On Modeling Approaches for the Predictive Simulation of Living Systems Dynamics

*Ivan Mura**

Fecha de recepción: 13 de septiembre de 2013
Fecha de aprobación: 20 de octubre de 2013
Pp. 101 a 124.

Resumen

Este artículo se propone presentar dos de los principales enfoques que están disponibles hoy en día para modelar y simular la evolución dinámica de sistemas vivos: el modelo determinístico continuo, que es dictado por los sistemas de ecuaciones diferenciales ordinarias, y el estocástico discreto, que encuentra su base en el algoritmo de simulación estocástica propuesto por Gillespie en 1976. El objetivo de esta comparación es proporcionar la información necesaria para apoyar la selección de un enfoque de modelaje, basado en un conjunto de criterios verificables. Para alcanzar este objetivo, se analizan los fundamentos de la modelación, se propone un ejemplo de modelado para un sistema de vida simple y se discuten las principales ventajas y desventajas de cada enfoque.

Palabras clave

Modelo, Simulación, Sistemas vivos, Simulación estocástica

* Doctor en Ingeniería Electrónica, Informatica y de Telecomunicaciones, Universidad de Pisa

Modelling approaches for the predictive simulation of dynamic living systems

Abstract

This research paper describes two main approaches that are currently available for modelling and simulating the evolution of dynamic living systems, namely the continuous-deterministic one, which is rendered by systems of ordinary differential equations and the discrete-stochastic one, which bases on the Stochastic simulation algorithm proposed by Gillespie in 1976. The aim of this comparison is to provide the necessary information to support the selection of a modelling approach, focusing on a set of verifiable criteria. For this reason, we review the rationale approach of modelling, proposing a modelling sample for a single living system and discussing the main advantages and drawbacks of each approach.

Key words

Modelling, Simulation, Living Systems, Stochastic Simulation.

À propos des modèles de simulation préventive de la dynamique des systèmes vivants

Résumé

Cet article présente les deux principales approches actuellement disponibles en termes de modélisation et simulation de l'évolution dynamique des systèmes vivants. Il s'agit du modèle continuum-déterministe qui rend compte des systèmes d'équations différentielles et du modèle discret-stochastiques qui trouve son fondement dans l'algorithme de simulation stochastique proposé par Gillespie en 1976. L'objectif de cette comparaison est de fournir l'information nécessaire permettant la sélection d'une approche de modélisation basée sur un ensemble de critères vérifiables. Pour atteindre cet objectif, nous examinerons la rationalité de la modélisation avant de proposer un exemple de modélisation d'un système vivant simple pour finalement analyser les principaux avantages et inconvénients de ces approches.

Mots-clés

Modélisation, Simulation, Systèmes Vivants, Simulation Stochastique.

Sobre abordagens de modelagem para a simulação preditiva da dinâmica de sistemas vivos

Resumo

Este artigo objetiva apresentar dois modelos principais que estão atualmente disponíveis para modelar e simular a evolução dinâmica de sistemas vivos, aquele conhecido como continuo-determinístico, que está sujeito por sistemas de equações diferenciais ordinárias, e o discreto-estocástico, que está baseado no algoritmo de simulação estocástica proposto por Gillespie em 1976. Esta comparação objetiva fornecer a informação necessária para sustentar a escolha de uma abordagem de modelado, baseado em um conjunto de critérios verificáveis. Para atingir este objetivo, é feita uma revisão da lógica da modelagem, propõe-se um exemplo de modelagem para um sistema vivo simples e discutem-se as principais vantagens e desvantagens de cada abordagem.

Palavras-chave

Modelagem, Simulação, Sistemas Vivos, Simulação Estocástica.

1. Introducción

The term model is perhaps one of the most commonly used words in science, and it takes a different meaning depending on the realm and the context that is being used. Even when we limit ourselves within the boundaries of biology, the science of living systems, the term model recalls many different meanings. For instance, the EME iron binding domain of hemoglobin is an example of a protein tertiary structure model; the common budding yeast *Saccharomyces cerevisiae* is a model, same as many other organisms that were selected as sample subjects of study for other species that are more difficult to study directly; the mechanism of action of a drug, for instance aspirin, is a model for the action of non-steroidal anti-inflammatory compounds.

Therefore, it is important to clarify what would be the intended meaning of the word model in our context. We consider a model to be a generalization and abstraction of some phenomenon or system, used to convey qualitative and/or quantitative information about the phenomenon or system it represents. To be more precise, in this paper we will limit ourselves to consider those models of living systems which possess the following properties:

- They include both qualitative and quantitative aspects of living systems.
- They can be used to determine the dynamical evolution of a phenomenon/system over time, starting from a known condition (initial state).
- They are amenable to simulation on a computer, which implies in computer-science terms that they have a single semantics, i.e. an unambiguous meaning.

One example of such a model would be the Lotka-Volterra model for the time dependent dynamical evolution of the preys and predators populations (Cooke et al., 1981).

Modeling biological systems is a significant task of systems biology (Kitano, 2002). Computational systems biology aims to develop and use efficient algorithms, data structures, and visualization and communication tools, with the goal of modeling biological systems and study their evolution through computation. The advantage of using computational models is manifold:

- Models can be built at different levels of abstraction, allowing to focus on the real object of interest of the study or investigation.

- Models permit to explore behaviors that would otherwise be difficult or impossible to observe.

- Model based studies consume much less resources, time and money than wet-lab experiments.

Of course, the points above do not mean that models should replace biological experimentation; rather, they are providing an effective companion tool that (a) complements and assists biology investigation through the analysis of what-if scenarios, (b) sustains/disproves experimental hypotheses, (c) drives experimental design.

Having said that, we come to the core of this paper, whose objective is to present and compare two main approaches to the modeling and simulation of living systems, namely the continuous deterministic and the discrete stochastic ones. There is an on-going debate on merits and drawback of the two in the systems biology community, quite often biased by the subjective preferences that are rooted in the background of the modelers; on the other hand, biologists that approach modeling are usually much more pragmatic and ready to experiment with both the continuous-deterministic and discrete-stochastic tools and methodology (Twycross et al., 2010), a strategy that can provide very reach predictions about system behavior (Mura and Csikász-Nagy, 2008).

This paper aims at providing the guideline information that should be used when selecting one modeling method. We present throughout the paper an example of modeling of a simple living system, from which we gather our conclusion about the important criteria that should guide a model selection, which very shortly are: the life-cycle of the model, i.e. whether a reuse/

extension is planned, the available experimental data, the relevance of noise for system behavior, the available time, and the measures of interest.

The rest of this paper is organized as follows: section 2 presents a very concise view of the abstraction processes required to extrapolate from the wealth of data that is available about living systems, and applies it to a simple case study example of predator-prey system. In Section 3 and 4 we introduce the continuous-deterministic and the discrete-stochastic modeling approaches, respectively, and we apply them to our case of study. Section 5 is devoted to the comparison of the methods. The results of such comparison are distilled in Section 6, which states the guidelines for model selection.

2. A modeling view of living systems

We define in this section the elements that are necessary to define a model of a living system, according to the specific definition of model we gave above. The kind of models we are interested in have the nice property of accommodating the most disparate levels of abstraction, which allows covering the wide range of spatial and temporal scales encompassed by biological investigation, from molecules to ecosystems.

When building a computational model amenable to simulation, we will be basically dealing with two types of information:

- Structural information, which includes the entities of the system, their evolution and interaction possibilities.

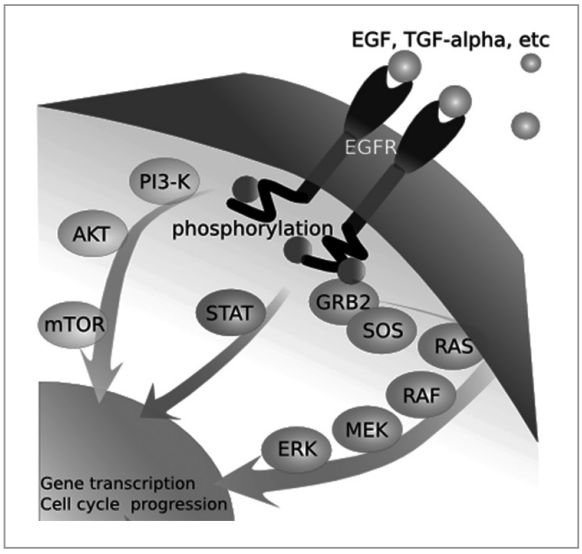
- Quantitative information, which provides the details about the speed with which the entities of the living system change their state and interact, as well as the multiplicity of the entities in the initial state of the system.

This information is to be distilled according to the objective of the modeling that defines the possible abstractions in which parts and behaviors are not relevant, and complete the projection from the total amount of information available. This point of detail is quite important when dealing with living systems, for which huge amounts of data are being generated.

2.1 Structural information

To better understand what we mean with the structural information of a model, we can imagine visualizing it as a network, where nodes are entities and arcs are relationships representing changes and interactions. Much of biology is described and explained through qualitative networks, often termed cartoons, where entities and their transformations/interactions are depicted in a graphical form. For instance, we show in Figure 1 a cartoon network for the EGFR signaling pathway. This network includes entities such as the EGF, EGFR, PI3-K and various other molecular species, interactions (in this case mainly phosphorylations) represented by arrows and finally compartments such as the extracellular space, membrane, cytosol and nucleus, specifying the localization of entities.

Figure 1. EGFR signaling pathway cartoon network



Source. Wikimedia COMMONS.

The example in Figure 1 provides a good idea of the type of information we expect to collect for defining the structural part of a model. The following list provides a better description of what needs to be extracted from the biological knowledge available:

- The list of entities participating in the phenomenon or system to be modeled. They can be molecular species such as proteins and enzymes, macromolecules such as genes and ribosomes, or even animals when we are dealing with food webs or entire ecosystems. When an entity may appear in different states or configurations, and they are relevant to the evolution of the system, we need to include all of them in the list. For instance, a protein may exist in multiple states of ubiquitination: if these species possess different levels of activity, they should be considered as different entities and therefore be included in the model.

- The compartments or physical locations where the entities can exist or move to. Molecules may or may not have the ability to cross membranes, so animals may move within different areas of the environment.

- The possible changes in the state, activity, availability or location of the entities. For example, a molecule can be degraded and therefore disappear from the system, or may move itself from one compartment to an adjacent one.

- The possible interactions between entities, i.e. those changes that result from the physical contact of two entities, such as molecular complexation, mating or killing of a prey by a predator.

To make a simple example, if we wanted to gather the structural information of a well-known prey-predator model (again, Lotka-Volterra), we would get the following:

- There are just two entities: prey and predator.
- There is a single physical space: the savanna environment.
- A prey reproduces with consuming the resources of the environment; a predator dies.
- A predator eats a prey and reproduces.

2.2 Quantitative information

The following list describes the quantitative details that need to be extracted to complement the structural information:

- The multiplicity or abundance of each entity listed in the structural part at the initial time (it may be 0 for some). This information defines the initial state of the model.

- The size (volumes and areas) of the compartments or physical spaces.

- The speed of the changes in the state, activity and availability, or location of the entities. In this case, how much does it take for a given amount of a molecule to be synthesized or degraded? How long does an animal survive?

- The frequency with which entities encounter each other and interact, obviously in a given physical space.

To continue with the prey-predator example we would require the following additional information:

- The density or number of preys and predators at time zero.

- The area where the preys and predators live and move.

- The rate at which preys reproduce and the time a predator survives in the environment.

- The rate at which predators eat preys and reproduce.

These two types of information are sufficient to build a model that is amenable to simulation, and that can predict the evolution of the prey-predator system over time. We will be using this specific example in the next two sections, when detailing the two modeling approaches we deal with in this paper.

3. The continuousdeterministic modeling approach

The continuous-deterministic approach to the modeling of living systems finds its basis in the continuous approximation of the multiplicity of the entities. When dealing with the high numbers typical of molecular counts, it is quite obvious to approximate discrete quantities with continuous variables. This comes even more naturally, given that experimental measurements about molecule abundances are normally taken in form of concentrations, i.e. continuous numbers.

In a continuous- deterministic model, the abundance of entities is represented by continuous variables. The changes that affect such abundance are represented in terms of the speed of variation of the variable, i.e. its derivative. Therefore, a continuous-deterministic model is usually a set of ordinary differential equations (ODEs). The name “deterministic” comes from the fact that the behavior of the model over-time, that is the solution of the ODEs, is totally determined by the initial state and the equations itselfthemselves. No stochastic fluctuations are considered, and any prediction of the model is perfectly reproducible (Ellner and Guckeheimer, 2006).

To give a practical example of how a continuous deterministic model would be built, we consider again in the following the predator prey example. There will be exactly two time-dependent continuous variables, one for each of the entities of the system. Let us denote such variables with prey and pred. The units of measure of the variables would be densities, i.e. number of animals divided by area.

Changes affecting such variables are represented as variations over time. The changes of variable prey are determined by two distinct phenomena:

- Births, which increase the variable, and occur with a rate that is proportional to the current size of the population.

- Deaths, which are always caused by predators, and are therefore proportional to the populations of preys and predators.

In a differential equation, this looks like this: prey'=α·prey- β·prey·pred, prey^'=α·prey- β·prey·pred where α and β are the rates at which the density changes because of the births and the deaths, respectively.

Similarly, we can write a differential equation for the evolution of the variable pred, as follows: pred'=γ·prey·pred- δ·pred, pred^'=γ·prey·pred-δ·pred where the first term accounts for the positive variations of density due to reproduction and the second one for the negative variation due to predators death. The γ and δ are again the rates at which the density changes because of the births and the deaths, respectively. The structural part of the model is represented by the system of the two coupled ODEs.

The quantitative part of the model assigns an initial state to the ODE system, and provides the values of the rates α, β, γ and δ. For instance, if the initial densities were 5/km^2 and 0.1/km^2 for preys and predators, and the following values of the rates were used[1].

α=0.2/day, β=0.02/(day*density), γ=0.02/(day*density), δ=0.01/day

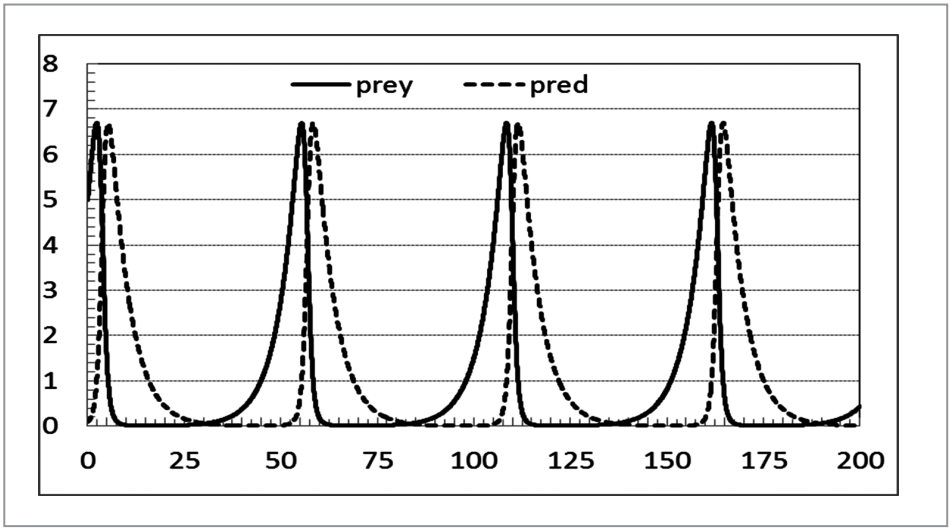
Then, the final model would be as follows:

prey'=0.2 · prey-0.2 · prey · pred
pred'=0.2 · prey · pred-0.2 · pred
prey(0)=5
prey(0)=0.1

[1] Notice the different units of measure for the various rates, depending on whether the term they are used in is of the first or second order.

A simulation of the model obtained through numerical integration would provide the time-dependent evolution of the variables shown in Source. It is interesting to notice the oscillations that result from this model simulation, which predict the fluctuations in the populations of preys and predators observed in nature. Oscillatory dynamical equilibriaequilibriums are quite commonly encountered in living systems dynamics.

Figure 2: Simulation of the predator-prey continuous-deterministic model. Time (days) is on the horizontal axis, density is on the vertical axis



Source. Simulation realized with the WinPP software package

4. The continuous-deterministic modeling approach

In 1976, a paperA document presented by Daniel T. Gillespie (Gillespie, 1977) proposed a novel computational approach to effectively analyze the time behavior of living systems through a discrete-stochastic approach. That paper provided an easy to implement algorithm for simulating the evolution of a system together with a theoretical justification of its applicability, grounded on statistical mechanics, of its applicability. The success of Gillespie's method for the study of biochemical systems dynamics is well demonstrated by the plethora of studies, papers and computational tools based on it that have appeared since the its original publication.

The main reasons for this widespread acceptance stem from the simplicity of the proposed algorithmic approach, which easily lends itself to straightforward implementations (Cao and Samuels, 2009), and from the clear link that is maintained with the intuitive descriptive language of chemical reactions (which also suits biochemistry). In fact, Gillespie's algorithm, called Stochastic Simulation Algorithm (SSA), can be seen as a formalization of the common intuitive understanding of how a chemical or a biochemical system described through chemical reactions would evolve over time.

Discreteness is at the core of the SSA. The very true nature of almost all the entities of living systems is discrete. Genes, proteins, cellules, and organisms can be counted as discrete numbers. Stochasticity is also inherent to the living system behavior; for instancein fact, it arises from the likelihood that molecules collide with each other due to their random motion in a medium, or that an animal dies or survives an encounter with a predator. The most important contribution of Gillespie was to reduce the complexity of an analytical treatment of discreteness and stochasticity, so that it can nicely fit into the well-known theory of Markov processes (Gillespie, 1992).

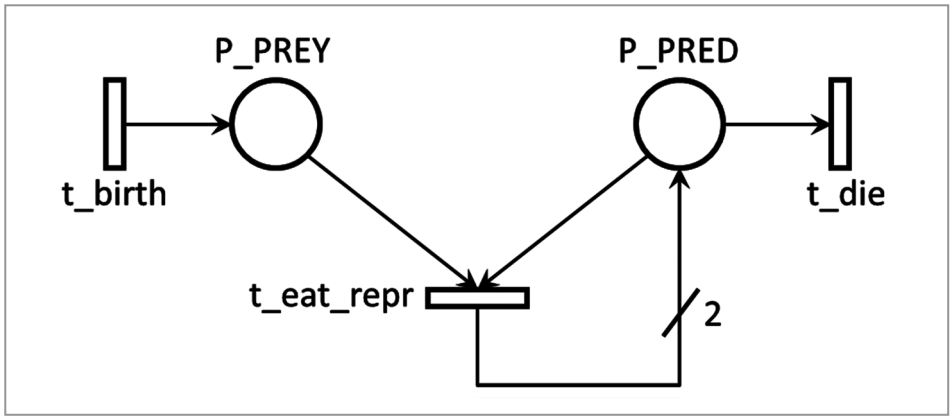
When approaching the discrete stochastic modeling of a system, we introduce a discrete variable for each entity. Therefore, for our predator-prey modeling example we would have two variables PREY and PRED (capital letters used not to confuse them with the variables of the continuous-deterministic model), which can take any value in the set of natural numbers. The initial values of these variables can be determined from the initial density value. We assumed that 5/km^2 and 0.1/km^2 are the initial values of for variables PREY and PRED, respectively. To convert these values into discrete numbers, we multiply by the area of the surface we intend to model, say 500km^2, getting the initial values PREY(0)=2500 and PREY(0)=50.

The other structural information can be rendered with various tools that support discrete-stochastic modeling. We consider in the following a very common and practical one, called Petri petri Nets nets (Mura, 2010). Petri Nets nets are a graphical modeling tools consisting of four elements:

- Named places, represented as white circles, which model entities.
- Tokens, represented as small dots, contained inside places, modeling the state or number of an entity.
- Named transitions, represented as empty bars, which model events.
- Arcs, which only link places to transitions and transitions to places, and model the flow of tokens in the net. Arcs have a weight (usually 1, not shown), to specify the number of tokens that flow through them.

A possible encoding of the structural information of the predator-prey system in a Petri petri Net net is shown in Figure 3. The number of tokens contained in places P_PREY and P_PRED represents the value of variables PREY and PRED of the model. Transition t_birth models the event “birth of a prey”, and adds one token to the place P_PREY. Transition t_eat_repr models the two events (a)“a predator kills a prey” and (b)“a predator reproduces”. It subtracts one token from place P_PRED and one from place P_PREY, and adds two tokens (notice the arc weight) to the place P_PRED. Therefore, the net balance of the flows associated with transition t_eat_repr models the removal of one prey and the birth of a predator. Transition t_die models the death of one predator, by removing one token from place P_PRED.

Figure 3: Petri Net of the predator-prey system (structural information)

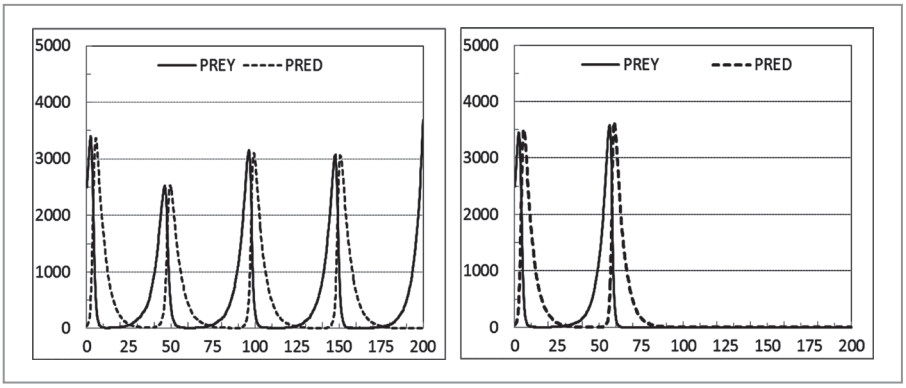


Source. by the author

The quantitative information of the system about the speed and frequency of change and interactions events is included into the Petri petri Net net model in the transition rate specification. The event associated with transition t_birth occurs 0.2 times per day (the same value we used in the continuous-deterministic model). This value is now interpreted as the rate of a negative exponential distribution, and defines the probability density function of the events that add tokens into place P_PREY. Similarly, we define the rates for the other two transitions.

The complete Petri petri Net net model can be simulated by using one of the many tools available. We report in Figure 4 the results of two simulation runs, executed by the Möbius software package (Clart et al., 2001). We show two simulation runs to demonstrate two different effects of stochasticity: first, each simulation run provides one of the possible evolutions of the modeled system, and second, the discrete stochastic model accounts for the extinction probability of extinction of thefor populations (see the right chartchart on the right).

Figure 4. Simulation results for the discrete stochastic model



Source. By the author

5. A comparison of approaches

In this section we will compare the continuous deterministic and the discrete stochastic modeling approaches for the prediction of living systems behavior, concerning the following aspects:

- Easiness of model creation, evaluated in terms of the time that is necessary to fully define the models from the biological information that is available.

- Understandability of the models, which we shall evaluate based on the closeness of the model to the standard biological representation of knowledge.

- Behaviors that are captured by the models referring to the quality of the predictions that can be made from simulation outcomes.

- Difficulty in the analysis of simulation outcomes, evaluated as the complexity of simulation data post-processing required to quantify the measures of interest, such as averages of abundance or sizes of populations, periods and amplitude of oscillations, time to achieve equilibrium, etc.

These set of criteria selected for the comparison does not pretend to be complete, rather we are focusing on the perceived experience of a modeler along the various phases of a modeling process.

5.1 Model creation

As it can be deduced from the example used throughout this paper, the steps to extract the structural and quantitative information are pretty independent from the selected modeling approach. A difference exists in the quantitative data required, in which the continuous deterministic modeling approach that naturally deals with continuous variables may easily obviate the necessity of knowing the exact dimensions of the compartment or geographical area object of the modeling. On the contrary, the discrete-stochastic approach requires an estimation of this quantitative information to determine the number of molecules of species given a concentration measure, as well as to perform a scaling of the rates for the interactions among two entities (this operations scaling was omitted in the paper not to burden the treatment of the example with and excessive level of detail).

The preference for an ODE based or a petri net base model is largely determined by the skills of the modeler. There is no difference in the time required to build them. The petri net modeling approach tends to hide the mathematical details of model formulation, and is usually easier to accept by beginners.

5.2 Model understandability

Once we have a model built, we may ask ourselves how intuitive it may be for a biologist who does not have a deep knowledge on the formalism we use. Here, we can fairly say that a petri net encoding of a model is for sure easier to understand, share and reuse than an ODE model. First of all, a petri net

represents all the events and interactions that lead to a system state change into separate and named graphical objects, whereas an ODE transforms everything into terms of a mathematical expression. Therefore, in a petri net a biologist can easily recover each aspect of the biological system that has been modeled, basically because the causality is preserved, whereas to perform such operations in a continuous-deterministic model requires a process of deconvolution of a potentially complex expression, where each term has to be analyzed to understand the piece of the biological system it relates to.

5.3 Behaviors captured by the model

Regarding the number and quality of the predictions that can be obtained by the two approaches, it is quite intuitive to understand that the discrete-stochastic one gains in the comparison. This is due to the fact that this modeling approach includes more information about the system, i.e. the discreteness and the stochasticity. The discrete nature of the model ensures that unfeasible states of the system with negative abundances are ever reached during a simulation. The stochastic characterization provides for the evaluation of averages, variances and whole distributions of the measures of interest, whereas the outcomes of a continuous-deterministic model only for average or mode values. In the predator-prey example we stated before, the inclusion of stochasticity in the model unveils a possible behavior of the system that passes totally unpredicted in the deterministic continuous approach, i.e. the possibility of population extinction. This occurs because the outcome of the ODE is only showing the most likely behavior of the system, without accounting for the unlikely events, such as when the predators kill all the preys. It is also important to mention that the outcomes of the two modeling approaches agree in terms of the most likely behaviors. The average period and the average amplitude of the oscillations computed from the simulation outcomes of the discrete-stochastic model are in agreement with the constant period and constant amplitude of the simulation outcome of the continuous-deterministic model.

5.4 Analysis of simulation outcomes

We consider here the process by which the simulation output is analyzed to obtain the information about the possible behaviors of the system. The predictions can be of two different types, qualitative and quantitative. Qualitative predictions about system behavior refer to the trends of system evolution, such as growth of populations or in the abundance of species, the existence of steady-states or oscillatory behaviors. Quantitative predictions refer to times to achieve equilibrium or the periods of oscillations, size of populations and concentrations.

When dealing with the outcomes of a continuous-deterministic model simulation, all the qualitative and quantitative predictions are easily made through the analysis of a single simulation run. On the contrary, when the discrete-stochastic modeling approach is adopted, the outcomes of multiple runs are to be aggregated to obtain a statistically meaningful evaluation of systems behaviors. This applies to both qualitative and quantitative behaviors. In a model where the abundance of entities is limited, for instance when studying with genes and their immediate products such as mRNAs, the stochastic noise can easily mask qualitative behaviors of systems in every single simulation run. Hence, to understand whether a discrete-stochastic model is predicting the existence of a steady state, we need to resort to the techniques for the statistical analysis of simulation outputs to compute estimates of averages and variances, together with their confidence intervals (Kelton, 1997). Depending on the variation coefficient of of the measure of interest, this analysis may require the aggregation of the output of a considerable number of simulation runs, up to thousands when we are interested in rare events such as extinctions.

6. Conclusions

We reviewed in the previous sections the necessary steps to create models and to analyze the outcomes of simulations for the continuous deterministic and discrete stochastic approaches, in their application to the prediction of living systems behavior.

When we are confronted to the selection of a modeling approach, we should primarily take into consideration the purpose of the modeling, in terms of the measures of interest. If we are only interested in the average behavior of a system, the continuous deterministic is usually providing a more suitable means to explore system dynamics. It also has the advantage of requiring less time in the analysis of simulation outcomes.

However, the characterization of the average behavior can sometimes be misleading, and obfuscate the real dynamics of a system, for instance when the measure of interest exhibits a bimodal distribution. In this case, the average of a measure may be quite far from any of the most likely behaviors of the modeled system.

If the objective of the modeling is the prediction of variances or distributions, we have to resort to the discrete stochastic modeling approach. This entails the necessity of gathering more data for model definition, as well as to spend additional time for the statistical aggregation of simulation outcomes. Undoubtedly, this approach can improve the level of detail of the predictions.

Another important consideration we have to make has to do with the life-cycle planned for the model. If we are building a standalone model that will hardly be reused or extended, the complexity of the mathematical treatment inherent to ODE will be obviated by the time savings. On the contrary, if the objective is to create a family of models, possibly refining or extending the model in a cycle of improvement, then the understandability and the preservation of causality that come with the discrete stochastic approach become of utter relevance.

As a conclusion, we can advocate the use of both modeling approaches, the continuous deterministic one as a first cut modeling tool , and the discrete stochastic to selectively refine the interesting model predictions. In line with this suggestion, various systems biology modeling tools, for instance COPASI (Hoopes et al., 2006), are nowadays providing the facility to specify the structural and quantitative modeling information and then to select in a second state the specific model formalism into which the modeled system will be represented and simulated.

7. References

Cao, Y., & Samuels, D.C., (2009). *Discrete stochastic simulation methods for chemically reacting systems, Methods in Enzymology,* 454, pp. 115-140, doi: 10.1016/S0076-6879(08)03805-6.

Clark, G., Courtney, T., et al., (2001). *The Möbius Modeling Tool, Proceedings of the 9th International Workshop on Petri Nets and Performance Models (PNPM'01) (PNPM '01).* IEEE Computer Society, Washington, DC, USA, pp. 241-250.

Cooke, D., Hiorns, R.W., et al., (1981). *The Mathematical Theory of the Dynamics of Biological Populations II*, Academic Press Inc.

Csikász-Nagy, A., & Mura, I., (2010). *Role of mRNA gestation and senescence in noise reduction during the cell cycle, In Silico Biology,* 10, pp. 81-88.

Ellner, S.P., & Guckenheimer, J., (2006). *Dynamic Models in Biology,* Princeton University Press.

Gillespie, D.T., (1977). *Exact stochastic simulation of coupled chemical reactions,* The Journal of Physical Chemistry, 81 (25), pp. 2340–2361.

Gillespie, D.T., (1992). A *rigorous derivation of the chemical master equation, Physica,* 188, pp. 404–425, doi:10.1016/0378-4371(92)90283-V.

Hoops, S., Sahle, S., et al., (2006). *COPASI: a COmplex PAthway SImulator,* Bioinformatics, 22, pp. 3067-3074.

Kelton, W.D., (1997). *Statistical analysis of simulation output, Proceedings of the 1997* Winter Simulation Conference, Atlanta, USA.

Kitano, H., (2002). *Systems Biology: a brief overview, Science*, 295, pp. 1662-1664, doi: 10.1126/science.1069492

Mura, I., (2010). *Stochastic Modeling, in I. Koch, W. Reisig and F. Schreiber (eds.), Modeling in Systems Biology, The Petri Net Approach,* Computational Biology 16, ISBN: 978-1-84996-473-97, Springer-Verlag London Limited, chapter 7.

Mura, I., & Csikász-Nagy, A., (2008). *Stochastic Petri Net extension of a yeast cell cycle model,* Journal of Theoretical Biology, 4, pp. 850-860, doi: 10.1016/j.jtbi.2008.07.019.

Twycross, J., Band, L.R., et al., (2010). *Stochastic and deterministic multiscale models for systems biology: an auxin-transport case study,* BMC Systems Biology, 4, doi:10.1186/1752-0509-4-34.