# A simple rule for the evolution of fast dispersal at the edge of expanding populations

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**Abstract**

Evolution by natural selection is commonly perceived as a process that favors those that replicate faster to leave more offspring; nature, however, seem to abound with examples where organisms forgo some replicative potential to disperse faster. When does selection favor invasion of the fastest? Motivated by evolution experiments with swarming bacteria we searched for a simple rule. In experiments, a fast hyperswarmer mutant that pays a reproductive cost to make many copies of its flagellum invades a population of mono-flagellated bacteria by reaching the expanding population edge; a two-species mathematical model explains that invasion of the edge occurs only if the invasive species' expansion rate, $v_2$, which results from the combination of the species growth rate and its dispersal speed (but not its carrying capacity), exceeds the established species', $v_1$. The simple rule that we derive, $v_2 > v_1$, appears to be general: less favorable initial conditions, such as smaller initial sizes and longer distances to the population edge, delay but do not entirely prevent invasion. Despite intricacies of the swarming system, experimental tests agree well with model predictions suggesting that the general theory should apply to other expanding populations with trade-offs between growth and dispersal, including non-native invasive species and cancer metastases.

## Introduction

Natural selection is commonly perceived as a process that favors species that grow in numbers by reproducing faster and surviving better. In expanding populations, however, species face a choice between allocating resources to growth or to disperse faster (1). Expanding populations consume local resources; the resource availability is highest outside the population range, which creates an advantage to being at the population margin (2). Fast-dispersing species take advantage of this spatial heterogeneity: they take over the edge, cutting-off competitors' access to growth-limiting resources (1, 3). The role of resource limitation in evolutionary processes, such as gene surfing (4) and the founder effect (5), has been analyzed before; however, crucial questions remain: When does natural selection favor dispersal over growth? How much growth can a fast-dispersing species forgo and still be favored by natural selection?

Invasiveness in expanding populations has global implications at many levels (6): non-native species invade due to human introduction and climate change (7), expanding tumors become invasive cancers (8) and antibiotic-resistant bacteria spread due to global travel (9). Predicting the evolution of expanding populations, however, is a non-trivial problem. Dispersal and growth are sometimes under an allocation trade-off where investing in one trait leaves fewer resources available for the other (10-13). Mathematical modeling could help address these complex questions by defining the conditions favoring fast dispersal.

Testing model predictions with field studies in traditional ecology is challenging and experimental manipulation of natural ecosystems is often impractical. The shortage of robust empirical systems to test mathematical models stalls the development of predictive theory. Laboratory experiments with microbes can provide rigorous tests of mathematical models (5, 14-17); microbes have large populations sizes, short generation times, affordable DNA sequencing and—in many cases—tools for genetic engineering. We recently discovered that experimental evolution in swarming colonies of the bacterium *Pseudomonas aeruginosa* leads to the spontaneous evolution of hyperswarmers (18). Hyperswarmer mutants have a single point mutation in a gene called *fleN;* their cells are multiflagellated, and therefore more dispersive. Importantly, the many flagella come at the cost of a slower growth (table S1). *P. aeruginosa* wild-type outcompete hyperswarmers in well-mixed liquid media; hyperswarmers, on the other hand, swarm faster on semi-solid surfaces (19) and outcompete the wild-type in swarming competitions (18).

Here we exploited the differences in growth rate and dispersal between the wild-type *P. aeruginosa* and its hyperswarmer mutant to motivate, and then experimentally test, a mathematical model of invasion in expanding populations. We based our analysis on a well-established principle of spatial expansion in growing populations: the traveling wave derived from the Fisher-Kolmogorov-Petrovsky-Piscunov (F-KPP) equation. The F-KPP equation, in its original form, describes a 1-D monospecies population (20-22). We expanded the F-KPP equation to investigate the conditions favoring invasion of a species with different dispersal and growth rates and solving that two-species system produced a simple rule to determine the outcome of invasion. Somewhat surprisingly, this simple rule had not been proposed before to

the best of our knowledge, despite much theoretical and experimental work in this field. Simulations reveal the conditions at which the rule is applicable, and the time-scales necessary for invasion in biologically relevant situations. Quantitative experiments with *P. aeruginosa* hyperswarmers indicated that our model may predict the evolution of expanding populations in a wide range of biological scenarios.

## Results

### *Modeling swarming in* P. aeruginosa *with the F-KPP equation*

*P. aeruginosa* populations swarm across agar gels containing nutrients and form branched colonies. Bacteria at the branch tips travel at a nearly constant rate by dividing and dispersing (19). Each growing tip consumes resources in its vicinity and thus forms a nutrient gradient (16) that drives a resource-limited growth similar to the F-KPP model. Knowing that cell sizes have a positive correlation with growth (23) we compared the size of cells collected from the tip of a branch with the size of cells collected behind the tip; cells at the tip were longer indicating faster growth at the edge of the population (Fig. S1). We then modeled *P. aeruginosa* swarming as a one-dimensional expanding population according to the F-KPP equation:

$$\frac{\partial u}{\partial t} = ru\left(1 - \frac{u}{K}\right) + D\frac{\partial^2 u}{\partial x^2} \quad (1)$$

where $x$ is space, $t$ is time, $u$ is the local population density, $K$ is the carrying capacity, $r$ is the maximum per-capita growth rate and $D$ quantifies dispersal. Growth and dispersal can obey different laws in nature; for generality the F-KPP equation assumes logistic growth, where the per-capita growth rate decreases linearly as the population density increases, leading to a decrease in resource availability, and assumes Fickian diffusion for dispersal. The F-KPP equation suits the common scenario where regions with excess of nutrients lie outside the population and determine the direction of expansion. The resource availability, represented by $1 - \frac{u}{K}$, is highest outside the population range and growth, represented by $ru(1 - \frac{u}{K})$, occurs mostly at the edge of the population. Eq. (1) has a traveling wave solution, $u(x,t) = u_0(x - vt)$, where the population front travels at a constant expansion rate $v = 2\sqrt{rD}$ and its density increases from the edge with a length-scale $\lambda = \sqrt{D/r}$ (Movie S1 and Fig. S1) (4, 24). In summary, the F-KPP equation—where low-density at the leading edge drives population expansion—has a traveling wave solution where the expansion rate depends on both growth and dispersal, but is independent of the carrying capacity.

### *A simple rule for the evolution of faster dispersal*

Hyperswarmers grow slower in well-mixed liquid media due the cost of synthesizing multiple flagella, but—thanks to their faster dispersal on semi-solid surfaces—outcompete the wild-type in spatially structured environments (18, 19). In semi-solid surfaces lacking spatial structure hyperswarmers are outcompeted, as expected (Fig. S2). At the micrometer scale, an expanding population of hyperswarmers displays patterns of active turbulence typical of dense bacterial suspensions, which is different from the wild-type where cells remain nearly static even

at the tips of swarming tendrils (Movie S2). To gain a better understanding of the competition dynamics in expanding swarming colonies we mixed wild-type bacteria (labeled with a red fluorescent protein) with hyperswarmers (labeled with a green fluorescent protein) at 10:1 ratio; we then used time-lapsed florescence imaging to film the swarming competition (Fig. 1A). The time-lapse showed that hyperswarmers quickly reached the population edge, increasing their dominance as the colony expanded to win the competition (Movie S3).

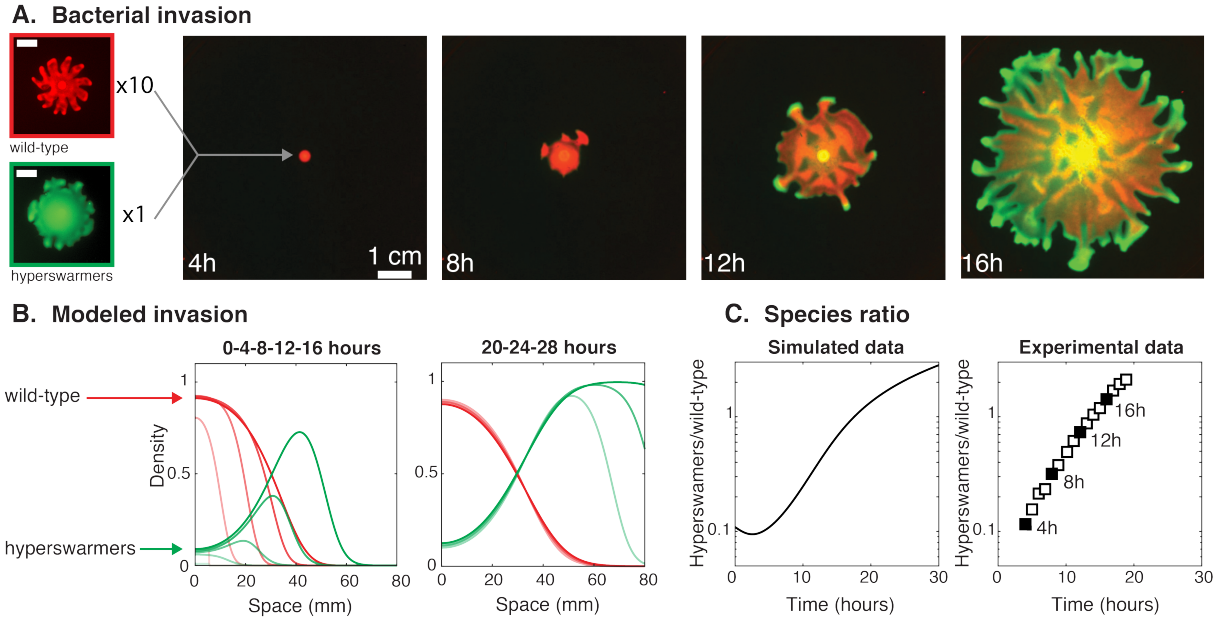


**Figure 1: Invasion dynamics in an expanding population of swarming *P. aeruginosa* bacteria.** A: Fluorescence time-lapse imaging shows a swarming competition in a mixed population of wild-type and hyperswarmers at 10:1 ratio and on an soft-agar gel. Leftmost panels: Monoclonal swarming colonies of wild-type (top) and hyperswarmer (bottom) imaged at $\boldsymbol{T} = \mathbf{12}$h (scale bars 1 cm). B: Snapshots of numerical simulations of Eq. 2 modeling competition of *P. aeruginosa* (red lines) and the hyperswarmer mutant (green lines) with initial ratio 10:1, using parameters extracted from experiments (Table S1). Left panel shows time points represented in panel A. Right panel shows later time points, where the wild-type population stalls while the hyperswarmer population keeps expanding. C: Ratio of hyperswarmer biomass over wild-type biomass. The experimental data is extracted from fluorescence signals in panel A (black squares represent time points shown in A).

To determine the conditions favoring evolution of faster dispersal we used an extension of the F-KPP equation for a two-species system. Species 1—representing the wild-type—has density function $u_1(x,t)$, disperses with coefficient $D_1$ and grows with a rate $r_1$; species 2—representing the hyperswarmer—has $u_2(x,t)$, $D_2$, and $r_2$. For simplicity, and according to data for the hyperswarmer system (18) (Fig. S3), we assumed that both species have the same carrying capacity, which we normalized to 1. We assumed that their dispersal rates, determined by $D_1$ and $D_2$, are independent.

In this model, the only traits that may be improved in species 2 are its dispersal ($D_2$) and growth ($r_2$). The two-species system has two coupled F-KPP equations (25-28):

$$\frac{\partial u_1}{\partial t} = r_1 u_1 (1 - u_1 - u_2) + D_1 \frac{\partial^2 u_1}{\partial x^2}$$
$$\frac{\partial u_2}{\partial t} = r_2 u_2 (1 - u_1 - u_2) + D_2 \frac{\partial^2 u_2}{\partial x^2} \quad (2)$$

Species 1 and 2 interact only by competing for the same resources, a feature implemented by the factor $1 - u_1 - u_2$. This framework is well established and it was used previously to investigate competition in various contexts of range expansion (17, 25-27, 29-36), including a linear trade-off between dispersal and growth (28). However, previous studies did not continue to derive a general rule to determine the invasion outcome for all possible values of dispersal and growth.

To derive that rule, we first investigated the conditions that allow an introduced population to invade and replace the resident population at the expansion front. We could determine analytically that invasion at the edge occurs only if the expansion rate of species 2 exceeds that of species 1 (see mathematical demonstration in *SI Appendix 2: Analytical solution for the condition of invasion*):

$$v_2 > v_1 \quad (3)$$

where $v_1 = 2\sqrt{r_1 D_1}$ and $v_2 = 2\sqrt{r_2 D_2}$ are the expansion rates of each species when grown alone.

Eq. 3 is a simple rule for invasion of the expansion edge. The intuition behind invasion dynamics is well illustrated in a simulation of the competition between an established species (species 1) and an invader with faster dispersal but slower growth (species 2), which we simulated (Fig. 1B) by numerically solving the system in Eq. 2 with parameters corresponding to the hyperswarmer system ($r_2/r_1 = 0.9$ and $D_2/D_1 = 2$, Table S1). Species 2, initially homogeneously mixed with species 1, outcompetes species 1 once it reaches the leading edge. The invasion succeeds despite the growth disadvantage of species 2 because its faster dispersal enables it to reach the low-density edge where it can take advantage of the resources available. Once species 2 dominates the edge, species 1 is left behind in the high-density region where growth has stopped. Over time, the global frequency of species 1—blocked by species 2 from reaching the edge and incapable of growing further—decreases in frequency whereas species 2 keeps increasing thanks to its edge domination (Fig. 1C, left). These simulation results are consistent with experimental tests conducted here (Fig. 1C, right) and also with the original experiment that led to evolution of hyperswarmers (18), which clearly showed that *fleN* mutants would outcompete the wild-type to extinction given sufficient competition time on swarming plates.

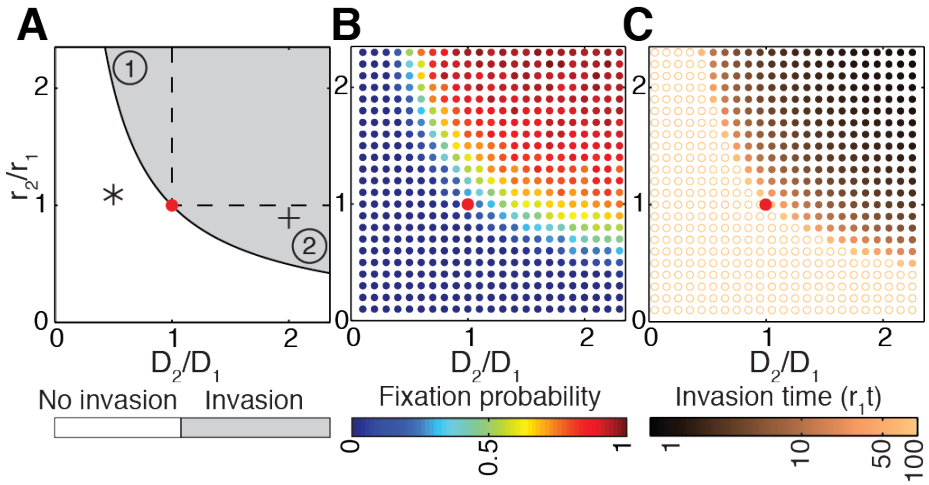


**Figure 2. Faster-dispersing species invade expanding populations despite growing slower as long as $v_2 > v_1$.** A: Phase diagram of invasion in $r_2/r_1$, $D_2/D_1$ space, with 2 subdomains of interest in the invasive domain: (1) is the domain of invasion with lower growth rate and faster dispersal. (2) is the domain of invasion with faster dispersal but lower growth rate. The (+) symbol represents the hyperswarmer phenotype with respect to wild-type phenotype. The (*) symbol represents the wild-type phenotype with respect to the hyperswarmer phenotype. B: Fixation probability obtained from the stochastic model with death rate (stochastic simulations performed with $S = 1$, $K = 100$, $L = 2\lambda_1$). C: Invasion time obtained from the deterministic model (simulations performed with $L = 2\lambda_1$ and $S = 0.2$). The empty orange circles represent absence of invasion within the duration of the simulation ($r_1 t = 180$). In A-C, the red dot depicts the reference point ($D_2 = D_1$ and $r_2 = r_1$).

The invasion rule obtained from the two species system (Eq. 3) states that the evolution of an expanding population is entirely determined from the growth and dispersal rates. Importantly, and similar to the expansion rate obtained for a monospecies traveling wave, the invasion rule is independent of the carrying capacity of each species (See Fig. S4 for confirmation with numerical simulations).

The invasion rule leads to a diagram that delineates the invasion outcome in a growth-dispersal space (Fig. 2A). This diagram shows two trivial domains: when both growth and dispersal of species 2 are lower ($D_2 < D_1$ and $r_2 < r_1$), invasion does not occur because $v_2$ is always lower than $v_1$. Numerical simulations explain that for very low values of $D_2$ and $r_2$ species 1 continues to expand and travel at constant expansion rate whereas species 2 spreads out and stalls (Movie S4, bottom left panel, and Fig. S5A). When growth and dispersal of species 2 are greater ($D_2 > D_1$ and $r_2 > r_1$), species 2 invades because $v_2$ is always higher than $v_1$: species 2 grows rapidly, moves to the front where it reaches the active layer and outcompetes species 1 (Movie S4, top right panel, and Fig. S5B).

The two domains where one trait is higher and the other is lower are less trivial, but arguably more relevant because of the trade-off between dispersal and growth commonly found in nature (6). Domain 1 of Fig. 2A shows that invasion occurs if species 2 disperses slower than species 1 as long as its growth rate is sufficiently higher ($r_2 > r_1\, D_1/D_2$, Movie S4, top left panel, and Fig. S5C). Domain 2 shows that species 2 invades even if—like the hyperswarmer—it grows slower than species 1 as long as it disperses fast enough such that $D_2 > D_1\, r_1/r_2$. A fast-

193 dispersing invader reaches the active layer where it can gain better access to growth-limiting
194 resources and outcompete the established species (Movie S4, bottom left panel, and Fig. S5D).
195 The simple invasion rule, therefore, makes quantitative predictions of invasion outcome
196 that hold true in our experimental system despite intricacies such as the large-scale branching
197 and small-scale turbulence. Hyperswarmers have a ~100% increase in dispersal ($D_2/D_1 \sim 2$) that
198 comes at a ~10% growth rate cost ($r_2/r_1 \sim 0.9$) (Movie S2 and Table S1). Therefore the
199 experimental system falls into domain 2 of the invasion diagram (cross symbol in Fig. 2A).
200 We measured the frequency of hyperswarmers within the first millimeter of the colony
201 from video frames and saw that it increased exponentially with a rate $\tilde{r} = 0.39 \pm 0.08\,\mathrm{h}^{-1}$,
202 which is in quantitative agreement with the mathematical model (Fig. S6, see Eq. S6 of *SI*
203 *Appendix 2: Analytical solution for the condition of invasion*). Hyperswarmers introduced into an
204 expanding wild-type colony spread within a wild-type branch (Fig. S7), reach the tip of the
205 branch, and take over the population (Movie S5A) resembling our simulations (Fig. S5D).
206 Hyperswarmers evolved from a wild-type swarming colony (18) and could take over the
207 ancestral population thanks to a greater dispersal. To test the invasion rule, we asked whether
208 this process was reversible: Could wild-type cells invade the edge of an expanding a
209 hyperswarmer colony thanks to their greater growth rate? Our model predicted that wild-type
210 cells would be unable to invade the hyperswarmer population edge since in this case $v_2 < v_1$
211 ($D_2/D_1 = 0.5$ and $r_2/r_1 = 1.1$, see star symbol in Fig. 2A). This was confirmed experimentally:
212 wild-type cells introduced in a hyperswarmer colony simply spread out and were rapidly
213 outpaced at the edge by the hyperswarmers (Movie S5B).
214
215 *Invasion rule valid despite phenotypic variability*
216 Even in mono-species systems, individuals with identical and defined genotypes can still
217 display phenotypic variation (19, 37). To study whether such variation had an effect on invasion
218 outcome, we introduced non-heritable fluctuations in birth and death events as well as in the
219 dispersal processes. These phenotypic variations were modeled as stochastic distributions around
220 the mean population value, which is determined by the strain's genotype (see *SI Appendix 4:*
221 *Stochastic Modeling*).
222 Our simulation results show that the invasion rule, $v_2 > v_1$, despite having been derived
223 from deterministic assumptions, holds valid even in stochastic situations. The transition at
224 $v_2 = v_1$ was, however, more gradual (Fig. 2B and S8): the zone of transition broadened as
225 stochasticity increased because, as expected from other stochastic studies (38, 39), stochasticity
226 allowed for a non-zero probability of deleterious mutants ($v_2 < v_1$) to invade and beneficial
227 mutants ($v_2 > v_1$) had a non-zero probability of failing to invade. Larger carrying capacities
228 lessened the stochastic effects and sharpened the transition zone, again as expected from
229 previous stochastic analyses (38, 39); importantly, however, the rule $v_2 > v_1$ could still predict
230 invasion of the population edge even with different carrying capacities (Fig. S9).
231 We confirmed the generality of the invasion rule further by carrying out evolutionary
232 simulations where mutations randomly arise at division. We considered two schemes: i)

mutations that change growth and/or dispersal relative to the ancestor phenotype but do so in an uncorrelated way; ii) mutations that change growth and dispersal considering that the two traits are linearly correlated (linear trade-off) but independent of the ancestor phenotype. In the case of uncorrelated mutations, populations evolved—on average—towards a greater expansion rate $v = 2\sqrt{rD}$ (Fig. S10C). In contrast, when the two phenotypes were constrained by a trade-off, evolution converged to the value along the trade-off line which maximized the expansion rate $v = 2\sqrt{rD}$ (Fig. S10D-G). In summary, we conducted several types of stochastic simulations that all showed that invasion of the population edge obeys the simple rule $v_2 > v_1$.

*The role of spatial structure and founder effect*

We then investigated whether our model would account for other factors that can affect invasion and competition in biologically relevant scenarios. For example, in most evolutionary scenarios where competition starts with a mutation the size of the invader population is initially very low (1 individual) whereas a competing species introduced by external processes (e.g. human intervention) can start at higher densities. Also, the initial location of the invader species matters because resources are not evenly distributed in nature, and an invading species may invade faster if it is introduced in the resource-rich leading edge than if it is introduced in deprived regions where it will take longer to grow to domination. In summary, species 2 should take longer to invade (i) when it is introduced further from the edge where resources are already limited or (ii) when its initial size is small. The invasion rule determines whether species 2 can invade (Eq. 3, Fig. 2A) but does not give us the time necessary for invasion.

To investigate how the time to invasion depends on the location and initial size of the invading population we modeled the introduction of species 2 into a traveling wave formed by species 1. We assumed an initial density $S$ across a small interval at a distance $L$ from the edge for species 2 *(SI Appendix 3: Time of invasion*), and we determined the time needed to outnumber species 1 at the front. Numerical simulations revealed that the general rule, $v_2 > v_1$ holds for all initial conditions given sufficient time (Fig. 2C). The time required, however, depends on the initial conditions, increasing approximately linearly with the distance $L$ from the front and decreasing sub-linearly with the initial density $S$ (Fig. S11 and S12).

To better distinguish the factors that influence the time-scale of invasion we considered two steps: first, we considered that species 2 disperses until it reaches the active layer. The time for species 2 to reach the edge depends on the distance from the introduction point to the front—a distance that increases constantly because species 1 is itself advancing—and also on the initial width of species 2. Second, once species 2 reaches the active layer it must grow to outnumber species 1. When the introduction is sufficiently far from the edge the time of invasion, $t_i$, is:

$$t_i \sim \alpha L - \beta \log(S) \tag{4}$$

where $\alpha$ and $\beta$ depend on the parameters $D_1$, $D_2$, $r_1$ and $r_2$ (see *SI Appendix 3: Time of invasion*). This analysis confirms simulation results that the time to invasion depends linearly on $L$ but only sub-linearly on $S$ (Fig. S11 and S12), highlighting that the distance to the edge is key to invasion.

We then tested these findings in our experimental system. We manipulated the distance to

the edge ($L$) and the density ($S$) of a small population of hyperswarmers introduced into an expanding wild-type population, and we compared the experimental results to the corresponding simulations. In simulations, the invasion outcome was calculated as the frequency $f$ of species 2 at the edge of the population 6 hours after implantation (Fig. 3A). In the experiments, we ranked the invasion outcome after 6 h of expansion as no invasion, scarce invasion, abundant invasion and complete invasion according to the amount of hyperswarmers visible at the edge (Fig. 3B, see details in *SI Appendix 1: Materials and Methods*). The experiments confirmed the dominant role of $L$ compared to $S$ in determining the time of invasion (Eq. 4), which is evident from the concave shape of the invasion scores (Fig. 3C, see *SI Appendix 5: Statistical analysis*).

The intuition behind the concave shape is that when the initial distance from the edge is too long then the invasive species may not be able to invade within biologically relevant time, even if its initial size is large. The shape of the iso-frequency contour lines can be calculated from the simplified two-step model of invasion described above and is given by

$$S \sim e^{L/L_0} \tag{5}$$

where $L_0 = 4D_2 v_1/(3v_1^2 + v_2^2)$ is the characteristic length of theses lines. In the case of *P. aeruginosa* and its hyperswarmers $L_0 = 1.1 \pm 0.07$ mm (SD). The results from our hyperswarmer experiments agree with the theoretical model (Fig. 3A and C; compare to lines of constant mutant frequency), indicating that the two-species F-KPP model, in spite of its simplifying assumptions and despite any intricacies of the experimental system (e.g. the swarming population is tri-dimensional; bacterial cells tend to lose motility as they lose access to resources inside the population, which freezes the spatial organization), is sufficiently general to describe invasion in swarming colonies.

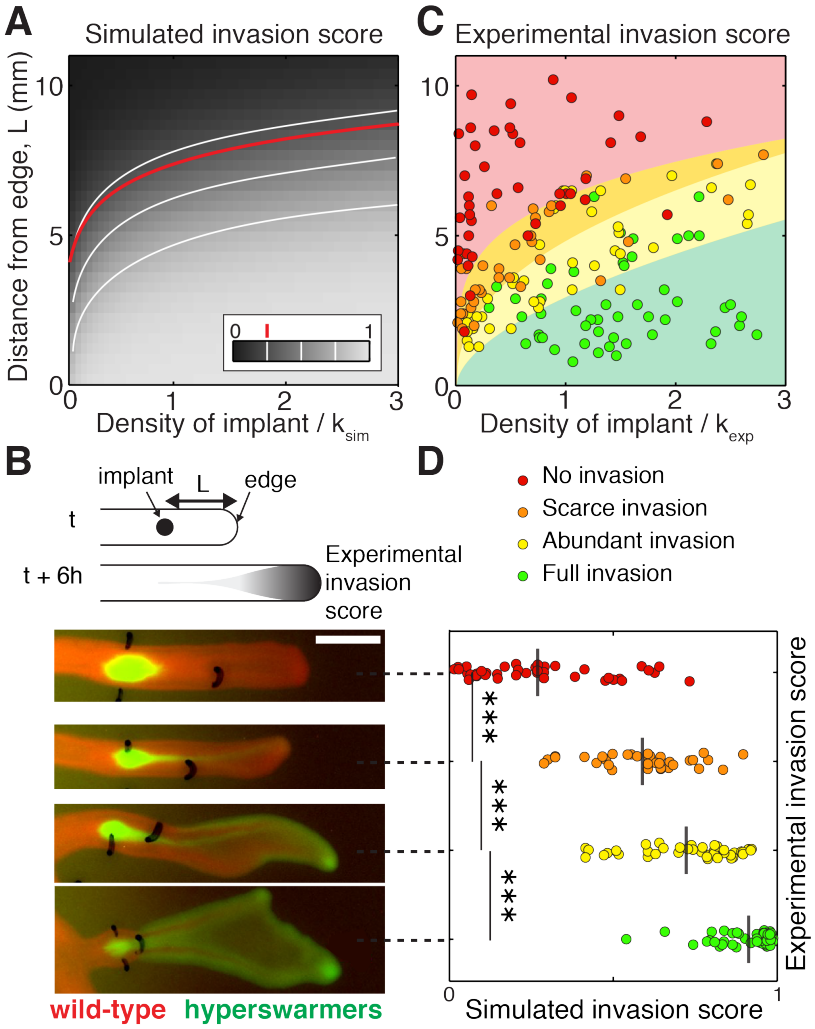


**Figure 3. Likelihood of invasion increases with the initial size of the invading population and its proximity to the population edge.** A: Simulation results of introducing species 2 into an expanding species 1. The color scale represents the frequency $f$ of the invading species (species 2 with $\boldsymbol{D_2 = 2D_1}$ and $\boldsymbol{r_2 = 0.9r_1}$) at the population edge $\boldsymbol{T = 6h}$ after introduction. White lines are iso-frequency lines for $f = 0.25$, 0.5, and 0.75. The red line is from Eq. (5) for $f$ =0.25. B: Invasion experiments where the hyperswarmer (in green) was introduced at varying initial densities and distances to the edge of an expanding swarm of wild-type *P. aeruginosa* (in red). Scale bar is 5 mm. Leftmost marks depict the location of the hyperswarmer introduction; rightmost marks locate the position of the front of the *P. aeruginosa* population at the time of hyperswarmer implantation. The four snapshots represent four experimental replicates. C: Experimental invasion success evaluated visually at 6 hours after hyperswarmer introduction. Background colors represent results from multinomial logistic regression (see details in *SI Appendix 5: Statistical analysis*). In agreement with the theory, invasion success is lower for large distances from the front and smaller initial densities. D: Comparison of simulated and experimental invasion scores for each experimental replicate. The grey vertical lines represent the average simulated invasion score and the p-values are $< 10^{-3}$ (Kruskal-Wallis test).

## Discussion

Since the modern synthesis unified principles of natural selection and genetics in the same mathematical framework, simple rules have provided important solutions to evolutionary puzzles (40). Many cornerstone concepts of evolutionary theory can be expressed as such rules, and their logic can be followed from simplifying assumptions. Real systems are often more complicated, but simple rules still provide useful "rules of thumb" to guide intuition. For instance, Hamilton's rule of kin selection (41), which among other feats determines conditions for the evolution of altruism, drives despite its simplifying assumptions an important body of work both theoretical and experimental in social evolution (42). The F-KPP is one such simple equation to describe the contributions of growth and dispersal to the spatial expansion of a mono-species population (20, 21). The F-KPP also defines conditions for a traveling wave which resembles expanding populations observed in nature, and allows calculating the traveling wave speed from the growth and dispersal rates.

Here we showed that extending the F-KPP equation to a multi-species situations produces a simple mathematical rule to predict the outcome of invasion in an expanding edge depending on the growth and dispersal rates of the competing species. The problem of invasion in an expanding population had been investigated, both theoretically (e.g. 43, 44) and empirically (e.g. 7). But its simple solution, the inequality $v_2 > v_1$, had not been—to the best of our knowledge—presented this way before, a fact that somewhat surprises us.

This simple rule derived analytically from the deterministic two-species F-KPP equation establishes the mutual contribution of growth and dispersal for the invasion outcome. Importantly, the outcome can be independent of the system's carrying capacity, particularly because the competition dynamics relies especially on the low-density of the population at the expanding edge. Further away from the edge, where population is denser, spatial structure and dispersal are less relevant; the evolutionary fate of an invader might then be determined by high-density dynamics where additional factors such as kin competition become important (36, 45). The combined effect of growth and dispersal was also tested experimentally using a laboratory system of swarming bacteria. The rule determined that a slower growing species could invade an established species as long as its dispersal rate is sufficiently faster and that was indeed the case in our experimental system. Hyperswarmer mutants paid a growth cost for synthesizing their multiple flagella but—without affecting their competitive ability—dispersed faster than wild-type bacteria (domain 2 of Fig. 2A).

Our experimental tests with hyperswarmers showed, then, that the invasion rule $v_2 > v_1$ applies to an empirical system despite intricacies that could violate the simplifying assumptions of the F-KPP. This is important, because nature abounds with examples like our experiments where invasive species speed up dispersal but at a cost to their growth rate: the invasion of the cane toads in Australia, a human-introduced species, is led by faster long-legged individuals with lower birth rates (46); the South African mountain fynbos is threatened by invasive pine trees with lighter pine seeds that disperse better (47) but produce weaker seedlings (48); metastatic cancer cells are more invasive due to a loss of contact inhibition of locomotion (49) that also

lowers their cellular proliferation rates (50-53). Additional examples include other plants (54-56), freshwater ciliates (57), bugula (58), fish (59), crickets (60), beetles (61, 62) and butterflies (63). Our invasive rule may potentially be applied to these systems as well, despite their specific intricacies, and used to make quantitative predictions of invasion outcomes.

We should note two exceptions to our findings. First, while our model predicts that a species with faster growth but slower dispersal should be able to invade (domain 1 of Fig. 2A), we did not observe examples of this in our experimental system. And, besides observations by another group in laboratory experiments with *Escherichia coli* (64), we could not find examples in nature either. The reason for not finding invasion of rapid growers that disperse slower may be population history: Empirical and theoretical studies of range expansions suggest that only dispersal can be improved in expanding populations (65-68); invasion of new niches is possibly a rare event, whereas competition within a confined, but relatively homogenous environment is more common. In such situations, the selection is not on dispersal but on growth, which means that—in most species—growth rates may already be close to their physiological limit (Fig. 4A). Individuals challenged to overcome spatial structure may only have dispersal-related traits left to improve. Second, while the trade-off between growth and dispersal may be found and seem logical, a comparative analysis of dispersal in terrestrial and semi-terrestrial animals suggested that dispersal and fecundity may be positively correlated (69).

The trade-off between growth and dispersal likely results from molecular, cellular and physiological constraints, whereas the invasion rule $v_2 > v_1$ specifies the outcome of invasion. The rule determines the maximal cost in growth $|\Delta r|_{\max}$ that the invader can afford to pay for faster dispersal and still be able to dominate the edge of the expanding population (Fig. 4B):

$$|\Delta r|_{\max} = r_1 \left(1 - \frac{D_1}{D_1 + \Delta D}\right) \tag{7}$$

where $\Delta D$ is the increase of dispersal in species 2 relatively to species 1. The evolutionary experiment that originally created the hyperswarmers always produced mutations in *fleN*, a gene involved in the regulation of flagellar synthesis (18). It is possible that other mutations could increase dispersal even more, but were not favored if they carried costs higher than $|\Delta r|_{\max}$.

The invasion rule $v_2 > v_1$ could be combined with quantitative knowledge on the physiological mechanisms of a trade-off between growth and dispersal and used to better predict the evolution of expanding populations. For example, if the trade-off can be represented by a line in $(r, D)$ space the slope of that line, $dr/dD$, represents the cost of dispersal. When the trade-off is subtle, the slope is shallow and we predict that the population will evolve to disperse faster with a lower growth rate (Fig. 4C). Conversely, when the trade-off is strong, the slope is steep and we predict that the population will evolve a higher growth rate and slower dispersal (Fig. 4D). According to this model, the *P. aeruginosa* system has a subtle trade-off: the improved dispersal advantage of hyperswarmers is ~100% but costs only ~10 % of their growth rate relatively to wild-type (18).

Using the simple invasion rule to make predictions about real world scenarios must take into account the appropriate time scales. Our analysis showed that in some conditions where the model predicts invasion, the invasion might take so long that it becomes irrelevant; other

processes—working on shorter time scales—would likely alter the system in the meantime. For example, the hyperswarmers may never invade a real world population of the wild-type if conditions change such that the bacteria need to suddenly form a biofilm. This is particularly important because the same *fleN* mutations that give multiple flagella to hyperswarmers also decreased their ability to form biofilm communities (18). Like Hamilton's rule and other cornerstone concepts of evolutionary theory, if the conditions change dramatically before a fast invader has the chance to dominate the edge of the expanding population then obviously the model predictions will not hold.

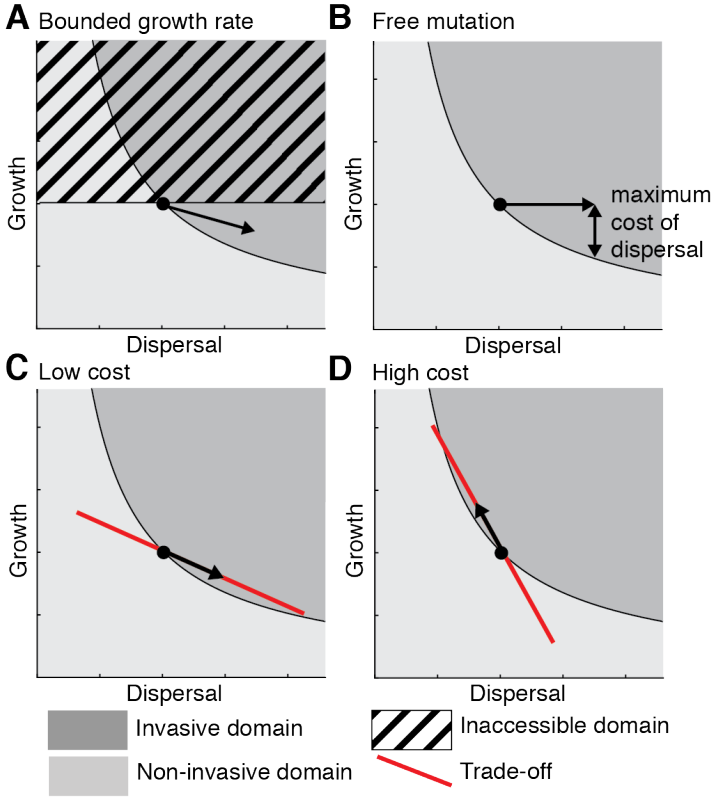


**Figure 4. Evolutionary predictions from the invasion diagram in four scenarios**. A: the growth rate is bounded by physiology. B: illustration of the maximal cost of an increased dispersal. C and D: linear trade-off between growth and dispersal that corresponds to a low cost of dispersal (C) and high cost of dispersal (D).

In summary, we found a simple rule for invasion that, expanded with trade-off constraints observed for each particular system, could be used to predict the fate of expanding populations. Every model requires simplifying assumptions, and ours is certainly not an exception. In those systems where the rule does hold, the invasion rule could be used to help control expanding populations. Systems in this category may include the growth of cancer tumors and invasion of non-native species in ecosystems.

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