Dynamics and evolution of stochastic bistable gene networks with sensing in fluctuating environments

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We study how cells can optimize fitness in variable environments by tuning the internal fluctuations of protein expression of a bistable genetic switch. We model cells as bistable toggle switches whose dynamics are governed by a delayed stochastic simulation algorithm. Each state of the toggle switch makes the cell more fit in one of two environmental conditions. Different noise levels in protein expression yield different fitness values for cells in an environment that randomly switches between the two conditions. We compare the behavior of two cell types, one that can sense the environmental condition and one that cannot. In fast changing environments both cell types evolve to be as noisy as possible while maintaining bistability of the toggle switch. In slowly changing environments, evolved nonsensing cells are less noisy while sensing cells evolve the same noise level as in fast changing environments. Sensing removes the need of genotypic changes to adapt to changes in the environment fluctuation rate, providing an evolutionary advantage in unpredictable environments.

PACS number(s): 87.16.Yc, 05.45.−a, 87.23.Kg, 87.18.Tt

I. INTRODUCTION

Gene expression is inevitably noisy but the noise level can be tuned and, thus, be subject to selection [1–5]. If evolvable, the evolutionary pathway of the noise of a gene's expression is bound to depend on the processes its proteins are involved in. Rather than minimizing or maximizing the noise level, gene networks might evolve specific noise levels adapted to their tasks and thereby, in many cases, to environmental conditions.

Studies suggest that the noise in the expression level of critical genes ought to be minimized since it is detrimental to organismal fitness. Essential genes of yeast and genes involved in circadian oscillators were found to have expression levels with relatively low noise [5,6].

However, noise in gene expression can be beneficial by creating phenotypic diversity in isogenic populations [7], a selective advantage in unpredictable environmental conditions [8]. Importantly, noise-driven genetic mechanisms can evolve [2]. *Bacillus subtilis* has transient and probabilistic differentiation. The noise in the expression level of ComK, a transcription factor that activates the expression of a set of genes necessary for competence, influences the number of cells that uptake DNA [9].

Recently, a *Saccharomyces cerevisiae* strain was engineered that switches between two phenotypes due to noise in gene expression [10]. Each phenotype is more fit to one of two environments. Comparing two populations with different switching rates, fast switchers outgrew slow ones in rapidly changing environments, while the opposite occurs in rarely changing environments, suggesting that it is advantageous for cells to tune noise-driven interphenotype switching rates to the environment switch rate [10]. The cells did not have mechanisms to sense the environment state. When these ex-

ist, they should play a key role in survival in fluctuating environments [8].

We investigate how cells can use noise in gene expression and sensing mechanisms of environmental conditions to adapt to noisy two-state environments. We model individual cells, each with a toggle switch (TS), whose dynamics is driven by a delayed stochastic simulation algorithm [11], in a randomly switching two-state environment. Each state of the TS is more fit to one of the two environment states. The environment effects are modeled by subjecting cells to toxins correspondent to the environment state. Each protein of the TS inhibits one of the toxins, mimicking the function of the gene responsible for resistance to tetracycline in *Escherichia coli* K-12 [12].

For comparison and model validation, we study the dynamics and evolution of two types of cells, differing in how the phenotypic switching is regulated. While both cell types have internal noise-driven switching, as in [10], only one of them can bias the switches by sensing the environment ("sensing cells"), i.e., the TS state of these cells is affected by the toxins' amounts, while in the other cell type it is not ("nonsensing cells").

Simulating the cells dynamics in rapidly and slowly switching two-state environments, we address the following questions: can cells increase fitness by tuning the noise level of the TS? How does the capacity to sense and act upon the environment state affect the cells' dynamics and evolutionary pathway? What advantages sensing mechanisms provide that justify its maintenance, even though there are significant energetic costs in doing so?

II. MODEL

Cell populations are simulated at the single cell level. The cell dynamics are driven by a delayed stochastic simulation algorithm (SSA) [11,13] based on the original SSA [14], and

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implemented in Stochastic Genetic Networks Simulator (SGNSIM) [15].
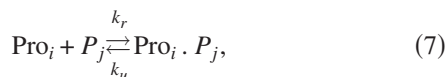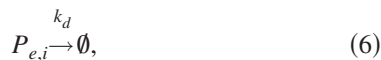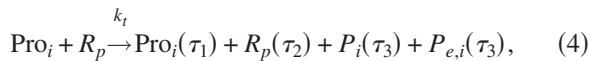
The model of gene expression [17] accounts for stochastic fluctuations and, being a multiple-time delayed reaction, for the fact that transcription and translation are multistep processes that take non-negligible time to complete once initiated. The model was validated [13] by matching measurements of gene expression at the single molecule level [18]. Time delayed reactions are represented as $A \rightarrow B + C(\tau)$. In this reaction, B is instantaneously produced and C is placed on a waitlist until it is released, after τ seconds [11].

We assume an unbiased two-state environment, with states 1 and 2, defined by the amounts of W_1 and W_2. At any moment during a simulation one of these two substances is present (in the amount of one molecule) while the other is absent. Each environment state subjects cells to a specific toxin (X_1 or X_2). Environment state transitions occur via reactions (1). The average switching frequency between the two states is W_{fr}. Toxins are introduced via reaction (2) and decay via reaction (3). c_x controls the expected amount of toxin in a cell. The symbol " *" in Eq. (2) indicates that the reactant is not consumed in the reaction. Finally, all chemical reactions apply to all $i = 1, 2$ (when only the index i is present), or to all $i, j = 1, 2$ with $i \neq j$ when both indices are present),

$$W_i \overset{W_{fr}^{-1}}{\underset{W_{fr}^{-1}}{\rightleftarrows}} W_j, \tag{1}$$

$$^*W_i \overset{c_x}{\rightarrow} X_i, \tag{2}$$

$$X_i \overset{d_x}{\rightarrow} \emptyset. \tag{3}$$

Another way to describe the environment dynamics is that X_i is produced (stochastically) at the rate c_x if the environment is in state W_i.

Gene expression is modeled by time-delayed reactions [17] where Pro_i is the promoter of gene i, and R_p is an RNA polymerase (4). The delays account for the time duration of the processes involved in transcription [19] and translation. Each promoter controls the expression of two proteins, P_i and $P_{e,i}$. P_i represses the other gene of the TS, while $P_{e,i}$ degrades a toxin X_i (9), as in [12]. Reactions (7) model the binding and unbinding of the repressor protein to the other gene's promoter, defining the TS. Proteins decay via reactions (5), (6), and (8),

$$\text{Pro}_i + R_p \overset{k_t}{\rightarrow} \text{Pro}_i(\tau_1) + R_p(\tau_2) + P_i(\tau_3) + P_{e,i}(\tau_3), \tag{4}$$

$$P_i \overset{k_d}{\rightarrow} \emptyset, \tag{5}$$

$$P_{e,i} \overset{k_d}{\rightarrow} \emptyset, \tag{6}$$

$$\text{Pro}_i + P_j \overset{k_r}{\underset{k_u}{\rightleftarrows}} \text{Pro}_i . P_j, \tag{7}$$

$$\text{Pro}_i . P_j \overset{k_d}{\rightarrow} \text{Pro}_i, \tag{8}$$

$$P_{e_i} + X_i \overset{k_{dx}}{\rightarrow} \emptyset. \tag{9}$$

As a side note, having two independent promoters, instead of one, controlling the expression of P_i and $P_{e,i}$, as long as the two promoters were equally affected by the repressor proteins [reaction (7)] and given the values set here for k_t and k_r, it would not cause significant changes in the dynamics since the repressor proteins exist in sufficient amount to repress both promoters simultaneously.

The cell's fitness is measured throughout their life. Each TS state is more fit to one of two environment states and both TS states have identical energetic costs, since they express an equal number of proteins and both proteins are assumed to have equal production costs. It is assumed that cells aim to simultaneously decrease the number of toxins and proteins since both are harmful (protein overproduction wastes resources [4]). The smaller these four quantities are the better for a cell. Combining these conditions, fitness is stochastically measured by 10. Since no substance is consumed and the product is not a substrate to any reaction, it does not affect the cell dynamics.
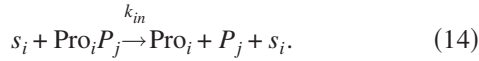
The propensity, P_r, i.e., the probability that the reaction will occur in the next infinitesimal time interval [14], determines the expected amount of fitness units (*fit*) produced and is computed by Eq. (11), in agreement with the assumptions of being "fit," but ensuring that P_r is never infinite. Namely, the propensity is, at each moment, inversely proportional to the number of molecules of X_1, X_2, P_{e_1}, and P_{e_2},

$$^*X_1 + {}^*X_2 + {}^*P_{e_1} + {}^*P_{e_2} \overset{c_{fit}}{\rightarrow} fit, \tag{10}$$

$$P_r(10) = c_{fit} \cdot \frac{1}{[X_1] + 1} \frac{1}{[X_2] + 1} \frac{1}{[P_{e_1}] + 1} \frac{1}{[P_{e_2}] + 1}. \tag{11}$$

For simplicity, we assume that the two toxins have identical toxicity. Thus, the two phenotypes are symmetric in fitness. For example, expressing P_{e_1} with X_1 present or expressing P_{e_2} with X_2 present is equally fit. When the environment state switches, the fitness associated to being in each phenotypic state changes accordingly, as the quantities of P_{e_i} and X_i change.

One cell type modeled has a sensing mechanism of the environment state that bias the TS toggles. Signaling molecules, s_i, are produced via Eq. (12) when X_i is present, and decay via Eq. (13). Reaction (14) models their effect on the TS. s_i unrepresses Pro_i, which expresses $P_{e,i}$, which degrades X_i. Thus, these cells bias the TS's toggles with the amount of toxin present, while maintaining the stochastic nature of the toggling. In the absence of toxins, unbiased toggles occur due to stochastic fluctuations of the proteins levels. In the presence of toxins, the state transitions of the TS of sensing cells are biased by the amounts of signaling molecules present, so that the TS is more likely to be in the state more fit to the environment,

$$* X_i \xrightarrow{k_{sig}} s_i, \tag{12}$$

$$s_i \xrightarrow{d_{sig}} \emptyset, \tag{13}$$

$$s_i + \mathrm{Pro}_i P_j \xrightarrow{k_{in}} \mathrm{Pro}_i + P_j + s_i. \tag{14}$$

It is noted that the toxins do not affect the amount of proteins P_i in the cell, which regulate the TS state. In non-sensing cells, the TS dynamics is independent of the toxins. In sensing cells, the toxins can indirectly affect which protein is produced, via the signaling molecules that can unrepress the promoters, but do not affect the mean level of the protein P_i being expressed, which is determined by k_t, k_d, and τ_1.

Rate constants (in s^{-1}) and time delays (in s) are set following the TS model in [13], taken from measurements in $E.$ $coli$, $\tau_1 = 1$, $\tau_2 = 20$, $\tau_3 = 100$, $k_t = 0.05$, and $k_d = 0.005$. These two rate constants values are varied to attain TS's with distinct levels of noise while maintaining the average proteins' quantities over time.

In each cell, the number of RNA polymerases (R_p) is 100, each Pro_i is 1 and other substances are initially absent. Other rate constants are $c_x = 1$, $d_x = 0.01$, $k_{dx} = 1$, $k_r = 0.1$, $k_u = 0.005$, $c_{fit} = 1$, $k_{sig} = 0.01$, $d_{sig} = 0.001$, and $k_{in} = 0.1$. These were set so that on average the number of toxins is ~ 100 and, if gene i is "on," $P_i \sim 150$ (when the TS is bistable, otherwise $P_1 + P_2 \sim 150$). The value of c_{fit} is arbitrary, not affecting the results qualitatively.

To simulate evolution, mutations are introduced in the model. In [20] it was shown that the variability of mRNA levels can be altered by mutations. When simulating mutations, we assume that these only affect k_t (transcription initiation) and k_d (protein decay), causing either its decrease or increase (equally likely) which changes the noise intensity in the proteins' levels. To this end, we set at 10% the probability that, at the beginning of a cell's lifetime, these rates are simultaneously multiplied or divided by a factor of 2 (depending on which mutation occurred). A mutation causes the increase (or decrease) of both k_t and k_d by a factor of 2. This change in the rate constants does not change significantly the proteins' mean levels, affecting mostly the noise level.

For example, increasing both k_t and k_d by a factor of 4 decreases the proteins' mean levels by $\sim 1\%$. Given the limited number of generations simulated, the maximum change in the mean level of proteins due to the accumulation of mutations was below $\sim 10\%$ in all cases where mutations were allowed. Such variations are not significant dynamically since the mutated cells of both cell types always produced sufficient proteins to cope with the toxins if the TS is in the appropriate state. More importantly, these changes in mean levels are, given identical mutations, identical in sensing and nonsensing cells. That is, the change caused by, e.g., an increase by a factor of 2 of k_t and k_d is identical in sensing and nonsensing cells, thus not introducing any bias favoring either cell type.

Selection occurs at the end of each generation, eliminating the 50% least fit cells while the others divide into two daughter cells, which inherit the (possibly mutated) values of

k_t and k_d of the mother cell, as well as all substances quantities (except fit units which are set to null) and all other rate constants values.

It is noted that in [10] an energetic cost is associated with phenotypic transitions, that is likely to limit the cells' transition rate. This is not the case here since the average number of proteins produced is not altered by switching phenotypic state, and it is assumed that both proteins have equal production costs. Also, we assume that each cell's dynamics is independent of the others, which would be false if the cells, e.g., competed for nutrients in each state or cooperated by sharing proteins or its byproducts, that inactivate the toxins. Finally, altering the fitness calculation formula, one could address other scenarios, not explored here, such as assuming one toxin more toxic than the other.

In the end of the next section, a cost in sensing is introduced so that sensing and nonsensing cells are equally fit for one environmental fluctuation rate, and afterwards compete in an environment with another fluctuation rate.

III. RESULTS

A. Noise and fitness for sensing vs nonsensing cell populations (fast environmental switching)

We first simulate nine cell populations per cell type (sensing and nonsensing), each with a unique set of values of k_t and k_d. Each population consists of 1000 cells independently simulated. A cell's lifetime is 500 000 s. This unrealistically long lifetime allows better statistics without affecting the results qualitatively (alternatively one could follow the dynamics of cell lines for many generations). A cell's state is measured every 1000 s. The first 10 000 seconds of a simulation are disregarded since cells are initialized without proteins.

The nine pairs of values, obtained by multiplying the original values by factors, all impose equal mean values of $(P_1 + P_2)$, ensuring that differences in noise level (and thereby in toggling rate and fitness) are not due to differences in mean values of $(P_1 + P_2)$. To obtain the values of k_t and k_d for the cells of each population we multiplied the original values ($k_t = 0.05$ and $k_d = 0.005$) for populations 1 to 9, respectively, by (0.01, 0.05775), (0.1, 0.4), (0.25, 0.65), (0.5, 0.85), (1, 1), (2, 1.1), (5, 1.175), (10, 1.205), and (100, 1.245).

These two rates cannot be varied by constant amounts each time to attain equal mean proteins' expression levels due to the delay τ_1, at each transcription event, that accounts for the promoter occupancy time by an RNA polymerase [19]. Its existence means that, e.g., constant increases in k_d require each time bigger increases in k_t up to a limit, beyond which it cannot be further compensated due to reaching the maximum transcription rate (1 transcription event per τ_1 seconds).

The environment state, initially randomly set, changes with an average switching frequency of W_{fr}^{-1}. We set $W_{fr} = 10\,000$ s (fast switching case).

As k_t and k_d increase, the TS dynamics changes. The TS's of nonsensing cells of populations 1 to 5 are bistable (Fig. 1), but of population 6 and beyond are not (Fig. 2). In these, the value of k_t is such that when the promoter is free it is more
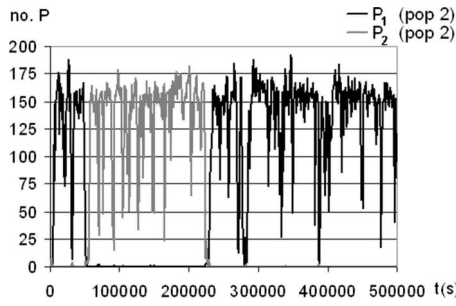
FIG. 1. Time series of P_1 and P_2 ($\langle P_1+P_2 \rangle \sim 150$) of a cell of population 2 (unable to sense the environment state).

likely for an RNA polymerase to bind to it than it is for a repressor protein to bind (if $[R_p] k_t > k_r$). Thus, the two mutually repressing genes of the TS can express simultaneously, destroying bistability. Nevertheless, the average value of (P_1+P_2) is identical in all populations (~ 150). In sensing cells this loss of bistability also occurs, but only beyond population 7.

Figure 3 shows the average noise level of the proteins' time series (P_1+P_2) of cells of each population (with and without sensing). Noise is measured by the standard deviation over the mean of (P_1+P_2) time series [3].

The noise level of nonsensing cells is independent of the environment switch rate since the proteins P_i do not interact with the toxin and, the proteins that do interact with the toxins, $P_{e,i}$, do not interact with any promoter or other proteins. Thus, the noise level in nonsensing cells (Fig. 3) is identical for any environment state switching frequency. The noise level of sensing cells depends on environmental fluctuations since the signals can induce toggles in the TS when the environment changes state. If the TS state is fit to the environment state, this interaction reduces the cells' noise level. Note that these cells' noise level, if no interaction with the environment existed, would equal the noise level of nonsensing cells with equal k_t and k_d. Due to this dependency on the environment, the effects of genotypic changes in sensing cells are easier to interpret by comparing fitness and noise levels with those of nonsensing cells.

Initially, the noise increases as k_t and k_d increase since the number of toggles of the TS increase. Beyond population 5 (for nonsensing cells), noise decreases as cells lose bistability and both genes express simultaneously, since transitions between the two noisy attractors [21] of the TS contribute
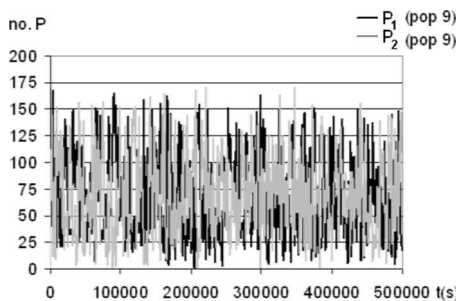

FIG. 2. Time series of P_1 and P_2 ($\langle P_1+P_2 \rangle \sim 150$) of a cell of population 9 (unable to sense the environment state).
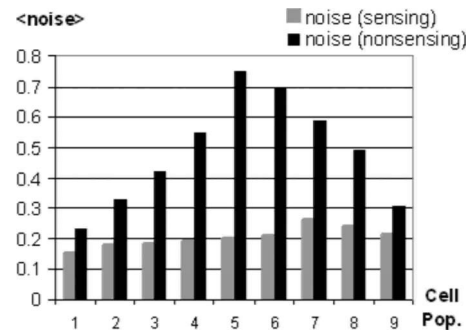

FIG. 3. Noise in the time series of P_1+P_2 of individual cells, averaged over 1000 cells per cell population. Cell populations of the two cell types (sensing and nonsensing cells) differ in the values of k_t and k_d. $W_{fr}=10\,000$ s.

more to noise than the stochastic fluctuations of the proteins' levels around a mean value. As the fraction of time that P_1 and P_2 are simultaneously present increases, noise decreases. For example, cells of population 9 are never bistable and have low noise, except when compared to population 1 (~ 1 switch per cell lifetime).

Sensing cells always have lower noise than nonsensing ones. The signaling molecules reduce the number of stochastic toggles between the two noisy attractors of the TS since, when the TS is in a state fit to the environment the signals reinforce the TS stability.

Importantly, in sensing cells, population 7 has the highest noise level while in nonsensing cells it is population 5. Sensing cells maintain bistability for a larger range of parameter values due to the stabilizing effect of the signaling molecules on the proteins' levels.

Information on the environment state stabilizes the TS when the TS state is fit to the environment state, but also destabilizes it if its unfit, allowing a switch to the fit state. Since the internal noise in cells of populations 8 and 9 is too high to be damped by this interaction, these cells are not bistable and thus their noise level is lower than in cells of population 7 (since once bistability is lost, the only contribution to noise is from the fluctuations of the proteins' levels around a mean value).

The fitness of the cell populations is shown in Fig. 4. From Figs. 3 and 4 one sees, for both cell types, a clear
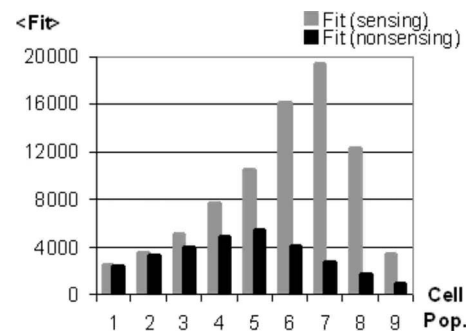

FIG. 4. Fitness of individual cells at the end of their lifetime, averaged over 1000 cells per cell population. Cell populations of the two cell types (sensing and nonsensing cells), differ in the values of k_t and k_d. $W_{fr}=10\,000$ s.
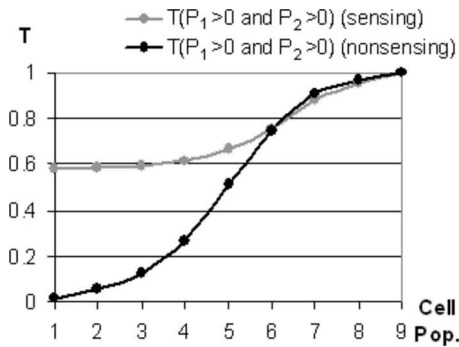
FIG. 5. The fraction of lifetime of individual cells during which both proteins are present (averaged over 1000 cells per cell population) for sensing and nonsensing cells. $W_{fr}=10\,000$ s.

correlation between noise level and fitness. The higher the noise the higher the fitness in rapidly changing environments. Beyond certain values of k_t and k_d, the internal noise disrupts the bistability (as exemplified in Fig. 2) and cells become unable to adapt to the environment, thus, fitness decreases since both proteins are always present. The measured noise also decreases due to the loss of bistability since, as said, "state switching" contributes more to noise than fluctuations of proteins levels around a mean value.

From Figs. 3 and 4 one observes that, in sensing cells, small variations in the noise level cause high variations in fitness while, comparatively, in nonsensing cells high variations in noise level cause small variations in fitness.

Within the regime where both cell types are bistable this is explained as follows. In nonsensing cells, the fitness increase with the increase of internal noise is not because these cells more often acquire a correct internal state in relation to the environment state but because during switches of the TS both proteins levels are low in absolute amounts, while still almost sufficient in quantity to cope with either toxin. In sensing cells, the increase in fitness is due to a decrease of the response time of the TS to changes in the environmental state. Therefore, identical variations in k_t and k_d cause higher increases in fitness of sensing cells than in nonsensing ones.

The increase in noise when varying k_t and k_d of sensing cells is much lower than in nonsensing ones due to the interaction between sensing cells and the environment. This interaction minimizes the number of toggles of the TS due to stochastic fluctuations. Sensing cells almost only toggle when the environment state changes. Because of this and since toggles of the TS are the events most contributing to noise, all populations of sensing cells that are bistable have similar noise levels. Sensing minimizes the noise level while still allowing response to environmental state changes (provided the noise is not sufficiently high to disrupt bistability).

To study the relation between noise, bistability, and fitness we plot in Fig. 5 the average fraction of lifetime (T) during which both genes of the TS are expressing and thus, both proteins are present. As noise increases, the number of transitions between the two noisy attractors increases, which increases T, that goes through a "phase transition" (in the sense that, dynamically, the TS goes from bistable to having both proteins expressed simultaneously). When this transition occurs and bistability is lost, the TS noise arises only from

fluctuations of the proteins' levels around a mean value.

The transition in T is caused by, as k_t and k_d increase, cells gradually being bistable for lesser time (one protein present and one absent), as the number of transitions between the two noisy attractors increases. Beyond some point (when $[R_p]k_t>k_r$), as k_t and k_d increase further, the two proteins become simultaneously present for longer periods of time, until the limit $T=1$, when both proteins are always present.

Note that the environment switches, on average, 50 times in a cell's lifetime. Sensing cells, if not too noisy internally, change their internal state following these switches. The combined effect of the number of environment switches and the average time needed for all proteins to decay once its production ceases results in $T \sim 0.58$ for sensing cell populations 1 to 3 (Fig. 5).

Figures 3–5 results indicate that in rapidly switching environments both cell types are optimally fit at the transition between bistability and its loss. The ability to sense the environment state provides higher fitness by increasing the probability that the TS adopts a correct state and the range of values for which the TS is bistable. For small values of k_t and k_d (low noise) sensing cells have higher T, since they switch in response to environmental switches, while the others rarely switch since they toggle only due to noise-driven fluctuations.

B. Evolutionary dynamics of sensing and nonsensing cells (fast environmental switching)

We now investigate the evolutionary dynamics of the two cell types (sensing and nonsensing). To this end, we simulate cell generations subject to selection and mutations, that change k_t and k_d. When a cell divides, all substances (except *fit*) are replicated and passed on to daughter cells [16]. This is an example of a possible realistic evolutionary pathway of cells evolving their noise level to attain better fit in fluctuating environments, and as expected from the previous results, shows that the cells evolve, in rapidly switching environments, to be in a phenotypic state that lies in the transition between bistability and its loss. Next, we compare the results to what occurs in slowly varying environments.

Cells of the first generation are of population 1. This choice does not affect the end results. Starting with another population only affects the number of generations necessary to reach the maximum fitness.

We simulate cell populations of 100 cells for 30 generations (30G). Effects of mutations in k_t and k_d and of selection on cells' average noise level are shown in Fig. 6, and on the average fitness in Fig. 7. After 20G, both cell types are optimally fit and the genotypes stabilize, since further changes cause fitness decrease. The populations of the two cell types evolved noise levels that match those previously found to be optimal, namely, ~ 0.75 (as population 5 in Fig. 3) for the nonsensing cells and ~ 0.25 (as population 7 in Fig. 3) for the sensing cells, i.e., in both cases in the transition between bistable and unstable.

Since most mutations are harmful, although the cells of the first generation are initialized as cells of population 1, their average fitness is lower than the average fitness of
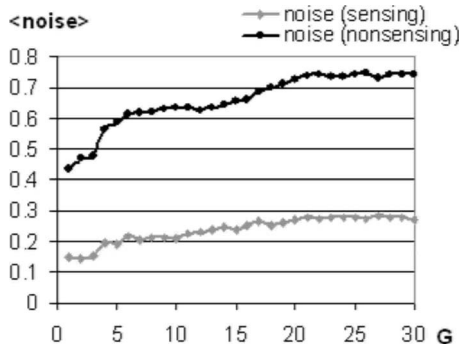
FIG. 6. Average noise level of P_1+P_2 time series of individual cells for sensing and nonsensing cells. 30 generations, 100 cells per generation. $W_{fr}=10\,000$ s. Changes in the mean levels of P_1+P_2 are below ~10% in both cell types.

"nonmutating" cells of this population. As selection acts, after ~4 generations, the average fitness overcomes the fitness of cells of population 1 (Fig. 4).

To confirm that the results are independent of the cells' initial genotype we simulated, for 100G, populations of 100 cells subject to mutation and selection, where initially, cells are from each of the 9 populations of sensing and nonsensing cells. The evolutionary pathways led in all cases, after a variable number of generations, to the same final noise level and fitness as in Figs. 6 and 7.

C. Effects of slow environmental switching on the evolutionary dynamics of cell populations

So far cells were in fast-switching environments. We now study the evolutionary dynamics in slowly switching environments. Setting $W_{fr}=500\,000$ s the environment state switches, on average, once per cell lifetime.

In slowly varying environments the optimal internal noise level of nonsensing cells is smaller than in fast-changing environments, in agreement with experimental results [10]. Population 3 is now the optimal one, while in the previous environment it was population 5 (shown for comparison) (Fig. 8). The best fit cells maintain a significant number of switches since the first noisy attractor reached by the TS might be unfit. A compromise is needed between being stable as long as possible when well adapted, and the need to
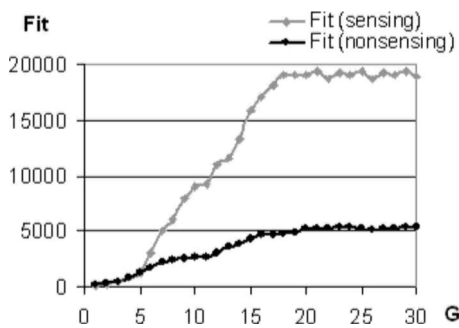

FIG. 7. Average fitness of sensing and nonsensing cells. 30 generations, 100 cells per generation. $W_{fr}=10\,000$ s.
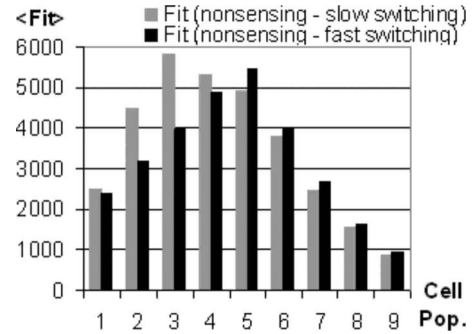

FIG. 8. Fitness at the end of the lifetime of nonsensing cells (averaged over 1000 cells per population) of cell populations with distinct values of k_t and k_d subject to fast ($W_{fr}=10\,000$ s) and slowly switching environments ($W_{fr}=500\,000$ s).

switch otherwise (thus populations 1 and 2 are not the fittest).

In contrast, the internal noise level that maximizes fitness of sensing cells (Fig. 9) is the same as before. Population 7 is still the fittest since these cells can, with its noise level, adapt to any environment change almost independently of the rate of change.

These results imply that there is an important advantage in having sensing mechanisms to inform on the environment state, although cells will have energetic costs associated to their maintenance and functioning. If the optimal phenotype is the same for different rates of changes of the environment state then, when changes occur, these cells have a selective advantage against nonsensing cells, since nonsensing cells need mutations to change their switch rate. On long time scales, such changes in the environment switch rate are bound to occur, explaining the selective advantage and maintenance of energetically expensive sensing mechanisms.

For example, assume that the two cell types coexisted in direct competition for a long time in an environment with a given switch rate, implying that they are equally and optimally fit there. Also, assume that when the environment switch rate suddenly changes, survival will not depend on mutations since there is not sufficient time to accumulate the necessary mutations. Since the genotype of sensing cells will still be optimal, unlike the genotype of nonsensing cells (no longer optimal), sensing cells are expected to prevail after a few generations.
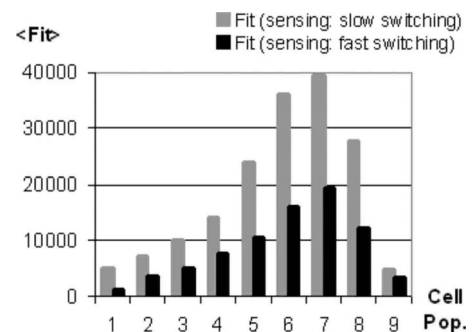

FIG. 9. Fitness at the end of the lifetime of sensing cells (averaged over 1000 cells per population) of cell populations with distinct values of k_t and k_d subject to fast ($W_{fr}=10\,000$ s) and slowly switching environments ($W_{fr}=500\,000$ s).

This can be tested by confronting directly sensing and nonsensing cells for several generations, in an environment with a given switch rate, assuming that the two cell types previously coexisted, and thus were equally fit, in an environment with another switch rate.

D. Effects of changing the environment fluctuation rate in sensing and nonsensing cells

A problem in confronting sensing and nonsensing cells is that they do not have equal absolute fitness even if both are optimally adapted to a given environment. While sensing cells have higher fitness in slowly and rapidly switching environments (Figs. 8 and 9), it is necessary to show that, if the two cell types had equal fitness when optimally adapted to some fluctuation rate, sensing cells would prevail if the fluctuation rate suddenly changed.

It is noted that increasing the environment switch frequency causes sensing cells fitness to decrease. This is due to an interesting difference in how individual sensing and nonsensing cells cope with a bistable environment. Assuming that the two cell types are optimally adapted, when the environment is in a given state, most sensing cells will be in the appropriate internal state while only ~50% of nonsensing cells will be in the appropriate internal state. Thereby, when the environment state changes, for a short period, most sensing cells will be unadapted while ~50% of nonsensing cells will already be in the correct state.

Thus, increasing the environment flip rate decreases mostly the fitness of sensing cells, although these cells fitness is always higher than the nonsensing ones for any limited environment flip rate. In the limit case, if the environment is always flipping (e.g., if $W_{fr}=1$ s), sensing and nonsensing cells fitness will be identical.

This limit case is, however, irrelevant, in the sense that if the environment flipped that fast, it would be unreasonable to use a bistable gene network to cope with it.

If confronting the two cell types given the fitness values previously found, and tuning only the fitness of sensing cells so that it would match the fitness of nonsensing cells for a given environment fluctuation rate, the results would depend on the initial environment flip rate one assumes the two cell types are equally well adapted to.

To confront the two cell types ability to cope with sudden changes in the environment flip rate, independently of the value of initial flip rate, and assuming identical fitness for sensing and nonsensing cells for that rate, one needs to adjust the fitness calculations so that two conditions are satisfied: (i) given a optimally adapted sensing cell and a optimally adapted nonsensing cell to a given environment switch rate, the two ought to have equal fitness, and (ii) given an optimally adapted sensing and/or nonsensing cell to a slowly varying environment and a optimally adapted sensing and/or nonsensing cell to a rapidly varying environment, the two ought to be equally fit.

The simplest way to fulfill these two conditions is to "normalize" the fitness values of each population of a given cell type such that the fitness of the optimally adapted population to a given environment equals 1. The fitness of the other
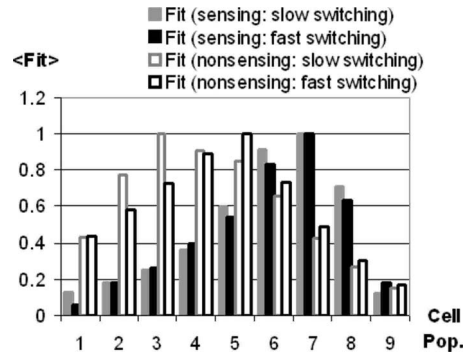

FIG. 10. Normalized fitness at the end of the lifetime of sensing and nonsensing cells (averaged over 1000 cells per population) of cell populations with distinct values of k_t and k_d subject to rapidly ($W_{fr}=10\,000$ s) and slowly switching environments ($W_{fr}=500\,000$ s).

populations (with different noise levels) in the same environment, normalized by the same factor, are thus always smaller than 1. The normalized fitness of each population of each cell type is shown in Fig. 10 for rapidly and slowly switching environments. The absolute fitness values (prior to normalization) equal the values in Figs. 8 and 9.

The inverse of the fitness value of the fittest population within the set of populations of a given cell type can be used as the multiplicative factor of c_{fit}, resulting in the fitness value of each population in Fig. 10.

Given these cell populations, we start with a population of cells such that 50% are sensing and 50% are nonsensing. Assuming that the two cell types have been in an environment with $W_{fr}=10\,000$ for a long time, their genotypes are optimal for that environment. Thus, nonsensing cells genotype is that of population 5, and sensing cells genotype is that of population 7, the ones best fit to $W_{fr}=10\,000$. Setting W_{fr} to 500 000, 100 independent simulations of 50 generations with 100 cells per generation were done (Fig. 11).

As seen in Fig. 11, in a slowly varying environment, sensing cells rapidly outcompete nonsensing cells (importantly, much faster than the time that would probably be needed for nonsensing cells to adapt via mutations). Although the fitness of the two cell types is identical for ($W_{fr}=10\,000$ s), the change of environment switch rate to $W_{fr}=500\,000$ s causes
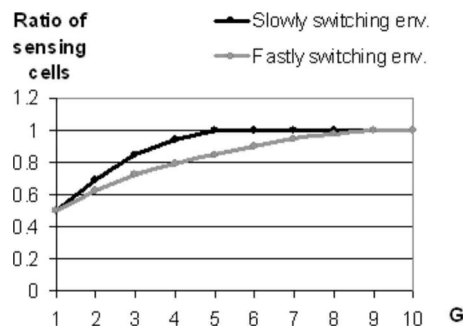

FIG. 11. Average of 100 simulations of the ratio of sensing cells in populations of 100 cells over ten generations in a slowly switching environment ($W_{fr}=500\,000$ s) and in a rapidly switching environment ($W_{fr}=10\,000$ s).

nonsensing cells to become less fit due to no longer being the best fit nonsensing cells.

In the opposite case, with both cell types equally well adapted to $W_{fr}=500\,000$ s and then confronted in an environment with $W_{fr}=10\,000$ s, we set as initial cell populations, population 3 for nonsensing cells and population 7 for sensing cells (the optimal ones for $W_{fr}=500\,000$ s). Again, sensing cells prevail (Fig. 11). Note that in this case it takes more generations for sensing cells to prevail, since the decrease in fitness of nonsensing cells due to the change in the environment flip rate (Fig. 10) is smaller than in the previous case.

As said, these results hold only if the environment is not always switching. If constantly switching, sensing cells would not benefit from their ability to adjust to the environment state. However, a mechanism of switching between two states is likely to evolve only when it would be beneficial (regardless of having a sensing mechanism or not), i.e., when there are two well-defined environment states, which implies a limited switching frequency.

IV. DISCUSSION

It was observed that, without sensing mechanisms, cells with fast interphenotype switching rate are more fit than slow ones in rapidly changing two-state environments, while the opposite is true in rarely changing environments [10]. Our results are in agreement. Also, it was hypothesized but not yet experimentally assessed that if cells sense the environment state they could evolve high response rates to switch as soon as a change is detected [4]. Our results also agree with this hypothesis. Namely, information on the environment state was shown to not remove the need to maximize internal noise, to allow fast phenotypic state switching to cope with unpredictable environmental changes as suggested in [8].

Both cell types able and unable to receive and act upon information on the environment state, in rapidly switching environments, evolved towards maximizing the TS switch rate. To achieve it, cells maximized the noise of the TS expression level. Sensing cells evolved towards a genotype whose phenotype bistability relies on the signals received from the environment, since these stabilize the TS state if this state is adapted to the environment state. This evolutionary pathway of increasing internal noise is, in both cell types, limited by the loss of bistability, since this loss implies inability to respond to environmental changes, which results in the existence of an optimal phenotype. The results indicate therefore that bounded maximization of noise within the space of gene networks accessible via genome evolution may play an important role in the natural selection of gene networks responsible for tackling unpredictable environmental changes.

The maintenance, by cells, of sensing mechanisms of the environment state is bound to be limited to the cases where it is energetically viable. Such energetic costs vary on a case by case basis and are likely to depend on many variables, such as on what specific information is being gathered (which determines what mechanisms can gather it) and the rate by which the environment conditions change. In the case here studied, the energetic cost to maintain the sensing mechanism is in the production of chemical signals able to unrepress a gene's promoter region, assumed as an energetically viable process.

Given the premise of low energetic costs in sensing and limited environmental state switching rate, importantly, a significant advantage was found that justifies the maintenance by cells of sensing mechanisms informing on the environment state, although there are energetic costs to do so. Namely, the optimal phenotype of cells that sense the environment state is independent of the environment variability rate. While cells without sensing need genotypic changes (mutations) to adapt to changes in the environment switch rate, sensing mechanisms remove this need and thereby provide an important selective advantage. In [8] it was shown that stochastic switching can be favored over sensing when the environment changes infrequently. However, in long time scales, not only the environment changes, but also the rate at which it changes can vary, and in this scenario, sensing provides a selective advantage if the energetic costs of sensing are sustainable.

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