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Social Interactions In Two Species Of Social Amoebae *Dictyostelium Discoideum* And *Dictyostelium Purpureum*

by

Chandra Nicole Jack

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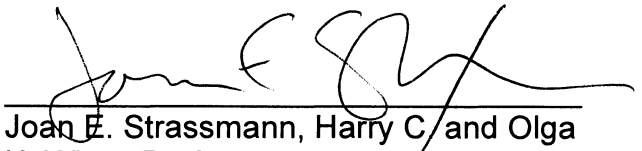

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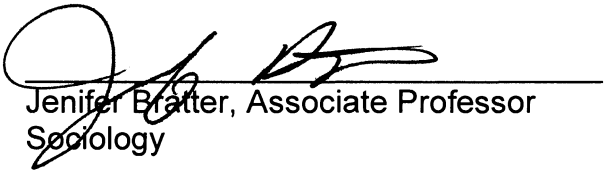
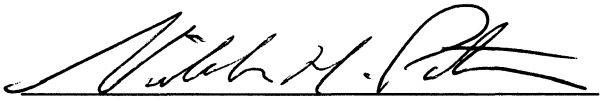
David C. Queller, Harry C. and Olga K. Wiess Professor Ecology and Evolutionary Biology (Co-Chair)



Jenifer Bratter, Associate Professor Sociology



Nicholas H. Putnam, Assistant Professor Ecology and Evolutionary Biology

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ABSTRACT

Social Interactions in Two Species of Social Amoebae *Dictyostelium discoideum* and *Dictyostelium purpureum*

By

Chandra Nicole Jack

The core of sociality and one of the key forces behind the transition to multicellularity is cooperation. The study of social behavior in microorganisms has gained considerable attention in the last decade as researchers have discovered that many of the cooperative social interactions found in higher organisms can also be found in microbes. The dictyostelids are particularly amenable to the study of social evolution because of the potential for conflict and cooperation during multicellular formation. The formation of the multicellular fruiting body may lead to conflict because all nearby cells aggregate together, which may be distinct clones, each trying to increase its own fitness. I first explored how D. discoideum and D. purpureum interact and if either species looks to cheat the other when they interact. I found that both species prefer being clonal but cooperate with each other when it seems the benefits outweigh the costs. Cooperating amoebae are able to make larger fruiting bodies,

which are advantageous for migration and dispersal, but both species suffer a cost in producing fewer spores per fruiting body. I next examined short-range social dispersal in the social amoebae, D. discoideum and D. purpureum. 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. In my final study, we examine the interaction of different clones of D. discoideum before and after migration. We show that chimerism and migration interact to produce fruiting bodies that have a proportionally higher spore allocation compared to clonal fruiting bodies after migration but were unable to determine whether the results that we see are an indication of clones defecting in a tragedy of the commons or more cooperation. With further study will be able to better explain the affects of cooperation on group dispersal and whether it can be used as a mechanism to reduce local competition.

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1 Introduction

1.1 Sociobiology and microbes

The core of sociality and one of the key forces behind the transition to multicellularity is cooperation (Maynard Smith & Szathmáry 1995; Crespi 2001). The study of social behavior in microorganisms has gained considerable attention in the last decade as researchers have discovered that many of the cooperative social interactions found in higher organisms can also be found in microbes (Crespi 2001). One example is that of cooperative swarming in *Myxococcus xanthus*, which is comparable to the cooperative hunting seen in wolf packs. Another social behavior found in both micro- and macroorganisms is a cooperative division of labor. The social amoeba *Dictyostelium discoideum* aggregates with nearby cells to form a fruiting structure where some individuals will reproduce and others will not is comparable to the eusocial wasp *Polistes dominulusi*, where several foundresses nest together and all but one give up their reproduction (Strassmann et al. 2004). Microorganisms have an advantage over larger organisms because of their small size and rapid generation times. They are also more easily manipulated genetically.

1.2 The social amoebae *Dictyostelium* as a model organism for social evolution.

The dictyostelids are particularly amenable to the study of social evolution. The most commonly studied species of the group is *Dictyostelium discoideum*. It is a model organism for the study of development because of its ability to for a multicellular structure. It is now being considered a model for the study of social evolution because of the potential for conflict and cooperation during multicellular formation (Strassmann et al. 2000). It is a unicellular amoeba that preys on soil bacteria (Raper 1984). When it runs out of food it sends out a signal of cAMP, causing all nearby cells tog aggregate and form a multicellular slug (Kessin 2001). At this point there is the potential for conflict because approximately 20% of the cells in the slug will altruistically forego reproduction to form a sterile stalk so that the remaining 80% can be held aloft as reproductive spores in a structure called a sorus. The formation of the multicellular fruiting body (stalk plus sorus) may lead to conflict because all nearby cells aggregate together, which may be clones of different genotypes (Fortunato et al. 2003), each trying to increase its own fitness. Several studies have found that *D. discoideum* is capable of recognizing kin from non-kin. The response to this recognition ranges from sorting out from aggregates containing other genotypes to cheating, where one of the genotypes will gain an unfair

advantage in reproductive spores (Fortunato et al. 2003; Ostrowski et al. 2008; Buttery et al. 2009). Its genome has been sequenced (Eichinger et al. 2005), which gives the potential to connect social behaviors to their underlying genes.

The other species I study in my thesis is *D. purpureum*. Its genome has recently been sequenced, although it is not yet fully aligned (Sucgang et al. 2011). It differs both developmentally and morphologically from *D. discoideum*. Comparative analysis of the two genomes finds that they are as different from each other as humans are from jawed fish (Sucgang et al. 2011). However, the two species are in the same major phylogenetic dictyostelid group based on small subunit RNA and α-tubulin sequences (Schaap et al. 2006). Phylogenetic analysis of morphology shows that *D. purpureum* is the older species (Schaap et al. 2006; Schaap 2007), characterized by its stalked migration and smaller spores.

1.2.1 Between species interactions of *Dictyostelium*

The first project in my thesis is about the mutualistic interaction of *D. discoideum* and *D. purpureum*. Mutualisms are interactions between different species where each gains a benefit out of partnership. It is a cooperative relationship without the benefit of relatedness, such as seen between cooperative interactions within a species. Because inclusive

fitness cannot be used as a mechanism for this behavior, there must other reasons why species would cooperate. One species might offer some public good or service that the other cannot obtain on its own. For example, the in the mutualism between the Senita cactus and Senita moth, the moth pollinates the cactus in exchange for a place to oviposit eggs (Holland & Fleming 2002). A host of literature on mutualisms and cooperative interactions mentions several methods that individuals use to prevent cheating, such as policing, tit-for-tat, sanctions (Axelrod & Hamilton 1981; Boucher 1985; Frank 1995). We know that both species can be found in the same soil sample and that they both aggregate to the same chemoattractant making it more than likely that they may find themselves in the same multicellular aggregate. In this chapter we explore how *D. discoideum* and *D. purpureum* interact and if either of the species looks to cheat the other when they interact.

1.2.2 Dispersal and *Dictyostelium*

The second part of my thesis is devoted to looking at the cost of dispersal in *D. discoideum* and *D. purpureum.* Dispersal is a life history trait that affects both ecological and evolutionary behaviors because of its effects on population structure and speciation (Johnson & Gaines 1990; Friedenberg 2003). While well studied in macroorganisms, it has not been nearly as well studied in microorganisms (Holekamp 1984; Cote & Clobert

2010). One reason may be the strength of the hypothesis proffered by Baas Becking in 1934, that "everything is everywhere, but, the environment selects" (Fierer 2008). According to this theory, 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). My aim was to quantify the costs of dispersal during the social migration phase of *Dictyostelium*. We know that as the slug migrates, it loses cell that must be replaced through dedifferentiation of the cell types within the slug. Additionally, we are using two species that migrate in different ways. *D. discoideum* migrates and then undergoes final differentiation into stalk and spore, while *D. purpureum* differentiates some of its cells to stalk as it migrates. Our goal was to explore the fitness costs associated with timing of stalk determination.

1.2.3 Cooperation and Dispersal in *D. discoideum*

My final project is to examine how migration affects the interaction of different clones of *D. discoideum*. Hamilton's theory of kin selection shows that cooperation can evolve if the recipient of the beneficial action has a high enough degree of relatedness to the actor to overcome the cost

involved, according to the inequality: rb-c > 0 (Hamilton 1964). In order for kin selection to favor altruism, relatedness must be sufficiently high. Hamilton proposed two mechanisms for this. One way is through kin discrimination, where organisms preferentially direct benefits towards kin whom they can recognize on some level (Hamilton 1964). The second mechanism is through limited dispersal (Hamilton 1964). Theoretical models suggest that it can be an important method for the evolution of cooperation but may also lead to the breakdown of cooperation because of increased local competition between kin (Taylor 1992; Queller 1994; Bourke & Franks 1995; West et al. 2006). We know that clones of *D. discoideum* will compete with each other to become reproductive spores (Fortunato et al. 2003). We also know that chimeric slugs do not travel as far as clonal slugs of the same size (Foster et al. 2002). However, we do not know the effect of simultaneous dispersal and cooperation on population structure. In this study, we examine the interaction of different clones of *D. discoideum* before and after migration.

1.3 Conclusion

Together, these chapters will help explain how the population structure of *Dictyostelium* is affected by interactions with other species and with other members of the same species. This work will give us further

understanding of how kin competition and cooperation may have evolved and remained stable.

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2 Segregate or cooperate- a study of the interaction between two species of *Dictyostelium*

Chandra N. Jack, Julia G. Ridgeway, Natasha J. Mehdiabadi, Emily I. Jones, Tracy A. Edwards, David C. Queller, Joan E. Strassmann

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2.1 Abstract

2.1.1 Background

A major challenge for evolutionary biology is explaining altruism, particularly when it involves death of one party and occurs across species. Chimeric fruiting bodies of *Dictyostelium discoideum* and *Dictyostelium purpureum* develop from formerly independent amoebae, and some die to help others. Here we examine co-aggregation between *D. discoideum* and *D. purpureum,* determine its frequency, which party benefits, and the extent of fair play in contribution to the altruistic caste.

2.1.2 Results

We mixed cells from both species in equal proportions, and then we analyzed 198 individual fruiting bodies, which always had either a *D. discoideum* or *D. purpureum* phenotype (*D. discoideum*- 98, *D. purpureum*- 100). Fifty percent of the fruiting bodies that looked like *D. discoideum* and 22% of the fruiting bodies that looked like *D. purpureum* were chimeric, though the majority of cells in any given fruiting body belonged to one species (*D. discoideum* fruiting bodies- 0.85±0.03, *D. purpureum* fruiting bodies- 0.94±0.02). Clearly, there is species level recognition occurring that keeps the cells mostly separate. The number of fruiting bodies produced with the *D. discoideum* phenotype increased

from225± 32 fruiting bodies when *D. discoideum* was alone to 486±61 in the mix treatments. However, the number of *D. discoideum* spores decreased, although not significantly, from $2.75e^7$ $\pm 1.29e^7$ spores in the controls to $2.06e^7$ $\pm 8.33e^6$ spores in the mix treatments. *D. purpureum* fruiting body and spore production decreased from 719± 111 fruiting bodies and $5.81e^7 \pm 1.26e^7$ spores in the controls to 394±111 fruiting bodies and $9.75e^6$ $\pm 2.25e^6$ spores in the mix treatments.

2.1.3 Conclusions

Both species prefer being clonal but cooperate with each other when it seems the benefits outweigh the costs. Cooperating amoebae are able to make larger fruiting bodies, which are advantageous for migration and dispersal, but both species here suffer a cost in producing fewer spores per fruiting body.

2.2 Background

Cooperative relationships between different species are common in nature (Boucher 1985; Maynard Smith & Szathmáry 1995; Herre et al. 1999). They can be found in every environment, from cactus pollinators in the desert (Holland & Fleming 2002) to microbial symbionts in the ocean (Nyholm & McFall-Ngai 2004). Although species cooperation has become recognized as important both ecologically and evolutionarily it is less well

studied than other interspecies relationships such as predator-prey interactions and competitive interactions (Bronstein 1994; Bergstrom et al. 2003). The evolution of cooperation presents a conundrum. How do these relationships evolve and remain stable over generations? Why would selection favor altruism and cooperation when cheaters could reap the benefits of an interaction without paying any of the associated costs (Bull & Rice 1991; Ferriere et al. 2002; Sachs et al. 2004; Foster et al. 2006)? Only in recent years have the worlds of microbiologists and evolutionary biologists merged to begin interdisciplinary studies on cooperation in microorganisms (Crespi 2001; West et al. 2006). The social amoebae of the genus *Dictyostelium* present ideal candidates for studying microbial interactions. All dictyostelid species spend the majority of their lifecycle as solitary amoebae living on the forest floor, eating bacteria. When the cells begin to starve, they send out a signal, which is cyclic AMP in many species, causing all nearby cells to aggregate together. In some species, such as *D. discoideum* (Figure 2-1a), the cells form a multi-cellular slug that then migrates to a new location. Once migration is complete, approximately one-fifth of the cells will altruistically die to form a sterile stalk to hold aloft the remaining cells, which have formed a sorus consisting of viable spores (Bonner 1967; Raper 1984). In other species, including *D. purpureum* (Figure 2-1b), the slug forms a sterile stalk as it

migrates to a new location. Once migration is complete, the stalk becomes vertical and the sorus forms. At this stage in the life cycle of *Dictyostelium* conflict may occur as the amoebae make the transition to multicellularity from individual cells. Some of the cells undergo the ultimate sacrifice of dying to form the sterile stalk leaving the majority to form fertile spores. This behavior will be favored by natural selection only if those cells are able to pass on their genes through relatives, who may often be clone mates. The higher the relatedness to spore cells, and the greater the advantage to having a stalk, the more the stalk cells will benefit from paying the cost and there will be less conflict between the two cell types.

The social behavior of *Dictyostelium discoideum* makes it an ideal model to study social evolution (Strassmann et al. 2000). It has been shown, both in the lab and in nature, that *D. discoideum* clones will form chimeras (Strassmann et al. 2000; Foster et al. 2002; Fortunato et al. 2003; Gilbert et al. 2007). In these chimeric fruiting bodies, conflict can occur. Foster *et al.* (Foster et al. 2002) reported that chimeric slugs migrate less far than clonal slugs of the same size indicating that some form of conflict is occurring within the slug between different clones. Additionally, some clones of *D. discoideum* have the ability to cheat other *D. discoideum* clones in chimeras by forcing them into the stalk, leading to an unequal

representation in the fruiting body (Strassmann et al. 2000). Recently it has been shown that clones of two other species of social amoeba, *D. purpureum* and *D. giganteum* form intraspecific chimeras (Kaushik et al. 2006; Mehdiabadi et al. 2006).

A single soil sample of a fifth of a gram may contain several clones and species (Fortunato et al. 2003). Yet, the formation of interspecific chimeras has not been carefully studied even though many of these species aggregate to the same chemo-attractant, cyclic AMP. Olive (Olive 1902) first looked for and failed to find chimeras of *D. purpureum* and *D. mucoroides,* followed by two other groups almost fifty years later (Raper & Thom 1941; Bonner & Adams 1958). Neither group managed to find chimeras under normal aggregation conditions. Another researcher, Hagiwara (Hagiwara 1992), made some preliminary interspecific mixtures while he explored whether aggregating streams of cells of three different genera of Dictyostelids mixed or overlapped in any way. His work verified that many species use the same chemo-attractants.

A molecular phylogeny of the Dictyostelids based on small subunit RNA and α-tubulin sequences shows subdivision of all known species into four major groups. *D. discoideum*, *D. purpureum*, and *D. giganteum* are all members of Group 4, a group where all of the studied species aggregate and respond to the same chemo-attractant, cAMP (Schaap et

al. 2006). Most of the studies exploring social behavior in the Dictyostelids have concentrated on within-species interactions in *D. discoideum*. Recent work in other species, such as *D. giganteum* and *D. purpureum* has shown interesting behavior between clone mates (Kaushik et al. 2006; Mehdiabadi et al. 2006). However, species interactions between members of Group 4 have not been closely studied, despite their similar biological properties. Here we test the hypothesis that *D. discoideum* and *D. purpureum* form chimeric fruiting bodies, and we test some of the costs and benefits.

2.3 Results

2.3.1 Chimerism of *D. discoideum* and *D. purpureum* fruiting bodies

We found chimeric fruiting bodies in 20 out of 21 trials where the initial cell suspension contained an equal number of cells of both species. All fruiting bodies in each experiment displayed either the *D. discoideum* phenotype or the *D. purpureum* phenotype. None of the fruiting bodies, including those that were chimeric, displayed an intermediate phenotype. Half of the *D. discoideum* fruiting bodies examined contained spores of *D. purpureum*, while only 22% of the *D. purpureum* fruiting bodies contained *D. discoideum* spores ($W_{14, 13}$=124, n=27, $p<0.05$).

The majority species in the chimeras, with a few exceptions, determined the phenotype. Chimeric *D. discoideum* fruiting bodies contained an average of 26.8± 4.4% *D. purpureum* spores per clone while chimeric *D. purpureum* fruiting bodies had 29.5±9.2 % *D. discoideum* spores ($W_{12, 8}$=51, n=20, p=0.851). However, when we include all fruiting bodies, chimeric and clonal, from the experimental plates, fruiting bodies with the *D. discoideum* phenotype contained a higher percentage of 'nonself' spores, than *D. purpureum* fruiting bodies, although it was not significant. ($DD_{nonself\ spores}$ =16.04±4.52%, $DP_{nonself\ spores}$ =5.44±1.90%, $W_{14, 13}$=125, n=27, p=0.101, Figure 2-2A-B).

2.3.2 Numbers of fruiting bodies with the morphology of *D. discoideum* vs. *D. purpureum*

We counted the number of fruiting bodies produced by each species on the control plates (each species alone) and on the experimental plates (50:50 mix of the two species) to compare the number of fruiting bodies produced after we standardized for the difference in cell number. We distinguished the fruiting bodies based solely on phenotype and not on whether the fruiting bodies may have contained spores of the other species. In the controls, *D. discoideum* produced 225± 32 fruiting bodies

per $2x10^7$ cells, while *D. purpureum* produced an average of 719± 111 fruiting bodies per $2x10^7$ cells, ($W_{14, 15}$=10, n=27, p<0.001).
The number of fruiting bodies with *D. discoideum* morphology significantly increased to 486± 61 when plated with *D. purpureum* when compared to the number of fruiting bodies produced when alone ($W_{14, 13}$=35, n=27, p<0.01, Figure 2-2C). Conversely, the number of fruiting bodies with *D. purpureum* morphology decreased significantly to 394±111 when plated with *D. discoideum* ($W_{13, 13}$=135, n=26, p<0.01, Figure 2-2D).

2.3.3 Spore production by D. discoideum and D. purpureum from cells
We determined the number of spores produced by each species after equal numbers of cells of each species were mixed together without food to determine if one species gained an advantage over the other. We corrected for germination efficiency and initial cell number when we compared control plates to experimental plates. *D. discoideum* produced the same number of spores from a given number of cells whether or not cells of *D. purpureum* were also present. ($DD\_spores_{exp}=2.06e^7 \pm 8.33e^6$, $DD\_spores_{ctrl}=2.75e^7 \pm 1.29e^7$, $W_{9, 9}$=29, n=18, p=0.331, Figure 2-2E). However, *D. purpureum* produced fewer spores when cells of *D. discoideum* were present compared to when *D. purpureum* cells were

alone ($DP\_spores_{exp}=9.75e^{6} \pm 2.25e^{6}$, $DP\_spores_{ctrl}=5.81e^{7} \pm 1.26e^{7}$, $W_{10, 10}=81.5$, n=20, p=<0.05, Figure 2F). We also calculated the number of spores produced per fruiting body for both *D. discoideum* and *D. purpureum* when alone and when mixed with each other as a measure of fruiting body size. Both *D. discoideum* and *D. purpureum* produced fewer spores per fruiting body in mixes when compared to the number produced when alone but neither was significant ($DD_{exp}=4.16e^{4} \pm 1.54e^{4}$, $DD_{ctrl}=6.15e^{4} \pm 1.55e^{4}$, $W_{9, 9}=60$, n=18, p=0.094, $DP_{exp}=3.65e^{4} \pm 6.74e^{3}$, $DP_{ctrl}=4.96e^{4} \pm 6.74e^{3}$, $W_{10, 10}=53$, n=20, p=0.853).

2.3.4 Relatedness

We defined relatedness (r) as the probability that two spores in the same fruiting body were from the same species. Relatedness was calculated in the experimental fruiting bodies using $p^2 + (1-p)^2$ where p was the proportion of *D. purpureum* spores in a fruiting body. This measures the degree to which clones experience their own type in the fruiting body, above the population expectation of near zero. Fruiting bodies with the *D. purpureum* phenotype had an average relatedness of r=0.943±0.014 which was not significantly higher than the relatedness of those fruiting bodies with a *D. discoideum* phenotype, r=0.89±0.022 ($W_{14, 13}=60.5$, n=27, p=0.140).

2.3.5 Time-lapse microscopy

We used time-lapse microscopy to determine when the cells of *D. discoideum* and *D. purpureum* aggregate together and when they begin to sort. We found that the cells aggregate through the mound stage, the point in the life cycle after aggregation when the cells are found in small mounds before they differentiate into slugs, and then a primarily *D. purpureum* slug migrates away, leaving behind a mound composed mostly of *D. discoideum* cells that eventually become a fruiting body (Figure 2-3).

2.4 Discussion

More than one-third of the fruiting bodies we examined were chimeric and 95% of the experiments contained at least one chimeric fruiting body. This shows that, at least in a lab setting, *D. discoideum* and *D. purpureum* cells can interact, aggregate, and form chimeric fruiting bodies, although the average percentage of one clone in any given fruiting body is 90%, which indicates that the two species prefer to segregate but do so imperfectly. Both species had an equivalent proportion of the other species in the chimeric fruiting bodies, but because chimeras were more frequent in *D. discoideum*, there were more foreign spores found in fruiting bodies with the *D. discoideum* morphology. We measured relatedness to determine how much mixing and sorting is happening between the two species. The higher the relatedness, the less intermixing that is occurring

between the two species. An r-value of 0.5 means that the cells are randomly mixing while an r-value of 1 means that the cells are completely sorting. Despite the presence of chimeric fruiting bodies, relatedness remained high within fruiting bodies of each phenotype (0.89 for *D. discoideum*, 0.94 for *D. purpureum*) on plates that began with an equal number of cells of each species.

It is remarkable that we found such a high incidence of chimerism for several reasons. First, *D. discoideum* and *D. purpureum* are not particularly closely related. In the current phylogeny (Schaap et al. 2006), the node separating the two species has 4 other species in the branch including *D. discoideum* and 17 others in the branch including *D. purpureum*. This phylogenetic distance is manifest in several developmental differences between the two species that may impact the level of sorting. *D. purpureum* forms a stalk as the slug migrates, while *D. discoideum* forms its stalk after the slug finishes migrating (Raper 1984). Additionally, *D. purpureum* develops faster than *D. discoideum.* As a result, cells of *D. purpureum* may differentiate first leading to an increase in sorting if the genes responsible for cell-type partitioning and development up-regulate at different times. We found evidence of this pattern when we used time-lapse microscopy. Cells of both species aggregate together for a short time, but then *D. purpureum* slugs break off

and migrate away from the initial mound, leaving mostly *D. discoideum* cells. A short time later, *D. discoideum* slugs begin to migrate and then form fruiting bodies. Interestingly, slugs contained cells from both species, indicating only partial disassociation.

Our chimerism result is also surprising because prior research failed to show chimerism despite mixing different species in a variety of ways. Raper and Thom (Raper & Thom 1941) first mixed spores of *D. discoideum* and *D. purpureum* and reported the absence of intermediate phenotypes, which was in accordance with our results, but does not preclude chimerism. They then mixed *D. discoideum* spores with spores of *D. mucoroides*, a species that is as equally distant phylogentically as *D. purpureum* (Schaap et al. 2006). They used the bacterium *S. marcescens* as a food source. *S. marcescens* contains a red pigment that *D. discoideum* is unable to digest, resulting in dyed cells (Raper 1937; Raper 1984) while *D. mucoroides* digests the pigment and remains white They found that the red cells initially aggregated together with the white cells but separated into red and white fruiting bodies. This shows that most cells segregated, but it is not clear if some individual cells of the wrong type might have been present.

Raper and Thom (Raper & Thom 1941) also tried making grafts between different portions of the slugs of *D. discoideum* and *D. purpureum*, but

were unsuccessful in getting the segments to permanently coalesce and form chimeric fruiting bodies. Using these data, they concluded that *Dictyostelium* species did not form chimeras. In one final experiment, they were able to obtain fruiting bodies with intermediate phenotypes by allowing cells of each species to form slugs and then crushing those slugs and mixing them (Raper & Thom 1941). These fruiting bodies contained spores from both species. However, those fruiting bodies that retained the phenotype of only one parent only produced fruiting bodies of that same phenotype, seemingly indicating that those fruiting bodies consisted of one species. Bonner and Adams (Bonner & Adams 1958) also failed to find chimeras after they completed a series of experiments where they attempted to make intermediate fruiting bodies by grafting different species together during the aggregation stage. Neither group reported the density of spores that they used.

Perhaps we were able to find chimeras while the others did not because we plated out individual spores from fruiting bodies carefully at a very low density so we could detect low levels of mixing. Overall, there was mostly sorting, but some mixing, which may have been missed if not looked for carefully. We also used multiple clones, and had we used only one pair, an unlucky choice (for example mix 10 between clones QS75 and

QSPu13 in Figure 2-2A-B) could have led us to the false conclusion that there was little mixing.

Finally, the finding of chimerism between species is surprising because both species apparently avoid chimerism even with other clones of their own species, Gilbert *et al.* (Gilbert et al. 2007) found that the relatedness for naturally occurring fruiting bodies collected in the wild that contained multiple clones of *D. discoideum* was 0.68, which was much lower than the overall relatedness of 0.98, because there were many clonal fruiting bodies. This result could be due either to sorting or to patchy distribution of clones. However, clear sorting was shown in fruiting bodies of *D. purpureum* when pairs of clones were mixed in 50:50 ratios; the result was an overall relatedness of 0.81 (Mehdiabadi et al. 2006). Recently, somewhat weaker sorting has also been demonstrated between *D. discoideum* clones (Ostrowski et al. submitted). Our relatedness values for the two species mixed 50:50, were 0.89 for *D. discoideum* and 0.94 for *D. purpureum.* The higher values indicate greater clonal sorting than within-species mixes.

Why do these two species cooperate, at least some of the time? In most cooperative interactions involving different species, each partner brings different goods or services to the association, such as between the Senita cactus and Senita moth, where the moth pollinates the cactus in exchange

for a place to oviposit eggs and the larvae to subsequently eat a portion of the seeds. That is not the case with these two dictyostelids because both species provide essentially the same services – migration and stalk formation. One possibility is that the mixing is a mistake. Each species may undergo its social lifecycle where certain cells altruistically form stalk cells as it would if in a clonal population. Cells of different species may aggregate and develop together because of their close proximity to each other and similar developmental characteristics. Another possibility is that although this interaction evolved to provide beneficial cooperation within species (or even within clones), different species are able to benefit from those services, such as protection from predators, migration, spore formation and dispersal when they would otherwise not be able to because of a cell number deficiency. When both species face the possibility of being unable to aggregate on their own because they lack sufficient cell number, the two species will aggregate together and form fruiting bodies, to their mutual benefit instead of dying out. Though we are unable to fully distinguish these hypotheses, we can provide an accounting of some of the costs and benefits that result from interspecies chimerism.

In any cooperative relationship, there are costs associated with each altruistic act. One such cost is that the altruistic act is not reciprocated,

which may lead to the exploitation of one partner by the other. Cheating is the greatest concern when there is an interaction between two individuals that are not genetically identical. Earlier research shows that clones of *D. discoideum* may cheat each other but prior experiments involving only *D. purpureum* clones show that the species maintains a high degree of kin discrimination by preferentially associating with kin without displaying a consistent pattern of cheating (Strassmann et al. 2000; Mehdiabadi et al. 2006). The stronger segregation seen in *D. purpureum* may have evolved as a way to prevent cheating between clones, but it also might mean that this species no longer has a need to maintain mechanisms of cheating, or other defenses against cheating. When the two species are mixed together, *D. discoideum*'s ability to cheat and *D. purpureum*'s lack of a cheating mechanism may be the reason *D. purpureum* was exploited.
It is possible that this association is kept stable and cheating to a minimum because the aggregates form only when necessary and that they are kept as pure as possible, as indicated by the much higher relatedness values we calculated when compared to those found in previous studies. Additionally, both species suffered in the production of spores per fruiting body, which may be why the two species tend to segregate from each other despite some of the benefits that may be gained from the interaction.

We did not observe a clear benefit to this interaction that might explain why it has persisted. In terms of spore production, *D. discoideum* maintained the number of spores it produces while *D. purpureum* decreased the number of spores produced. However, additional possible benefits result from larger slug size that are not measurable using spore production, the metric we tested. One possible benefit for cells from both species is protection from predators. By aggregating together, the amoebae can initiate mechanisms to avoid soil predators such as nematodes. Kessin *et al.* (Kessin et al. 1996) showed that *Caenorhabditis elegans* feeds on individual amoebae up through early aggregation. However, in late aggregation the cells form a polysaccharide sheath that the nematodes are unable to penetrate. This sheath protects the amoebae as they migrate as a multicellular slug. Once the fruiting body is formed, *C. elegans* may ingest the spores, but they are unable to digest them. Kessin *et al.* (Kessin et al. 1996) found an additional benefit in *D. purpureum*: at high cell densities, it is able to repel nematodes. Therefore, it may be beneficial to both species to aggregate together when cell numbers are low, especially in the presence of predators.

Migration distance is another potential benefit of forming a larger slug. Foster *et al.* (Foster et al. 2002) found that larger slugs of *D. discoideum* traveled further than slugs containing half the number of cells. Also, they

found that larger chimeric slugs traveled further than smaller clonal slugs. When slugs are traveling to a new location because the current one has run out of bacteria, larger slugs are more likely, over both smaller slugs and solitary cells, to reach a new patch of bacteria (Foster et al. 2004; Kuzdzal-Fick et al. 2007).

A final possible benefit to co-aggregation is for spore dispersal purposes. To successfully disperse spores, they must be held aloft on a stalk of sufficient height. If there are too few cells in the aggregate, a fruiting body may not form at all. Or, even if a small fruiting body is able to form, it may be at a disadvantage relative to larger fruiting bodies, making it less likely to disperse due to contact from passing invertebrates.

There may be other benefits that both species gain from cooperating and these benefits may override the cost to *D. purpureum* in spore production and any conflict that may arise as a result of the chimerism.

2.5 Conclusions

The surprising finding that *D. discoideum* and *D. purpureum* can cooperate to form chimeric fruiting bodies cannot be explained by increased spore production. It may simply be a mistake or it may be making the best of a bad job. Both species seem to prefer being clonal, but a fraction of cells cooperate with other clones and even other species, perhaps when benefits are high enough to overcome those costs.

Cooperating amoebae are able to make larger fruiting bodies, which is advantageous for migration and dispersal, but these benefits will need to be quantified to assess their importance.

2.6 Methods

2.6.1 Clones

We used fourteen genetically distinct wild clones of *D. discoideum* and thirteen clones of *D. purpureum* isolated from different soil samples collected at the Houston Arboretum, Texas (Table 2-1).

2.6.2 Cell preparation

We plated out $3x10^5$ spores from each clone with 300μl of the bacteria *Klebsiella aerogenes* (KA) as food on SM/5 agar plates (Sussman 1966). After approximately 38 hours, we harvested the cells while they were in log growth before multi-cellular development occurred with cold standard KK2 buffer (3.8mM K_2HPO_4, 16.5mM KH_2PO_4). The cells were then centrifuged three times at 1000 rpm for three minutes to remove any remaining bacteria and set at a concentration of 10^8 cells per milliliter in KK2 buffer.

2.6.3 Experiment set-up

For each of the 21 experiments, we tested one *D. discoideum* clone with one *D. purpureum* clone. We filled each well of a 6-well tissue culture plates (3.5cm in diameter) with 7ml of non-nutrient agar (14.9g agar per liter KK2 buffer). We designated four of the wells, from here forward called plates, as control plates. We labeled the final two plates as experimental plates. For the control plates, we added $4x10^7$ cells in 400μl of KK2 buffer for each clone. For the experimental plates, we added together $2x10^7$ cells of each clone in 200μl of KK2 buffer. After thoroughly mixing the cells, we spread 400μl of the cell suspension on a plate. Thus, we had a replicate of the control and experimental plates. We used one set to assess mixing and the other to assess spore production. We used both sets to assess fruiting body production.

2.6.4 Data collection and analyses

2.6.4.1 Fruiting body assessment:

In order to determine the number of fruiting bodies present on all plates, we created a circular grid 3.5cm in diameter that exactly fit the bottom of the tissue culture plates. Each square in the grid had an area of $0.25cm^2$. Before the start of the experiment we randomly selected eight of the squares to be the counting squares so that the same squares were used

consistently for all 21 of the experiments. In these squares, we counted all of the fruiting bodies, and in the case of the experimental plates, whether they had a *D. discoideum* or *D. purpureum* phenotype using a set of established criteria such as sorus color, presence of a basal disc, and stalk type (Figure 2-4). We examined the remaining squares for the presence or absence of fruiting bodies of each species. We then calculated the number of fruiting bodies by multiplying the average number of fruiting bodies over the eight squares by the area of the plate.

2.6.4.2 Spore production assessment:

For one of each control and experiment plate, we collected all of the fruiting bodies in 1ml of KK2 buffer to count the number of spores that were produced. We used a hemacytometer to count the spores. Additionally, we plated out a dilute sample of the spores from the experiment on five 60 cm Petri plates containing SM/5 agar to determine the proportion of spores produced for each species. By plating a diluted concentration of the spores, we were able to determine where on the plate cells were released from individual spores and were then able to determine the identity of the spore and calculate the proportion of spores of each species. After adjusting for germination efficiency, we were able to determine the number of spores produced by each species on the experimental plates by multiplying the proportion of spores of each

species previously calculated by the total number of spores that were collected from the experimental plate.

2.6.4.3 Germination efficiency:

For each clone, we plated out approximately 30 spores per plate over six 60 cm Petri plates containing SM/5 agar with 300ul of KA. After three days, we began scoring the plates for germinated spores, indicated by clearings in the bacteria. We replicated this procedure twice to get an average number of spores that germinate for each species. We also plated out an equal known number of spores from both species together at low density to see if the spores of one species inhibited the other and prevented them from germinating.

When plated at low density, the average germination rate for *D. discoideum* was 17.7% (SE=0.022) while the average rate of germination for *D. purpureum* was 50.3% (SE=0.033) ($F_{1, 58}$=59.41,n=60, p<0.001). We also tested the germination efficiency of each species alone and when plated with the other species to ensure that the spores of one species were not inhibiting spores of the other. We found that there was no difference in the germination efficiency for either *D. discoideum* ($F_{1, 16}$=0.31, n=18, p=0.585) or *D. purpureum* (($F_{1, 15}$=0.31, n=17, p=0.361) in mixes as compared to pure clones. Based on these results, we adjusted

spore numbers to reflect the greater spore germination rate of *D. purpureum.*

2.6.4.4 Chimera assessment:

From the other set of experiment plates, we collected five fruiting bodies that had a *D. discoideum* phenotype and five fruiting bodies that had a *D. purpureum* phenotype. We placed each fruiting body individually in 40μl of KK2 buffer and plated out a dilute sample of the spores with on SM/5 plates with KA. We tallied the number of spores of each species that hatched from the fruiting bodies to determine if the fruiting body was chimeric and what percentage of the spores in the fruiting body were of the other species' phenotype after again adjusting for germination.

2.6.4.5 Timelapse Florescence Microscopy:

We observed the different stages of development in one pair, (Experiment #4: QS71 and QSPu16) by labeling QS71with CellTracker™ Green CMFDA. We followed the manufacturer's recommended protocol to label the cells, except that we used 50μM of CellTracker™ Green CMFDA. We created the timelapse using a Nikon™ E1000 florescent microscope and MetaMorph® imaging software.

2.6.4.6 Analyses:

We ran Wilcoxin rank sum tests on all our data except the germination efficiency results. The data were analyzed after grouping by clone, although when the data were grouped by experiment, the results were comparable. The germination efficiency data was analyzed using ANOVAs. We ran all analyses on our data using R (Team 2006). All of the data are reported as the mean ± standard error. The graphs were created using Microsoft Excel version 11.3.5.

2.7 Authors' contributions

CNJ, DCQ, JES, and NJM designed and conducted the research, analyzed the data, and wrote the manuscript. CNJ, DCQ, JES, NJM, and EIJ designed and conducted research on the initial experiment. CNJ, NJM, JGR, and TAE conducted the experiments. All authors read and approved the final manuscript.

2.8 Acknowledgements

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Table 2-1 Table of *D. discoideum* and *D. purpureum* clone used in the experiment

EXPERIMENT #	D. discoideum clone ID	D. purpureum clone ID
1	QS68	QSPu16
2	QS69	QSPu16
3	QS70	QSPu16
4	QS71	QSPu16
5	QS72	QSPu16
6	QS73	QSPu15
7	QS73	QSPu16
8	QS73	QSPu17
9	QS74	QSpu18
10	QS75	QSPu13
11	QS76	DP12
12	QS71	QSPu14
13	QS77	QSPu8
14	QS77	QSPu19
15	QS78	QSPu10
16	QS78	QSPu11
17	QS79	QSPu20
18	QS78	QSPu20
19	QS80	QSPu20
20	QS81	QSPu12
21	QS81	QSPu14

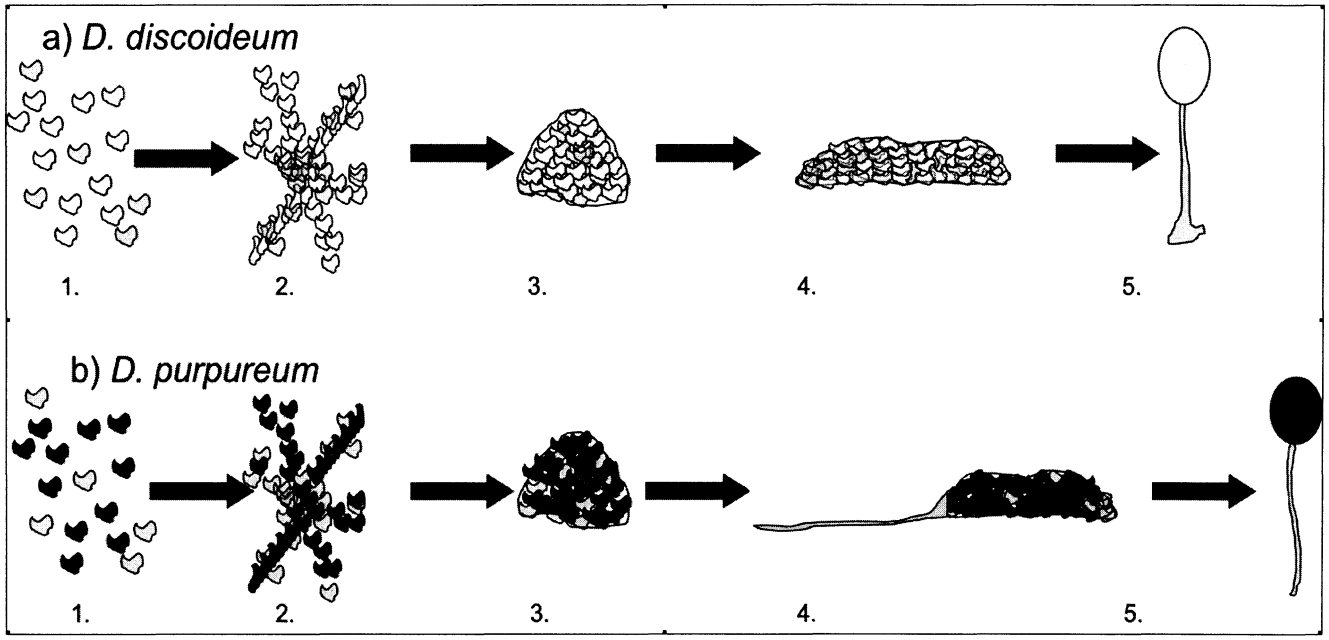

Figure 2-1 The lifecycle of the two *Dictyostelium* species

Both species have a similar developmental lifecycle until the slug stage. The darker cells are the spore cells and the lighter cells are the stalk cells. 1) The cells eat bacteria and reproduce asexually. 2) Upon starvation, the cells begin to aggregate together using cyclic AMP as a chemo-attractant. 3) In late aggregation, the cells form a mound. 4) The cells form a multicellular slug. In *D. discoideum*, the slug migrates to a new location, forms a stalk, and then completes development. In *D. purpureum*, the slug forms a stalk as it migrates to a new location and then completes development. 5) The final fruiting body stage where some of the cells have become sterile to form a stalk and hold up the reproductive spore body.

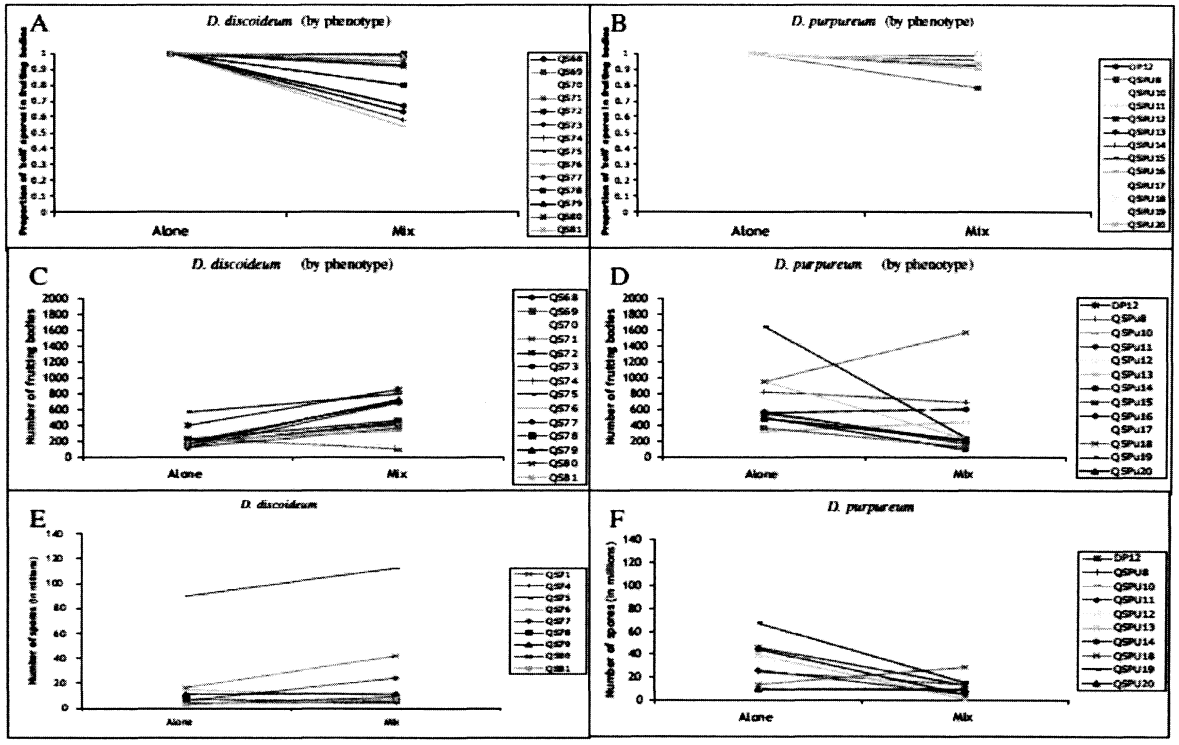

Figure 2-2 The effects of the interaction of *D. discoideum* and *D. purpureum*

The graphs show the results of analyses of *D. discoideum* and *D. purpureum* by clone, both when alone and when mixed with each other. A-B: The composition of the fruiting bodies of each species, alone and mixed, where the higher the percentage means the more clonal the fruiting body. C-D: Fruiting body production of each species, alone and when mixed, after being standardized for the number of cells of each species added to a plate. E-F: The number of spores produced by each species, alone and mixed after being standardized for the number of cells of each species added to a plate.

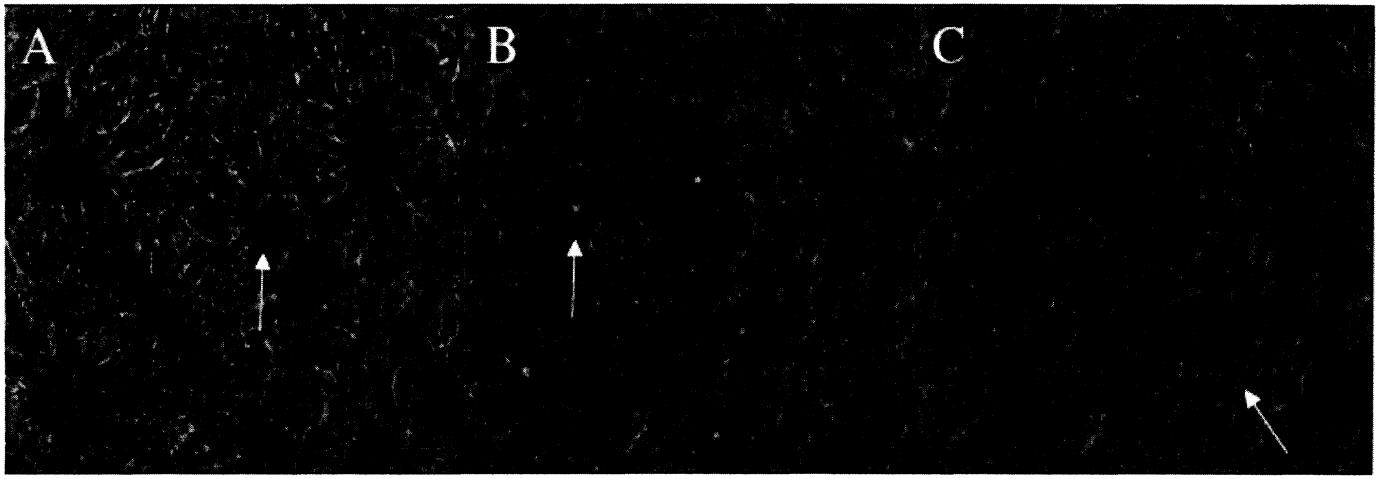

Figure 2-3 Slugs of *D. discoideum* and *D. purpureum* show partial sorting due to developmental differences

Although cells of both species aggregate together (A), the majority of cells of *D. purpureum* forms a slug first and migrates away, taking some *D. discoideum* cells (indicated by the green) with it (B). The majority of the cells of *D. discoideum* stays in the mound to later form a slug and migrate (C).

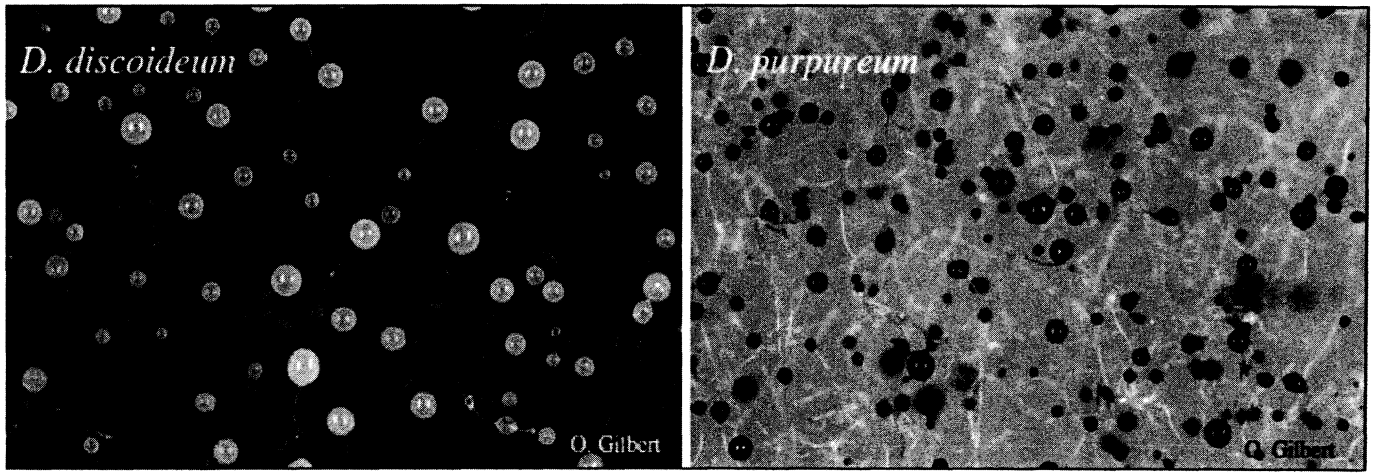

Figure 2-4 Characteristics used to determine *Dictyostelium* phenotype

D. discoideum is characterized by having a basal disc; thick, straight stalk, and white/clear sorus. *D. purpureum* is characterized by its lack of basal disc; thin, wavy stalk; and purple sorus.

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

Chandra N. Jack, Boahemaa Adu-Oppong, Michael Powers, David C. Queller, & Joan E. Strassmann

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3.1 Abstract

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.

3.2 Introduction

Dispersal is a life history trait that affects both ecological and evolutionary behaviors because of its effects on population structure and speciation (JOHNSON & 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 approximately 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, personal communication). 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 comprised 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 are 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.

3.3 Materials and Methods

3.3.1 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.

3.3.2 Cell Preparation

We plated out spores from each clone with 300µl 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 (3.8mM K2HPO4, 16.5mM KH2PO4). We then centrifuged the cells three times at 1300 rpm for three minutes to remove any remaining bacteria and prepared a concentration of 10^8 cells per milliliter in KK2 buffer.

3.3.3 Experimental Setup

We placed buffered non-nutrient agar Petri plates (72.7mM KH_2PO_4, 12.54mM $Na_2H_2PO_4$, 20 g agar) in a laminar flow hood for 30 minutes prior to use to remove all excess moisture from their surface. We then drew a line on the bottom of the plates that was 1cm 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-hour light, and left for one 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.

3.3.4 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 one 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.

3.3.5 Data Collection and analyses

We assessed migration distance by dividing each Petri plate into zones (z). Four of the zones were migration zones 2cm 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 1mL of 20mM 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 2011) 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.

3.4 Results

3.4.1 Migration distances

In the migration treatment, we found that *D. discoideum* slugs moved an average of 2.46 ± 0.19cm while *D. purpureum* slugs moved an average of 1.04 ± 0.06cm. None of the *D. purpureum* slugs in the no migration treatment moved past the start line (Figure 3-1). The *D. discoideum* slugs in the no migration treatment moved slightly, on average 0.12 ± 0.02cm, 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.*

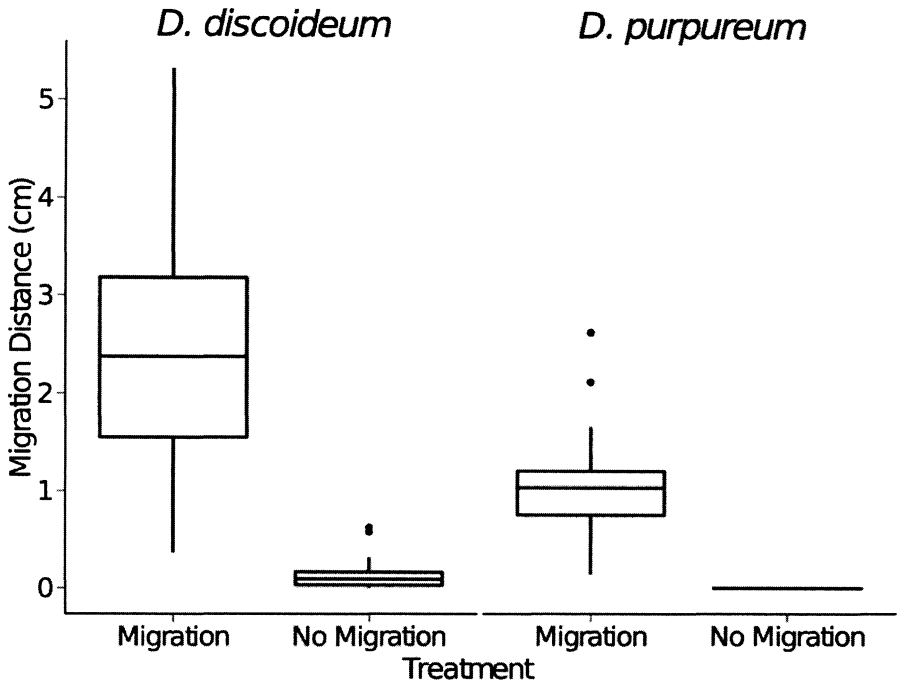

Figure 3-1 The distance each species migrated in the migration treatment compared to the no migration treatment.

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.

purpureum No Migration treatments, which did not differ from each other ($F_{3, 27} = 0.79$, $p = 0.378$).

3.4.2 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 (*D. discoideum* - 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; $F_{1,14} = 5.66$, $N = 15$, $p < 0.05$, Figure 3-2). 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, $F_{1, 28} = 0.019$, $p = 0.892$).

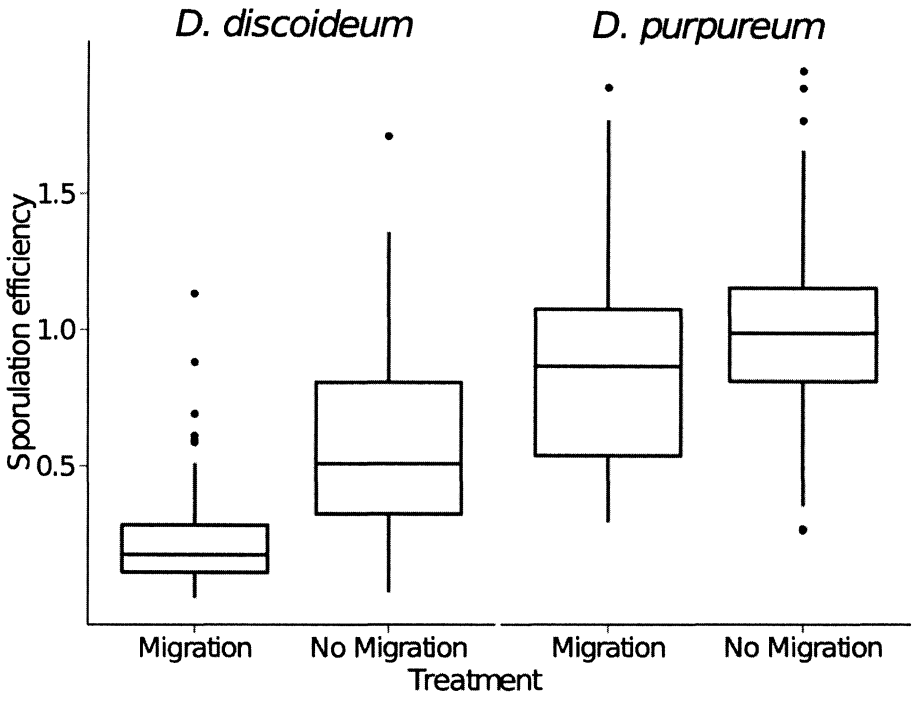

Figure 3-2 The sporulation efficiency of *D. discoideum* and *D. purpureum* in each treatment

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.

3.4.3 Spores per fruiting body

We looked at the average number of spores per fruiting body for each species to determine if the sori of the fruiting bodies from the Migration treatment were smaller than those in the No Migration treatment (Figure 3-3). 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, $p = 0.482$).

3.5 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 to active migration in

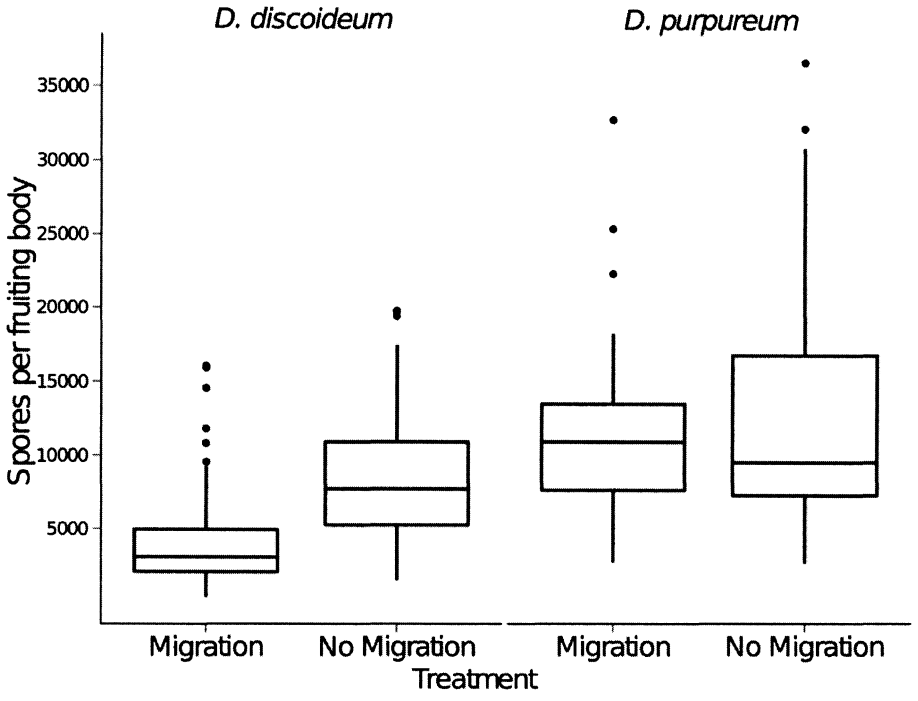

Figure 3-3 The number of spores per fruiting body produced by *D. discoideum* and *D. purpureum* in both treatments

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* suggests that it may be changing its spore:stalk allocation when it migrates.

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 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 bodies 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 *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 morphologic traits onto a molecular phylogenetic tree. *D. discoideum* has a stalk length of 3-7mm and an average spore volume of between 50-80μm^3. Compare this to *D. purpureum*, which has a much taller stalk (> 7mm) and smaller spores (< 50μm^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, personal communication). *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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4 The interaction of chimerism and migration in the *Dictyostelium discoideum*

4.1 Abstract

Hamilton's theory of kin selection predicts that cooperation can evolve through either kin discrimination or limited dispersal. Theoretical studies of limited dispersal suggest that it can either increase local competition or lead to cooperation. There have been very few experimental studies that have tested this idea. The social amoeba *Dictyostelium discoideum* is an ideal organism to study these ideas. There have been many studies that have looked at cooperation in *D. discoideum* but very few that have added dispersal. In our study we look at the effect of migration on fitness and cooperation. We found that chimeric slugs traveled a shorter distance than expected compared to their clonal counterparts. We did not find a difference in cheating when we compared the No migration and Migration treatments using the traditional measure of cheating. When we looked at facultative change, whether a clone increases its spore allocation in the presence of a partner or changed its partner's spore allocation, we found that clones that migrated show a greater change in their behavior when in chimera compared to when they did not migrate in a chimera. This may show that both clones are cheating, but one is more successful. Both clones may be decreasing their cooperation leading to reduced stalk production in a tragedy of the commons.

4.2 Introduction

The evolution of cooperation is key to understanding the transition from single cells to multicellular organisms (Maynard Smith & Szathmáry 1995). This process creates a conundrum for evolutionary biologists because it requires individuals to seemingly decrease their own fitness to provide some benefit to another. These individuals would also be susceptible to cheaters, selfish individuals who benefit from the cooperation of others, but who themselves do not pay any costs. An individual who can increase its reproductive fitness by not cooperating can rapidly spread through a population leading to an overall decrease in altruistic behavior.

Hamilton's theory of kin selection shows that cooperation can evolve if the recipient of the beneficial action has a high enough degree of relatedness to the actor to overcome the cost involved, according to the inequality: $rb-c > 0$ (Hamilton 1964). In order for kin selection to favor altruism, relatedness must be sufficiently high. Hamilton proposed two mechanisms for this. One way is through kin discrimination, where organisms preferentially direct benefits towards kin whom they can recognize on some level (Hamilton 1964). The second mechanism is through limited dispersal (Hamilton 1964). Under this mechanism, altruism is directed indiscriminately towards all nearby individuals because those receiving the benefits are likely to be relatives. The spread of cooperation using kin discrimination is readily understood. Relatives help their kin and both benefit through inclusive fitness. This has been demonstrated in *Dictyostelium*

purpureum, where cooperative fruiting structures are mostly composed of kin, the result of actual sorting (Mehdiabadi et al. 2006).

However, there is some dispute as to how limited dispersal alone can lead to the evolution of cooperation. Theoretical models suggest that it can be an important method for the evolution of cooperation but may also lead to the breakdown of cooperation because of increased local competition between kin (Taylor 1992; Queller 1994; Bourke & Franks 1995; West et al. 2006). According to Kümmerli et al (2009), there have been very few empirical reports on how population structure and cooperation interact. Their recent work found that limited dispersal leads to an increase in local competition and disfavored cooperation. They also found that by dispersing in groups, cooperation would be favored because it would maintain high relatedness while reducing local competition (Kümmerli et al. 2009).

The social amoeba, *Dictyostelium discoideum,* is an excellent organism for studying the effects of cooperation and competition in local patches. It is a unicellular organism found in the soil of deciduous forests (Bonner 1967; Raper 1984). They prey on bacteria and reproduce mostly asexually, though they do have a sexual stage. When conditions deteriorate and they begin to starve, they send out a signal of cAMP, which causes all nearby cells to aggregate into a multicellular structure. After aggregation, the resulting slug will migrate to a new location where approximately 20% of the cells will die to form a sterile stalk to hold the remaining cells as reproductive spores. If all of the neighboring cells are related, then cooperating to form the fruiting body is expected because cells that

give up their reproductive rights will increase their inclusive fitness through the reproduction of clones. However, if there are multiple clones present, then conflict between reproductive fates can lead to the breakdown of cooperation.

We know that multiple clones and multiple species are found in the same soil samples (Fortunato et al. 2003; Jack et al. 2008) and that they form chimeras both in the lab and in nature (Strassmann et al. 2000; Gilbert et al. 2007). The resulting struggle between genotypes can lead to cheating, where one genotype gains an unfavorable advantage over the other by producing more than its fair share of spores (Fortunato et al. 2003; Buttery et al. 2009). A recent experimental study forced global dispersal upon a population composed of 12 distinct clones of *D. discoideum* and found that low relatedness allowed cheaters to persist in the population (Saxer et al. 2010).

Some dispersion beyond that expected of an amoeba is a normal part of the *D. discoiduem* lifecycle. As part of this lifecycle, the multicellular slug will migrate to a more favorable location to form a fruiting body. This local dispersal is very costly because the slug loses a considerable number of cells as it travels (Jack et al. 2011). However, this dispersal is necessary if the slug is to find a location more suitable for later reproduction because slugs can travel greater distances than single cells(Kuzdzal-Fick et al. 2007). Additionally, traveling within a slug is also a security measure against predation (Kessin et al. 1996). Foster et al (2002) found that chimeric slugs traveled less far when compared to clonal slugs of the same size but that chimeric slugs would travel further than a smaller clonal slug. Despite these studies, we do not know the effect of

simultaneous dispersal and cooperation on population structure. In this study, we examine the interaction of different clones of *D. discoideum* before and after migration. We predict that migration will lead to an increase in cheating because the inherent conflict between clones will be protracted giving the dominant clone more time to cheat.

4.3 Methods

4.3.1 Clone growth and development

To study social interactions and migration in *D. discoideum,* we used 5 clones that have been much studied previously, NC28.1, NC34.1, NC63.2, NC85.2, and NC105.1. John Eisenberg collected these clones from Little Butt's Gap, west of Mount Mitchell, North Carolina (Francis & Eisenberg 1993). For each replicate, we started by growing spores stored at -80°C on SM plates (Sussman 1966) with *Klebsiella aerogenes* (*Ka*) for food.

4.3.2 Transformation of wild clones with red fluorescent protein (*rfp)*

We needed a visual label to observe interactions among clones through chimera formation and migration, so we labeled them with a gene for red fluorescent protein, (*rfp)* on an actin-15 promoter. To do this, we collected actively growing and dividing cells from the edges of plaques grown in association with *Ka* on SM agar plates and transferred them to HL5 axenic medium (5g proteose peptone, 5g thiotone E peptone, 10g glucose, 5g yeast extract, 0.35 g $Na_2HPO_4 \cdot 7H_2O$, 0.35 g KH_2PO_4 per liter (Watts & Ashworth 1970)) + 1% PVS (100,000 units of penicillin, 100mg streptomycin sulphate, 200ug folate, 600ug vitamin B12 per

liter). We removed the spent medium daily and replaced it with fresh medium until no bacteria were visible in the culture dishes. We then harvested the cells and washed them twice by centrifugation and resuspended them in cold standard KK2 buffer (3.8mM K2HPO4, 16.5mM KH2PO4). We transformed the clones with the actin15-*rfp* plasmid by electroporation (Pang et al. 1999). We then transferred the cells to culture dishes containing HL5+1%PVS and left them for 24 hours before replacing with fresh medium containing 20μg/ml G418. After five days of selection, we transferred the amoebae to SM agar with *Ka*. We transferred red plaques to G418-SM agar plates (30μg/ml G418) in the presence of G418 resistant *Ka* for a final round of selection.

4.3.3 Determination that RFP-labeled clones behave similarly to wild type

After selection, we tested each transformant for neutrality against its ancestor by making 50:50 mixes of cells and allowing them to develop on a 1.5% water agar plate without bacteria. We examined spores from individual sori using fluorescent microscopy to ascertain that the two clones were not sorting out from each other. We collected spores from the whole plate to ensure that one clone was not producing a disproportionate number of spores. We also made mixes of cells at a ratio of 5:95 *rfp*:ancestor and looked for sorting during the slug stage. We chose the most stable isolates that displayed no sorting or cheating behavior (NC28.1R2, NC34.1R3, NC63.2R2, NC85.2R2, and NC105.1R2).

4.3.4 Cell Preparation for experiments

We plated out spores from each clone with 400μl of a stationary phase culture of *Ka* on SM agar plates. We harvested the cells while they were in log growth, but

before multi-cellular development occurred, and suspended them in cold standard KK2 buffer. We then centrifuged the cells four times at 1300 rpm for three minutes to remove any remaining bacteria and prepared a concentration of 10^8 cells per milliliter in KK2 buffer. To measure the effect of social competition on migration, we made 10 chimeric mixes at 50:50 (each *rfp* clone against all other ancestor clones). We also had clonal treaments of ancestor strains and the *rfp*-transformants

4.3.5 Social Migration Assay

We placed 1.5% water agar Petri plates (size: 150 x 15mm) in a laminar flow hood for 45 minutes, prior to use, to remove all excess moisture from their surface. We then drew a line on the underside of the plates that was 2cm from the edge of the plate. For each treatment, we carefully pipetted 1×10^7 cells on the agar behind the line on each of two Petri plates. We then left the plates in the laminar flow hood for one hour so that any excess buffer dried on the plates leaving a film of cells, after which we divided the plates into two different treatments: migration and non-migration. For the migration condition, cells were exposed to a unidirectional light source. The plates were stacked with paper circles between them and aligned so that all of the cells were all aligned at one end. The plates were then wrapped in aluminum foil, leaving a small opening at the end of the plates opposite to the cells. This provided a directional light gradient for the aggregates to phototax toward. The non-migration treatment consisted of subjecting cells to overhead light, causing them to produce fruiting bodies without migration. Although previous experiments show that the amount

of light did not affect spore production (Jack et al. 2011), we wanted to standardize the amount of light received in both treatments. The remaining plate of each clone or mix was wrapped individually in foil with a 0.5cm wide slit cut over the cells and then placed in the incubator so that they received light from above. Each mix and clone was replicated five times.

4.3.6 Assessment of the distance slugs traveled

We assessed migration distance on both the clonal and mix plates by dividing each Petri plate into seven zones (z). Six of the zones were migration zones 2cm in width starting from the initial cell line. Zone 0 was counted as 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: $\sum_{z=0}^{6} F_z 2z \Big/ \sum_{z=0}^{6} F_z$

4.3.7 Initial and final clone representation in chimeric fruiting bodies

We determined the initial (cells) and final (spores) proportion of each clone in each mix by using a Ziess Axioplan microscope with DIC and CY3 filters and MetaMorph™ Imaging Software. We also measured the percentage of fluorescent cells and spores for the clonal RFP clones so that we could correct for loss of fluorescence in the chimeric mixes.

4.3.8 Calculating spore allocation in clonal fruiting bodies

We calculated the spore allocation (a_i) of our clones 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 represented by the equation $a_i = \frac{\text{Number of spores } (i)}{\text{Number of cells}(i)}$. All fruiting bodies on each agar plate were collected in one Eppendorf tube containing KK2 buffer. We used a hemacytometer to count the number of spores produced.

4.3.9 Calculating spore allocation chimeric fruiting bodies

The chimeric spore allocation of a clone *i* in a mix with clone *j* (a_{ij}) was calculated using the equation $a_{ij} = \frac{\text{Number of spores } (ij) * \text{ Proportion of spores } (i)}{\text{Number of cells}(ij) * \text{Proportion of cells } (i)}$

This was calculated for each clone in a mix for both treatments. This allowed us to calculate the deviation of a clone in chimera from its clonal spore allocation (d_{ij}):

$$d_{ij} = \frac{\left(a_{ij} - a_{ii}\right)}{a_{ii}}$$

4.3.10 Social success of clones in chimera

Social success is defined as how well a clone does when in chimera and is used to determine if a clone produces more spores in chimera than it would clonally. We calculated several different measures of social success for both the migration and non-migration treatments:

4.3.10.1 *Cell-to-spore change*

This answers the most basic definition of cheating: does the proportion of clones in a mix change from the cell to the spore stage? This is calculated using $\Delta p = p_{(t+1)} - p_t$ where p_t is proportion of cells of the clone in the mix and $p_{(t+1)}$ is the proportion of spores of the clone in the mix. If Δp is greater than 0, then the clone is considered a cheater under this broad definition of cheating. If Δp is less than 0, then the clone is considered a loser. We chose one clone from each mix and looked at its change in frequency. If that clone had a value over 0, then its partner must have a value below 0. To ensure that we were not only analyzing the affect of *rfp* labeling, we randomly chose one clone from each pair for analysis. We averaged the clones to get an overall idea of cheating.

4.3.10.2 *Mechanism behind social success*

Self-promotion- a clone is a good self-promoter in chimeras if it increases its spore allocation in chimeras, averaged across all partners. It is calculated as:

$$\bar{d}_i = \frac{\sum_{i \neq j} d_{ij}}{n-1}$$

Ability to coerce- this is a clone's ability to decrease its partner strain's spore allocation and is calculated similarly:

$$\bar{c}_i = -\frac{\sum_{i \neq j} d_{ji}}{n-1}$$

4.3.10.3 *Facultative change and migration*

We measured the interaction of migration and facultative change by mapping the clones on a mean d_i-mean c_i coordinate plane for each treatment similar to

Buttery *et al* (2009). We then calculated each clones Euclidean distance from the origin and compared the distances between the No Migration and Migration treatments. The higher the value, the more a clone is changing its behavior. This is a good measure of social success because it allows us to consider not only the end result of an interaction between clones, but the way in which the clones interact (i.e. are the clones changing their behavior in response to their partners (self-promotion) or, are the clones having their behavior changed by their partners (coercion)?).

4.3.11 R- statistical software program

All data were analyzed using R version 2.12.2 (R Development Core Team 2011) and the nlme package was used to create the models that we analyzed (Pinheiro et al. 2009). All figures were created using ggplot2 (Wickham 2009).

4.4 Results

4.4.1 Clonal Measurements

4.4.1.1 Migration distances

We did not find a significant difference between the RFP and Ancestor clones for distance migrated, which allowed us to combine the data from both to analyze the migration distances (one-way Nested ANOVA; $F_{1, 4} = 1.54$, $p = 0.28$). We did find that the clones migrated significantly different distances (one-way nested ANOVA; $F_{4, 34} = 7.55$, $p < 0.001$, Figure 4-1).

4.4.1.2 Spore allocation

As with the clonal migration distances, we also did not find a significant difference between the clonal spore allocation between the RFP and Ancestor clones (one-way Nested ANOVA; $F_{1, 47} = 3.16$, $p = 0.082$). We found, in accordance with Jack et al (2011), that clones that migrated had a lower spore allocation compared to clones that did not migrate (one-way nested ANOVA; No migration: $\mu = 0.324 \pm 0.021$ spores per cell; Migration: $\mu = 0.102 \pm 0.009$ spores per cell; $F_{1, 48} = 160.05$, $p < 0.001$, Figure 4-2). We did not find a significant difference between the different clones (one-way nested ANOVA; $F_{8, 83} = 0.56$, $p = 0.81$); although the spore allocations did match the linear hierarchy found in Buttery et al (2009). However, we are only interested in the broad differences in behavior with migration, not specific differences between the different clones.

4.4.2 Chimeric Measurements

4.4.2.1 Migration distances

We found that, on average, chimeric slugs traveled shorter distances than we expected based on clone composition ($\mu_{Obs} = 5.50 \pm 0.24$ cm, $\mu_{Exp} = 6.19 \pm 0.20$ cm; one-way Nested ANOVA: $F_{1, 49} = 17.86$, $p < 0.001$; Figure 4-3).

4.4.2.2 Spore allocation

We found that overall, chimeras showed a decrease in spore allocation when the Migration treatment was compared to the No Migration treatment similar to the single clones but we also saw a slight increase in their spore allocations in both the No Migration and Migration treatments compared to the single clones that

was nearly significant (two-way ANOVA; Clone vs. Chimera: $F_{1, 73} = 2.76$, $p = 0.066$; Treatment: $F_{1, 73} = 133.85$, $p < 0.001$; Figure 4-2). When we calculated the decrease in spore allocation per centimeter traveled, we did not find a significant difference between the clonal group ($\mu = -0.040 \pm 0.004$ spores per cell/cm) and the chimera group ($\mu = -0.046 \pm 0.005$ spores per cell/cm; one-way ANOVA; $F_{1,93} = 0.83$, $p = 0.365$; Figure 4-4).

4.4.3 Social success and cheating with and without migration

4.4.3.1 Measuring cheating using cell-to-spore change

When all 10 mixes are compared together for both treatments in a nested ANOVA, we find that there is a significant interaction between the change in frequency of clone *i* between development stages and the clone in the mix, although each on its own was not significant (Cell stage vs. Spore stage: $F_{1, 144} = 1.4077$, $p = 0.24$; Clone: $F_{4, 16} = 2.6064$, $p = 0.075$; Development stage x Clone: $F_{4, 144} = 3.5252$, $p < 0.01$). This indicates that some clones are cheaters and some are losers. We did not find a significant difference between the No Migration and Migration treatments ($F_{1, 144} = 0.4019$, $p = 0.53$), Figure 4-5.

4.4.3.2 Facultative change

We found that facultative change, a combination of a clone's ability to promote itself and its ability to coerce other clones was significantly different between treatments (one-way nested ANOVA; No Migration vs. Migration: $F_{1, 24} = 7.62$, $p < 0.05$; Figure 4-6).

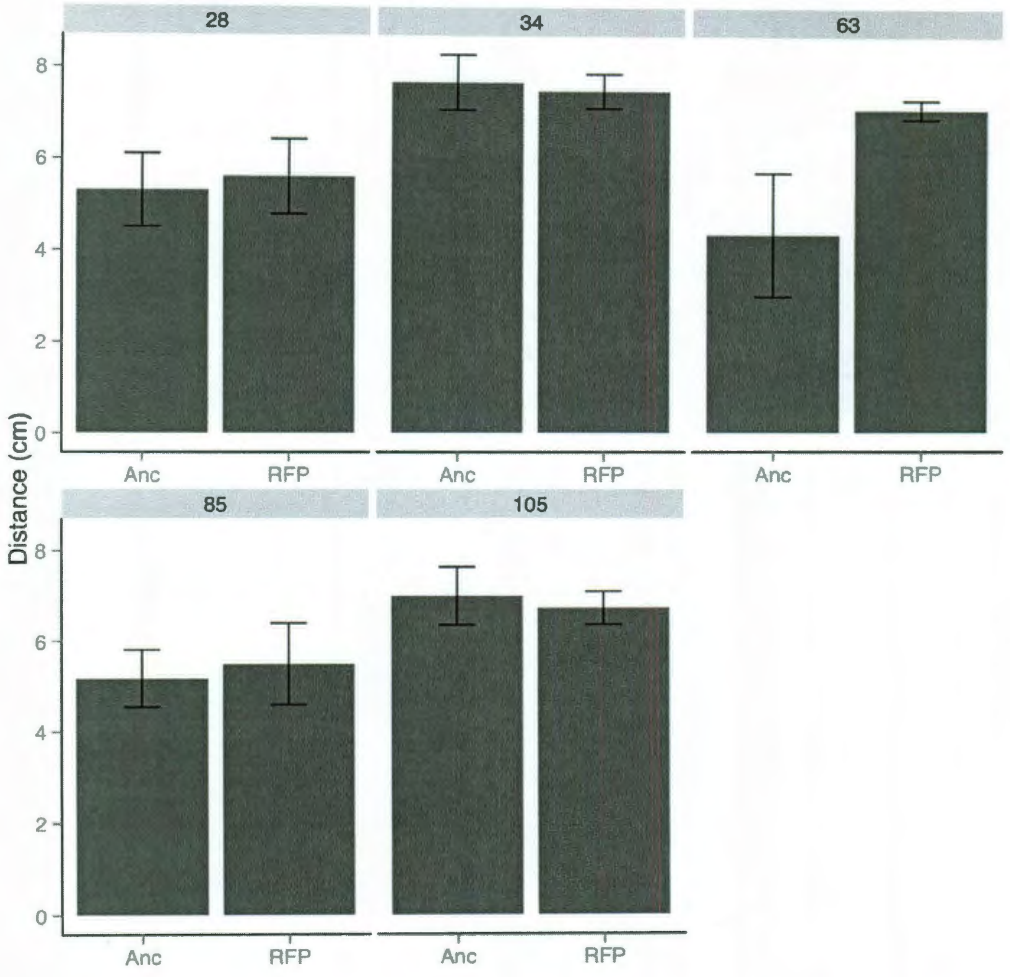

Figure 4-1 The average distance the clones migrated were significantly different

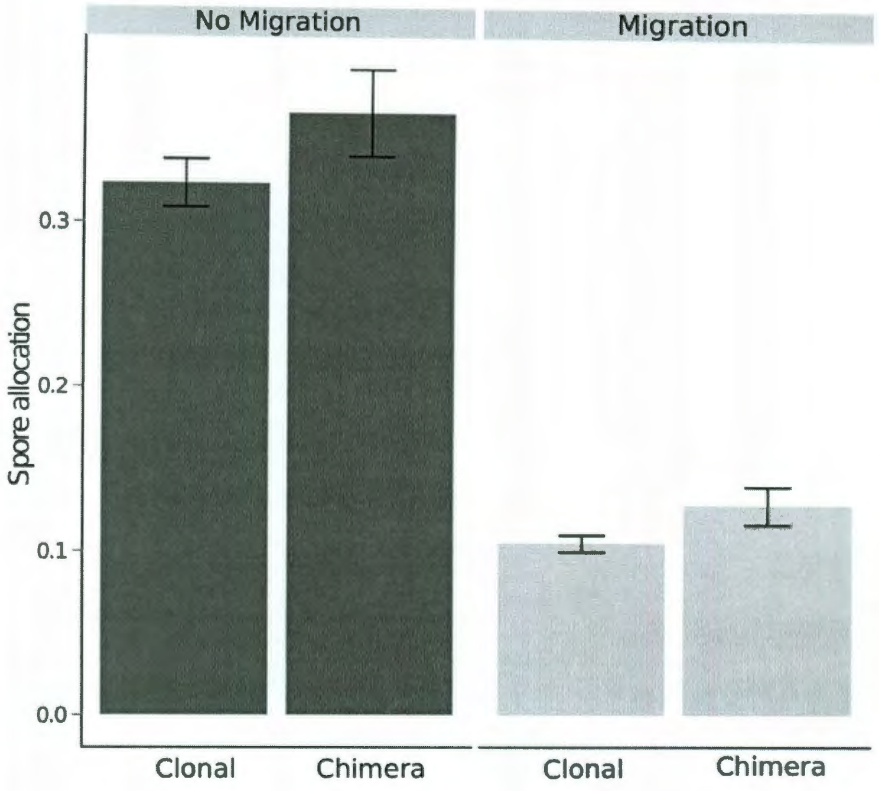

Figure 4-2 Comparison of migration and no migration treatments for the clonal slugs versus the chimeric slugs.

Clones that migrated had a lower spore allocation compared to clones that did not migrate (No migration: $\mu = 0.324 \pm 0.021$ spores per cell; Migration: $\mu = 0.102 \pm 0.009$ spores per cell; $F_{1, 83} = 118.71$, $p < 0.001$, Fig 2). Chimeras increased their spore allocation in both the No Migration and Migration treatments compared to the single clones, but they showed a similar decrease in spore allocation when the Migration treatment was compared to the No Migration treatment (Clone vs. Chimera: $F_{1, 73} = 2.76$, $p < 0.066$; Treatment: $F_{1, 73} = 133.85$, $p < 0.001$

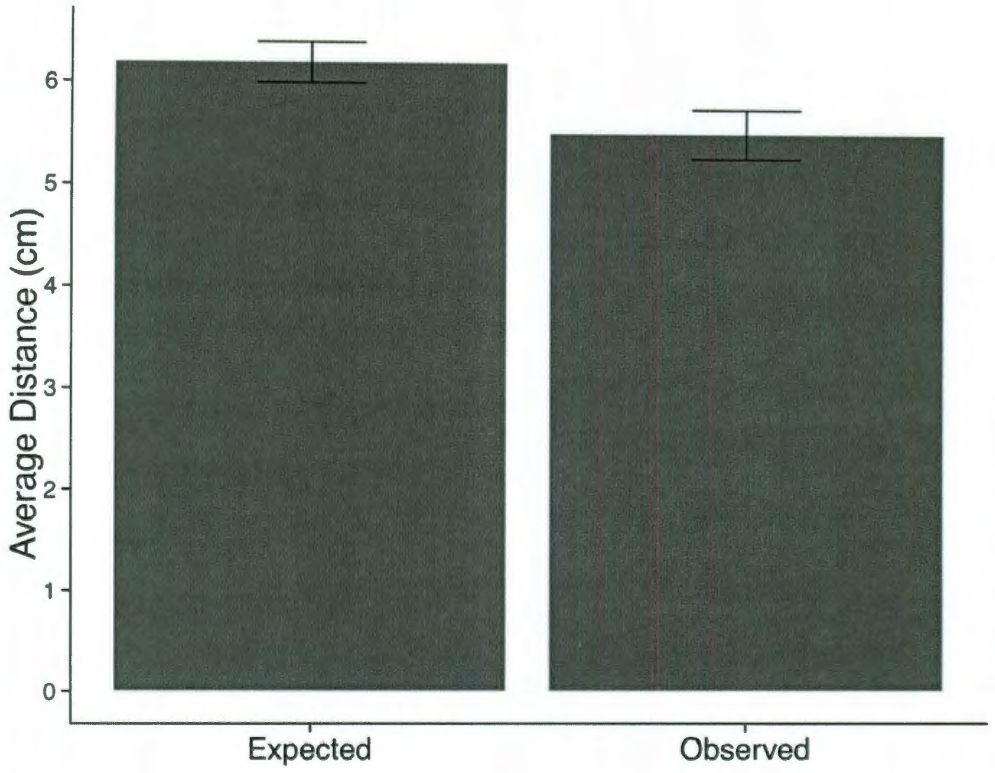

Figure 4-3 Chimeric slugs traveled a shorter distance than expected based on clone composition

(μ_{Obs} = 5.50 ± 0.24 cm, μ_{Exp} = 6.19 ± 0.20 cm; one-way Nested ANOVA: $F_{1, 49}$ = 17.86, $p < 0.001$.

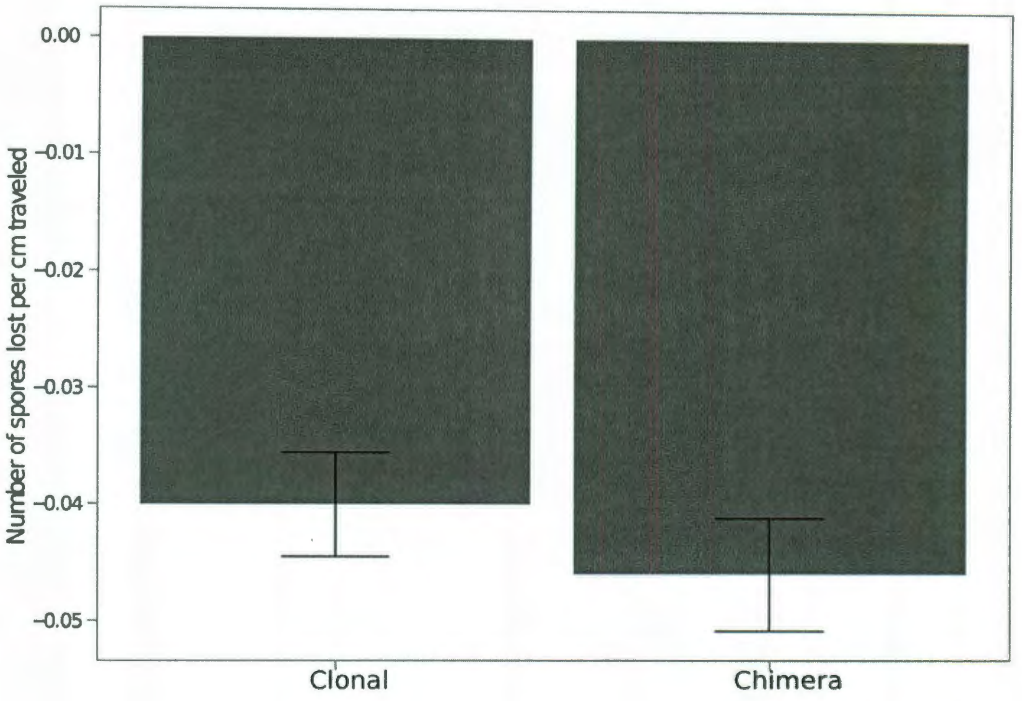

Figure 4-4 Clonal and chimeric slugs lost approximately the same number of spores per cell after standardizing for distance traveled.

When we calculated the decrease in spore allocation per centimeter traveled, we did not find a significant difference between the clonal group (μ = -0.040 ± 0.004 spores per cell/cm) and the chimera group (μ = -0.046 ± 0.005 spores per cell/cm; one-way ANOVA; $F_{1, 93} = 0.83$, $p = 0.365$).

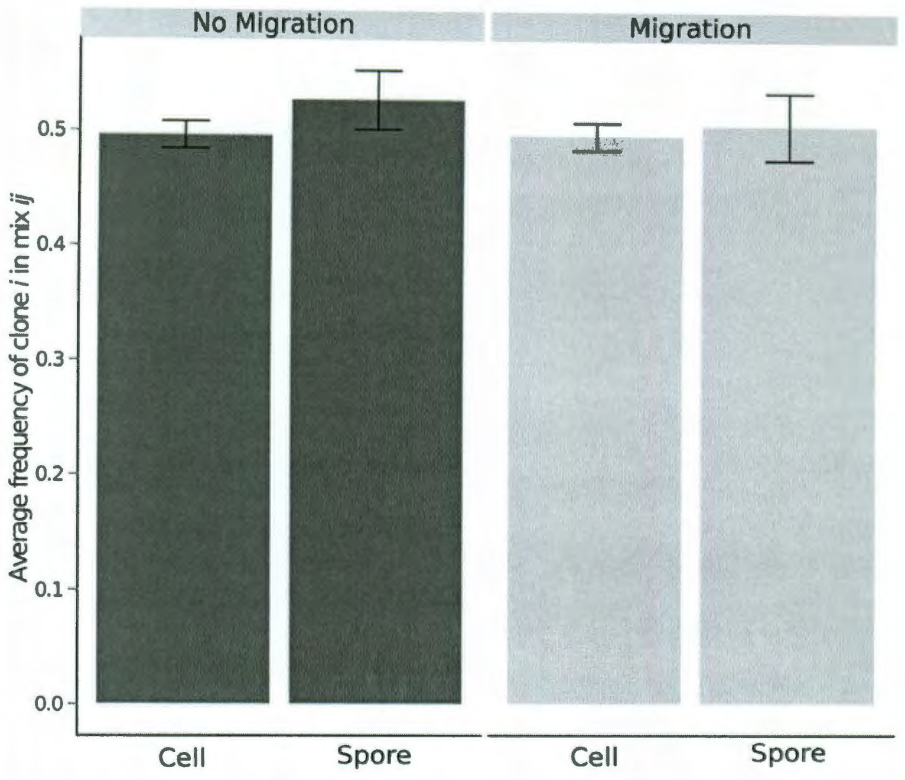

Figure 4-5 Does the proportion of clones in a mix change from the cell to the spore stage? Between treatments?

If there is no difference between the proportions, then there should not be a difference between the frequency of clone *i* in the cell stage and its frequency in the spore stage. We find that there is a significant interaction between the change in frequency of clone *i* between development stages and the clone in the mix (Development stage x Clone: $F_{4, 144} = 3.5252$, $p < 0.01$). This indicates that some clones are cheaters and some are losers. We did not find a significant difference between the No Migration and Migration treatments ($F_{1, 144} = 0.4019$, $p = 0.53$)

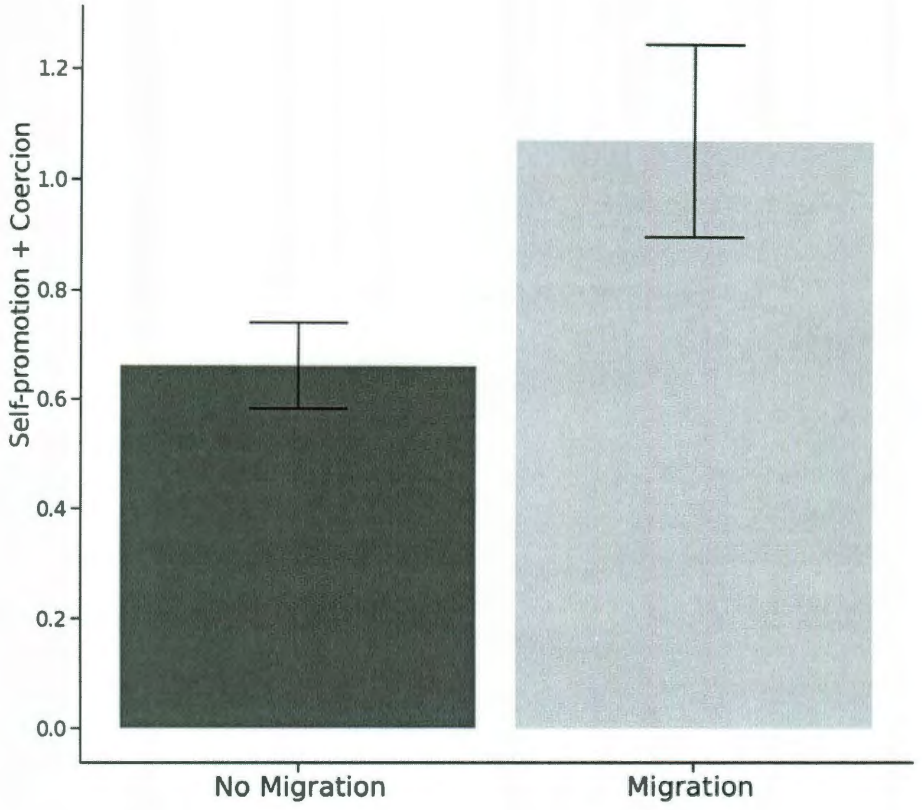

Figure 4-6 Clones in the migration treatment showed more facultative change than in the No migration treatment

We found that social success, a combination of a clone's ability to promote itself and its ability to coerce other clones was significantly different between treatments (one-way nested ANOVA; Treatment: $F_{1, 24}= 7.62$, $p < 0.05$)

4.5 Discussion

4.5.1 Clonal Migration Behavior

In a previous study on slug migration, slugs of *D. discoideum* only averaged a migration distance of 2.5cm (Jack et al. 2011). In order to maximize the potential affect of migration on chimerism we switched to a larger Petri plate and decreased the percentage of solutes in the agar (Bonner & Shaw 1957). This resulted in longer migration distances and allowed us to find discernible differences between clone migration distances. We also confirmed the result from our earlier paper that migration leads to a decrease in spore allocation, as cells that are sloughed off as the slug migrates must be replaced.

4.5.2 Chimeric Migration behavior

We used the same set of clones from two previous studies that found a linear hierarchy among the clones when they were tested pair-wise against each other (Fortunato et al. 2003; Buttery et al. 2009). For this reason and because we are more interested in the global affect of migration, we did not look for hierarchies in this experiment. Although our results were not as extreme as Foster et al (2002), we did find that chimeric slugs traveled a shorter distance than expected based on the clones that composed them. There are a number of possibilities for why

this is the case. It is possible that the chimeric slugs can only travel as far as the clone that migrates the shortest distance. It could also be that there is some recognition mechanism that is causing conflict within the slug. One additional possibility is that the slug is not traveling as far because there are fewer prestalk cells. Buttery et al (2009) found that clones increased their spore allocation in chimera compared to when they were clonal, and we found similar results in both our migration and no migration treatments. The prestalk region of the slug is "motor" of the slug and is responsible for forward movement. If this region is small, then it is possible a slug would not travel as far as a slug with a larger prestalk region.

4.5.3 Competitive interactions and migration

We examined cheating using two different methods. The first method is the traditional method to compare the initial frequency of cells of clone in a mix to its final frequency of spores in a mix after the cells have developed. Using this method, we found a significant interaction effect between the average frequency of the clones in the initial cell stage and the final spore population. This indicates that some of our clones are cheaters and some are losers. However, we did not find a significant difference in the change of clone frequency between the migration and no migration treatments as we expected.

Our first method of cheating showed that clones cheat but it does not show how they do so. We used the method of Buttery et al (2009) to determine the method of facultative cheating the clones were using. We looked to see if the clones were using self-promotion (changing their own spore allocation in response to a partner) or were using coercion (forcing their partner to change its spore allocation). We found that most clones are increasing their own spore allocation rather than changing their partner's behavior. Using this method, we found some support for our prediction of an increase in cheating with migration. However, it was not in the method that we predicted, which was the dominant clone increasing its competitive advantage. Instead, we found that both clones are cheating, but one is more successful. An analogy can be made using five runners in a race. A racer can cheat by taking steroids to improve his own performance (self-promotion) or by hindering his competitors (coercion). Our predicted hypothesis would be that the racer, who would win at one distance by cheating, would have an even greater advantage at a longer distance. Using this same analogy, our results suggest another scenario. We would again have our dominant runner, but we would also have another cheater. Normally this runner would come in last place, but the increased distance allows him to come in third. The runner has not won the race, but has done better than expected.

A recent paper found that wild isolates of *D. discoideum* vary in their production and sensitivity to diffusible stalk-inducing factors (StIFs)(Parkinson et al. 2011). The prespore cells produce this diffusible signal molecule known as DIF-1 (Differentiation inducing factor),which induces differentiation of cells into prestalk cells (Kay & Thompson 2001). They were able to predict the outcome of competitive interactions between clones when they were not migrating using differences in the production and response to these StIFs. They also found that there was an inverse relationship between StIF production and response within clones. They suggest that this is a tradeoff between passive dispersal (formation of a longer stalk) and fecundity (greater spore allocation); and that facultative outcomes are not the result of facultative changes.

One point that is not addressed is why we generally see higher spore allocations in chimera, regardless of the outcome of the interaction. Also, why do we see proportionally higher spore allocation in chimeric slugs that have migrated compared to those that have not. A possible explanation is that clones are defecting from participating in production of the stalk, a problem known as tragedy of the commons (West et al. 2006). This is where the group would benefit from everyone cooperating, but where individuals can gain by pursuing their own self-interests, leading to

a decrease in cooperation and unwillingness to enter the prestalk region. This could manifest itself in the shorter migration distances that we found and also the higher spore allocations. Clones are cooperating less and are producing more spores at the expense of stalk production. Another possibility that may explain higher spore allocation is that migrating, which we know is energetically taxing, causes the clones to produce less DIF-1, which may result in higher spore allocations after migration, but would not explain the spore allocation increases without migration. It is a question of whether it is a variable trait or simply a maladaptive response to increased slug migration.

4.6 Conclusion

We have shown that chimerism and migration interact to produce fruiting bodies that have a proportionally higher spore allocation compared to clonal fruiting bodies after migration. We have also shown that we see a greater change in clonal behavior after migration, which suggests that migrating leads to more cheating by clones, and less cooperation. The next step is to parse out whether the results that we see are an indication of clones defecting in a tragedy of the commons, or if the results may be somewhat more mechanistic due to StIF production and response. Once we have these answers, we will be able to better explain the affects of

cooperation on group dispersal and whether it can be used as a mechanism to reduce local competition

4.7 References

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