Stochastic Gene Expression in Fluctuating Environments

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ABSTRACT

Stochastic mechanisms can cause a group of isogenic bacteria, each subject to identical environmental conditions, to nevertheless exhibit diverse patterns of gene expression. The resulting phenotypic subpopulations will typically have distinct growth rates. This behavior has been observed in several contexts, including sugar metabolism and pili phase variation. Under fixed environmental conditions, the net growth rate of the population is maximized when all cells are of the fastest growing phenotype, so it is unclear what fitness advantage is conferred by population heterogeneity. However, unlike ideal laboratory conditions, natural environments tend to fluctuate, either periodically or randomly. Here we use a stochastic population model to show that, during growth in such fluctuating environments, a dynamically heterogenous bacterial population can sometimes achieve a higher net growth rate than a homogenous one. By using stochastic mechanisms to sample several distinct phenotypes, the bacteria are able to anticipate and take advantage of sudden changes in their environment. However, this heterogeneity is beneficial only if the bacterial response rate is sufficiently low. Our results could be useful in the design of artificial evolution experiments and in the optimization of fermentation processes.

PERHAPS the most apparent manifestation of stochastic mechanisms in gene expression is the heterogeneity of cell populations. In the simplest case, the concentration of a constitutively expressed protein could show some variability from cell to cell (Elowitz *et al.* 2002; Ozbudak *et al.* 2002; Blake *et al.* 2003); more interestingly, a bacterial population could split into two or more groups, each of which is characterized by a distinct state of gene expression. This multistability, the existence of multiple stable states of gene expression in a given environment, can arise due to autocatalytic loops in cell regulatory networks (Ferrell 2002). Multistability has been predicted or observed in a growing number of metabolic systems (Siegele and Hu 1997; Biggar and Crabtree 2001; Thattai and Shraiman 2003), as well as in other types of networks. Several decades ago, Novick and Weiner (1957) found that the *lac* network of *Escherichia coli* was multistable: under intermediate concentrations of an external inducer, the bacterial population consists of cells that are either fully induced for *lac* expression or not induced at all, with individual cells switching stochastically between these states (Carrier and Keasling 1999). Furthermore, the two cell types exhibit different growth rates because of the metabolic burden imposed by futile gene expression. Similarly, cells of *E. coli*, upon being infected by phage-λ, are stochastically driven toward one of two possible fates (Arkin *et al.* 1998): they can either be lysed by phage particles or survive as lysogens, with the phage DNA incorporated into their chromosomes. The bias between these two outcomes depends sensitively on environmental and nutritional conditions. As a final example, the switch between the expression and nonexpression of cell surface pili during infection of the urinary tract by *E. coli* occurs in a stochastic fashion (Low *et al.* 2001; Wolf and Arkin 2002). The expression of certain types of pili is thought to trigger an immune response; however, pili also facilitate the colonization of the urinary tract surface, helping prevent bacterial removal by urine flow (Hernday *et al.* 2002). Cells in different states of pilus expression therefore proliferate at different rates.

It is clear that diverse systems are capable of generating multistability of gene expression states in cell populations. However, all of the systems discussed above share three important characteristics. First, cells are able to switch stochastically between the different expression states, generating a heterogenous population. Second, the rates of these transitions are functions of the environmental conditions, so the distribution of cells between the various states can vary as a function of time. Third, cells in different states of gene expression exhibit distinct growth rates, so the population distribution of states affects overall fitness. Under these circumstances, we describe the cell population as being "dynamically heterogenous." (This is in contrast to a "statically heterogenous" population, in which the transitions between states are not influenced by environmental conditions.) In a *fixed* environment, only one of the several subpopulations of a heterogenous population can exhibit the highest growth rate, with the others lagging behind. The net growth rate of the population will be maximized

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if the individuals are all in this fittest state; it might therefore seem surprising that cells have not evolved mechanisms to suppress transitions into the less fit states. However, we argue that in more realistic, *fluctuating* environments, dynamic heterogeneity might actually be beneficial.

The idea that heterogeneity might enable a population to better cope with an uncertain future has a long history. Indeed, natural selection itself is able to operate only once variation has arisen by mutation. More subtly, it is possible that certain sources of increased variation, such as mutator responses, sexual reproduction, and genetic recombination, can enhance survival during periods of rapid environmental change (Burger 1999; Tanaka *et al.* 2003). These ideas have been supported by the discovery of various mechanisms that increase mutation rates in organisms under stress (Rosenberg 2001). Heterogeneity can also be beneficial on more rapid timescales. A compelling example is the dormancy response, which occurs in several organisms including plants (Cohen 1966), insects (Menu *et al.* 2000), and viruses (Stumpf *et al.* 2002). In these organisms, each generation, a small fraction of the population remains in a protected dormant state, emerging for growth only after some delay, thus enhancing survival through unfavorable environmental epochs. Note that in all the cases discussed in this paragraph, the populations are statically heterogenous; the main conclusion to be drawn from these cases is that, given a broad but static distribution of phenotypes, the chances are increased that some of these will remain viable after a sudden environmental change. However, as we mentioned previously, bacterial populations are often dynamically heterogenous: cells cope with external changes primarily by generating specific internal responses designed to enhance fitness; any fitness advantage that might be gained from heterogeneity is secondary. We wish to determine the precise conditions under which dynamic heterogeneity is beneficial.

We approach this problem by constructing a general model of stochastic gene expression and population growth, one that captures all the essential features of the diverse systems discussed above. In our analysis, we allow environmental conditions to fluctuate, either periodically or stochastically, and determine the net growth rates of the cell populations in each case. We find that, when cells are able to respond much more quickly than external conditions vary, a homogenous population is most fit. However, if the cell response rate is comparable to or lower than the rate of environmental variations, dynamic heterogeneity can actually enhance fitness. The degree to which heterogeneity is beneficial depends on the penalty incurred for being caught in an unfit state. For intermediate penalties, heterogeneity is favored, while at extremely high or extremely low penalties, homogeneity can be preferable. These effects are more pronounced for periodically varying environments as compared to stochastically varying ones: in the latter case, heterogeneity is favored over a smaller parameter range. Interestingly, cells grow faster in a stochastic environment than in a periodic one of the same mean frequency: since cells themselves respond stochastically, they are able to ignore brief stochastic variations in their surroundings, modifying their states only in response to persistent external changes. In each case, cell populations are able to enhance their net growth rates by dynamically anticipating and exploiting environmental changes, perpetuating cellular states that seem disadvantageous at one time, but that will prove advantageous at later times.

ANALYSIS

Modeling the growth of stochastic populations: We assume that each bacterial cell in the population is capable of two distinct states of gene expression, as is common in many systems of interest; we label these cellular states as c_a and c_b. The cellular states have growth rates γ_a and γ_b, respectively, and stochastic transitions between these states occur with rate $k_{a\to b}$ from c_a to c_b, and $k_{b\to a}$ in the reverse direction. Experimental measurements suggest that these transitions should be regarded as Poisson processes, occurring with a constant probability per unit time (Low *et al.* 2001; Isaacs *et al.* 2003). For any particular system, the rates of stochastic transitions, and to some extent the different rates of growth, can be derived from a sufficiently detailed model of the underlying regulatory network (Bialek 2001; Kepler and Elston 2001); more practically, these various rates could also be determined experimentally (Siegele and Hu 1997). To go beyond the details specific to particular systems, we take the growth and transition rates to be free parameters and investigate the behavior of the system over all possible parameter values.

We are interested in circumstances involving fluctuating environments. Typically, all of the rates mentioned above are functions of environmental conditions. For example, consider the classic *diauxie* experiment (Monod 1966) in which cells of *E. coli* are grown in various types of glucose-lactose mixtures. As discussed earlier, cell populations under these circumstances sometimes consist of two cell types, those uninduced for *lac* expression and those fully induced; the presence of lactose tends to promote transitions into the induced state, while the presence of glucose tends to promote the reverse transitions. In lactose-rich media, induced cells grow faster since they are able to metabolize lactose; in glucose-rich media, although both cell types are able to metabolize glucose, induced cells grow slower since they must bear the burden of extra enzyme synthesis. Similarly, the transition rates between states of pilus expression and nonexpression during pili phase variation depend on external parameters such as temperature (Wolf and Arkin 2002). There is also evidence for

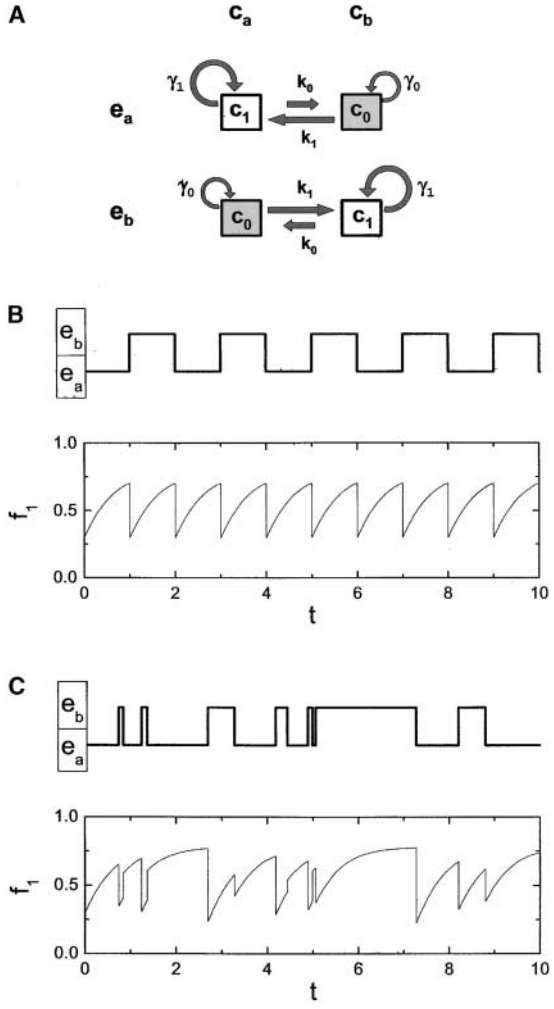

FIGURE 1.—A stochastic bacterial population. (A) Each cell can be in one of two states, c_a and c_b; correspondingly, the environment can switch between two states e_a and e_b. In environmental state e_a, cell state c_a is the fit state, labeled c_1 (open box), and cell state c_b is the unfit state, labeled c_0 (shaded box); when the environmental state flips, the two cell states exchange their properties. The growth rate of the fit state is γ_1, and that of the unfit state is $\gamma_0 < \gamma_1$; Poisson transitions into the fit state occur with rate k_1, and those into the unfit state occur with rate k_0. Typically, bacteria will tend to transition into the fit state, so k_1 will usually be higher than k_0. (B) Growth in a periodic environment. The environment cycles between the two states, spending a time $T = 1$ in each state. The fraction f_1 of cells in the fit state is plotted as a function of time, as predicted by Equation 4, for $k_0 = 0.5$, $k_1 = 1.0$, $\Delta\gamma = 1.0$. While the environment is fixed, more cells tend to transition into than out of the fit state, so f_1 increases. When the environment flips, cells that were in the fit state now find themselves in the unfit state, so $f_1 \rightarrow (1 - f_1)$. After this event, cells again begin to switch into the newly fit state, and so on. (C) Growth in a stochastic environment. The time spent by the environment in a given state is now exponentially distributed, with mean value T; this results in several brief environmental epochs, interspersed with a few periods in which the environment is more persistent. We generated the time course shown using a Monte Carlo simulation, then used Equation 4 to determine the time evolution of f_1, for the same parameter values as in Figure 1B. We see that cells are able to attain much higher fitness values during the extended environmental periods than in a periodic environment.

growth rate differences between these states under different environmental circumstances (HERNDAY *et al.* 2002). For example, during urine flow, pilus-expressing cells are able to survive by anchoring themselves to urinary tract surfaces, while cells without pili are flushed out. The latter cell type can be described as having a negative growth rate, a constant probability per unit time of being removed from the cell population. In the absence of urine flow, pilus-expressing cells are able to harvest resources less efficiently and may also become targets of an immune response; such cells then have the lower, possibly even a negative, growth rate.

To introduce these environmental influences into our model, we assume that the environment can cycle between two different states, e_a and e_b; this can be achieved by alternating between glucose-rich and lactose-rich media in the *diauxie* experiment or between the presence and absence of urine flow for bacteria colonizing the urinary tract. In environmental state e_a, cellular state c_a is most fit, with a similar correspondence between states e_b and c_b. We now make the simplifying assumption that the situation is completely symmetric under the interchange of the two environmental states; that is, when the state of the environment flips, the two cellular states simply exchange their properties. Under a certain environmental condition, one of the cellular states will then be the fit state labeled c_1, with growth rate γ_1, and the other will be the unfit state labeled c_0, with growth rate $\gamma_0 < \gamma_1$; the Poisson transition rate from c_0 to c_1 is k_1, and that from c_1 to c_0 is k_0 (Figure 1A). (In laboratory experiments under fixed conditions, a population with $k_1 > 0$ but $k_0 = 0$ would *eventually* become homogenous, while one with $k_1 > 0$ and $k_0 > 0$ would remain heterogenous. We therefore loosely use the term "heterogenous" to mean "$k_0 > 0$".)

The assumption of symmetry makes our analysis simpler to present, but our main results will hold even in the case of asymmetric parameters. Potentially more serious limitations of the model are the following. First, we have assumed that the duration of a cellular transition is much shorter than the time separation between transitions. In reality, cellular transitions do cost time and energy; however, for low enough transition rates, these costs can be ignored. This is a reasonable description of actual systems: during pili phase variation, for example, transitions occur about once per 10^5 generations per cell, but are executed within a single generation. We must simply be careful, during our analysis, not to let the switching rates take on arbitrarily high values. Second, we have assumed that the rate of growth in any particular cellular state is independent of the number of cells in that or in any other state. This assumption breaks down, for example, during growth under metabolite-limited conditions in which the different subpopulations compete with each other for nutrients (SMITH and WALTMEN 1995); similarly, it does not apply when the subpopulations cooperate with each other in any way, such as

during biofilm formation (Shapiro 1998). However, barring situations of competition or cooperation, our results are still broadly applicable.

Dynamics of population growth: We construct our dynamical equations in terms of the number of cells n_1 in the fit state c_1 and the number n_0 in the unfit state c_0. We take the population to be large enough that fluctuations in cell numbers can be ignored. These numbers can vary either due to growth of each subpopulation or due to transitions between them. Thus,

$$\frac{d}{dt}n_0 = \gamma_0 n_0 - k_1 n_0 + k_0 n_1$$

$$\frac{d}{dt}n_1 = \gamma_1 n_1 + k_1 n_0 - k_0 n_1. \quad (1)$$

The first term in each equation describes the growth of the unfit and fit cell populations, with growth rates γ_0 and γ_1, respectively; the remaining terms describe switching into the fit state with rate constant k_1 and into the unfit state with rate constant k_0 (Figure 1A). Let $n = n_0 + n_1$ represent the total number of cells, and define $f_0 = n_0/n$ and $f_1 = n_1/n$ as the fraction of cells in each state. The time evolution of the total number of cells is given by summing the two parts of Equation 1:

$$\frac{d}{dt}n = \gamma_0 n_0 + \gamma_1 n_1 = (\gamma_0 f_0 + \gamma_1 f_1)\, n \equiv \gamma(t)\, n. \quad (2)$$

Here we have introduced the population-averaged growth rate $\gamma(t)$, which is itself a function of time since it depends on the time-dependent fraction of cells in each state. This equation is easily solved to give $n(t) = n(0)e^{\int_0^t \gamma(t')dt'} \equiv n(0)e^{\langle\gamma\rangle t}$, where we have used $\langle . \; . \; . \rangle$ to represent time averaging. Thus, the net growth rate of the population over long time periods is simply given by the time-averaged quantity $\langle\gamma(t)\rangle$. Setting $\Delta\gamma \equiv \gamma_1 - \gamma_0 > 0$, this effective growth rate can be written as

$$\langle\gamma\rangle = \gamma_0 + \Delta\gamma\langle f_1\rangle \equiv \gamma_0 + \Delta\gamma f, \quad (3)$$

where we have defined $f \equiv \langle f_1\rangle$ as the time-averaged fraction of cells in the fit state. Consider a specific example: Figure 1, B and C, explicitly shows the time evolution of $f_1(t)$, for typical cases of periodic and stochastic environments. We can see that the time-averaged quantity $f \equiv \langle f_1\rangle$ will be higher in the second case than in the first, so the net growth rate of the population will be correspondingly higher. As the value of f is varied between zero and one, this net growth rate varies linearly between γ_0 and γ_1; f is therefore the natural measure of population fitness.

To calculate f, we must first determine the dynamics of f_1 by applying Equation 1. This gives

$$\frac{d}{dt}f_1 = k_1 + (\Delta\gamma - k_0 - k_1)f_1 - \Delta\gamma f_1^2. \quad (4)$$

In a fixed environment, Equation 4 can be solved analytically to produce an expression for $f_1(t)$. Whenever the environmental state changes, cells that were previously in the fit state now find themselves in the unfit state, and vice versa. Therefore, if the fraction of cells in the fit state just before the environmental change is f_1, then that fraction just after the change will be $1 - f_1$. We consider two types of environmental time variations: periodic and stochastic (Figure 1, B and C). In the periodic case, the environment cycles between states e_a and e_b, spending a fixed time T in each state; under these circumstances, the model can be solved analytically. In the stochastic case, the time spent in each state has mean value T, but can be made more or less variable; a purely periodic environment is, of course, a limiting case of this. For stochastic environments, population fitness can be determined by performing Monte Carlo simulations. In either case, given the environmental state as a function of time, we can calculate the net fitness f of the population. It is convenient to choose the environmental period as our time unit, so that $T = 1$; all growth and transition rates are therefore measured per environmental cycle. With this choice of units, we are left with fitness as a function of three parameters, namely, the cellular transition rates into the unfit and fit states and the growth rate difference between these states: $f \equiv f(k_0, k_1, \Delta\gamma)$.

Bacterial response strategies: The switching rates k_0 and k_1, determined by some underlying regulatory network, embody the bacterial response strategy to changes in environmental conditions. We sometimes refer to k_1, the rate of transitions into the fit state, as the bacterial response rate. The quantity $\Delta\gamma$, measuring the growth rate difference between the two cellular states, should be thought of as the penalty for being caught in the unfit state. It is likely that, over evolutionary time, those response strategies that confer some fitness advantage to cell populations growing under time variations and penalty conditions characteristic of their natural environments have been selected (Wolf and Arkin 2003).

Consider a passive bacterium, one that is unable to switch its cellular state. In our model, since the environment spends half its time in each of the states e_a and e_b, such an organism would have a fitness $f = 0.5$. In contrast, an active bacterium would be able to increase its fitness above this level by some appropriate choice of transition rates k_0 and k_1. It is clear that increasing the rate k_1 of transitions into the fit state will be beneficial; however, it is not clear how much benefit can be derived by allowing a rate k_0 of transitions into the unfit state. In the following section we explore, for different types of fluctuating environments and different penalties $\Delta\gamma$, the transition rates k_0 and k_1 that maximize fitness.

RESULTS

Growth in periodic environments: Figure 2 shows, for population growth in a periodic environment, the cir-

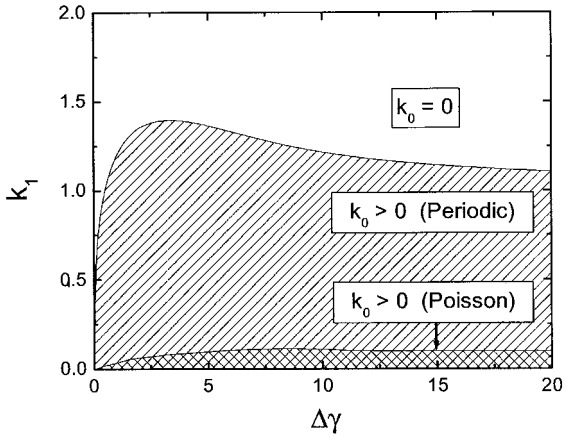

FIGURE 2.—Beneficial heterogeneity. Our central result is that transitions into the unfit state, at some rate $k_0 > 0$, can enhance cell fitness in certain circumstances. This can be seen by exploring the behavior of the fitness, $f(k_0, k_1, \Delta\gamma)$, as the cell response rate, k_1, and the fitness penalty, $\Delta\gamma$, are varied. The hatched region shows, for growth in periodic environments, those parameter values for which $k_0 > 0$ produces a fitness increase relative to $k_0 = 0$. The cross-hatched region shows, for growth in Poisson environments, those parameters values for which a fitness increase of at least 0.01 is attained for some $k_0 > 0$. This region was determined by performing Monte Carlo simulations (see Figure 4). We see that heterogeneity enhances fitness over a larger parameter range in a periodic environment than in a Poisson environment. Note that, if the cell response rate is sufficiently high ($k_1 > 1.4$), then $k_0 = 0$ is always preferred.

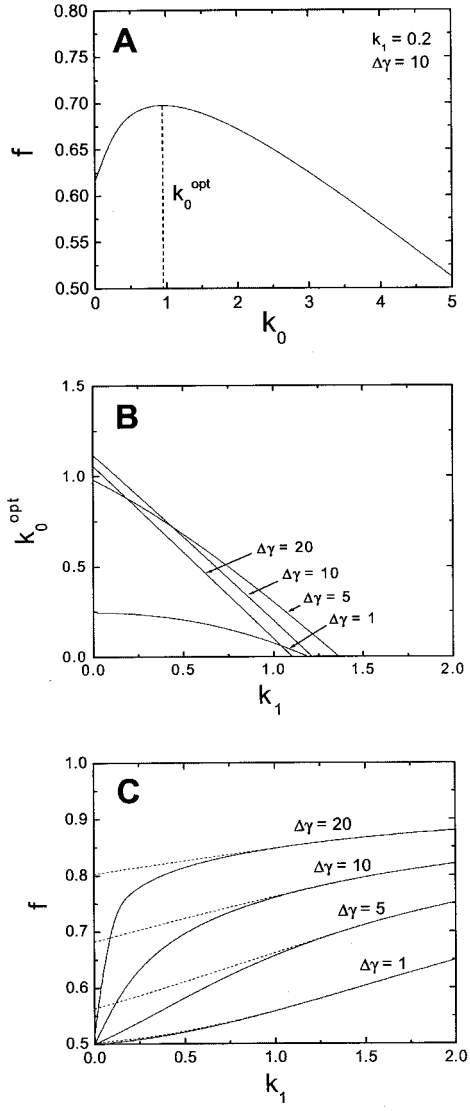

FIGURE 3.—Growth in periodic environments. (A) We plot the fitness, f, as a function of the transition rate into the unfit state, k_0, for $k_1 = 0.2$, $\Delta\gamma = 10$. This allows us to determine the optimal transition rate k_0^{opt} that maximizes fitness. (B) k_0^{opt} is shown for various values of k_1 and $\Delta\gamma$. Note that, beyond some value of k_1, $k_0^{opt} = 0$. (C) The fitness f obtained at $k_0 = k_0^{opt}$ (dotted line) is compared with that obtained at $k_0 = 0$ (solid line). The two curves are identical beyond the point at which $k_0^{opt} = 0$; the curves diverge for those parameters k_1 and $\Delta\gamma$ that are hatched in Figure 2. The fitness enhancement is considerable for high values of the penalty $\Delta\gamma$, but negligible for low values.

cumstances under which transitions into the unfit state enhance fitness. We see that, for $k_1 > 1.4$, $k_0 = 0$ is always the optimal solution. For $1.4 > k_1 > 1$, the situation becomes more interesting. For very low penalties, it still does not pay to maintain an unfit subpopulation in anticipation of environmental changes; for extremely high penalties, cells that transition to the unfit state are lost almost immediately, so it becomes wasteful to maintain an unfit subpopulation; for intermediate penalty values, however, heterogeneity is actually preferred. As the response rate drops even lower, for $k_1 < 1$, heterogeneity is always preferred even at very low or high penalties.

We can now ask how much benefit may be derived from making the best possible choice of k_0 (Figure 3A). That is, given k_1 and $\Delta\gamma$, we can ask which optimal transition rate k_0^{opt} maximizes fitness (Figure 3B) and compare the fitness attained at this optimal value (Figure 3C, dotted line) to that attained at $k_0 = 0$ (Figure 3C, solid line). We again see that, for $k_1 > 1.4$, $k_0 = 0$ is preferred, while for $k_1 < 1$, transitions into the unfit state are always beneficial. However, the benefit in the latter case becomes more significant at higher penalty values. It is interesting that, in the limit $k_1 \to 0$, a bacterium that makes transitions purely into the *unfit* state ($k_0 > 0$, $k_1 = 0$, $f > 0.5$) has a fitness advantage over a passive bacterium ($k_0 = 0$, $k_1 = 0$, $f = 0.5$). Of course, it is because the unfit state will soon become the fit state that such transitions enhance fitness.

Growth in stochastic environments: In the simplest case, we can model environmental transitions as Poisson processes of rate $1/T$, occurring with a constant probability per unit time. The time t spent in a given state will then be exponentially distributed, with mean value $\langle t \rangle = T$ and relative standard deviation $\sigma_t = 1$. More

generally, we can assume that each environmental transition is a process involving n_e steps, each occurring at rate n_e/T. We will then have $\langle t \rangle = T$, but $\sigma_t = 1/\sqrt{n_e}$, so transitions will occur with greater regularity. Thus $n_e = 1$ produces a Poisson environment, with $\sigma_t = 1$, while in the limit $n_e \to \infty$ we recover a purely periodic environment, with $\sigma_t = 0$.

For a Poisson environment, the range of parameters over which $k_0 > 0$ is favored is very small, and the fitness gain due to such a response strategy is negligible (Figure 2). Our results will therefore be essentially unchanged if we assume that transitions into the unfit state do not occur at all. Much more significant and interesting are effects that arise due to the fact that transitions into the fit state still occur stochastically: we find that population fitness actually *increases* as the environment grows more irregular (Figure 4A). When we compare fitness in a Poisson environment to that in a periodic one we find that, although fitness is always enhanced for the stochastic case, at a certain response rate k_1 this enhancement is greatest (Figure 4B).

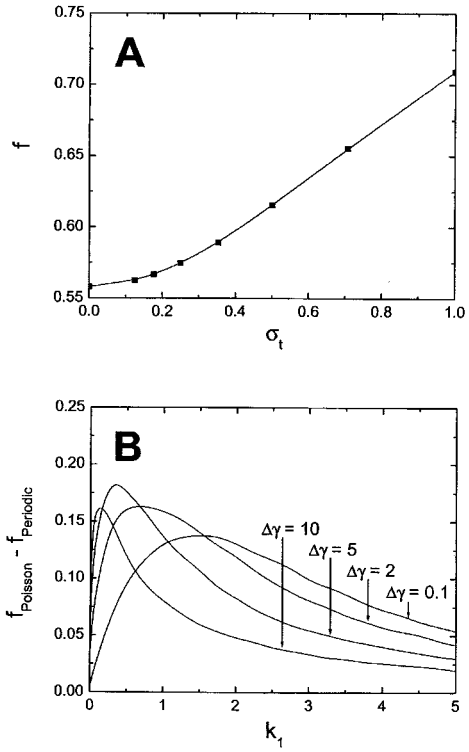

FIGURE 4.—Growth in stochastic environments. We used Monte Carlo simulations to generate stochastic environmental time courses; given each time course, we used Equation 4 to determine the time evolution of f_1 and time averaged the result over 20,000 environmental epochs to determine the fitness $f = \langle f_1 \rangle$. (A) The distribution of times spent by the environment in a given state has mean value $\langle t \rangle = T$ and relative standard deviation $\sigma_t = 1/\sqrt{n_e}$. Here we plot the fitness for $k_0 = 0.0$, $k_1 = 1.0$, $\Delta\gamma = 1.0$, for successive values $n_e = 1$, 2, 4, 8, 16, and 32. Thus we are able to explore environments characterized by a range of values of σ_t. We see that, at $\sigma_t = 0$, the curve tends to the limit $f = 0.56$ predicted for a periodic environment. Surprisingly, fitness is seen to increase as the irregularity of the environment, σ_t, is increased. (B) Again setting $k_0 = 0$, we show the fitness enhancement achieved in a Poisson environment compared to a periodic one, for various values of k_1 and $\Delta\gamma$. We see that fitness is always higher in the stochastic environment, but this enhancement is greatest at intermediate values of k_1.

DISCUSSION

When faced with a fluctuating environment, a bacterial cell that is able to track the environmental state, matching external changes with appropriate internal responses, would achieve the highest possible growth rate. A brute force solution to this problem would be for the cell to have an extremely high response rate, causing it to switch as soon as any external change was detected. However, the same result could be achieved with more finesse if the cell were able to *anticipate* environmental changes, changing its own state preemptively. Such a strategy would have the added benefit of minimizing the inherent time and energy costs of switching, which we have ignored in our model. Evolution would tend to select cells that have this capability, cells whose intrinsic switching rates somehow corresponded to those of their natural environments.

For growth in a perfectly periodic environment, it would seem ideal for a cell to have an internal oscillator that could be entrained to the external frequency. However, there would be some drawbacks. First, the biochemical implementation of a reliable oscillator usually requires several components, and we must ask if the outcome is worth the complexity. Second, such oscillators can typically be entrained over only a certain range of frequencies (STROGATZ 1994), which is a problem if the environmental period is rather variable. Third, the intrinsic noise of biochemical reactions would cause the oscillator to perform less than ideally in any case (BARKAI and LEIBLER 2000). In contrast, the cell could perform nearly as well if it were regulated by the kind of stochastic bistable system we have considered here. Bistability occurs generically in a variety of systems; the response rates and entrainment properties of a bistable switch are easily adjustable; and the system would be driven by noise rather than hampered by it. Cells would achieve greatest fitness by tuning their switching rates k_0 and k_1 appropriately, so that the average frequency of internal transitions matched those of external transitions.

When cells are grown in stochastic environments, they are able to achieve a higher net fitness than they were able to achieve under periodic conditions; moreover, this enhancement is peaked as a function of k_1. The explanation for this behavior is the following: as the environment is made more irregular, the distribution of times spent in a given environmental state becomes broad; for a Poisson environment, as mentioned earlier,

the distribution is exponential. This results in several short-lived environmental epochs, interspersed with a few in which environmental states persist for longer times (Figure 1C). Consider a cell that is initially in the fit state, but suddenly finds itself in the unfit state because of an environmental change. Since transitions into the fit state occur stochastically, the cell will tend to remain in this unfit state for a short period of time, of average length $1/k_1$. If the environment returns to its original state within this time, the cell will once again be in the fit state, with no effort on its part. It therefore benefits a cell to ignore brief environmental variations, but to switch only in response to persistent external changes. If the cell response rate is too high, it will tend to track the environment, switching its state whenever the environment does; if this rate is too low, the cell will tend to ignore even persistent changes, and its growth will be impaired as a result. The balance between these false-positive and false-negative responses is achieved at some optimal response rate k_1. Although fitness is always a monotonically increasing function of k_1, increases in k_1 beyond this optimal value provide diminishing returns. In principle, a cell could achieve $f = 1$ if it were to respond infinitely fast; in practice, it can get very close to $f = 1$ simply by setting k_1 at or slightly above the optimal level. When the additional costs of switching are considered, this argument becomes even more compelling: it is very likely optimal for a cell to tune its response rate to match the rate of natural environmental variations.

We have seen that, during growth in both periodic and stochastic environments, heterogeneity can be beneficial; however, the benefit is substantial over only a small parameter range, and it is worth understanding why this is the case. A statically heterogenous population copes with an uncertain future by hedging its bets, generating a broad distribution of phenotypes in the hope that some of these will remain viable after an external change. In contrast, a dynamically heterogenous population has a much more reliable strategy: individuals in such populations sense and respond to external changes by actively switching into the fit state. The benefit of a heterogenous response is therefore diminished. Indeed, if the cell response rate is sufficiently rapid compared to the rate of environmental fluctuations, as is the case in many real systems, then transitions into the unfit state are actually detrimental. If heterogeneity is nevertheless observed in such systems, then it must be due to factors more complex than those we have considered here, such as interactions between the different cell populations. We hope that, by placing limits on the circumstances under which heterogeneity can be easily justified, our results will prompt a deeper investigation into the role of heterogeneity in dynamic cell populations.

The analysis of cellular systems in the context of their natural environments often yields valuable insight into certain aspects of their structure and function. For example, apparently equivalent bacterial gene regulatory mechanisms are seen to be more or less robust, depending on time variations in external nutrient concentrations (Savageau 1998). When such external time variations are coupled to the dynamics of cell populations, the results can sometimes be unexpected. This is especially true when stochastic mechanisms in the underlying regulatory networks are considered, since these are often known to influence population dynamics (Paulsson 2002). Here we have shown that, during growth in fluctuating environments, cells are able to exploit the intrinsic stochastic nature of biochemical reactions to maximize their own fitness. However, this fitness advantage is obtained over only a limited range of cell response rates and environmental perturbation rates. These results suggest that time-dependent conditions ought to play a central role in the design and interpretation of laboratory experiments. Thus, experiments that seek to understand the behavior of natural networks should try, as nearly as possible, to mimic the natural time variations to which those networks would be subject. Conversely, standard experimental protocols such as cell growth in batch culture, which often necessitate time variations in growth conditions, might be more susceptible to side effects of these variations than what is usually assumed. Studies of external time variations on stochastic biological populations will have several useful applications, reducing the detrimental effects of external fluctuations on cell cultures, providing more effective protocols for artificial evolution experiments, and even increasing the yield of industrial fermentation processes.

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