Image-based Parameter Inference for Spatio-temporal models of Organogenesis

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Abstract—Advances in imaging technology now provide us with detailed 3D data on gene expression patterns in developing embryos. This information can be used to build predictive mathematical models of embryogenesis. Current modelling approaches are, however, limited by lack of methods to automatically infer the regulatory networks and the parameter values from the image-based information. Here we make a first step to the development of such methods. We use limb bud development as a model system. For a given regulatory network we developed a decision tree based algorithm to automatically determine parameter values for which the model reproduces the expression patterns. Starting from this parameter set, local optimization was performed to further reduce the chosen goodness-of-fit measure. This approach allowed us to recover the target expression patterns, as judged by eye, and thus provides a first step towards the automated inference of parameter values for a given regulatory network.

1. Introduction

Developmental processes are controlled by complex regulatory networks. Decades of genetic experiments have defined the core regulatory proteins for most developmental processes and many regulatory links. The resulting regulatory networks are complex and the regulatory interactions change dynamically as the embryo is developing. As a consequence, our understanding of the regulatory logic that controls patterning in time and space remains limited. Mathematical modelling offers the opportunity to integrate the experimental information into a consistent framework and to define the underlying regulatory mechanisms [1].

To obtain a mathematical model with predictive value the model must be firmly rooted in experimental data. Available experimental data mainly consists of images that show the spatial distribution of mRNAs as a measure of gene expression at the different stages of development. This information is not quantitative, but provides a qualitative indication of expression patterns. Current models of the spatio-temporal processes in the embryo are largely hand-tuned to reproduce the experimental data both in wild type and mutants [2]. Computational methods are largely missing for the image-based inference of the the biological network architecture and the parameter values, even though methods for the estimation of parameter values for partial differential equation (PDE) models have been established. An important limitation are the higher computational costs for the simulations, which renders many approaches computationally infeasible for models of organogenesis. Here, we will focus on a model for mouse limb bud development to illustrate the challenges in inferring parameter values from the available data.

Limb buds grow out of the flank at about day 9 of mouse embryonic development [3]. Importantly, the limb bud consists of different tissue domains, and the expression of some of the proteins is restricted to particular subdomains, i.e. to either the mesenchyme (Fig.1A, red part), the apical ectodermal ridge (AER) (Fig.1B, blue part) or the ectoderm (Fig.1B, green and blue part). We will focus on the
early regulatory interactions, and we will thus restrict ourselves to the core regulatory interactions between fibroblast growth factor (FGF) 10, WNT3, FGF8 and BMP. Decades of experiments have defined the core regulatory interactions (Fig 1b). Thus, WNT2B from the flank induces the expression of Fgf10 in the mesenchyme. FGF10 signalling induces the expression of Wnt3 in the ectoderm. WNT signalling is necessary for the development of the AER, which expresses Wnt3 and other Fgfs. FGF8 diffuses into the mesenchyme and maintains the expression of Fgf10. FGF8 together with WNT3 also induce the expression of Bmps and BMPs supports the development of the AER.

In the following, we will build a mathematical model that represents these regulatory interactions. The focus will then be on the inference of the parameter values. To test the approach we will use simulated in silico data rather than experimental gene expression data as obtained from in situ hybridisation.

2. Results & Discussion

2.1. The Model

To keep the computational costs to a minimum we will limit ourselves to a 2D limb bud domain (Fig 1b). To simulate the regulatory network (Fig 1b) with n components on a limb bud domain we use a set of coupled partial differential equations of reaction-diffusion type, i.e.

\[ \frac{\partial c_i}{\partial t} = D_i \Delta c_i + R(c_1, \ldots, c_n) \tag{1} \]

where \( c_i \) denotes the concentration of species \( i \), with diffusion constant \( D_i \) and reaction term \( R(c_1, \ldots, c_n) \). As network components we include the morphogens FGF10 (FGF10), FGF8 (FGF8), BMP, WNT and the structure AER with diffusion constant \( D \) as well as the respective receptor-ligand-complexes with FGF10, denoted F10R, with FGF8, denoted F8R, with BMP, denoted BRa, and with WNT, denoted WFZ with diffusion constant DR. The reaction terms are set to

\[
\begin{align*}
R(F10) &= \rho_{F10} p_{FGF10} - d F10 \\
&\quad + (1 - \mathbb{1}_{\text{Mes}})(k_{off} F10R - k_{on} F10(RT_{F10} - F10R)) \\
R(F8) &= \rho_{F8} p_{FGF8} - d F8 \\
&\quad - \mathbb{1}_{\text{Mes}} k_{on} F8(RT_{FGF8} - F8R) + \mathbb{1}_{\text{Mes}} k_{off} F8R \\
R(WNT) &= \rho_{WNT} p_{WNT} - d WNT \\
&\quad - k_{on} WNT(RT - WFT) + k_{off} WFZ \\
R(BMP) &= \rho_{BMP} p_{BMP} - d BMP \\
&\quad - k_{on} BMP(RT_{BMP} - BRa) + k_{off} BRa \\
R(F10R) &= k_{on} F10(RT_{F10} - F10R) - (k_{off} + dF10R)F10R \\
R(F8R) &= k_{on} F8(RT_{FGF8} - F8R) - (k_{off} + dF8R)F8R \\
R(WFZ) &= k_{on} WNT(RT - WFT) - (k_{off} + dWFZ)WFZ \\
R(BRa) &= k_{on} BMP(RT_{BMP} - BRa) - (k_{off} + dBRa)BRa \\
R(AER) &= \rho_{AER} \left( \mathbb{1}_{AER} \left( \frac{WFZ}{WFZ + K_{WFZ,WFZ}} + \frac{BRa}{BRa + K_{BRa,WFZ}} \right) \right) \\
&\quad - d_{AER} \frac{K_{WFZ,WFZ}}{WFZ + K_{WFZ,WFZ}} \frac{K_{BRa,WFZ}}{BRa + K_{BRa,WFZ}} AER
\end{align*}
\]

with production terms

\[
\begin{align*}
p_{FGF10} &= \mathbb{1}_{\text{Mes}} + \frac{p_{FGF10}^2}{K_{FGF10,F10} + 1} \\
p_{FGF8} &= \mathbb{1}_{\text{AER}} \frac{WFZ^2}{WFZ^2 + K_{WFZ,WFZ}^2} \frac{BRa^2}{BRa^2 + K_{BRa,WFZ}^2} \frac{K_{WFZ,WFZ}^2}{K_{BRa,WFZ}^2 + K_{BRa,WFZ}^2} AER \\
p_{WNT} &= \frac{F10R^2}{F10R^2 + K_{F10R,F10}} (\mathbb{1}_{\text{Ect}} + AER \mathbb{1}_{\text{Ect}}) \\
p_{BMP} &= \left( \mathbb{1}_{\text{Mes}} \frac{p_{BMP}^2}{p_{BMP}^2 + K_{BMP,BMP}} + (1 - \mathbb{1}_{\text{Mes}}) \frac{WFZ^2}{WFZ^2 + K_{WFZ,WFT}} \right) \frac{K_{BMP,BMP}^2}{K_{BMP,BMP}^2 + K_{BMP,BMP}^2} AER
\end{align*}
\]

The indicator function \( \mathbb{1}_X \) denotes that the corresponding reaction only take place in domain \( X \), as the expression of the morphogens and receptors is restricted to different parts of the limb bud domain. Here, \( \text{lowMes} \) denotes the lower half of the Mesenchyme, \( \text{Mes} \) the Mesenchyme, \( \text{Ect} \) the Ectoderm, and \( \text{AER} \) the AER. To account for the inhibitory and activating effects on gene expression as displayed in the regulatory network (Fig 1b) the model uses Hill kinetics with maximum production rates \( \rho_c \) and Hill constants \( K_{xx} \).

2.2. Parameter Inference

To prepare for the inference of the parameter values, \( \theta \), we first generate in silico data with the parameter set, \( \theta_0 \), in Table 1. These result in the expression patterns in Fig. 1c. As starting values for parameter inference we then use diffusion constants (in \( \mu m^2 h^{-1} \)) in Table 1, as their physiological range is typically rather well known. For the other parameters we set all maximal production rate to 2, all Hill constants and maximum receptor capacities to 1, and all binding and degradation rates to 0.01. These initial parameter values result in the expressions patterns shown in Fig. 2.

Starting from those, we aim at finding a set of parameter values, with which we can reproduce the expression patterns in Fig. 1c.

Quantitative, spatial expression data is currently not available in the limb bud. Therefore, the model only needs to match the observed patterns qualitatively. To quantify the goodness-of-fit of the simulated expression patterns, we used a mathematical formulation for the constraints that describe the desired patterns. Thus, based on the image data we require \( p_{FGF10} \) to be present in the mesenchyme with a gradient, \( p_{FGF8} \) to be uniformly present in AER, \( p_{BMP} \) to be uniformly present in the ectoderm and AER as well as in the mesenchyme with a gradient and \( p_{WNT} \) to be uniformly present in the ectoderm. The absence of WNT and F8 production in the mesenchyme and (in case of F8) in the ectoderm is already hard-coded by the indicator-functions in the PDEs. As the production rates range from 0 to 1 we choose by eye 0.3 as a threshold for presence, and a penalty term enforces presence of substance \( c \) in domain \( X \), i.e.

\[ \int_X \max(0.3 - c, 0)^2 d\mu. \tag{4} \]

For uniform production, we added the penalty term

\[ \int_X (c - \max(c))^2 d\mu. \tag{5} \]
The constraint given by a gradient was approximated using a smoothed step function \( \varphi_c(x) \) in x-direction, i.e.

\[
\int_X (0.3 - c)^2 \varphi_c(x) + (c - 0.1)^2 (1 - \varphi_c(x)) \, du. \tag{6}
\]

Adding all these constraints provides us with a fitness function \( f(\theta) \) as a measure for the goodness-of-fit. For the in silico generated expression pattern (Table 1) its value is \( f(\theta_0) = 3852 \), whereas for the initial parameter values in our optimisation we obtain \( f(\theta_{\text{start}}) = 13989 \). Only the relative, but not the absolute value of \( f(\theta) \) matters.

A sensitivity analysis revealed that the expression patterns are sensitive to all parameters except for \( K_{\text{BRA, AER}} \) and \( K_{\text{BRA, AER1}} \). The parameters in the model are highly correlated and the vast dimension of the parameter space as well as the non-smooth fitness function render standard optimization methods such as Coordinate Search, gradient-based methods like SNOPT adjoint method, or random algorithms such as the Particle Swarm Optimization unable to improve the value of the fitness function and to arrive at a parameter set for which the model reproduces the patterns in Fig. 1c. We note that the repeated simulation of the PDEs is computationally very expensive.

### 2.3. A decision tree approach for the sequential inference of parameter values

To overcome the difficulties described above, we developed a decision tree that also uses prior knowledge and that optimises parameter values sequentially given that most parameter values affect the patterning process only at certain time intervals. To determine the sequence, in which parameters are identified, we start with the initial conditions and the constitutive production rates and check which production rates are controlled by those factors that are initially present, here FGF10 production in the lower part of the mesenchyme. All production rates except for the direct downstream targets are set to zero. For each of these steps only a subset of the original parameters has a direct influence. This subset is tuned with help of a decision tree that checks whether the currently considered components are present in the correct domains. If all important features are reproduced, the algorithm goes on to check the next component activated downstream; if not, the parameters which have direct influence on the considered feature are doubled or halved depending on the sign of their influence and then the procedure of the decision tree in the current step is repeated until the constraint is fulfilled or a maximum number of calls is reached. For the model considered here this approach results in the steps

1. F10 production → check F10, F10R
2. F10 and WNT production → check WNT, WZF in Ectoderm (Ecto)
3. F10, WNT, BMP, AER production → check BMP, BRA in Ecto, check AER, check WNT, WZF in Ecto
4. F10, WNT, BMP, AER, F8 production → check F8, F8R, check BMP in Mesenchyme, check F10 in Mesenchyme

The decision tree for the second step of the algorithm is illustrated in Fig. 3. Starting with the parameter values \( \theta_{\text{start}} \) in a set of parameters \( \theta_{\text{dec}} \), for which the model yields expression patterns (Fig. 2) close to the original patterns (Fig. 1c), and the fitness function \( f \) reduces from \( f(\theta_{\text{start}}) = 13989 \) to \( f(\theta_{\text{dec}}) = 5598 \).
Figure 3: The decision tree performed by the parameter inference algorithm in step 2.

2.4. Local optimization with SNOPT

The parameter values recovered from the decision tree approach are used as initial values to perform local optimization using the SNOPT algorithm implemented in COMSOL 4.4, a gradient-based algorithm that uses sequential quadratic programming (SQP) methods and which requires the least computation time for this problem among the COMSOL methods. To calculate the gradient, the adjoint method was used, as it is most efficient in case of many parameters. At this step of our approach we compare the optimised patterns (Fig. 5) to the in silico generated patterns (Fig. 1c) directly, i.e. as objective function \( J(\theta) \) we integrate the difference between the in silico generated expression values and the model output, while adding scaling factors \( \lambda_1, \lambda_2, \lambda_3, \lambda_4 \) as additional parameters as we aim at reproducing the patterns and no absolute values, i.e.

\[
J(\theta) = \int_{AER} (p_{FGF8} - \lambda_1\hat{p}_{FGF8})^2 \, d\mu + \int_{AER,Ect} (p_{WNT} - \lambda_2\hat{p}_{WNT})^2 \, d\mu + \int_{all} (p_{BMP} - \lambda_3\hat{p}_{BMP})^2 \, d\mu + \int_{all} (p_{FGF10} - \lambda_4\hat{p}_{FGF10})^2 \, d\mu
\]

This formulation allows us to evaluate the recovered parameter values with statistical measures, i.e. calculate the profile likelihood. To reduce computational costs we exclude the Hill constants, which are correlated in particular with expression and decay rates, from the optimization, and we reduce the accuracy of the simulation. Thereby, we can reduce the computation time from 24 hours to approx. 2 hours for one optimization. Within 100 iterations the algorithm halves the value of the objective function and recovers parameter values \( \theta_{opt} \) with a fitness function value \( f(\theta_{opt}) = 4526 \) (Fig. 5), reasonably close to the fitness function value of the target pattern, \( f(\theta_0) = 3852 \) (Fig. 1).

3. Methods

The model was solved with COMSOL Multiphysics using the Optimization Toolbox (version 4.4) and the MATLAB Livelink (version 4.3b) as described before [4, 5].

Figure 4: Expression patterns after the decision-tree based parameter inference.

Figure 5: Expression patterns after the optimization with SNOPT, using the parameters recovered by the decision tree algorithm as initial values.

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