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Exploiting New Terrain: an Advantage to Sociality in the Slime Mold *Dictyostelium discoideum*

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ABSTRACT

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in the Slime Mold Dictyostelium discoideum

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Identifying benefits to cooperation is important in studying social evolution. When the social amoebae of Dictyostelium discoideum starve, they aggregate to form multicellular slugs that migrate towards the soil surface to form fruiting bodies. Multicellularity in D. discoideum is thought to help provide protection from predators and aid in the dispersal of reproductive spores. Here we show an additional benefit of local dispersal. Utilizing D. discoideum’s phototactic behavior, we induced slug migration over bacteria and show that this passage results in the removal of bacteria. Time-lapse video revealed that slugs do not dissociate on contact with bacteria, rather cells sloughed from the migrating slugs consume the prey and reproduce. Using an aggregation mutant, we show that the cells slugs shed are able to reach and colonize food sources solitary amoebae cannot. We propose that the exploitation of local food patches is an important selective benefit favoring multicellularity in D. discoideum.
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INTRODUCTION

The evolution of cooperation among genes, cells and organisms is a major theme in the history of life (Maynard Smith and Szathmáry, 1995). Understanding the evolution of cooperation requires the identification of processes such as kinship that suppress within-group conflict (Hamilton, 1964) and the ensuing conflict reduction from mechanisms like enforcement (Frank, 1995; 2003) and conventions (Seppä et al., 2002). However, it is equally important to identify the factors that select for cooperation over a solitary existence. Several general advantages to sociality have been proposed including defense, increased foraging efficiency, and cooperative brood care (Alexander, 1974; Wilson, 1975; Krebs and Davies, 1993). For example, grouping benefits social insects like the paper wasp Polistes by increasing productivity and providing survival benefits, such as superior recovery from nest destruction in larger colonies (Strassmann and Queller, 1989). Meerkat sentinels keep post looking for predators, which protects the group and allows increased foraging (Clutton-Brock et al., 2002). Advantages to grouping in social insects have been categorized into fortress defense and life insurance (Queller and Strassmann, 1998). Fortress defenders like termites, naked mole rats, social shrimp, gall dwelling thrips, and aphids defend a protected home that typically contains their food. By contrast, life insurers forage in high-predation environments and have young that will die if the food providers die. Grouping means another can take over rearing the young if one dies (Queller, 1996).

It is increasingly appreciated that micro-organisms have social systems amenable to analysis in ways initially applied to multicellular animals (Crespi, 2001; Travisano and
Velicer, 2004). Though attributes favoring sociality like relatedness are likely to be the same as in animals, there may be different kinds of costs and benefits associated with sociality. Here we explore this in a social protist, *Dictyostelium discoideum*. Two advantages to social behavior in the slime mold, or social amoeba, *Dictyostelium discoideum* have been suggested: protection from predators (Kessin et al., 1996) and long-range spore dispersal (Bonner, 1982; Huss, 1989; Kessin, 2001). When their bacterial prey become scarce, the normally unicellular amoebae aggregate and form a differentiated multicellular organism containing thousands of cells (Bonner, 2001). The amoebae first form a slug that moves towards heat and light, and away from ammonia (Bonner et al., 1950; Bonner, 1967; Foster et al., 2002). The slime sheath coating of the slug prevents nematode predators from eating the amoebae (Kessin et al., 1996). Furthermore, the slug takes the cells to the soil surface where around a fifth terminally differentiate into dead stalk cells that hold aloft a sorus of spores (Loomis, 1982). This is thought to enable the spores' dispersal from the soil surface by water and passing invertebrates (Bonner, 1982; Huss, 1989; Kessin, 2001; Queller, et al. 2003).

We predict that there might be an additional advantage of sociality in *D. discoideum*. Specifically, the migration of the slug may enable the cells in the slug to reach and exploit patchy local food sources. *D. discoideum* slugs are surrounded by a thin extracellular matrix of protein and cellulose which, as the slug moves forward, is left behind as a collapsed tube containing some live cells (Smith and Williams, 1979; Morrisey, 1982; Sternfeld, 1992; Wilkins and Williams, 1995; Alexander, 1997; Kessin, 2001). Our prediction is that these cells might be able to exploit bacterial food sources in the path of the slug migration. Consistent with this hypothesis, artificially disaggregated
cells in *D. discoideum* are able to dedifferentiate from prestalk or prespore cells and become amoebae (Katoh et al., 2004). Alternatively, the occurrence of dedifferentiation in *D. discoideum* could mean that slugs are able to break up on contact with a new food source. Previous work suggested that slug migration across bacteria does not lead to exploitation of the bacteria by amoebae (Raper, 1940), but laboratory observations led us to reexamine this finding.

We investigated the following questions: Are slugs able to exploit new bacterial food sources? If they are, is this due to cell sloughing or slugs disaggregating on contact with bacteria? Can slugs and the cells they slough off reach places that solitary amoebae cannot?

**METHODS**

*Experiment 1: do cells from slugs eat bacteria?*

We tested whether slugs moving over bacteria resulted in the bacteria being eaten. In order to direct slugs towards and across a strip of bacterial slurry, we utilized the phototactic behavior of *D. discoideum* slugs (Bonner et al., 1950; Bonner, 1967; Foster et al., 2002). We obtained this slurry by growing *Klebsiella aerogenes* on standard medium plates (10 g glucose, 10 g peptone, 10 g yeast extract, 1 g MgSO₄•7H₂O, 1.9 g KH₂PO₄, 1 g K₂HPO₄, 20 g agar, 1000 mL H₂O) for 4-5 days, scraping the cells into tubes with KK2 buffer (2.25g anhydrous KH₂PO₄, 0.67g anhydrous K₂HPO₄, 1L deionized water, pH 6.1), centrifuging at 10,000 rpm for 10 minutes, and recovering the pellet. We applied a 1.5 cm wide by 6 cm long strip of the bacteria slurry to one edge of a nutrient free
'starving’ plate (0.36 g K₂HPO₄, 2 g KH₂PO₄, 20 g agar, 980 mL H₂O), and used a toothpick to mix in spores from 3-7 fruiting bodies (Figure 1a). We used twelve genetically distinct clones of *D. discoideum* collected from Virginia, USA (56A1, 56A2, 56C1, 77B1, 301B1, 301B2, 319B3, 327A1, 330D2, 342B2, 336B1, 337C1, Fortunato et al., 2003). We added one clone per plate and made three replicate plates per clone giving a total of 36 experimental plates. Additionally, we prepared three control plates with no *D. discoideum*, just bacteria.

We stacked the plates with disks of black opaque card between each, and surrounded them with a black opaque cylinder that had a 1 cm wide slit opposite to the bacteria. This slit provided a unidirectional light source towards which the slugs migrated (Bonner et al., 1950; Bonner, 1967; Foster et al., 2002). Amoebae hatched from the spores, consumed the bacteria in the slurry at one end of the plate, starved, aggregated into slugs and began to migrate across the plate towards the light. When the slugs were half way across the plate, we laid down a second strip of *Klebsiella aerogenes* slurry 1.5 cm wide by 9 cm long in front of them. We restacked the plates in the same orientation with respect to the light, and removed and analyzed them three days later.

*Experiment 2: do slugs break up on contact with bacteria?*

We examined whether slugs dissociate and dedifferentiate on contact with bacteria by using time-lapse video of slugs crossing bacteria. As an additional control, we transferred slugs to new plates to be absolutely certain that slugs were the only possible source of *Dictyostelium* amoebae. We obtained slugs using the same protocol as experiment 1. We transferred 8 to 19 slugs each of six genetically different clones (77B1,
301B1, 319B3, 330D2, 336B1, 337B1, a total of 74 slugs) onto new starving plates. Transfers were made with pins, and transferred slugs were oriented in their original direction toward the light source. After transfer, we pipetted a 1.5 cm wide by 9 cm long strip of bacteria between the slugs and a dim electric lamp towards which the slugs moved (Figure 1b). We videotaped the slugs with a Sony CCD-TRV66 Handycam using night vision mode and a Panasonic AG-6750A time-lapse recorder, with one image captured every four seconds.

Experiment 3: Are slugs more successful than amoebae at traversing soil to locate bacterial food?

We examined whether D. discoideum slugs were more successful than amoebae at crossing soil to find bacteria. We prepared soil plates by placing a 0.5 cm thick, 6 cm wide layer of autoclaved topsoil on top of a water agar plate (10 g agar in 1 L water), leaving 1.5 cm strips of exposed agar on opposite sides of the plate. On one side, we applied a 1 cm wide by 6.5 cm long strip of bacterial slurry to the agar, and on the other agar side we delivered $1 \times 10^7$ D. discoideum cells to the plate (Figure 1c). The D. discoideum wild type strain AX4 and aggregation mutant CAP2 were raised axenically, without bacteria, in HL5 liquid medium. We prepared the HL5 as described previously (Spudich, 1982), with peptic peptone substituted for proteose peptone, and added trace minerals and salts (8 mg/L NaOH, 1.7 mg/L NaHCO₃, 5.4 mg/L NH₄Cl, 0.29 mg/L CaCl₂•2H₂O, 8.1 mg/L FeCl₃•6H₂O, 0.48 mg/L Na₂EDTA, 0.06 mg/L NaB₄O₇•10H₂O, 0.01 mg/L CoCl₂•6H₂O, 0.015 mg/L CuSO₄•5H₂O, 0.01 mg/L (NH₄)6Mo₇O₂₄•4H₂O, 0.05 mg/L MnCl₂•4H₂O, 0.23 mg/L ZnSO₄•7H₂O) before autoclaving. After
sterilization, we added 0.5 g/L streptomycin, 0.1 g/L penicillin, 0.6 mg/L vitamin B12, and 0.2 mg/L folic acid. We centrifuged each strain twice for three minutes at 1,000 rpm, diluted them with KK2 buffer to $1 \times 10^7$ cells/ml, and delivered one milliliter of each strain’s cell solution to ten plates. We obtained the adenylyl cyclase null aggregation mutant CAP2 from the Dictyostelium Stock Center where Carole Parent had deposited it (www.dictybase.org). Adenylyl cyclase null mutants fail to produce the cAMP signal required for amoebae to aggregate (Pitt et al., 1992) and exhibit defective movement in response to cAMP gradients (Stepanovic et al., 2005). However, the mutant shows no other deficits in cell movement (Pitt et al., 1992; Stepanovic et al., 2005) and performs cytokinesis normally (Pitt et al., 1992). We chose the CAP2 mutant for analysis of vegetative movement, in which cAMP does not play a role. After leaving the plates in a dark drawer for five days, we examined them to see if the bacteria had been removed, something that would require crossing 6 cm of soil. Unlike the other experiments, there was no light source since this would attract slugs but not cells. By not using a light source we avoided a potential bias in the study.

RESULTS

Experiment 1: do cells from slugs eat bacteria?

The movement of *D. discoideum* slugs through a strip of bacteria resulted in the disappearance of the bacteria within three days on all experimental plates ($N = 36$ plates, 3 plates for each of 12 genetically distinct clones, Figure 2). Bacteria did not disappear on the three control plates that had no *D. discoideum* spores added.
Experiment 2: *do slugs break up on contact with bacteria?*

Time-lapse video revealed that no slugs disassociated when they contacted the bacteria. Of 74 slugs observed, 65 moved straight through the bacteria and 8 slugs split into two slugs while in contact with the bacteria. Two slugs disassociated immediately after they split from a single slug, but before they reached the bacterial strip. As in experiment 1, the passage of *D. discoideum* slugs caused the bacteria to be removed in all cases (Figure 2). This finding confirmed that amoebae from slug cells eat the bacteria rather than amoebae that never joined an aggregation, because these slugs had been transferred from their original plates.

*Experiment 3: Are slugs more successful than amoebae at traversing soil to locate bacterial food?*

After five days, *D. discoideum* slugs had crossed the field of soil and the bacterial strip, which resulted in the removal of spots of bacteria on all 10 plates with AX4 wild type clones. On eight of the plates with the aggregation-minus CAP2 mutant clones, there was no evidence that the amoebae had aggregated, and the bacterial strip remained completely intact showing that no amoebae were able to cross the soil and reach the bacteria to consume it. Amoebae on two of the plates where we plated out CAP2 mutants actually formed slugs that reached the other side of the plate and crossed the bacterial strip. As expected, amoebae from these slugs ate the bacterial strip. (These slugs may have been formed by wild type contamination or from the CAP2 aggregation-minus phenotype not being fully penetrant.) Together, these results are consistent with the hypothesis that slugs can travel across stretches of soil better than amoebae, and in nature
amoebae that are sloughed off of slugs can reach places that a solitary amoebae could not travel to alone. Despite the fact that slugs formed on two of the plates containing CAP2 cells, AX4 cells formed slugs on significantly more plates than CAP2 cells did (Fisher’s exact test $p = 0.0007$).

DISCUSSION

When slugs of *D. discoideum* move across bacteria, the bacteria are subsequently eaten (Figure 2). By transferring slugs to starving plates with no solitary amoebae, we confirmed that it was cells shed from slugs and not solitary amoebae causing the removal of bacteria. Figures 2c and 2d clearly illustrate that *D. discoideum* cells have colonized the bacteria, because there is no other way to explain the formation of new slugs. In addition, we showed that solitary amoebae with a mutation preventing slug formation did not cross a soil barrier that was easily crossed by normal slugs, which supports the idea that slugs provide a dispersal distance advantage not available to an asocial amoeba. However, the cells in the slug also benefit from the cells in the slime trail colonizing food sources, because members of all clones in the slug also have members in the slime trail. Every cell in the slug gains indirect fitness benefits from slime trail cells consuming bacteria and reproducing. After starving, these cells aggregate and assemble into new slugs that can migrate (Figure 2c & 2d) and eventually form fruiting bodies.

Our results contrast with Raper’s (1940) work that showed when undisturbed *D. discoideum* slugs encountered bacteria, the bacteria remained uneaten. The difference can be reconciled because Raper only followed slugs for 24 hours, which did not allow
the cells in the slime trail enough time to dedifferentiate and consume the bacterial prey, something that took 72 hours in our study. However, our data support Raper’s conclusion that slugs do not respond to bacteria by disassociating, because the slugs in our study remained intact on encountering bacteria. This suggests that the bacteria are colonized by the cells that are known to be continuously sloughed from *D. discoideum* slugs (Smith and Williams, 1979; Morrisey, 1982; Sternfeld, 1992; Wilkins and Williams, 1995; Alexander, 1997; Kessin, 2001). Interestingly, Raper (1956) noted that another species of slime mold, *Dictyostelium polycephalum*, often loses amoebae from migrating slugs. However, Raper (1956) did not show that *D. polycephalum* slugs leave a trail of amoebae while migrating like *D. discoideum* slugs. Rather, the delicate slime sheath of *D. polycephalum* easily becomes torn as the slugs migrate, and the amoebae posterior to the gaps in the sheath either form new migrating slugs, or dissociate into solitary amoebae that can colonize bacteria if it is available (Raper, 1956). This may potentially serve as a method of local dispersal to food patches in *D. polycephalum*, but this mechanism is markedly different from that of *D. discoideum*.

The evolution of multicellularity in *D. discoideum* has been explained through the ability of slugs to provide safe passage to the soil surface for long distance dispersal of spores by water or passing invertebrates (Bonner, 1982; Huss, 1989; Kessin et al., 1996; Kessin, 2001; Foster et al., 2002; Queller et al., 2003). Here, we have shown that slug migration also allows local food patches to be exploited as the slug moves through the substrate. Some local migration would also be possible by solitary amoebae, but they move a great deal more slowly and travel much shorter distances than slugs. Single vegetative cells move at 9.8-14.8 μm/minute on agar (Rifkin and Goldberg, 2006) and
aggregating cells generally travel 1 cm at most, which is the size of a large aggregation territory (Kessin, 2001). In contrast, slugs traveling on agar move 1-2 mm/hr (Raper, 1940), or 16.7-33.3 μm/minute, and can cover distances of 10-20 cm in a matter of days (Kessin, 2001).

The distance that amoebae can travel in the soil is likely to be further restricted by the need to cross air gaps between soil particles, which slugs readily cross (Kessin et al., 1996). In support of this, we found that the solitary cells of the aggregation-minus mutant CAP2 did not cross soil to reach bacteria that were reached by multicellular slugs. This suggests that slug formation provides opportunities for local dispersal that would not be possible in an asocial state. Furthermore, the slug provides protection from nematode predators (Kessin et al., 1996) and exhibits extreme sensitivity to light and shallow heat gradients that facilitate movement towards favorable environments (Kessin, 2001). We propose, therefore, that local dispersal represents a previously unrecognized advantage that favored the evolution of multicellularity in D. discoideum. By leaving a trail of cells as it moves, cells sloughed from the slug can exploit nearby food sources without the slug sacrificing the benefits of the slug stage, migration and ultimate spore production.
Figure 1. Setup for experiments 1, 2, and 3. a) In experiment 1, we mixed *D. discoideum* spores into a *Klebsiella aerogenes* bacterial slurry applied to one edge of a starving plate. After the resulting slugs had migrated halfway across the plate towards the light source, we added a second line of bacteria in front of them. b) In experiment 2, we transferred slugs to a starving plate and added bacterial slurry between them and the light source. c) In experiment 3, we placed a 6 cm wide layer of topsoil on top of a water agar plate. We pipetted cells onto the exposed agar on one side of the plate and applied a strip of bacteria to the agar on the opposite side. In one treatment the cells were *D. discoideum* wild type and would aggregate into slugs, while in the other treatment the cells were CAP2 mutants that could not aggregate.
Figure 2. Slug movement follows the arrow from the left towards a light source at the right. The white strip running from top to bottom is a strip of the bacteria *Klebsiella aerogenes*, which is approximately 1.5 cm wide, on an agar starving plate (see methods). The small circles in the bacteria are air bubbles produced by pipetting the bacteria. a) Slugs immediately after transfer from another plate. b) At 12 hours, the migrating slugs have passed through the bacteria. c) At 72 hours, the bacteria are being removed and the first new aggregations form. d) At 96 hours, most of the bacteria are consumed and many new slugs are produced.
REFERENCES


