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Slow and Steady Wins the Race: A Bacterial Exploitative Competition Strategy in Fluctuating Environments

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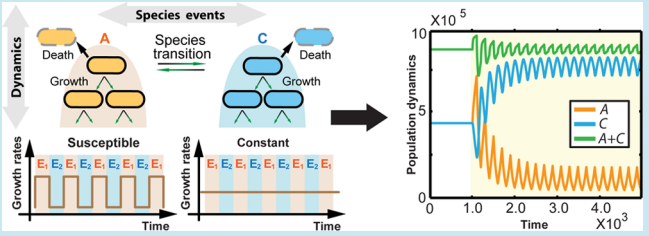
Supporting Information

ABSTRACT: One promising frontier for synthetic biology is the development of synthetic ecologies, whereby interacting species form an additional layer of connectivity for engineered gene circuits. Toward this goal, an important step is to understand different types of bacterial interactions in natural settings, among which competition is the most prevalent. By constructing a two-species population dynamics model, here, we mimicked bacterial growth in nature with resource-limited fluctuating environments and searched for optimal strategies for bacterial exploitative competition. In a simple game with two strategy options (constant or susceptible growth), we found that the species playing the constant growth strategy always outplays or is evenly matched with its competitor, suggesting that constant growth is a "no-loss" good bet. We also showed that adoption of sophisticated strategies enables a species to maximize its fitness when its competitor grows susceptibly. The pursuit of fitness maximization is, however, associated with potential loss if both species are capable of strategy adjustment, indicating an intrinsic risk-return trade-off. These findings offer new insights into bacterial competition and may also facilitate the engineering of microbial consortia for synthetic biology applications.



KEYWORDS: *bacterial exploitative competition, strategic games, fluctuating environments, limited resources, risk and return*

Rapid advances in synthetic biology have illustrated both its power in fostering new understanding of biology and its potential for real world applications.[1−3] Over the past decade, a large set of functional engineered gene circuits have been successfully created,[4−9] among which most have been constructed for single cellular species. Recently, interest has also emerged in engineering microbial consortia,[10] such as predator−prey ecosystems,[11] intra- and inter- species kingdoms,[12] and yeast cooperation populations.[13] The construction of microbial consortia is a natural solution for creating more sophisticated cellular functions as species communities are often robust to environmental fluctuations and versatile for function programming.[10] However, due to the intrinsic complexity of microbial communities, consortia engineering is also more challenging compared with circuits for single cellular species. To facilitate the construction of synthetic microbial consortia, a deeper understanding of how natural bacterial species interact is thus instrumental.

Bacteria are single cell organisms but are present dominantly in nature with the form of complex communities, such as biofilms and the microbiome.[14−17] As one of the major domains of life on earth, they exist in diverse habitats including soil, water, organic matter, and the live bodies of plants and animals.[18,19] Due to the limited resources provided by these natural environments, bacteria face constant battles within communities.

The understanding of bacterial competition has been a focal point for biologists.[20,21] Started by illustrating the relationship between limiting nutrients and bacterial growth,[22,23] the field has advanced significantly over the decades through the discovery of fundamental competition principles,[20,24,25] exploration of basic dynamics between competing organisms,[26−30] investigation of representative game scenarios,[31−33] and examination of contributing factors such as spatial heterogeneity[34,35] and cellular communication.[12] In order to acquire limited resources, bacteria may compete with their neighbors via various direct or indirect fashions,[20,21,36] among which exploitative competition is one of the main forms. In exploitative competition, one species either more efficiently uses or reduces a shared resource, thus depleting the availability of the resource for others.[23,37]

Recent studies have discovered that bacteria may be capable of playing sophisticated exploitative strategies during battles to maximize their gain.[38,39] For instance, bacteria can use a version of "prisoner's dilemma" through chemical communication to consider their options and decide on a course of action when

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faced with life-or-death situations,[40] adopt the time delay effect of quorum sensing to provide an advantageous strategy to regulate their costly but beneficial public goods,[41] and play a resource self-reservation strategy to outperform 'cheater cells' in a snowdirft game.[33]

In addition to resource restrictions, natural environments are often subject to periodic or irregular fluctuations, including variations of nutrient, temperature, pH, and toxin that are all critical to cellular physiology and hence population growth.[42−44] Bacteria, therefore, survive in complex situations where they interact with each other, compete among species, and are simultaneously subject to external environmental fluctuations.

Previous studies have considered the effects of fluctuations and limited resources on phenotypic switching and fitness maximization for a microbial species.[42,43,45−48] Here, we focus on the impact of fluctuating, resource-limited environments on competition between species that can alter their respective growth rates through environmental cues. Specifically, we address the question: How can individual species play exploitative competition strategies to outplay others in such natural environments? Although population dynamics has been a classical topic in evolution,[49,50] systematic understanding of this question, particularly in the context of bacterial strategic games, has remained obscure.

In order to address the question from a quantitative, systematic, and integrative strategic game perspective, we construct a two-species population dynamics model to mimic bacterial competitive growth in resource limited, fluctuating environments and employ it to search for optimal exploitative competition strategies. We first consider two simple strategies: constant growth (environment-insensitive) and susceptibleness (environment-dependent growth), and analyze the outcome of exploitative competition in different strategic scenarios. We then explore optimal strategies that maximize species' fitness and discuss the inherent risk−return trade-off associated with optimization. We conclude by summarizing our findings and discussing their biomedical implications.

RESULTS AND DISCUSSION

Simple Strategies for Exploitative Competition in a Bacterial Community. We began our exploration of bacterial competition strategies by considering a two-species community, as shown in Figure 1A. Here, the species A and C are supplied with a shared limited nutrition source, and thereby, their proliferations follow a logistic growth fashion.[51] The two species also die over time obeying first-order chemical kinetics. In addition, they are mutually switchable, representing a common cellular interaction form. Examples of such a cellular interaction include genotypic transitions, such as gene island excision and integration in *N. gonorrheae*[52] and *fim*BE-controlled DNA inversion in *E. coli*,[53] as well as phenotypic switching, such as the formation of bacterial persistence[54] and bistable transitions in natural and synthetic gene circuits.[4,55,56] Thus, the two species are not required to have different genomes. The detailed biochemical description of the cellular events of this model is presented in Figure 1B.

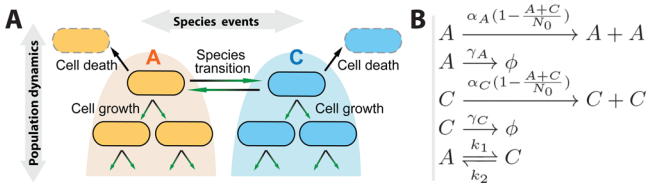

Figure 1. Bacterial community model. (A) Schematic of cellular events occurring within the community. The species A (orange) and C (blue) are both capable of proliferation, apoptosis, and interspecies transition. (B) Kinetic representation of cellular events illustrated in part A. The two species have a growth rate of $\alpha_A(1 - (A + C)/N_0)$ and $\alpha_C(1 - (A + C)/N_0)$, a death rate of γ_A and γ_C, and a transition rate of k_1 and k_2, respectively. N_0 is the carrying capacity of the community.

The population dynamics of this competing community can be described through the following set of coupled differential equations:

$$\frac{dA}{dt} = \alpha_A A\left(1 - \frac{A + C}{N_0}\right) - k_1 A + k_2 C - \gamma_A A$$
$$\frac{dC}{dt} = \alpha_C C\left(1 - \frac{A + C}{N_0}\right) + k_1 A - k_2 C - \gamma_C C \quad (1)$$

where t is time, A and C are the population numbers of the species A and C, α_A and α_C are their maximal growth rates, k_1 and k_2 correspond to the rates of transitions between the species, and γ_A and γ_C are the death rates. In addition, N_0 refers to the carrying capacity of the community constrained by nutrient limitation, and the first terms on the right hand side of the equations represent the logistic growth of each species. A special case of this model, $k_1 = k_2 = 0$, corresponds to the classical competitive Lotka−Volterra equations.[57,58]

Under a static environment where all rates remain constant, the community always achieves a final steady state regardless of its initial condition: the two species are equally represented when all of their rates are identical but the species with a larger growth rate dominates when their rate constants are different (see details in Supporting Information (SI) section 1).

However, the community structure can become sophisticated in realistic cellular niches where environments fluctuate. This is because environmental fluctuations are typically associated with the variation of nutrient, toxin, temperature, and other factors that are critical to cellular growth and survival.[59] To mimic such environments, we assumed the community is subject to two environments, say E_1 and E_2, that alternate with an equal duration time $T/2$ in each environment.

How can one bacterial species play strategies to outcompete the other in such environments? The simplest strategies that cells can play would include environment-susceptible growth, where the growth rate oscillates with changing environments, and, alternatively, constant growth, where the growth rate is independent of environmental perturbations, as shown in Figure 2A. This naturally leads to another question: *Which strategy is better between susceptibleness and constant growth?*

To answer this question, we described these two strategies with a mathematical formulation. Specifically, we assume the growth rate of a species adopting the susceptibleness strategy has an expression as

$$\alpha(t) = \alpha_o(1 + \Delta \cdot \Phi(t)) \quad (2)$$

where t is time, α_o is the growth coefficient, $\Delta \in [0,1]$ is the amplitude of growth rate fluctuation (GRF), and $\Phi(t)$ is a unit periodic square function reflecting the environmental dependence of growth: $\Phi(t) = 1$ in environment E_1 $(0 \le [t/T] < T/2)$ and $\Phi(t) = -1$ in environment E_2 $(T/2 \le [t/T] < T)$, where T is the period of environmental fluctuations and $[t/T]$ is the

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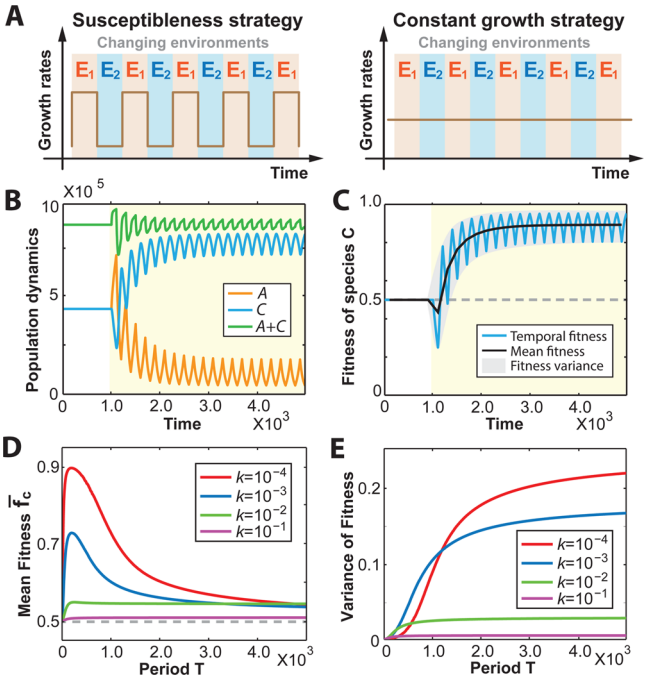

Figure 2. Simple strategies for exploitative competition in resource-limited fluctuating environments. (A) Two strategies for coping with changing environments: susceptibleness strategy (left) where cellular growth depends on the environment and constant growth strategy (right). (B) Representative population dynamics of cellular species in the competition case 3 where the species A and C adopt the susceptibleness and constant growth strategy, respectively. Cellular environments are switched from a constant environment to a fluctuating environment at $t = 1.0 \times 10^3$. (C) The fitness of species C f_c in the competition case 3. The temporal fitness (blue line) has a periodic variation but its average over single periods gives rise to a well-defined mean fitness (black line) and variance (gray shading). (D, E) The period dependence of the mean and variance of species C's fitness. The mean (D) may have a bell-like or sigmoidal shape depending on the parameters, while the variance (E) increases monotonically with the period. The corresponding parameters are given in SI section 7.

residue of t/T. The mean cellular growth rate $(\overline{\alpha(t)})$ is hence equal to α_o. In contrast, the growth rate of the cellular species adopting the constant strategy remains unchanged despite environmental fluctuations, that is,

$$\alpha(t) = \alpha_o \tag{3}$$

where α_o is a constant identical to the α_o in eq 2. These two strategies therefore have the same mean growth rate but different cellular responses to environmental fluctuations. In addition, the death and transition rates of the species are assumed environment-independent and symmetrical for simplicity, that is, $\gamma_A = \gamma_C = \gamma$ and $k_1 = k_2 = k$.

As each of the species has the option to play either of the two strategies, there are a total of four possible strategic scenarios for our two-species community (A, C):

(1) (constant growth, constant growth),
(2) (constant growth, susceptibleness),
(3) (susceptibleness, constant growth), and
(4) (susceptibleness, susceptibleness),

among which the species have equal fitness for both case 1 and case 4 due to the symmetrical structure of the community where reactions rates and responses to external environments are all identical (See SI section 2). In addition, cases 2 and 3 have equivalent dynamics under interchange of the bacterial species; thus, we need only consider case 3.

To evaluate the merit of the competition strategies, we employed species fitness as our metric, which is mathematically defined as the relative abundance of cell populations,[60] that is, $f_a = A/(A + C)$ and $f_c = C/(A + C)$.

For case 3, we assigned species A and C with a growth coefficient in the form of eqs 2 and 3, respectively, and then integrated the community model eq 1 computationally. Figure 2B shows the representative time evolution of cellular populations where fluctuating environments are activated at $t = 1.0 \times 10^3$. The corresponding fitness is obtained in Figure 2C with the blue and black lines corresponding to the temporal and averaged fitness of species C. Interestingly, we found that the mean fitness is higher than 0.5 when the community populations are at steady oscillations, regardless of the community's initial conditions (See SI section 8), suggesting that constant C outperforms susceptible A although they have an identical mean growth rate.

To systematically examine this finding, we performed a set of *in silico* competition assays for different environmental fluctuation periods. We found that, for the entire spectrum we explored, the mean fitness $\overline{f_c}$ is always larger than 0.5 (Figure 2D) although it depends on fluctuation period and may have a maximum at a finite period in certain parameter settings. In contrast, the variance of the fitness $\overline{f_c^2} - \overline{f_c}^2$ increases monotonically with period and achieves a plateau in the long period limit (Figure 2E).

To identify the conditions under which a maximal mean fitness arises at a finite period, we proceeded to conduct a wide spectrum of *in silico* competition experiments by varying the GRF amplitude of species A (Δ_A) and the transition rate (k). We chose these two parameters because the former reflects the strength of growth response to environmental fluctuations and the latter is strongly associated with interspecies flow. Figure 3A shows the maximal mean fitness of species C with respect to Δ_A and k within the period spectrum (0,2000] we scanned. Figure 3B is the corresponding characteristic environmental fluctuation period at which C's maximal fitness is achieved. Clearly, the characteristic period has a phase separation with the existence of a maximal fitness at a finite period in the blue region.

All of our assays converge to the same conclusion that species C outperforms species A in case 3 where C has constant growth and A is susceptible. From a strategic game perspective, this result, along with the equivalence between case 2 and case 3 and the even match of the species in both case 1 and case 4 where they employ the same strategies, suggests that constant growth is a "no-loss" good strategy for competition. Furthermore, in certain circumstances, the constant growth strategy can enable a species to dominate the community even though its average growth rate is smaller than its competitor (see SI section 9), suggesting that the constant growth species, which is "slow and steady", can win even in unfair competitions.

Analytical Investigation of Competition: Constant Growth versus Susceptibleness. To determine if our finding that constant growth is a good strategy holds true in general regardless of parameter settings, we pursued an analytical analysis of the strategic game (case 3) through a perturbation expansion (detailed in SI sections 2–6).

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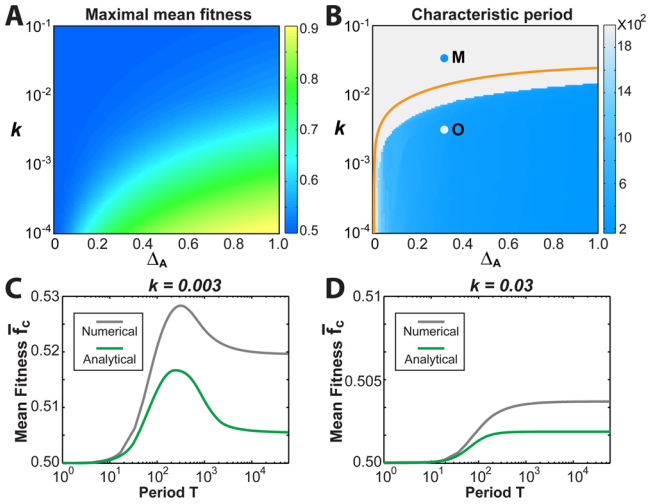

Figure 3. Maximal mean fitness of species C in case 3 competition where A and C adopt the susceptibleness and constant growth strategy correspondingly. (A) Maximal mean fitness with respect to the GRF amplitude Δ_A and transition rate k. (B) The characteristic period of environment fluctuations at which C's mean fitness achieves a maximum. The plot shows two distinct domains with the white domain corresponding to a characteristic period of 2000 (maximal numerically screened period) and the blue domain corresponding to a period of a few hundreds. The orange line is the phase boundary derived from analytical calculation using an approximation to the unit periodic square function, and the two dots (M and O) are representative cases of the two phases. (C–D). Comparisons for the case of $\Delta_A = 0.3$ and $k = 0.003$ (C) and the case of $\Delta_A = 0.3$ and $k = 0.03$ (D), corresponding to the dots O and M in panel B, respectively. The green and gray lines are the results from analytical calculation and numerical simulation accordingly.

We first rewrote the community dynamics model eq 1 in terms of the variables $X = A + C$ and $Y = A - C$ as

$$\frac{dX}{dt} = (\alpha - \gamma)X\left(1 - \frac{X}{N_0\left(1 - \frac{\gamma}{\alpha}\right)}\right) + \alpha\Delta\Phi\left(\frac{X+Y}{2}\right) \times \left(1 - \frac{X}{N_0}\right)$$

$$\frac{dY}{dt} = (\alpha - \gamma)Y\left(1 - \frac{X}{N_0\left(1 - \frac{\gamma}{\alpha}\right)} - \frac{2k}{\alpha - \gamma}\right) + \alpha\Delta\Phi\left(\frac{X+Y}{2}\right)\left(1 - \frac{X}{N_0}\right) \quad (4)$$

where X is total cell population and Y is population difference.

As the GRF amplitude $\Delta \in [0,1]$, we proposed an asymptotic expansion in the long time limit in terms of Δ by plugging in X and Y as $X = X_0 + \Delta X_1 + \Delta^2 X_2 + \ldots$ and $Y = Y_0 + \Delta Y_1 + \Delta^2 Y_2 + \ldots$. By collecting terms according to the order of Δ, we found that the equations satisfied by X_0 and Y_0 have exact solutions with a well-defined long time limit while the remaining orders (X_i and Y_i, $i \geq 1$) in the perturbation expansion have the following form:

$$\frac{d}{dt}\begin{pmatrix} X_i \\ Y_i \end{pmatrix} = \begin{pmatrix} -(\alpha - \gamma) & 0 \\ 0 & -2k \end{pmatrix}\begin{pmatrix} X_i \\ Y_i \end{pmatrix} + \begin{pmatrix} g(\Phi) \\ h(\Phi) \end{pmatrix} \quad (5)$$

where $g(\Phi)$ and $h(\Phi)$ are functions that depend on lower orders of the perturbation series. Notice that the solution to the homogeneous differential equation for every order decays to zero in the long time limit; therefore, we only need to pursue a specific solution based upon the form of Φ. For the case of the unit periodic square function (our model), Φ can be represented as an infinite sum over sines and cosines. Accordingly, the perturbations terms also take the form of sines and cosines with coefficients to be determined by the specific form of g and h.

By determining the first and second order corrections to X and Y, we have an expression for the average fitness of species C as

$$\bar{f}_c \equiv \left\langle \frac{C}{A + C} \right\rangle \approx \frac{1}{2}\left(1 - \frac{\langle Y_1 \rangle}{X_0}\Delta + \left(\frac{\langle X_1 Y_1 \rangle}{X_0^2} - \frac{\langle Y_2 \rangle}{X_0}\right)\Delta^2\right) \quad (6)$$

Here, the first order term averages to zero (See SI sections 3–4), but the second order term yields the first nonzero contribution to fitness, leading to the final result

$$\bar{f}_c \approx \frac{1}{2}\left(1 + \sum_{\text{odd } n} \frac{\gamma\Delta^2}{n^2\pi^2 k}\left(\frac{(\alpha - \gamma)^2}{\left(\frac{2\pi n}{T}\right)^2 + (\alpha - \gamma)^2}\right)\right) \quad (7)$$

As all values of α, γ, k, Δ, T, and n are nonnegative, the second term of eq 7 is nonnegative definite, suggesting that the fitness f_c has a value equal or larger than 0.5 always. This result hence demonstrates that the constant growth strategy is a no-loss good strategy to the order of Δ^2.

It is worthy of notice that the above conclusion holds true for any periodic, zero-mean fluctuating environment (i.e., not limited to square wave-like fluctuating environments). This is because any periodic signal is essentially a superposition of a set of sine and cosine functions (Fourier series), and every sine and cosine function contributes positively to the overall fitness (See SI section 5 for details).

In addition to supporting the main finding, our analytical calculation also enables us to explore secondary features associated with higher orders of Δ in the expansion, including the period dependence of the average fitness. By extending the serial expansion of Δ to the fourth order, we can determine the fitness as

$$\bar{f}_c \approx \frac{1}{2}\left(1 + \left(\frac{\langle X_1 Y_1 \rangle}{X_0^2} - \frac{\langle Y_2 \rangle}{X_0}\right)\Delta^2 + \left(\frac{\langle X_1^3 Y_1 \rangle}{X_0^4} + \frac{\langle X_1 Y_3 \rangle}{X_0^2} + \frac{\langle X_2 Y_2 \rangle}{X_0^2} + \frac{\langle X_3 Y_1 \rangle}{X_0^2} - \frac{\langle X_1^2 Y_2 \rangle}{X_0^3} - \frac{2\langle X_1 X_2 Y_1 \rangle}{X_0^3} - \frac{\langle Y_4 \rangle}{X_0}\right)\Delta^4\right) \quad (8)$$

where a normalized, first order approximation to the square function is used (see SI section 3).

As the fitness maximum occurs when the derivative of $\bar{f}_c$ with respect to the period T equals zero, we can search for the existence of a maximum by finding the characteristic period, which is equivalent to solving for the roots of a polynomial equation in T (see SI section 4). Our calculation gives rise to a boundary (orange line in Figure 3B) that separates the maximal fitness into two regimes: The fitness achieves a maximum at a finite value of T below the boundary; above the boundary, the maximum occurs at $T \to \infty$. Furthermore, we plotted a

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comparison of the analytical and computational results with given sets of k and Δ in Figure 3C–D, showing qualitative agreement in the period dependence of maximal fitness.

Searching for Optimal Competition Strategies. Both our analytical calculations and computational simulations have shown that constant growth is a good competition strategy in coping with changing environments. But, *is constant growth the optimal strategy*?

To answer this question, we conducted a systematic survey of more sophisticated competition strategies by enabling the species to respond differently to fluctuating environments and evaluating how the modulations of growth parameters impact the outcome of competition. Mathematically, this can be implemented by assigning the growth of both species (A and C) with a generalized form of susceptible growth (eq 2), that is, $\alpha_s(t) = \alpha_0(1 + \Delta_s\Phi_s(t))$ (s = A or C) but allowing their coefficients to be tunable. Here, Δ_s is the GRF amplitude of the species s, and $\Phi_s(t)$ is the corresponding unit growth fluctuation function that has a periodic expression as

$$\Phi_s(t) = \begin{cases} 1, & \text{if} \quad 0 \le \left[\frac{1}{T_s}\left[\frac{t}{T_s}\right] + \frac{\phi_s}{2\pi}\right] < 1/2 \\ -1, & \text{if} \quad 1/2 \le \left[\frac{1}{T_s}\left[\frac{t}{T_s}\right] + \frac{\phi_s}{2\pi}\right] < 1 \end{cases}$$

where T_s is the period of growth fluctuation, ϕ_s is its initial phase, and the bracket "[•]" refers to the residue of the term "•".

To search for optimal competition strategies, we examined the mean fitness of species C ($\bar{f}_c$) by systematically altering the amplitudes (Δ_A, Δ_C), phases (ϕ_A, ϕ_C), and periods (T_A, T_C), as illustrated in Figure 4A accordingly. We chose to examine the role of amplitude modifications due to the fact that biological organisms and their underlying genetic networks have often evolved to generate appropriate cellular response amplitudes for coping with fluctuating environmental stimuli such as nutrients, pH value, and temperature.[44,61] In addition, architectural features of signaling and genetic regulatory networks, such as cascade size, can influence the time needed for detection and propagation of an external signal, resulting in variable phase lags in response to environments.[62] Thus, we were motivated to examine how the adjustment of phase lag would affect competition outcomes. To give a complete picture of competition strategies, we also considered tuning the period of the response for environmental fluctuations, although this is less biologically applicable.

Figure 4B shows C's mean fitness profile with respect to the GRF amplitudes of the two species. This profile has a contour of $\bar{f}_c = 0.5$ along the diagonal ($\Delta_A = \Delta_C$), suggesting a neutral competition under this situation. This corresponds to case 4 in the simple competitions discussed earlier and the equal fitness arises from the identity of the two competing species in terms of their growth, death and transition rates. The symmetry argument also holds true at the origin ($\Delta_A = \Delta_C = 0$) corresponding to case 1 where both species employ the constant growth strategy. In addition, the lower horizontal borderline ($\Delta_A = 0$) and the left vertical borderline ($\Delta_C = 0$) represent case 2 (constant growth, susceptibleness) and case 3 (susceptibleness, constant growth) in the simple competitions accordingly. Moreover, the fitness landscape is split by the line ($\Delta_A = \Delta_C$) into two domains with the one above the diagonal having a fitness higher than 0.5 and the one below having a fitness lower than 0.5, leading to the conclusion that the species with a smaller GRF amplitude always wins in the competing community.

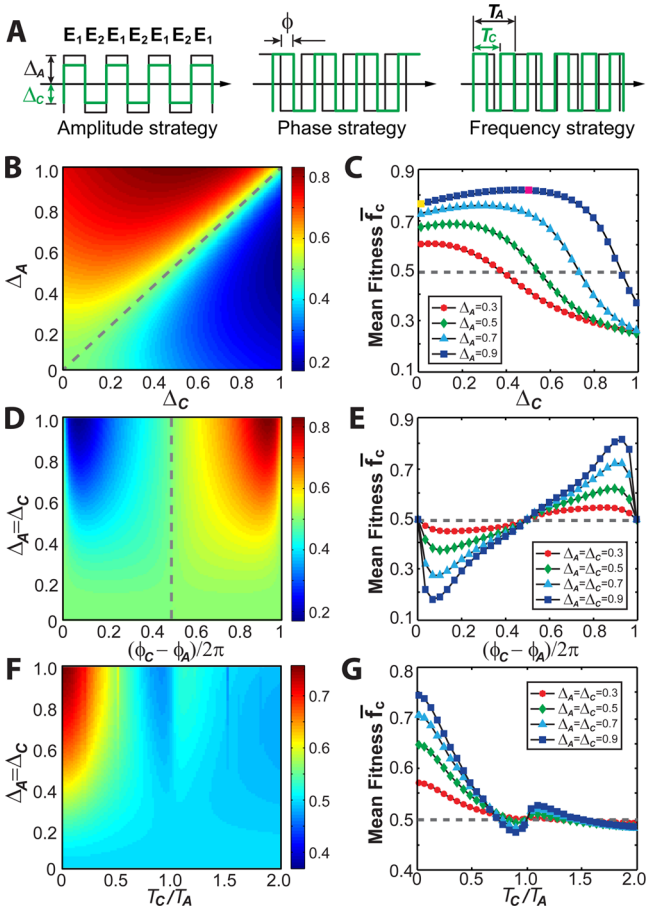

Figure 4. Searching for optimal competition strategies. (A) Three possible strategies for optimizing competition: fine-tuning of the amplitude, phase lag, and period of growth rate fluctuations (GRF). (B) The mean fitness $\bar{f}_c$ as a function of the GRF amplitudes Δ_A and Δ_C. (C) Horizontal cross sections of panel B at different values of A's GRF amplitude. The nonmonotonic dependence of the fitness indicates that the species C can optimize its gain with appropriate tuning of its GRF amplitude. (D) The mean fitness $\bar{f}_c$ with respect to the phase lag $\phi_C - \phi_A$. (E) Horizontal cross sections of panel D at different amplitudes. The species C has an elevated fitness when the phase lag is within $(\pi,2\pi)$. (F) The mean fitness $\bar{f}_c$ as a function of the ratio of the periods (T_C/T_A). (G) Horizontal cross sections of panel F at different common amplitudes. Parameters are detailed in SI section 7.

Figure 4C illustrates the horizontal cross sections of the mean fitness landscape at different GRF amplitudes of the species A (Δ_A). Interestingly, C's mean fitness is not necessarily a monotonically decreasing function of its GRF amplitude (Δ_C) but instead could increase for a given GRF amplitude of the species A. For example, the fitness at $\Delta_C = 0.5$ (pink square) is higher than the fitness at $\Delta_C = 0$ (yellow square) for the case of $\Delta_A = 0.9$. This suggests that, adoption of susceptible growth with an appropriately chosen GRF amplitude may confer a fitness higher than that of constant growth ($\Delta_C = 0$). This also means that, although the constant growth strategy is a "no-loss" good strategy, it may not be optimal. Instead, an adapted

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version of the susceptibleness strategy may produce an augmented fitness.

In addition to GRF amplitudes, GRF phases can also impact the species' fitness. As depicted in Figure 4D, the mean fitness ($\bar{f}_c$) varies significantly as the phase lag (the difference of the phases, i.e., $\phi_C - \phi_A$) changes, although the GRF amplitudes of both species remain the same. However, the fitness ($\bar{f}_c$) remains at 0.5 for $\phi/2\pi$ = 0,0.5,and 1 despite their GRF amplitudes. The evenness of the fitness at $\phi/2\pi$ = 0 and $\phi/2\pi$ = 1 comes from the complete symmetry of the competition while the evenness at $\phi/2\pi$ = 0.5 is due to the fact that the two species' strategies are opposite in phase and the average of the fitness over a period results in a symmetrical structure as well. In addition, the line $\phi/2\pi$ = 0.5 serves as a separatrix that divides the fitness landscape into two domains: A fitness lower than 0.5 in the left domain and higher than 0.5 in the right, suggesting that a winning strategy needs to have a phase lag within $(\pi,2\pi)$. The profile of the landscape further suggests that, for a given amplitude, the fitness can be potentially maximized: it can be augmented considerably with appropriate fine-tuning of the phase lag (e.g., $\phi/2\pi \simeq 0.93$ for the case of $\Delta_A = \Delta_C = 0.9$) as shown in Figure 4E.

Moreover, GRF periods (frequencies) also affect competitions. Figure 4F, G shows the species C's fitness with respect to the ratio of the periods (T_C/T_A). As the magnitude of population number fluctuations decreases with GRF period and diminishes when the period approaches zero, the boarder line (T_C/T_A = 0) corresponds effectively to the case of constant growth of species C. As the modulation of GRF period generally reduces the species' fitness (Figure 4G) and both of the left border lines of Figure 4B and Figure 4F correspond to case 3 (susceptibleness, constant growth) in the simple competitions, comparison of the two panels suggests that modulation of GRF amplitude, rather than period, is favored for strategy optimization.

Risk Association of Fitness-Maximized Strategies. Our survey has shown that, compared with the simple constant growth strategy, an increased fitness may be conferred by sophisticated strategies through appropriate GRF modulation. On the other hand, it is widely acknowledged in social and economic sciences that maximal gain is often associated with increased risk. The similarity of bacterial competition with social and economic settings naturally leads us to ask another question: *Is there any potential risk associated with greed (fitness maximization) of bacteria in competition?*

To answer this question, we revisited the optimal competition strategies explored in Figure 4. Clearly, appropriate modulation of GRF factors, such as amplitude and phase, can give rise to a fitness higher than that from the constant growth strategy; however, arbitrary strategy adjustment does not necessarily aid in the increase of fitness. Indeed, the species C's mean fitness is lower than 0.5 in half of the GRF amplitude space (the half below the diagonal of Figure 4B) as well as half of the phase lag space (the $(0,\pi)$ domain of Figure 4D). This suggests potential loss during maximization: the fitness of a species employing sophisticated strategies can be lower than the 'safe bet' (constant growth strategy) if its GRF is not correctly tuned.

The risk of potential loss can be exemplified in situations where both competing species are capable of sensing each other's strategy and adjusting their own, particularly when the response time required for GRF modulation is species dependent. To illustrate this idea, we considered a simple competition scenario where species A and C are employing the susceptibleness (Δ_A = 0.9) and constant growth (Δ_C = 0.0) strategies respectively (Figure 5A, light green window) but A

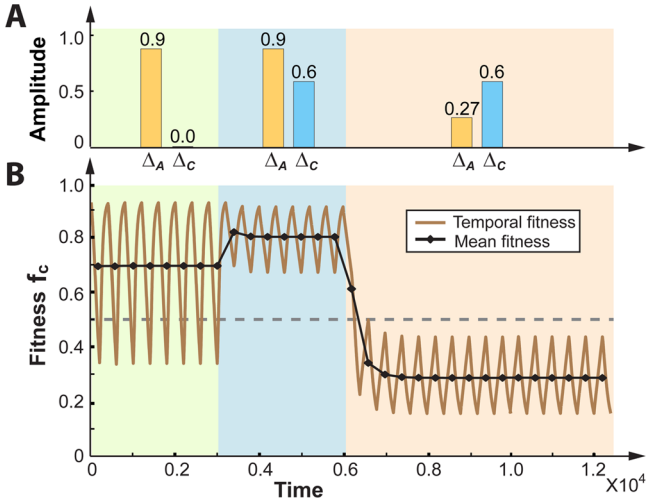

Figure 5. Possible loss during the pursuit of maximal fitness. (A) Dynamic strategy adjustments via GRF amplitude modulation in a competition where both species are capable of sensing each other's strategy and adjusting their own. (B) Corresponding temporal (brown line) and mean (black line) fitness of species C. During a game started with species A being susceptible and C utilizing constant growth (Δ_A = 0.9, Δ_C = 0) (light green window), species C can further increase its mean fitness from 0.70 to 0.81 by adjusting its strategy from constant growth to susceptibleness with a GRF amplitude of 0.6 (light blue window). C's pursuit of maximal fitness, however, may lead to its loss if the competing species A is able to quickly adjust its own GRF amplitude to 0.27 after sensing the strategic change of C (light orange window), resulting in a mean fitness of 0.29 for species C.

has a much shorter response time than C. In this case, C outperforms with a mean fitness of 0.70 (Figure 5B). If C pursues a maximal gain by turning its strategy into sophisticated susceptibleness with a GRF amplitude of Δ_C = 0.6, its fitness can be elevated to 0.81 accordingly (light blue window). However, because of its short response time, species A can quickly adjust its own strategy by using a smaller GRF amplitude (Δ_A is tuned from 0.6 down to 0.27), which will cause a fitness loss for species C ($\bar{f}_c$ = 0.29) in a long time run (light orange window).

This example illustrates that optimal strategies that maximize species fitness are associated with potential loss. Conversely, the constant growth strategy has a lower fitness gain but no risk for loss. These facts collectively reveal an intrinsic high risk-high return relationship in bacterial strategic games and also suggest the need for rational balance between gain maximization and risk minimization during competition.

To explore a more realistic dynamic population competition, we simulated a two-species competition game with each strain having an equal capability for adaptive strategy adjustment. For simplicity, we assumed that both species have two adaptive strategy options: increasing amplitude by 0.05 or decreasing amplitude by 0.05. In addition, at the end of every single period, both species can adjust their strategies depending on their fitness: a species adopts the same strategy as in the previous period if its fitness increases through the previous period; otherwise, the opposite strategy is adopted. Interestingly, our simulations (SI section 10) show that the fitness for

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both species approaches 0.5 over time, suggesting an equilibrium of the population competition.

Conclusion and Outlook. Bacteria face frequent battles for resources provided by environments where they inhabit. To explore how bacteria play strategies to outperform others, we have herein systematically evaluated different strategies by using a two-species population dynamics system as a community model. Our study showed that constant growth is a good strategy that is always unbeatable (conferring a fitness of 0.5 or higher). However, it may not be optimal. In the case when one species grows susceptibly, its competitor can acquire a fitness higher than that from the constant growth strategy by employing an optimized version of the susceptibleness strategy. The pursuit of fitness maximization is, however, associated with potential loss if susceptibleness is not appropriately tuned. Moreover, the risk of loss can be exemplified when both species are capable of strategy adjustment. In contrast, persistence has no risk of loss although it confers a smaller fitness. These results hence illustrate an intrinsic risk-return trade-off and suggest a guideline for strategic decision-making: a species shall take a 'safe bet' (constant growth) if risk minimization is mandatory but adopt the optimal strategy (finely tuned susceptible growth) when maximal gain is required.

The above findings are important for understanding bacterial communities, such as biofilms and the human microbiome, by revealing their establishment, compositional evolution, and functionality, and may also benefit in deciphering the development of infectious diseases where pathogenic bacteria populate in patients. Moreover, the importance of appropriate strategy selection further suggests that there may be a potential solution for treating diseases and reducing the rise of antibiotic resistance by employing commensal bacteria to outcompete target pathogens.

With our bacterial community model, we have caught a glimpse of the alluring world of cellular competition. In addition to the exploitative competition illustrated here, bacteria also combat through different fashions, such as contest competitions that involve direct antagonistic interactions.[20] Moreover, stochasticity and spatial heterogeneity of population growth can considerably shape cellular competition and corresponding outcomes as well.[33,34,63,63−65]

Beyond theoretical exploration, experimental validation of our theory has also become possible due to rapid advances in synthetic biology. For instance, DNA invertase systems[66] that confer digital DNA reconfiguration in cellular logic computation circuits[67,68] can be potentially adopted for the implementation of differential cellular responses to environmental fluctuations. In addition, cutting-edge microfluidic platforms have enabled dynamic control of cellular microenvironments[44] as well as long-term observations of bacterial populations,[69] which collectively offer an ideal testbed for monitoring bacterial competition. It will thus be feasible and highly interesting to test our theoretical predications using synthetic bacterial populations in the future.

Through both computational and analytical studies, we have uncovered good and optimal competition strategies, illustrated the importance of strategic decision-making, and further revealed the intrinsic association between risk and return, shedding light on the complex but fascinating world of bacterial competition. In addition, the knowledge acquired from these findings will also facilitate the advance of synthetic biology as the field moves from the engineering of gene circuits in single species to microbial consortia for the innovation of sophisticated biological functionality.

METHODS

Numerical Methods and Parameters. Numerical simulations of this work were implemented based on the mathematical framework, eq 1. ODE45 in Matlab was used to solve the differential equations for various parameters. The following parameter values were adopted throughout the paper unless otherwise noted: $N_0 = 10^6$, $A_0 = 1$, $C_0 = 1$, $\alpha_0 = 0.1$, $\gamma = 0.02$, and $T = 200$.

In Figure 2B, C, the initial condition $A_0 = C_0 = 4 \times 10^5$ and the GRF amplitude $\Delta_A = 0.8$ are used along with the default parameters. The cellular environment was constant at the beginning but was switched to be fluctuating at $t = 1.0 \times 10^3$. The mean fitness $\bar{f}_c$ is acquired by averaging over single periods when the community is in steady oscillation. In Figure 2D, E, the mean and variance of species C's fitness as functions of the period of environmental fluctuations were plotted for the cases of $k = 0.0001$, 0.001, 0.01, and 0.1. Here, $\Delta_A = 0.8$ is used.

In Figure 3A, B, we calculated the maximum fitness $\bar{f}_c$ and its corresponding fluctuating period for changing environments in terms of the parameters k and Δ_A. We considered square waves Φ with periods in the range (0,2000]. In Figure 3C, D, we plotted the mean fitness of species C from analytical calculation and numerical simulation. $\Delta_A = 0.3$ and $k = 0.003$ for panel c, and $\Delta_A = 0.3$ and $k = 0.03$ for panel d, corresponding to the two dots O and M in Figure 3B respectively.

In Figure 4, we illustrated possible strategy optimizations through the fine-tuning of the amplitudes, phases, and periods of the GRFs. For the case of amplitude strategy (panels b and c), we scanned the parameter regimes of $\Delta_A \in [0,1]$ and $\Delta_C \in [0,1]$ with $T_A = T_C = 200$. For the case of phase strategies (panels d and e), we explored the parameter space of $\Delta_A = \Delta_C \in [0,1]$ and $\Phi/2\pi \in [0,1]$ with $T_A = T_C = 200$. For the frequency strategies (panels f and g), the stable period in the steady state is the least common multiple of T_A and T_C; therefore, the mean fitness is calculated by averaging over this stable period. We explored the parameter space of $T_C/T_A \subseteq (0,2)$ and $\Delta_A = \Delta_C \in [0,1]$ with $T_A = 200$. $k = 0.001$ was used for all panels of Figure 4.

In Figure 5, we explored the potential loss during the pursuit of maximal fitness when both species use an amplitude strategy and are capable of sensing their competitor's strategy and adjusting their own. The community was modeled with $\Delta_A = 0.9$ and $\Delta_C = 0$ in the light green window, followed by a second window (light blue) with $\Delta_A = 0.9$ and $\Delta_C = 0.6$, and a third time window (light orange) with $\Delta_A = 0.27$ and $\Delta_C = 0.6$. The corresponding mean fitness is $\bar{f}_c = 0.70$, 0.81, and 0.29 respectively. $T = 400$.

Analytical Results. We employed an asymptotic approach (expansion in Δ) for our model and obtained approximate results for the mean fitness of both species in fluctuating environments. The details of the calculations for the terms in the expansion, the expected regime of validity, and the time averaged fitness can all be found in SI sections 1−6.

ASSOCIATED CONTENT

Supporting Information

Detailed explanations of the simulations and analytical calculations along with the parameters used in the figures. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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