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Cost of movement in the multicellular stage of the social amoebae *Dictyostelium discoideum* and *D. purpureum*

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One of the challenges of microbial life is that the best location for feeding and growth may not be the best location for dispersal. This is likely to be the case for the social amoebae *Dictyostelium discoideum* and *Dictyostelium purpureum* that feed on soil bacteria in the amoeba stage, but then group into a multicellular slug that moves towards light before forming a fruiting body. Here we examine this short-range social dispersal in the social amoebae, *Dictyostelium discoideum* and *D. purpureum*. We predicted *D. purpureum* would have higher migration costs and travel less far because it forms a dead stalk from living cells as it moves, while *D. discoideum* delays stalk formation until movement ceases. We found that *D. purpureum* migrated shorter distances than *D. discoideum*, in accord with our prediction. *D. discoideum* slugs moved an average of 2.46 ± 0.19 cm while *D. purpureum* slugs moved an average of 1.04 ± 0.06 cm. In both species, migration incurred a cost in reduced spore production, compared to experimental conditions where slugs did not migrate. *D. discoideum* under the no migration treatment produced 0.55± 0.05 spores per cell and under the migration treatment produced 0.25 ± 0.04 spores per cell. *D. purpureum* under the no migration treatment produced 1.01± 0.06 spores per cell and under the migration treatment produced 0.85 ± 0.06 spores per cell. We also found that *D. discoideum* produced fruiting bodies with fewer spores after migrating while *D. purpureum* did not. It appears that the evolutionary loss of stalked migration gives *D. discoideum* cells the advantage of delaying specialization and the ability to colonize more distant locations, but has significant costs due to migration distance, such as the fraction of cells that become fertile spores.

KEY WORDS: migration, *Dictyostelium*, conflict, sociality, cooperation, microbes.

INTRODUCTION

Dispersal is a life history trait that affects both ecological and evolutionary behaviors because of its effects on population structure and speciation (JOHNSON &

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GAINES 1990; FRIEDENBERG 2003). The costs of dispersal must be outweighed by the benefits of this potentially dangerous and energetically costly activity. The cost of dispersal has been studied extensively and modeled in macro-organisms (HOLEKAMP 1984; SCHTICKZELLE et al. 2009; COTE & CLOBERT 2010). However, it has not been examined nearly as extensively in microorganisms. One reason may be the strength of the hypothesis proffered by Baas Becking in 1934, that "everything is everywhere, but, the environment selects" (VAN dER GUCHT et al. 2007; FIERER 2008). This theory assumes that microorganisms have high dispersal and therefore are found, without population structure, in all types of environments. If there is high, random dispersal, then the assumption is that costs are not limiting and do not factor into dispersal distances (ROUSSET & GANDON 2002). However, many of these studies were done on microorganisms that disperse passively (FINLAY 2002). More recent research on active dispersers has introduced new theory that suggests that dispersal is non-random (MARTINY et al. 2006; JENKINS et al. 2007), but this area is still developing and is just beginning to include microorganisms that have cooperative dispersal (VOS & VELICER 2008; SCHTICKZELLE et al. 2009).

The eukaryote social amoebae *Dictyostelium discoideum* and *D. purpureum* are microorganisms where both cooperation and dispersal can be studied. Both species spend most of their life cycles as single-celled organisms eating soil bacteria (RAPER 1984). However, when their prey becomes scarce, the cells aggregate together to form a multicellular slug. The slug can then move to a better location where it will form a fruiting body. The fruiting body consists of a sterile stalk composed of aproximately 20% of the cells which hold aloft the other cells as fertile spores in a ball called a sorus (BONNER 2001). The two species are in the same major phylogenetic dictyostelid group based on small subunit RNA and α-tubulin sequences (SCHAAP et al. 2006). However, within this group they are not particularly closely related, and their genomes are as disparate as humans and fish (N. PUTNAM pers. comm.). Both species aggregate to the same chemical stimulant, cAMP (BONNER 1967), but then largely, but not entirely, sort into species-specific slugs (JACK et al. 2008).

There are several developmental and behavioral differences between the two (BONNER 1957; FOSTER et al. 2002; BONNER & LAMONT 2005; MEHDIABADI et al. 2006). The most relevant difference for the purpose of this study is the timeline for when cells fully differentiate into different cell types. The slugs of both species contain two main cell types: prespore cells in the posterior portion of the slug and prestalk cells in the anterior of the slug. When there is no migration, the cells in both species are totipotent. However, the two species behave differently during migration. *D. discoideum* forms a stalk composed of dead cells only when it has ceased moving. This means all cells in *D. discoideum* are totipotent during slug movement. On the other hand, *D. purpureum* cells head towards their fate much earlier in development. They produce a stalk horizontally along the entire migratory path, which means cells in the slug are continually dying to form the stalk and must be replaced by prespore cells. A much earlier work by Bonner shows that the prespore and prestalk regions of slugs of *D. discoideum* and *D. mucoroides*, a stalked migrator similar to *D. purpureum*, maintain constant proportions throughout migration (BONNER 1957). This may mean that movement is more costly for *D. purpureum* because many cells that would otherwise become reproductive spores in a non-migrating slug must dedifferentiate into prestalk cells to make up for the cells that become stalk as the slug travels.

Experimental work has shown that *D. discoideum* leaves cells behind in a slime trail, although not as many as would be expected in *D. purpureum* (BONNER et al. 1953; KUZDZAL-FICK et al. 2007). Some of the cells in the slugs that are left behind are likely to be sentinel cells that served as the slug's waste removal organ. These immune-like

cells engulf bacteria that may infect the slug and are then sloughed off as the slug migrates (CHEN et al. 2007). Whether they die, or are still able to eat bacteria if they encounter them, and proliferate, is not known. Therefore, we expect to see a cost in both *D. discoideum* and *D. purpureum* because they are not only losing cells that could become spores, but that they are also investing energy into constantly reallocating cells to retain the proper proportion of prespore to prestalk cells within the migrating slug in addition to the increased energy required for moving. Our goal is to explore the fitness costs associated with timing of stalk determination. We predict that *D. purpureum* will pay a higher cost and travel less far because it continually produces a dead stalk as it migrates while *D. discoideum* delays stalk formation until migration is finished.

MATERIALS AND METHODS

Clones

We used 15 clones each of genetically distinct *Dictyostelium discoideum* (QS68, QS69, QS70, QS71, QS73, QS74, QS75, QS76, QS79, QS80, QS81, QS175, QS176, QS177, QS178) and *D. purpureum* (QSPU1, QSPU2, QSPU3, QSPU6, QSPU7, QSPU8, QSPU9, QSPU11, QSPU12, QSPU13, QSPU15, QSPU16, QSPU18, QSPU19, QSPU20) that we isolated from soil in natural, undisturbed areas of the Houston Arboretum and Nature Center, Houston, TX.

Cell preparation

We plated out spores from each clone with 300 μL of a saturated culture of *Klebsiella aerogenes* as food on SM/5 agar plates (SUSSMAN 1966). We harvested the cells while they were in log growth, well before multi-cellular development occurred, and suspended them in cold standard KK2 buffer (3.8 mM K_2HPO_4, 16.5 mM KH_2PO_4). We then centrifuged the cells three times at 1300 rpm for 3 min to remove any remaining bacteria and prepared a concentration of 10^8 cells per milliliter in KK2 buffer.

Experimental setup

We placed buffered non-nutrient agar Petri plates (72.7 mM KH_2PO_4, 12.54 mM $Na_2H_2PO_4$, 20 g agar) in a laminar flow hood for 30 min prior to use to remove all excess moisture from their surface. We then drew a line on the bottom of the plates that was 1 cm from the side of the plate. Cells of each clone were spread on the agar behind the line on two Petri plates. The plates were once again left in the laminar flow hood so that any excess buffer dried on the plates leaving a film of cells. After the plates dried, they were divided into two different treatments, placed in an incubator at 22 °C with 24-hr light, and left for 1 week. The first treatment was a unidirectional light treatment, which we refer to as our migration treatment. The plates were stacked with black paper circles between them and aligned so that all of the cells were on one end. The plates were then wrapped in aluminum foil, leaving a small opening at the end of the plates opposite the cells. This provided a directional light gradient. These two species of *Dictyostelium* are phototactic so the slugs will migrate towards the light source. Our second treatment was the overhead light treatment, which we refer to as the no migration treatment. The remaining plate of each clone was placed as is in the incubator so that they received light from above. Each clone was replicated twice.

Light intensity versus spore production

To ensure that any change we saw in the number of spores was a result of migration and not the light intensity on a plate, we set up a small experiment where we tested four clones under

six different light intensities using a light meter to measure relative light intensity. Two of the treatments closely resembled the treatments used in the migration experiment. We had a treatment with overhead light, which was set at a light intensity measurement of 1 and we had a foil treatment that blocked some light and was standardized to a value of 0.03 after we converted the f-stop number to a linear scale. The other four treatments were created by placing tinted nylon stockings around a plate and measuring the light intensity and subsequent spore production. Their values ranged from 0.31 to 0.79 after standardizing. These results indicate that light intensity did not affect spore the number of spores produced (treatment: $F_{6,18} = 1.625$, $P = 0.197$), so we can be confident that any results are due to slug movement, not quality of the light.

Data collection and analyses

We assessed migration distance by dividing each Petri plate into zones (z). Four of the zones were migration zones 2 cm in width in front of the initial cell line. A zone 0 was used for the area where the cells were initially placed. All fruiting bodies in each zone (F_z) were counted. We then used the following formula to determine the average distance fruiting bodies traveled on each plate: $\sum_{z=0}^{4} F_z 2z \Big/ \sum_{z=0}^{4} F_z$.

We used sporulation efficiency as our measure of fitness to determine the cost of migration. This is defined as the proportion of initially plated cells that become reproductive spores. All fruiting bodies on each plate were collected in one Eppendorf tube containing 1 ml of 20 mM EDTA in buffer. We used a hemacytometer to count the number of spores produced.

All data were analyzed using R version 2.11.1 (R Development Core TEAM 2010) and the nlme package was used to create the models that we analyzed (PINHEIRO et al. 2009). We used species and treatment as fixed effects predictors and clone as a random effect to look at the response variables migration distance, sporulation efficiency, and spores per fruiting body. All figures were created using ggplot2 (WICKHAM 2009). The boxplots are Tukey boxplots where the box ends are the 1st and 3rd quartiles, the middle line represents the median, the whiskers extend to the farthest point that is no more than 1.5 times the interquartile range and the dots represent outliers beyond those values.

RESULTS

Migration distances

In the migration treatment, we found that *D. discoideum* slugs moved an average of 2.46 ± 0.19 cm while *D. purpureum* slugs moved an average of 1.04 ± 0.06 cm. None of the *D. purpureum* slugs in the no migration treatment moved past the start line (Fig. 1). The *D. discoideum* slugs in the no migration treatment moved slightly, on average 0.12 ± 0.02 cm, not different from 0 ($t_{1,27} = 1.26$, $P = 0.219$). We found that all comparisons between treatments and species were significantly different from each other (*P*-values less than 0.001) except for the *D. discoideum* and *D. purpureum* no migration treatments, which did not differ from each other ($F_{3,27} = 0.79$, $P = 0.378$).

Sporulation efficiency

We found that both *D. discoideum* and *D. purpureum* produced fewer spores from the initial cells after migration as compared with no migration (Fig. 2) (*D. discoideum*

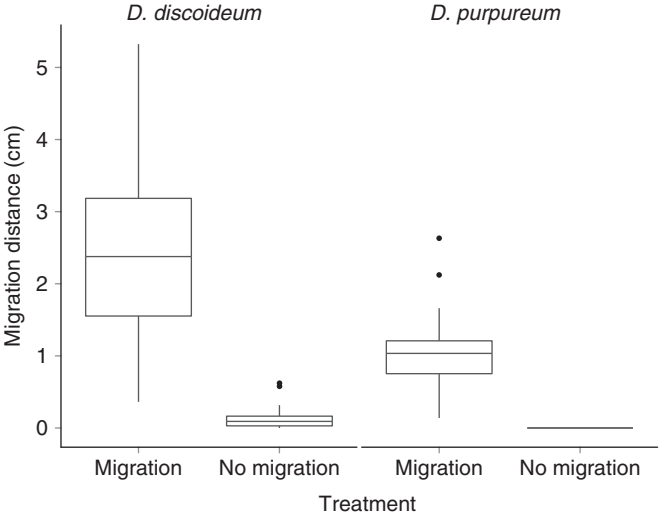

Fig. 1. — The distance each species migrated in the migration treatment compared to the no migration treatment. All interactions were significantly different from each other (*P*-values less than 0.001) except for the *D. discoideum* and *D. purpureum* no migration treatments ($F_{3,27} = 0.79$, $P = 0.378$). The distance traveled during the migration treatment was greater and varied more amongst the *D. discoideum* clones compared to the *D. purpureum* clones.

no migration: 0.55 ± 0.05 spores per cell; migration: 0.25 ± 0.04 spores per cell; $F_{1,14} = 15.05$, $n = 15$, $p < 0.01$) and *D. purpureum* (no migration: 1.01 ± 0.06 spores per cell; migration: 0.85 ± 0.06 spores per cell; $F_{1,14} = 5.66$, $n = 15$, $P < 0.05$). We also found that *D. discoideum* has a lower sporulation efficiency under all treatments than *D. purpureum* ($F_{1,28} = 42.44$, $P < 0.001$). Interestingly, when we standardized for distance traveled by dividing the change in sporulation by the average distance traveled, we found that *D. discoideum* and *D. purpureum* showed a similar decrease in sporulation efficiency (*D. discoideum*: − 0.211 ± 0.056 spores per cell per cm; *D. purpureum*: − 0.201 ± 0.053 spores per cell per cm, $F_{1,28} = 0.019$, $P = 0.892$).

Spores per fruiting body

We looked at the average number of spores per fruiting body for each species to determine whether the sori of the fruiting bodies from the Migration treatment were smaller than those in the no migration treatment (Fig. 3). We found a mixed result. *D. discoideum* produced smaller sori after migrating (migration: 4390 ± 611 spores per fruiting body; no migration: 8511 ± 755 spores per fruiting body; $F_{1,14} = 12.514$, $n = 15$, $P < 0.01$). However, *D. purpureum* did not produce fruiting bodies that were significantly different in sorus size between treatments (migration: 11,240 ± 874 spores per fruiting body; no migration: 12,510 ± 1244 spores per fruiting body; $F_{1,14} = 0.523$, $n = 15$, $P = 0.482$).

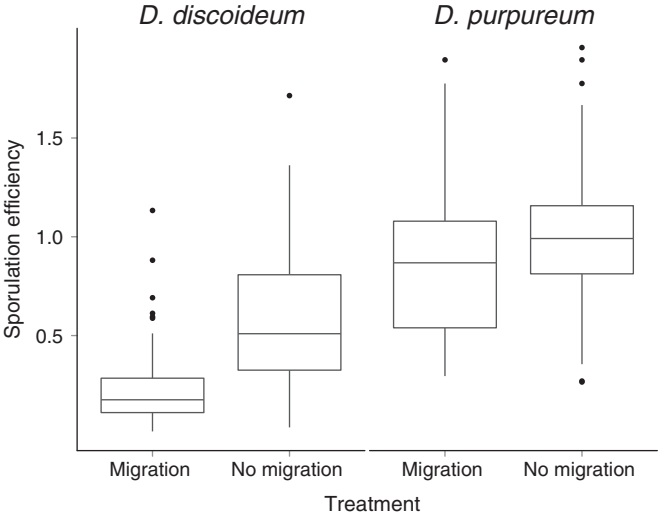

Fig. 2. — The sporulation efficiency of each species was lower in the migration treatment compared to the no migration treatment (*D. discoideum*: $F_{1,14} = 15.05$, $n = 15$, $P < 0.01$; *D. purpureum*: $F_{1,14} = 5.66$, $n = 15$, $P < 0.05$). Overall, *D. purpureum* had a higher sporulation efficiency and was less affected by migration. The presence of outliers and data over 1 suggest that some clones may have undergone a late-stage cell division.

DISCUSSION

We found that migration in both species led to lower sporulation efficiency (percent spores resulting from a starting number of cells) and resulted in fruiting bodies with sori that contained fewer reproductive spores as compared to the no migration treatments. Our results demonstrate that there is a cost to migration in reproductive fitness in both species of slime molds, despite their different methods of migration. To our knowledge, this is one of the first papers to quantify actual costs of active migration in microbes. While there is some work done on dispersal on microbes, the focus is generally population structure and microbial diversity with emphasis on passive dispersal (MARTINY et al. 2006). Our results are similar to what is found in the literature on macroorganisms, where there are tradeoffs between fecundity and migration (RANKIN & BURCHSTED 1992; ROFF & FAIRBAIRN 2001; JOHNSON et al. 2009)

Our *a priori* hypothesis was that *D. purpureum* would pay a higher cost and travel less far than *D. discoideum* because we thought that stalkless migration would be a cost saver. If very few cells are sloughed off, then this manner of migrating to a more favorable location should be favored over stalked migration, where many cells must be lost to form the stalk. However, that is not what we found. Instead, we found that migration seems to have a larger cost in *D. discoideum*, resulting in drastically fewer spores per fruiting body and depressed sporulation. We found reduced sporulation in *D. purpureum* but did not find fruiting bodies with significantly smaller sori. This suggests that *D. purpureum* changes its spore:stalk ratio when migrating. When we correct for the distance migrated, we found that there was no difference in spore loss between

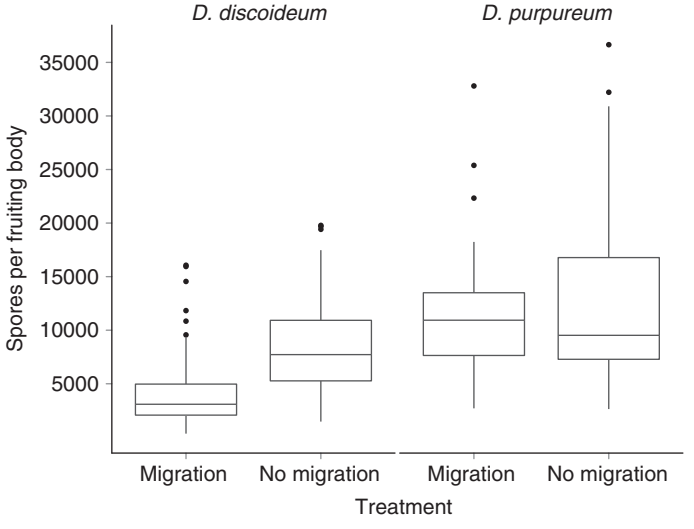

Fig. 3. — The number of spores produced per fruiting body by each species was lower in the migration treatment compared to the no migration treatment, but it was only significant for *D. discoideum* ($F_{1,14} = 12.514$, $n = 15$, $P < 0.01$) and not for *D. purpureum* ($F_{1,14} = 0.523$, $n = 15$, $P = 0.482$). In both species there was higher variance (but fewer outliers) in the no migration treatment, suggesting a constraint on spore production in the migration treatment. The lack of significance in *D. purpureum* coupled with the decrease in sporulation suggests that it may be changing its spore:stalk allocation when it migrates.

D. discoideum and *D. purpureum*, because *D. purpureum* traveled only half the distance that *D. discoideum* traveled. Nevertheless, it remains true that stalkless migration does not show the cost reduction expected from not having to allocate cells to a stalk. Some of the cell loss may be due to the sentinel cells as these cells engulf bacteria that may infect the slug and are then sloughed off as the slug migrates (CHEN et al. 2007). Additionally, there may be some cell mortality due to the exhaustion of energy stores as the slug moves.

A partial explanation for our finding may be found in a recent paper that looked at benefits to sociality in *D. discoideum*. KUZDZAL-FICK et al. (2007) found that cells that were sloughed off of migrating slugs were able to colonize local bacteria patches, which could lead to more fruiting bodies after a period of time (KUZDZAL-FICK et al. 2007). This could mitigate the loss of cells in individual migrating slugs and could actually be a benefit that leads to increased migration. Like *D. discoideum*, sloughed off cells of *D. purpureum* are also able to colonize bacteria patches (data not shown). However, the majority of lost cells that ends up as part of the reproductively dead stalk cannot contribute to this function. So, although there is no difference in cells lost per distance traveled, most cell loss in a stalked migrator is absolute, while most cells lost in stalkless migration may have the potential to colonize, especially since more cells being sloughed off as the slug moves gives it a higher likelihood of encountering a bacterial patch.

Perhaps the adaptation of *D. discoideum* represents a tradeoff in dispersal traits that led to more reliance on active dispersal compared to *D. purpureum*. SCHAAP et al.

(2006) mapped all well-documented morphological traits onto a molecular phylogenetic tree. *D. discoideum* has a stalk length of 3–7 mm and an average spore volume of between 50 and 80 μm^3. Compare this to *D. purpureum*, which has a much taller stalk (> 7 mm) and smaller spores (< 50 μm^3). A taller stalk allows the fruiting body to rise up farther off the ground, which may make it easier to be dispersed by passing invertebrates or the wind. Conversely, a shorter stalk in *D. discoideum* means that it has to travel farther to find a suitable location while *D. purpureum* may be able to fruit in more locations. This information combined with the distance that each species traveled may indicate that although each species uses both passive and active dispersal, they may be better adapted to one method over the other. *D. purpureum* has stalked migration, which could be a more costly method of travel over long distances. However, it could compensate by not traveling as far and making structures that are better suited for passive dispersal to lower its costs of migration. An additional benefit for *D. purpure*um is that migrating slugs with stalks can cross gaps in soil and leaf litter while those that migrate without them are unable to do so (O. GILBERT pers. comm.). *D. discoideum*, on the other hand, has adapted its method of migration in such a way that it allows migration and propagation while reducing some of the costs normally associated with migration. It may lose more cells as it travels longer distances, but there is no reason these cells could not colonize bacteria patches since none have died to form stalk cells. This would lead to more reproductive spores, which would go unaccounted in our experimental setup. This could be a recent adaptation as only five species of *Dictyostelium* have been found to show stalkless migration: *D. discoideum*, *D. citrinum*, *D. intermedium*, *D. dimigraformum*, and *D. polycephalum*, representing two origins of the trait, since the first four species are each other's closest relatives, and are separated from *D. polycephalum* by many stalked migrating species (BONNER 1982; SCHAAP 2007).

More work is necessary to fully explore the benefits and costs of microbial dispersal but this work indicates that there is a cost to migration in some microbes, much as there is for macroorganisms, which can limit the extent of movement. However, it also indicates that species are able to get around those costs in different ways.

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