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Exploiting new terrain: an advantage to sociality in the slime mold *Dictyostelium discoideum*

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Understanding the ecological benefits of social actions is central to explaining the evolution of social behavior. The social amoeba *Dictyostelium discoideum* has been well studied and is a model for social evolution and development, but surprisingly little is known about its ecology. When starving, thousands of the normally solitary amoebae aggregate to form a differentiated multicellular organism known as a slug. The slug migrates toward the soil surface where it metamorphoses into a fruiting body of hardy spores held up by a dead stalk comprising about one-fifth of the cells. Multicellularity in *D. discoideum* is thought to have evolved to lift the spores above the hazards of the soil where spores can be picked up for long-distance dispersal. Here, we show that multicellularity has another advantage: local dispersal to new food sources. We find that cells shed by *D. discoideum* slugs during migration consume and remove bacteria in the path of the slug, although slugs themselves do not breakup. We also show that slugs are adept at local dispersal by comparing migration of slugs with migration of individual cells of the mutant, CAP2, which cannot aggregate and so rely only on cellular movement. In particular, the solitary cells of the aggregation mutant are unable to cross a soil barrier, easily crossed by slugs. We propose that the exploitation of local food patches is an important selective benefit favoring multicellular cooperation in *D. discoideum*. *Key words:* altruism, *Dictyostelium discoideum*, multicellularity, social evolution, sociality. *[Behav Ecol 18:433–437 (2007)]*

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 suppresses 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, meerkat sentinels keep post looking for predators, which protects the group and allows increased foraging (Clutton-Brock et al. 2002). Grouping benefits social insects like the paper wasp *Polistes* by increasing productivity particularly by providing survival benefits, such as superior recovery from nest destruction in larger colonies (Strassmann et al. 1988; Strassmann and Queller 1989). 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 microorganisms have social systems amenable to analysis in ways initially applied to multicellular animals (Crespi 2001; Travisano and Velicer 2004; West et al. 2006). 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. For instance, the bacterium *Myxococcus xanthus* travels better on soft wet surfaces by grouping during S-motility (Shi and Zusman 1993). Interestingly, *M. xanthus* strains incapable of producing the pili required for S-motility can evolve new beneficial group motility mechanisms (Velicer and Yu 2003).

Here, we explore benefits to sociality in a social amoeba, *Dictyostelium discoideum*. Two advantages to social behavior in the slime mold *D. 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 toward 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 toward 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 by water and passing invertebrates (Bonner 1982; Huss 1989; Kessin 2001; Queller et al. 2003).

We predicted that there might be an additional advantage of sociality in *D. discoideum*. Slug migration may enable the cells in the slug to reach and exploit patchy local food sources that are more distant than those that could be reached by a solitary amoeba. *Dictyostelium 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

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some live cells (Smith and Williams 1979; Morrissey 1982; Sternfeld 1992; Wilkins and Williams 1995; Alexander 1997; Kessin 2001). Our prediction was 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 breakup 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 toward and across a strip of concentrated *Klebsiella aerogenes* bacterial slurry, we used the phototactic behavior of *D. discoideum* slugs (Bonner et al. 1950; Bonner 1967; Foster et al. 2002). We applied a 1.5-cm wide by 6-cm long strip of bacterial slurry to one edge of a nutrient-free "starving" plate (see supplementary material for slurry and media recipes) and used a toothpick to mix in spores from 3 to 7 fruiting bodies (Figure 1a). We used 12 genetically distinct clones of *D. discoideum* collected from the University of Virginia's Mountain Lake Biological station, USA (56A1, 56A2, 56C1, 77B1, 301B1, 301B2, 319B3, 327A1, 330D2, 342B2, 336B1, 337C1, Fortunato et al. 2003). We used one clone per plate and made 3 replicate plates per clone for a total of 36 experimental plates. We also prepared 3 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 toward 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 toward the light. When the slugs were halfway across the plate, we laid down a second strip of *K. 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 3 days later.

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

We examined time-lapse video of slugs crossing a bacterial strip to determine whether slugs dissociate and dedifferentiate on contact with bacteria and if the slugs or the cells they shed are responsible for the removal of 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 6 genetically different clones (77B1, 301B1, 319B3, 330D2, 336B1, 337B1, a total of 74 observed 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 bacterial slurry between the slugs and a dim electric lamp toward 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 4 s. We analyzed videos of 2 clones to create a time course of percent bacteria removed in relationship to when the slugs crossed.

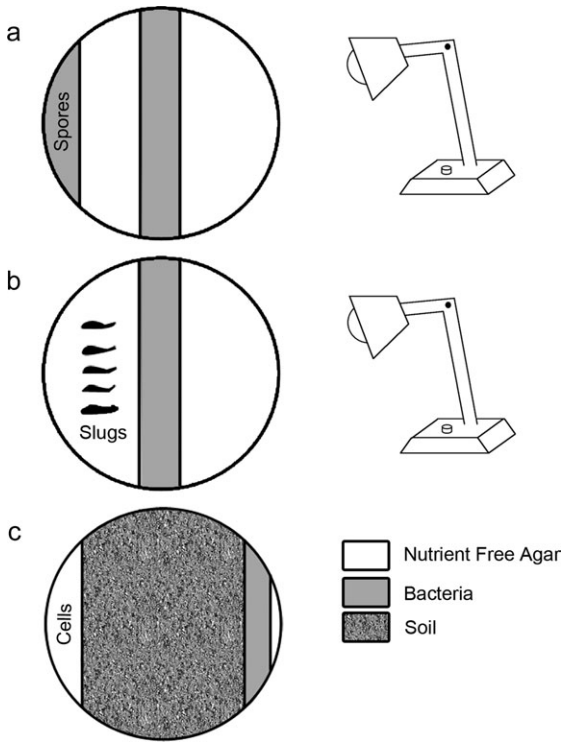


Figure 1
Setup for experiments 1, 2, and 3. a) In experiment 1, we mixed *D. discoideum* spores into a *K. aerogenes* bacterial slurry applied to one edge of a starving plate. After the resulting slugs had migrated halfway across the plate toward the light source, we added a second line of bacteria in 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, whereas in the other treatment the cells were CAP2 mutants that could not aggregate.

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, 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 1×10^7 *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 centrifuged each strain twice for 3 min at 1000 r.p.m., diluted them with KK2 buffer to 1×10^7 cells/ml, and delivered 1 ml of each strain's cell solution to 10 plates. We obtained the adenylyl cyclase null aggregation mutant

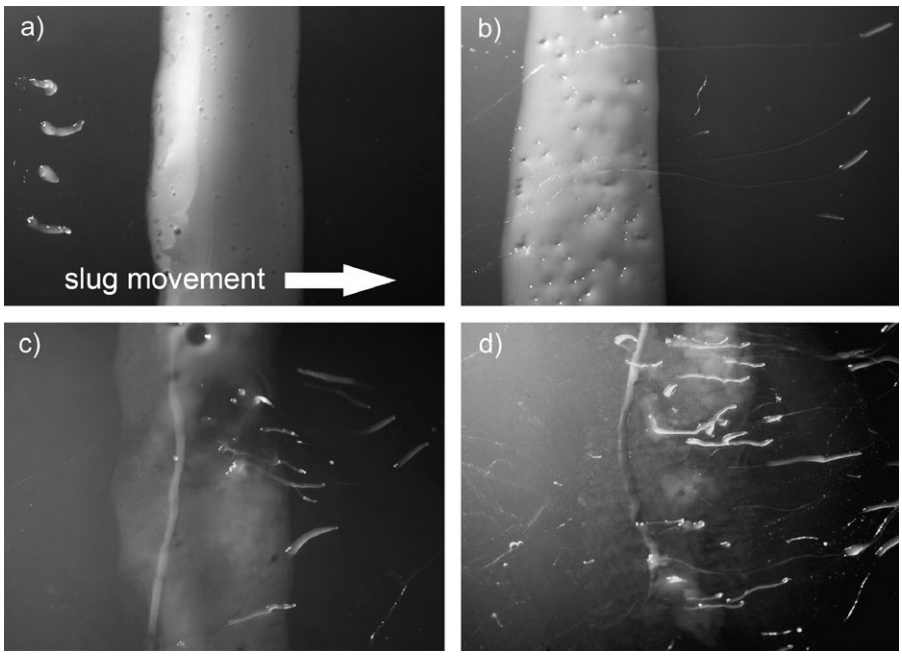


Figure 2
Slug movement follows the arrow from the left toward a light source at the right. The white strip running from top to bottom is a strip of the bacteria *K. aerogenes*, which is approximately 1.5 cm wide, on a starving agar plate. The small circles in the bacteria are air bubbles produced by pipetting the bacteria. a) Slugs immediately after transfer from another plate. b) At 12 h, the migrating slugs have passed through the bacteria. c) At 72 h, the bacteria are being removed and the first new aggregations form. d) At 96 h, most of the bacteria are consumed and many new slugs are produced.

CAP2 from the Dictyostelium Stock Center where Carole Parent had deposited it (www.dictybase.org). Adenylyl cyclase null mutants fail to produce the cyclic adenosine monophosphate (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 5 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 because 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 3 days on all experimental plates ($N = 36$ plates, 3 plates for each of 12 genetically distinct clones). Bacteria did not disappear on the 3 control plates that had no *D. discoideum* spores added.

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

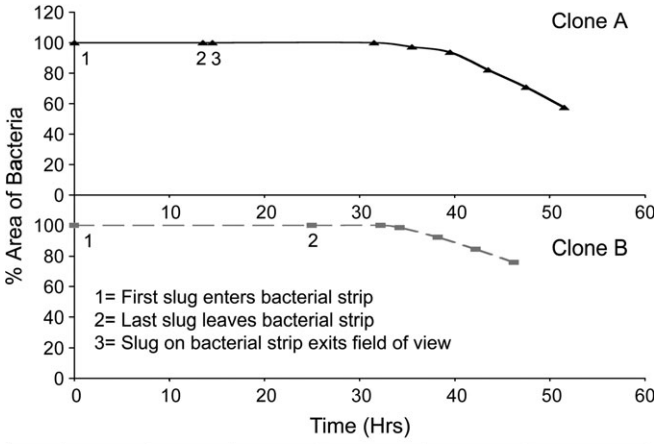
Time-lapse video revealed that no slugs disassociated when they contacted the bacteria. Of 74 slugs observed, 65 moved straight through the bacteria and 8 slugs split into 2 slugs while in contact with the bacteria. Two slugs disassociated immediately after they split from a single slug but before they 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). Time-course data revealed that the bacteria were not visibly removed until several hours after the last slug crossed the bacterial strip (approximately 9.25–22 h, Figure 3), indicating that the cells in the slime trail consumed the bacteria, not the cells remaining in the slug. New slugs formed after the cells in the slime trail cleared spots of bacteria (Figures 2 and 3). This experiment allowed us to eliminate the effect of amoebae that never joined an aggregation on the bacteria because these slugs had been transferred from their original plates where they aggregated.

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

After 5 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 8 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 2 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 2 of the plates containing CAP2 cells, AX4 cells more successfully crossed the soil gaps than CAP2 cells did (Fisher's Exact test $P < 0.001$).

DISCUSSION

Contrary to earlier reports (Raper 1940), we have shown that 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 from slugs and not solitary amoebae causing the removal of bacteria. Time-lapse video revealed that the bacterial slurry remains visibly intact until many hours after the slugs have finished crossing it, indicating that cells in the slime trail, not the slug, consume the bacteria (Figure 3). Figure 2c,d clearly illustrates that *D. discoideum* cells have colonized the



Clone	# Slugs enter bacteria	# Slugs exit bacteria	# Slugs exit field of view	Time (Hrs) between 1st slug entering bacteria and bacteria removal	Time (Hrs) between last slug leaving bacteria and bacteria removal	Time (Hrs) between 1st slug entering bacteria and the first new slug moving
A	13	12	1	35.5	22	39.5
B	9	9	0	34.25	9.25	43.25

Figure 3
We analyzed time-lapse videos of transferred slugs (see experiment 2 of Methods) from 2 *D. discoideum* clones to create time courses showing the percent area of bacteria present in relationship to the position of the slugs. For clone A, bacteria were not visibly removed until approximately 22 h after the last slug had exited the bacterial strip. The first new slug formed and started migrating 39.5 h after the first transferred slug entered the bacterial strip. For clone B, bacteria were not visibly removed until approximately 9.25 h after the last slug exited the bacterial strip. The first new slug formed and started migrating 43.25 h after the first transferred slug entered the bacterial strip.

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 their clone mates in the slime trail colonizing food sources. 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 further migrate (Figure 2c,d) and eventually form fruiting bodies.

Raper's (1940) work showed that when undisturbed *D. discoideum* slugs encountered bacteria, the bacteria remained uneaten. The difference with our results can be reconciled because Raper only followed slugs for 24 h, which did not allow the cells in the slime trail enough time to dedifferentiate and consume the bacterial prey, something that took approximately 35 h 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. Figures 2 and 3 illustrate that bacteria are not visibly removed until long after the slugs have passed through the bacterial strip, indicating that cells in the slime trail, and not the slug, are colonizing the bacteria. Although we cannot exclude the possibility that slugs preferentially shed more cells in the presence of bacteria, bacteria are not needed to induce cell sloughing. Cells are known to be continuously shed from *D. discoideum* slugs even in the absence of bacteria (Smith and Williams 1979; Morrissey 1982; Sternfeld 1992; Wilkins and Williams 1995; Alexander 1997; Kessin 2001), which allows the slug to act as a mobile distributor of cells to local areas.

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 elevate spores from 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/min 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/h (Raper 1940), or 16.7–33.3 μm/min, 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. Although we could not control for turning frequency, this experiment 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 toward 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.

Group actions are an almost ubiquitous feature of microbial life. The social evolution perspective allows new insights into microbial systems and, in particular, how selection for cooperation and conflict shapes social traits (West et al. 2006). In this case, we have discovered a new and immediate advantage to grouping and sociality, the facilitation of local migration. Dispersal, then, is pivotal to the evolution of sociality in *D. discoideum* and may be important in many other species. However, dispersal will not apply across the board because many microbial groups are sessile, including biofilms. The challenge now is to provide ultimate explanations for the wide variety of group behaviors seen across microbial taxa. Only then, can we hope to fully understand the mysteries of their social life.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

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