Context, competition, and symbiont-induced bet-hedging between *Dictyostelium discoideum* and *Paraburkholderia*

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Abstract

Symbiotic interactions change with environmental context. We investigated context-dependence and bet-hedging in the symbiosis between social amoeba hosts and *Paraburkholderia* bacteria, where the context is the abundance of host food bacteria. *Paraburkholderia* have been shown to harm hosts dispersed to food-rich environments, but aid hosts dispersed to food-poor environments by allowing hosts to carry food bacteria. Through measuring symbiont density and host spore production, we show that this food context matters in three other ways. First, it matters for symbionts, who suffer a greater cost from competition with food bacteria in the food-rich context. Second, it matters for host-symbiont conflict, changing how symbiont density negatively impacts host spore production. Third, data-based simulations show in some cases this context-dependence can lead to a symbiont-induced bet-hedging advantage for hosts. These results show how food context can have many consequences for the *Dictyostelium*-Paraburkholderia symbiosis and suggest that symbionts can induce bet-hedging in hosts.

Introduction

Context-dependence, where the environment can change the sign or magnitude of one partner's effect on the other, is common in symbioses (Bronstein 1994; Thompson 1994; Chamberlain *et al.* 2014). These context-dependent effects on partners can be crucial to understanding the nature of symbiotic interactions (Keeling & McCutcheon 2017; Iwai 2019). For example, in the symbioses between *Paramecium bursaria* hosts and their *Chlorella* endosymbionts, hosts benefitted from symbiosis in light environments, but were harmed in the dark. For *Chlorella*, the effects of symbiosis were negative in co-culture, indicating that hosts exploited their endosymbionts for the benefits hosts receive in light conditions (Lowe *et al.* 2016). However, in the context of an environment with a *Chlorella* competitor, hosts benefited their symbionts by eating these competitors (Iwai 2019). This example illustrates that understanding how partners affect each other across multiple contexts can change our view of the interaction, sometimes from one of exploitation to one of mutual benefit.

Measures of interaction effects in individual contexts may miss beneficial interactions in symbioses if benefits accrue across contexts at the expense of benefits in any individual context. These benefits that occur across contexts are an example of bet-hedging, where organisms adapt to uncertain environments by avoiding the worst effects of harsh contexts while being suboptimal in more favorable contexts (Slatkin 1974; Philippi & Seger 1989; Starrfelt & Kokko 2012). This reduces the variance in fitness across contexts (increasing geometric mean fitness) at the expense of the conventional, arithmetic mean fitness (Seger & Brockmann 1987). For example, in *Lobelia inflata,* limiting the timing of reproduction may reduce the variance in fitness from unpredictable

70 season lengths while being suboptimal in any individual year (Simons & Johnston 2003).
71 Bet-hedging is thus defined as an adaptation that involves a trade-off between geometric
72 mean fitness and arithmetic mean fitness.

73 Bet-hedging is commonly non-social, but joining together with others may be a
74 powerful way to hedge one's bets against harsh conditions. Cooperative breeding in birds
75 (Rubenstein 2011) and plant-fungus mutualisms (Lekberg & Koide 2014) have now been
76 suggested as bet-hedging phenotypes. Symbiont-induced bet-hedging may be common
77 because symbionts often give hosts novel traits that allow hosts to gain access to new,
78 sometimes harsh, environments (Moran 2007; Oliver *et al.* 2010; DiSalvo *et al.* 2015).
79 For example, endosymbiotic bacteria in aphids play important roles in whether hosts can
80 withstand heat (Montllor *et al.* 2002) or survive on different host plants (Tsuchida 2004).
81 These novel traits may be more likely to involve bet-hedging if symbionts induce a cost
82 in other contexts so geometric mean fitness is increased at the expense of arithmetic mean
83 fitness. However, symbiont-induced bet-hedging in context-dependent symbioses
84 remains relatively unexplored.

85 Context-dependence is important in the lifecycle of the social amoeba
86 *Dictyostelium discoideum*. Amoebae need edible bacteria to grow and proliferate (Raper
87 1937), but the abundance (Young 2004; Vos *et al.* 2013) and quality (Kuserk 1980;
88 Brock *et al.* 2018) of food bacteria in the soil is known to vary at smaller scales. This
89 results in a patchy environment where some patches are food-rich and other patches are
90 food-poor. In response to starvation, amoebae aggregate and form a multicellular fruiting
91 body to disperse resistant spores to new environments (smith *et al.* 2014). The patchy soil

environment is considered an important selection pressure for this fruiting body structure (Bonner 1982; Kessin 2001).

D. discoideum interacts with three species of mostly inedible *Paraburkholderia* bacterial symbionts — *P. agricolaris*, *P. hayleyella*, and *P. bonniae* (Brock *et al.* 2020). Throughout this paper, we will use "*Paraburkholderia"* or "symbionts" as shorthand for the three symbiotic *Paraburkholderia* species. Hosts infected with *Paraburkholderia* have been isolated from multiple locations in the United States with around 25% of screened hosts being infected by at least one species (Haselkorn *et al.* 2019). *Paraburkholderia* are able to enter and live inside *D. discoideum* cells and spores, but can also proliferate, albeit sometimes only slowly, without their hosts (DiSalvo *et al.* 2015; Shu *et al.* 2018a; Brock *et al.* 2020) unlike the obligate endosymbionts that are also found in *D. discoideum* (Haselkorn *et al.* 2021). There is some evidence consistent with coevolution between hosts and symbionts (Brock *et al.* 2016; Shu *et al.* 2018a; Garcia *et al.* 2019; Brock *et al.* 2020). For example, host clones naturally infected with *P. hayleyella* are harmed less by infection with this symbiont than host clones that were not infected in the wild (Shu *et al.* 2018a) indicating that *P. hayleyella* hosts have adaptations favoring symbiosis. Symbionts also have the ability to move towards hosts (Shu *et al.* 2018b), suggesting that being able to find hosts is beneficial.

The symbiosis with *Paraburkholderia* bacteria impacts the growth and proliferation of *D. discoideum*. Having symbionts allows hosts to carry food bacteria inside the spore-containing part of fruiting bodies called the sorus (DiSalvo *et al.* 2015). Whether this novel trait is advantageous or not depends on the presence of food bacteria after dispersal. When food is abundant, having symbionts can be costly, as shown by

115 infected amoebae producing fewer spores than uninfected amoebae (Brock *et al.* 2011;
116 DiSalvo *et al.* 2015). In food-poor environments, the cost of having *Paraburkholderia* is
117 compensated by hosts gaining the ability to carry food bacteria (in addition to inedible
118 *Paraburkholderia*) in dispersing spores. This allows amoebae to disperse and grow where
119 they ordinarily could not (Brock *et al.* 2011; DiSalvo *et al.* 2015). These context-
120 dependent effects on the host could be extremely important in the natural soil
121 environment, where food-poor patches arise frequently (Kessin 2001).

122 Less is known about how symbionts are affected across food contexts. Gaining
123 the ability to disperse to new locations may be a major reason for symbionts to seek out
124 social amoeba hosts (Garcia & Gerardo 2014), but could also make the context of host
125 food bacteria in the new environment important for symbionts. A benefit from being
126 dispersed to patches with few bacteria could be that symbionts face reduced competition.
127 If few bacteria are present, symbionts will mostly compete with food bacteria that were
128 also carried in the sorus. This should be a relatively low competition situation because
129 symbionts outnumber food bacteria in sori (Khojandi *et al.* 2019). Having few
130 competitors should advantage symbionts while environments with plentiful bacteria
131 could strongly limit symbiont growth because of their relatively slow growth rates, at
132 least as measured in the lab (Brock *et al.* 2020). We will use "food-rich" and "food-poor"
133 to describe newly colonized patches with many and few bacteria, respectively, of the sort
134 edible by *D. discoideum*. These categories reflect the relationship to *D. discoideum* and
135 could be called high and low competition in terms of their effect for *Paraburkholderia*.

136 It is also unclear how the number of extracellular *Paraburkholderia* in the
137 environment impacts hosts since previous studies have focused on intracellular

138 *Paraburkholderia* (Shu *et al.* 2018b; Miller *et al.* 2020). When they are outside the
139 amoebae, *Paraburkholderia* could affect *D. discoideum* fitness through interactions with
140 food bacteria perhaps by reducing the amount of food for hosts through competition or by
141 releasing diffusible toxins that affect amoebae. Thus host food context could also affect
142 the relationship between symbiont density and host spore production.

143 It is also not known whether symbionts can induce a bet-hedging phenotype in
144 hosts. This phenotype would give hosts the ability to evade the harsh effects of food-poor
145 contexts at the expense of reduced fitness in food-rich contexts. This could increase host
146 success across contexts (measured by geometric mean fitness) by reducing the variance in
147 fitness across contexts, but will appear maladaptive according to measures of arithmetic
148 mean fitness (Seger & Brockmann 1987; Starrfelt & Kokko 2012). Infected hosts could
149 alternatively gain an outright advantage from carrying symbionts without a trade-off
150 between arithmetic and geometric mean fitness.

151 To understand context-dependence in the symbiosis between amoebae and
152 *Paraburkholderia*, we used *D. discoideum* infected with either *P. agricolaris* or *P.*
153 *hayleyella* — the two most common and best-studied species of *D. discoideum*
154 symbionts. We investigate whether *Paraburkholderia* benefit from reduced interspecific
155 competition when dispersed to food-poor contexts, how symbiont density and food
156 context impact host spore production, and whether symbionts induce a bet-hedging
157 phenotype in their hosts when food conditions vary across soil patches.

158

159 **Methods**

160

161 To understand the effects of symbiosis across food contexts, we used 4 naturally
162 uninfected *D. discoideum* clones, 4 clones naturally infected with *P. agricolaris*, and 4
163 clones naturally infected with *P. hayleyella* (Figure 1). We cured the infected clones and
164 re-infected them with their native symbionts to equalize infection density. Uninfected
165 clones were left uninfected, but were otherwise treated the same as infected clones. This
166 resulted in three host infection conditions: uninfected, infected with *P. agricolaris*, and
167 infected with *P. hayleyella*. To mimic natural dispersal, we collected sori and transferred
168 them to food-rich (with additional *K. pneumoniae* bacteria) or food-poor (KK2 buffer
169 with no *K. pneumoniae*) nutrient plates. Bacteria appear on food-poor plates only if
170 transferred sori contain bacteria, as expected for infected samples. We grew replicate
171 experimental sets involving all conditions beginning on two separate dates, July 13 and
172 22, 2020, and followed up with additional experiments (see results) beginning on January
173 26 and April 23, 2021.
174
175 *Paraburkholderia isolation*
176 To isolate *Paraburkholderia* from their hosts, we grew wild collected *D.*
177 *discoideum* clones on SM/5 plates (2 g glucose (Fisher Scientific), 2 g Bacto Peptone
178 (Oxoid), 2 g yeast extract (Oxoid), 0.2 g $MgSO_4$ * $7H_2O$ (Fisher Scientific), 1.9 g
179 KH_2PO_4 (Sigma-Aldrich), 1 g K_2HPO_4 (Fisher Scientific), and 15 g agar (Fisher
180 Scientific) per liter). Wild *D. discoideum* clones were grown with *K. pneumoniae* food
181 bacteria that were suspended in KK2 buffer (2.25 g KH_2PO_4 (Sigma-Aldrich) and 0.67 g
182 K_2HPO_4 (Fisher Scientific) per liter). After wild clones completed the social cycle
183 (feeding, starvation, and fruiting body formation), we collected sori with pipette tips and

184 placed them on SM/5 plates. We allowed the bacteria and amoebae contained within to
185 proliferate and then streaked out the resulting bacteria to get single colonies.
186
187 *Paraburkholderia removal*
188 To generate uninfected clones, we treated infected *D. discoideum* clones with
189 antibiotics by plating on 30 μg/mL tetracycline SM/5 plates with 200 μL of 1.5 optical
190 density (OD_{600}) tetracycline-resistant *K. pneumoniae* suspended in KK2 buffer. After
191 passage on SM/5 plates without tetracycline to let the amoebae recover from any effects
192 of the antibiotic, we collected single sori with a pipette tip and placed ten of them in
193 different locations on SM/5 plates to confirm that we had successfully removed the
194 bacteria. If bacteria are present, these spot tests will show bacterial growth and
195 *Dictyostelium* proliferation as the spores hatch and eat the bacteria (Brock *et al.* 2011).
196 Without bacteria, amoebae cannot proliferate and the spot will stay blank. We considered
197 a clone to be cured if no bacteria showed up in spot tests. We similarly treated naturally
198 uninfected hosts with tetracycline to control for any effect of curing on our results.
199
200 *Paraburkholderia re-infection*
201 We re-infected cured *D. discoideum* clones with their native *Paraburkholderia*
202 isolates by plating 200 μL $2x10^5$ spores with 200 μL of 0.1% *Paraburkholderia* solution.
203 This solution consisted of 1.5 OD_{600} *Paraburkholderia* and 1.5 OD_{600} *K. pneumoniae* in a
204 1:1000 ratio. To confirm re-infection (and also successful isolation), we performed spot
205 tests as above, where successful re-infection was inferred when bacteria grew on 8 or
206 more spots out of the 10 we put down.

207

208 *Artificial dispersal to food-rich and food-poor plates*

209 To obtain sori to transfer to food-rich and food-poor plates, we started by growing
210 *D. discoideum* clones from frozen stock, as described above, on 200 μL 1.5 OD *K.*
211 *pneumoniae* expressing green fluorescent protein (GFP). We obtained GFP-expressing *K.*
212 *pneumoniae* (strain ID DBS0349837) from the Dicty Stock Center at dictyBase (Fey *et*
213 *al.* 2013). This initial growth period is to remove freezer effects and ensure that food
214 bacteria that are carried to new plates are GFP-expressing since stocks were fed non-GFP
215 bacteria before freezing. After six days of growth, we used pipette tips to collect sori
216 from mature fruiting bodies. We counted spores using a hemocytometer and diluted
217 spores to a concentration of $2x10^5$ per mL, and then plated them on plates with (food-
218 rich) or without (food-poor) an additional 200 μL of the GFP-expressing food bacterium
219 *K. pneumoniae* (Figure 1). In order to survive on food-poor plates, the host must carry
220 food bacteria from the previous plate. We grew food-rich and food-poor plates for six
221 days unless otherwise stated, enough time for mature fruiting bodies to form.

222

223 *Measurement of bacteria density*

224 To measure *Paraburkholderia* density, we measured the quantity of bacteria left
225 on plates after *D. discoideum* formed fruiting bodies. We first collected plate contents by
226 washing plates with 15 mL of KK2 buffer. To remove fruiting bodies and bacteria
227 associated with fruiting bodies, we centrifuged wash solutions for three minutes at 13000
228 rpm. We measured bacteria using optical density measured at 600 nm (OD_{600}), a

229 frequency at which bacteria commonly scatter light. Because the OD_{600} is due to both
230 *Paraburkholderia* and *K. pneumoniae*, we used GFP fluorescence measurements (with an
231 excitation wavelength of 485 and emission wavelength of 515 nm) and a standard curve
232 relating *K. pneumoniae* fluorescence to its OD_{600} to subtract out the component due to
233 GFP-expressing *K. pneumoniae*. Both OD_{600} and fluorescence measures were performed
234 in a 96 well plate with a Tecan Infinite 200 Pro microplate reader.

235 To validate our standard curve, we compared predicted OD_{600} of *P. agricolaris*
236 and *K. pneumoniae* to colony forming unit (CFU) counts from the same samples. Linear
237 regression revealed that predicted OD_{600} measurements explained most of the variation in
238 CFUs, showing that our assay is reliable (Figure S1). We also checked our standard curve
239 for significant quadratic terms, which can cause measurement errors when combining
240 OD_{600} and fluorescence measures at high densities (Meyers *et al.* 2018), but our curve did
241 not have a significant quadratic term.
242
243 *Host spore production*
244 To measure host spore production, we estimated spore concentration in the
245 supernatants from washed plates using a hemocytometer. We then calculated the total
246 number of spores per plate by multiplying by the volume of wash solution.
247
248 *Spore production simulations*
249 To test whether *Paraburkholderia* symbionts induce a bet-hedging phenotype in
250 hosts, we simulated repeated bouts of growth and dispersal across soil patches with
251 different probabilities of having food bacteria. Bet hedging occurs when the best

252 phenotype in terms of arithmetic mean fitness is not the same as the best phenotype in
253 terms of geometric mean fitness (Seger & Brockmann 1987). We separately modeled
254 three host phenotypes: (1) unifected (2) infected with *P. agricolaris*, or (3) infected with
255 *P. hayleyella*. Infections with both species at once are possible, but are rare in nature
256 (Haselkorn *et al.* 2019) so we exclude coinfections from our analysis.

257 We assumed that environments consisted of x discrete soil patches. In each round
258 of the simulation, patches are randomly assigned as food-rich with probability p or food-
259 poor with probabity 1-p. Thus, in simulations with one patch, populations experience
260 variation in food abundance that is completely temporal. With additional patches,
261 variation can also occur across space. This will reduce temporal variation because fitness
262 at a given time will be summed over multiple patches.

263 Initially all patches are colonized. Each patch produces a number of spores,
264 drawn from the distribution of our empirical spore production values, according to
265 whether it is a food-rich or food-poor patch. Infected hosts have been found to have
266 reduced spore production in food-rich environments that depends on infection density of
267 *Paraburkholderia* endosymbionts (Brock *et al.* 2011; DiSalvo *et al.* 2015; Miller *et al.*
268 2020). We did not detect this cost in our data, perhaps because we infected with fewer
269 *Paraburkholderia*. Since these costs appear to vary and could be an important factor
270 affecting fitness in the soil environment when food abundance varies, we included them
271 as a variable. To model costs, we penalized host spore production in food rich
272 environments by multiplying spore production values by $1 - c$, where c is the proportion
273 of spores lost because of the cost of infection. When c is 0, we model the scenario
274 observed in this study, with no infection cost. These cost-adjusted spore production

values are summed to get the total spore production across all patches. This is divided by $2x10^5$, a rough estimate of the number of spores in a typical sorus, to get the total number of sori, which we are assuming to be the dispersal unit.

The global pool of sori is used to seed the next round. Patches are assumed to be empty and are randomly reassigned as food-poor or food-rich. Each sorus is randomly assigned to a patch and it successfully germinates with probability *g* (we assume g = 0.5 in the main text; other values (0.1 and 0.9) result in similar outcomes (Figure S2&S3). We assumed that patches colonized by multiple sori were the same as singly colonized patches for the purposes of determining their subsequent spore production. Some patches may remain unfilled.

We let cost of infection *c* and the frequency of food-poor patches *p* vary from 0 to 1 in increments of 0.1 and simulated environments with one, 10, and 100 soil patches. We simulated dispersal to new patches for 100 rounds of growth and dispersal using 500 replicates for each combination of probability of *p* and *c* for each phenotype. At the end of the 100 rounds, we calculated the total spore production per round and calculated geometric mean spore production from these values. To calculate arithmetic mean spore production, we sampled our spore production values from food-rich and food-poor plates according to the number of patches and the value of *p*. We used t tests to compare uninfected hosts' spore production to that of either *P. hayleyella* or *P. agricolaris* infected hosts for a given parameter combination. If there was no significant difference between infected and uninfected, we categorized results as "no best phenotype". Because we used many parameter combinations, we corrected p-values for multiple comparisons

297 using false discovery rate at the level of different numbers of patches and different
298 infection statuses (at the level of individual grids in Figure 4).
299
300 *Statistical Methods*
301 We performed statistics in R version 3.6.3 (R Core Team 2020). To compare
302 bacteria density and spore production, we used linear mixed models (LMM) with the *lme*
303 function in the nlme package (Pinheiro & Bates 2006). To account for random variation
304 from replicate clones and effects of dates when experiments were performed, we included
305 clone and the date the experiment was performed — along with each variable on its own
306 — as random effects. To select the best model of random effects, we used AICc, a
307 sample-size-corrected measure of model fit that balances predictive ability and model
308 complexity (Burnham & Anderson 2004). Many of our models showed different
309 variances between treatments. To account for these differences in variance, we weighted
310 models with the *varIdent* function in nlme (Pinheiro & Bates 2006). We used the
311 emmeans package (Lenth *et al.* 2018) to perform contrasts.
312 To understand how *Paraburkholderia* density affects host spore production across
313 food conditions, we fit a LMM using only infected hosts that included symbiont density
314 leftover on plates and whether the plate was food-rich or food-poor, along with the
315 interaction between these variables. We included random effects for clone, date, and both
316 crossed effects and selected the best random effect structure with AICc. We determined
317 whether the interaction was important by comparing AICc of the model including the
318 interaction with models including the other variables but lacking the interaction.
319

Results:

Paraburkholderia dispersed by Dictyostelium sori have lower growth when host food bacteria are abundant

The context of a food-poor environment is known to be important for *D. discoideum* hosts. It is not known how *Paraburkholderia* are affected by this same context, but reduced competition with food bacteria seems likely. We tested this by growing infected sorus contents on food-poor and food-rich nutrient plates and measuring the density of *Paraburkholderia* after *D. discoideum* fruiting body formation (Figure 1). After infected hosts formed fruiting bodies, *Paraburkholderia* densities were lowest in food-rich conditions (Figure 2A), as expected if they compete with food bacteria. There was around five times more *P. agricolaris* on food-poor than food-rich plates (LMM, $p < 0.001$). *P. hayleyella* growth was higher in food-poor conditions than food-rich, but this difference was not significant after 6 days (LMM, $p = 0.416$). Because *P. hayleyella* grows slowly, we performed two more experiments with *P. hayleyella* with 8 and 12-day growth periods (Figure 2B). Allowing for longer incubations did not result in significantly higher density of *P. hayleyella* (LMM, $p = 0.633$), suggesting that *P. hayleyella* reach their maximum density at or before 6 days, but including these additional experiments gave us enough power to find a significant increase in *P. hayleyella* density in food-poor conditions relative to food-rich (LMM, $p = 0.027$). These results show that symbiont density is context-dependent.

Higher symbiont density harms hosts, but less so in food-poor contexts

342 The host-food context may affect the relationship between symbiont density and
343 host spore production and therefore the degree of conflict or cooperation between them.
344 To investigate this, we also measured total host spore production from plates where we
345 measured the growth of *Paraburkholderia* symbionts (Figure 1). We used uninfected
346 hosts as a baseline for fitness without symbionts. We confirmed prior studies (Brock *et*
347 *al.* 2011; DiSalvo *et al.* 2015) showing that infected hosts could carry food bacteria and
348 proliferate on food-poor plates, while uninfected host could not (Figure 3A).
349 Surprisingly, we did not observe a cost of being infected in food-rich conditions ($p > 0.5$
350 for both species) which has been seen in previous studies (Brock *et al.* 2011; DiSalvo *et*
351 *al.* 2015; Shu *et al.* 2018a). This is likely a result of our lower infection dosage of 0.1%.
352 While having some symbionts is essential for hosts to be able to carry food and
353 survive in food-poor conditions, higher symbiont densities may nevertheless harm hosts,
354 perhaps in ways that depend on food context. We found that larger populations of
355 symbionts as measured by OD_{600} were associated with lower host spore production, but
356 this harm was reduced in food-poor conditions. Lower host spore production was
357 associated with being in a food-poor environment ($\beta_{food\text{-}poor} = -3.283$, se = 0.853) and
358 symbiont density ($\beta_{density} = -10.317$, se = 6.364), but the interaction between food scarcity
359 and symbiont density showed that the harmful effect of higher symbiont densities was
360 lessened on food-poor plates ($\beta_{food\text{-}poor*density} = 8.078$, se = 6.381; Figure 3B). These
361 results indicate that symbiont density may come at the expense of host spore production,
362 but that this cost decreases in food-poor environments.
363
364 *Symbionts can induce a bet-hedging phenotype in hosts*

365 Because *Paraburkholderia* helps hosts in food-poor contexts, we hypothesized
366 that they could give hosts a bet-hedging advantage that increases geometric mean fitness
367 at the expense of arithmetic mean fitness across spatial and temporal variation in food
368 abundance in the soil. We modeled this by using our empirical spore production values to
369 simulate 100 rounds of growth and dispersal across environments with one, 10, and 100
370 soil patches. We modeled three symbiosis phenotypes: (1) uninfected, (2) infected with
371 *P. agricolaris*, and (3) infected with *P. hayleyella* (Figure 4A, Animations S1-3) in their
372 own separate environments and then compared their geometric and arithmetic mean spore
373 productions.

374 Soil patches were either food-rich or food-poor and changed randomly in each
375 round. By changing the number of patches, we are able to understand how host fitness is
376 affected when variation in food abundance is more temporal (few soil patches) or spatial
377 (many soil patches). We varied the frequency of food-poor soil patches and calculated a
378 phenotype's success as the geometric or arithmetic mean spore production. We included
379 the cost of infection observed in other studies (DiSalvo *et al.* 2015; Shu *et al.* 2018a;
380 Haselkorn *et al.* 2019; Miller *et al.* 2020) by subtracting some proportion of spores from
381 our empirical measures for infected hosts in food-rich conditions. We classified a
382 phenotype as a bet-hedger if the best phenotype in terms of geometric mean fitness
383 differed from the best phenotype in terms of arithmetic mean fitness (Figure 4B).

384 We found that uninfected hosts were favored by both geometric and arithmetic
385 means when costs of infection were high, food was abundant, and food abundance
386 variation was spatial (Figure 4C&D; pink). Associating with *Paraburkholderia*,
387 especially *P. hayleyella*, provided an advantage in terms of arithmetic mean fitness across

388 a wide range of parameters, even with fairly large costs (Figure 4C&D; blue). Symbionts
389 were most beneficial when variation in food abundance was temporal (one soil patch). As
390 the number of patches in the environment increased, the benefits of having symbionts
391 decreased, with *P. agricolaris* being beneficial only when the probability of food was
392 high. This lowered benefit of *P. agricolaris* is especially apparent when fewer potential
393 dispersers manage to colonize new patches (Figure S2).

394 We found that bet-hedging is likely when variation in food abundance is more
395 temporal (one patch), when food-poor patches are common, and when costs of infection
396 are high (Figure 4C&D; green). This bet-hedging advantage in more temporally varying
397 environments is because uninfected populations go extinct when generations without
398 food occur (as illustrated in Figure 4A with a single soil patch, Animation S1). Bet-
399 hedging becomes rarer as the number of patches increases (middle and rightmost panels
400 in Figure 4C&D) and the worst consequences of food-poor patches (extinction) become
401 rare due to the presence of some patches with food. Bet-hedging occurs across a broader
402 range of parameters in *P. agricolaris* simulations (Figure 4C). These results show that
403 symbionts can provide an outright benefit for hosts in many conditions and can induce a
404 bet-hedging phenotype in some conditions.

405

406 **Discussion**

407 Our results show how the context of host food abundance affects the
408 *Dictyostelium-Paraburkholderia* symbiosis beyond the previously demonstrated
409 advantage to hosts when food is rare (Brock *et al.* 2011). We found evidence that both
410 *Paraburkholderia* species benefit from reduced competition when they are carried to

411 food-poor environments (Figure 2). Symbiont density negatively affected host spore
412 production, but symbionts harmed hosts less in food-poor conditions (Figure 3B).
413 Symbionts gave hosts an outright or a bet-hedging advantage across a range of
414 parameters in patchy soils (Figure 4).

415 Our finding that symbionts benefit from reduced competition when dispersed to
416 food-poor environments fits in with other findings of competitive benefits for symbionts
417 (Iwai 2019). Competition between symbionts and food bacteria may also be the cause of
418 context-dependent effects of symbiont density on host spore production. Our spore
419 production results showed that higher symbiont densities resulted in lower host spore
420 production, indicating that symbionts are harmful to hosts. However, higher symbiont
421 densities are less harmful in food-poor conditions when competition is lower (Figure 3B).
422 The reduced harm for hosts could be the result of less antagonism between bacteria,
423 which results in less collateral damage to amoebae through secreted toxins or other
424 competitive interactions between food bacteria and symbionts. The generality of our
425 results is limited somewhat by only using one species of food bacteria. While using a
426 single food bacterium is more experimentally tractable, amoebae encounter multiple
427 bacteria species in their natural environments (Brock *et al.* 2018). Different species, or
428 combinations of species, could change competition with symbionts and affect host spore
429 production in different ways.

430 *Paraburkholderia* symbionts benefit hosts by giving hosts the ability to carry food
431 to food-poor contexts (Brock *et al.* 2011; DiSalvo *et al.* 2015). Using simulations, we
432 found that this benefit was prevalent across a range of costs, food probabilities, and
433 number of soil patches according to measures of arithmetic mean spore production

434 (Figure 4). Hosts could also benefit across contexts through bet-hedging, where
435 geometric mean fitness trades off with arithmetic mean fitness (Seger & Brockmann
436 1987). Bet-hedging occurred more often in our simulations when costs imposed by the
437 symbiont were relatively high and food abundance varied temporally (few soil patches).
438 Temporal variation disadvantaged uninfected amoebae because they went extinct in food-
439 poor patches while infected amoebae weathered these harsh contexts. These results are
440 interesting when paired with the finding that infected hosts constitute about 25% of the
441 population (Haselkorn *et al.* 2019). This may mean that symbiosis is not universally
442 favored — if so, *Paraburkholderia* infection would be fixed in the population — but
443 instead depends on ecological conditions.

444 Our simulations were necessarily simplified to make them tractable. Two notable
445 limitations from this are that soil patches are discrete and equally sized and that host
446 spore production as measured in the lab may not accurately reflect nature. Though not
447 unique to our model (Starrfelt & Kokko 2012), our finding that bet hedging is most likely
448 in temporally varying environments also limits the scope of our results. However, such
449 temporal variation may result naturally in the soil from rainfall variation and has been
450 shown to impact soil bacteria abundance (Barnard *et al.* 2013). Though it remains
451 uncertain how much *Paraburkholderia* induces host bet-hedging in the wild, our model
452 illustrates the potential for a symbiotic partner to induce bet-hedging.

453 Our simulation results suggest that bet-hedging may be an important, but
454 underappreciated, consequence of some symbioses. Bet-hedging has been suggested in
455 some symbioses (Lekberg & Koide 2014), but has not been tested in detail. Though our
456 simulations were tailored to the symbiosis between *D. discoideum* and *Paraburkholderia*,

they should apply to other cases of context-dependent symbiosis with costs and temporal variation. Since context-dependence is common in symbioses (Bronstein 1994), we expect that bet-hedging that involves symbiosis may be relatively common. Accounting for geometric mean fitness in symbiotic interactions will be crucial to determining the true extent of bet-hedging in symbioses.

Bet-hedging may be especially relevant in symbioses where hosts gain novel phenotypes from symbionts (Moran 2007; Oliver *et al.* 2010) because they can allow symbionts to survive in new, previously harsh, contexts (Montllor *et al.* 2002; Tsuchida 2004). However, it is important to note that bet-hedging will not evolve unless these harsh contexts occur probabilistically. If the new harsh environment is constant, symbiosis will be favored without any bet-hedging. If the harsh environment is too predictable, plasticity will be favored instead of bet-hedging (Botero *et al.* 2015). Thus an understanding of the ecological contexts will also be important for identifying symbiont-induced bet-hedging.

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Authorship statement: TS, DQ, and JS designed the study and wrote the manuscript. TS performed the experiments and simulations and analyzed the data.

Data Statement: Data will be archived at Dryad upon acceptance. Data and code for simulations and analysis will be available at www.gitlab.com/treyjscott/farmerBH.

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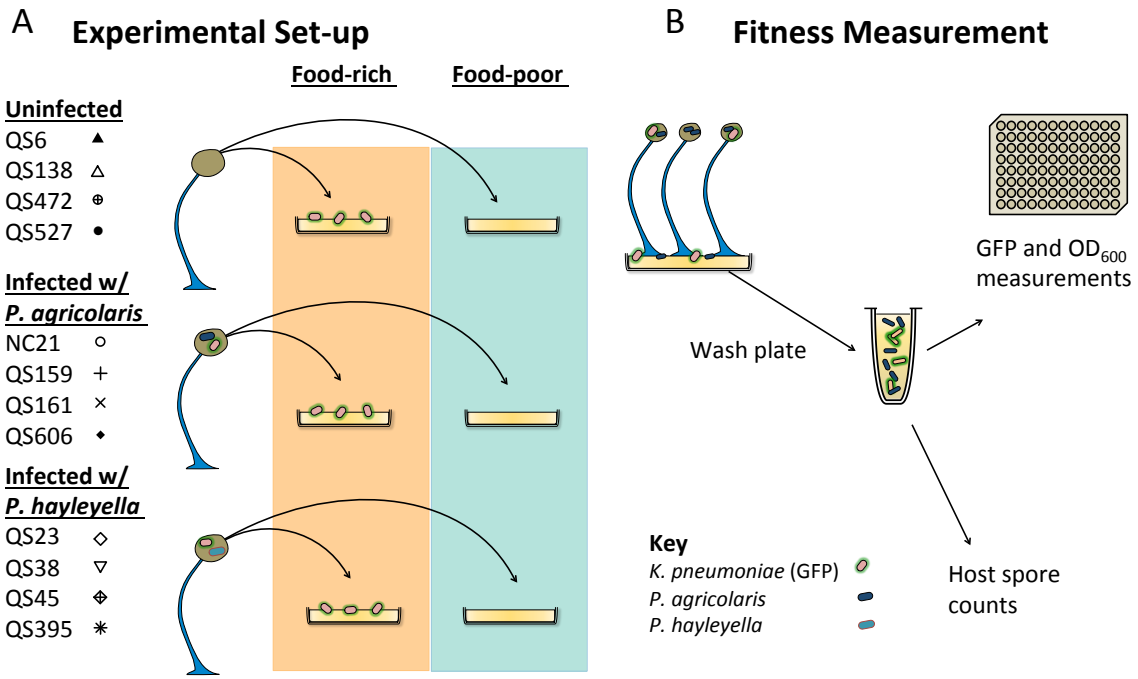
606 **Figure Legends**



607

608 **Figure 1:** Schematic of experimental design. (A) Uninfected and infected *D. discoideum*

609 fruiting bodies are collected and plated on food-rich and food-poor plates (after one

610 passage on GFP-expressing *K. pneumoniae* food bacteria). These plates are grown for six

611 days and then washed for bacterial measurement and spore counting (B). Bacteria are

612 measured by calculating GFP fluorescence and optical density (see Methods).

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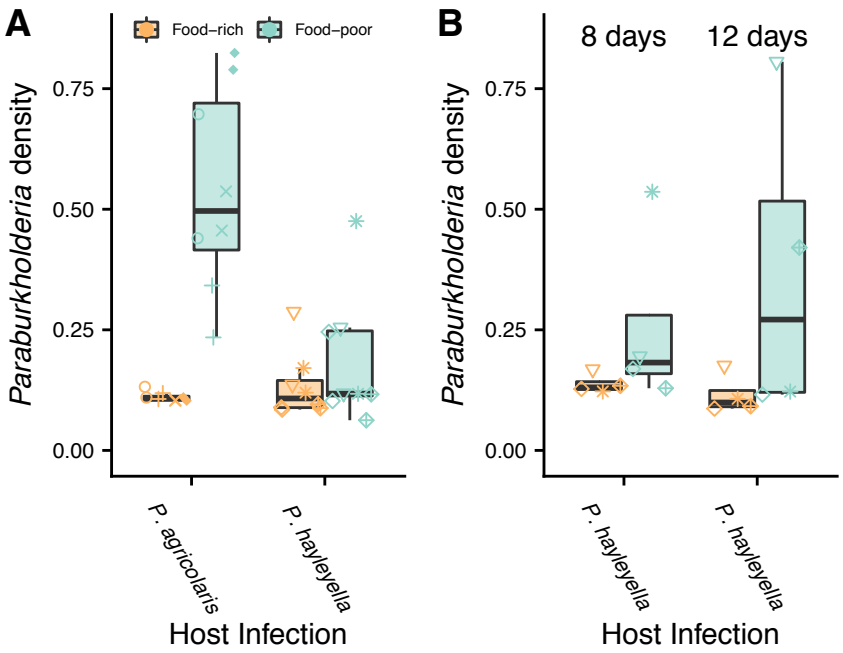

Figure 2: More *Paraburkholderia* were recovered from plates after fruiting body formation from food-poor plates (those that had not received additional *K. pneumoniae*). (A) *Paraburkholderia* density after 6 days. (B) *P. hayleyella* density after 8 and 12 days. Point shapes show individual clones 4 of each (see Figure 1).

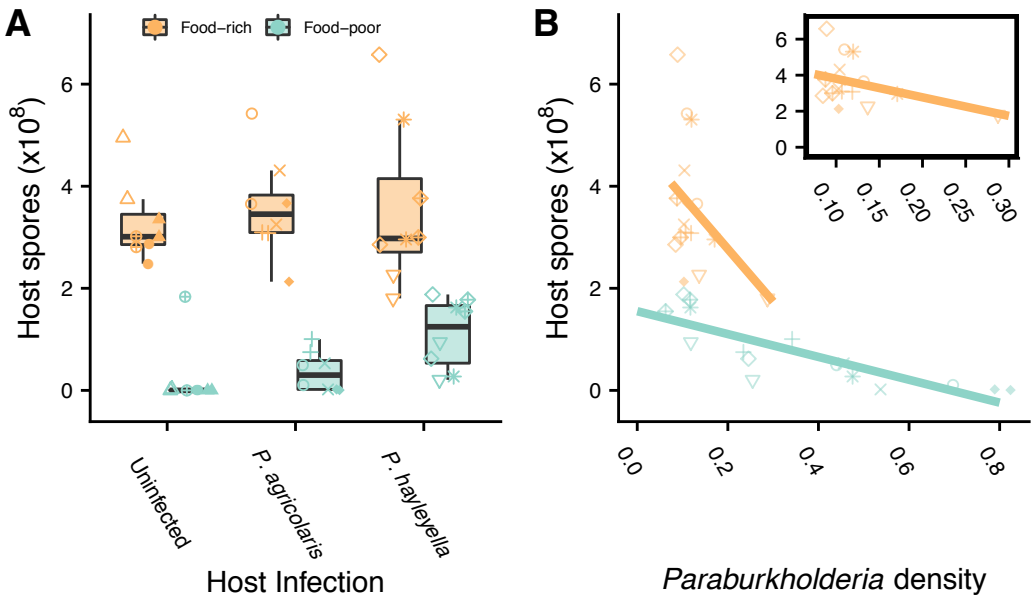

Figure 3: Effects of *Paraburkholderia* infection and density on host spore production. (A) Spore production of hosts from food-rich and food-poor plates for uninfected, *P. agricolaris* infected, and *P. hayleyella* infected hosts. (B) Interaction between measured *Paraburkholderia* density (OD_{600}) and food environment on host spore production. This interaction model explained 95% of the variance in spore production. Inset shows food-rich results on smaller scale. Point shapes show individual clones (see Figure 1).

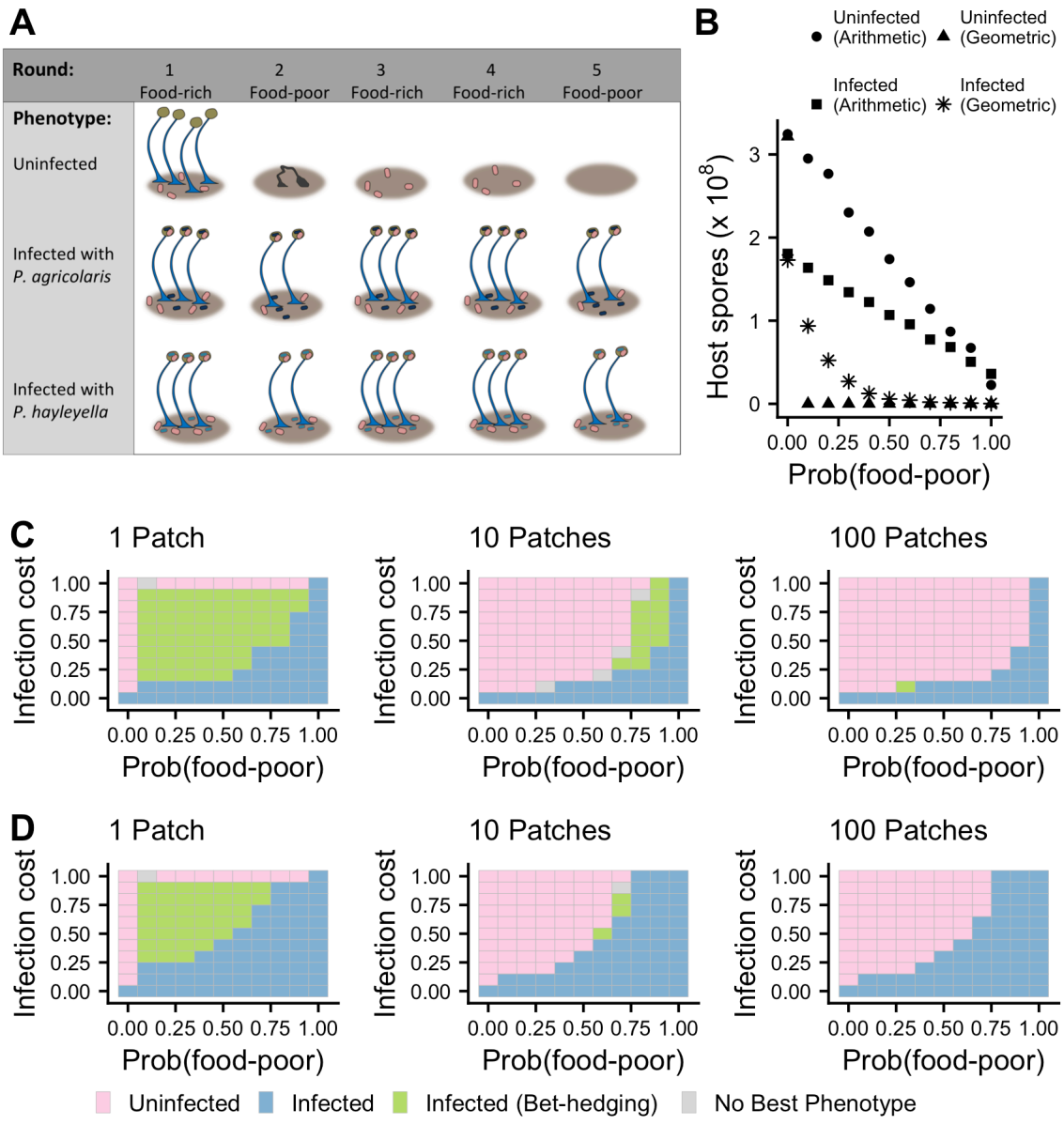

Figure 4: Benefits of associating with *Paraburkholderia* symbionts depends on variation in food availability, fitness costs, and number of soil patches. (A) Toy example of simulations with five rounds of growth and dispersal where spores are dispersed to a single soil patch each generation. Lack of food could lead to the extinction of uninfected clones. (B) Illustration of bet-hedging trade-off between geometric and arithmetic mean spore production in a single soil patch. Bet-hedging occurs when the food-poor probability is between 0.1 and 0.7 (note how uninfected hosts have the highest arithmetic

mean while infected hosts have the highest geometric mean). Infected hosts suffered a 50% reduction in spore production in these simulations. Performance of *P. agricolaris* (C) and *P. hayleyella* (D) relative to uninfected for 1, 10, and 100 soil patches. Pink shows when uninfected hosts have higher arithmetic and geometric mean spore production; blue shows when infected hosts have higher arithmetic and geometric mean spore production; green shows when arithmetic fitness is reduced for higher geometric mean fitness (bet-hedging).

Supplemental Material

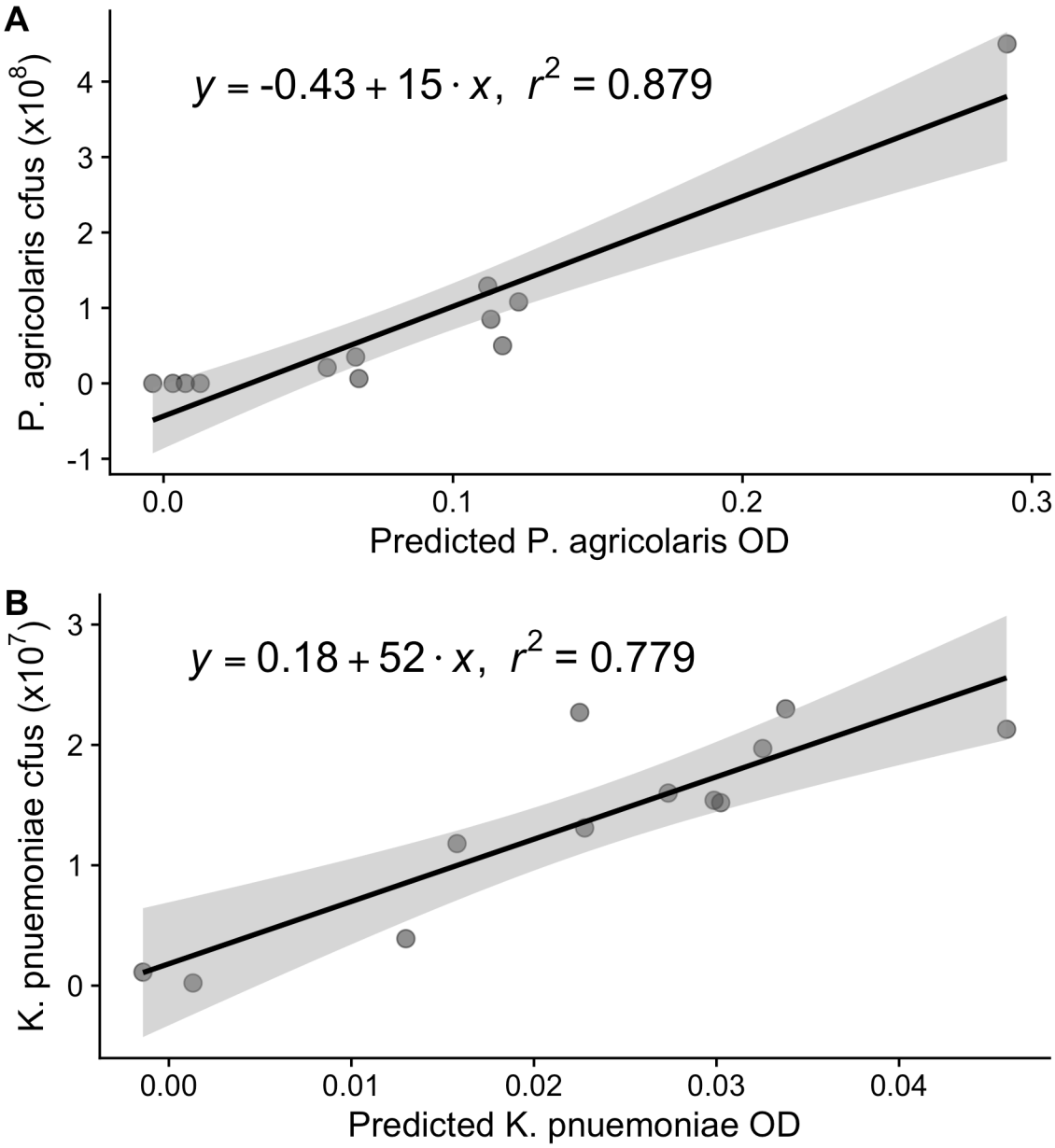

Figure S1: Linear regression results relating estimated optical density (predicted from standard curve) to colony counts from serial dilutions of the same *P. agricolaris* (A) and *K. pneumoniae* (B) samples.

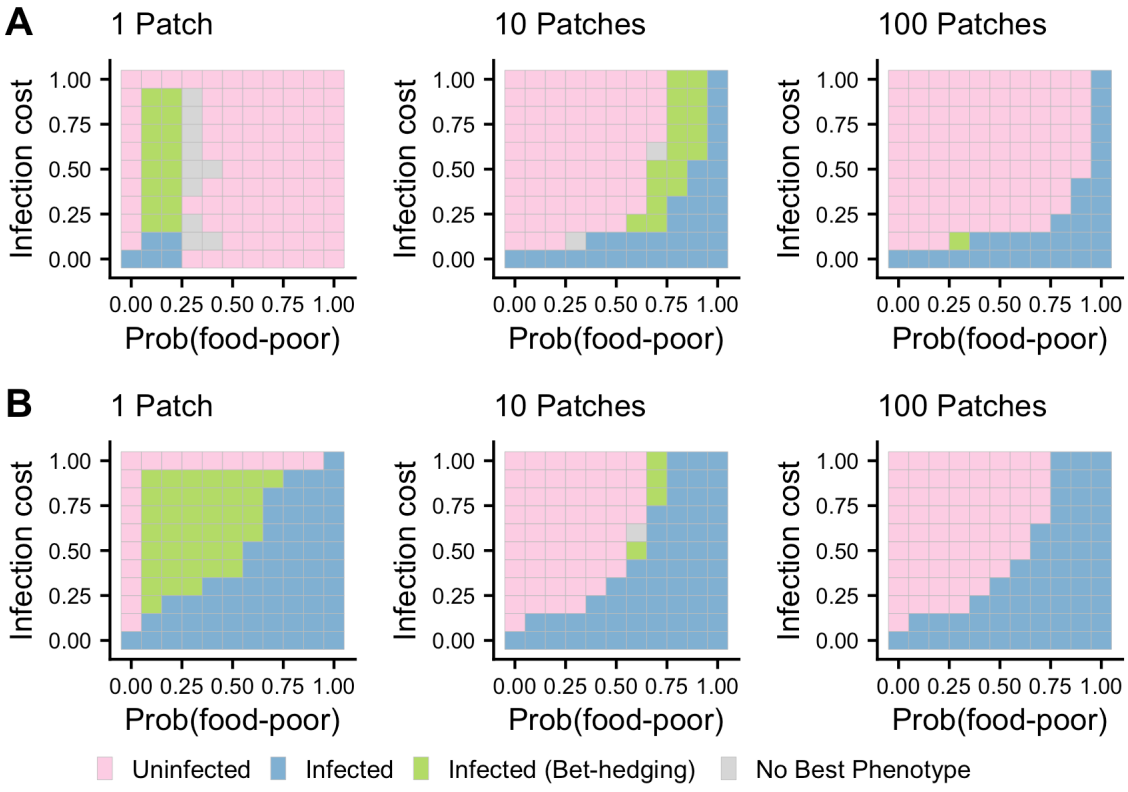

Figure S2: Plot of the best phenotype for a given probability of being in a no food environment and cost of having symbionts. These are the same as Figure 4C and D with 10% of sori successfully germinating.

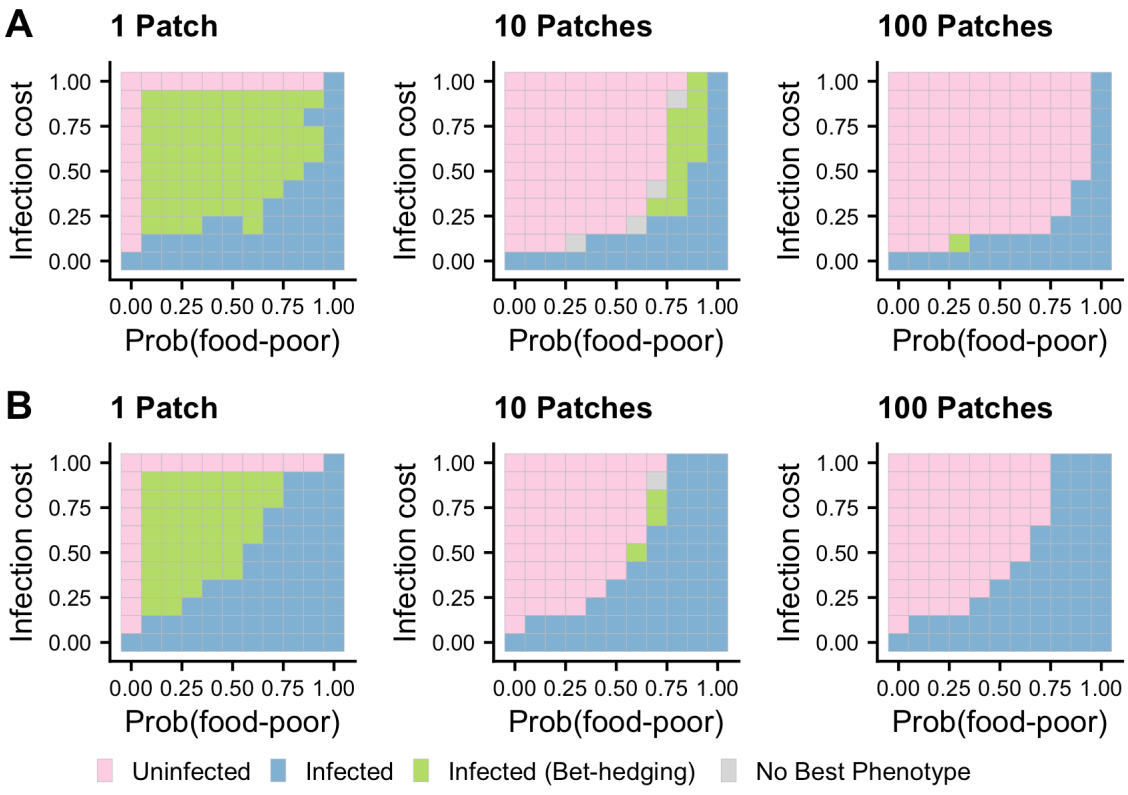

Figure S3: Plot of the best phenotype for a given probability of being in a no food environment and cost of having symbionts. These are the same as Figure 4C-H with 90% of sori successfully germinating.

Animation S1: Example simulation with 100 rounds of growth and dispersal with one soil patch, a 50% cost of infection, and a food-poor frequency of 0.3.

Animation S2: Example simulation with 100 rounds of growth and dispersal with 10 soil patches, a 50% cost of infection, and a food-poor frequency of 0.3.

Animation S3: Example simulation with 100 rounds of growth and dispersal with 100 soil patches, a 50% cost of infection, and a food-poor frequency of 0.3.