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Context-dependence in the symbiosis between *Dictyostelium discoideum* and *Paraburkholderia*

Short Title: Context in an amoebae-bacteria symbiosis

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Abstract

Symbiotic interactions change with environmental context. Measuring these context-dependent effects in hosts and symbionts is critical to determining the nature of symbiotic interactions. We investigated context-dependence in the symbiosis between social amoeba hosts and their inedible *Paraburkholderia* bacterial symbionts, 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 that symbiosis often provides a long-term fitness advantage for hosts after rounds of growth and dispersal in variable food-contexts, especially when conditions are harsh with little food. These results show how food context can have many consequences for the *Dictyostelium-Paraburkholderia* symbiosis and that both sides can frequently benefit.

Impact Statement

Many organisms form symbiotic relationships with other species. These symbioses often exhibit context-dependence, where the sign or magnitude of one partner's effect on the other will change in different environments. Context-dependent effects make it difficult to assign interactions to categories like mutualisms or antagonisms because they involve both benefits and costs depending on the environment. However, in some cases, accounting for context-dependence can clarify an interaction so that it more easily fits a mutualism or antagonism. We investigated context-dependence using the symbiosis between *Dictyostelium discoideum* and two symbiotic *Paraburkholderia* species. In this symbiosis, *Paraburkholderia* bacteria allow hosts to carry food bacteria to food-poor contexts, where hosts rarely survive without food, but reduce host fitness in the more hospitable food-rich contexts. The effect of food context on P*araburkholderia* symbionts is unknown. We show that *Paraburkholderia* symbionts are also affected by this context, through facing reduced competition after being dispersed by hosts to food-poor contexts. We also identify a new way that symbionts affect hosts, where symbiont density reduces host fitness, but less so in food-poor contexts. Finally, we use simulations to show that infected hosts benefit in the long-term across variable food contexts, especially in the harshest environments with little food. These results show that context-dependence in symbiosis can have many consequences for hosts and symbionts, though in general for *D. discoideum* and *Paraburkholderia*, both are likely to benefit.

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,

69 1994; Chamberlain *et al.*, 2014). These context-dependent effects on partners can be
70 crucial to understanding the nature of symbiotic interactions (Keeling & McCutcheon,
71 2017; Iwai, 2019). For example, in the symbioses between *Paramecium bursaria* hosts
72 and their *Chlorella* endosymbionts, hosts benefitted from symbiosis in light
73 environments, but were harmed in the dark. For *Chlorella*, the effects of symbiosis were
74 negative in co-culture, indicating that hosts exploited their endosymbionts for the benefits
75 hosts receive in light conditions (Lowe *et al.*, 2016). However, in the context of an
76 environment with a *Chlorella* competitor, hosts benefited their symbionts by eating these
77 competitors (Iwai, 2019). This example illustrates that understanding how partners affect
78 each other across multiple contexts can change our view of the interaction, sometimes
79 from one of exploitation to one of mutual benefit.

80 Context-dependence is important in the lifecycle of the social amoeba
81 *Dictyostelium discoideum*. Amoebae need edible bacteria to grow and proliferate (Raper,
82 1937), but the abundance (Young, 2004; Vos *et al.*, 2013) and quality (Kuserk, 1980;
83 Brock *et al.*, 2018) of food bacteria in the soil is known to vary. This results in a patchy
84 environment where some patches are food-rich and other patches are food-poor. In
85 response to starvation, amoebae aggregate and form a multicellular fruiting body to
86 disperse resistant spores to new environments (smith *et al.*, 2014). The patchy soil
87 environment is considered an important selection pressure for this fruiting body structure
88 (Bonner, 1982; Kessin, 2001).

89 *D. discoideum* interacts with three species of mostly inedible *Paraburkholderia*
90 bacterial symbionts — *P. agricolaris*, *P. hayleyella*, and *P. bonniae* (Brock *et al.*, 2020).
91 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 (and inedible *Paraburkholderia*) 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 infected amoebae producing fewer spores than uninfected amoebae (Brock *et al.*, 2011; DiSalvo *et al.*, 2015). In food-poor environments, the cost of having *Paraburkholderia* is compensated by hosts gaining the ability to carry food bacteria in dispersing spores. This allows amoebae to disperse and grow where they ordinarily could not (Brock *et al.*, 2011; DiSalvo *et al.*, 2015). These context-dependent

effects on the host could be extremely important in the natural soil environment, where food-poor patches arise frequently (Kessin, 2001).

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

It is unclear how the number of extracellular *Paraburkholderia* in the environment impacts hosts since previous studies have focused on intracellular *Paraburkholderia* (Shu *et al.*, 2018b; Miller *et al.*, 2020). When they are outside the amoebae, *Paraburkholderia* could affect *D. discoideum* fitness through interactions with food bacteria perhaps by reducing the amount of food for hosts through competition or by

releasing diffusible toxins that affect amoebae. Thus host food context could also affect the relationship between symbiont density and host spore production.

The fitness effects of symbiosis for hosts have been tested only in food-poor and food-rich contexts individually. The benefits of symbiosis could pay out over the long-term across different food contexts in the soil. Growth rates in temporally variable contexts are best captured by geometric mean fitness rather than arithmetic mean fitness because only the geometric mean captures the lasting effects of periods of low fitness (Sæther & Engen, 2015). Ignoring geometric means can lead to incorrect assessments of the adaptive value of strategies in variable environments. One example of an adaptation that is only apparent from geometric mean fitness measures are bet-hedging phenotypes, 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 lowers the variance in fitness across time and results in higher geometric mean fitness at the expense of lower arithmetic mean fitness.

Bet-hedging is suspected to play a role in explaining observations of disadvantageous partnerships in plant-fungus mutualisms (Lekberg & Koide, 2014; Veresoglou *et al.*, 2021). If bet-hedging occurs, short-term costs are acceptable if partnerships increase geometric mean fitness. Alternatively, symbiosis could increase both geometric and arithmetic mean fitness across contexts without the need for bet-hedging. In this case, the benefits of symbiosis simply outweigh the costs as in more traditional descriptions of symbioses (Douglas, 2010). However, these alternatives have not been tested in detail.

To understand context-dependence in the symbiosis between amoebae and *Paraburkholderia*, we used *D. discoideum* infected with either *P. agricolaris* or *P. hayleyella* — the two most common and best-studied species of *D. discoideum* symbionts. We investigate whether *Paraburkholderia* benefit from reduced interspecific competition when dispersed to food-poor contexts, how symbiont density and food context impact host spore production, and whether symbiosis is beneficial for hosts when food conditions vary.

Methods

To understand the effects of symbiosis across food contexts, we used 4 naturally uninfected *D. discoideum* clones, 4 clones naturally infected with *P. agricolaris*, and 4 clones naturally infected with *P. hayleyella* (Figure 1). We cured the infected clones and re-infected them with their native symbionts to standardize infection density. Uninfected clones were left uninfected, but were otherwise treated the same as infected clones. This resulted in three host infection conditions: uninfected, infected with *P. agricolaris*, and infected with *P. hayleyella*. To mimic natural dispersal, we collected sori and transferred them to food-rich (with additional *K. pneumoniae* bacteria) or food-poor (KK2 buffer with no *K. pneumoniae*) nutrient plates. Bacteria appear on food-poor plates only if transferred sori contain bacteria, as expected for infected samples. We grew replicate experimental sets involving all conditions beginning on two separate dates, July 13 and 22, 2020, and followed up with additional experiments (see results) beginning on January 26 and April 23, 2021.

183

184 *Paraburkholderia isolation*

185 To isolate *Paraburkholderia* from their hosts, we grew wild collected *D.*
186 *discoideum* clones on SM/5 plates (2 g glucose (Fisher Scientific), 2 g Bacto Peptone
187 (Oxoid), 2 g yeast extract (Oxoid), 0.2 g $MgSO_4$ * $7H_2O$ (Fisher Scientific), 1.9 g
188 KH_2PO_4 (Sigma-Aldrich), 1 g K_2HPO_4 (Fisher Scientific), and 15 g agar (Fisher
189 Scientific) per liter). Wild *D. discoideum* clones were grown with *K. pneumoniae* food
190 bacteria that were suspended in KK2 buffer (2.25 g KH_2PO_4 (Sigma-Aldrich) and 0.67 g
191 K_2HPO_4 (Fisher Scientific) per liter). After wild clones completed the social cycle
192 (feeding, starvation, and fruiting body formation), we collected sori with pipette tips and
193 placed them on SM/5 plates. We allowed the bacteria and amoebae contained within to
194 proliferate and then streaked out the resulting bacteria to get single colonies.

195

196 *Paraburkholderia removal*

197 To generate uninfected clones, we treated infected *D. discoideum* clones with
198 antibiotics by plating on 30 μg/mL tetracycline SM/5 plates with 200 μL of 1.5 optical
199 density (OD_{600}) tetracycline-resistant *K. pneumoniae* suspended in KK2 buffer. After
200 passage on SM/5 plates without tetracycline to let the amoebae recover from any effects
201 of the antibiotic, we collected single sori with a pipette tip and placed ten of them in
202 different locations on SM/5 plates to confirm that we had successfully removed the
203 bacteria. If bacteria are present, these spot tests will show bacterial growth and
204 *Dictyostelium* proliferation as the spores hatch and eat the bacteria (Brock *et al.*, 2011).
205 Without bacteria, amoebae cannot proliferate and the spot will stay blank. We considered

206 a clone to be cured if no bacteria showed up in spot tests. We similarly treated naturally
207 uninfected hosts with tetracycline to control for any effect of curing on our results.

208

209 *Paraburkholderia re-infection*

210 We re-infected cured *D. discoideum* clones with their native *Paraburkholderia*
211 isolates by plating 200 μL $2x10^5$ spores with 200 μL of 0.1% *Paraburkholderia* solution.
212 This solution consisted of 1.5 OD_{600} *Paraburkholderia* and 1.5 OD_{600} *K. pneumoniae* in a
213 1:1000 ratio. To confirm re-infection (and also successful isolation), we performed spot
214 tests as above, where successful re-infection was inferred when bacteria grew on 8 or
215 more spots out of the 10 we put down.

216

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

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

229 food bacteria from the previous plate. We grew food-rich and food-poor plates for six
230 days unless otherwise stated, enough time for mature fruiting bodies to form.

231

232 *Measurement of bacteria density*

233 To measure *Paraburkholderia* density, we measured the quantity of bacteria left
234 on plates after *D. discoideum* formed fruiting bodies. We first collected plate contents by
235 washing plates with 15 mL of KK2 buffer. To remove fruiting bodies and bacteria
236 associated with fruiting bodies, we centrifuged wash solutions for three minutes at 13000
237 rpm. We measured bacteria using optical density measured at 600 nm (OD_{600}), a
238 frequency at which bacteria commonly scatter light. Because the OD_{600} is due to both
239 *Paraburkholderia* and *K. pneumoniae*, we used GFP fluorescence measurements (with an
240 excitation wavelength of 485 and emission wavelength of 515 nm) and a standard curve
241 relating *K. pneumoniae* fluorescence to its OD_{600} to subtract out the component due to
242 GFP-expressing *K. pneumoniae*. Both OD_{600} and fluorescence measures were performed
243 in a 96 well plate with a Tecan Infinite 200 Pro microplate reader.

244 To validate our standard curve, we compared predicted OD_{600} of *P. agricolaris*
245 and *K. pneumoniae* to colony forming unit (CFU) counts from the same samples. Linear
246 regression revealed that predicted OD_{600} measurements explained most of the variation in
247 CFUs, showing that our assay is reliable (Figure S1). We also checked our standard curve
248 for significant quadratic terms, which can cause measurement errors when combining
249 OD_{600} and fluorescence measures at high densities (Meyers *et al.*, 2018), but our curve
250 did not have a significant quadratic term.

251

252 *Host spore production*

253 Spore production is a standard fitness measure in *D. discoideum* (Buttery *et al.*,
254 2009; Hall *et al.*, 2013; Gruenheit *et al.*, 2017). To measure host spore production, we
255 estimated spore concentration in the supernatants from washed plates using a
256 hemocytometer. We then calculated the total number of spores per plate by multiplying
257 by the volume of wash solution.

258

259 *Spore production simulations*

260 To test whether infected hosts benefit across variable food contexts, we simulated
261 rounds of growth and dispersal across soil patches with different probabilities of having
262 food bacteria. We separately modeled three host phenotypes: (1) uninfected, (2) infected
263 with *P. agricolaris*, and (3) infected with *P. hayleyella*. Co-infections are possible, but
264 are rare in nature (Haselkorn *et al.*, 2019) so we exclude them from our analysis.

265 We assumed that environments consisted of 100 discrete soil patches. Patches
266 were either food-poor or food-rich (we investigated continuous amounts of food and
267 found similar results; see Supplemental file 1). Food-poor patches at time t were drawn
268 from a binomial distribution with probability p_t. Food-rich patches were drawn with
269 probabity $1\text{-}p_t$. To allow temporal variation, the value of p_t in each generation was drawn
270 from a beta distribution with mean p and variance v_{temp}. High values of v_{temp} resulted in
271 more temporally variable environments. For low values, most of the variation was spatial.

272 Initially all patches were colonized. Each patch produced a number of spores,
273 drawn from the distribution of our empirical spore production values, according to

whether it was a food-rich or food-poor patch. To model costs, we penalized host spore production in food rich environments by reducing spore production by a percentage *c*. When *c* is 0, we modeled the scenario observed in this study, with no infection cost. We did not detect a cost of infection in food-rich contexts, but numerous other studies have documented this cost (Brock *et al.*, 2011; DiSalvo *et al.*, 2015; Miller *et al.*, 2020). It is likely that we did not detect a cost because we infected hosts with fewer *Paraburkholderia*. Because these costs have been demonstrated repeatedly in other studies and because of the importance of costs to bet-hedging (Lekberg & Koide, 2014; Veresoglou *et al.*, 2021), we included them as a variable. We summed cost-adjusted spore production values 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. New patches are assumed to be empty and dispersal is assumed to be global such that sori from one patch can disperse to any other patch with equal probability. Dispersal is likely efficient in *D. discoideum* as sori can be dispersed long distances by arthropods (smith *et al.*, 2014) and possibly even by birds (Suthers, 1985). Each sorus is randomly assigned to a patch and it successfully colonizes that patch *g*% of the time. Because the value of *g* for natural hosts is unknown, we investigated three values of *g* (50%, 5%, and %0.5) that range from cases where there are many more sori successfully establishing than available patches to cases where each patch produces around one sorus. 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 (though this is

297 unlikely when g = 50%). We also assume that infection status is not associated with
298 different rates of colonization as those differences are better captured by our empirical
299 spore production values, which will include differences in growth efficiency or spore
300 germination rate.

301 We vary the average probability of food-poor patches p from 0.1 to 0.9 and
302 simulate four different cost regimes reflecting variation found in different
303 *Paraburkholderia* isolates (Miller *et al.*, 2020). We simulated dispersal to new patches
304 for 100 rounds of growth and dispersal using 100 replicates for each combination of p,
305 v_{temp}, and c for each phenotype. Within each replicate, all three phenotypes experience the
306 same environment. At the end of the 100 rounds, we calculated the total spore production
307 per round and calculated geometric and arithmetic mean spore production from these
308 values across the 100 rounds. Within each replicate, we determined whether infected
309 hosts had higher geometric or arithmetic mean fitness for each individual simulation and
310 whether any phenotype went extinct.

311 We assigned outcomes for each parameter combination by calculating the
312 frequency that infected or uninfected hosts had higher geometric mean fitness or
313 arithmetic mean fitness. Infected and uninfected hosts were assigned as winners if they
314 had higher geometric mean fitness in 75% of replicates. We assigned an outcome as bet-
315 hedging when infected hosts won and more than half of the winning replicates did so
316 with lower arithmetic mean fitness. Extinctions occurred in some simulations and were
317 treated as a distinct outcome. We assigned mixed outcomes when neither infected nor
318 uninfected hosts were able to have higher geometric mean fitness in 75% of replicates.

319 Some mixed outcomes involved individual replicates where infected hosts were found to
320 bet-hedge.
321
322 *Statistical Methods*
323 We performed statistics in R version 3.6.3 (R Core Team, 2020). To compare
324 bacteria density and spore production, we used linear mixed models (LMM) with the *lme*
325 function in the nlme package (Pinheiro & Bates, 2006). To account for random variation
326 from replicate clones and effects of dates when experiments were performed, we included
327 clone and the date the experiment was performed — along with each variable on its own
328 — as random effects. To select the best model of random effects, we used AICc, a
329 sample-size-corrected measure of model fit that balances predictive ability and model
330 complexity (Burnham & Anderson, 2004). Many of our models showed different
331 variances between treatments. To account for these differences in variance, we weighted
332 models with the *varIdent* function in nlme (Pinheiro & Bates, 2006). We used the
333 emmeans package (Lenth *et al.*, 2018) to perform contrasts.
334 To understand how *Paraburkholderia* density affects host spore production across
335 food conditions, we fit a LMM using only infected hosts that included symbiont density
336 leftover on plates and whether the plate was food-rich or food-poor, along with the
337 interaction between these variables. We included random effects for clone, date, and both
338 crossed effects and selected the best random effect structure with AICc. We determined
339 whether the interaction was important by comparing AICc of the model including the
340 interaction with models including the other variables but lacking the interaction.
341

342 **Results:**

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

345 The context of a food-poor environment is known to be important for *D.*
346 *discoideum* hosts. It is not known how *Paraburkholderia* are affected by this same
347 context, but reduced competition with food bacteria seems likely. We tested this by
348 growing infected sorus contents on food-poor and food-rich nutrient plates and measuring
349 the density of *Paraburkholderia* after *D. discoideum* fruiting body formation (Figure 1).
350 After infected hosts formed fruiting bodies, *Paraburkholderia* densities were lowest in
351 food-rich conditions (Figure 2A), as expected if they compete with food bacteria. There
352 was around five times more *P. agricolaris* on food-poor than food-rich plates (LMM, $p <$
353 0.001). *P. hayleyella* growth was higher in food-poor conditions than food-rich, but this
354 difference was not significant after 6 days (LMM, $p = 0.416$). Because *P. hayleyella*
355 grows slowly, we performed two more experiments with *P. hayleyella* with 8 and 12-day
356 growth periods (Figure 2B). Allowing for longer incubations did not result in
357 significantly higher density of *P. hayleyella* (LMM, $p = 0.633$), suggesting that *P.*
358 *hayleyella* reach their maximum density at or before 6 days, but including these
359 additional experiments gave us enough power to find a significant increase in *P.*
360 *hayleyella* density in food-poor conditions relative to food-rich (LMM, $p = 0.027$). These
361 results show that symbiont density is context-dependent.

362

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

364 The host-food context may affect the relationship between symbiont density and
365 host spore production and therefore the degree of conflict or cooperation between them.
366 To investigate this, we also measured total host spore production from plates where we
367 measured the growth of *Paraburkholderia* symbionts (Figure 1). We used uninfected
368 hosts as a baseline for fitness without symbionts. We confirmed prior studies (Brock *et*
369 *al.*, 2011; DiSalvo *et al.*, 2015) showing that infected hosts could carry food bacteria and
370 proliferate on food-poor plates, while uninfected host could not (Figure 3A).
371 Surprisingly, we did not observe a cost of being infected in food-rich conditions ($p > 0.5$
372 for both species) which has been seen in previous studies (Brock *et al.*, 2011; DiSalvo *et*
373 *al.*, 2015; Shu *et al.*, 2018a). This is likely a result of our lower infection dosage of 0.1%.
374 While having some symbionts is essential for hosts to be able to carry food and
375 survive in food-poor conditions, higher symbiont densities may nevertheless harm hosts,
376 perhaps in ways that depend on food context. We found that larger populations of
377 symbionts as measured by OD_{600} were associated with lower host spore production, but
378 this harm was reduced in food-poor conditions. Lower host spore production was
379 associated with being in a food-poor environment ($\beta_{food\text{-}poor} = -3.283$, se = 0.853) and
380 symbiont density ($\beta_{density} = -10.317$, se = 6.364), but the interaction between food scarcity
381 and symbiont density showed that the harmful effect of higher symbiont densities was
382 lessened on food-poor plates ($\beta_{food\text{-}poor*density} = 8.078$, se = 6.381; Figure 3B). These
383 results indicate that symbiont density may come at the expense of host spore production,
384 but that this cost decreases in food-poor environments.
385
386 *Symbiosis is often beneficial for hosts across variable contexts*

387 Because symbiosis helps hosts in food-poor contexts, we hypothesized that
388 infected hosts would gain a long-term benefit across contexts compared to uninfected
389 hosts. If infected hosts increased their geometric mean fitness at the expense of arithmetic
390 mean fitness, infected hosts could even gain a bet-hedging advantage. We modeled this
391 by using our empirical spore production values to simulate 100 rounds of growth and
392 dispersal across environments where the number of food-poor patches was determined by
393 the mean frequency (p) and the temporal variance (v_{temp}; more detail can be found in the
394 methods). Because the natural conditions of this symbiosis are mostly unknown, we
395 simulate a wide-range of parameter space to determine which conditions favor symbiosis.
396 The supplement includes animations of representative simulations.
397 We first describe the results when dispersing sori successfully colonize new
398 patches 5% of the time. When there was no cost of infection, we found that infected hosts
399 were favored in every condition we tested (Figure 4; blue). We also simulated costs of
400 infection because those have been found in other studies (Brock *et al.*, 2011; DiSalvo *et*
401 *al.*, 2015; Miller *et al.*, 2020). As the cost of infection in food-rich contexts increased,
402 infected hosts were favored in the most food-poor environments while uninfected hosts
403 were favored when food was abundant (Figure 4; orange). *P. hayleyella* was favored
404 across more environments than *P. agricolaris*.
405 Bet-hedging in this symbiosis appears to be rare (Figure 4; green and yellow).
406 Infected hosts had a bet-hedging advantage when costs were added and food was
407 intermediately rare. More temporally variable environments had a weak effect on
408 increasing the likelihood of bet-hedging.

When dispersing sori successfully colonize new patches 50% of the time (each patch produces enough sori to completely fill the patches in the next generation), we found similar results (Figure S2). When only 0.5% were successful (each patch may only produce one or two sori for dispersal), we again found similar results except in the most food-poor conditions, where both uninfected and *P. agricolaris* infected hosts tended to go extinct (Figure S3A). *P. hayleyella* infected hosts were able to survive in these food-poor contexts (Figure S3B).

The natural environment of hosts is unlikely to involve food patches that are binary. Variation in the environment is also often auto-correlated, with the state of the environment at one time more often resembling the state of the environment in the near future (Ruokolainen *et al.*, 2009). To determine whether our results were robust to variable environments with continuous food and temporal correlations, we ran additional simulations (described in detail in Supplemental file 1) where the amount of food varied from 0 to 1 (Figure S4) depending on a continuous resource that allowed us to tune autocorrelations (Figure S5). These additional simulations broadly supported our conclusions from the simpler simulations (Figure S6-8).

Discussion

Our results show how the context of host food abundance affects the *Dictyostelium-Paraburkholderia* symbiosis beyond the previously demonstrated advantage to hosts when food is rare (Brock *et al.*, 2011). First, we found evidence that both *Paraburkholderia* species benefit from reduced competition when they are carried to food-poor environments (Figure 2). Second, symbiont density negatively affected host

spore production, but symbionts harmed hosts less in food-poor conditions (Figure 3B). Third, infected hosts had an advantage over uninfected hosts in simulations when food conditions were harsh or when the cost of symbiosis was low (Figure 4).

Our finding that symbionts had higher growth when dispersed to food-poor contexts shows that *Paraburkholderia* symbionts experience parallel context-dependence as hosts. These results highlight the importance of context-dependence for both partners. *Paraburkholderia* may benefit from reduced competition when hosts bring them to food-poor environments because symbionts interact with fewer competitors or because hosts eat competitors. This, together with our finding that hosts can benefit across contexts, points to a relationship of mutual benefit in this symbiosis. Our results also fit with other findings of competitive benefits for symbionts (Iwai, 2019). Other benefits of symbiosis for *Paraburkholderia* remain to be tested.

Competition between symbionts and food bacteria may also be responsible for the context-dependent effects of symbiont density on host spore production. Our spore production results showed that higher symbiont densities resulted in lower host spore production, indicating that symbionts are harmful to hosts. However, higher symbiont densities are less harmful in food-poor conditions when competition is lower (Figure 3B). The reduced harm for hosts could be the result of less antagonism between bacteria, which results in less collateral damage to amoebae through secreted toxins or other competitive interactions between food bacteria and symbionts. The generality of our results is limited somewhat by only using one species of food bacteria. While using a single food bacterium is more experimentally tractable, amoebae encounter multiple bacteria species in their natural environments (Brock *et al.*, 2018). Different species, or

455 combinations of species, could change competition with symbionts and affect host spore
456 production in different ways.

457 Symbiosis benefits amoeba hosts by giving hosts the ability to carry food to food-
458 poor contexts (Brock *et al.*, 2011; DiSalvo *et al.*, 2015). Using simulations, we showed
459 that this ability resulted in higher fitness across variable contexts when costs were low
460 and food was rare (Figure 4). Under conditions with plentiful food and high costs, being
461 uninfected was advantageous. In nature, about 25% of clones are infected (Haselkorn *et*
462 *al.*, 2019), suggesting that symbiosis is not universally favored. This indicates that our
463 finding of no cost to hosts in the symbiosis may be unrepresentative of many natural
464 infections. On the other hand, a 25% infection rate is high if the symbiosis is generally
465 harmful. This indicates that the prevalence of symbiosis could reflect a balance of forces
466 where *D. discoideum* is not strongly selected to fight *Paraburkholderia* infection in a
467 geographic mosaic of coevolution (Thompson, 1994). Unfortunately, the natural
468 conditions of this symbiosis are the biggest unknowns in this system as it is difficult to
469 study this symbiosis, and microbes more generally (Kraemer & Boynton, 2017), in
470 nature.

471 Hosts could also benefit across contexts through bet-hedging, where geometric
472 mean fitness trades off with arithmetic mean fitness (Seger & Brockmann, 1987). It is
473 suspected that costly symbioses may be able to evolve because they are advantageous
474 over the long-term even if they are not advantageous in the short term (Lekberg & Koide,
475 2014; Veresoglou *et al.*, 2021). We found that bet-hedging was rare in our simulations.
476 Our finding that bet-hedging occurs between where conditions favor infected over
477 uninfected hosts hints at the possibility that bet-hedging could facilitate the evolution of

478 symbiosis where benign environments transition to harsh environments. However, as our
479 simulations also reveal, symbiosis is more often favored without the need for bet-hedging
480 even with costs. Our results thus weaken the case that costly symbiosis in some contexts
481 are necessarily examples of bet-hedging since symbiosis was more often favored outright
482 than by bet-hedging.

483 Symbiotic interactions may play a larger role in adaptation to variable
484 environments than previously understood, even without bet-hedging. Symbioses are
485 known to result in novel phenotypes that allow partners to survive in harsh conditions
486 (Moran, 2007; Oliver *et al.*, 2010). Rarely do studies incorporate environmental variation
487 and long-term fitness. We investigated the long-term effects of context-dependence in the
488 symbiosis between *D. discoideum* and *Paraburkholderia* and found that hosts frequently
489 benefited from symbiosis in the harshest conditions. An understanding of the ecological
490 contexts along with long-term measures of fitness will be important for understanding the
491 evolutionary consequences of context-dependent symbioses.

492

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499

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

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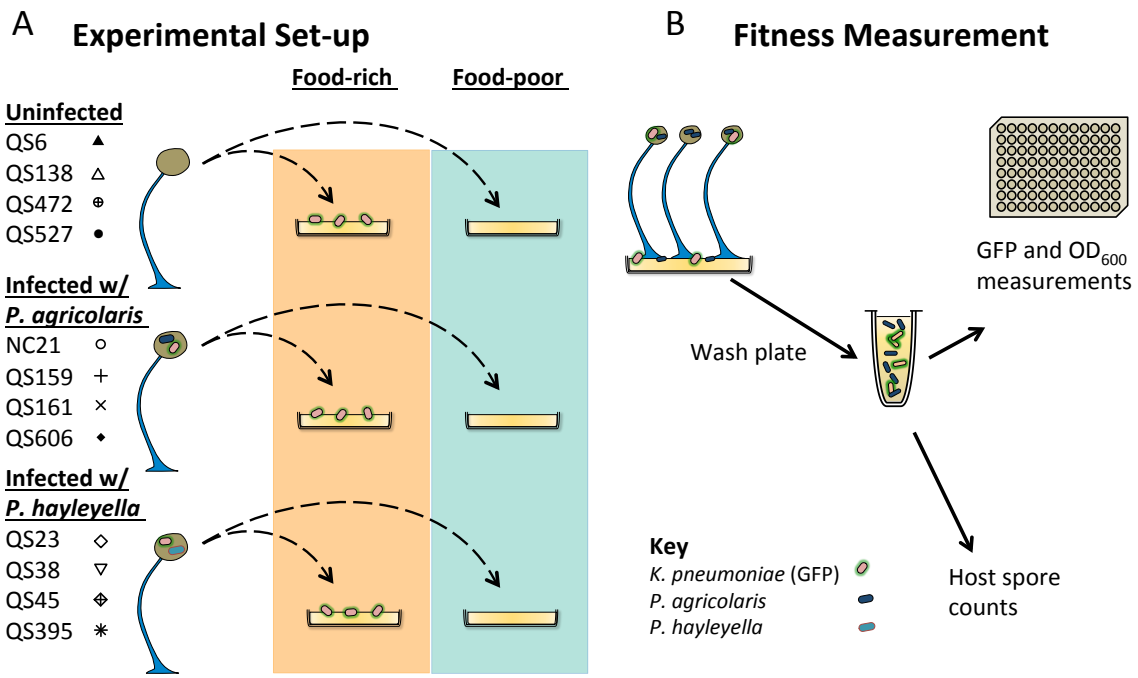
638 **Figure Legends**



639

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

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

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

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

644 measured by calculating GFP fluorescence and optical density (see Methods). Host spore

645 production is measured from washed plates.

646

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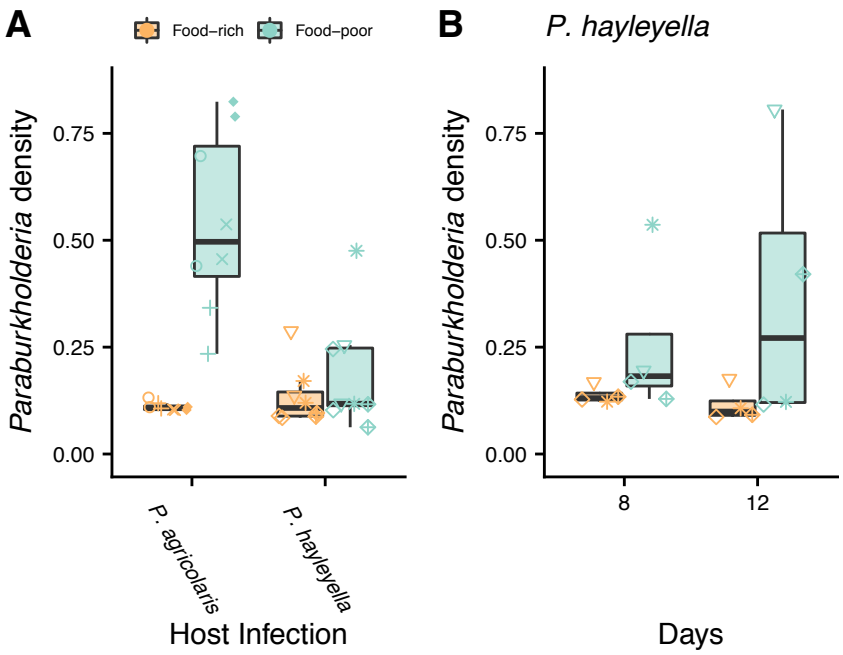

Figure 2: More *Paraburkholderia* were recovered from plates after fruiting body formation from food-poor plates (those that had not received additional *K. pneumoniae*). (A) *Paraburkholderia* density after 6 days. (B) *P. hayleyella* density after 8 and 12 days. Point shapes show individual clones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

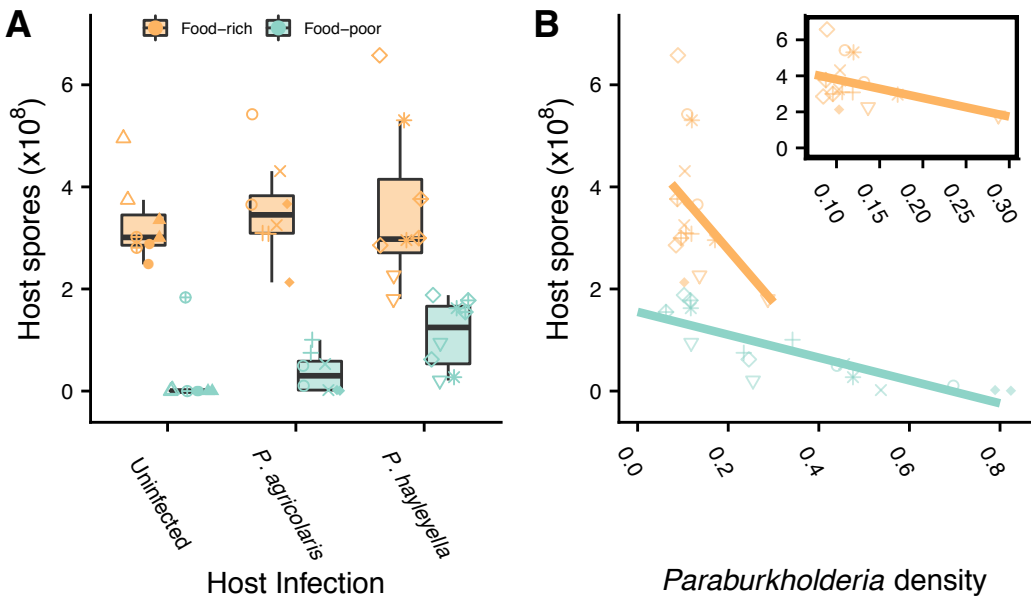

Figure 3: Effects of *Paraburkholderia* infection and density on host spore production. (A) Spore production of hosts from food-rich and food-poor plates for uninfected, *P. agricolaris* infected, and *P. hayleyella* infected hosts. (B) Interaction between measured *Paraburkholderia* density (OD_{600}) 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).

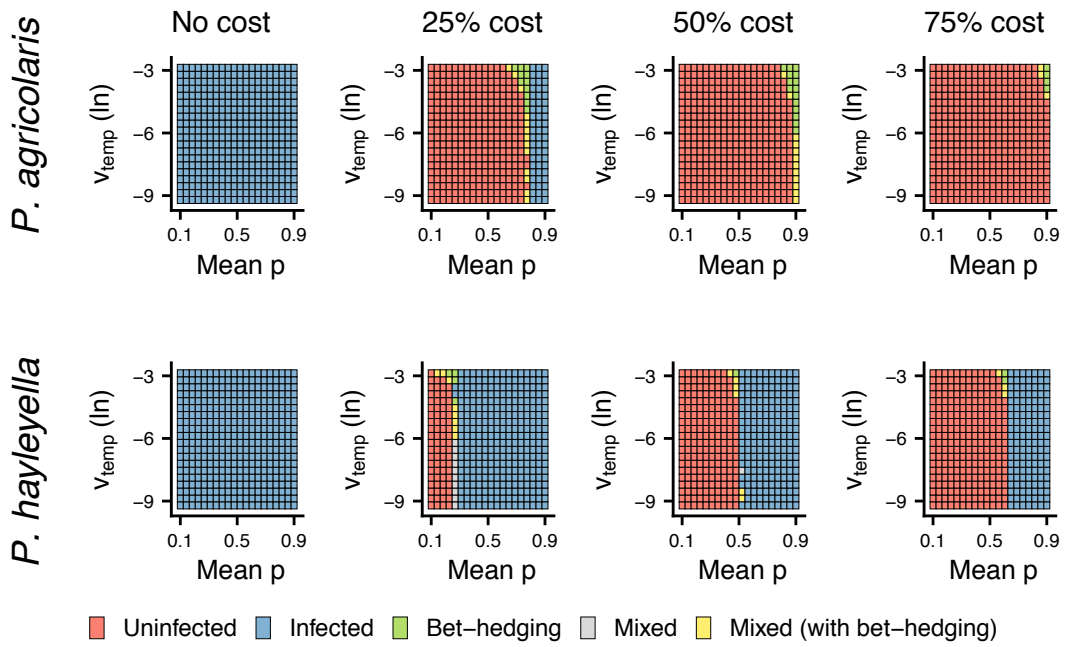

Figure 4: Benefits of symbiosis depends on variation in food availability and fitness costs. Winning phenotypes of *P. agricolaris* (top) and *P. hayleyella* (bottom) relative to uninfected for different costs of infection with a 5% probability of colonization. Orange shows when uninfected hosts have 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); gray shows areas where both infection strategies can win; yellow shows where both strategies can win and where infected hosts bet-hedge.