Disentangling Direct and Indirect Fitness Effects of Microbial Dormancy

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abstract: Disentangling individual selection from kin selection is one of the greatest challenges of evolutionary biology. Even solitary organisms that do not interact directly with conspecifics may interact indirectly with them through competition for resources. As a result, traits that appear to affect individual fitness alone can also modify the fitness of relatives nearby and thus may evolve partially through these cryptic indirect fitness effects. Here we develop a method to quantitatively separate direct and indirect fitness consequences when some microbes become dormant, while neighbors of the same genotype remain active. Dormant microbes typically survive stresses that kill metabolically active cells, but dormancy also has a social side effect, sparing resources that may be used by nondormant individuals for growth. In structured populations, spared resources may be preferentially consumed by nondormant clonemates, providing an indirect benefit. Without population structure, however, exploitation by a never-dormant competitor imposes an indirect fitness cost on dormant cells. Cryptic indirect fitness effects may play a significant role in the evolution of many ostensibly asocial traits.

Keywords: cooperation, social evolution, reproductive restraint, spatial structure, indirect effects.

Introduction

Understanding the relative contributions of individual and kin selection to evolutionary processes has long been a central aim of evolutionary biology. Traits in social organisms evolve through their effects on inclusive fitness, the combination of their direct effect on the fitness of the focal individual and their indirect effect on the fitness of nearby conspecifics (Hamilton 1964*a*, 1964*b*; Michod 1982; Frank 1998; Gardner et al. 2011). Canonical examples of social evolution, such as the altruism described by Hamilton (1964*a*, 1964*b*), can be attributed to kin selection even without quantitative analysis of costs and benefits, because the persistence of individually costly traits can be explained only by benefits to relatives. Examples include alarm calling (Mateo 1996), reproductive restraint (Strassman et al. 2000), and suicidal antibiotic production (Gardner et al. 2004; Kerr 2007). In contrast, some behaviors may benefit the focal individual as well as nearby kin. This may be especially common in microbes, where many compounds are excreted extracellularly, potentially benefitting both the producer and others nearby. Examples include the production of siderophores for mineral acquisition (Griffin et al. 2004), structural components of biofilms (Hansen et al. 2007), and digestive enzymes (Greig and Travisano 2003).

Quantitatively disentangling direct and indirect fitness effects for traits that enhance both has been extremely challenging. In particular, empirical tests of models that separate direct and indirect fitness components are rare and have so far largely remained limited to population genetic models that describe statistical relationships between individual- and group-level fitness (Wolf 2003; Bijma et al. 2007; Bergsma et al. 2008; Ellen et al. 2008). These models are general and have broad utility, but they rarely allow researchers to examine the direct and indirect fitness consequences of specific traits. As a result, there has been a longstanding tendency either to ignore the indirect fitness effects of traits that also have a clear individual benefit or to treat direct and indirect benefits as mutually exclusive hypotheses (Redfield 2002). Given the ubiquity of social interactions among organisms, however, indirect fitness effects may be common side effects of traits that also benefit the focal individual. Understanding the contribution of cryptic indirect fitness effects to inclusive fitness may be essential for accurate evolutionary predictions.

Here we examine the role of cryptic kin selection in the evolution of microbial dormancy by measuring both direct and indirect effects. Many microbes form dormant resting

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stages (e.g., spores, cysts, and "persisters") that can improve survival when conditions are poor (Lewis 2010; Lennon and Jones 2011). Heterogeneous dormancy occurs when a single genotype forms a mixture of dormant and reproductively active phenotypes. Although dormant individuals survive some stresses that would kill nondormant individuals, they pay an opportunity cost in delayed reproduction if the environment becomes favorable for growth (Gardner et al. 2007; Veening et al. 2008; Lennon and Jones 2011).

This opportunity cost may be ameliorated by the benefits of dormancy to kin. For example, dormant spores do not use external resources, thus sparing them. If potentially reproductive clonemates of dormant spores preferentially use these resources for additional growth, then genes for dormancy can increase in frequency through this indirect effect (Gardner et al. 2007). Alternatively, if spared resources are consumed preferentially by a nonsporulating competitor, then this indirect effect may reduce the relative fitness of the spore former. In this article, we use the yeast *Saccharomyces cerevisiae* to disentangle the direct and indirect effects of dormancy.

Model

Effects of Dormancy on Relative Fitness

When starved of carbon or nitrogen, diploid yeast can form a tetrad containing four dormant haploid spores. Spore formation is expected to increase survival during periods of environmental stress, such as long-term starvation or antibiotic exposure. When conditions allow growth, however, dormant individuals will usually pay an opportunity cost in delayed reproduction. Any social effects are in addition to these individual costs and benefits. Dormant yeast do not consume spared resources, increasing the reproduction of nondormant yeast in the same population. When those resources are preferentially consumed by clonemates, this indirect effect increases fitness; otherwise, it results in a relative fitness cost.

To quantify these contrasting effects of dormancy, we modeled competition between two strains of *Saccharomyces cerevisiae* differing in sporulation ability. We considered a population with one spore-forming strain (strain SF) and one nonsporulating, grower-only strain (strain GO). Because natural isolates of *S. cerevisiae* exhibit heterogeneous dormancy and vary in the percentage of cells that form dormant spores under sporulation-inducing conditions (Gerke et al. 2006), we modeled SF spore formation rates from 0% to 100%. We modeled a fluctuating environment where first some fraction of the cells form spores, then growers have access to a small, finite amount of an exogenous resource, such as glucose, and finally the mixture of spores and growers faces an environmental stress to which the dormant spores are more resistant than growing cells. We calculate SF's per capita fold change in population size for a given initial SF spore frequency (R_{SF}) after one round of growth and antibiotic exposure as

$$R_{SF} = \frac{(SF_g + \tau_{SF})s_g + SF_d s_d}{SF_g + SF_d}, \quad (1)$$

where SF_d and SF_g are the initial number of SF-strain dormant spores and growers, respectively; s_d and s_g are the surviving fraction of spores and growers after antibiotic exposure, respectively; and τ_{SF} is the number of offspring the SF strain produces before exposure to antibiotics. Here we are calculating SF's per capita change as the ratio of final SF cells (nondormant cells plus spores; numerator) to initial SF cells (denominator). The final number of SF cells includes any of the original spores and nondormant cells that survive the antibiotic plus new nondormant cells from reproduction, corrected for any antibiotic-induced death of those new cells.

In allocating resource use for reproduction between the two strains, we calculate SF's reproduction, τ_{SF}, as the sum of two terms: reproduction using only its proportional share of original exogenous resources, αSF_g, and additional reproduction from resources spared by dormant spores, $\tau_{SF,d}$. That is,

$$\tau_{SF} = \alpha SF_g + \tau_{SF,d}, \quad (2)$$

where

$$\tau_{SF,d} = \frac{SF_g}{GO_g(1-\gamma) + SF_g}\beta. \quad (3)$$

Here α is the starting resource supply per initial cell in units of per-cell fecundity, GO_g is the initial number of GO-strain cells (all growers), β is the number of offspring that can be produced using resources spared by dormant SF spores ($\beta = \alpha SF_d$), and γ scales the extent to which positive assortment among SF cells limits use of spared resources by GO cells. Specifically, γ is the proportion of GO cells that cannot consume resources spared by SF spores and ranges from $\gamma = 0$, for a well-mixed population in which there is no preferential assortment between SF spores and SF growers, to $\gamma = 1$, for assortment that completely prevents use by GO of resources spared by SF spores. Positive values of γ would occur when the population is spatially structured (Fletcher and Doebeli 2009). Reproduction from spared resources comes from the same pool of resources that, in the absence of spores, would have been used by growers. So τ_{SF} can never exceed $\alpha(SF_g + SF_d)$. We calculate GO's per capita fold change in population size in a similar manner. Let

$$R_{GO} = \frac{(GO_g + \tau_{GO})s_g}{GO_g}, \quad (4)$$

where τ_{GO} is the number of offspring GO produce before antibiotic exposure, calculated as

$$\tau_{GO} = \alpha GO_g + \beta - \tau_{SF,d}. \quad (5)$$

Finally, to measure the fitness of SF relative to GO, we calculate the difference in each strain's per capita fold change over one round of growth and antibiotic exposure, R_{SF-GO}:

$$R_{SF-GO} = R_{SF} - R_{GO}. \quad (6)$$

So when $R_{SF-GO} = 1$, each initial SF cell is represented by one more surviving descendent than each starting GO cell.

Measuring Direct and Indirect Effects of Dormancy

To measure the relative importance of direct and indirect fitness effects, we decompose equations (1) and (4) into two components. First we calculate the direct effects of spore formation: dormant spores have enhanced survival during antibiotic stress but cannot reproduce using exogenous resources, thus imposing a growth-opportunity cost:

$$R_{SF,dir} = \frac{SF_g s_g + SF_d s_d + \alpha SF_g s_g}{SF_g + SF_d}, \quad (7)$$

$$R_{GO,dir} = \frac{(GO_g + \alpha GO_g)s_g}{GO_g}. \quad (8)$$

Second, we calculate the indirect effect of dormancy, the average number of offspring that survive antibiotic exposure (per starting SF or GO cell), produced from resources that were spared by dormant SF spores:

$$R_{SF,ind} = \frac{\tau_{SF,d} s_g}{SF_g + SF_d}, \quad (9)$$

$$R_{GO,ind} = \frac{\beta - \tau_{SF,d} s_g}{GO_g}. \quad (10)$$

Note that these terms can be summed to yield equations (1) and (4): $R_{SF} = R_{SF,dir} + R_{SF,ind}$ and $R_{GO} = R_{GO,dir} + R_{GO,ind}$.

By contrasting the contribution of direct and indirect effects to SF's survival and reproduction relative to GO, we quantitatively separate direct and indirect fitness effects of dormancy and examine the conditions under which each provides a benefit to SF. We calculate the relative direct effect of dormancy as the difference between each strain's direct effects:

$$\delta_{dir} = R_{SF,dir} - R_{GO,dir}. \quad (11)$$

So if $\delta_{dir} = 1$, then direct effects of dormancy would result in each starting SF cell being represented by an average of one more surviving cell than each starting GO cell.

We assess the relative indirect effect of dormancy in a similar manner, calculating the difference between SF's and GO's reproduction using resources spared by SF spores:

$$\delta_{ind} = R_{SF,ind} - R_{GO,ind}. \quad (12)$$

As before, if $\delta_{ind} = -1$, then each starting SF cell would produce an average of one fewer surviving offspring from resources spared by dormant spores than each starting GO cell. Note that δ_{dir} and δ_{ind} represent a simple decomposition of R_{SF-GO}, such that $R_{SF-GO} = \delta_{dir} + \delta_{ind}$. Equations (11) and (12) calculate the difference (not the ratio) of partitioned effects because this allows us to compare the size of direct effects to indirect effects.

Experimental Methods

We experimentally tested the predictions of our model for conditions under which SF and GO were thoroughly mixed so there was no assortment of SF cells ($\gamma = 0$). Strains differing only in the fraction of cells that form spores were not available. We therefore used phenotypic manipulation (sensu Partridge and Harvey 1988) to experimentally simulate different SF strains that vary in the fraction of cells that are dormant. This was accomplished through controlled mixing of two nearly isogenic *Saccharomyces cerevisiae* strains with different fluorescent labels and unlabeled spores (fig. 1). The link between spores and SF growers, which would normally occur via direct production of spores from those growers, was created by substituting spores for the designated fraction of SF growers. To prevent the designated GO strain from forming spores and SF's spores from germinating, yeast were grown in conditions that prevented both spore formation and germination.

To construct "SF" yeast with a given percentage of dormant cells, we mixed *S. cerevisiae* strain Y55 yeast marked with blue fluorescent protein (BFP; Δlys2 Δura3 dORF(SWH1)::yEBFP) and spore tetrads obtained by culturing diploid Y55 in sporulation media (per L: 20 g potassium acetate, 2.2 g yeast extract, 870 mg amino acid mixture, 0.5 g glucose) for 4 days. The "GO" strain consisted of *S. cerevisiae* strain Y55 yeast marked with green fluorescent protein (GFP; Δlys2 Δura3 dORF(SWH1)::yEGFP) and no spores. Through this three-way controlled mixing, we generated populations of phenotypically SF and GO yeast that varied only at loci coding for fluorescent labels. We varied the percentage of SF that were spores from 0% to 100% in 10% intervals, with SF initially either rare (10% of the population) or common (90% of the population). The starting spore frequency of SF was de-

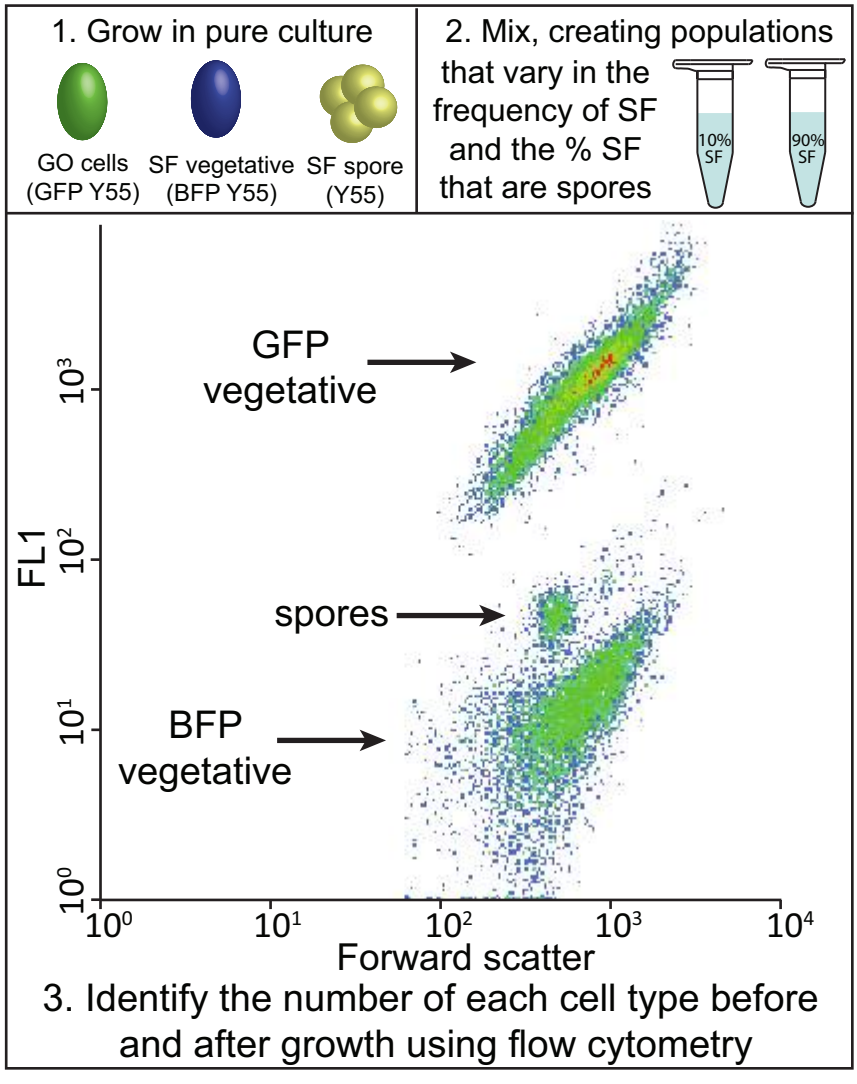

Figure 1: Experimental approach. Populations of *Saccharomyces cerevisiae* strain Y55 were constructed with three cell types that differed only at loci coding for fluorescent markers. Yeast labeled with green fluorescent protein (GFP) were used as the grower-only (GO) strain; all cells were nondormant. Nondormant cells of the spore-forming (SF) strain were labeled with blue fluorescent protein (BFP), and dormant spores (a specified fraction of the SF population) were unlabeled Y55 tetrads. Via controlled mixing of these cells, populations that varied in the frequency of the SF strain (10% or 90%) and in the percentage of SF cells that are spores were constructed. Fitness was measured by counting the number of SF and GO yeast before and after growth by flow cytometry.

termined empirically when it was composed of both spores and growers. Because each strain contains markers discernible by flow cytometry (fig. 1), we were able to measure the fitness of these simulated SF and GO strains as we varied both the frequency of SF (growers and dormant spores) in the population and the fraction of SF cells that were spores.

Five experimental replicates were established per treatment combination. Yeast were grown at 30°C for 2 days in yeast nitrogen base (YNB) minimal media (Sigma-Aldrich) with 50 mg/L glucose, a concentration that allowed a fivefold increase in population size (initially 50,000 cells per mL) and did not cause spores to break dormancy. Tetrads, which contain four haploid spores that fuse to form two diploid nondormant cells upon germination, were counted as two cells for fitness estimates. At time zero and after 48 h of growth, subsamples were analyzed flow cytometrically, and the number of "SF" spore tetrads, "SF" BFP cells, and "GO" GFP cells were counted in each population, allowing us to calculate SF's relative fitness.

The parameters of the model were based on independent preliminary experiments. These were conducted to deter-

mine the concentration of glucose and yeast that would result in a fivefold increase in population size ($\alpha = 5$) without causing spores to germinate. The variables SF_d, SF_g, and GO_g were set by controlled mixing. Antibiotic susceptibility of spores and actively growing cells was determined by incubation of sporulated or nondormant Y55 (taken from a 24-h stationary-phase culture grown in YNB with 10 g/L glucose) in miconazole (50 μg/mL) for 30 minutes. Viability was determined by plate counting.

Results

Direct Effects of Dormancy

When a spore-forming strain is only a small fraction of the population, SF spores spare only a small fraction of the total resources used by the population, so the indirect effects of dormancy are small (fig. 2). This case was considered first. Figures 3*a* and 3*b* show predictions and data when SF was 10% of the population.

Without a source of extrinsic mortality that preferentially kills nondormant cells, such as antibiotic exposure, dormancy reduced the relative fitness of the spore-forming SF genotype. Our model predicts a decline in the relative fitness of the SF strain as the fraction of cells that are dormant spores (and therefore do not grow) increases (fig. 3*a*). Our experimental test of the modeled conditions gave a quantitatively similar result (correlation between expected and observed fitness = 0.96, $F_{1,12} = 149$, $P < .0001$; raw data available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.hf792; Ratcliff et al. 2013).

We next examined the fitness of SF yeast when growth as above was followed by exposure to an environmental stress to which spores were relatively resistant. We found that 57% of spores survived treatment with miconazole, in contrast to only 6% of actively growing cells. Our ex-

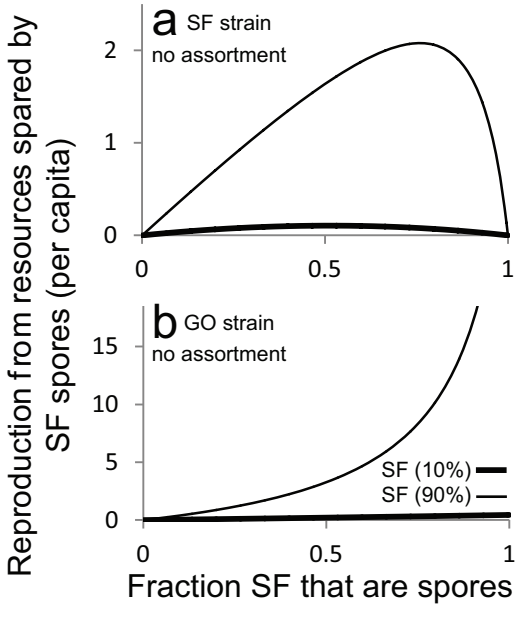

Figure 2: Higher population frequencies of the spore-forming (SF) strain increase the magnitude of the indirect effect. *a*, The per capita reproduction by SF due to consumption of resources spared by SF spores, $\tau_{SF,d}/(SF_g + SF_d)$. *b*, The per capita reproduction of the grower-only (GO) strain from resources spared by SF spores, calculated as $(\beta - \tau_{SF,d})/GO_g$. Both SF and GO yeast produce substantially more offspring from spared resources when SF is common (90%) than when SF is rare (10%). Here it is assumed that there was no positive assortment among SF cells ($\gamma = 0$), no antibiotic exposure, and enough resources to facilitate an overall fivefold increase in population size ($\alpha = 5$).

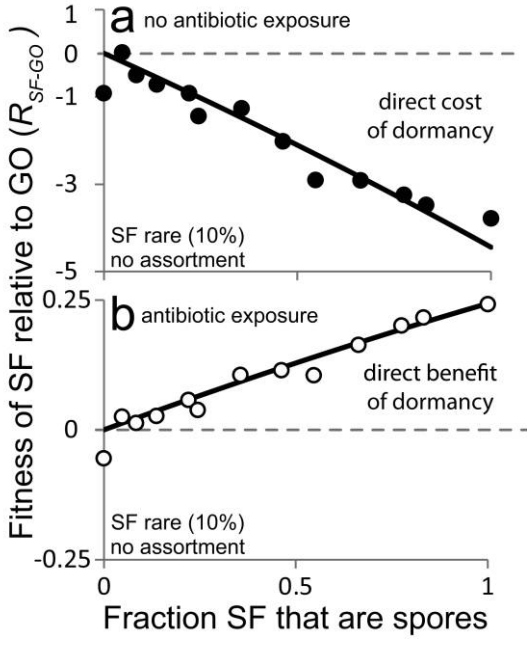

Figure 3: Dormancy provides a direct fitness benefit only when antibiotics are encountered. Because dormant yeast do not reproduce using external resources, their fitness declines with increased spore formation when antibiotics are not present. However, spores gain a direct survival advantage during antibiotic exposure, improving fitness when growth is followed by miconazole treatment. *a*, Predictions from our model (solid line) and experimental measurements (circles) of the fitness of the spore-forming (SF) strain relative to the grower-only (GO) yeast when it is initially 10% of the population so that the total amount of resources spared by SF spores is low. SF and GO have identical fitness when $R_{SF-GO} = 0$ (dashed line). Yeast were grown in minimal media with enough glucose for a fivefold increase in total numbers and no antibiotic exposure. *b*, Predictions from our model (solid line) and growth data from *a* (10% SF, so little resource sparing) modified by separately determined survival of vegetative and sporulated cells after miconazole exposure (open circles; $s_g = 0.06$, $s_d = 0.57$).

perimental protocol for measuring the fitness of SF and GO yeast depends on our ability to recognize GFP-labeled yeast via flow cytometry. Cell death, however, resulted in rapid loss of GFP fluorescence, making killed nondormant GO and SF cells indistinguishable in the flow cytometer. We were therefore unable to measure fitness after miconazole exposure directly. Instead, we estimated the fitness of SF yeast when growth was followed by antibiotic exposure by applying the separately determined survival rate for spores (57%) and growing cells (6%) to the flow-cytometric data from figure 3*a*. For example, a population that contained 500 spores, 1,000 BFP growers, and 1,000 GFP growers after growth would be estimated to have 285 spores, 60 BFP growers, and 60 GFP growers remaining alive after antibiotic exposure.

When growth was followed by antibiotic exposure, the greater relative survival of spores more than compensated for the reproductive opportunity cost of forming spores (fig. 3*b*). Our model predicts that when SF is initially rare (10% of the population) and growth is followed by our antibiotic treatment, SF fitness increases with the fraction of SF that are initially spores. Our experimental estimates for SF yeast fitness after antibiotic exposure quantitatively matched these predictions (fig. 3*b*; $r = 0.98$, $F_{1,12} = 240$, $P < .0001$).

Indirect Effects of Dormancy

The direct benefits of dormancy—higher survival during antibiotic exposure—do not depend on the frequency of SF in the population: dormant cells are more resistant to antibiotics than nondormant cells at any frequency. In contrast, the indirect effects of dormancy are frequency dependent. When SF is initially rare, only a minority of the individuals in the population will be dormant (even if all SF cells are spores) and so resource sparing is minimal. When SF is initially common, however, a large fraction of the population can be dormant, increasing the amount of resources spared. As a result, the indirect fitness effects of dormancy are larger when SF is at a higher frequency (fig. 2). As the fraction of SF that are dormant increases, SF spare more resources, increasing the number of offspring that nondormant SF and GO produce. SF's reproduction from spared resources declines rapidly, however, as the fraction of SF that are spores approaches one, because few SF growers are present to consume spared resources (fig. 2*a*).

The indirect effect of dormancy is costly in an unstructured environment. Because all GO cells are capable of consuming resources spared by SF spores while only nondormant SF cells can consume spared resources, GO will consume proportionally more of this public good than SF. As a result, the indirect effect reduces SF's relative fitness in unstructured environments (fig. 4*a*). Our experimental estimates of relative fitness matched our model's predictions of SF's fitness when SF was initially 90% of the population ($r = 0.97$, $F_{1,12} = 175$, $P < .0001$).

Spatial structure can change this pattern. Strong assortment among SF yeast spores and growers ($\gamma = 0.9$) can allow SF growers to preferentially consume resources spared by SF spores, increasing SF's fitness relative to GO (fig. 4*b*) over most of the range of spore formation.

Quantitatively Separating Direct and Indirect Fitness Effects

The relative importance of direct and indirect fitness depends on conditions. We plot the results of δ_{dir} and δ_{ind} when SF is initially rare (10%) and common (90%) in

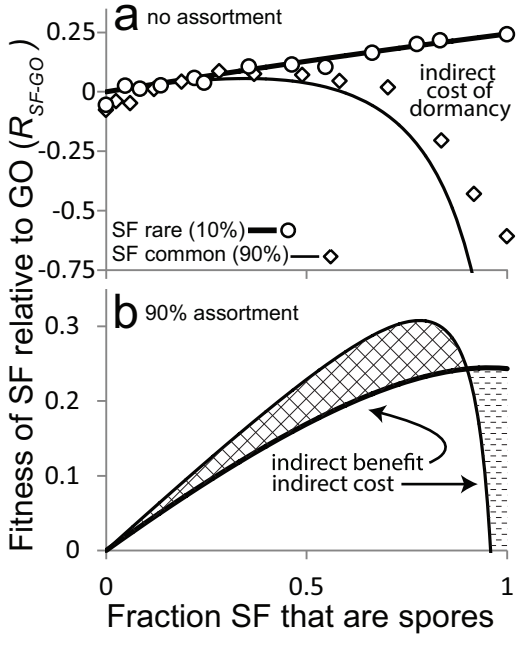

Figure 4: The indirect effect of dormancy is beneficial only in structured populations. When the spore-forming (SF) strain is common (90%), a large fraction of the population can be dormant, sparing many resources. Without population structure, spared resources are preferentially consumed by the competing grower-only (GO) strain, reducing SF's relative fitness. This is seen as a reduction in SF fitness when common (90%) and more resources are spared. SF fitness when rare (10%) is redrawn from (fig. 3*b*). *a*, Model predictions (solid lines) and experimental results modified by separately determined survival of spores and vegetative cells after exposure to miconazole ($s_g = 0.06$, $s_d = 0.57$). *b*, Assortment among SF spores and growers reduces social exploitation by GO. For most of the range of spore formation, SF's relative fitness is higher when SF is common and more resources are spared, compared to when SF is rare (diamond shading).

both unstructured ($\gamma = 0$) and highly structured ($\gamma = 0.9$) environments (fig. 5) with antibiotic exposure. This analysis demonstrates several key results. First, the direct benefit of dormancy is not affected by either SF frequency or assortment. In contrast, higher frequencies of SF (fig. 5; thinner line) increase the magnitude of the indirect effect. Second, dormancy has an indirect fitness effect under all conditions considered, but indirect effects may be negligible when SF is rare and there is little population structure. Finally, dormancy provides an indirect fitness benefit only in structured populations in a way that depends on the degree of structure, specifically when the fraction of spores formed by SF is less than the assortment parameter γ (see fig. A1 for explanation).

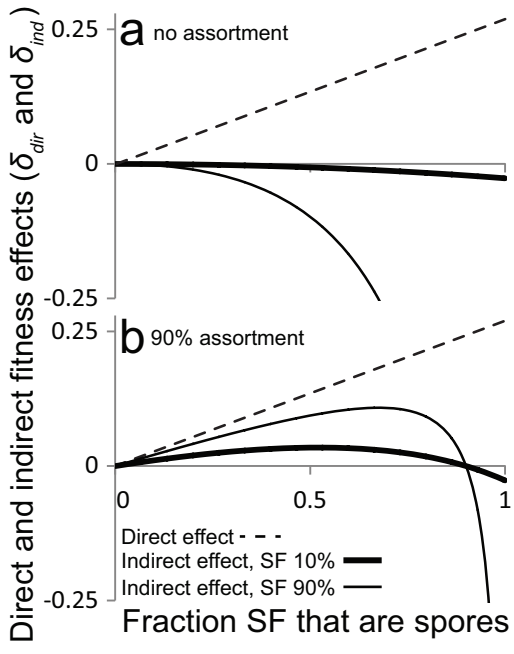

Figure 5: Net consequences of direct (higher survival during antibiotic exposure but fewer growers to reproduce) and indirect (reproduction using resources spared by spores) effects of dormancy for the spore-forming (SF) strain. Specifically, plotted are the relative survival and reproduction of SF to the grower-only (GO) strain due to direct and indirect effects, δ_{dir} and δ_{ind}. While the direct effect of dormancy does not depend on the population frequency of SF or assortment of SF cells, the indirect effect is costly over all ranges of spore formation without assortment (*a*) but beneficial when the fraction of SF that are spores is below SF's degree of assortment, γ (*b*).

Discussion

Since Hamilton's (1964*a*, 1964*b*) insights into inclusive fitness, evolutionary biologists have attempted to disentangle the roles of individual and kin selection in behavioral evolution. This has been especially challenging for traits where there is a clear individual benefit but also an effect on the fitness of conspecifics. While inclusive fitness models acknowledge the need to include such indirect fitness effects (Griffin and West 2002; Lehmann et al. 2007), these indirect "side effects" are typically ignored. Here we show that sporulation in *Saccharomyces cerevisiae* provides an individual fitness benefit during antibiotic exposure while simultaneously affecting the fitness of other yeast nearby. Dormant spores do not consume external resources, allowing nearby nondormant cells to produce more offspring than they otherwise would have. In this article, we quantitatively disentangle individual- and kin-level effects of dormancy, demonstrating that indirect effects can profoundly affect the fitness of a spore-forming strain in ways that depend on its frequency in the population, the fraction of it that are spores, and the level of assortment among its cells (see table A1).

Importantly, dormancy had an indirect fitness effect in all conditions we examined. Without assortment, sporulation was costly, facilitating proportionally more reproduction in a nondormant grower-only competitor. When SF cells preferentially assort with each other, however, resources spared by spores may be consumed mainly by nondormant kin, resulting in an indirect fitness benefit. Such assortment can be generated by population spatial structure (Kerr et al. 2006; Kümmerli et al. 2009; Driscoll and Pepper 2010; Nadell et al. 2010), which is common in natural *Saccharomyces* populations (Johnson et al. 2004; Koufopanou et al. 2006).

Because SF spores cannot themselves use the resources they spare, higher frequencies of spore formation reduce the fraction of spared resources utilized by SF, preferentially making these resources available to the GO (grower-only) competitor. We find that the maximum amount of dormancy SF can support while still preferentially providing spared resources to kin (positive δ_{ind}; figs. 5, A1) depends on the degree of assortment among SF: indirect effects increase SF's relative fitness as long as the fraction of SF cells that are dormant is less than the degree of assortment (γ). Our model, therefore, predicts that in natural populations of heterogeneously dormant organisms, optimal rates of dormancy will depend on the degree of population spatial structure.

Heterogeneous dormancy has evolved in diverse taxa, including plants (Nilsson et al. 1994; Dyer 2004; Gremer et al. 2012), insects (Mousseau and Dingle 1991), and bacteria (Veening et al. 2008; Ratcliff and Denison 2010). This can be problematic for human management of pests and disease-causing microbes. Dormant persister microbes can survive antibiotics and other environmental stresses (Lewis 2010), while dormancy in seeds and insects is thought to allow weeds and pests to avoid exposure to

herbicides and insecticides, respectively (Carriére et al. 1995; Batlla and Benech Arnold 2007). Strong selection by humans may contribute to the prevalence of heterogeneously dormant pests (Naylor and Jana 1976; Carriére et al. 1995) and pathogens (Gefen and Balaban 2009; LaFleur et al. 2010).

Our results suggest that management strategies for heterogeneously dormant organisms should consider cryptic indirect effects. Because indirect effects are minimal when the frequency of a dormant genotype is low, an initially rare heterogeneously dormant mutant under strong selection will increase in frequency only to the extent that dormancy enhances higher individual survival. As a genotype with heterogeneous dormancy becomes more frequent, however, it begins to experience the indirect effects of dormancy. Without population structure, the indirect effects of dormancy will be costly (fig. 5*a*), slowing or even halting its evolution. In highly structured populations (such as pathogens with low within-host diversity), the indirect benefit of dormancy on relative fitness (fig. 5*b*) may accelerate its evolution.

Cryptic indirect fitness effects may play a widespread role in the evolution of traits traditionally considered nonsocial. In general, any trait subject to ecological feedbacks whereby that trait's expression affects the fitness of conspecifics can have a cryptic indirect fitness component. The trait considered in this article, heterogeneous microbial dormancy, is an example of how competition for a finite resource can create such an ecological feedback. Similar feedbacks must be common in nature, as many organisms compete for limiting resources such as food, territory, or mating opportunities. Limiting resources are by definition zero-sum: if an individual obtains more of the resource, then others will necessarily obtain less. In these situations, any change in an individual's resource consumption affects the fitness of conspecifics, creating an indirect fitness effect. Similarly, interactions with antagonists (i.e., predators, pathogens, competitors) may create an ecological feedback, where the result of an individual's interaction with the antagonist affects the fitness of conspecifics. For example, plants that shade competitors (perhaps by making more leaves than would otherwise be optimal) increase the focal individual's access to light, water, and soil nutrients (Denison et al. 2010; Weiner et al. 2010; Dybzinski et al. 2011). This trait creates a local public good, however, providing the same benefits to nearby conspecifics. The extent to which this indirect effect provides a fitness benefit or cost to the focal individual depends on the probability that the neighbors are relatives, which in turn depends on the degree of population structure.

Elucidating the role of cryptic indirect effects in the evolution of traits that provide a direct fitness benefit will require creative theoretical and experimental approaches, custom tailored to individual study systems. But with the potential for such work to improve evolutionary predictions and our expectation that cryptic indirect effects are widespread, we are confident the effort will prove worthwhile.

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APPENDIX

Supplementary Information

Table A1: Key factors influencing the direct and indirect fitness effects of dormancy

Factor	Direct fitness consequence	Indirect fitness consequence
Absence of antibiotics	Fitness cost	Fitness cost
Presence of antibiotics	Fitness benefit	Cost without population structure Benefit if the fraction of SF that are spores $<\gamma$, cost otherwise
SF assortment (γ)	No effect	Higher values of γ improve SF fitness
Higher frequency of SF	No effect	Magnitude of indirect effect (either cost or benefit) increased

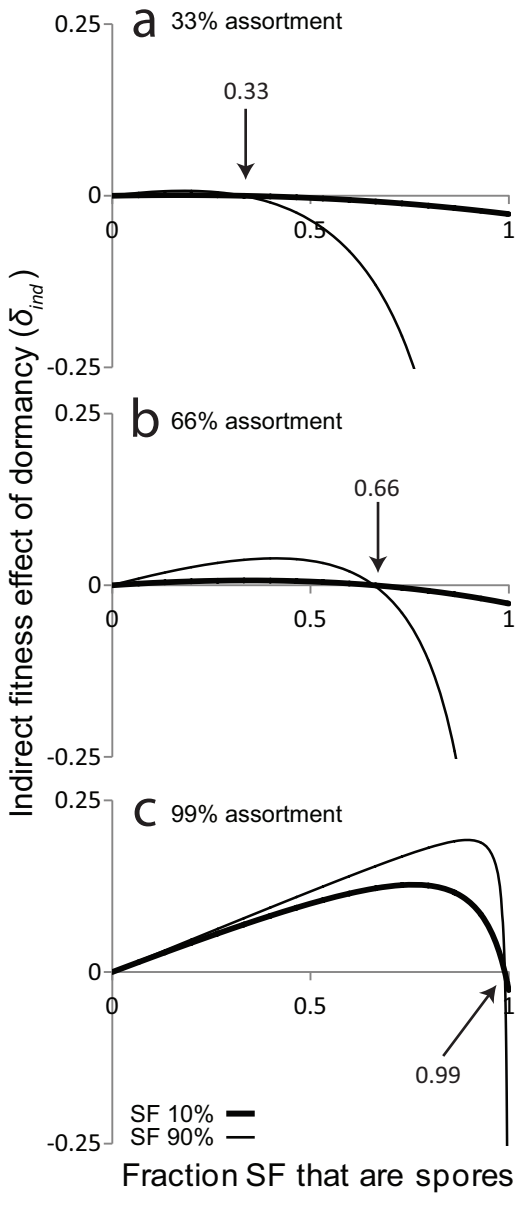

Figure A1: Effects of dormancy on spore-forming SF's survival and reproduction relative to grower-only GO (δ_{ind}) under different levels of assortment (γ). The indirect effect of dormancy increases SF's reproduction more than GO's reproduction when the fraction of spores formed by SF < γ. When the fraction of SF cells that are spores > γ, GO growers utilize proportionally more of the resources spared by SF than SF does (resulting in a negative δ_{ind}). This can be seen by δ_{ind}'s change in sign when the fraction of SF that are spores exceeds (*a*) 0.33, (*b*) 0.66, and (*c*) 0.99. Higher population frequencies of SF generate a larger public good, increasing δ_{ind} when the fraction of SF that are spores < γ but decreasing δ_{ind} when the fraction of SF that are spores > γ.

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