Fitness tradeoffs between spores and nonaggregating cells can explain the coexistence of diverse genotypes in cellular slime molds

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Cellular slime molds, including the well-studied *Dictyostelium discoideum*, are amoebae whose life cycle includes both a single-cellular and a multicellular stage. To achieve the multicellular stage, individual amoebae aggregate upon starvation to form a fruiting body made of dead stalk cells and reproductive spores, a process that has been described in terms of cooperation and altruism. When amoebae aggregate they do not perfectly discriminate against nonkin, leading to chimeric fruiting bodies. Within chimeras, complex interactions among genotypes have been documented, which should theoretically reduce genetic diversity. This is however inconsistent with the great diversity of genotypes found in nature. Recent work has shown that a little-studied component of *D. discoideum* fitness—the loner cells that do not participate in the aggregation—can be selected for depending on environmental conditions and that, together with the spores, they could represent a bet-hedging strategy. We suggest that in all cellular slime molds the existence of loners could resolve the apparent diversity paradox in two ways. First, if loners are accounted for, then apparent genotypic skew in the spores of chimeras could simply be the result of different investments into spores versus loners. Second, in an ecosystem with multiple local environments differing in their food recovery characteristics and connected globally via weak-to-moderate dispersal, coexistence of multiple genotypes can occur. Finally, we argue that the loners make it impossible to define altruistic behavior, winners or losers, without a clear description of the ecology.

Dictyostelium discoideum | cooperation | dispersal | coexistence | variable environments

The cellular slime molds, of which the most studied is *Dictyostelium discoideum*, arise from starving amoebae. Upon exhausting their local supply of food, amoebae initiate a developmental program, joining with neighbors to form an aggregate. The culmination of development is a fruiting body made of stalk and spores (1–4). In nature, there is significant diversity and coexistence of multiple species and genotypes of cellular slime molds (5, 6). Moreover, chimeras (aggregates consisting of at least two genotypes) occur naturally (5–8), which implies that amoebae do not discriminate perfectly in the process of aggregation. These chimeras are functional and viable: Their aggregation results in a fruiting body in which the multiple genotypes participate both in stalk formation and in spore production, although not necessarily in equal measures, which is known as reproductive skew (7). Certain genotypes are disproportionately represented in the spores despite being equally represented in the initial population of starving amoebae, and those are considered to be stronger competitors. Studies to date have found significant reproductive skew in chimeras of a variety of cellular slime molds (9), with perhaps the greatest skew being registered for *D. discoideum*, where linear hierarchies of competitors have been described (10, 11).

In the absence of additional frequency-dependent processes that maintain coexistence, these findings point toward a decrease

in genetic diversity that is inconsistent with the immense diversity and coexistence among strains in nature (9, 10). An explanation for coexistence that was suggested but unexplored both for *D. discoideum* (10) and for other cellular slime molds (9) is that strains that are at a disadvantage in chimeras have an advantage at a different stage in their life cycle, i.e., that there is a tradeoff between sporulation efficiency and other fitness-related traits. To date, analyses of genotypical fitness have focused on spore contribution as the sole fitness indicator. However, during the social phase, not all cells aggregate; some cells stay behind. We refer to such cells as nonaggregators or loners. Loner cells have been generally ignored because they were assumed to simply die (10), but recent results show that loner cells of *D. discoideum* are viable, meaning that they can eat and divide if food is replenished in the environment, and that therefore the loners can be an important component of *D. discoideum* fitness (12).

This finding fits into a long-standing theory of spatially and/or temporally heterogeneous (variable) environments leading to bet-hedging or long-term optimization strategies including dormancy versus dispersal, persistence versus normal growth versus dormancy, and exploitation versus exploration, well established in ecology from studies of plants (13–19) to those of bacteria (20–22), planktonic copepods (23), and even social insects (24). In the case of cellular slime molds, loner cells can act as a form of exploitation strategy: Certain environments may become

Significance

Cellular slime molds, including *Dictyostelium discoideum*, are amoebae whose life cycle includes both single-cellular and multicellular stages, the latter achieved when individual amoebae aggregate upon starvation. In the (not necessarily clonal) aggregate, there is strong selection to be represented in the reproductive spores. This would lead to a reduction in overall genotypic diversity inconsistent with the great diversity found in nature. We suggest that cells that fail to aggregate provide an additional fitness component that can resolve the inconsistency: Strong selection for aggregation only occurs in environments where food is slow to replenish. Otherwise, there is strong selection for unicellularity. These tradeoffs allow a multitude of genotypes to coexist when many environments with different food-recovery characteristics are connected via weak-to-moderate dispersal.

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advantageous quickly and, unlike spores that take time to germinate, loner cells can begin to eat and divide instantaneously, thus giving their genotype a head start. Then, environments where food replenishes faster (henceforth fast-recovery environments) will select for genotypes that are more likely to invest in loners, whereas environments where food replenishes slower (henceforth slow-recovery environments) will select for genotypes that are more likely to invest in spores (12).

Here we suggest that for cellular slime molds for which loners exist and are indeed part of the survival strategy of a genotype, they can contribute toward the understanding of chimeric interactions and genotypic diversity in two ways. First, we claim that the reproductive skew observed within the spores of chimeras might be only apparent and simply due to different investments into loners versus aggregating cells, and not due to chimeric interactions between genotypes. Second, assuming that there are no chimeric interactions among genotypes, we claim that if both spores and loners are part of cellular slime mold survival strategies, then many diverse genotypes can coexist in an ecosystem consisting of multiple local environments with different food-recovery characteristics, connected via weak-to-moderate dispersal. This claim relies on principles already established in ecology: When fitness has multiple components, tradeoffs between them can lead to coexistence of strategies in spatially or temporally heterogeneous environments (reviewed in refs. 25–29). In addition to these main results, we also independently confirm the viability of *D. discoideum* loners via an experimental setup different from that in ref. 12.

Experimental Results

We used cells left behind during the aggregation of starving *D. discoideum* from a naturally isolated [as opposed to axenic (12)], clonal population that were allowed to form fruiting bodies on nonnutrient agar (experimental details in *Materials and Methods*). The fruiting bodies were then removed and fresh bacteria were added as a food source. This facilitated the nonaggregating cells to regrow and deplete the bacteria as expected from ref. 12 and reaggregate and go on to form normal fruiting bodies, leaving behind a population of nonaggregating cells themselves (Fig. 1). In addition to viability, this experiment confirms that there are no longer-term effects of starvation or an epigenetic effect that would prevent these nonaggregating cells from aggregating in the future under starvation conditions. Therefore, the loners can indeed constitute an important component of *D. discoideum* fitness.

Theoretical Results

Chimeric Interactions. Existing work shows no interaction between different coexisting genotypes before the aggregate stage (9). Therefore, in agreement with refs. 1 and 30, we suggest that a natural first hypothesis is that of neutrality, i.e., that no interaction occurs at the aggregate stage either, such that genotypes behave in a chimera exactly as they would in a clonal fruiting body. Then, if different genotypes have different investments in loners depending on the environments encountered, in a 50:50 initial mix of starving amoebae, genotypes that invest more in spores and less in loners will be more represented in the spores of the chimera and vice versa (Fig. 2). Because current experimental work on chimeras only counts spores (loners are generally ignored and stalk cells are experimentally hard to count), such pairings of genotypes with different loner investments would be classified as reproductively skewed and chimeric interactions would have to be invoked to explain the skew (11). We do not suggest that chimeric interactions do not occur, but only that (i) they are not necessary to explain the existence of a reproductive skew and (ii) should they exist, they cannot be inferred unless all cells are counted, including loners, cells left behind in slug trails, stalk cells, and spores. In the absence of

Fig. 1. “Loner” cells left behind after aggregation are fully viable and capable of aggregation during future starvation cycles. (A) Freshly starved cells plated on agar (day 0). (B) Two fruiting bodies formed from starving cells in A (day 2). (C) Fruiting bodies removed from B, leaving behind only stalk and loner cells (day 2). (D) Bacteria added to loner cells left on agar in C (day 2). (E) New fruiting bodies resulting from viable loner cells from B–D (day 5). (Scale bar, 1 mm.) At this scale, individual cells cannot be observed. This image is representative of multiple replicates run with different genotypes.

such data, given the lack of interaction before the aggregate stage, it is natural to assume that there are no interactions in the aggregate either.

Genotypic Diversity. Next, starting from the hypothesis of no chimeric interactions, we construct a well-mixed model of resource competition similar to that of ref. 12 and extend it to explore the effect of multiple environments on genotypic diversity. Because we assume that genotypes behave in chimeras exactly as they behave when clonal, it is not necessary to model chimeras explicitly: Whether chimeras are formed or not (and how many of them are formed) becomes irrelevant to the dynamics (Fig. 2).

In our model, for simplicity, we equate genotype and phenotype. A genotype is characterized by a scalar α , which represents the fraction of cells that aggregate; the remaining $1 - \alpha$ constitute the fraction of nonaggregating cells, or loners. Thus, if $\alpha = 0$, a monoculture of genotype α does not undergo an aggregation phase; if $\alpha = 1$, a monoculture only produces aggregates and leaves no loner cells behind. Intermediate α values represent a mixed strategy, where some cells aggregate and others do not. Out of the cells that aggregate, only a fraction will become spores; the remaining cells are not viable; or they die and contribute to the formation of stalk, as is the case for *D. discoideum*; or, under environmental conditions where a migrating slug is formed, they get shed during migration. In this paper we are not concerned with the selective forces that shape the stalk-to-spore investment ratio or the average length of slug migration and therefore, for simplicity, we assume that the cell

Fig. 2. Chimeric interactions are not necessary to produce reproductive skew in spores. Genotype A (blue) invests a fraction α_1 in aggregation and $(1-\alpha_1)$ in loners; genotype B (red) invests α_2 in aggregation and $(1-\alpha_2)$ in loners. Of the aggregating cells, 20% become stalk and 80% spores. Then, (i) if A is clonal (N initial cells), we should observe: $(1-\alpha_1)N$ loners and a fruiting body with $0.2\alpha_1N$ stalk cells and $0.8\alpha_1N$ spore cells; (ii) if B is clonal (N initial cells), we should observe: $(1-\alpha_2)N$ loners and a fruiting body with $0.2\alpha_2N$ stalk cells and $0.8\alpha_2N$ spore cells; and (iii) if N A cells and N B cells are plated together, and assuming there are no interactions in the chimera so that the two genotypes contribute to the stalk and spores exactly as they would within clonal aggregates, then we should observe: $(1-\alpha_1)N$ A + $(1-\alpha_2)N$ B loners and a chimeric fruiting body with stalk = $(0.2\alpha_1N_A + 0.2\alpha_2N_B)$ and a spore mass = $(0.8\alpha_1N_A + 0.8\alpha_2N_B)$. Unless $\alpha_1 = \alpha_2$, the chimeric spore investment appears skewed, but the same skew is also present in the stalk and is simply accounted for by the differences in loner versus spore investment between genotypes.

loss during migration and the investment in the stalk are fixed and identical for all genotypes of a species. Consequently we focus our analysis solely on the fraction of spores versus loner cells.

The model we describe depends on the ecology that influences the lifecycle of the cellular slime molds. For simplicity, we are not concerned with soil type, light, or moisture and assume those to be the same across environments; the property of interest is the ability of food to replenish in a given environment or, in other words, the starvation times (times between the onset of starvation and the next resource pulse) experienced in that environment.

One environment (patch). In the first part of the model we assume that the ecosystem is comprised of a single environment, in which food replenishment can be either deterministic (certain) or stochastic (uncertain). This model is similar to that in ref. 12 but it is more general in that it explicitly accounts for resource competition among different genotypes. The amoebae consume resources, reproduce freely, and grow at a rate governed by Michaelis–Menten kinetics (details in *SI Appendix*). There is density dependence because different genotypes compete indirectly through the existing resources, but we assume no other frequency dependence (i.e., we assume that intrinsic parameters such as the growth and aggregation rates of different genotypes are independent of the composition of the population, consistent with observations in ref. 9). We further assume that amoebae die at rate μ . Resources are depleted by the growing amoebae until they are no longer able to sustain growth, after which the amoebae enter a starvation phase. During this phase, a fraction α of cells of genotype α aggregate with the purpose of forming spores, whereas the remaining $1 - \alpha$ stay as starving loners. The nonaggregating (loner) cells stop consuming resources, stop reproducing, and decay at rate μ until the next resource pulse. Of the aggregating cells, a fraction s become viable spores; we assume that spores are very resistant to environmental stress, but that they nevertheless incur a small decay rate δ ; therefore, we assume that $\delta < \mu$.

When the starvation period is over, food is reintroduced in one resource pulse, R_0 . Then the surviving loner cells start consuming

resources and reproducing immediately, whereas spores undergo a delay period τ , which is the time required to activate the metabolic machinery necessary for resource consumption. The longer the delay τ , the more cost will be incurred by spores in an environment where loner cells are already consuming the resource while the spores undergo the germination process. Therefore, genotypes that can leave behind some loners can have a head start and be favored. If resources get depleted before the germination period is over, we assume that spores return to dormancy, without incurring any cost associated with the abortion of the germination process. In reality, in addition to the costly delay of germination, certain species like *D. discoideum* also experience a costly delay of sporulation: After only 6 h, just as individual amoebae are beginning to aggregate, they are irreversibly committed to continuing with sporulation for the remaining 18 h of the process (31). Because we are trying to show that in certain environments loners can be selected for, an additional cost for the spores will only make the selection for loners stronger and reinforce our results. Therefore, for simplicity, we do not include in our model the additional cost due to the irreversibility of the sporulation process. The dynamic equations describing the spores and loners are presented in *SI Appendix*.

In a single environment as described above, with competing cellular slime mold genotypes and instantaneous, identical resource pulses arriving at random times, we explore how the lengths of the starvation periods (time between the onset of starvation and the next resource pulse) determine the winning genotype. Our results agree with those in ref. 12 and in general with well-established results in the bet-hedging literature. In a deterministic environment (i.e., when the starvation times are always of the same length) there is selection for one of the pure strategies: We find a critical threshold starvation time T_{cr} such that for $T < T_{cr}$, the winning genotype is one that never produces any aggregates ($\alpha = 0$), whereas for $T > T_{cr}$, the winning genotype is one that always aggregates to produce spores ($\alpha = 1$). When the environment is stochastic such that successive starvation times are independent and exponentially distributed with rate $1/\lambda_T$, we find that mixed strategies can be selected for: If on average the environment is a fast-recovery one (low λ_T), then the mixed strategy invests more in loners than in spores; indeed, for sufficiently low λ_T , only loners will survive. Conversely, if the environment is a slow-recovery one (high λ_T), then the mixed strategy invests more in spores, and for sufficiently high λ_T , only spores will persist (Fig. 3A and *SI Appendix*, Fig. S2A). We confirm that the winning strategy is continuously stable (32): It cannot be invaded by any rare mutant and can invade any resident monoculture from rare initial levels (*SI Appendix*, Fig. S2B). Here we assume an exponential distribution of starvation times; assuming uniformly distributed starvation times leads to qualitatively similar results (12). In the future, it will be interesting to explore other distributions (e.g., normal); however, we generally expect similar results to hold.

The effects of the model parameters on the evolutionarily stable genotype are intuitive: The higher the consumption rate c , and implicitly the reproductive rate of solitary amoebae, the longer the costly spore germination delay τ , and the higher the death rate of spores, δ , the more the loners will be favored. Conversely, the higher the spore success rate s or the death rate of solitary cells μ , the more spores will be favored. Finally, varying the fixed resource pulse R_0 in a stochastic environment has little effect on the winning genotype. A detailed sensitivity analysis can be found in *SI Appendix*, Figs. S3 and S4.

Multiple environments (patches). In this part, we explore whether the extension of our model to a spatially heterogeneous ecosystem with multiple local environments connected via spore dispersal can, under certain conditions, favor the coexistence of a diverse range of genotypes. We consider $M = 25$ stochastic environments evenly spanning the range of average starvation

Fig. 3. Different environments connected via weak-to-moderate dispersal, D , can maintain coexistence of genotypes. Mean genotype frequency of 21 genotypes ($\alpha = 0.05i$; $i = 0, \dots, 20$) in 25 environments. Average is taken over 60 replicates after 1,500 growth/starvation cycles in the slowest environment. (A) Without dispersal, stochastic starvation times can select for mixed strategies but no coexistence. In each environment, there is only one evolutionarily stable strategy. The surviving genotype in the simulations alternates between both highlighted genotypes in the figure due to the discretization in α . The actual winning strategy is one in between (details in *SI Appendix*). (B–E) In multiple environments, if food recovery is stochastic and the environments are sufficiently different, coexistence between a multitude of strategies is possible for weak-to-moderate dispersal. (F) For high dispersal, coexistence will tend to be lost— one winning genotype emerges, which bet hedges over all existing genotypes. Parameters are as in *SI Appendix, Table S1*. The colors correspond to frequencies such that dark blue = 0–0.0075; blue = 0.0075–0.025; green = 0.025–0.05; yellow = 0.05–0.0875; orange = 0.0875–0.125; magenta = 0.125–0.375; red = 0.375–0.625; and dark red = 0.625–1. Transitions between colors are given by gradients.

times from $\lambda_T = 80$ (which in the absence of dispersal selects for all loners) to $\lambda_T = 2,000$ (which in the absence of dispersal selects for all spores) (Fig. 3A). Each environment is governed by the same dynamics as above, but they receive and exhaust resources independently of each other (asynchronously); the only element that couples the dynamics of the environments is the dispersal of spores. When starvation occurs in an environment, a fraction $1 - D$ of the spores remains in the home environment, whereas a fraction D is dispersed uniformly across the other environments. Because the dynamics in the environments are desynchronized, when spores get moved to a new environment they may immediately find food and start the germination process, or they may be lying dormant until food gets introduced into that environment.

In the absence of dispersal, each environment will have its winner, as discussed above in *One Environment (Patch)*; and the more different the environments, the more different the respective winning genotypes (Fig. 3A). When dispersal connects the environments, for low values of D , a multitude of genotypes coexists in almost all environments; this is consistent with theory (25). The most abundant genotypes in each environment are those close to the genotype for which that environment selects in the absence of dispersal; however, many other diverse genotypes coexist, albeit at lower abundances (Fig. 3B and C and *SI*

Appendix, Figs. S7–S10). Overall, almost all genotypes are present in the whole ecosystem. The only environments with little-to-no coexistence are the very fast recovery environments, in which, as is the case in the absence of dispersal, the all-loner strategy is by far the most dominant genotype present. This is because in such environments, the food recovers quickly enough that the resident loners can (almost) completely consume it before any immigrant spores can finish their germination process (Fig. 3B and C).

As dispersal increases, the environments get increasingly more connected. For intermediate dispersal, the winning strategies segregate into two subsets: one subset with higher loner investment dominating the fast-recovery environments and one subset with higher spore investment dominating the slow-recovery environments (Fig. 3D). When dispersal becomes high enough, there is sufficient transfer between all environments for a new successful genotype to emerge that is selected to bet hedge over the average of all existing environments (Fig. 3F); the two winning subsets from the intermediate dispersal region now merge (Fig. 3E) and coexistence is reduced only to a subset of genotypes neighboring the dominant bet hedger. These genotypes now coexist in more or less all environments, although they are still poorly represented in the fast-recovery environments where loners continue to dominate (Fig. 3F). Throughout, we

measure cumulative genotype frequency including both spores and loners (a breakdown can be found in *SI Appendix*, Fig. S6). Eventually, as dispersal continues to increase, we expect coexistence to be lost. In general, the dispersal range for which coexistence is maintained depends on how many and how different the environments are, with more similar environments losing coexistence at lower levels of dispersal. Our results agree with general theoretical predictions on the effect that global dispersal has on coexistence (reviewed in ref. 29).

Discussion

We argue that if loners (nonaggregating cells) are part of the cellular slime mold survival strategy, recognizing this contributes to an understanding of chimeric dynamics and genotypic diversity in two ways. First, it shows that chimeric interactions between genotypes are not necessary to produce reproductive skew in the spores of chimeras. Under the assumption of neutral interactions, the skew is accounted for by a skewed investment in loners by the two genotypes. In this case, the location of a genotype in the competitive hierarchy based on spore investment in chimeras is inversely related to the genotype's investment in loners.

Second, in the context of a richer ecology (variable food-recovery environments connected via weak-to-moderate dispersal), the loners can provide an explanation for the great genotypic diversity observed in nature. The loners—an exploitation strategy—can also be seen as fulfilling the role of local dispersal. By contrast, the spores—an insurance against prolonged starvation—also fulfill the role of global dispersal. Because environments with different food-replenishment characteristics select for different investments in spores versus loners, we showed that weak-to-moderate dispersal between faster-recovery and slower-recovery environments can allow for the coexistence of multiple genotypes (Fig. 4 provides a schematic description of our argument for two environments).

So far, only *D. discoideum* loners have been demonstrated to be viable here and elsewhere (12) but one can expect similar findings in other cellular slime molds. The viability of the loners does not demonstrate that they are part of the slime mold survival strategy and much remains to be done empirically in this direction, including work to uncover the mechanisms by which a genotype mediates the amount of loner cells left behind. The viability of loners, however, is consistent with this hypothesis, especially in the context of the vast existing theoretical and empirical literature on bet-hedging strategies in microbes.

Because quorum sensing is instrumental in the decision to aggregate in *D. discoideum* (reviewed in ref. 3), one possibility is to search for mechanistic hypotheses there. We hypothesize two related mechanisms by which a *D. discoideum* genotype could lead to mixed investment in loners and spores when grown in a monoculture: (i) direct, signal-mediated quorum activation and (ii) indirect, resource co-mediated quorum activation. The former posits

the genotypes have varying and heritable sensitivities directly to the autoinducer; the latter posits that resource availability mediates a cell's probability of responding to an autoinducer and that the genotypes have varying resource starvation tolerances (hence resource co-mediated quorum activation). As resources are depleted, small-scale spatial heterogeneity can lead to some cells of the same genotype sensing abundant resources in their local environment and others sensing sparse resources. For a given level of resources and a given degree of spatial heterogeneity, a fraction of the cells may initiate their developmental program and move toward aggregation, and that fraction can vary across genotypes due to variation in the sensory and transcriptional machinery involved in detecting local resource density. Recent work (12) supports the plausibility of such a mechanism, but as loners still remain even in homogenous food conditions it is unresolved if this is the only mechanism at work in loner formation. We suggest that dose-response experiments examining different genotypes' responsiveness to the autoinducer at various resource concentrations could test this hypothesis and assess the validity of our model.

It is furthermore important to note that the spores and loners are not the only components of the amoeba fitness: Both stalk allocation and cells left behind in the trail of the slug need to be included, because the former plays a crucial role in dispersal and the latter have been shown to remain viable (33). However, because slugs are not always formed and because they travel different distances depending on the environment (therefore shedding different numbers of cells), a more careful analysis and further experiments are necessary to determine exactly how to include these additional components. Here we showed that a very simple mechanism allows for great diversity. We expect that, provided more empirical evidence, further extensions of the model to include stalk- and trail-shedding allocations and additional elements of the ecology of different environments are likely to allow for even richer dynamics and greater coexistence.

Here we have made a theoretical case for explaining the great genetic diversity of cellular slime molds through considering the nonaggregating cells. We further suggest however that, if the loners are indeed shown to be selected for, other existing analyses of cellular slime molds and in particular *D. discoideum* need to be revisited. For example, because stalk cells undergo apoptosis, *D. discoideum* has been used as a powerful model organism to explore the evolution and maintenance of altruism (reviewed in ref. 3). However, whereas before the fitness of a genotype was well defined as the number of spores it produced, because of the loners' contribution, fitness becomes a relative quantity that strongly depends on the environments that genotype will encounter. Therefore, in this context, a cheater, whether it be in a clonal or chimeric context, is much more challenging to define, motivating a comprehensive consideration of an organism's life history and ecological context when looking for problems of altruism.

Materials and Methods

Experiments. Clonal, natural strains NC34.1, NC105.1, and NC85.2 of *D. discoideum*, originally from Little Butts Gap, North Carolina (34) were obtained from dictyBase (35) and maintained on *Klebsiella aerogenes* lawns grown on SM agar plates (36). For growth of amoebae, spores of each strain were inoculated in SorMC buffer (15 mM KH_2PO_4 , 2 mM Na_2HPO_4 , 50 μM MgCl_2 , and 50 μM CaCl_2 , pH 6.0) supplemented with *Klebsiella* to an OD_{600} of 8 and shaken at 180 rpm. For starvation experiments, vegetative cells were harvested from these shaking cultures, washed, and resuspended at $1\text{--}2 \times 10^7$ cells per milliliter in developmental buffer (10 mM K/Na_2 phosphate buffer, 2 mM MgSO_4 , 200 μM CaCl_2 , pH 6.5). A total of 1–2 μL of this cell suspension was placed on a nonnutrient agar plate and allowed to aggregate. To test the viability of cells left behind after aggregation, spores were removed using tweezers, and 5 μL of *Klebsiella* at an OD_{600} of 8 in SorMC was added to the remaining cells. The results shown in Fig. 1 are for strain NC34.1.

Fig. 4. Fast-recovery environments select for investment in loners; slow-recovery environments select for investment in spores. Fast- and slow-recovery environments connected via weak-to-moderate dispersal allow for coexistence of strategies—each strategy dominates its home environment but dispersal allows for it to be present in the other environment as well.

Simulations. We performed numerical simulations of $M = 25$ patches undergoing desynchronized growth–starvation cycles. The patches were chosen with mean starvation times $\lambda_T = 80i$, $i = 1, \dots, 25$. The spectrum of genotypes was discretized: We used 21 strategies ($\alpha_i = 0.05i$, $i = 0, \dots, 20$) corresponding to a regular discretization of step 0.05 (Fig. 3). Initial abundances of each genotype were independently drawn from a standard log-normal distribution and subsequently normalized so that the entire population contained 10^8 cells in every environment. The cells of the different genotypes were then split into spores with probability α_i and loners with probability $1 - \alpha_i$. An initial resource pulse of magnitude 10^8 was added and the trajectories governed by *SI Appendix, Eq. 1* were integrated using finite-differences numerical methods. Dispersal took place at the end of the growth phase in a given environment. First, the population of each genotype was divided into spores and loners depending on α_i ; afterward, a fraction D of the successfully formed spores was equally distributed among the rest of the environments. The starvation phase started then in the dispersing environment, with a duration T_k drawn from the exponential distribution of that environment, with mean λ_T . During this period spore populations decayed exponentially at rate δ , whereas loners decayed exponentially at

rate μ , such that $\delta < \mu$. At the end of the starvation time we measured the abundance of each genotype and a new resource pulse of size 10^8 arrived. To obtain Fig. 3, 60 realizations were run for 1,500 growth/starvation cycles in the slowest patch, which means, on average 3×10^6 h. Dispersed spores arriving to depleted patches remained dormant until a new pulse of resources arrived. If resources were still present in the new environment, the spores started germinating and became active amoebae after time τ . If resources disappeared before the germination process could be completed, then the spores aborted germination at no cost to them.

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