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# Modeling the evolution of gene regulatory networks for spatial patterning in embryo development

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## Abstract

A central question in evolutionary biology concerns the transition between discrete numbers of units (e.g. vertebrate digits, arthropod segments). How do particular numbers of units, robust and characteristic for one species, evolve into another number for another species? Intermediate phases with a diversity of forms have long been theorized, but these leave little fossil or genomic data. We use evolutionary computations (EC) of a gene regulatory network (GRN) model to investigate how embryonic development is altered to create new forms. The trajectories are epochal and non-smooth, in accord with both the observed stability of species and the evolvability between forms.

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## 1. Introduction

Biological species can generally be distinguished by their unique physical shapes. These shapes are created through developmental processes, from the fertilized egg to the adult form. Development is guided by the unique genetic signature of a species, but the genetics must operate within physical and chemical constraints, such as tissue mechanical properties, reaction rate laws and molecular transport properties. In addition, organisms develop within an environment and must be robust to environmental perturbations, i.e. produce reliable outcomes despite variable conditions.

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Robustness, however, must be balanced against evolvability: extreme robustness could produce very stable species, for a time, until environmental changes caused a drop in the species' fitness, leading to extinction. By being continuously subjected to mutation, a species' genome maintains an evolvability which allows for adaptation to new or changing environmental conditions.

The developmental programme of a species is governed not only by the DNA sequences of its genome, but by the gene regulatory networks (GRNs) governing the dynamics of how, when and where genes are expressed in developing organisms to create specific cell types. These networks are created by highly interconnected gene-gene interactions, which are characterized by a great deal of feedback and nonlinearity.

Evolution from one form or species to another frequently involves mutations which alter the GRNs controlling particular stages or events in development. Understanding evolution, therefore, ultimately requires an understanding of the dynamics of GRNs: how they spatially and temporally control gene expression to create developmental tissues; how they are made robust to environmental perturbations; and how mutation can drive the development of new patterns of gene expression to create new forms and species.

A specific aspect of how forms evolve from one to another is how discrete numbers of structures change from one to another. For instance, it is common to see differences between species in numbers of structures such as vertebrate digits, body segments in arthropods, or wing spots in butterflies. What is the evolutionary process through which these numbers of units change? How is it that we observe discrete changes in unit number, despite small or incremental changes in the genetics? To address this question, both the response of the GRNs to environmental stress and the inherent variability in GRN dynamics must be considered. It is insufficient to think of the genome as an isolated encoding of traits or forms.

In pioneering work, Goldschmidt, Waddington and others began to consider how genetically similar offspring could respond slightly differently to environmental stress. One of the examples Waddington [1] studied was the bithorax-like response to environmental stress seen in fruit flies (*Drosophila*; Fig. 1A). Flies are normally dipteran (2-winged), but in response to environmental stress (e.g. ether treatment), some (not all) offspring develop repeated thoracic segments, producing 4 wings. Such an environmentally caused phenotype, mimicking changes typically seen under genetic mutation, is termed a phenocopy. Waddington [1,2,3] developed a theory that if such phenocopies conferred a selective advantage (had higher fitness) in the environment (especially in a changing environment), mutations in the genomes of their descendants might eventually lock these morphological changes in, i.e. become 'genetically assimilated'. From diverse starting points the new developmental process would come to produce the new trait with high reproducibility, or 'canalize'. Goldschmidt termed transitional forms 'hopeful monsters' (see [4]). It has been a matter of debate how reproductively viable such 'monsters' might be, but recent work is showing that genetic mutation can lead to rapid development of highly fit new forms and species (see review [5]).

Since these ideas were first developed, a huge amount of work has gone into understanding evolutionary processes in molecular terms. It is becoming increasingly well understood how the evolution from species to species depends on changes in GRN architecture and regulatory dynamics (which genes affect which other genes, and how they do so). A great deal of this work is comparative – studying the relations between genomes in related species. But a newer and complementary approach is to simulate the evolution of GRN models. This approach has the advantage of being able to explore the evolutionary dynamics in time; for instance, it allows for exploring the role of transitory forms which may not have a fossil record or a presently available genome.

In the present work we develop a framework for modelling GRN evolution, especially focusing on the transitions between discrete numbers of units. We develop the approach on the biologically well-characterized system, in terms of molecular and comparative biology, of anterior-posterior (AP) segmentation of the arthropod body; but the technique should be applicable to GRN evolution in general. Development of AP arthropod pattern depends on molecular signals both from the mother and from the embryo's own genome. In *Drosophila*, which we focus on, the primary maternal signal is of the Bicoid (Bcd) protein, which emanates from an anterior-high distribution of mRNA. Bcd is a transcriptional regulator, binding DNA in the nuclei of

the embryo, and activating embryonic segmentation genes (so called *gap* genes), which form broad domains of expression. Different gap genes are activated at different Bcd concentrations, spatially organizing the AP axis (Fig. 1B). Bcd is a classic example of a morphogen gradient in developmental biology [6], in which a concentration profile specifies positional information in the embryo (early development of this idea was due to Wolpert [7, 8]; retinoic acid is another classic morphogen, found widely in vertebrate development [9, 10]).

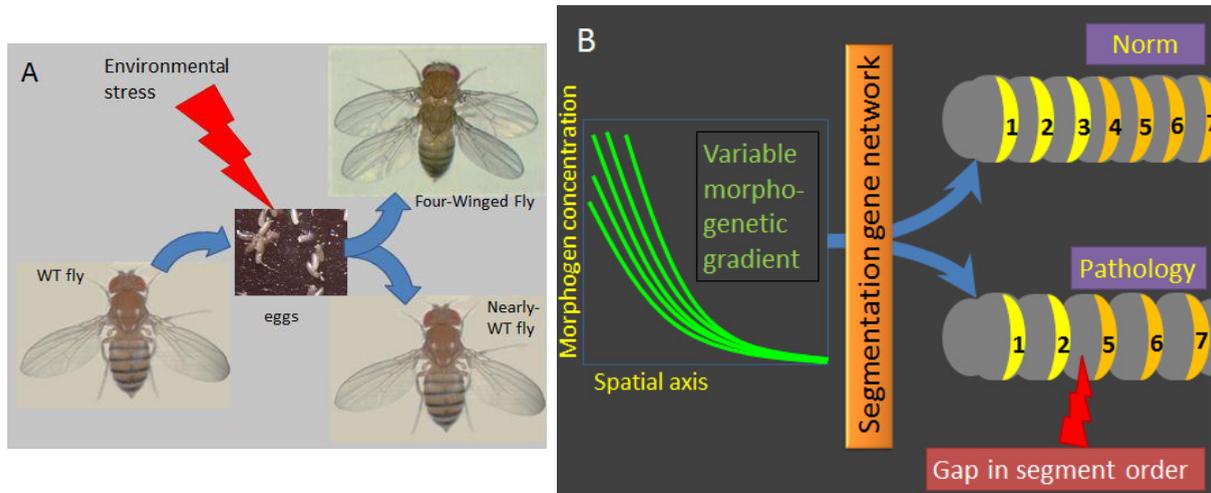


Fig. 1. (A) Environmental stress can cause sudden changes in the body plan of flies, and the response varies between flies, with some appearing unaffected (2 wings) and some undergoing discrete changes in number of structures (4 wings). (B) AP segmentation in *Drosophila*. Left (green), Bcd forms an anterior high gradient. Bcd is a transcriptional regulator which activates expression of embryonic segmentation genes (*gap* genes). Gap genes, in turn, control downstream gene expression, positioning the body segments. Bcd is highly variable, and normal development requires the gap GRN to form reliable pattern despite this variability. In pathological, non-robust cases (bottom) extreme Bcd gradients would cause non-normal gap patterns, affecting the number of segments. As shown (right), segment deletions could be due to natural maternal variability in the same genetic background (i.e. could be a ‘phenocopy’ rather than from a mutation).

A number of studies have focused on the robustness of the trunk *gap* genes (see Fig. 1B) to Bcd variability [11,12,13,14,15,16,17,18]. Within such a specific GRN, one can begin to ask some of the broader questions raised above: if the gap GRN is robust to Bcd variability, how has this allowed it to adapt over evolutionary time; and by what means do robust GRNs evolve?

Here, we focus on gap patterning in the head region. The diversity of arthropod head segments is rooted in the diversity of the number of gap gene expression domains in the head. How does a distinct, robust number of head segments in a particular species evolve into a different distinct, robust number of segments in a derived species? How is robustness affected during this evolutionary transition? Do multiple forms co-exist? Reductions in the number of head segments have occurred in arthropod evolution [19]. We are interested in simulating how these transitions might have occurred. The detailed data available for head gap gene expression in *Drosophila* makes it the best choice for developing a head GRN model; but once formulated, we use the model to study arthropod head evolution in general.

Instead of a general environmental stress, in this work we focus on the well quantified variability of the Bcd gradient. In this case, the different, intermediate forms would be in response to very high or very low Bcd gradients experienced by embryos (as in Fig. 1B). In dynamic terms, Bcd gradient level would control bifurcations in the GRN solutions, producing different head gap patterns. GRNs which can produce variable outcomes can be highly evolvable, by producing alternate forms which may have increased fitness to a

changing environment. As in Waddington's concept, mutation and selection can then modify the GRN to produce the new form without the environmental stress (or gradient variability). With simulations we can track robustness through such an evolutionary transition; in particular, does robustness go through a minimum in the transitional phase, illustrating the trade-off between evolvability and robustness?

Fig. 2 shows expression for 3 head gap genes that are key for development of the fly head [20,21,22,23,24]: *knirps* (*kni*); *cap' n' collar* (*cnc*) and *empty spiracles* (*ems*). These genes correspond to the 3 genes in our model (see Methods); all are transcriptionally regulated by Bcd.

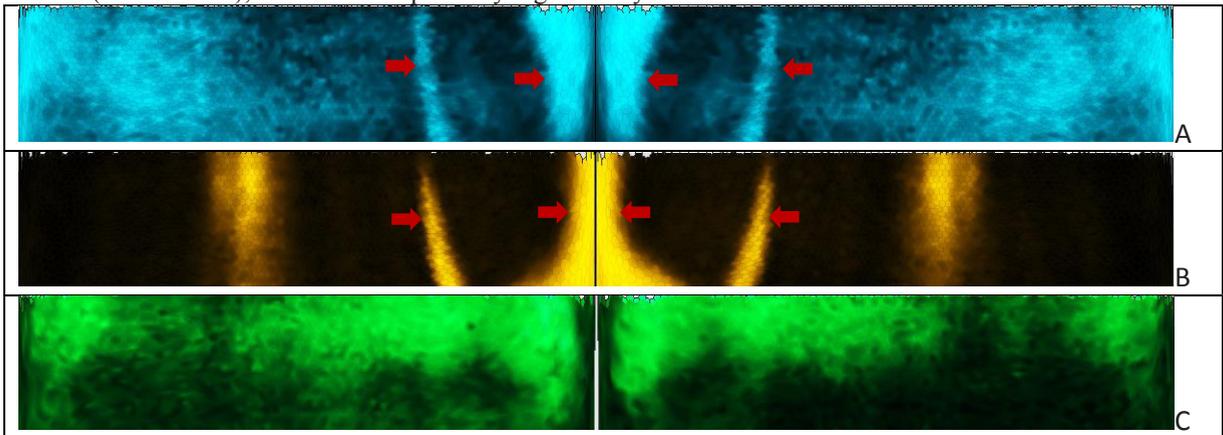


Fig. 2. Expression of three key genes involved in early patterning of the fruit fly head: *cap' n' collar* (*cnc*) (A); *knirps* (*kni*) (B); and *empty spiracles* (*ems*) (C). Red arrows highlight the expression domains (peaks) which we model. Data courtesy of the BDTNP (<http://bdtnp.lbl.gov/Fly-Net/bioimaging.jsp>). The images are made by the PointCloudXplore software (<http://bdtnp.lbl.gov/Fly-Net/bioimaging.jsp?w=pcx>). Colour intensity corresponds to the amount of gene product (RNA); the horizontal axis is centred at the anterior pole, extending right and left to the posterior of the embryo; the vertical axis is dorsal-ventral position.

In this work, we combine the dynamics of a maternal morphogen with those of three downstream genes into a reaction-diffusion (RD) model. This class of models has been studied since the time of Turing [25] for their ability to spontaneously form spatial patterns. Here, we use the RD system to model pattern formation, and then subject it to evolutionary computations to simulate biological evolution. We develop the model on the particular case of fly head segmentation, but the approach applies to the general question of how evolutionary transitions between discrete numbers of units occur.

RD pattern formation is a well-developed field in its own right, but in this project we use it within the context of the computer science field of evolutionary computations (EC). This fits within the emerging area of systems biology known as *evolution in silico* [26,27,28,29], in which genetic algorithms (and other EC techniques) are used for optimizing GRN models and simulating network evolution.

With simulations on this relatively small GRN, we are able to quantify network robustness (to maternal gradient variability) and to observe how robustness changes through evolutionary transitions which change the number of units. This gives insight into the network dynamics which produce both the robustness of particular species and the evolvability which allows for new forms and species.

## 2. Methods and Approaches

The RD model in this paper was originally developed by Shvartsman and co-authors [30,31,32] for development of respiratory appendages in the *Drosophila* egg; specifically, to understand how the number of appendages is limited to 2 in wild-type flies. RD models have long been studied for their ability to

spontaneously form spatial patterns for particular values of reaction parameters (e.g. controlling the rates of interaction between two or more patterning chemicals) and diffusion parameters [25]. Pattern formation in classical Turing systems depends on differential diffusivities between the patterning chemicals, or what has been termed the ‘diffusion-driven instability’. (The Muratov-Shvartsman model is capable of this type of pattern formation, though the original authors used it in a parameter regime which did not require the diffusive instability for pattern formation.) Here, we use the Muratov-Shvartsman mechanism to model head patterning, with an ‘M’ maternal gradient (e.g. Bcd) modulating pattern formation of three model variables A (e.g. kni), B (e.g. cnc), and C (e.g. ems):

$$\frac{\partial A}{\partial t} = D_A \frac{\partial^2 A}{\partial x^2} - k_A A + g_A C \quad (1)$$

$$\frac{\partial B}{\partial t} = D_B \frac{\partial^2 B}{\partial x^2} - k_B B + g_B \sigma \left\{ \frac{A - \alpha B + \beta M - \gamma_B}{\delta_B} \right\} \quad (2)$$

$$\frac{\partial C}{\partial t} = -k_C C + g_C \sigma \left\{ \frac{A - \alpha B + \beta M - \gamma_C}{\delta_C} \right\} \quad (3)$$

In this system of PDEs (partial differential equations) A, B, and C are proteins; coded by genes a, b, and c. The proteins are diffusible transcription factors, which would biologically affect the other protein concentrations by acting on the corresponding genes.  $x$  is the spatial coordinate, centered at the anterior pole of the embryo;  $t$  is time.  $\sigma\{X\}$  is a sigmoidal function  $\sigma\{X\} = X^2 / (X^2 + 1)$ , characterized by offset and steepness ( $\gamma$ 's and  $\delta$ 's, respectively). Transcription factors A, C and M act as activators, while B is a repressor.

The maternal gradient M is specified as a time-invariant spatial gradient, centred and maximal at the anterior pole. We represent M by a Gaussian function (see [30]):

$$M(x) = M_0^* \exp(-x^2/x_0^2) \quad (4)$$

To study the effect of M variability on GRN solution robustness, we vary M by

$$M_0^* = M_0 + \Delta M(k - 1) \quad (5)$$

where  $k$  is the number of M profiles used for each GRN ( $k=4$  in this paper) and  $\Delta M=0.02$ . This creates 4 M profiles, with maxima ranging from 0.55 to 0.61 arbitrary units (about 11% relative change; see Fig. 3A,B). This variability is sufficient to study robustness, but is lower than some observed variability levels [11,12,15,16,33]. Robust GRN solutions are those which produce the same pattern (specifically, number of peaks) over this range of M input gradients (Fig. 3D,F).

Following [30] we used a non-dimensionalized form of eqns. (1-3):

$$\tau_a \frac{\partial a}{\partial t} = \varepsilon^2 \frac{\partial^2 a}{\partial x^2} - a + c \quad (6)$$

$$\tau_b \frac{\partial b}{\partial t} = \frac{\partial^2 b}{\partial x^2} - b + \lambda \sigma \left\{ \frac{a - b + M - \Gamma_b}{\Phi_b} \right\} \quad (7)$$

$$\frac{\partial c}{\partial t} = -c + \sigma \left\{ \frac{a - b + M - \Gamma_c}{\Phi_c} \right\} \quad (8)$$

*Numerical integration of the RD model:* The PDE model (eqns. (6-8)) was integrated by spatial discretization into cells; in each of the 100 cells the continuous system becomes a set of finite difference equations, solved by the standard Euler method. RD models can be computationally expensive, with small time steps frequently needed to maintain stability. This can be challenging for evolutionary searches. We found that 50,000 time steps for each integration ( $\Delta t = 0.0005$ ) gave a good balance between calculation costs and achieving stable patterns.

*Implementation of the evolutionary search:* The model (6-8) has 10 parameters; the aim of the EC is to find particular parameter sets that produce the desired spatial pattern (the PDE system (6-8) solution) despite variability in the maternal gradient  $M$ . Examples of such patterns are shown in Fig. 3 D,F. Using a standard GA (Genetic Algorithms) approach, each trial parameter set was represented by a floating-point chromosome (array), with each floating-point element corresponding to a particular value of a given parameter in the model.

*Initial population:* The computational population was usually 400 species (parameter sets). Instead of beginning from a standard random collection of parameter sets, we started from parameter sets known from [31, 32, 32] to produce desired patterns. We then generated populations from these through small random increments or decrements to one (or more) of the parameter values.

*Mutations:* During the evolutionary searches, parameters were changed one at a time (simple GA mutation). Crossover, in which multiple elements of a chromosome are changed per generation, was not used. Mutations produced small inheritable adjustments of the RD-model parameters. In theoretical terms, the EC searches are through a 10-dimensional parameter space, which is likely to be multi-stable (see Shvartsman analysis). Most parameter changes would be expected to yield little change, but those which crossed boundaries between different stable states could produce large quantitative pattern changes. In this paper, we performed an evolutionary search on only two parameters,  $\lambda$  (eq. 7, controlling regulatory input to the ‘a’ species) and  $x_0$  (eq. 4, spatial scaling of the  $M$  gradient). The other 8 parameters were kept fixed.

*Evaluating the solutions:* A common strategy for reverse engineering gene networks is to evaluate model solutions by the sum of squared differences from the desired patterns (i.e. experimental data; [e.g. 34]). In the present work, however, we were interested in the number (count) of broad expression domains, and this is not properly captured by a difference summed from every position (cell). In other words, we did not want to evaluate the shifting of the expression domains: their count is important, but not their exact positions. In particular, our aim was to find profiles with two or four domains but with freedom to shift position (while keeping number constant). For this, we devised an evaluation based on pairs of steep slopes, i.e. ‘up-hill’ and ‘down-hill’. Moving along a concentration profile in the spatial dimension, a single domain (peak) will show a steep rise (minimum to maximum) followed by a steep drop (maximum to minimum). For two domains, there are two such pairs of up and down slopes. We devise an evaluation measure in which each such pair of up and down slopes adds a predetermined score value of  $N$  (=20 in this paper). In this work, we only use the profile of ‘C’ for the fitness evaluations. We also predefine a threshold domain height, ignoring domains which are lower than 40% of the global maximum.

To investigate the robustness (or non-robustness) of particular parameter sets of eqns. (6-8), we solve each parameter set with 4 different  $M$  gradients (shown in Fig. 3B). Each solution is scored, and a composite score is calculated as the sum of these individual scores. Robust parameter sets produce the same number of domains (peaks) for all four  $M$  gradients (while the exact heights and positions of the profiles can vary, see Fig. 3D,F). Non-robust cases might produce, for example, 4-domain solutions with the lowest  $M$  gradient, 2-domain solutions with the highest  $M$  gradient, and 3-domain solutions with intermediate  $M$  gradients (see Fig. 4).

*GRN evolution in silico:* As described above, parameter sets were encoded in floating-point chromosomes, and these were altered to form a varied initial population. Each parameter set in the population was solved numerically and scored according to the number of domains produced. An average score was then calculated for all the chromosomes. A portion (25%) of the chromosomes with worse-than-average scores were replaced by randomly-chosen chromosomes with better-than-average scores. Offspring underwent a standard mutation operation, changing one or more of the chromosome values. The complete generation cycle of PDE solution, scoring, replacement of below-average chromosome sets, and mutation was repeated until the score converged above a set threshold. This typically took 100 – 200 generations. The model was implemented in Delphi (Windows) and Free Pascal (Linux). The source code is available from the authors upon request.

### 3. Results and discussion

In this work, we focus on 4-domain to 2-domain evolutionary transitions. These numbers are applicable to head segmentation and other developmental phenomena, but our main aim is to present a framework for the evolutionary transition between any numbers of units.

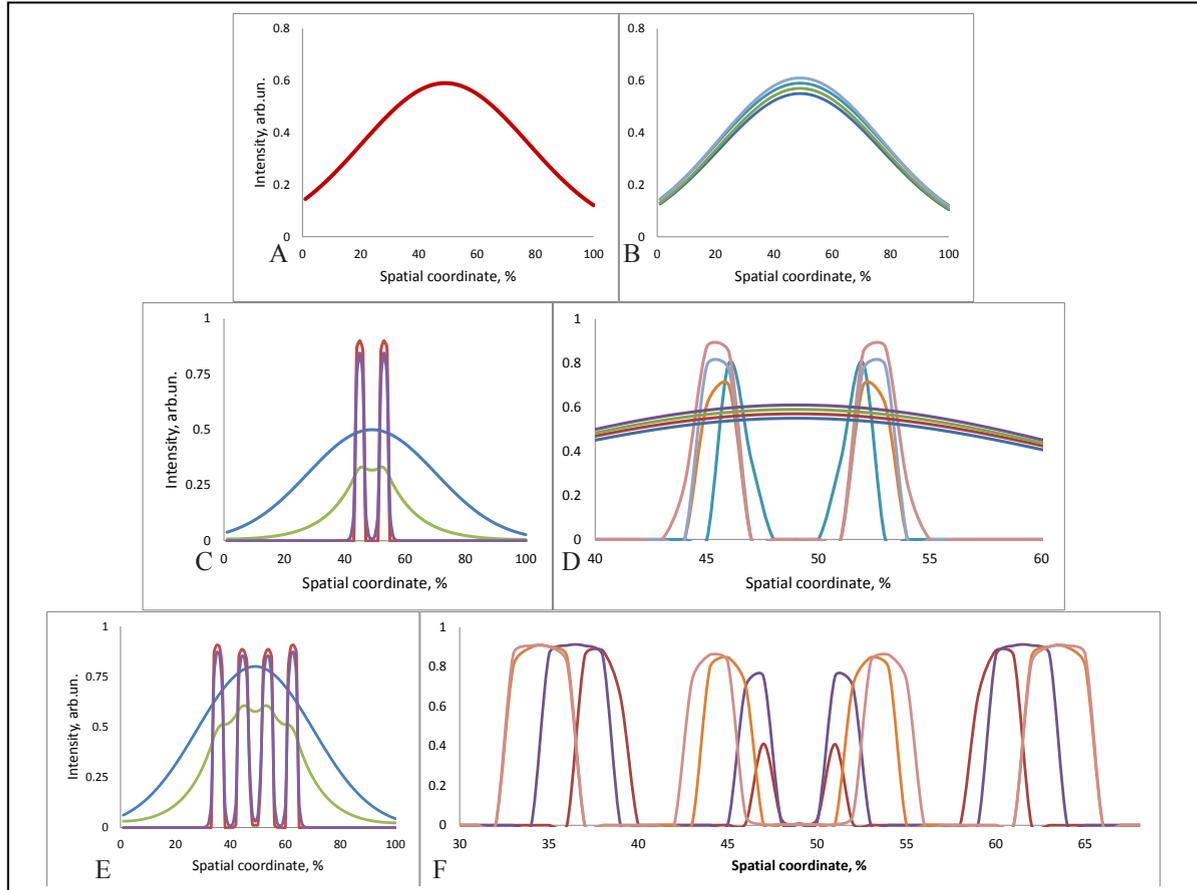


Fig. 3. Behavior of the reaction-diffusion system (eqns. 6-8). A, B) input M gradients: A) an ideal non-varying M gradient; B) the more realistic case of varying M input, representing the different maternal gradients that different embryos experience. C, E) solutions which are non-robust to changes in the M gradient: C) 2-domain solution with a low M gradient; E) 4-domain solution with a high M gradient. D, F) Solutions which are robust to M variability: D) stable 2-domain solutions, despite a range of M inputs; F) stable 4-domain solutions, despite a range of M inputs. M is specified by eqs. (4-5), with  $X_0=2.92$ ,  $M_0=0.8$ .

#### 3.1. Modeling evolution: from 4-domain to 2-domain patterns

**Initial population:** Starting from a 4-domain pattern robust to M variability (Fig. 3F; magnitude of M variability shown in Fig. 3B), we are interested in studying the evolutionary transition to 2-domain patterns.  $x_0$  and  $\lambda$  parameters were varied as described to create a population of individual parameter sets. Individuals were scored on the number of domains (peaks) produced (each domain adding 20 to the total score, see Methods). For instance, each 4-domain pattern costs 80, so summing over all 4 input M profiles, an initial robust 4-domain solution (such as Fig. 3F) produces a score of  $80+80+80+80=320$ . Since a 2-domain pattern evaluates

as 40, a robust 2-domain solution has a total score of  $40+40+40+40=160$  (see Fig. 3D). The aim of the evolutionary search is to find trajectories which take the initial score of 320 and reduces it to 160.

**Selection:** In successive generations, individuals continued to be mutated by small increments/decrements in the  $x_0$  and  $\lambda$  parameters. Mutant solutions which produced 2-domain patterns gained selective advantage by lowering their scores.

**Representative case of in silico evolution:** There is enough diversity in the initial population that there is frequently one (or more) individual with score lower than 320 (i.e. it already has at least one or two 3-domain profiles or even a 2-domain one). Early into an evolutionary experiment mutations start to create non-robust individuals, in which 4- 3- and 2-domain patterns (examples shown in Fig. 4) are produced by the range of input M profiles. Though they produce variable responses to the M gradients, such individuals (GRNs) are at a selective advantage due to their reduction of peak number. Continued evolution produces robust 2-domain individuals (in which 2-domain solutions are achieved for all 4 M gradients, Fig. 3D).

The simulations show the feature of evolutionary epochs (also reported in other studies with evolution in silico [e.g. 35]): long times pass with very slow (or even no) changes to the population, punctuated by bursts of new forms. For example, a first sudden jump can be seen when the evolutionary search finds the score of  $80+80+40+40$  (corresponding to two M profiles producing 4-domain solutions and two M profiles producing 2-domain solutions). Some time later, solutions appear with, for example, one 4-domain, one 3-domain and two 2-domain patterns. The next evolutionary jump finds solutions with scores of  $80+40+40+40$ , and so on. Epochs last from 50 to 100 generations and take from 15,000 to 50,000 new mutants to evaluate.

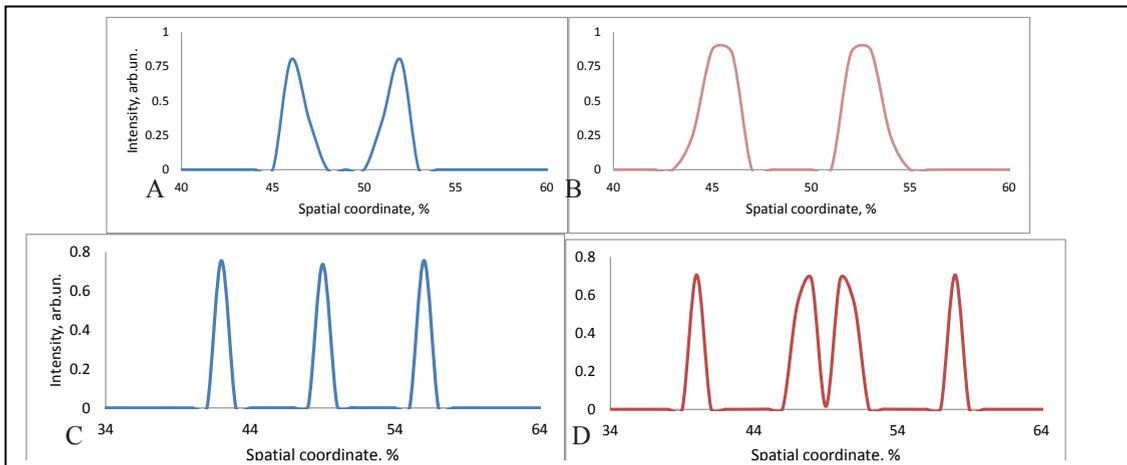


Fig. 4. Non-robustness of spatial patterns of the reaction-diffusion model (6-8) to variability of the external input (profile of factor M; Fig. 3B). (A - D) the diversity of patterns induced by the M profile variability.

With a cost associated with domain number, our computational experiments on the RD model can produce rapid evolution which changes discrete units of the body plan. We are specifically interested in embryo segmentation, but the model could easily be applied to other cases of arthropod (generally) and insect (particularly) morphogenesis.

Interestingly, simulations of the reverse evolutionary process, from 2- to 4-domain solutions (optimizing fitness to a score of 320), look and behave very much like the forward 4-to-2 transition. Overall, the computations can explain not only a decrease in the number of pattern elements through evolution, but also their increase.

These simple dynamic evolutionary models capture some of the crucial features expected by evolutionary biologists for developmental mechanisms [e.g. 36]. In particular, these computational experiments show transitional and highly variable patterns which connect old and new robust patterning processes.

#### 4. Conclusions

Using evolutionary computations on a model for spatial pattern formation, we have been able to follow the dynamics of transitions between discrete numbers of units. Though the model has been developed for the evolution of head segmentation in flies, we address the general problem of how a developmental GRN which robustly produces a particular number of units in a particular species evolves to create a new form or species with a different number of units. We have focused on a fundamental type of developmental robustness: the ability of a pattern forming GRN to produce the same patterns despite variable inputs from signaling gradients (in our case, maternal positional information gradients, such as Bcd). Our simulations show that GRNs robust to a particular number of peaks (e.g. 4) can evolve to new number-of-unit solutions if fitness conditions change (e.g. 2 domains become more fit than 4; which could arise from a change in environment). Our simulations eventually become robust to variable gradient input with the new fitness criteria, but we were quite intrigued that the, for example, 4→2 transition occurred via a collection of intermediate forms – a by-definition non-robust state. However, in terms of the earlier ideas of Waddington and others, the ability of a GRN to produce this selection of ‘hopeful monsters’ is key to achieving the new robust form. Early concerns about the ‘hopeful monster’ idea were that these individuals would be too different in fitness from their ancestors to survive. Our simulations indicate that a diversity of forms can be a natural part of optimizing GRNs to new fitness criteria to achieve a new robust form. We also find an ‘epochal’ structure in the evolutionary course of the transition, characterized by discrete ‘jumps’ in fitness. Such jumps would be expected for the sudden appearance/disappearance of forms or species. Our model, therefore, presents a mechanism for a ‘punctuated equilibrium’ style of evolution [37], with discrete jumps between species forms rather than a gradualist approach.

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