Modeling the Shoot Apical Meristem in *A. thaliana*: Parameter Estimation for Spatial Pattern Formation

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Abstract. Understanding the self-regulatory mechanisms controlling the spatial and temporal structure of multicellular organisms represents one of the major challenges in molecular biology. In the context of plants, shoot apical meristems (SAMs), which are populations of dividing, undifferentiated cells that generate organs at the tips of stems and branches throughout the life of a plant, are of particular interest and currently studied intensively. Here, one key goal is to identify the genetic regulatory network organizing the structure of a SAM and generating the corresponding spatial gene expression patterns.

This paper addresses one step in the design of SAM models based on ordinary differential equations (ODEs): parameter estimation for spatial pattern formation. We assume that the topology of the genetic regulatory network is given, while the parameters of an ODE system need to be determined such that a particular stable pattern over the SAM cell population emerges. To this end, we propose an evolutionary algorithm-based approach and investigate different ways to improve the efficiency of the search process. Preliminary results are presented for the Brusselator, a well-known reaction-diffusion system.

1 Motivation

Ordinary differential equations (ODEs) represent a common approach to model genetic regulatory networks [1]. Such models are on the one hand used to quantitatively understand the interactions of multiple genes controlling specific cellular processes and on the other hand applied to make predictions about the cell behavior. One important and challenging problem in this context is the determination of the model parameters that lead to the desired temporal dynamics. For single cell networks, there has been a lot of work on parameter estimation using analytical as well as heuristic methods [13]; in particular, several studies make use of evolutionary algorithms to find suitable parameter settings [8,9,11].

This paper considers a slightly different problem where the focus is on multicellular systems, in particular the shoot apical meristems (SAMs) in the plant *Arabidopsis thaliana*. The main goal is to identify an ODE system that is capable of producing an (experimentally observed) spatial gene expression pattern

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across the cell population, assuming that gene products can cross cell borders via diffusion. Starting with a given set of gene interactions in terms of an ODE system, we address the problem of model parameter determination for such a spatial scenario. In comparison to previous studies on parameter estimation, there are several differences with respect to the scenario under investigation:

- Instead of a single cell, multiple interacting cells are considered which requires a prespecified spatial cell structure and a cell interaction model;
- Instead of achieving a particular temporal behavior, we are interested in obtaining a stable, i.e., non-oscillating system state in which a particular gene expression pattern emerges over the spatial cell structure;
- Instead of considering absolute gene product concentrations as target values, the gene expression patterns are rather defined qualitatively since quantitative measurements in space are scarcely available.

It is an open question of how to efficiently search for model parameters in such a scenario and how to formalize spatial patterns in terms of an objective function.

In the following, we present a preliminary study for this problem where a more general goal is taken as a basis: we do not assume a given target pattern, but aim at finding parameter settings that produce arbitrary, non-chaotic patterns. We first propose a general modeling framework which allows to simulate genetic regulatory networks within multicellular systems. Secondly, for a simple reaction-diffusion system with two genes that has been part of a previously published model for the shoot apical meristem by Jönsson et al. [6], we investigate the issue of parameter estimation. To this end, we introduce and apply an evolutionary approach based on the Covariance Matrix Adaption Evolution Strategy (CMA-ES) [3,4] and investigate different ways to improve the efficiency of the search.

2 Background

2.1 The Shoot Apical Meristem (SAM)

A shoot apical meristem (SAM) consists of multiple dividing, undifferentiated cells and is located at the tips of stems or branches of a plant. It is responsible for generating organs throughout the life of a plant and determines the number, type and position of the resulting lateral organs. A SAM has a particular internal organization that is preserved through its existence and its position at the tip of the stem or a branch remains fixed, although the plant is growing. Therefore, a fundamental question in meristem research is what this structure looks like and how it is maintained.

In various experimental studies, a number of genes and gene interactions have been identified that are involved in the organization of a SAM. At the heart of preserving the organization and functioning of a SAM is a negative feedback loop with two critical elements, the transcription factor gene WUS and the CLAVATA (CLV) genes, which encode components of a ligand/receptor complex.

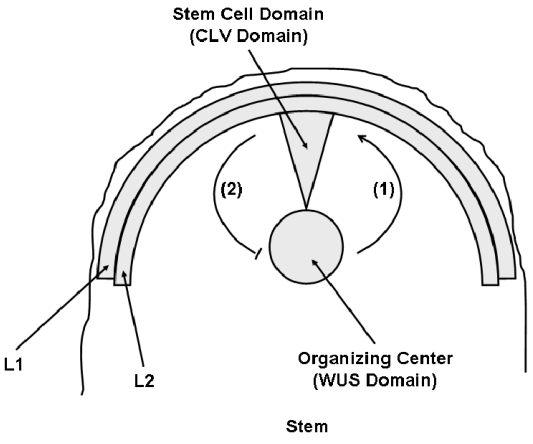

Fig. 1. A sketch of the SAM summarizing the known structural constituents and showing the WUS-CLV feedback loop. In the middle of the model, the organizing center is located. Directly on top of this domain the triangular shaped CLV stem cell domain begins and stretches up to the outermost cell layers L_1 and L_2. This setup remains stable throughout the life of the plant. During growth, cells from the CLV stem cell area move laterally, differentiate and thereby contribute to the plants growth. In regulating the maintenance of this spatial pattern the WUS-CLV feedback loop indicated by (1) and (2) plays a central role. Starting from the organizing center it promotes its own growth and the regeneration of the CLV domain (1) which lost cells due to differentiation. To prevent the system from over stimulating growth in CLV domain and organizing center, in turn CLV3 produced in the topmost layers of the CLV domain (L_1, L_2) gives negative feedback to the WUS organizing center (2). As a result of this interplay both, CLV domain and organizing center can maintain a stable size.

This negative-feedback loop elegantly corrects transient aberrations in stem-cell number. Besides these relatively well-characterized regulators, a range of other elements has been identified. In many cases their function in the meristem is unclear and, so far, there is no overall picture of the genetic regulatory networks in a SAM. Fig. 1 schematically summarizes the main constituents of a SAM that are currently known.

A current limitation in meristem research is the resolution of the measurements. Ideally, for each gene the gene product concentration within each cell of the meristem separately would be known, but it is obvious that such type of measurements are utopian for the near future. For this reason, data-driven modeling approaches where genetic regulatory networks are inferred from quantitative data are currently infeasible. Instead, a knowledge-driven approach is pursued where the topology of the network is determined by hand based on previous knowledge, and novel hypotheses are tested by slightly modifying the existing network and validating it with regard to phenotypic data.

2.2 Pattern Formation

One approach to study the regulation mechanisms enabling plants to maintain these spatial SAM patterns is to use reaction-diffusion systems, a well understood system to produce spatial patterns in general, dating back to work by Turing [12]. He investigated the influence of diffusion as a spatial component on systems described by coupled non-linear differential equations. In contrast to the predominant opinion, he found out that systems which converge to a homogeneous steady state without diffusion can be perturbed in such a way that they form either spatially stable patterns over time or temporally stable patterns in the spatial domain. Using similar systems many pattern forming dynamics in sea shells [7], development of animals like hydra [2] or drosophila [5] have been investigated.

In the context of SAM modeling, Jönsson et al. [6] employed reaction-diffusion systems to simulate the domain formation and maintenance in the SAM. In their work, the authors used a two dimensional model only considering the WUS-CLV feedback loop extended by an additional activator substance; the reported results in simulating phenotypic observations in SAM development and maintenance are promising. As to model parameter determination, their model consisted only of few constituents and therefore it was possible to tune the parameters by hand. Considering the fact that these systems are sensitive to either start conditions and parameters like coupling constants, degradation rates and production rates, it is likely that tuning more complex models by hand becomes intractable. Therefore we here present a method which, using a model similar to the one from Jönsson et al., (1) optimizes parameters of the system in such a way that spatial patterns are formed and (2) thereby can be used to explore the pattern formation capabilities of that given setup.

3 A SAM Modeling Framework

In the following, we present a modeling framework for multicellular systems in general and SAMs in particular that serves two goals: hypothesis testing and hypothesis exploration. On the one hand, it should be testable whether a given system of interacting factors can form certain spatial patterns by finding the necessary parameter settings and simulating the system. On the other hand, based on the parameter optimization, predictions on the possible patterns of novel interactions resulting from novel intracellular and intercellular interactions shall be made.

3.1 Model Structure

The model proposed here is defined by the following core components:

Cells: The model consists of spatially discrete units, the cells. They are used as autonomous units. We assume that all cells are similar to each other in design, in particular regarding the underlying genetic regulatory network, and only differ in their states.

Gene products: The state of a cell v_i is characterized by the concentrations of the gene products produced in the cell. The gene product concentrations are represented by a real valued vector. The term 'gene product' in this case not only refers the the product but has to be understood synonymous for gene products, gene expression levels and all processes on the way from gene to gene product. Since there exists a mapping between expression levels and the resulting amount of gene products, the gene product concentrations are representing the gene expression levels.

Cell structure: The cells are grouped according to a spatial neighborhood defining which cells share common cell surface areas. In this model only a two dimensional horizontal cut through the SAM is considered. We assume that the cells are hexagonal and the cell plane is arranged in rings around a central cell. A schematic picture of the plane is given in Fig. 2. Internally the cell neighborhood is represented by a graph $G(V, E)$ consisting of a set of cells V. Contacts or interaction pathways between the cells are represented by edges $e_{i,j} \in E$ between two cells v_i and v_j.

Cell communication: To form spatial heterogeneous patterns, spatial interactions, namely diffusion, between the constituting components are mandatory. In this model diffusive interactions are possible along the edges between the cells. Therefore implicitly zero flux boundary conditions are used on the boundaries of the cell plane.

The framework is implemented in Java and for the graph representation the JUNG library is used.

3.2 Model Dynamics

During the simulation process the states of the cells change according to (1) intracellular interaction between genes or gene products and (2) the intercellular diffusion. In a formal description the state change of a cell v_i follows a transition function $\delta(q_i, N(v_i))$ depending on the current state q_i of the cell and the states of its interaction partners given by the neighboring vertices $N(v_i)$. Each iteration in the simulation corresponds to calculating the transitions made for every cell based on the status quo.

The reaction equations describing the intracellular interactions can easily be transformed to ordinary differential equations, using the reaction rates from the reaction equations as parameters. The time course of ODE systems can be simulated by numerical integration. Since the intracellular interactions are already represented by ODEs, it is convenient to express the diffusion by ODEs as well. The used ODE approximation for diffusion is given in Eq. 1,

$$\frac{dx_{i,j}}{dt} = \sum_{k \in N(v_i)} D_j(x_{k,j} - x_{i,j}) \tag{1}$$

where $x_{i,j}$ is the concentration of gene product j in cell v_i, $N(v_i)$ encompasses all cells in contact with v_i, and D_j is the diffusion constant for the type of gene

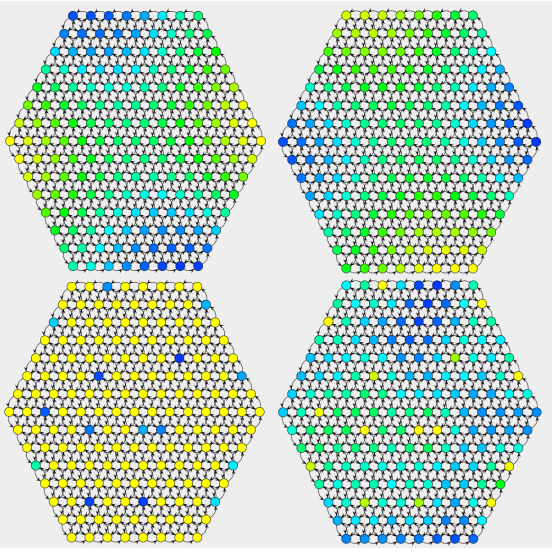

Fig. 2. Activator inhibitor patterns resulting from the Brusselator reaction-diffusion system for two different parameter sets on two dimensional cell planes with a hexagonal lattice. Each vertex represents a cell, each edge indicates an interaction pathway between cells. The cells are colored according to the concentrations of a single gene product. The activator patterns are shown in the left column and the corresponding inhibitor patterns in the right column. Gene product concentration levels are relative and range from low (light color) to high (dark color). The first row shows the patterns simulated using the parameters recorded by Jönsson et al. [6] and the second row shows patterns resulting from parameter optimization using our framework. The difference in size of the patches with high activator concentrations between both parameter sets stems from the difference in the activator diffusion constant D_A. For the optimized parameter set it is smaller and therefore the activator peaks are more local.

product. For our two dimensional meristem simulations, the system is integrated for 5000 steps using a fourth order Runge Kutta integrator with fixed step size $\Delta_t = 0.1$.

Additionally to reaction rates and diffusion constants, the starting conditions or initial gene product concentrations can be considered as a third group of parameters. Due to the non-linearity of the considered system, already slight changes in any of the parameter settings can result in drastic changes in the system behavior whilst the system can be highly robust with respect to other variations. To illustrate this fact, in Fig. 3 two simulation runs of a one dimensional reaction-diffusion system with slightly varying parameter settings are shown. This system, namely the Brusselator, was introduced in 1968 by Prigogine and Lefever [10] and ranges among the best studied reaction-diffusion systems. It is defined by the two equations:

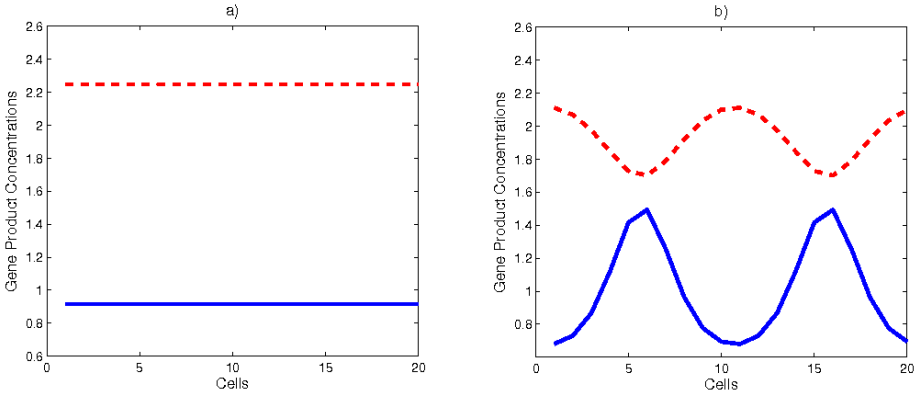

Fig. 3. Two simulations using the Brusselator activator (solid lines) inhihibitor (dashed lines) reaction-diffusion system with similar parameters but one. One diffusion constant (D_b) is changed from 0.69 (a) to 0.7 (b) which results in a state change of the system from spatial homogeneous to spatial heterogeneous waves for both gene products. The other parameter settings were: $a = 0.1$, $b = 0.2$, $\beta = 0.1$, $c = 0.1$, $D_a = 0.1$.

$$\frac{dA}{dt} = a - (b + \beta)A + cA^2B + D_a\nabla^2 A \tag{2}$$

$$\frac{dB}{dt} = bA - cA^2B + D_b\nabla^2 B \tag{3}$$

3.3 A Clavata-Wuschel Model

As mentioned in Section 2.1, the feedback loop between CLV produced in the L_1 layer and WUS produced in the organizing center is one of the key regulation mechanisms for maintaining a stable SAM. Jönsson et al. [6] simulated this feedback loop complemented by a an activator inhibitor reaction-diffusion system. They decided to use the Brusselator model explained in Sec. 3.2 for this task and following this suggestion we use the same system for this study. With help of this system, Jönsson and coworkers were able to reproduce similar pattern formation for the considered horizontal cut through the SAM when compared to the *in vivo* SAM either unperturbed or after laser ablation of the WUS producing organizing center (cf. Fig. 1). Since in our study we are only interested in pattern formation in general, we reduce their model to the Brusselator equations.

4 Model Parameter Estimation

This study is concerned with investigating ways to optimize parameters for the SAM model based on reaction-diffusion systems. The considered optimization problem can be summarized by the following design parameters:

- Search space $X \subseteq \mathbb{R}^n$, for the Brusselator $n = 6$,
- objective space $Z = \mathbb{R}$,
- objective function $f(x) : \mathbb{R}^n \to \mathbb{R}$ evaluating the resulting patterns for the given parameter set considering the two aspects (1) stability of the pattern over time and (2) significance of the heterogeneity of the resulting pattern.

In the following, we present two types of objective functions used during optimization with the Covariance Matrix Adaption Evolution Strategy (CMA-ES) developed by Hansen and Ostermeier in 1996 [3,4] – a state of the art stochastic optimization method already successfully applied to several real valued optimization problems. The first type of objective functions is designed to avoid using any domain knowledge. Therefore it represents a baseline approach for optimizing reaction-diffusion systems. Secondly, we consider a set of methods to incorporate domain knowledge into the objective function in order to improve the quality of the patterns found.

4.1 Baseline Approach

Method. For the baseline approach we used both spatial heterogeneous gene product concentration distribution and convergence of the gene product concentrations over time and aggregated them into a single objective $f(x)$ as follows:

$$f(x) = \sum_{i \in gp} \left(\max(\delta_t - \Delta_{s_i}, 0) + \Delta_{t_i}\right), \tag{4}$$

where gp are all gene products, Δ_{s_i} is the maximal difference in gene product i measured over all cells at the end of the simulation and δ_t is a threshold value which is used to decide if a given spatial heterogeneity is significant. For our simulations $\delta_t = 0.5$ was used. Δ_{t_i} is the largest change in gene product concentration i in the last integration step. The first term in the fitness function can be seen as a penalty term on parameter settings that fail to generate a stable pattern. In effect, the second term penalizes settings for which the simulation does not converge within the given number of integration steps.

Results. Using the described fitness function we made eleven optimization runs using the CMA-ES. Due to runtime constraint, one optimization run took up to 4 hours, for each variant only eleven runs were conducted. The used (4, 9)CMA-ES parameter values are given in Tab. 1 and the results are shown in Fig. 4.

The undertaken optimization runs failed to converge to an optimum within 1000 objective function evaluations. After investigating which parts of the parameter space had been explored during the optimization runs, it turned out that only 3 percent of the tested settings had relations between the activator diffusion constant D_a and the diffusion constant of the inhibitor D_b of $\frac{D_a}{d_b} \leq \frac{1}{7}$. Although it is known from literature that pattern formation using reaction-diffusion systems only takes place if for the relation of the diffusion constants $\frac{D_a}{d_b} \leq \frac{1}{7}$ holds, the idea behind this base approach was to avoid using domain knowledge and thereby testing the feasibility of our parameter optimization approach on general ODE systems.

Table 1. Parameter settings for CMA-ES

Parameter	Value
Initial ODE Parameters $[a, b, \beta, c, D_a, D_b]$	$[0.45, 0.45, 0.45, 0.45, 0.8, 0.8]$
Initial Standard deviation for the Parameters	$[0.25, 0.25, 0.25, 0.25, 0.7, 0.7]$
Maximal Number of Objective function Evaluations	1000

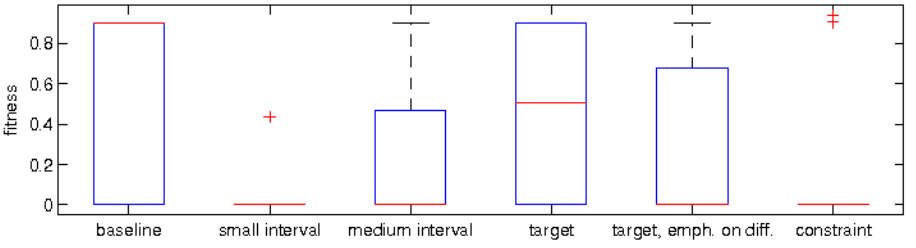

Fig. 4. The results of all conducted runs are shown in boxplots. 'baseline' refers to the first variant not incorporating any domain knowledge, 'interval small' and 'interval medium' both refer to the variant were the optimization process operated on a predefined search interval, 'target' refers to the variant where the domain knowledge was integrated by guiding the search to the vicinity of a known solution, 'target, emph. on diff.' denotes the runs using a target setting with an emphasize on the diffusion relation and finally 'constraint' refers to the variant where knowledge about the dependency of the diffusion constants was used.

4.2 Integration of Domain Knowledge

Method. Considering the difficulties in optimizing the parameters without domain knowledge, we decided to include domain knowledge into the optimization process. We tested three different approaches:

1. Restricting the initial search interval of the CMA to a smaller interval which is known or suspected to contain good parameter settings,
2. introducing a term pointing to a region that it is known to be good and thereby generating bias towards this region,
3. constraining the parameters considering known dependencies between parameters like the relation between diffusion constants.

The first approach is trying to increase the probability of identifying a good solution by simply regarding a smaller search space.

Since a study using a sampling grid on the diffusion constants and fixing all other parameters showed that the fitness landscape for the screened part of the parameter space consists of mainly two plateaus, a small sink containing the pattern forming settings and a large plateau of settings for which the system converges to a spatially homogeneous state (cf. Fig. 5), we introduced the latter

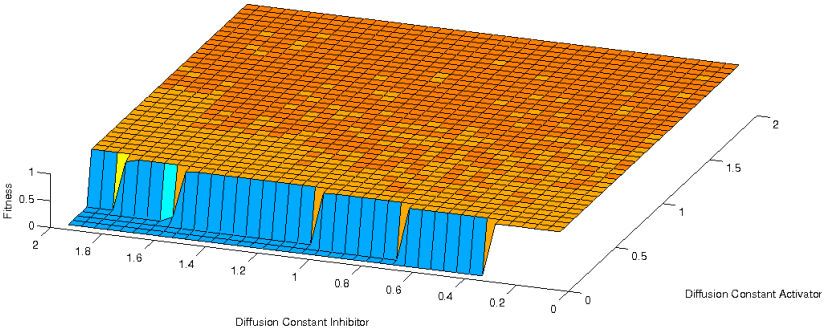

Fig. 5. Slice of the fitness landscape resulting from the objective function given in Eq. 4: For this slice only two of the six parameters of the Brusselator reaction-diffusion system (cp. Eq. 3) are considered, namely the diffusion constants D_A and D_B. They were sampled in the interval $[0.05, 1.95]$ using 0.05 steps while the other parameters are fixed. It can be seen that only for small activator diffusion constants there are pattern forming sets and therefore for most of the tested settings no pattern formation takes place. Further on it can be seen that the transition between pattern forming settings and non-pattern forming settings is ridge like.

two approaches. Both aiming at reshaping the fitness landscapes to become easier to optimize. By integrating new terms in the fitness function, higher plateaus are slightly inclined to point at pattern forming parameter regions. The second approach to this end uses a term penalizing distance to a known promising region. The resulting objective function reads as follows:

$$f(x) = \begin{cases} \sum_{i \in gp} (\max(\delta_t - \Delta_{s_i}, 0) + \Delta_{t_i}) + \|x - x_t\| & \text{if } \|x - x_t\| > \delta_d, \\ \sum_{i \in gp} (\max(\delta_t - \Delta_{s_i}, 0) + \Delta_{t_i}) & \text{else,} \end{cases} \tag{5}$$

where x_t is the target parameter vector and δ_d is a minimal length of the difference vector of x and x_t.

The third approach follows a more general idea: It exploits the knowledge about the necessary relation between the two diffusion constants D_A and D_B. Whenever the relation between both constants exceeds a threshold of 0.1, the actual relation is added to the function value. In effect, the search space is constraint and the resulting objective function reads as follows:

$$f(x) = \begin{cases} \sum_{i \in gp} (\max(\delta_t - \Delta_{s_i}, 0) + \Delta_{t_i}) + \frac{D_A}{D_B} & \text{if } \frac{D_A}{D_B} > 0.1, \\ \sum_{i \in gp} (\max(\delta_t - \Delta_{s_i}, 0) + \Delta_{t_i}) & \text{else.} \end{cases} \tag{6}$$

Results. For all mentioned approaches we did eleven optimizations runs each. Using the two different interval sizes around a parameter set found using the optimization framework ($a = 0.3, b = 0.05, \beta = 0.05, c = 0.25, D_A = 0.075, D_B = 1.525$) and σ settings for the corresponding search distribution in the CMA-ES

of $\sigma \in \{0.1, 0.3\}$, for the small σ reproducibly good solutions were found whereas already for the medium σ the results became significantly worse. Therefore the successes have to be contributed to the small size of the explored search space rather than to an effective optimization.

Using the parameter vector ($a = 0.1, b = 0.2, \beta = 0.1, c = 0.1, D_A = 0.1, D_B = 1.5$) as a target vector generating search direction towards a good region in parameter space (for the CMA-ES the parameter settings in Tab. 1 were used), the obtained results were reasonable but still the runs did not converge to a setting with an objective value below $1 * 10^{-14}$, the convergence threshold used by the CMA-ES. This can be attributed to the fact that by taking the euclidean distance between the parameter vector describing the desired parameter vector and the actual parameter vector, all parameters equally contribute to the distance between the two vectors. Since D_A is an important parameter that is measured in smaller scale than the other parameters, its contribution to the search direction is overpowered by the others and the generated signal is blurred. And in fact, emphasizing the diffusion relation improved the convergence.

Coping with this problem brings us to our last approach. Here a desired minimal relation of $\frac{D_A}{D_B} = 0.1$ is used as a constraint (for the CMA-ES the parameter settings in Tab. 1 were used). Compared to all other approaches, it was only outperformed by the approach searching in a small already known region. Schematic pictures of the resulting patterns are given in Fig. 2. When again looking at the number of evaluated settings having a suitable diffusion constant relation, it turned out that for this last setup more than 50 percent were sufficiently small. The results for all approaches are shown in Fig. 4.

5 Conclusions

In this paper, we have studied the problem of parameter estimation for ODE models of genetic regulatory networks in order to generate spatial gene expression patterns over a population of cells. We have tested variants from two types of objective functions, one abandoning all domain knowledge and three objective functions integrating domain knowledge in different ways.

Already for small systems like the considered Brusselator with six parameters, the first approach failed to identify suitable parameter settings. A naive variation of this method drastically restricting the search space to a region known to be promising in principle failed as well. Only for very small parts of the decision space it was possible to identify good solutions, indicating that no real optimization took place but mere sampling.

The last two variants produced promising results. Both have in common that the search process is guided towards a region of in principle good solutions. Following this direction both approaches succeeded in identifying good parameter sets. The two variants are (1) using a single point which is known to be good as an attractor for the search process and (2) using knowledge about dependencies between parameters to guide the search process, with variant (2) producing the better results and therefore beeing the method of choice for the given problem.

Additionally, it might be interesting to combine the used approaches and thereby further improve the method.

As key results of this study it can be concluded that on the one hand side optimization on the given problem domain without having additional domain knowledge seems to be intractable. If domain knowledge becomes available on the other hand there are strategies allowing to identify good solutions to the problem.

This study only represents preliminary work. The focus of our work is on dealing with more complex networks both when considering the number of involved species and the number of cells in the simulated system. Additionally it is planned to expand the model to three dimensional setups. For these systems we are not only interested in the mere pattern formation but in the formation of specific patterns visible in our real world target *Arabidopsis thaliana*.

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