbrace{b_2, \ldots, b_2}_{k_2}, \ldots, \underbrace{b_r, \ldots, b_r}_{k_r}, b_{r+1}).$$

Second, suppose f is a $\{\sigma : \mathbb{S} : \beta\}$ NCF, where $\mathbb{S} = (S_1, S_2, \ldots, S_n)$ and $\beta = (b_1, b_2, \ldots, b_{n+1})$, with $b_n \neq b_{n+1}$. Then there exist k_i, $i = 1, \ldots, r$, $k_1 + \cdots + k_r = n$, $k_i \geq 1$, such that

$$b_1 = \cdots = b_{k_1} =: C_1,$$
$$b_{k_1+1} = \cdots = b_{k_1+k_2} =: C_2$$
$$\vdots$$

$$b_{k_1+\cdots+k_{r-1}+1} = \cdots = b_n =: C_r,$$

$$b_{n+1} =: C_{r+1},$$

$$\text{and } C_j \neq C_{j+1} \text{ for all } j = 1, \ldots, r$$

Let $B_1 := C_1, B_2 := C_2 - C_1, \ldots, B_{r+1} = C_{r+1} - C_r$. Hence, $B_1 \in \mathbb{F}, B_2, \ldots, B_{r+1} \in \mathbb{F} - \{0\}$, and $f(x) = M_1(M_2(\cdots(M_{r-1}(B_{r+1}M_r + B_r) + B_{r-1})\cdots) + B_2) + B_1$, which shows that any NCF can be written as in Equation (4.1).

Finally, we need to show that each NCF has a unique polynomial representation. Let f be written as in Equation (4.1). Then all the variables $x_{\sigma(1)}, \ldots, x_{\sigma(k_1)}$ of M_1 are canalizing variables of f with common canalized output B_1. To prove the uniqueness of M_1 and B_1, we will now show that f has no other canalizing variables. All variables of M_2, $x_{\sigma(k_1+1)}, \ldots, x_{\sigma(k_2)}$, are canalizing variables of the subfunction $f_1 := M_2(\cdots(M_{r-1}(B_{r+1}M_r + B_r) + B_{r-1})\cdots) + (B_2 + B_1)$. Since $B_1 \neq B_1 + B_2$, $x_{\sigma(k_1+1)}, \ldots, x_{\sigma(k_2)}$ are not canalizing variables of f. In the same manner, all variables of M_3 are not canalizing variables of f_1 and thus not canalizing variables of f either. Iteratively, we can prove that $x_{\sigma(1)}, \ldots, x_{\sigma(k_1)}$ are the only canalizing variables of f, which proves the uniqueness of M_1 and B_1. In the same way, the uniqueness of $M_2, \ldots, M_r$ and $B_2, \ldots, B_{r+1}$ follows.   □

Corollary 4.3. *Any choice of r, k_i, A_i, B_j as in Theorem 4.2 results in an NCF. Thus, this formula can be used to generate NCFs with desired properties.*

Remark 4.4. In the Boolean case, each M_i in Theorem 4.2 is an extended monomial, and, since $B_{r+1} + B_r = 1 + 1 = 0$, k_r is greater than 1. Thus, Theorem 4.2 reduces to its Boolean version, already stated as Theorem 4.2 in [26].

Example 4.5. The function f from Example 2.4 can be written as $f(x_1, x_2) = Q_{S_1}(x_1)(2Q_{S_2}(x_2) + 2) + 2$, where $S_1 = \{1, 2\}, S_2 = \{2\}$.

Because each NCF can be uniquely written in the form of Equation (4.1), the number r is uniquely determined by f, and can be used to specify the class of NCFs as in the Boolean case [26]. We can therefore use the structure of this polynomial form to define an additional structure of NCFs, which might shed light on the dynamic behavior of NCF-governed networks.

Definition 4.6. For an NCF f, written in the form of Equation (4.1), let the number r be called its *layer number*. Essential variables of M_1 are called most dominant variables (canalizing variables), and are part of the first layer of f. Essential variables of M_2 are called second most dominant variables, and are part of the second layer, etc.

Remark 4.7. Just like in the Boolean case, Equation (4.1) allows the use of Corollary 4.8 in [26]: The layer number of any NCF can be determined by counting the number of changes in the canalized output values. For example, if $p = 3$ and if f is nested canalizing with canalized output values $\beta = (1, 0, 2, 2, 0, 1)$ ($n = 5$), then the layer number of f is 4. The nested canalizing function f from Example 2.4 has layer number 2.

We now derive some technical results needed in the construction of a closed formula for the number of NCFs.

Lemma 4.8. *Let a, b be any nonzero elements of $\mathbb{F}$, and let S be any segment of $\mathbb{F}$. The number of different functions $f = bQ_S(x) + a$, which cannot be written as $cQ_{S'}(x)$, where $c \neq 0$ and S' is a segment of $\mathbb{F}$, is $(p-1)^2(p-2)$.*

Proof. See Appendix.   □

Lemma 4.9. *Given $a, b \neq 0$ and segments $S_i, i = 1, \ldots, k$ with $k \geq 2$, then*

1. *$f(\mathbf{x}) = f(x_1, \ldots, x_k) = b\prod_{j=1}^{k} Q_{S_j}(x_j) + a$ cannot be written as $c\prod_{j=1}^{k} Q_{S'_j}(x_j)$, where $c \neq 0$ and all S'_j are segments, $j = 1, \ldots, k$.*
2. *There are $2^k(p-1)^{k+2}$ different functions of the form $b\prod_{j=1}^{k} Q_{S_j}(x_j) + a$.*

Proof. See Appendix.   □

Let $\mathbb{NCF}(n)$ denote the set of all NCFs in n variables.

Theorem 4.10. *For $n \geq 2$, the number of NCFs is given by*

$$|\mathbb{NCF}(n)| = 2^n p(p-1)^n \sum_{r=1}^{n} (p-1)^r r! \left[S(n, r) - \frac{np}{2} S(n-1, r) \right] \tag{4.2}$$

in terms of the Stirling numbers of the second kind

$$S(n,r) = \frac{1}{r!}\sum_{t=0}^{r}(-1)^t\binom{r}{t}(r-t)^n = \frac{1}{r!}\sum_{\substack{k_1+\cdots+k_r=n \\ k_i\geq 1, i=1,\ldots,r}}\frac{n!}{k_1!k_2!\cdots k_r!}$$

Proof. If $r=1$, then by Theorem 4.2 any NCF f can be written as $f = B_2M_1 + B_1$. Similar to Lemma 4.9, the number of such functions is $(2(p-1))^n(p-1)p = 2^n(p-1)^{n+1}p$, since $B_1 \in \mathbb{F}$ can be arbitrarily chosen, and $k_1 = n$.

For $r > 1$, Equation (4.1) yields that for each choice of $k_1,\ldots,k_r$, $k_i \geq 1$, $i = 1,\ldots,r$, there are $(2(p-1))^{k_j}\binom{n-k_1-\cdots-k_{j-1}}{k_j}$ ways to form M_j, $j = 1,\ldots,r$. For those NCFs with $k_r = 1$, by Lemma 4.8, there are $(p-1)^2(p-2)$ different functions of the form $B_{r+1}M_r + B_r$ with $B_r, B_{r+1} \neq 0$. For the remaining NCFs, i.e., those with $k_r > 1$, Lemma 4.9 yields that there are $(p-1)^2(2(p-1))^{k_r}$ ways to form $B_{r+1}M_r + B_r$, with $B_r, B_{r+1} \neq 0$.

Note that there are $p - 1$ choices for each B_i, $2 \leq i \leq r$, p choices for B_1, and $2(p-1)$ choices for each canalizing input segment. Hence, the total number of NCFs with $r > 1, k_r = 1$, can be given by

$$N_1 = \sum_{r=2}^{n}\sum_{\substack{k_1+\cdots+k_{r-1}=n-1 \\ k_i\geq 1, i=1,\ldots,r-1}}(2(p-1))^{k_1+\cdots+k_{r-1}}\binom{n}{k_1}\binom{n-k_1}{k_2}\cdots\binom{n-k_1-\cdots-k_{r-2}}{k_{r-1}}(p-1)^2(p-2)(p-1)^{r-2}p$$

$$= 2^{n-1}p(p-2)\sum_{r=2}^{n}\sum_{\substack{k_1+\cdots+k_{r-1}=n-1 \\ k_i\geq 1, i=1,\ldots,r-1}}(p-1)^{n+r-1}\frac{n!}{(k_1)!(n-k_1)!}\frac{(n-k_1)!}{(k_2)!(n-k_1-k_2)!}\frac{(n-k_1-\cdots-k_{r-2})!}{k_{r-1}!(n-k_1-\cdots-k_{r-1})!}$$

$$= 2^{n-1}p(p-2)\sum_{r=2}^{n}(p-1)^{n+r-1}\sum_{\substack{k_1+\cdots+k_{r-1}=n-1 \\ k_i\geq 1, i=1,\ldots,r-1}}\frac{n!}{k_1!k_2!\cdots k_{r-1}!}$$

$$= 2^n p(p-1)^n\sum_{r=1}^{n-1}(p-1)^r\left(\frac{p-2}{2}\right)nr!S(n-1,r)$$

where the last step follows by shifting the index of the sum. Similarly, the total number of NCFs with $r > 1, k_r > 1$ is

$$N_2 = \sum_{r=2}^{n-1}\sum_{\substack{k_1+\cdots+k_r=n \\ k_i\geq 1, i=1,\ldots,r-1,k_r\geq 2}}(2(p-1))^{k_1+\cdots+k_r}\binom{n}{k_1}\binom{n-k_1}{k_2}\cdots\binom{n-k_1-\cdots-k_{r-1}}{k_r}(p-1)^2(p-1)^{r-2}p$$

$$= 2^n p\sum_{r=2}^{n-1}\sum_{\substack{k_1+\cdots+k_r=n \\ k_i\geq 1, i=1,\ldots,r-1,k_r\geq 2}}(p-1)^{n+r}\frac{n!}{(k_1)!(n-k_1)!}\frac{(n-k_1)!}{(k_2)!(n-k_1-k_2)!}\cdots\frac{(n-k_1-\cdots-k_{r-1})!}{k_r!(n-k_1-\cdots-k_r)!}$$

$$= 2^n p\sum_{r=2}^{n-1}(p-1)^{n+r}\sum_{\substack{k_1+\cdots+k_r=n \\ k_i\geq 1, i=1,\ldots,r-1,k_r\geq 2}}\frac{n!}{k_1!k_2!\cdots k_r!}$$

$$= 2^n p(p-1)^n\sum_{r=2}^{n-1}(p-1)^r\left[r!S(n,r) - n(r-1)!S(n-1,r-1)\right]$$

We can extend the upper limit to n and shift the index of the second sum

$$N_2 = 2^n p(p-1)^n\left[\sum_{r=2}^{n}(p-1)^r r!S(n,r) - \sum_{r=1}^{n-1}(p-1)^{r+1}nr!S(n-1,r)\right].$$

The $r = 1$ term was previously calculated and neatly corresponds to a $r = 1$ term in the first sum, so, by combining all three groups of NCFs, the total number of NCFs in n variables is

$$|\mathbb{NCF}(n)| = 2^n(p-1)^{n+1}p + N_1 + N_2$$

$$= 2^n p(p-1)^n\sum_{r=1}^{n}(p-1)^r r!\left[S(n,r) - \frac{np}{2}S(n-1,r)\right]. \quad \square$$

Note that for $p = 2$, we get the same formula as in [26]. However, we are now also able to explicitly compute the number of multistate NCFs. For example, when $p = 3$ and $n = 2, 3, 4$, we get 192, 5568, 219468, respectively; when $p = 5$ and $n = 2, 3, 4$, we get 5120, 547840, 78561280, respectively. A recursive formula for the number of multistate NCFs was already described in [29], and the examples here are consistent with those results. Theorem 4.10 therefore yields an explicit solution for the recursive sequence of [29].

Corollary 4.11. *For the nonlinear recursive sequence*

$$a_2 = 4(p-1)^4, a_n = \sum_{r=2}^{n-1} \binom{n}{r-1} 2^{r-1}(p-1)^r a_{n-r+1} + 2^{n-1}(p-1)^{n+1}(2+n(p-2)), n \geq 3$$

it holds that

$$\left|\mathbb{NCF}(n)\right| = pa_n,$$

and the explicit solution for a_n is given by

$$a_n = 2^n(p-1)^n \sum_{r=1}^{n} (p-1)^r r! \left[S(n, r) - \frac{np}{2} S(n-1, r) \right].$$

We are now in a position to derive an asymptotic formula for the number of NCFs, from a generating function.

Corollary 4.12. *The exponential generating function of the number of multistate NCFs is*

$$G_p(s) = \sum_{n=2}^{\infty} \frac{|\mathbb{NCF}(n)|}{n!} s^n = \frac{p - p^2(p-1)s}{p - (p-1)e^{2(p-1)s}} - p - p(p-1)(p-2)s. \tag{4.3}$$

Proof. We first rewrite Equation (4.2)

$$|\mathbb{NCF}(n)| = 2^n p(p-1)^n \left[A_n(p-1) - \frac{np}{2} A_{n-1}(p-1) \right] \tag{4.4}$$

in terms of an auxiliary sum

$$A_n(z) = \sum_{k=0}^{n} k! S(n, k) z^k.$$

The exponential generating function of $A_n(z)$ is

$$H(y) = \sum_{n=0}^{\infty} \frac{A_n(z)}{n!} y^n = \sum_{n=0}^{\infty} \sum_{k=0}^{n} \frac{k! S(n, k) z^k}{n!} y^n.$$

Since $S(n, k) = 0$ when $k > n$, we can extend the upper limit of the sum over k and exchange the sums

$$H(y) = \sum_{n=0}^{\infty} \sum_{k=0}^{\infty} \frac{k! S(n, k) z^k}{n!} y^n = \sum_{k=0}^{\infty} \sum_{n=0}^{\infty} k! \frac{S(n, k) y^n}{n!} z^k.$$

Using the exponential generating function of $S(n, k)$ with fixed k

$$\sum_{n=0}^{\infty} \frac{S(n, k)}{n!} y^n = \frac{(e^y - 1)^k}{k!}$$

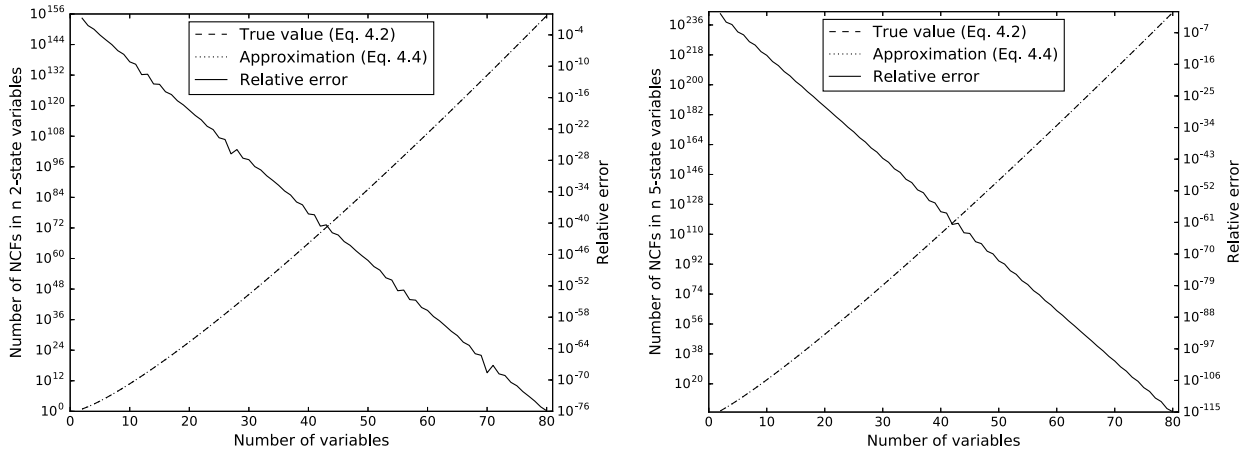the exponential generating function of $A_n(z)$ then simplifies to

$$H(y) = \sum_{k=0}^{\infty} (e^y - 1)^k z^k = \frac{1}{1 + z - ze^y}. \tag{4.5}$$

The case $z = 1$ corresponds to the ordered Bell numbers [38].

Using Equation (4.4), the generating function for NCFs is

$$\sum_{n=0}^{\infty} \frac{|\mathbb{NCF}(n)|}{n!} s^n = p \sum_{n=0}^{\infty} \frac{A_n(p-1) 2^n (p-1)^n s^n}{n!} - p^2(p-1)s \sum_{n=1}^{\infty} \frac{A_{n-1}(p-1) 2^{n-1}(p-1)^{n-1} s^{n-1}}{(n-1)!}$$

Fig. 1. The true number of NCFs (Equation (4.2)) and the approximated number of NCFs (Equation (4.6)) as well as the relative error of the approximation are shown for $n = 2, \ldots, 80$ and $p = 2$ (left panel), $p = 5$ (right panel).

since the $n = 0$ term is zero for the second sum. By shifting that sum's index, we then obtain

$$\sum_{n=0}^{\infty} \frac{|\mathbb{NCF}(n)|}{n!} s^n = pH\big(2(p-1)s\big) - p^2(p-1)sH\big(2(p-1)s\big)$$

evaluated at $z = (p-1)$. Substituting the simplified form of H from Equation (4.5) provides the generating function for NCFs of the form

$$\sum_{n=0}^{\infty} \frac{|\mathbb{NCF}(n)|}{n!} s^n = \frac{p - p^2(p-1)s}{p - (p-1)e^{2(p-1)s}}.$$

Finally, terms for $n = 0$ and $n = 1$ are subtracted to obtain the expression in Equation (4.3). □

From the generating function, one can obtain the number of NCFs by Taylor expansion or by performing a contour integral (using Cauchy's differentiation formula)

$$|\mathbb{NCF}(n)| = \frac{n!}{2\pi i} \oint \frac{G_p(s)}{s^{n+1}} ds.$$

We can evaluate the integral analogously to the example of the ordered Bell numbers treated in [38]. Essentially, we start to deform the contour around the simple pole $\frac{1}{2(p-1)} \ln\left(\frac{p}{p-1}\right)$, which is the only real pole, so that the residue there provides the leading order asymptotic approximation for the number of multistate NCFs for large n. Successive subleading corrections then arise from the complex poles.

Corollary 4.13. *The number of NCFs is approximately given by*

$$|\mathbb{NCF}(n)| \approx \left[1 - \frac{p}{2} \ln\left(\frac{p}{p-1}\right)\right] 2^n (p-1)^n n! \left[\ln\left(\frac{p}{p-1}\right)\right]^{-(n+1)}. \tag{4.6}$$

Fig. 1 shows the excellence of this approximation for $p = 2$ and $p = 5$.

We now study the number of equivalence classes of NCFs under permutation of variables. There has been recent interest in the study of various symmetries in the input variables of NCFs and the choice of representatives from different equivalence classes; see, e.g., [33,34].

Definition 4.14. Given two functions $f(x_1, \ldots, x_n)$ and $g(x_1, \ldots, x_n)$ over $\mathbb{F}$. We call f and g permutation equivalent if there exists a permutation σ such that $f(x_1, \ldots, x_n) = g(x_{\sigma(1)}, \ldots, x_{\sigma(n)})$.

Equivalent functions share many properties. For example, two equivalent Boolean NCFs have the same average sensitivity [26,39] as well as the same average c-sensitivity (see Theorem 3.3 and [25]).

Theorem 4.15. *For $n \geq 2$, the number of different equivalence classes of NCFs under permutation of variables is*

$$N = 2^{n-1}(p-1)^{n+1}p^n.$$

Proof. See Appendix. □

The number of different equivalence classes of NCFs under permutation of variables is much lower than the number of NCFs. For example, when $p = 3$ and $n = 2, 3, 4$ we get 144, 1728, 20736 equivalence classes respectively, compared to the totals of 192, 5568, 219468 NCFs; when $p = 5$ and $n = 2, 3, 4$ we get 3200, 128000, 5120000 respectively, compared to 5120, 547840, 78561280.

5. Discussion

For a class of functions to be a good representative of the mechanisms at work in gene regulation, it should be sufficiently large to capture many possible logic rules that appear, but should, at the same time, be small enough to endow networks with special properties that can be discerned. In our case, this necessitates a study of the class of general NCFs. There are a few ways to generalize the concept of Boolean nested canalization. We treated a fairly restrictive but simple generalization, in which each variable can only appear once in the canalizing order. One limitation of this approach is that some Boolean NCFs are no longer nested canalizing when generalizing them to the multistate case. For instance, MIN and MAX, which can be seen as natural generalizations of the Boolean AND and OR functions, are not multistate NCFs. Another less restrictive but more complicated generalization would allow variables to appear in the canalizing order up to $p - 1$ times, each time with a different canalizing input. This approach would, however, lead to uniqueness issues of the canonical parametric polynomial form. For instance, the layer number would no longer correspond to the number of changes in the canalized output vector.

While much work remains to be done in order to fully characterize the class of multistate NCFs, this paper provides a collection of tools for a more in-depth study of systems governed by NCFs. The presented formula of the normalized average c-sensitivities of a multistate NCF allows the computation of Derrida values of NCF-governed networks, a commonly used metric of network stability. In the process, we have extended the definition of Derrida value from the Boolean to the multistate context. A very appealing closed form formula for the number of NCFs in a given number of variables is derived, in terms of the Sterling numbers of the second kind. A canonical parametric polynomial form of NCFs is derived that has important consequences: (i) it allows the easy construction of such functions, useful for simulation studies; (ii) it helps to derive an asymptotic formula for the portion of NCFs among all functions; (iii) this polynomial form suggests a division of variables into layers which appear related to robustness properties captured by the Derrida values. Despite recent progress in [25], the precise connection remains to be elucidated. Our work builds in part on prior work done in the Boolean case [20,23,26].

As in [32,36], the results in this paper show the utility of the polynomial algebra viewpoint of discrete dynamical systems in general, and Boolean networks in particular, for the study of discrete dynamical systems in biology and elsewhere. Viewing polynomial algebra as a nonlinear version of linear algebra, the connection might not be surprising, and we believe that we have only scratched the surface in finding new applications of algebra to the study of nonlinear dynamical systems.

Acknowledgements

Appendix

Proof of Theorem 3.3. Let f be a $\{\sigma : \mathbb{S} : \beta\}$ NCF, as in Definition 2.3, with n essential variables. Let $\mathbf{x} = (x_1, \ldots, x_n), \mathbf{y} = (y_1, \ldots, y_n) \in \mathbb{F}_p^n$ be two system configurations that differ at c of the n positions. Let $\tilde{\sigma}$ be the restriction of σ on the variables where $\mathbf{x}$ and $\mathbf{y}$ differ,

$$\tilde{\sigma} = \{\sigma(i) | x_{\sigma(i)} \neq y_{\sigma(i)}\}.$$

The order of the elements in $\tilde{\sigma}$ is the same as in σ.

(i) As an NCF, f is evaluated in an iterative process. If in the evaluation of $f(\mathbf{x})$ the most important value $x_{\sigma(1)}$ is in the canalizing set S_1, the evaluation is completed. If not, the second most important value, $x_{\sigma(2)}$, is considered, etc. In the Boolean case, whenever $\mathbf{x}$ and $\mathbf{y}$ differ at a variable $\sigma(i)$, $x_{\sigma(i)} \in S_i$ and $y_{\sigma(i)} \notin S_i$ or $x_{\sigma(i)} \notin S_i$ and $y_{\sigma(i)} \in S_i$. This implies that after evaluation of $\tilde{\sigma}(1)$, the most important variable where $\mathbf{x}$ and $\mathbf{y}$ differ, $\mathbf{x}$ and $\mathbf{y}$ always follow different paths in the evaluation process, and all less important variables do not matter when determining the probability that $f(\mathbf{x})$ and $f(\mathbf{y})$ differ. This is no longer true in the multistate case. For instance, if $p = 3, S_1 = \{0\}, x_{\sigma(1)} = 1, y_{\sigma(1)} = 2$, then the second most important variable $\sigma(2)$ needs to be considered even though $\mathbf{x}$ and $\mathbf{y}$ differ at the most important variable $\sigma(1)$. Let $\phi_1(p)$ denote the probability that, given $x_{\sigma(i)} \neq y_{\sigma(i)}$, the next important variable, $\sigma(i + 1)$, needs to be considered in order to determine whether $f(\mathbf{x})$ and $f(\mathbf{y})$ differ. That is,

$$\phi_1(p) := \mathbb{P}\Big([x_{\sigma(i)} \in S_i \wedge y_{\sigma(i)} \notin S_i] \vee [x_{\sigma(i)} \notin S_i \wedge y_{\sigma(i)} \in S_i]\Big| x_{\sigma(i)} \neq y_{\sigma(i)} \wedge S_i \in \mathcal{S}\Big),$$

where

$$\mathcal{S} = \Big\{ \{0\}, \{0,1\}, \ldots, \{0,\ldots,p-2\}, \{p-1\}, \{p-2,p-1\}, \ldots, \{1,2,\ldots,p-1\} \Big\}$$

is the set of all possible canalizing input segments. Clearly, $|\mathcal{S}| = 2(p-1)$, and there exist two segments with one element, two with two elements, etc. Moreover, there are $\binom{p}{2}$ pairs of $x_{\sigma(i)}$ and $y_{\sigma(i)}$ so that the two values are different. For a given S_i,

$$\mathbb{P}\Big([x_{\sigma(i)} \in S_i \wedge y_{\sigma(i)} \notin S_i] \vee [x_{\sigma(i)} \notin S_i \wedge y_{\sigma(i)} \in S_i] \Big| x_{\sigma(i)} \neq y_{\sigma(i)} \Big) = \frac{|S_i|(p - |S_i|)}{\binom{p}{2}}.$$

Thus,

$$\phi_1(p) = \sum_{S_i \in \mathcal{S}} \frac{|S_i|(p - |S_i|)}{2(p-1)\binom{p}{2}} = \frac{\sum_{i=1}^{p-1} i(p-i)}{(p-1)\binom{p}{2}} = \frac{p+1}{3(p-1)}.$$

(ii) If the jth evaluation step is needed to determine the probability that $f(\mathbf{x})$ and $f(\mathbf{y})$ differ, and if $x_{\sigma(j)} \neq y_{\sigma(j)}$, then the probability that $\mathbf{x}$ and $\mathbf{y}$ follow different paths in the evaluation process at step j, i.e., the probability that no more steps are needed, is $\phi_1(p)$. If $j = n$ is the least important variable, then $f(\mathbf{x})$ and $f(\mathbf{y})$ differ for sure because $b_n \neq b_{n+1}$ in Definition 2.3. If $j < n$, then $b_j \neq b_q$ with probability $\frac{p-1}{p}$ for any $j < q < n$, so that

$$\phi_2(j,n,p) := \mathbb{P}\Big(f(\mathbf{x}) \neq f(\mathbf{y}) \Big| [x_{\sigma(j)} \neq y_{\sigma(j)}] \wedge [x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \ \forall q, 1 \leq q < j] \Big) = \begin{cases} \phi_1(p)\frac{p-1}{p} & \text{if } j < n \\ \phi_1(p) & \text{if } j = n \end{cases}$$

(iii) When determining the probability that $f(\mathbf{x})$ and $f(\mathbf{y})$ differ, the jth evaluation step is only needed if $x_{\sigma(q)} \notin S_q$ and $y_{\sigma(q)} \notin S_q$ for all $1 \leq q < j$. For any q, the probability that the qth step does not determine whether $f(\mathbf{x})$ and $f(\mathbf{y})$ differ (given that S_q is unknown) is given by

$$\mathbb{P}\big(x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \big| S_q \in \mathcal{S} \big) = \begin{cases} \mathbb{P}\big(x_{\sigma(q)} \notin S_q \big| S_q \in \mathcal{S} \big) & \text{if } x_{\sigma(q)} = y_{\sigma(q)} \\ \mathbb{P}\big(x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \big| x_{\sigma(i)} \neq y_{\sigma(i)} \wedge S_q \in \mathcal{S} \big) & \text{if } x_{\sigma(q)} \neq y_{\sigma(q)} \end{cases}$$

$$= \begin{cases} \frac{1}{2} & \text{if } x_{\sigma(q)} = y_{\sigma(q)} \\ \frac{1}{2}\big(1 - \phi_1(p)\big) & \text{if } x_{\sigma(q)} \neq y_{\sigma(q)} \end{cases}$$

If $\sigma(j)$ is the ith most important variable where $\mathbf{x}$ and $\mathbf{y}$ differ (i.e., if $\sigma(j) = \tilde{\sigma}(i)$), then

$$\phi_3(i,j,p) := \mathbb{P}\Big(x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \ \forall q, 1 \leq q < j \Big| \sigma(j) = \tilde{\sigma}(i) \Big) = \prod_{q=1}^{j-1} \mathbb{P}\big(x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \big| S_q \in \mathcal{S} \big)$$

$$= \left(\frac{1 - \phi_1(p)}{2} \right)^{i-1} \left(\frac{1}{2} \right)^{j-i}$$

(iv) The probability that the jth most important variable $\sigma(j)$ is the ith most important variable, when only considering those variables where $\mathbf{x}$ and $\mathbf{y}$ differ, is equal to the probability that a c-subset of $\{1,2,\ldots,n\}$ contains j as its ith lowest element. There are $\binom{n}{c}$ c-subsets of $\{1,2,\ldots,n\}$. If j is the ith lowest element, then there are $\binom{j-1}{i-1}$ choices for the $i-1$ lower elements and $\binom{n-j}{c-i}$ choices for the $c-i$ higher elements. Thus, for $1 \leq i \leq c, i \leq j \leq n - c + i$,

$$\phi_4(i,j,c,n) := \mathbb{P}\big(\sigma(j) = \tilde{\sigma}(i) \big) = \frac{\binom{j-1}{i-1}\binom{n-j}{c-i}}{\binom{n}{c}}$$

(v) Only variables where $\mathbf{x}$ and $\mathbf{y}$ differ matter when deciding whether $f(\mathbf{x})$ equals $f(\mathbf{y})$. We therefore only consider these c variables in the calculation of $\mathbb{E}[s_c^f]$. The probability $\phi_4(i,j,c,n)$ describes how likely the ith most important variable where $\mathbf{x}$ and $\mathbf{y}$ differ, $\tilde{\sigma}(i)$, occurs at position j when considering all variables. Therefore, $1 \leq i \leq c$ and $i \leq j \leq n + i - c$. Thus,

$$\mathbb{E}[s_c^f] = \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{y}) \mid d(\mathbf{x},\mathbf{y}) = c \big)$$

$$= \sum_{i=1}^{c} \sum_{j=i}^{n+i-c} \mathbb{P}\Big(\sigma(j) = \tilde{\sigma}(i) \Big) \cdot \mathbb{P}\Big(x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \ \forall q, 1 \leq q < j \ \Big| \ \sigma(j) = \tilde{\sigma}(i) \Big) \cdot$$

$$\cdot \mathbb{P}\Big(f(\mathbf{x}) \neq f(\mathbf{y}) \mid [x_{\sigma(j)} \neq y_{\sigma(j)}] \wedge [x_{\sigma(q)} \notin S_q \wedge y_{\sigma(q)} \notin S_q \ \forall q, 1 \leq q < j] \Big)$$

$$= \sum_{i=1}^{c} \sum_{j=i}^{n+i-c} \phi_4(i,j,c,n)\phi_3(i,j,p)\phi_2(j,n,p)$$

$$= \phi_4(c, n, c, n)\phi_3(c, n, p)\phi_2(n, n, p) + \sum_{i=1}^{c} \sum_{j=i}^{\min(n+i-c, n-1)} \phi_4(i, j, c, n)\phi_3(i, j, p)\phi_2(j, n, p)$$

$$= \phi_1(p)\left[\frac{c2^c}{n2^n}\left(\frac{1-\phi_1(p)}{2}\right)^{c-1} + \frac{p-1}{p}\sum_{i=1}^{c}\sum_{j=i}^{\min(n+i-c, n-1)} \phi_4(i, j, c, n)\phi_3(i, j, p) \right]$$

$$= \phi_1(p)\left[\frac{c2^c}{n2^n}\left(\frac{1-\phi_1(p)}{2}\right)^{c-1} + \frac{p-1}{p}\binom{n}{c}^{-1}\sum_{i=1}^{c} q_i(c, n)\left(\frac{1-\phi_1(p)}{2}\right)^{i-1} \right],$$

where

$$q_i(c, n) = \sum_{j=i}^{\min(i+n-c, n-1)} \binom{n-j}{c-i}\binom{j-1}{i-1}\left(\frac{1}{2}\right)^{j-i}, \ 1 \le i \le c$$

can be expressed in terms of hypergeometric functions

$$q_i(c, n) = \binom{n-i}{c-i}{}_2F_1\left[i, c-n; i-n; \frac{1}{2}\right] - \delta_{c,i}\binom{n-1}{c-1}{}_2F_1\left[1, n; n+1-c; \frac{1}{2}\right]. \quad \square$$

Proof of Lemma 4.8. If a function $f(x) = bQ_S(x) + a$ can be written as $cQ_{S'}(x)$, then

$$f = bQ_S(x) + a = \begin{cases} a & x \in S \\ a+b & x \in S^c \end{cases} = \begin{cases} 0 & x \in S' \\ c & x \in S'^c \end{cases} = cQ_{S'}(x).$$

Since a and c are nonzero, $a + b = 0 \Leftrightarrow a = -b$ must hold for such a function. Since $\mathbb{F}$ contains $p - 1$ nonzero numbers, there are $p - 1$ choices for b and $p - 2$ choices for a, to obtain a function that cannot be written as $cQ_{S'}(x)$. Moreover, there are $2(p - 1)$ different segments S, but only half of them lead to a different function since every function can be expressed in two different ways:

$$bQ_S(x) + a = b(1 - Q_{S^c}(x)) + a = -bQ_{S^c}(x) + (a + b).$$

Thus, there are $(p - 1)^2(p - 2)$ different functions $f = bQ_S(x) + a$ that cannot be written as $cQ_{S'}(x)$. $\quad \square$

Proof of Lemma 4.9. (1) Assume a function $f(\mathbf{x}) = b\prod_{j=1}^{k} Q_{S_j}(x_j) + a$ can be written as $c\prod_{j=1}^{k} Q_{S'_j}(x_j)$, then

$$b\prod_{j=1}^{k} Q_{S_j}(x_j) + a = \begin{cases} a & \exists j : x_j \in S_j \\ a+b & \forall j : x_j \in S_j^c \end{cases}$$

$$= \begin{cases} a & \mathbf{x} \in (S_1 \times \mathbb{F}^{k-1}) \cup \ldots \cup (\mathbb{F}^{k-1} \times S_k) \\ a+b & \mathbf{x} \in S_1^c \times \ldots \times S_k^c \end{cases}$$

$$= \begin{cases} 0 & \mathbf{x} \in (S'_1 \times \mathbb{F}^{k-1}) \cup \ldots \cup (\mathbb{F}^{k-1} \times S'_k) \\ c & \mathbf{x} \in S_1'^c \times \ldots \times S_k'^c \end{cases}$$

$$= c\prod_{j=1}^{k} Q_{S'_j}(x_j).$$

Since $a, c \ne 0$, $a + b = 0$ must hold. Hence,

$$S_1^c \times \cdots \times S_k^c = (S'_1 \times \mathbb{F}^{k-1}) \cup \cdots \cup (\mathbb{F}^{k-1} \times S'_k).$$

This last statement is however impossible. Thus, there is no function $f(\mathbf{x}) = b\prod_{j=1}^{k} Q_{S_j}(x_j) + a$ that can be written as $c\prod_{j=1}^{k} Q_{S'_j}(x_j)$.

(2) The nonzero constants a, b can be arbitrarily chosen, with $p - 1$ choices each. Contrary to the previous lemma, each choice of segments $S_1, \ldots, S_k$ leads to a different function because $S_1^c \times \ldots \times S_k^c \ne (S_1 \times \mathbb{F}^{k-1}) \cup \ldots \cup (\mathbb{F}^{k-1} \times S_k)$ and because $a \ne a + b$. For each segment there are $2(p - 1)$ choices, so that altogether there are $(p - 1)(p - 1)(2(p - 1))^k = 2^k(p - 1)^{k+2}$ different functions of the form $b\prod_{j=1}^{k} Q_{S_j}(x_j) + a$. $\quad \square$

Proof of Theorem 4.15. In order to find the number of different equivalence classes of NCFs, we need the following combinatorial result [40, p. 70]:

Given n, r and s_i, $i = 1, \ldots, r$ and $s = s_1 + \cdots + s_r \leq n$. Then the number of integer solution of the equation $k_1 + \cdots + k_r = n$, where $k_i \geq s_i$, is

$$\sum_{\substack{k_1 + \cdots + k_r = n \\ k_i \geq s_i, s = s_1 + \cdots + s_r \leq n}} 1 = \binom{r + n - s - 1}{r - 1}.$$

The number of different equivalent classes of NCFs equals the number of different NCFs with a fixed canalizing variable order σ in Equation (4.1). Thus, we can follow the same enumerative approach as we did in the proof of Theorem 4.10, except that we do not consider the permutation of the variables. Hence,

$$N = 2^{n-1} p(p-2) \sum_{r=2}^{n} (p-1)^{n+r-1} \sum_{\substack{k_1 + \cdots + k_{r-1} = n-1 \\ k_i \geq 1, i=1,\ldots,r-1}} 1$$

$$+ 2^n p \sum_{r=1}^{n-1} (p-1)^{n+r} \sum_{\substack{k_1 + \cdots + k_r = n \\ k_i \geq 1, i=1,\ldots,r-1, k_r \geq 2}} 1$$

$$= 2^{n-1} p(p-1)^{n+1} \left[(p-2) \sum_{r=2}^{n} (p-1)^{r-2} \binom{n-2}{r-2} + 2 \sum_{r=1}^{n-1} (p-1)^{r-1} \binom{n-2}{r-1} \right]$$

$$= 2^{n-1} p(p-1)^{n+1} \left[(p-2) p^{n-2} + 2p^{n-2} \right]$$

$$= 2^{n-1} (p-1)^{n+1} p^n,$$

where we used the above mentioned lemma to eradicate the inner sums in the first equality and the binomial theorem to simplify the sums in the second equality. $\square$

References

[1] M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Stochastic gene expression in a single cell, Science 297 (2002) 1183–1186.
[2] D. Volfson, J. Marciniak, W.J. Blake, N. Ostroff, L.S. Tsimring, J. Hasty, Origins of extrinsic variability in eukaryotic gene expression, Nature 439 (2006) 861–864.
[3] S.H. Strogatz, Exploring complex networks, Nature 410 (2001) 268–276.
[4] S. Boccaletti, V. Latora, Y. Moreno, M. Chavez, D.-U. Hwang, Complex networks: structure and dynamics, Phys. Rep. 424 (2006) 175–308.
[5] H. Kitano, Biological robustness, Nat. Rev. Genet. 5 (2004) 826–837.
[6] S.A. Kauffman, Metabolic stability and epigenesis in randomly constructed genetic nets, J. Theoret. Biol. 22 (1969) 437–467.
[7] R. Thomas, Boolean formalization of genetic control circuits, J. Theoret. Biol. 42 (1973) 563–585.
[8] Z. Sun, X. Jin, R. Albert, S.M. Assmann, Multi-level modeling of light-induced stomatal opening offers new insights into its regulation by drought, PLoS Comput. Biol. 10 (2014) e1003930.
[9] M. Brandon, B. Howard, C. Lawrence, R. Laubenbacher, Iron acquisition and oxidative stress response in aspergillus fumigatus, BMC Syst. Biol. 9 (2015) 19.
[10] R. Poltz, M. Naumann, Dynamics of p53 and NF–κB regulation in response to DNA damage and identification of target proteins suitable for therapeutic intervention, BMC Syst. Biol. 6 (2012) 125.
[11] A. Mbodj, G. Junion, C. Brun, E.E. Furlong, D. Thieffry, Logical modelling of Drosophila signalling pathways, Mol. Biosyst. 9 (2013) 2248–2258.
[12] F. Li, T. Long, Y. Lu, Q. Ouyang, C. Tang, The yeast cell-cycle network is robustly designed, Proc. Natl. Acad. Sci. USA 101 (2004) 4781–4786.
[13] C. Fretter, A. Szejka, B. Drossel, Perturbation propagation in random and evolved Boolean networks, New J. Phys. 11 (2009) 033005.
[14] E. Balleza, E.R. Alvarez-Buylla, A. Chaos, S. Kauffman, I. Shmulevich, M. Aldana, Critical dynamics in genetic regulatory networks: examples from four kingdoms, PLoS ONE 3 (2008) e2456.
[15] B. Derrida, G. Weisbuch, Evolution of overlaps between configurations in random Boolean networks, J. Phys. 47 (1986) 1297–1303.
[16] C.H. Waddington, Canalization of development and the inheritance of acquired characters, Nature 150 (1942) 563–565.
[17] S. Kauffman, The large scale structure and dynamics of gene control circuits: an ensemble approach, J. Theoret. Biol. 44 (1974) 167–190.
[18] S. Kauffman, C. Peterson, B. Samuelsson, C. Troein, Random Boolean network models and the yeast transcriptional network, Proc. Natl. Acad. Sci. USA 100 (2003) 14796–14799.
[19] S. Kauffman, C. Peterson, B. Samuelsson, C. Troein, Genetic networks with canalyzing Boolean rules are always stable, Proc. Natl. Acad. Sci. USA 101 (2004) 17102–17107.
[20] A.S. Jarrah, B. Raposa, R. Laubenbacher, Nested canalyzing, unate cascade, and polynomial functions, Phys. D 233 (2007) 167–174.
[21] F. Karlsson, M. Hörnquist, Order or chaos in Boolean gene networks depends on the mean fraction of canalyzing functions, Phys. A 384 (2007) 747–757.
[22] N. Kochi, M.T. Matache, Mean-field Boolean network model of a signal transduction network, Biosystems 108 (2012) 14–27.
[23] D. Murrugarra, R. Laubenbacher, Regulatory patterns in molecular interaction networks, J. Theoret. Biol. 288 (2011) 66–72.
[24] K. Jansen, M.T. Matache, Phase transition of Boolean networks with partially nested canalyzing functions, Eur. Phys. J. B 86 (2013) 1–11.
[25] C. Kadelka, J. Kuipers, R. Laubenbacher, The influence of canalization on the robustness of Boolean networks, arXiv preprint, arXiv:1607.04474.
[26] Y. Li, J.O. Adeyeye, D. Murrugarra, B. Aguilar, R. Laubenbacher, Boolean nested canalizing functions: a comprehensive analysis, Theoret. Comput. Sci. 481 (2013) 24–36.
[27] L. Layne, E. Dimitrova, M. Macauley, Nested canalyzing depth and network stability, Bull. Math. Biol. 74 (2012) 422–433.

[28] Q. He, M. Macauley, Stratification and enumeration of Boolean functions by canalizing depth, Phys. D 314 (2016) 1–8.

[29] D. Murrugarra, R. Laubenbacher, The number of multistate nested canalyzing functions, Phys. D 241 (2012) 929–938.

[30] W. Just, I. Shmulevich, J. Konvalina, The number and probability of canalizing functions, Phys. D 197 (2004) 211–221.

[31] S.E. Harris, B.K. Sawhill, A. Wuensche, S. Kauffman, A model of transcriptional regulatory networks based on biases in the observed regulation rules, Complexity 7 (2002) 23–40.

[32] A. Veliz-Cuba, A.S. Jarrah, R. Laubenbacher, Polynomial algebra of discrete models in systems biology, Bioinformatics 26 (2010) 1637–1643.

[33] C.O. Reichhardt, K.E. Bassler, Canalization and symmetry in Boolean models for genetic regulatory networks, J. Phys. A: Math. Theor. 40 (2007) 4339.

[34] M. Marques-Pita, L.M. Rocha, Canalization and control in automata networks: body segmentation in Drosophila melanogaster, PLoS ONE 8 (2013) e55946.

[35] R. Thomas, Regulatory networks seen as asynchronous automata: a logical description, J. Theoret. Biol. 153 (1991) 1–23.

[36] R. Laubenbacher, F. Hinkelmann, D. Murrugarra, A. Veliz-Cuba, Algebraic models and their use in systems biology, in: Discrete and Topological Models in Molecular Biology, Springer, 2014, pp. 443–474.

[37] M. Nykter, N.D. Price, M. Aldana, S.A. Ramsey, S.A. Kauffman, L.E. Hood, O. Yli-Harja, I. Shmulevich, Gene expression dynamics in the macrophage exhibit criticality, Proc. Natl. Acad. Sci. USA 105 (2008) 1897–1900.

[38] H.S. Wilf, Generating Functionology, Academic Press, 1994.

[39] Y. Li, J. Adeyeye, Sensitivity and block sensitivity of Boolean nested canalizing functions, arXiv preprint, arXiv:1209.1597.

[40] C.A. Charalambides, Enumerative Combinatorics, CRC Press, 2002.