International Journal of Computational Intelligence in Control, 7(1), 2015

PROBING STOCHASTIC PHENOTYPE SWITCHING AS A SURVIVAL STRATEGY IN FLUCTUATING ENVIRONMENTS

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ABSTRACT: We investigate the fitness of stochastic mechanisms of cellular phenotypic determination in fluctuating bistable environments. We model cell populations at the single cell level and compare the fitness of stochastic versus deterministic choice of phenotype, for varying rates of fluctuation of the environment and biases in the choice of environmental state. We find that only when the stochastic choice of phenotype is biased by information on the state of the environment is the stochasticity selectively advantageous. Next, we model mutations that affect the rate of stochastic phenotypic switching and subject the cells to selection. The evolved phenotypic switching frequency is found to depend on the environment conditions. The results give insights on the conditions under which stochastic mechanisms to face environmental fluctuations are advantageous.

Keywords: Gene Expression; Stochasticity; Phenotype Distribution; Adaptability

1. INTRODUCTION

The phenotypic diversity of a cell population emerges from the genotypic diversity of the cells, e.g. due to mutations, from the stochasticity of the cellular functioning, and from the stochasticity in the interactions between cells and the environment [13,14]. Physiologic noise is present in most biological processes such as gene expression [19], interactions between proteins, between cells, and between organisms and the environment [5]. These interactions affect even the most complex phenotypic traits [2], and evidence suggests that some phenotypes are only expressed in certain conditions [15].

B. subtilis has probabilistic and transient differentiation, dependent on the environment [15]. Noise in ComK expression can cause cells to become competent for uptaking DNA, each choice being independent of previous events. Reduction of this noise decreases the number of competent cells [7] suggesting that noise-driven mechanisms can evolve [15]. Reversible differentiation between two phenotypes was also observed in λCI8B7 [8]. This transition is both spontaneous and affected by the environment (temperature), and is an example of a transmissible ability of physiological change without genetic change.

Stochasticity in gene expression is known to be useful in generating phenotypic diversity in microbial populations, improving their adaptability to sudden environmental changes [3, 17]. Evidence exists that bacteria possess mechanisms that allow transient increases in heterogeneity under stress conditions, e.g. by enhancing the fluctuations in the levels of some proteins [3].

One common strategy of bacteria for coping with unpredictable environmental conditions appears to be based on genetic circuits that have two or more stable regimes. Since several environmental conditions can be considered "bistable", for example, temperature can be within or outside normal ranges, a toxic can be present or absent, nutrients can be abundant or scarce, etc, it is no surprise that many of such genetic circuits are bistable [18]. Bistability is sufficient to trigger a specific defense strategy (e.g. halting cell division), depending on whether the environmental conditions require it.

These bistable circuits, in some cases, allow stochastic switching between two phenotypes, giving rise to bimodal phenotypic distributions [7, 13], as in the case of *Mycobacterium tuberculosis*, where persistence is determined by a bistable genetic circuit based on a positive feedback mechanism [16].

Studies of stochastic models of gene regulatory networks (GRN) have shown that they possess "noisy attractors", i.e., confined regions of the state space where the system tends to remain once reaching it [11]. These models also have "ergodic sets", i.e., sets of noisy attractors from which, once reached, the system does leave due to internal fluctuations, unless externally perturbed [11].

We investigate the fitness value of inherent phenotypic variability and of the ability to switch between phenotypes to cope with environmental changes. For that, we model cells with one ergodic set with two noisy attractors. Noise in protein levels can induce transitions between the attractors, each assumed to express a phenotype better fit to one of the two possible environmental states. Environmental state transitions are also modeled as being stochastic. The cells are given the ability to mutate the rate constants controlling state transitions between the noisy attractors.

We address the following questions: is there any advantage in switching between phenotypes in a stochastic bistable environment without any information on the environment state? Provided that information, how does the bias in being in one environmental state and the frequency of switching between environmental states affect the advantage of stochastic versus deterministic phenotype determination? Finally, we study the evolvability of this mechanism of phenotypic determination in a biased bistable environment to determine how this genetic system adapts to environmental biases.

2. MODELS AND SIMULATION

We aim to model cells, each with a genetic circuit with two noisy attractors [11]. Each noisy attractor corresponds to a specific phenotype. Noise in gene expression causes probabilistic transitions between the two attractors. One example of a delayed stochastic model of a bistable genetic circuit can be found in [9]. Here, we aim to model only the bistability and its transient and probabilistic nature, rather than the specific circuit that exhibits this dynamics. Due to that, our model regulating in which noisy attractor a cell is in is a simplified version of that in [9]. Namely, the genes, RNA and proteins are not explicitly modeled. Instead, only the phenotypic states and the mechanisms of phenotypic switching are modeled explicitly.

The environment is also bistable, for simplicity, and aims at modeling, as described before, environmental conditions that are well approximated by two states. Environmental state transitions are intended to be (and thus modeled as) stochastic events. Our model of bistable environment is similar to the one recently proposed in [10]. Finally, in one of the models, cells possess a mechanism that detects the environment state and biases the switching between the noisy attractors so as to increase the probability of switching to, and then remain in, the appropriate noisy attractor. This mechanism is also modeled as proposed in [10], and based on the studies by Kussel and Leibler [6].

In general, the models here proposed aim solely to capture the stochastic nature of phenotype switching in the cells, the stochastic nature of switching between two environment states, and the stochastic nature of cellular mechanisms of sensing the environment state. Since we aim to simulate events that are inherently probabilistic in nature, the dynamics of the simulations follows the Stochastic Simulation algorithm (SSA) [4] and is implemented in SGNSim [12]. From the biological point of view, this aims at mimicking the stochastic nature of phenotype determination, and the detection and response to environmental changes, found to occur in bacteria [6, 17].

Each cell is independent of the others, interacting only with the environment. In each simulation, multiple generations of cells are simulated, each of which with N cells. In each generation, the fitness of each cell is measured.

The best 50% are duplicated and their daughters simulated in the next generation. The others are eliminated.

In model 1, we simulate two distinct cell types (reactions 1 to 8). Generation 1 (G1) consists of N identical cells, each with equal chances of being of type 1 or 2. Daughters inherit the type from the mother. Cells of type 2 cannot switch between its two possible states. The phenotypic variability of its population is created at G1 alone. Cells of type 1 can stochastically switch between its two phenotypes at any moment of their life. In model 1, none of the cell types are able to regulate its internal state as a function of environment state.

$$W \xrightarrow{init} W_A, W \xrightarrow{init} W_B \quad (1)$$

$$W_A \xrightarrow{CW_A} W_B,\ W_B \xrightarrow{CW_B} W_A \quad (2)$$

$$\mathrm{S_{init}} \xrightarrow{init} Si_a,\ \mathrm{S_{init}} \xrightarrow{init} Si_b \quad (3)$$

$$\mathrm{Si_a} \xleftrightarrow{flip(i)} Si_b \quad (4)$$

$$*\mathrm{Si_a} + *\mathrm{W_A} \xrightarrow{r} fit_i \quad (5)$$

$$*\mathrm{Si_b} + *\mathrm{W_B} \xrightarrow{r} fit_i \quad (6)$$

$$*\mathrm{Si_b} + *\mathrm{W_A} + fit_i(\min:1) \xrightarrow{p} \varnothing \quad (7)$$

$$*\mathrm{Si_a} + *\mathrm{W_B} + fit_i(\min:1) \xrightarrow{p} \varnothing \quad (8)$$

Reactions (1) initialize the environment state (which can be either $\mathrm{W_A}$ or $\mathrm{W_B}$). Reactions (2) allow environmental state transitions, i.e., switching between $\mathrm{W_A}$ and $\mathrm{W_B}$. Reactions (3) initialize the state of the cells of G1. Index *i* indicates the cell type (1 or 2). Reactions (4) allow cells to switch their phenotype between $\mathrm{S_a}$ and $\mathrm{S_b}$ (a fixed phenotype is attained by setting these reactions rate constants to zero).

The fitness of a cell equals its number of $\mathrm{fit_i}$ units at the end of its simulation, computed by reactions (5) to (8). Reactions (5) and (6) allow gaining fitness units, when cell and environment states are both either "A" or "B". If environment and cell are in opposite states, fitness is lost via reactions (7) or (8). The number of fit units does not affect the penalization for being in an "incorrect" state, since the propensity of reactions 7 and 8 does not depend on the number of $\mathrm{fit_i}$. The *X notation means that the substance X is not consumed in the reaction. The X(min:1) notation means that if X is absent the reaction does not occur, and if present (regardless of the amount) the reaction occurs but X does not contribute to the calculation of the propensity.

When a cell divides, all substances are duplicated and equally shared by the daughter cells, including $\mathrm{fit_i}$. The weak dependence of a cell's fitness on the initial conditions is meant to mimic the fact that fitter mother cells ought to provide a small initial advantage to their daughters. The cell lifetime is set to sufficiently long so that the initial state is not critical to the survival of the cell, which depends mostly on the ability to cope with the environment during its lifetime.

In model 2, the state transitions of the cells, when occurring, depend on the environment state. Besides reactions (1-3) and (5-8), we add reactions (9-13) Signaling molecules, $\mathrm{sig_i}$, are generated via (9), and model cell sensors informing about the environment state. These molecules decay via reactions (10). Signaling molecules carrying information contrary to the environment state (due to a change in the environment state posterior to their creation) are further degraded by (11). Reactions (12) and (13) allow the signals to regulate state transitions (replacing reaction (4) in model 1):

$$*\mathrm{W_A} \xrightarrow{sig} sig_A,\ *\mathrm{W_B} \xrightarrow{sig} sig_B \quad (9)$$

$$sig_A \xrightarrow{dsig} \varnothing,\ sig_B \xrightarrow{dsig} \varnothing \quad (10)$$

$$*W_B + sig_A \xrightarrow{D} \varnothing\ *W_A + sig_B \xrightarrow{D} \varnothing \quad (11)$$

$$\mathrm{Si_a} + *sig_b \xrightarrow{flip(i)ab} Si_b \quad (12)$$

$$\mathrm{Si_b} + *sig_a \xrightarrow{flip(i)ba} Si_a \quad (13)$$

In model 3, the cell can regulate its state transitions as in model 2. A mutation mechanism affecting the value of the rate constants of reactions (12) and (13) is introduced in cell type 1. As the fitter cells are chosen, these rates ought to acquire local optimum values. To mutate the rate constants at run-time we

introduce "virtual" substances ($Ad_{w,z}$), two for each rate constant such that: if $Ad_{ab,up}$ quantity increases, $flip_{ab}$ increases linearly. If $Ad_{ab,down}$ quantity increases, $flip_{ab}$ decreases linearly. Creation (15) and decay (14) reactions of these substances are introduced, both independent of the substance quantities at any moment in time. Let $Ad_{w,z}$ be such that w is either ab or ba, and z is either up or down.

$$*Ad_{w,z} \xrightarrow{k_{down}} \varnothing \quad (14)$$

$$\xrightarrow{k_{up}} Ad_{w,z} \quad (15)$$

Finally, in model 3, the propensity P of (12) and (13) are calculated according to:

$$P^{4a} = \frac{flip(i)_{ab}.[Si_a].[sig_b].[Ad(ab,up)]}{[Ad(ab,down)]} \quad (16)$$

$$P^{4b} = \frac{flip(i)_{ba}.[Si_b].[sig_a].[Ad(ba,up)]}{[Ad(ba,down)]} \quad (17)$$

As the quantities of the virtual substances change, via reactions (14) and (15), so will the propensity of reactions (12) and (13). As the fitter cells are selected at each generation, one can observe which values of $\frac{flip(i)_{ab}.[Ad(ab,up)]}{[Ad(ab,down)]}$ and $\frac{flip(i)_{ba}.[Ad(ba,up)]}{[Ad(ba,down)]}$ locally optimize the fitness in a given environment.

We note that we do not focus on the underlying GRN required to express the phenotypes. Thus, no evolution is possible on how well a phenotype is adapted to the environment. Only the probability of the choice of each phenotype changes with the mutations.

3. RESULTS

We simulate 1000 cells per generation. Cell lifetime is 1000 seconds. Unless stated otherwise, the rate constants (units in s-1) are: init = 10^9, $CW_a = CW_b = 0.01$, $flip_{ab}(1) = flip_{ba}(1) = 0.01$, $flip_{ab}(2) = flip_{ba}(2) = 0$, r = 1, p = 0.1. The rate constant init is set to a large number for technical reasons. Optimally, it would be infinite so that the initialization of the state of the environment would be instantaneous.

The values of the rates CW_a and CW_b determine the frequency of switches between environmental states from A to B, and B to A, respectively. We set these rates so that this frequency is of the same order of magnitude of the frequency of phenotypic switching in the cells where this is possible, determined by $flip_{ab}(1)$ and $flip_{ba}(1)$. This has been found to maximize the fitness under such conditions [1]. If one would, e.g., multiply these four rates constants by the same amount, qualitatively there would be no changes in comparison to the results and conclusions presented here. The value chosen (0.01) was set empirically, taking in account the cell's lifetime. On average, this value allows 10 state transitions per cell lifetime. This was found to be sufficient so that most cells of the population would be subject to at least one switching in the environment during its lifetime (thus allowing comparison between the fitness of the various cell models).

Finally, the rates r and p determine, respectively, how fast a cell gains fitness when in a phenotypic state adapted to the environment, and how fast it loses fitness otherwise, respectively. Again, multiplying these two rates by a constant will not alter the results qualitatively. However, as explained below, the same is not true concerning the ratio between these two variables.

We first compare the fitness of types 1 and 2, using model 1, where cells have no information on the environment. Type 2 (fixed phenotype) almost always wins. In biased environments (e.g., 75% of the time as W_A), it is even more likely that type 2 wins. Thus, we conclude that in this case, the ability to stochastically change phenotype is not advantageous without knowing the environmental state as previously found in [6]. While both cell types have, on average, 50% of its cells adapted to the environment state at any time, any small disadvantage (such as due to stochastic fluctuations of the phenotype in cell type 1) is sufficient to unbalance the unstable equilibrium, since one is simulating finite populations for finite times. This result depends on how fast a cell gains fitness when in the "appropriate" state, and how fast it loses fitness

otherwise. If these two processes are identical in speed as in our model, random switching is not advantageous (at the population level) since during the time that the transitions need to be completed once initiated there is no increase (or, at best, slower increase) in fitness.

Fig. 1A shows a time series of model 2 in an unbiased bistable environment. The stochastic phenotypic transitions are advantageous since they follow the environment switches.

We now analyze the dynamics in biased environments. To model a biased environment we set CW_a and CW_b to different values. The ratio between them determines the expected time in each state. Fig. 1B shows the outcomes in various biased environments. Cell type 2 (fixed state) wins for highly biased environments since in these the environment is in one state most of the time, thus, the cells that randomly picked the correct state at the beginning have an advantageous over any cells that have a non-null probability of switching state at any moment.

The rate at which the environment state changes also affects the outcome. We simulated model 2, for varying values of $CW_a = CW_b$ (Fig. 1C). The value of CW_a above which cell type 1 wins is 0.001, i.e., on average the environment only changes state once per cell lifetime. Below this value, on average, a cell will face the same environment during its entire lifetime, making unnecessary for survival the ability to switch phenotype. Therefore, a cell with fixed phenotype, initially well adapted, will most likely remain well adapted throughout its life.

Using model 3, where cells have mutations that alter the values of the rate constants of the reactions responsible for phenotypic state transitions (12) and (13), we observe the evolutionary pathway of cells of type 1 when facing a biased environment. We compare two cell populations (simulated separately). In one there are mutations (adaptive), while in the other there are not (non-adaptive). In cells of G1, reactions (12) and (13) have the same propensity.

We set $CW_b = 5\ CW_a$ so that, on average, the environment is in state A 85% of the time. Interestingly, in the population of cells able to mutate, the variance of the fitness of the cells decreases over time (Fig. 2A), while their mean fitness increases as the propensity to go from state S_b to S_a becomes, by selection, much higher than the opposite, allowing the cells to remain in S_a most of the time (Fig. 2B).

Fig. 2C shows the average fraction of time the cells able and unable to mutate spent in state S_a. Interestingly, in the cells able to mutate, this quantity stabilizes at ~95%, while the environment state is W_a only ~85% of the time. The solution adopted by these cells was to, in general, ignore the transient environment changes. However, a small degree of stochasticity in phenotypic determination is maintained, rather than being completely nullified.

4. CONCLUSIONS

We investigated selective advantages of stochastic phenotypic determination in fluctuating environments. While using a simplistic model, where the genetic circuit determining the phenotype is not explicitly modeled, the results agree with experimental observations. Namely, probabilistic phenotypic determination provides selective advantages if the transitions between the phenotypes is biased by environmental conditions [6]. This is the case in *B. subtilis* differentiation [15] (a memoryless process), or lactose usage in *E. coli* [14] (where transitions depend on previous states).

In the absence of a sensing mechanism that provides the cell information about the environment state, we found the stochastic selection of phenotype to be disadvantageous. Moreover, when the environmental switching rate is very slow or one of the states of the environment is strongly preferred then the stochastic switching of phenotypes is also disadvantageous. Only for near-unbiased environmental switches (highly unpredictable) and provided a mechanism for sensing the environment state was the stochastic switching found to be a good strategy. Finally, we found that, in highly biased worlds, the cells able to switch phenotype and to mutate evolved

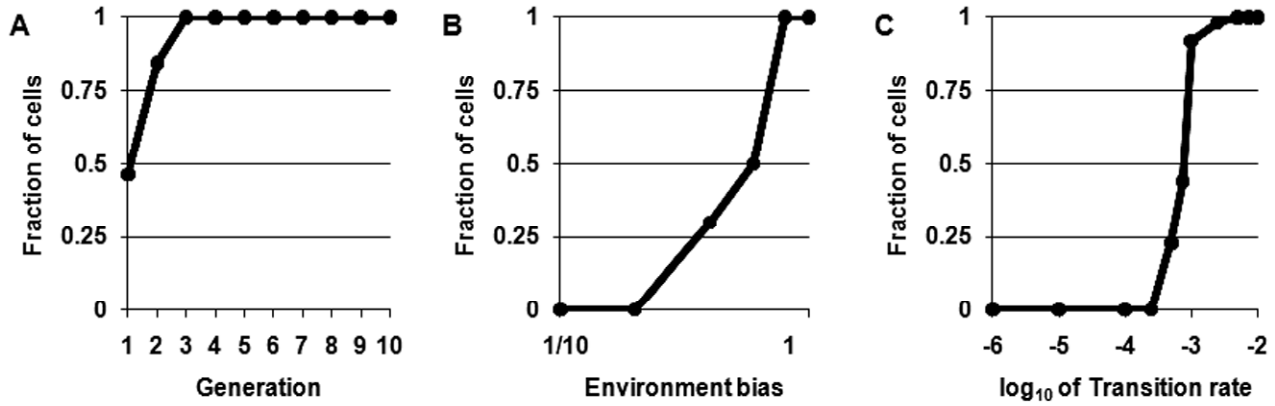

Figure 1: (A) Fraction of cells of type 1 in each generation in one simulation (model 2). (B) Fraction of wins for cells of type 1 for various environment state biases (W_a/W_b); 100 simulations per data point. (C) Fraction of wins for cells of type 1 for various state transition rates ($CW_a = CW_b$); 100 simulations per data point

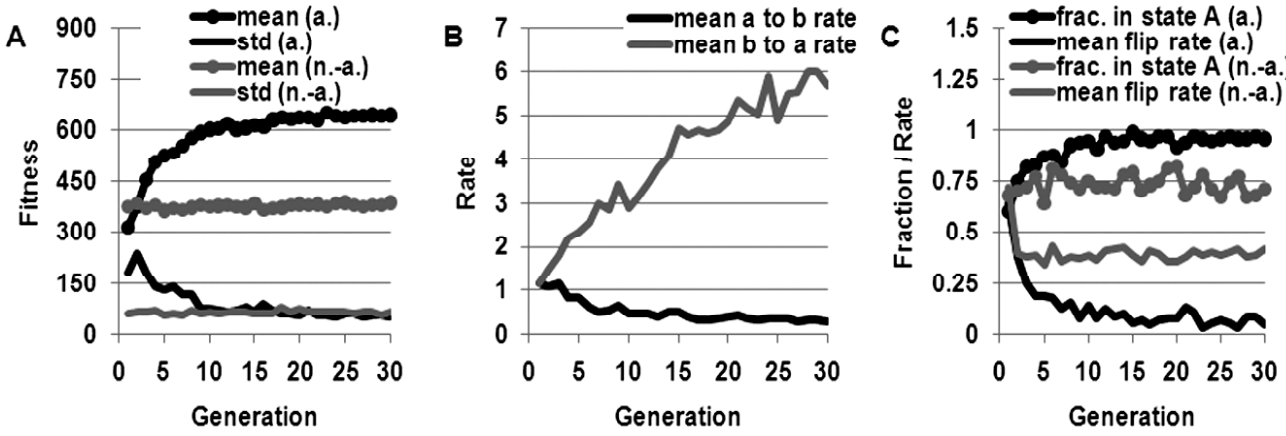

Figure 2: (A) Mean and standard deviation of fitness across the generations in populations of cells able (black) and unable to mutate (gray). (B) Evolution the values of the cell switching parameters (Si_a and Si_b) due to mutations and selection. (C) Evolution of the fraction of time cells spend in state A and of the mean switching frequency of cells able (black) and unable to mutate (gray)

towards becoming almost non-reactive to transient environmental changes.

Finally, we note that the improvement of the mean fitness of the cells due to selection is not trivial in this model, in that the optimal rates of phenotype switching of the cells do not equal the switching rate of the environment, due to a multitude of factors, including the speed at which the signals cause the cells to change state. Nevertheless, the results show that adaptation is possible by mutation. Importantly, we verified that stochasticity in the phenotype determination provides robustness to environmental changes in highly unpredictable environments.

Acknowledgements

We thank the Academy of Finland and Tekes for financial support.

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