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Paraburkholderia symbionts isolated from *Dictyostelium discoideum* induce bacterial carriage in other *Dictyostelium* species

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The social amoeba *Dictyostelium discoideum* engages in a complex relationship with bacterial endosymbionts in the genus *Paraburkholderia*, which can benefit their host by imbuing it with the ability to carry prey bacteria throughout its life cycle. The relationship between *D. discoideum* and *Paraburkholderia* has been shown to take place across many strains and a large geographical area, but little is known about *Paraburkholderia*'s potential interaction with other dictyostelid species. We explore the ability of three *Paraburkholderia* species to stably infect and induce bacterial carriage in other dictyostelid hosts. We found that all three *Paraburkholderia* species successfully infected and induced carriage in seven species of *Dictyostelium* hosts. While the overall behaviour was qualitatively similar to that previously observed in infections of *D. discoideum*, differences in the outcomes of different host/symbiont combinations suggest a degree of specialization between partners. *Paraburkholderia* was unable to maintain a stable association with the more distantly related host *Polysphondylium violaceum*. Our results suggest that the mechanisms and evolutionary history of *Paraburkholderia*'s symbiotic relationships may be general within *Dictyostelium* hosts, but not so general that it can associate with hosts of other genera. Our work further develops an emerging model system for the study of symbiosis in microbes.

1. Introduction

Symbiotic interactions between species are crucial to understanding most organisms. Species of all sizes interact with one another in a great diversity of ways, ranging from beneficial partnerships to deadly exploitations, and these interactions can have major impacts on the evolutionary course and fate of their participants. The processes by which organisms come together as symbionts or leave one another behind are therefore of central interest to evolutionary biologists.

One very intimate type of symbiosis of particular importance to many familiar organisms involves the relationship between eukaryotic hosts and intracellular bacterial symbionts. The most famous and extreme examples are the mitochondria found in virtually all eukaryotic cells, which are believed to have evolved from the relationship between a large proto-eukaryotic cell and one or more intracellular bacteria [1]. Their bacterial ancestors—possibly in the course of being engulfed as prey—managed to evolve a lifestyle of stable coexistence within their hosts' cells. The benefits of such symbioses can be enormously impactful—the evolution of mitochondria likely paved the way for the rise of complex eukaryotes [2], and chloroplasts radically changed the composition of the Earth's atmosphere [3,4]. However, while important, the origins of these ancient symbioses are difficult to study because they have become so intimate that their participants behave as a single organism in many respects. Younger intracellular symbioses, where partners still have the opportunity to enter or

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leave the symbiosis or move from one partner to another, are therefore useful in studying how symbioses begin and are maintained.

The interaction between *Dictyostelium discoideum* and three intracellular bacteria in the genus *Paraburkholderia* is an emerging model system that combines the utility of lab tractable microbes with a complex symbiosis including both cooperative and antagonistic elements. *D. discoideum* is a social amoeba with an elaborate, facultatively multicellular life cycle. Usually solitary predators of soil bacteria, when food sources are exhausted *D. discoideum* cells aggregate to produce multicellular fruiting bodies consisting of a sorus of durable spores held aloft by a narrow stalk [5–7]. Spores within fruiting bodies remain dormant to await dispersal to a new environment with sufficient food [8,9].

Three species within the genus *Paraburkholderia* (*Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea*) have been found to infect *D. discoideum* hosts as endosymbionts. Members of all three species are capable of forming long-term associations with their hosts and can persist within *D. discoideum* cells throughout repeated solitary and social stages of its life cycle [10,11]. *Paraburkholderia* infection has both negative and positive consequences for its host's fitness: infected *D. discoideum* generally produce fewer spores, but nonetheless can benefit from *Paraburkholderia's* presence under food-limited conditions [12]. While *Paraburkholderia* itself is not edible to *D. discoideum*, infection by *Paraburkholderia* enables *D. discoideum* to carry other, more edible bacteria through its dispersal stage. The mechanism by which carriage is induced is not fully known, but potentially involves inducing the host to produce a lectin, discoidin, that binds bacteria and facilitates their transport [13]. Food bacteria carried within the sori of infected *D. discoideum* allow them to disperse to environments lacking available prey, seeding new prey populations in a process that has been likened to primitive agriculture [12].

The genus *Paraburkholderia* is known to include both soil-dwelling and symbiotic members, but a screen of a diverse selection of *Paraburkholderia* strains found that only *Pa. agricolaris*, *Pa. hayleyella*, and *Pa. bonniea* could establish persistent infections within *D. discoideum* that were not cleared after a single social generation [11]. Phylogenetic evidence suggests that the *Dictyostelium*/*Paraburkholderia* association evolved at least twice independently—once for *Pa. agricolaris* and at least once more for the two sister species *Pa. hayleyella* and *Pa. bonniea* [11,14]. Though the age of these relationships is unknown, evidence suggests that *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea* have adapted to specialize as endosymbionts. This is especially apparent in *Pa. hayleyella* and *Pa. bonniea*, which show substantially reduced genome sizes (approx. 4.1 Mbp compared to the approx. 8.7 more typical of free-living congeners) as well as heightened GC content and loss of the ability to use a variety of carbon-based compounds used by non-symbiont *Paraburkholderia* [14,15]. Loss of non-essential genes is a common signature of endosymbionts [16] and these losses suggest these *Paraburkholderia* species have experienced a reduction in effective population size and/or a relaxation of selection typical for bacteria living inside of larger hosts [17,18]. By contrast, *P. agricolaris*' genome size and organization is much more typical of other non-symbiotic *Paraburkholderia* species, which may reflect it representing a more recently evolved association or it having adapted to a less obligately symbiotic lifestyle. Nonetheless, while they are capable of surviving independently of their hosts in the laboratory, thus far *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea* have not been isolated from nature except in association with amoeba hosts.

While *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea* may have adapted to specialize as symbionts, relatively little is known about their potential relationships with hosts outside of *D. discoideum*. *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea* have been isolated from *D. discoideum* over a wide geographical area and readily infect different *D. discoideum* strains in the laboratory [11]. In addition to the well-studied *D. discoideum*, however, there are dozens of dictyostelid species found in forest soils around the world, all of which might be potential hosts for *Paraburkholderia*. *Dictyostelium* is an ancient and diverse genus, containing members as divergent from one another as humans are from bony fish (which diverged from one another at least 400 million years ago) [19]. Despite this diversity, however, one study found sequences aligning to the genus *Paraburkholderia* within two thirds of the fruiting body microbiomes of sampled dictyostelids representing at least six amoeba species across a large geographical area [20].

Because symbiotic partners can exert strong selective pressures on one another, often hosts and symbionts will evolve to specialize on one another, to the exclusion of other potential partners. This sort of specialization could enable multiple species ostensibly within the same niche (like the three *Paraburkholderia* species) to persist by specializing on different hosts. Conversely, research in a variety of taxa suggests that partner switching has had a major impact on the evolution of many symbioses [21–23]. Opportunities for a symbiont to switch to a partner of a different species, genus, or even phylum seem to be infrequent [24,25], but can have major consequences on the nature of the symbiosis. This is especially apparent in host-pathogen relationships – most of the most significant outbreaks of infectious disease in human populations have resulted from rare events in which a pathogen jumps from its usual host into one or more new hosts in which its virulence is drastically increased [26,27]. Examples of host switching in mutualisms are less dramatic but nonetheless widespread, with many plant/symbiont relationships involving generalist interactions between guilds of species [28–30].

The available diversity at both the strain and the species level in both *Dictyostelium* and *Paraburkholderia* makes their interaction a particularly useful model for the study of symbiosis between microbes. Past work has explored the differences between *Paraburkholderia* species, but thus far the consequences of diversity within *Dictyostelium* have not been studied. In this study, we explore the potential interaction between *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea* with a diversity of host species. We assessed the ability of all three symbionts to infect and induce stable bacterial carriage within seven *Dictyostelium* species and one from the sister genus *Polysphondylium*. In addition, we assessed the fitness consequences of *Paraburkholderia* infection on different hosts through their ability to enable host growth in food scarce environments and their impact on total host fecundity.

2. Methods

(a) *Paraburkholderia*, *Dictyostelium* and *Polysphondylium* strains used

We tested seven *Dictyostelium* species, as well as a single species from the closely related genus *Polysphondylium*. Note that here

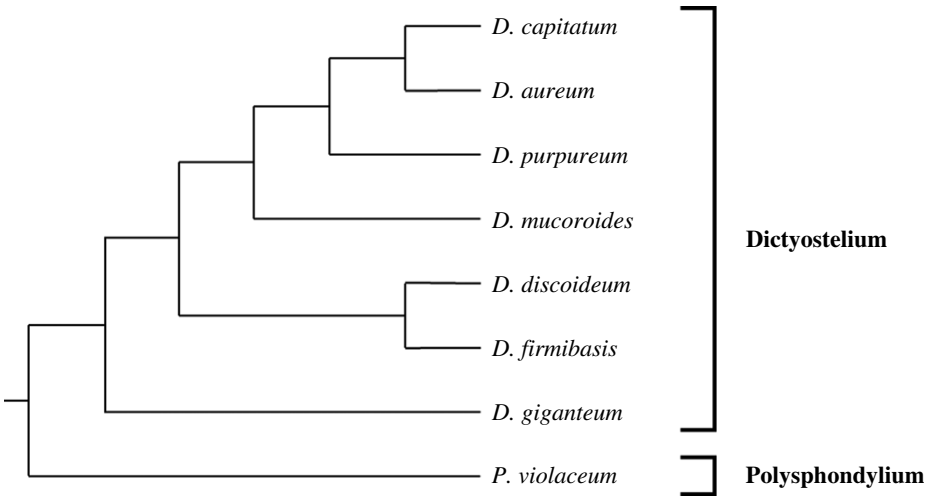

Figure 1. Phylogeny of host species used. Seven species from the genus *Dictyostelium* and one from the genus *Polysphondylium* were tested for carriage of food bacteria and spore production when infected by *Paraburkholderia* agricolaris, *Pa. hayleyella*, or *Pa. bonniea.* The genus *Dictyostelium* represents considerable variation and has been estimated to have split from its sister genus *Polysphondylium* more than 400 million years ago [19]. Phylogeny based on [31].

Table 1. *Paraburkholderia* and dictyostelid strains used. Eight different dictyostelid species were tested. Three strains of each species were tested when available. Each dictyostelid was tested against two strains each of three different *Paraburkholderia* species originally isolated from *D. discoideum*.

Paraburkholderia agricolaris	bQS159	Virginia Mountain Lake Biological Station
	bQS70	Texas—Houston Arboretum
Paraburkholderia hayleyella	bQS21	Virginia Mountain Lake Biological Station
	bQS11	Virginia Mountain Lake Biological Station
Paraburkholderia bonniea	bQS433	Virginia Mountain Lake Biological Station
	bQS859	Virginia Mountain Lake Biological Station
Dictyostelium discoideum	QS154	Virginia Mountain Lake Biological Station
	QS157	Virginia Mountain Lake Biological Station
	QS160	Virginia Mountain Lake Biological Station
Dictyostelium mucoroides	TAB16D	Texas—Armand Bayou Pasadena
	GMII19	Heidelberg, Germany
Dictyostelium giganteum	DG TAB19B	Texas— Armand Bayou Pasadena
	DG TAB13D1	Texas—Armand Bayou Pasadena
Dictyostelium purpureum	Y93	Texas—Houston Arboretum
	Y61	Texas—Houston Arboretum
	Y5	Texas—Houston Arboretum
Dictyostelium firmibasis	326	unknown (provided by Pauline Schaap)
Dictyostelium aureum	SL1	unknown (provided by Pauline Schaap)
Dictyostelium capitatum	327	unknown (provided by Pauline Schaap)
Polysphondylium violaceum	PVV80-A15	Virginia Mountain Lake Biological Station
	PVV7-C12	Virginia Mountain Lake Biological Station
	PV GVV5	Heidelberg, Germany

we use the taxonomy proposed by Sheikh *et al.* [31], applying the genus *Dictyostelium* only to species previously called Group 4 *Dictyostelium.* A phylogeny of the eight tested host species is shown in figure 1 [31,32].

When available, we chose three representative strains for each host species. We tested each host species against two strains each of three *Paraburkholderia* species known to be symbionts of *D. discoideum*: *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea*. All species and strains employed are listed in table 1.

For each dictyostelid strain used, we resuspended frozen material from freezer stocks in KK2 buffer (2.25 g KH_2PO_4 (Sigma-Aldrich) and 0.67 g K_2HPO_4 (Fisher Scientific) per liter deionized water) and used a hemocytometer to determine spore concentration. We plated 2×10^5 spores on SM/5 nutrient agar plates [33] (2 g glucose (Fisher Scientific), 2 g BactoPeptone (Oxoid), 2 g yeast extract (Oxoid), 0.2 g $MgCl_2$ (Fisher Scientific), 1.9 g $KHPO_4$ (Sigma-Aldrich), 1 g K_2HPO_5 (Fisher Scientific) and 15 g agar (Fisher Scientific) per liter deionized water). As food for

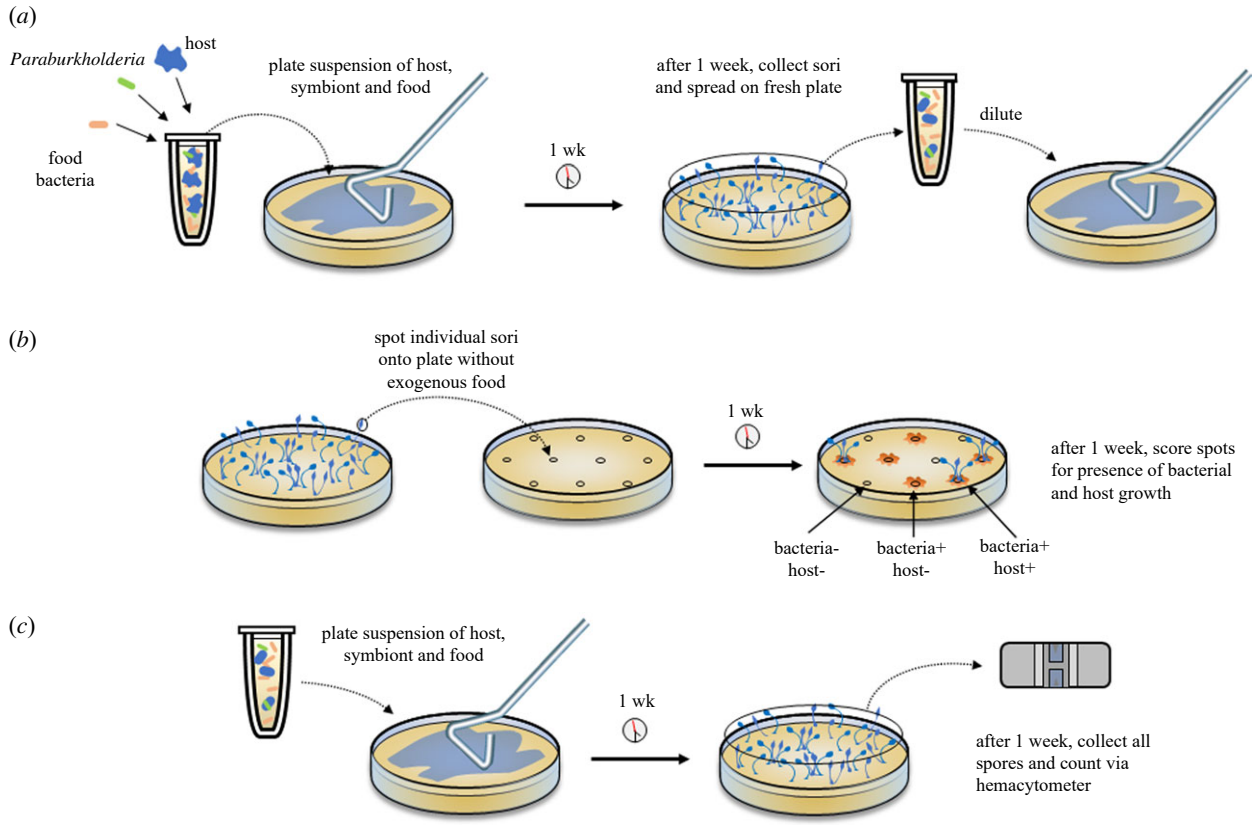

Figure 2. Diagram of experimental techniques. (*a*) Hosts were artificially infected with *Paraburkholderia* symbionts and plated on nutrient agar plates along with edible food bacteria. Hosts consume food, begin to starve, and undergo social development into fruiting bodies. Fruiting body sori contain spores, *Paraburkholderia* symbionts and unconsumed food bacteria. Sori were collected and plated onto fresh nutrient agar plates without additional food bacteria. Each such transfer constitutes a 'social generation', and was performed onto duplicate plates for use in the spot test and spore count assays. (*b*) Spot tests assay carriage of food bacteria within sori. Individual sori were collected and spotted onto fresh nutrient agar plates without exogenous food. After 1 week, each spot was assayed for the presence of a bacterial plaque and/or host fruiting bodies. (*c*) Spore counts assay consequences of infection on host fecundity. Plate contents were collected, diluted to a known concentration, and plated onto a fresh plate with food bacteria. After 1 week, entire plate contents were collected and counted.

dictyostelids, we also plated 200 µl of an $OD_{600} = 1.5$ suspension of *Klebsiella pneumoniae* in KK2.

To confirm the identity of each dictyostelid sample, we isolated DNA from spores and compared their ribosomal 17S sequences to online libraries. Once each of the dictyostelids formed fruiting bodies, spores were collected, and their genomic DNA extracted using the Chelex/Proteinase K protocol (Biorad Hercules, CA). We performed PCR using dictyostelid-specific primers and the closest GenBank relatives for each isolate were determined by aligning resolved sequences against the curated 17S ribosomal RNA sequence database in the National Center for Biotechnology Information database. The PCR amplification was done using a Gene Amp kit from Applied Biosystems (Roche Basel, Switzerland).

(b) Experimental infection of dictyostelids and serial transfer

For each dictyostelid, we plated 2×10^5 spores onto SM/5 nutrient agar plates as described above. Each dictyostelid was plated with 200 µl of either an $OD_{600} = 1.5$ suspension of *K. pneumoniae* food bacteria or a 99 : 1 (vol:vol) mixture of $OD_{600} = 1.5$ *K. pneumoniae* suspension and an $OD_{600} = 1.5$ suspension of one of six *Paraburkholderia* strains (figure 2*a*). We then incubated the plates at room temperature (22°C) until fruiting bodies formed.

To assess the stability of bacterial carriage, we passaged the samples through two additional social life cycles. For each passage, we collected spores from each plate using a sterile filter tip, suspended them in KK2 buffer, and determined their concentration using a hemocytometer. We then plated 2×10^5 spores on nutrient agar plates with 200 µl of *K. pneumoniae* with an OD_{600} of 1.5. As before, we incubated the plates at room temperature (22°C) until fruiting bodies formed. This process was repeated so that we had a total of three rounds: initial plating, first passage and second passage.

We performed all transfers onto replicate plates to use in subsequent assays (each assay was performed on a separate plate to ensure independence).

(c) Detection of bacterial carriage within dictyostelid sori (spot test assay)

To detect the carriage of bacteria within dictyostelid fruiting body sori, we performed spot test assays (figure 2*b*). After formation of fruiting bodies, individual sori were collected with a sterile 10 ul filter pipette tip, transferred onto an isolated spot on the surface of a sterile nutrient agar plate, and incubated at room temperature for one week. Twenty random sori were collected from each control and host/symbiont combination. After 1 week, we visually assessed each spot for the presence of bacterial growth, and recorded bacterial carriage as a percentage of sori that carried enough bacteria to have resulted in visible growth. In addition, we noted whether new dictyostelid fruiting bodies had grown at each spot, indicating that the dictyostelid had been able to survive exposure to infection and had carried enough edible bacteria in the sorus to support new dictyostelid growth. Each host–symbiont combination was assayed three times on three separate days.

In addition to testing after each round of growth (initial infection, first passage, and second passage), we performed spot tests on all dictyostelids immediately after initial plating to verify that bacteria had not contaminated freezer stocks.

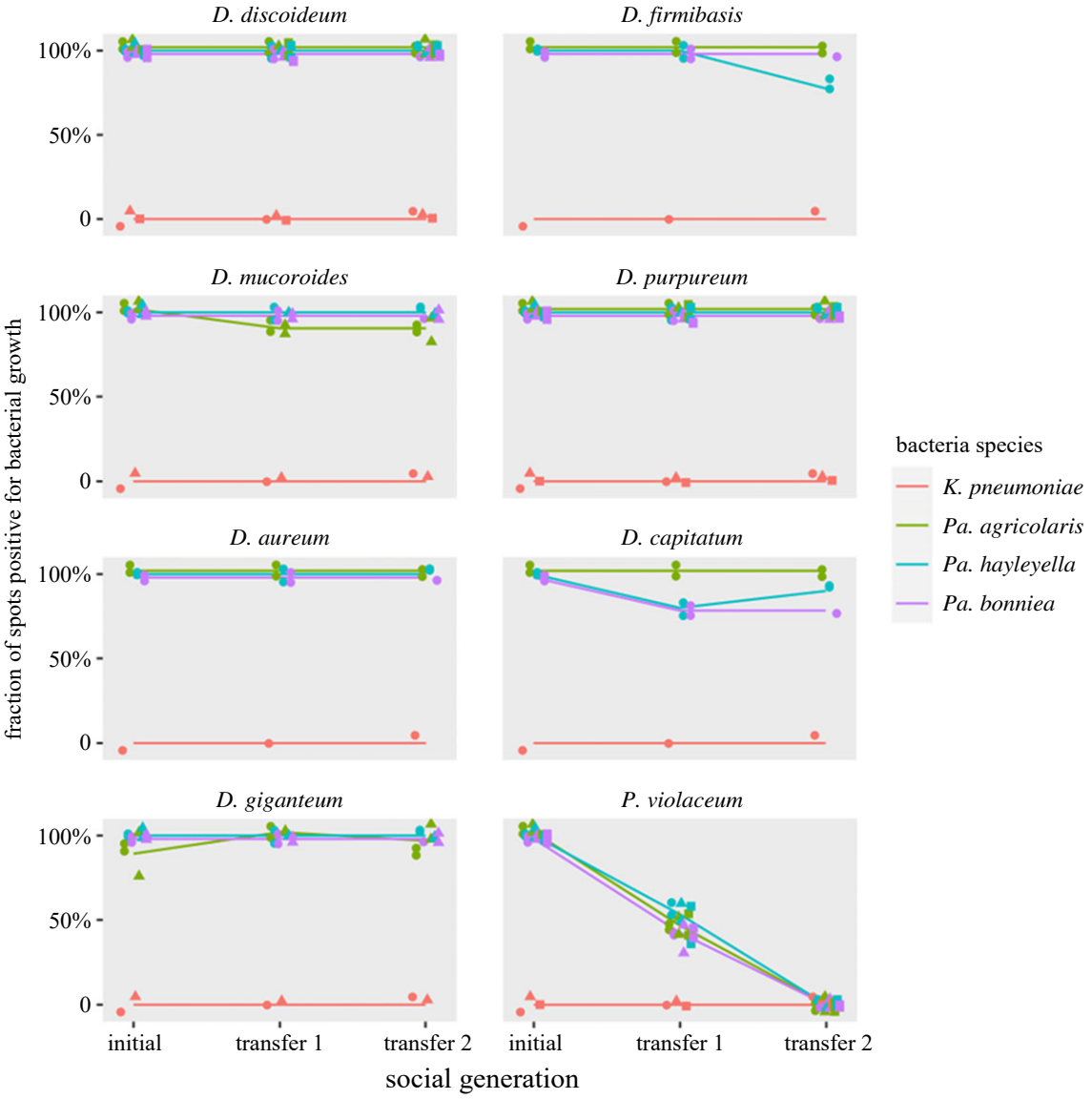

Figure 3. *Paraburkholderia* persistently infects all *Dictyostelium* hosts, but not *Polysphondylium* hosts. Percentage of fruiting body sori carrying sufficient bacteria to produce a visible bacterial plaque. Most sori from all tested *Dictyostelium* hosts carried food bacteria when infected by *Pa. agricolaris*, *Pa. hayleyella* or *Pa. bonniea*. Carriage remained stable near 100% over multiple social generations for most hosts. By contrast, infection of *Polysphondylium* was unstable and resulted in host extinction within two social generations. Point shapes indicate host strain identity. Lines connect the means of all replicates.

(d) Estimation of fitness effects of infection on dictyostelid hosts

To test whether *Paraburkholderia* causes similar fitness effects in other dictyostelids as in *D. discoideum*, we performed total spore count assays. Hosts and symbionts were plated onto fresh SM/5 plates with food bacteria as described above. After 1 week, we harvested spores by flooding each plate with 10–12 ml of starvation buffer + 0.1% NP-40 Alternative (CalBioChem, La Jolla CA) and collecting the full volume into a 15 ml Falcon tube. We then performed a 1 : 20 dilution using KK2 buffer and counted the spores using a hemocytometer. We compared total spore production of uninfected hosts, infected hosts immediately after infection and infected hosts after two social generations. By comparing spore production of uninfected and infected dictyostelids, we estimated the consequences of infection on host spore production. Each host/symbiont combination was assayed three times on three separate days.

(e) Statistical analysis

We performed statistics in R version 3.6.3 [34]. To analyse the results of spot test assays measuring bacterial carriage, we used a generalized linear model (*glm* function) with a binomial distribution. We tested models incorporating social generation, host species, and symbiont species and all possible interactions between them as fixed effects. We made pairwise comparisons of all possible models and selected models with minimized AICc, a measure which weights both goodness of fit and model complexity. To analyse the results of spore production assays, we used a linear model (*lm* function) with a Gaussian distribution.

3. Results

(a) *Paraburkholderia* persistently infects all *Dictyostelium* hosts, but not *Polysphondylium* hosts

We performed spot test assays to determine the ability of infected dictyostelid hosts to carry bacteria through the social stage of their life cycles.

Hosts within the genus *Dictyostelium* behaved similarly with regard to bacterial carriage over multiple social generations (figure 3). As expected, *Dictyostelium* hosts exposed only to the non-symbiont food bacterium *K. pneumoniae* did not carry sufficient bacteria within their sori to result in visible bacterial growth in spot test assays. By contrast, most

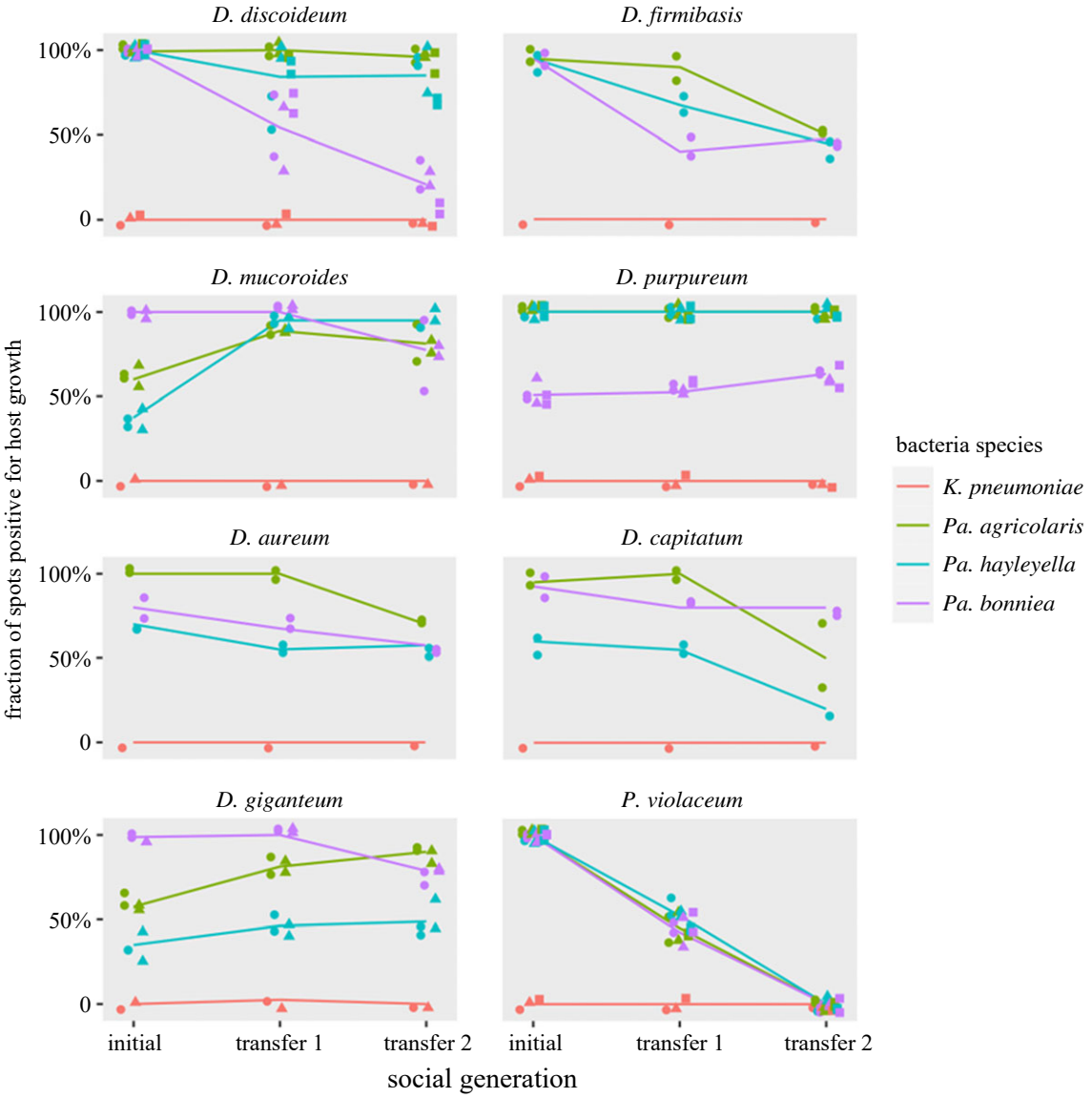

Figure 4. *Paraburkholderia* infection induces sufficient food bacteria carriage to support host growth. Percentage of fruiting body sori carrying sufficient bacteria to support host amoeba growth and fruiting. At least some sori from all tested *Dictyostelium* hosts carried enough food bacteria when infected by *Pa. agricolaris*, *Pa. hayleyella* or *Pa. bonniea* to support new host growth and fruiting without exogenous host food. Infection of *Polysphondylium* was unstable and resulted in host extinction within two social generations. Point shapes indicate host strain identity. Lines connect the means of all replicates.

Dictyostelium hosts infected by any of *Pa. agricolaris*, *Pa. hayleyella* or *Pa. bonniea* carried bacteria within 100% of tested sori. When assayed immediately after infection, carriage was detectable in all sori. We did not detect significant differences in carriage between different *Dictyostelium* host species, between different *Paraburkholderia* symbiont species, or in the interaction between the two. However, *P. violaceum* hosts infected by any *Paraburkholderia* species showed high initial carriage, confirming that infection was possible, but rapid decline over subsequent generations. In all cases, infected *P. violaceum* did not survive beyond the first social generation.

(b) *Paraburkholderia* infection induces sufficient food bacteria carriage to support dictyostelid growth

We additionally scored the spot test assays for the presence of dictyostelid growth. The presence of host fruiting bodies in these experiments indicates that not only were food bacteria carried within the sori, but they were carried in sufficiently high numbers to establish populations that would support the growth of new hosts in an otherwise food-limited environment.

When we plated sori from uninfected hosts (hosts initially provided only with non-symbiotic *K. pneumoniae* food bacteria) onto nutrient plates with no exogenous host food, we consistently did not detect the growth of new host fruiting bodies (red lines in figure 4), regardless of host species or strain used. Just like *D. discoideum*, other uninfected dictyostelids do not carry food within their sori, and so cannot grow without exogenous food. By contrast, sori of hosts treated with *Pa. agricolaris*, *Pa. hayleyella* or *Pa. bonniea* (in addition to *K. pneumoniae*) often carried enough edible bacteria within a sorus to seed new prey populations and support the growth of new hosts. Patterns of dictyostelid growth indicated a significant interaction between host species and symbiont species ($F_{12213} = 749.25$, $p < 0.0001$). In some combinations, such as *D. purpureum* infected by *Pa. agricolaris*, food carriage was high and stable from generation to generation. Other combinations showed evidence of decreasing food carriage over time, indicating potential instability. Still others, like *D. giganteum* infected by *Pa. agricolaris*, only some sori carried

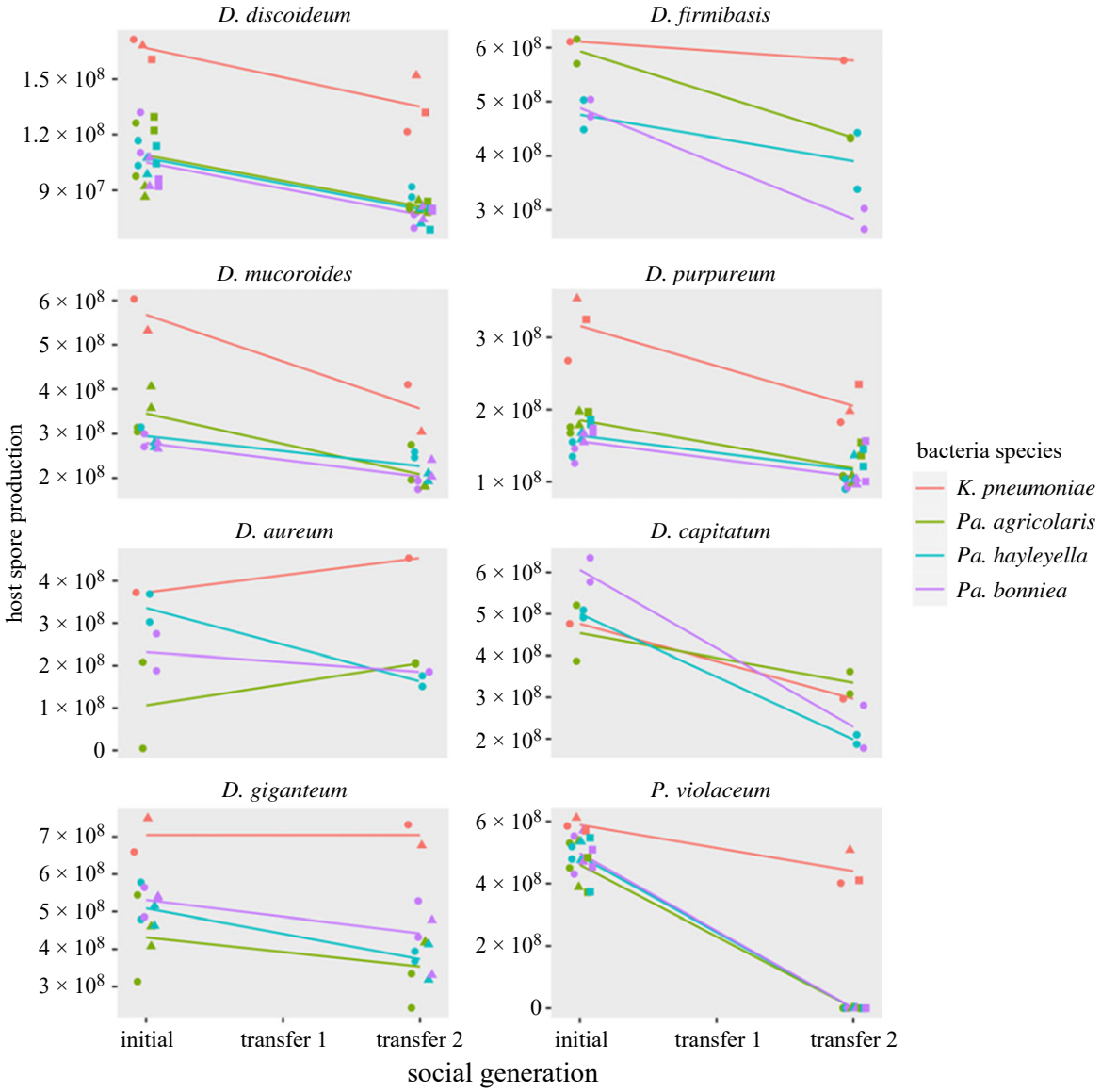

Figure 5. *Paraburkholderia* infection results in reduced host spore production. Spore production of infected hosts at initial plating and after two social generations. Infection by *Pa. agricolaris*, *Pa. hayleyella* or *Pa. bonniea* tended to reduce host spore production, though the degree of the reduction depended on the host and symbiont species used. Spore production tended to decrease after two social generations, especially in *Polysphondylium*.

sufficient bacteria to support host growth, but carriage seemed to increase after social generations.

Infections of *P. violaceum* hosts by any of the *Paraburkholderia* symbionts were highly unstable, and no host growth was detected after the first social generation.

(c) *Paraburkholderia* infection results in reduced host spore production

To more quantitatively judge the effects of symbiont infections on host fitness, we measured total spore production of hosts infected by *Paraburkholderia* symbionts after initial infection and after two social transfers.

Patterns of spore production in dictyostelid hosts infected by *Paraburkholderia* species over two social generations indicate significant three way interactions between host species, symbiont species, and time ($F_{30114} = 1.29$, $p < 0.0001$) (figure 5). Relative to uninfected hosts, hosts infected by *Paraburkholderia* produced significantly fewer spores ($t_{126} = 14.1$, $p < 0.0001$), but different host/symbiont combinations differed in the severity of the effect and its behaviour over multiple social generations.

4. Discussion

Many *Paraburkholderia* species are known to be symbionts of larger host organisms [35], and exploring host breadth within this genus can offer insight into the process by which organisms become symbionts.

Previous research paints a complex relationship between *D. discoideum* and its *Paraburkholderia* symbionts, which may act as beneficial symbionts or pathogens depending on the environment and the specific strains being considered [10,36–41]. Infection by *Paraburkholderia* can reduce host fitness as would be expected of an intracellular pathogen, but can also benefit the host under some conditions. *D. discoideum* cannot survive on a diet of *Paraburkholderia* alone, but infection by *Paraburkholderia* enables carriage of other, more edible bacteria. *Paraburkholderia*-induced carriage can facilitate its host's establishment in food-limited environments, and so the degree to which the *D. discoideum–Paraburkholderia*

interaction is mutualistic or antagonistic is likely to depend at least in part upon food availability. *Paraburkholderia* infection may also benefit its host's resistance to environmental toxins [37] and ability to compete with unrelated conspecifics [42].

In our study, we looked at infection, carriage of bacteria within host fruiting bodies, ability of hosts to grow on carried bacteria alone, and effects on host spore production across a variety of host and symbiont species. We infected strains from seven congeneric *Dictyostelium* species and one *Polysphondylium* species with three *Paraburkholderia* species isolated from wild *D. discoideum* fruiting bodies, and assayed symbiont presence and host spore production over two host life cycles. We found that these *Paraburkholderia* stably infected all tested *Dictyostelium* species, but with small but significant differences between some host–symbiont combinations. *Paraburkholderia* infection tended to reduce host spore production, but enabled carriage of food bacteria that facilitated host growth in food limited conditions. By contrast, *Paraburkholderia* was too destructive to induce stable infection in *Polysphondylium violaceum* hosts.

Infection by *Pa. agricolaris*, *Pa. hayleyella* or *Pa. bonniea* had broadly similar consequences on different *Dictyostelium* host species as have been observed in previous studies of *D. discoideum.* While all host/symbiont combinations (except *P. violaceum*) resulted in bacterial carriage (figure 3), there was much more obvious variation in which host/symbiont combinations resulted in enough carriage to support new *Dictyostelium* growth (figure 4). For instance, *Pa. bonniea* infection resulted in consistently high carriage in *D. giganteum*, but not in *D. purpureum*, while the opposite was true for *Pa. hayleyella*. Most of the host/symbiont combinations tested in this study have not been explored before, and so the long-term fate of infections beyond the two social generations we tested is unclear. In previous studies involving *D. discoideum*, infections of many different *Paraburkholderia* strains were found to be highly stable, to the point that nearly 100% of sori successfully carried enough bacteria for host growth after two social generations [11]. Further, a small number of strains have been shown to be stable for at least five social generations [10]. The fact that a few of our combinations showed loss of any carriage at all may therefore be meaningful.

Differences in the behaviour of different host/symbiont combinations may suggest that some *Paraburkholderia* symbionts may have adapted to specific *Dictyostelium* hosts (or vice versa). If so, such specialization could enable niche partitioning, which could in turn explain the coexistence of multiple *Paraburkholderia* species living in the same geographical areas [20]. Differences in symbiont species' effects on spore production were less obvious, with most symbionts appearing to reduce most hosts' spore production by a similar degree (figure 5). This reduction suggests that under the conditions of these assays, *Paraburkholderia* generally behaved as a pathogen of *Dictyostelium*. Spore production is, however, only one component of host fitness and it is possible that symbionts with similar effects on spore production might nonetheless affect different hosts' fitnesses in different ways not captured by our assay.

Though some host/symbiont combinations differed in their effects, many combinations showed surprisingly similar patterns to one another. *D. discoideum* and *D. purpureum*, for example, showed almost identical bacterial carriage and total spore counts, despite having an estimated last common ancestor over 400 million years ago and protein sequence divergence comparable to that between bony fishes and mammals [19]. As in *D. discoideum*, *Paraburkholderia* could stably infect hosts of other *Dictyostelium* species. Infection resulted in carriage of food bacteria that could support host growth in environments where food was scarce (spot test assays) but also reduced host spore production (spore production assays). The fact that *Paraburkholderia* can infect and induce stable carriage in many different *Dictyostelium* species suggests that while some strains or species may have adapted specificity to their partners, the mechanisms by which *Paraburkholderia* infects and persists within *Dictyostelium* are general enough to function across different species. It may be that *Paraburkholderia* symbionts are horizontally transmitted between *Dictyostelium* hosts—including between species—rather than the strict vertical transmission common in many more specialized endosymbionts. Horizontal transmission between hosts may further help explain why *Paraburkholderia's* relationship with *Dictyostelium* includes both cooperative and antagonistic elements.

While *Polysphondylium* could be successfully infected with *Paraburkholderia*, the infection could not persist longer than a single social cycle due to toxic effects on the host. Successful infection in the laboratory may mean that horizontal transmission from *Dictyostelium* hosts to *Polysphondylium* hosts in the wild is possible, but that *Paraburkholderia* behaves more like a pathogen for *Polysphondylium*, to the extent that long-term symbiosis is unlikely. Differences in the outcome of infection for *Dictyostelium* and *Polysphondylium* presumably reflect differences in the two species' ability to survive toxicity, perhaps by moderating their endosymbionts' growth. Alternately, the difference may reflect differences in the *Paraburkholderia* species' past adaptation to *Dictyostelium* hosts, which may have allowed them to attenuate their virulence. More detailed investigation of the microbiota and pathogen resistance of *Dictyostelium* and *Polysphondylium* in nature could help explain the mechanism by which *Paraburkholderia* can behave as a symbiont to one but not the other.

One feature of *D. discoideum* and *Paraburkholderia*'s relationship that has made it a useful model system for the study of symbiosis in previous studies is the existence of multiple *Paraburkholderia* species to compare with each other. In this study, we explore the equivalent possibility with the amoeba hosts. We found that *Paraburkholderia* infections imposed broadly similar consequences on many different host species within the genus *Dictyostelium.* Past studies have focused on *D. discoideum*, the original host from which *Pa. agricolaris*, *Pa. hayleyella* and *Pa. bonniea* were initially isolated, but our results indicate that the relationship between *Paraburkholderia* and *Dictyostelium* may be more generalist, at least within the genus level. Our results have implications about the evolutionary history of *Dictyostelium* and *Paraburkholderia*, the mechanism by which *Paraburkholderia* infects its hosts, and about the evolution of specificity within a symbiotic relationship that includes both cooperative and antagonistic elements. With the potential to infect a variety of different host species that may live in a variety of different contexts, *Paraburkholderia* in nature may find itself routinely changing its stripes—perhaps it behaves more like a pathogen when infecting some hosts and more cooperatively when infecting others. Future work in this system could explore how each host and symbiont species is geographically distributed to look for evidence that some symbionts preferentially associate with certain hosts and to better estimate how frequently symbionts may have the opportunity to switch host species. Applications of lessons

learned in the *Paraburkholderia–Dictyostelium* system may further understanding of generalism within beneficial and antagonistic symbioses in other systems, and of how new symbioses become established and then maintained.

Data accessibility. Data can be accessed on Dryad Digital Repository: https://doi.org/10.5061/dryad.3ffbg79pj [43].

Authors' contributions. R.V.M.: formal analysis, investigation, methodology; T.J.L.: formal analysis, visualization, writing—original draft, writing—review and editing; D.A.B.: conceptualization, investigation, methodology, project administration, supervision; D.C.Q.: conceptualization, funding acquisition, supervision, writing—review and editing; J.E.S.: conceptualization, funding acquisition, supervision, writing—review and editing.

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Conflict of interest declaration. We declare we have no competing interests.

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