Preprints of the 17th IFAC Symposium on System Identification
Beijing International Convention Center
October 19-21, 2015. Beijing, China

Identifiability Challenges in Mathematical Models of Viral Infectious Diseases ⋆

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Abstract: Nowadays, infections by viral pathogens are one of the biggest health threats to mankind. The development of new avenues of thinking to integrate the complexity of infectious diseases and the immune system is urgently needed. Recently mathematical modelling has emerged as a tool to interpret experimental results on quantitative grounds providing relevant insights to understand several infectious diseases. Nevertheless, modelling the complex mechanisms between viruses and the immune system can result in models with a large number of parameters to be estimated. Furthermore, experimental measurements have the problem to be sparse (in time) and highly noisy. Therefore, structural and practical identifiability are key obstacles to overcome towards mathematical models with predictive value. This paper addresses the identifiability limitations in the most common mathematical model to represent viral infections. Additionally, numerical simulations reveal how initial conditions of differential equations and fixing parameter values can alter the profile likelihood.

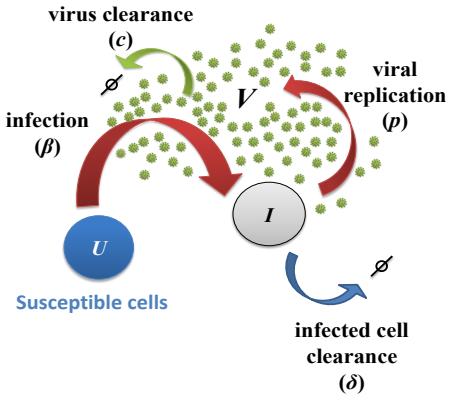
Keywords: parameter estimation, identifiability, viral infections

1. INTRODUCTION

Viruses are infectious agents that can replicate only inside of living cells, internalizing and releasing the genetic material (DNA or RNA) in the machinery of the host cell. After a successful infection, the infected cell can realize thousands of viruses which will be able to infect other target cells. Meanwhile, the host immune system is activated generating different responses to fight back the infection [Carter and Saunders, 2013].

Several viruses have the potential to evade the immune system promoting lethal diseases. Mathematical modelling has been introduced as a crucial tool to reveal underlying mechanisms for different viral infections. A huge amount of work has been invested to HIV, for which several models were proposed to understand the relation between HIV and the immune system [Kirschner et al., 1998, Perelson and Nelson, 1999, Nowak and May, 2000, Yates et al., 2007, Hernandez-Vargas and Middleton, 2013, Hernandez-Vargas et al., 2013]. Furthermore, many works have been attempting to quantify the dynamics of influenza virus infection [Möhler et al., 2005, Baccam et al., 2006, Beauchemin and Handel, 2011, Hernandez-Vargas et al., 2014, Pawelek et al., 2012] and Ebola virus infection [Nguyen et al., 2015]. The majority of these mathematical models have been based on the target cell-limited model [Nowak and May, 2000], see Fig.1. Even though the target cell-limited model can represent different infectious diseases (*e.g.* HIV, influenza, hepatitis and Ebola), this model possesses only the elemental infection interactions between viruses and a host.

Before rigorous parameter estimation methods can be applied to estimate the model parameters using experimental data, a critical obstacle to overcome is the verification of the model parameters. Previous works from [Xia, 2003] and [Miao et al., 2011] studied the structural identifiability properties to the model presented in Fig.1. However, a model that is structurally identifiable may still be practically non-identifiable if the amount and quality of the data are insufficient and the data manifest large deviations [Raue et al., 2009]. In viral infections, a large variability is presented from one host to another host, limiting the prediction value of mathematical models and estimated parameters. Using influenza virus infection as an



$$\dot{U} = -\beta UV \tag{1}$$
$$\dot{I} = \beta UV - \delta I \tag{2}$$
$$\dot{V} = pI - cV \tag{3}$$

Fig. 1. **Viral infection model.** Host cells can be either susceptible (U) or infected (I). Virus (V) infects susceptible cells with constant rate β. Once cells are infected, they release virus at rate p and virus particles are cleared with rate c. Infected cells can die with rate δ either by cytopathic viral effects or by the immune response.

⋆ This work was supported by iMed - the Helmholtz Initiative on Personalized Medicine. Corresponding author E.A. Hernandez-Vargas (e-mail: esteban.vargas@helmholtz-hzi.de)

example, this paper presents the identifiability limitations of the target cell-limited model considering the maximum likelihood principle.

2. IDENTIFIABILITY CONCEPTS REVIEW

Throughout this paper, $\mathbb{R}$ denotes the field of real numbers, $\mathbb{R}^n$ stands for the vector space of all n-tuples of real numbers, $\mathbb{R}^{n\times n}$ is the space of $n\times n$ matrices with real entries. Identifiability concepts introduced in this section are taken from Miao et al. [2011]. Consider the following general system

$$\begin{aligned}\dot{x} &= f(x(t),\theta)\\ y &= g(x(t),\theta)\\ \dot{\theta} &= 0\end{aligned} \tag{4}$$

where $x(t)\in\mathbb{R}^m$ is the vector of state variables with initial condition $x(0)=x_0$ and $y(t)\in\mathbb{R}^d$ is the output vector. For the parameter estimation problem, $\theta\in\mathbb{R}^q$ is a vector of constant parameters that can be estimated using experimental data. The initial condition x_0 is assumed to be known, not an equilibrium of the system and independent of θ. Additionally, we assume there are no model uncertainties.

Definition 1. Identifiability: The system (4) is identifiable if θ can be uniquely determined from the measurable output $y(t)$; otherwise, the system is unidentifiable.

A system that is controllable and observable has strong connections among input, states and output variables; such strong connectivity may indicate that the system is identifiable [Anguelova, 2007]. Furthermore, Ljung and Glad [1994] introduced the differences between global and local identifiability:

Definition 2. Global identifiability: The system of equations (4) is globally identifiable if for any two parameters vectors θ_1 and θ_2 in the parameter space $\Theta\subseteq\mathbb{R}^q$, $y(\theta_1)=y(\theta_2)$ holds if and only if $\theta_1=\theta_2$.

Note that the concept of global identifiability is much too strong for practical use. For biological applications, the concept of local identifiability is more appropriate.

Definition 3. Local identifiability: The system (4) is locally identifiable if for any θ within an open neighbourhood of some point θ^* in the parameter space $\Theta\subseteq\mathbb{R}^q$, $y(\theta_1)=y(\theta_2)$ holds if and only if $\theta_1=\theta_2$.

These two definitions imply one-to-one mapping between parameters and output. More restrictive for differential equations can be when the initial condition is given Tunali and Tarn [1987]:

Definition 4. Local strong identifiability (x_0-identifiability): The system structure is considered locally strong identifiable if for a given initial state $x_0=x(t_0)$, which is independent of θ and not an equilibrium point, and if there exists an open set Θ^0 within the parameter space Θ such that for any two different parameter vectors θ_1, $\theta_2\in\Theta^0$, the solutions $x(t,\theta)$ exist on $[t_0,t_0+\varepsilon]$ $(t_0<\varepsilon\leq t_1-t_0)$ for both θ_1 and θ_2, and $y(\theta_1)\neq y(\theta_2)$ on $[t_0,t_0+\varepsilon]$, and if there exists $\varepsilon\subset(0,\infty)$.

Furthermore identifiability definitions based on one-to-one mapping between system parameters and system output was proposed by Ljung and Glad [1994] developing the definition of identifiability based on the algebraic equations:

Definition 5. Algebraic identifiability: Based on algebraic equations of the system state and output, the system structure is considered to be algebraically identifiable if a real analytic function

$$\phi=\phi(\theta,y,\dot{y},...,y^{(k)}),\ \phi\in\mathbb{R}^q$$

can be constructed after a finite number of steps of algebraic calculations such that $\phi=0$ and the matrix $\frac{\partial\phi}{\partial\theta}$ is non-singular holding in the time range of interest $[t_0,t_1]$ for any (θ,x_0) in an open and dense subset of $\Theta\times M$, where k is a positive integer, M is an open set of initial states and $y,\dot{y},...,y^{(k)}$ are the derivatives of y.

2.1 Structural identifiability in viral infection models

Structural identifiability techniques verify identifiability by exploring the model structure. Xia [2003] proposed a method based on the implicit function theorem. This reads as follows:

Theorem 1. Let $\phi:\mathbb{R}^{d+q}\to\mathbb{R}^q$ denote a function model parameter $\theta\in\mathbb{R}^q$, system output $y\in\mathbb{R}^d$, and their derivatives, that is

$$\phi=\phi(\theta,y,\dot{y},...,y^{(k)})$$

where k is a non-negative integer. Assume that ϕ has continuous partial derivatives with respect to θ. A system is said to be locally identifiable at θ_*, if there exist a point $(\theta_*,y_*,\dot{y}_*,...,\dot{y}_*^{(k)})\in\mathbb{R}^{d+q}$ such that

$$\phi(\theta_*,y_*,\dot{y}_*,...,y_*^{(k)})=0$$

and the identification matrix

$$\left.\frac{\partial\phi}{\partial\theta}\right|_{\theta=\theta_*} \tag{5}$$

is non-singular. Using the Taylor expansion of ϕ at θ_*

$$\phi\approx\phi(\theta_*)+(\theta-\theta_*)\left.\frac{\partial\phi}{\partial\theta}\right|_{\theta=\theta_*}$$

since $\phi(\theta_*)=0$ and $[\frac{\partial\phi}{\partial\theta}|_{\theta=\theta_*}]^{-1}$ exists, a unique solution of θ can be found and the system is locally identifiable at θ_*.
The proof is provided in Xia [2003]. □

The system is identifiable if the identification matrix (5) is non-singular. This methodology has been applied by Miao et al. [2011] to the viral infection model presented in Fig.1. Considering the parameter set $\theta=[\beta,\delta,p,c]$ and assuming that the viral load V is the only measurement available, the system (1)-(3) is algebraically identifiable if there exists a function ϕ such that $det[\frac{\partial\phi}{\partial\theta}]\neq 0$ and $\phi(\theta,V,\dot{V},...,V^k)=0$ holds on $[0,t]$. Using high order derivatives in the system (1)-(3), Miao et al. [2011] obtained the following function:

$$\begin{aligned}\phi_1=(V^{-1}\dot{V}-\beta V)\left(\ddot{V}+\delta cV+(\delta+c)\dot{V}\right)\\ -(\delta+c)\ddot{V}-c\delta\dot{V}\end{aligned} \tag{6}$$

Note that the identification equation (6) does not depend on the unobservable states. Thus, further identifiability studies can be performed.

Remark: Previous works by Miao et al. [2011] and Wu et al. [2008] showed that the identification equation (6) does not contain the parameter p, therefore, this parameter is not structurally identifiable. However, Wu et al. [2008] remarked that the parameter p can be identified if the initial conditions of the system (x_0) are known. In a similar way, further structural

identifiability properties could be achieved using a structural polynomial and rational systems as those presented in Nemcova [2010]. Thus, initial conditions for the mathematical model (1)-(3) will be assumed to be known in the following sections. □

3. PRACTICAL IDENTIFIABILITY

A system that is algebraically identifiable may still be practically non-identifiable if the amount and quality of the data is insufficient and manifest large variability. The novel approach shown in Raue et al. [2009] exploits the profile likelihood to determine both structural and practical non-identifiability.

The idea of this approach is to explore for each parameter in the direction of the least squares ($\chi^2(\theta)$), which writes as follows:

$$\chi^2(\theta) = \frac{1}{T}\sum_{i=1}^{T}\frac{[\log(y_i) - \log(y(\theta,t_i))]^2}{\sigma_i^2} \tag{7}$$

where y_i denotes the experimental measurement at time-point t_i, σ_i^2 is the corresponding measurement errors, T is the total number of data points and $y(\theta,t_i)$ is the simulated output by the parameter set θ for the time point t_i. The parameters are estimated numerically by

$$\hat{\theta} = \arg\min[\chi^2(\theta)] \tag{8}$$

Note that when the output noise is normally distributed $\varepsilon \sim N(0,\sigma^2)$, χ^2 is equivalent to the maximum likelihood estimate (MLE) of θ, that is

$$\chi^2(\theta) = \text{const} - 2\log(L(\theta)) \tag{9}$$

where $L(\theta)$ is the likelihood.

Redundant parametrization in mathematical models provides structural non-identifiability, due to an insufficient mapping in the function g of the internal model states x to the output measurements y. Ambiguous parameters $\theta_{sub} \subset \theta$ may be varied without changing the output y resulting in constant values for χ^2. For practical identifiability, it is necessary to identify in which directions χ^2 flattens out.

3.1 Experimental data for influenza virus infection

The recent outbreaks of H1N1 (swine flu), H5N1 (bird flu) and H7N9 have underlined the impact of influenza A infections as a major threat to human health. The mechanisms responsible for viral control during influenza virus infection remain subject to discussion. Whereas much research has been done on the pathogen, our understanding of the host response and the host-pathogen interaction is still very limited.

Mathematical models have been proposed to capture the dynamics of influenza infection to understand the interaction of the virus with the immune system cells [Möhler et al., 2005, Baccam et al., 2006, Beauchemin and Handel, 2011, Pawelek et al., 2012, Hernandez-Vargas and Meyer-Hermann, 2012]. Nevertheless, the majority of these works have not shown identifiability studies, hence parameters estimation should be interpreted with caution.

To test the practical identifiability and reveal the parameter limitations, the mathematical model shown in Fig.1 is considered together with the murine experimental data presented in Toapanta and Ross [2009]. The mouse model has proven to be invaluable in exploring pathogenesis of influenza infection. Mice were infected with influenza virus A/Puerto Rico/8/34 (H1N1) at 12–16 weeks of age (young) or 72–76 weeks of age (aged). Lung virus titres were determined by plaque assay and reported as plaque forming units per ml (pfu/ml). Experimental methods and settings are described in detail in Toapanta and Ross [2009].

In short, mice were anaesthetized and intranasally instilled with 50–100 pfu. Following infection, mice were monitored daily for morbidity and mortality. Lungs were harvested on days 0, 1, 2, 3, 5, 7, 9, 11, 15 and 19 post-infection. Lungs were homogenized and the supernatants used to determine virus titers as well as cytokine and chemokine concentrations. The number of animals varied from 3 to 6 per time point per experiment. For some specific time points, a higher number of animals were used to confirm the results; further details can be found in Toapanta and Ross [2009].

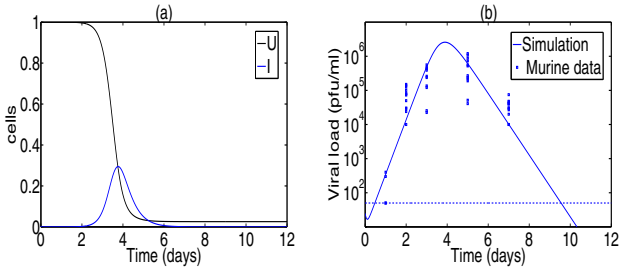


Fig. 2. **Best parameter fitting.** Cell dynamics are shown in panel (a). Viral titer data from Toapanta and Ross [2009] and simulation results are shown in panel (b). Best fitting values are $\beta = 7.22 \times 10^{-7}$, $p = 9.95$, $c = 7.12$ and $\delta = 3.07$. Experiments showed that the viral titer was not detectable at day 9 for 6 mice (detection levels, less than 50 PFU/ml, are shown with a horizontal dashed line in panel (b)).

The model parameters (β, p, δ and c) are fitted using the experimental data from Toapanta and Ross [2009]. The minimization of $\chi(\theta)$ is performed using the Differential Evolution (DE) algorithm presented in Storn and Price [1997]. Note that several optimization solvers were considered, including both deterministic (fmincon Matlab routine, and Pattern Search algorithm) and stochastic (Genetic algorithm) methods. Nevertheless, DE global optimization algorithm was robust to initial guesses of parameters and converges faster than the other mentioned methods.

Best fitting to the experimental data is shown in Fig.2, where panel (a) presents the typical cell dynamics during a viral infection that is a fast depletion of susceptible cells while infected cells peak between day 3-4 post infection. Simulations for the viral titer portrayed in Fig.2(b) fit reasonably well to the experimental data. These parameters are consistent with previous mathematical works [Möhler et al., 2005, Baccam et al., 2006, Beauchemin and Handel, 2011, Pawelek et al., 2012]. However, further identifiability studies may reveal the accuracy of parameter estimates.

4. NUMERICAL RESULTS

In this section, the practical identifiability is studied based on the approach proposed by Raue et al. [2009]. The idea

behind this approach is to explore the parameter space for each parameter θ_i re-optimizing the $\chi(\theta)$ with respect to all parameters $\theta_{j\neq i}$. The main task is to detect directions where the likelihood flattens out.

Numerical results in Fig.3 (blue lines) reveal that there are no flat valleys infinitely extended. Thus, the model parameters can be considered structurally identifiable. Although, the four parameters could possess a unique minimum, the flattening out of χ that continues along the functional relation may provide the intuition of practical non-identifiability. This can be mainly attributed to the large variation of the experimental data, which is a common variation in animal experiments. Note that this variation can be even larger in humans due to the presence of different antigens, lifestyle and the environment. Therefore, any viral infection model attempting to estimate the whole parameter set solemnly in the viral load should be interpreted with caution.

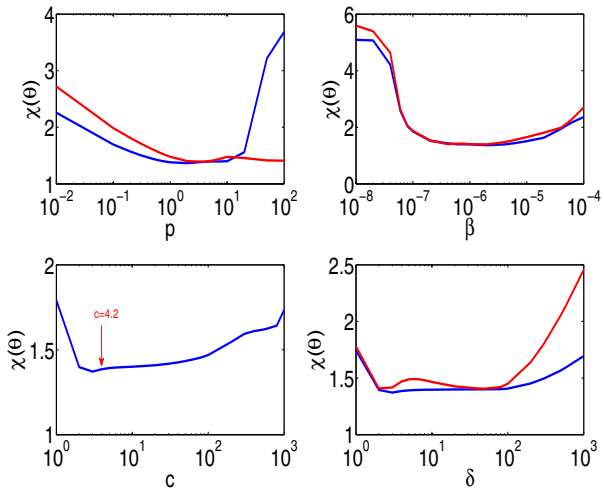


Fig. 3. **Practical identifiability.** The blue line represent the likelihood for each parameter θ_i re-optimizing the $\chi(\theta)$ with respect to the other parameters $\theta_{j\neq i}$. The red line is the likelihood fixing the parameter c.

Mathematical models with reduced number of parameters able to predict experimental data are required to provide more reliable estimations. For instance, Saenz et al. [2010] and Pawelek et al. [2012] proposed two different influenza infection models to represent the same data from 6 ponies. The model proposed by Pawelek et al. [2012] with 5 ordinary differential equations (ODEs) and 9 parameters improves the fits in several aspects respect to the model in Saenz et al. [2010] with 8 ODEs and 13 parameters. Nevertheless, the work developed by Pawelek et al. [2012] did not present any identifiability studies.

Non-identifiability of the parameter p is a considerable limitation. Miao et al. [2011] revealed that because of (6) does not involve the parameter p, the parameter is not identifiable suggesting that p could be fixed not affecting the other parameters. However, practical identifiability studies show that parameter p has an important role on the other parameters as can be observed in Fig.4. In fact, there is a linear correlation between parameter p and β ($R^2 = 0.956$), therefore one parameter can rescale the others providing flattening out the profile likelihood. Furthermore, the parameter p can impact the estimations in other parameters (δ and c). Previous studies showed that using singular value perturbation to the differential equation (3) can be approximated as an algebraic equation ($V = \frac{p}{c}I$) if the viral clearance is larger than one day, that is $V >> 1$ [Barão and Lemos, 2007]. This can be the explanation for the strong dependence of parameters c and p observed in Fig.4.

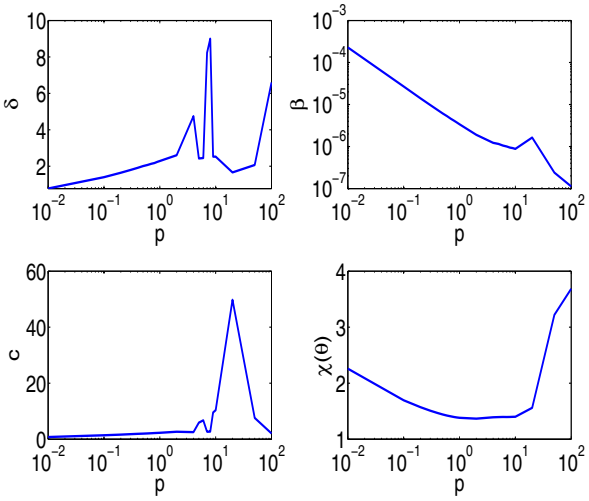


Fig. 4. **Profile likelihood for parameter p and the influence to other parameters.**

4.1 Does fixing a parameter help to improve estimations?

A common practice in theoretical biology is that in case of identifiability problems, it is believed that fixing parameters could help to estimate better the remaining parameters [Pinilla et al., 2012]. Even though this might apply to several cases, it is not always true. As a counter example provided here, the scenario simulation fixing the parameter $c = 4.2$ as reported by Miao et al. [2010] and then checking the profile likelihood for the other parameters is tested in Fig.3 (red line). On the one hand, the profile likelihood for the parameter δ presents a parabolic shape, suggesting an identifiability improvement for δ. On the other hand, the results for the parameter p in Fig.3 (red line) reveal that the profile likelihood is flattening out more in the right, implying serious identifiability problems. For the case of the parameter β, no change in the profile likelihood is observed. Therefore, in case that fixing parameters is opted, the profile likelihood can help to select which parameter is more appropriate to be fixed.

4.2 Does initial ODEs conditions affect the identifiability?

For initial conditions in our biological problem, the estimated number of epithelial cells (U_0) can be experimentally fixed, which is considered as 10^7 cells reported by Toapanta and Ross [2009]. Initial values for infected cells (I_0) are taken as zero. The initial viral titre V_0 in the majority of the works is constrained to be below the detection limit (less than 50 *ffu/ml*). Previous modelling works suggests using half of the detection limits or less [Thiébaut et al., 2006].

Nevertheless, the arbitrary selection of the initial condition for the viral load (V_0) can affect not only the parameter values but also the identifiability properties of the model. To test the effect of the initial viral load V_0, three different initial

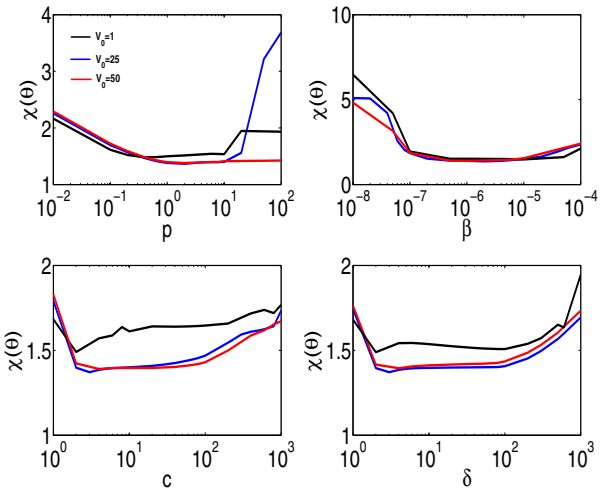


Fig. 5. **Parameter identifiability for different initial conditions of viral titer (V_0).**

conditions below the detection limit were selected as is shown in Fig.5. A low initial viral load ($V_0 = 1$ in black lines) provides relatively no changes in the profile likelihood shapes for all the parameters but slightly shifting the minimum values for $\chi(\theta)$. However, an initial viral load on the limits of detection ($V_0 = 50$ in red lines) can promote notorious identifiability problems for the parameter p that is flattening out the curve on the right. For the other parameters (β, δ and c) likelihood profiles remain the same. Numerical results in Fig.5 suggest that the initial viral load will have a strong implications on the parameter p value and its profile likelihood.

5. CONCLUSIONS

Modelling the dynamics of viruses and the immune system can result in models with a large number of parameters to be estimated. Here, a mathematical model that has been used to represent basic interactions in several viral infectious diseases is considered. Numerical results suggest that the parameter estimation is a key obstacle to overcome using experimental data. Solemnly viral load measurements are not enough to estimate all parameters and initial conditions in this viral infection model. Additionally, different initial conditions and fixing parameters can largely degrade the minimum in the profile likelihood and consequently identifiability properties.

Algebraic identifiability is a necessary condition to fulfill, providing important hints to the possible challenges that could appear during parameter estimations. However, this must be complemented by practical identifiability studies. Therefore, before any parameter estimation and of course interpretation of estimates, rigorous identifiability studies are required.

Future work on the experiments design strategy to decide the sample size and time points is needed to improve parameter estimations. Furthermore, Bayesian approaches, polynomial and rational systems are showing promising features on the parameter identifiability and should attract more attention in the mathematical biology field.

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