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A microbial modified prisoner's dilemma game: how frequency-dependent selection can lead to random phase variation

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Abstract

Random phase variation (*RPV*) is a control strategy in which the expression of a cell state or phenotype randomly alternates between discrete 'on' and 'off' states. Though this mode of control is common for bacterial virulence factors like pili and toxins, precise conditions under which *RPV* confers an advantage have not been well defined. In Part I of this study, we predicted that fluctuating environments select for *RPV* if transitions between different selective environments cannot be reliably sensed (J. Theor. Biol. (2005)). However, selective forces both inside and outside of human hosts are also likely to be frequency dependent in the sense that the fitnesses of some bacterial states are greatest when rare. Here we show that *RPV* at slow rates can provide a survival advantage in such a frequency-dependent environment by generating population heterogeneity, essentially mimicking a polymorphism. More surprisingly, *RPV* at a faster 'optimal' rate can shift the population composition toward an optimal growth rate that exceeds that possible for polymorphic populations, but this optimal strategy is not evolutionarily stable. The population would be most fit if all cells randomly phase varied at the optimal rate, but individual cells have a growth-rate incentive to defect (mutate) to other switching rates or non-phase variable phenotype expression, leading to an overall loss of fitness of the individual and the population. This scenario describes a modified Prisoner's Dilemma game (Evolution and the Theory of Games, Cambridge University Press, Cambridge, New York, 1982, viii, 224pp.; Nature 398 (6726) (1999) 367), with random phase variation at optimal switching rates serving as the cooperation strategy.

Keywords: Random phase variation; Game theory; Frequency-selective environment; Bacteria; Prisoner's dilemma

1. Introduction

Random phase variation (*RPV*) is a control strategy in which the expression of a cell state or phenotype randomly alternates between discrete 'on' and 'off' states. For example, individual *Escherichia coli* cells randomly alternate between being densely covered with pili—adhesive organelles mediating human infection—and being unpiliated (Abraham et al., 1985). Other examples of phase variable phenotypes include the expression of type IV pilus varieties in *Neisseria gonorrhoeae* and *N. meningitidis* (Abraham et al., 1985; Howell-Adams and Seifert, 2000; Power et al., 2003); toxin production, fimbriae, lipopolysaccharide variants, and restriction-modification genes in *Mycobacterium pulmonis* (Dybvig et al., 1998); outer membrane proteins in *Dichelobacter nodosus* (Moses et al., 1995); flagellum in *Salmonella typhimurium* (Bonifield and Hughes, 2003); phage growth limitation machinery in *Streptomyces coelicolor* (Sumby and Smith, 2003); and many others (Henderson et al., 1999; Hallet, 2001).

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Though the molecular and systemic mechanisms for random phase variation have been worked out for many model organisms (Henderson et al., 1999; Hallet, 2001; Wolf and Arkin, 2002), the evolutionary origins and fitness consequences of this expression strategy are not well understood. Random phase variation is believed to aid survival within a host by allowing bacteria to evade the immune system or search a host's receptor space (Henderson et al., 1999; Hallet, 2001). Evasion of the host immune system is thought to be facilitated by molecular mimicry of host structures by phase-variable lipopolysaccharides in *Helicobacter pylori* and *Campylobacter* (Moran and Prendergast, 2001), and by the astounding level of antigenic diversity produced by different pilus varieties in *N. gonorrhoeae* (used for host attachment and the uptake of exogenous DNA (Fussenegger et al., 1997)), whereas phase-variable *opa* genes in *Neisseria* are believed to orchestrate the recognition of different host receptors and result in tissue tropism (Hauck and Meyer, 2003). Phase variation between a small number of phenotypes (e.g. type 1 pili expression) does not fit neatly into these categories, and for the most part, the experiments and theory needed to test these hypotheses have yet to be done.

In Part I of this study, we took a game theoretic approach to investigate the precise conditions under which random phase variation or other diversification strategies confer a survival advantage (Wolf et al., 2005). We showed that if cells are unlikely to sense environmental transitions or are subject to long signal transduction delays relative to the time-scale of environmental change, a *time varying* environment could select for phase varying phenotype expression, *if* different environmental states select for different phenotypes. Within a single host, time-varying selection is at work as the immune system cycles through the process of identifying antigens and producing antibodies (on the order of 4–7 days) (Eigen, 1971; Nowak, 1992; De Clercq, 2001), and as the population moves from host compartment to compartment over the course of an infection. Environments outside a host are marked by fluctuations in nutrient levels, oxygen exposure, temperature, and pH. Variability among potential hosts also creates time varying selection on slow scales, as populations move from host to host. However, selective forces both inside and outside human hosts are also likely to be frequency dependent in the sense that the fitnesses of some bacterial states are greatest when rare. For example, an antigenic variant is more likely to find a new host naïve of that variant if it is rare in the population, thus making the fitness conferred by any particular variant a function of its frequency in the population. This view is supported by an analysis by Ancel-Meyers et al. of the role of phase shifting in *N. meningitides* pathogenicity (Meyers et al., 2003).

In this paper, we complement our treatment of time-varying environments (Wolf et al., 2005) with a brief study of the impact of frequency-dependent selection on the evolution of random phase variation. We show that random phase variation at slow rates can provide a survival advantage in such an environment by generating population heterogeneity, essentially mimicking a polymorphism. More surprisingly, random phase variation at rapid rates can shift the population composition toward an optimal growth rate that exceeds that possible for polymorphic populations, but this 'optimal' strategy is not evolutionarily stable. Defectors that do not phase vary, or phase vary at non-optimal rates, can stably coexist with 'optimal' random phase variants in such a manner that the overall population growth rate is sub-optimal. This scenario describes a modified Prisoner's Dilemma game (Maynard Smith, 1982; Nowak and Sigmund, 1999), with random phase variation at optimal switching rates serving as the cooperation strategy. The population would be most fit if all cells randomly phase varied at the optimal rate, but individual cells have a growth-rate incentive to defect (mutate) to other switching rates or non-phase variable phenotype expression, leading to an overall loss of fitness of the individual and the population.

2. Results

To study the impact of frequency-dependent selection on the evolution of random phase variation, we followed an approach similar to the one we took in (Wolf et al., 2005). Fig. 1a illustrates an abstraction of a frequency-selective environment where the fitnesses of cell-states are greatest when rare. Our hypothetical cells can be in two possible cell states, x and y (e.g. piliated and unpiliated). Cells in state x grow at rate g_x, and cells in state y grow at rate g_y. These growth rates depend on the composition of the population f_x, ($f_x = (\#$ cells expressing $x)/(\text{total } \#$ cells)). If the population consists mostly of y cells, x cells have a growth advantage. However, if the population consists mostly of x cells, y cells have a growth advantage. Such an environment has been shown to support probabilistic strategies or polymorphisms (Maynard Smith, 1982, p. 23). More precisely, if the average growth rate g_x of a bacterium in state x (say, with pili) is a decreasing function of the fraction of the population in cell state x, f_x, and if the average growth rate g_y of a bacterium in state y (no pili) is an increasing or flat function of f_x, and if these two curves cross, then the evolutionarily stable composition of the population occurs at that that point on the f_x axis where the two growth rate functions intersect (Fig. 1a). At this composition of the population, f_{ESS}, the average growth rates of x and y cells in the population are equal. The composition f_{ESS} is called evolutionarily stable (*ESS* denotes an evolutionarily stable strategy) because it is the stable composition at which the population

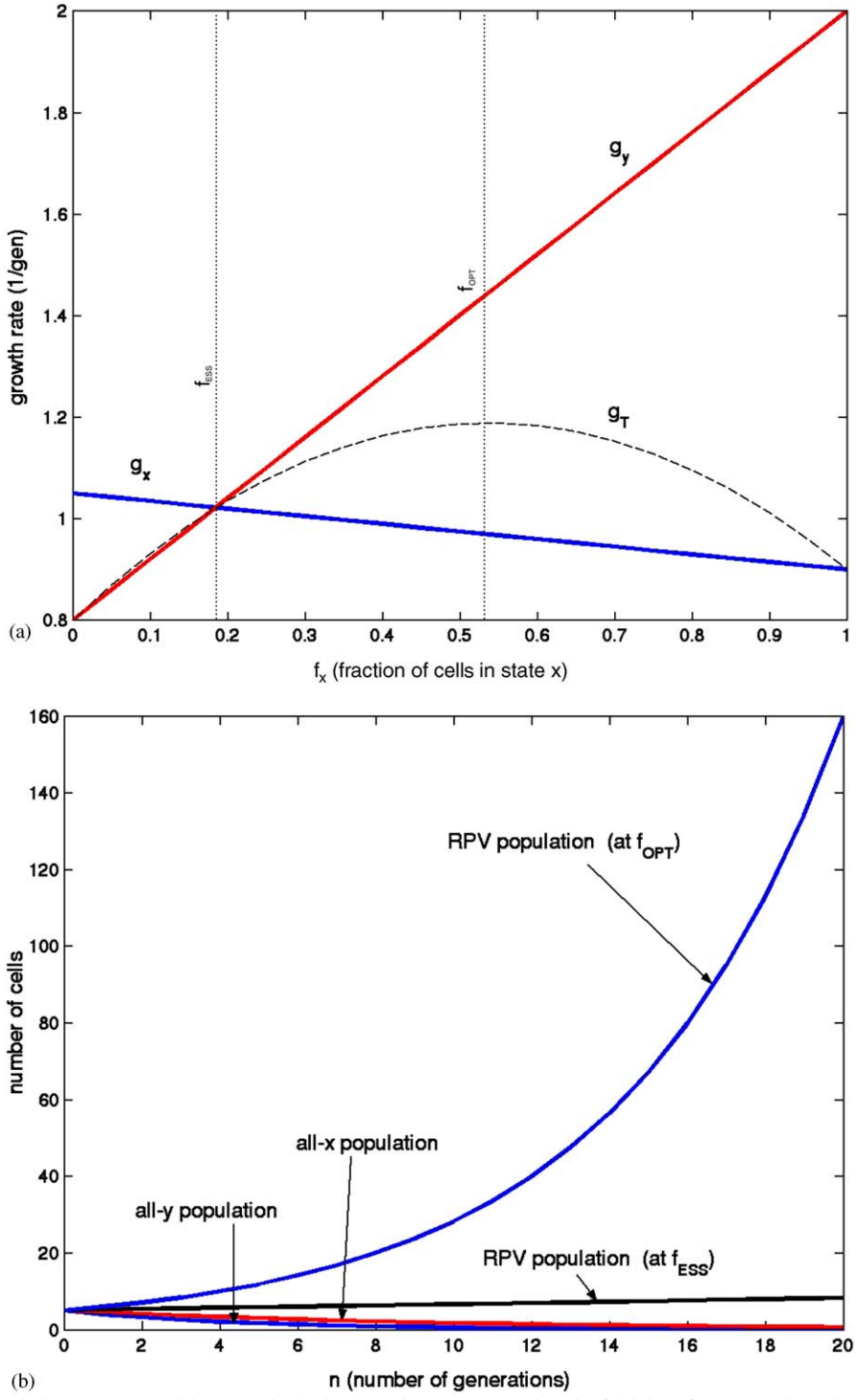

Fig. 1. Random phase variation (RPV) provides a survival advantage in a game 'playing the field' in a frequency-dependent environment: (a) If the growth rate of cells in cell-state y exceeds that of cells in cell-state x when y is rare ($g_y > g_x$ at $f_x = 1$), and vice versa, the environment supports a steady state *ESS* population composition at the point of intersection between g_x and g_y ($g_x(f_{ESS}) = g_y(f_{ESS})$). However, the *ESS* composition may not be growth-rate optimizing ($f_{ESS} \neq f_{OPT}$). In this example the optimal growth rate—the composition at which the total growth rate of the population g_T is maximum—occurs at $f_{OPT} \approx 0.53$, far from the *ESS* composition $f_{ESS} \approx 0.18$. (b) Random phase varying populations with switching rates such that the population composition is at f_{OPT} grow faster than randomly phase varying populations or polymorphic populations at the *ESS* composition f_{ESS}, which in turn grow faster than a pure population of either x or y cells. Pure x or pure y populations die out to extinction. Frequency-dependent growth rates in Eq. (1) are as follows: $g_x = 1.05 - 0.15 f_x$; $g_y = 0.8 + 1.2 f_x$; where $f_x = (\#\text{ cells expressing } x)/(\text{total } \#\text{ cells})$.

comes to rest. This scenario defines a game 'playing the field', in evolutionary game theory parlance, with a frequency-dependent fitness function producing a mixed, evolutionarily stable strategy (Maynard Smith, 1982, p.. 23). Frequency-dependent fitness functions in games playing the field have been used to explain the sex ratio (Fisher, 1930), sequential hermaphroditism (Leigh et al., 1976), and fig wasp male dimorphism (Hamilton, 1979), among others (Maynard Smith, 1982).

As shown below, random phase variation can provide a survival advantage in such an environment by generating population heterogeneity, or by shifting the population composition toward an optimum distinct from the f_{ESS}.

2.1. RPV generates population heterogeneity

Cells following a random phase variation strategy alternate between different cell states (e.g. piliated and unpiliated) according to a probabilistic rule, with transition probabilities that may be influenced by environmental sensor information. Thus phase variation, even at very low probabilities, generates a mixture of cells in the population, and mixed populations grow at a faster rate than would a pure x or pure y population so long as the growth at f_{ESS} exceeds the growth rate of x at $f_x = 1$ and the growth rate of y at $f_x = 0$, as in Fig. 1a. An extreme case is shown in Fig. 1b, wherein pure x or y populations become extinct but randomly phase varying populations proliferate.

These simulations employ the following simple population model, essentially a digitized, sensorless version of the general model in Wolf et al. (2004):

$$\begin{bmatrix} x_{k+1} \\ y_{k+1} \end{bmatrix} = \begin{bmatrix} g_x - sx & sy \\ sx & g_y - sy \end{bmatrix} \begin{bmatrix} x_k \\ y_k \end{bmatrix}. \quad (1)$$

In Eq. (1), x_k is the number of cells in state x at time step k, y_k is the number of cells in state y at time step k, and sx and sy are the x-to-y and y-to-x switching probability rates per generation of each cell, respectively. As described in Section 4, Eq. (1) is simulated with frequency-dependent growth rates $g_x = a - bf_x$; $g_y = c + df_x$; $f_x = x/(x+y)$; and constants $a > c$, $c + d > a - b$ to mimic the frequency-dependent environment illustrated in Fig. 1a.

2.2. RPV can shift the population composition toward the optimum

A subtler potential source of survival advantage from phase variation has to do with the possibility of advantageously shifting the population composition away from the f_{ESS} value. In a frequency selective environment generating mixed populations, the f_{ESS} composition—occurring at the 'point of indifference' between x and y phenotypes—generally does not occur at the optimal population composition for growth. The point of indifference occurs at f_{ESS} such that $g_x(f_{ESS}) = g_y(f_{ESS})$, whereas the optimal composition f_{OPT} satisfies Eq. (2) below, derived by differentiating the total growth rate of the population $g_T(f_x) = g_x(f_x)f_x + g_y(f_x)(1 - f_x)$ with respect to composition f_x and setting the expression to zero:

$$g_x(f_{OPT}) - g_y(f_{OPT}) = \left[\frac{\partial g_y(f_{OPT})}{\partial f} - \frac{\partial g_x(f_{OPT})}{\partial f}\right] f_{OPT} - \frac{\partial g_y(f_{OPT})}{\partial f}. \quad (2)$$

Clearly, if the right-hand side of Eq. (2) does not equal zero, $f_{ESS} \neq f_{OPT}$ (See Fig. 1a). In the linear case, for example, $f_{ESS} = f_{OPT}$ only if $g_y(1) = g_x(0)$.

Phase variation can shift the population composition away from f_{ESS} and toward f_{OPT} because the steady state composition of a phase varying population does *not* generally equal f_{ESS}, the intersection of the two growth curves. Rather, the composition f_{RPV} satisfies Eq. (3) below, where sx and sy are the x-to-y and y-to-x switching probability rates per generation of each cell, respectively:

$$g_x(f_{RPV}) - g_y(f_{RPV}) = sx(1/(1 - f_{RPV})) - sy(1/f_{RPV}). \quad (3)$$

This equation was derived by writing out population Eq. (1) and setting $x_{k+1}/y_{k+1} = x_k/y_k$ to solve for the steady state population composition. If the switching probability rates sx and sy of a random phase varying population satisfy Eq. (3), with $f_{RPV} = f_{OPT}$ as defined by Eq. (2), then the population will achieve the optimum growth rate. Note that Eq. (3) provides a single constraint on two parameters, sx and sy, and thus defines a curve rather than a single point. There are an infinite number of solutions to this equation, if any.

How is it that random phase variation can shape a steady state population composition different from f_{ESS}? Intuitively, one can think of two forces acting on the population composition. The frequency-dependent environment exerts one force, pushing the composition toward f_{ESS}, the point of growth rate indifference between x and y cell states. A second force is exerted by random phase variation, which pushes the population toward the composition $f_x' = sy/(sx + sy)$. These two forces balance out by generating a steady state composition between f_{ESS} and f_x', denoted f_{RPV}. If random phase variation is very slow, with $sx + sy << 1$, the composition will be much closer to f_{ESS} than f_x', whereas if the cells phase vary very rapidly, the composition will approach f_x'. By selecting a switching rate ratio sx/sy that places $f_x' > f_{OPT}$ (assuming $f_{OPT} > f_{ESS}$), finite, reasonable switching rates can generate this

new, 'compromise' steady state f_{RPV} at the optimal composition f_{OPT} (Fig. 1a,b).

2.3. Optimal but not ESS—a modified prisoner's dilemma

Maynard Smith defined an Evolutionarily Stable Strategy (ESS) to be one that is uninvadable by a rare mutant with access to same set of game 'moves', or cellular states (Maynard Smith, 1973). Random phase variation with switching rate probabilities satisfying Eq. (3) and with $f_{RPV}=f_{OPT}$ is the optimal strategy, but it is *not* evolutionarily stable. To see why not, consider for a moment a game playing the field with $f_{OPT}>f_{ESS}$. At f_{OPT}, the growth rate of cells in state y exceeds those in state x (and those that are optimally phase varying, as they alternate between x and y cell states). Thus, a pure-y mutant will grow faster than the dominant phase varying population, as will any phase varying mutant that spends more time in the y state on average than does the dominant strain. If a pure-y mutant is introduced, it will continue to grow faster than the phase variable cells until the composition f_{ESS} is reached. Once the f_{ESS} has been reached, all cells, phase variable and pure-y strategists, will grow at the same (slower than optimal) rate (Figs. 2a,b). This set-up defines a modified Prisoner's Dilemma (Maynard Smith, 1982; Nowak and Sigmund, 1999), with phase variation at optimal rates playing the role of the cooperator, and a pure-y or phase varying strategy with greater probability of being in a y cell state playing the role of the defector. The incentive to the individual is to defect, but this defection results in an overall decrease in the payoff to each individual and the population as a whole in the form of lower growth rates.

2.4. So what is the ESS?

Unlike a true Prisoner's Dilemma, the ESS in such a frequency-selective environment supports a number of different polymorphisms, rather than having a unique, all-defector solution (simulations described in the Section 4, data not shown). It is tempting to say that random phase variation at switching rates such that $f_{RPV}=f_{ESS}$ is the *ESS* strategy, since no single mutant can invade such a population, and since phase variable cells of this type can invade all-x or all-y populations, or phase variable populations with $f_{RPV}\neq f_{ESS}$. However, if two mutant cells are introduced in short succession, one with a pure-x strategy and one with a pure-y strategy, phase variable and pure strategies can coexist polymorphically, all growing at the same rate once f_{ESS} is reached (data not shown). In fact, as long as there are some pure-x and pure-y cells, all possible phase variable strategies can coexist at equal growth rate, albeit in relative amounts dictated by the f_{ESS} composition constraint. Furthermore, given a population with a composition equal to f_{ESS}, a phase varying mutant with $f_{PRV}=f_{ESS}$ can become established in the population, but does not out-compete its fellows if there are pure-x and pure-y cells in the mixture. Thus, the ESS is best defined in terms of the steady state population composition f_{ESS} rather than as a unique individual strategy.

3. Discussion

Is the scenario described above, a game playing the field with a frequency-dependent payoff function, reasonable for a pathogen? Not only is the fitness conferred by any particular variant a function of its frequency in the population because of the survival advantage in finding a new host naïve to that variant, but there is some evidence for frequency-dependent immune reactivity within a single host. Type 1 pili expression in uropathic *E. coli* might be such an example. Expression of type 1 pili, the very factor that allows UPEC to attach to and invade epithelial tissue in the bladder, also stimulates a concomitant response from the immune system. And if IL-6 production is any indication, this immune reactivity is frequency dependent, becoming markedly (nonlinearly) more reactive as the number of piliated cells in an infecting population exceeds a threshold (Schilling et al., 2001; Mulvey, 2002). Is frequency-dependent immune system reactivity the reason why type 1 pili expression, or any other virulence factor, phase varies, or are there other forces at work? This study suggests the combined forces exerted by a time-varying (Wolf et al., 2005) and frequency-dependent immune response can conspire to select for *RPV* as a survival strategy in mammalian pathogens, though in this case the 'selected' cell state is 'immune naiveté' and perhaps 'receptor specificity'.

Though we have constructed a framework for understanding some of the environmental and cell-sensory conditions that can give rise to *RPV* and other diversification strategies, experiments similar to those of Turner and Chao to reconstruct the RNA phage game (Turner and Chao, 1999, 2003) are needed to sift through hypotheses of how phase variation functions over the lifecycle of a particular organism. In such experiments, payoff functions inside and outside the host and in different host compartments are explicitly measured and interpreted in a game theoretic framework. If, for example, phase variation or another diversification strategy is primarily a response to frequency-dependent selection in an environment, an experiment that mixes phase-locked x and y cells will show that the population settles to a steady-state composition, with neither x out-competing y to extinction nor the other way around. Furthermore, a mixture of x and y cells will grow faster in this environment than

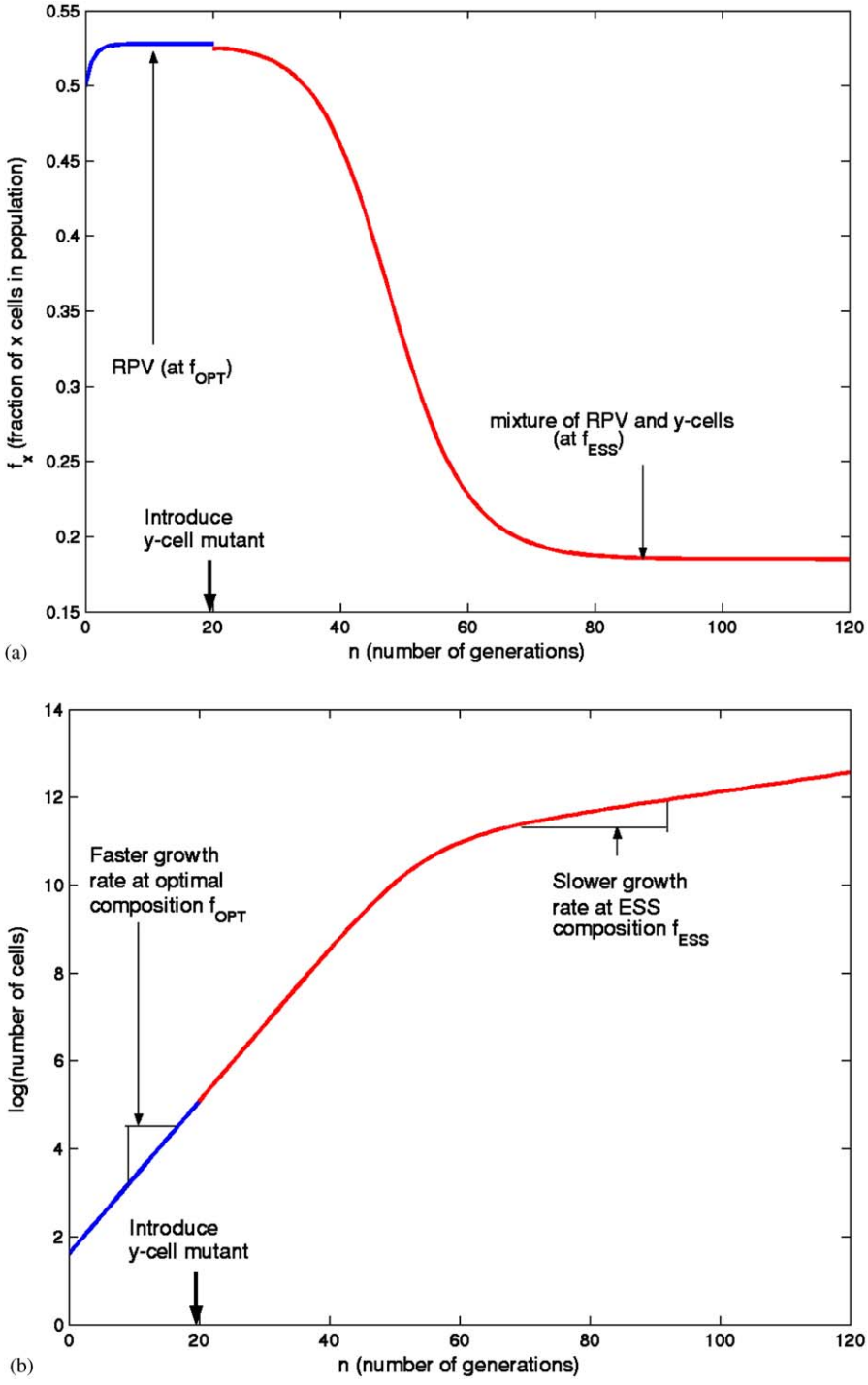

Fig. 2. RPV at switching rates such that the steady-state population composition is the optimal f_{OPT} rather than the *ESS* value f_{ESS} plays the cooperation role in a game of modified Prisoner's Dilemma. Introduction of a pure y cell-state mutant (defector) to a population of f_{OPT} random phase variers (cooperators) at time $n = 20$ has the effect of shifting the population composition from optimal f_{OPT} to ESS f_{ESS} (see (a)), bringing about an associated loss of fitness of the population in the form of lower growth rates (see (b)). Growth rate parameters are as in Fig. 1.

does either a pure-x or a pure-y population. In the unlikely event that *RPV* is at optimal rates, playing the cooperation strategy in the game of modified Prisoner's Dilemma, the addition of a few phase-locked x and y 'defector' cells to a wild type *RPV* population will result in a change in the steady state population composition and a decrease of overall fitness of the population.

Though in this paper and its companion (Wolf et al., 2005) we confine ourselves to the study of diversification strategies on the abstract phenotype, cell, and population

levels, a more complete understanding calls upon us to focus 'down' a level of abstraction to study the similarities and differences among diversification mechanisms across pathways and species, and 'up' a level to define and compare the ecological composition of particular niches. The integration of analysis on all three levels in an evolutionary context is necessary if we hope to understand why some *RPV* mechanisms are based on DNA rearrangement, whereas others employ slipped strand mispairing, DNA shuffling by gene conversion and allele replacement, or epigenetic mechanisms, and the similarities and differences of network designs in each category (Henderson et al., 1999; Hallet, 2001). Are these diverse designs merely evolutionary spandrels (Gould and Lewontin, 1979; Gould, 1997)? Or are distinct mechanisms a result of differences in the physics of the environmental factors being sensed, the intracellular signaling molecules transducing environmental signals, pathway cross-regulation, or the 'game' of survival played by each microbe? To distinguish between these possibilities there is challenging theory and experiment to be done such as experimentally measuring dynamics in single cells, developing consistent theories of regulatory network design and evolution, tracking population heterogeneity under varying conditions, quantifying fitness, and designing experiments to test and generate a broad range of game theoretic hypotheses.

4. Methods

We wrote a Matlab (Mathworks, Natick, Massachusetts, United States) program to simulate population growth in a frequency-dependent environment. This program updates the population vector [x y]' according to Eq. (1) with frequency-dependent growth rates $g_x = a - bf_x$; $g_y = c + df_x$; and $f_x = x/(x+y) = (\#$ cells expressing x at time step $k)$/(total # cells at time step k), where a,b,c,d are non-negative constants. Another program simulates mixed populations comprised of up to four subpopulations adopting different strategies (e.g. x cells, y cells, *RPV* at rates (sx_a, sy_a), and *RPV* at rates (sx_b,sy_b)). This program expands the state space to accommodate all subpopulations, with frequency-dependent growth rates that take the composition of the entire population into account. The former program was used to generate the plot in Fig. 1b, and the latter to generate the plots in Fig. 2. In our simulations, $2 \leqslant a,b,c,d \leqslant 3$ with $a > c$ and $c+d > a-b$.

Contact mailto:dmwolf@lbl.gov for our Matlab m-files.

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