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Mind the gap: a comparative study of migratory behavior in social amoebae

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Abstract

Social amoebae aggregate to form a multicellular slug that migrates some distance. Most species produce a stalk during migration, but some do not. We show that *D. giganteum*, a species that produces stalk during migration, is able to traverse small gaps and utilize bacterial resources following gap traversal by shedding live cells. In contrast, we found *D. discoideum*, a species that does not produce stalk during migration, can traverse gaps only when in the presence of other species' stalks, or other thin filaments. These findings suggest production of stalk during migration allows traversal of gaps, as commonly occur in soil and leaf litter. Considering the functional consequences of a stalked migration may be important for explaining the evolutionary maintenance or loss of a stalked migration.

Keywords cellular slime mold; *Dictyostelium*; development; inclusive fitness

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Introduction

One of the most striking examples of microbial altruism is the stalk formation of the amoeba *Dictyostelium discoideum* (Raper 1984; Bonner 2009). In this species, cells aggregate to form a freely-migrating mass of amoebae (Raper 1984). This mass of cells resembles a slug, and can migrate across or through natural substrates following gradients of light, heat or gas (Raper 1984; Bonner and Lamont 2005; Castillo et al. 2005). In response to environmental stimuli such as increased light or a drop in humidity, the multicellular slug ceases migration and begins to form a vertical fruiting body (Raper 1984). The construction of the fruiting body involves differentiation of some cells to produce a dead stalk, which lifts other cells aloft as fertile spores (Raper 1984). A potential benefit of this stalk formation is an increased ability to be dispersed by highly mobile animals found above the surface of the soil (Suthers 1985; Stevenson and Landolt 1992; Bonner and Lamont 2005; Gilbert et al. 2007).

While the production of a stalked fruiting body has been a central focus of evolutionary studies of altruism (Gilbert et al. 2007), all species of social amoebae produce a stalked fruiting body (Raper 1984). For construction of hypotheses based on comparative data, it is useful to identify a trait that varies between species (Crespi 1996). A candidate trait is the production of stalk during migration (Raper 1984). Some species always form dead stalk as they migrate, others facultatively produce a dead stalk during migration depending on environmental conditions, and yet others exhibit a completely stalkless migration (Bonner 1982; Raper 1984; Kaushik and Nanjundiah 2003). The two stalkless-migrating species, *Dictyostelium polycephalum* and *D. discoideum* lost the

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stalked migration independently (Schaap et al. 2006). In both species, live cells are often left behind migrating slugs, which may form fruiting bodies or regain vegetative growth (Raper 1935; Raper 1956; Kuzdzal-Fick et al. 2007). Given this advantage correlated with a stalkless migration, the question must be raised why most species migrate with a stalk.

One hypothesis to explain a stalked migration is it allows slugs to traverse gaps that commonly occur in soil and leaf litter (Bonner 1982). We here test this hypothesis by comparing two large and robust species, the stalked-migrating *Dictyostelium giganteum* and the stalkless-migrating *D. discoideum* (Raper 1984). Both species produce slugs of similar size that migrate toward light and large fruiting bodies with a single spore head (Raper 1984). To test the effect of stalked migration on traversing gaps, we provided directional light that stimulates both species to migrate across a 3-mm gap cut in an agar substrate (Fig. 1). This obstacle presents a challenge to a slug that is a maximum of about 3-mm in length (Raper 1984).

If a stalk-migrating species has a superior gap-traversing ability under this condition, this suggest producing stalk during migration allows slugs to traverse gaps. This raises the question of why some species lack this behavior. A first hypothesis to explain why some species lack a stalked migration is stalkless-migrating species can leave live cells behind as they migrate (Raper 1956; Kuzdzal-Fick et al. 2007), which compensates for a reduced ability to traverse gaps (Bonner 1982). For this hypothesis to hold, it must also be true that stalk-migrating species cannot exploit bacterial resources during migration. To test this hypothesis, we tested the ability of migrating *D. giganteum* slugs to exploit bacterial resources by shedding live cells. We also asked whether *D.*

giganteum slugs can exploit bacterial resource on the opposing side of a gap. If *D. giganteum* can also leave live cells behind as it migrates, then the ability to exploit bacterial resources during migration is not correlated with a stalkless migration.

An alternative explanation for why some species lack a stalked migration is only some species require their own stalks to traverse gaps. This could be the case, for example, if some species have invented new ways to migrate or an ability to use other species' stalks. For example, the stalkless-migrating *D. polycephalum* produces long and thin slugs capable of soaring above a substrate and climbing between fungal stalks (Raper 1956). This suggests *D. polycephalum* might not require its own stalks to traverse gaps. Likewise, *D. discoideum* can attach to stalks of members of its same species using its basal disc as an anchor (Raper 1935). This suggests *D. discoideum* might use the stalks of other species to traverse gaps or fruit. To test this hypothesis, we placed *D. discoideum* in a position to use the stalks of *D. giganteum* to traverse a gap or fruit. We also placed *D. discoideum* in a position to use small sections of line to traverse a gap.

Materials and methods

Gap-crossing performance

To examine the gap-crossing performance of the two species, we manipulated spatial arrangements with respect to a gap on an agar plate (Fig. 1). We placed the two species next to each other (Fig. 1a), *D. discoideum* behind *D. giganteum* (Fig. 1b) or *D. discoideum* in front of *D. giganteum* (Fig. 1c). To ensure that our comparison reflects

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species-specific rather than clone-specific differences, we used three clones of each

species. We used three *D. discoideum* clones, QS17, QS11, and QS4, isolated near

Mountain Lake Biological Station, VA (coordinates of isolation: 37°22′287″, N,

80°31′04.2″ W for all). These clones were distinguished as genetically distinct based on

five microsatellite loci (Fortunato et al. 2003). We isolated three *D. giganteum* clones,

QSgi25, Qsgi26 and QSgi27 from locations at least 13 km apart in or near Houston, TX

(coordinates of isolation: 29° 45' 45.949' N, 95° 26' 48.31' W for QSgi25; 30° 2′

13.7040′ N, 95° 46′ 27.2281′ W for Qsgi26; and 30° 8′ 0.0600′ N, 95° 40′ 31.7639′ W

for QSgi27). We performed three replicates of each of the nine possible combinations of

clones for each spatial arrangement (81 plates total).

To construct gaps, we poured 35 mL of buffered agar (1.98 g KH_2Po_4, 0.35 g

Na_2HPO_4, 20 g agar per L ddH20) into a 10-cm diameter plastic petri dish. Using a pair

of sterilized forceps, we cut a section of agar from the middle of the plate 3-mm wide at

the top and 11-mm wide at the base, and we placed 1 mL of mineral oil into the gap (Fig.

1d). The mineral oil served as a moat, ensuring that any slugs that fell into the gap were

unable to reach the opposite side. We then deposited a 75 μL elongated oval strip of the

spore and bacteria mix parallel to the gap for each clone (5.0 X 10^6 *Dictyostelium* spores

and 1/30 plate of *Klebsiella aerogenes* bacteria grown on 35 mL SM (Sussman 1966)

agar for 4 days), about 5-mm from the gap. After depositing spore / bacteria solutions,

we allowed plates dry open in a laminar flow hood for two hours. We then replaced the

lid and wrapped the plates with aluminum foil. We cut a 1-mm diameter hole in the foil

in the middle of the plate on the opposing side of the gap from where the spore / bacteria

solutions were placed. We stacked these plates 45-90 cm from a 100-watt incandescent

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light bulb with the holes facing the light source. The directional light stimulated slugs of

both phototactic species to traverse the gap.

We unwrapped the plates after six days and counted the number of *D. giganteum*

slugs to have traversed the gap based on the number of stalks bridging the gap. We

counted the number of *D. discoideum* fruiting bodies to have traversed the gap or fruited

on *D. giganteum* stalks based on the unique phenotype of *D. discoideum,* including the

basal disk, tapered stalk, and stalkless migration (Raper 1984). We also deposited each

D. discoideum clone in the presence or absence of 4 strands of 0.08-mm diameter Climax

8X nylon line bridging the gap (Climax Systems, Cortland, NY), with two replicate plates

for each clone.

To directly observe whether *D. discoideum* slugs used *D. giganteum* stalks to

traverse the gap, we took a time lapse video with *D. discoideum* positioned behind *D.*

giganteum using clones QS11 and QSgi26 (Video [1]). To capture the video, we placed a

plate constructed as in Fig. 1b in a plastic petri dish bag, and sealed the bag around the

objective of a Nikon SMZ-1500 stereoscopic microscope using masking tape. We placed

a fiber optic light guide powered by a M1-150 illuminator at 1/4 power 1.5 M from the

plate in an otherwise dark room. We took one photograph each minute with a

Photometrics coolsnap *cf* digital camera. We used these photos to make a movie, which

we edited using iMovie HD 6.0.4 (Apple Computer) to highlight the *D. giganteum* and *D.*

discoideum slugs (Video [1]).

Statistical analysis

Using JMP v. 7.0.2, we performed an analysis of variance with species, placement relative to other species (in front of, next to, or behind), species x placement interaction and clone nested within species as factors. We used the Box-Cox transformed average number of slugs to traverse the gap for each clone pair / treatment replicate as the response variable of this ANOVA model (averaging across the three replicate plates for each clone pair / treatment combination, for a total $n = 54$). The distribution of the residuals was not significantly different from normal (Shapiro-Wilks $W = 0.97$, $P = 0.21$, $n = 54$). We also tested whether the average number of slugs to traverse the gap or to fruit on the stalks of the other species was no greater than 0 for each species using a one-tailed *T*-test (averaging across the three clones of the other species and the three clones of the same species, for $n = 3$ for each species).

Exploitation of bacterial resources in *D. giganteum*

We tested for live cells behind migrating *D. giganteum* slugs by depositing spore and bacteria solutions of *D. giganteum* on buffered agar plates (recipe given above) free of bacteria with a directional light source. We used a pair of sterilized forceps to cut out section of agar (25–100 mm^2) with stalks from 1–2 mm behind migrating slugs (Fig. 1e). We collected five sections of agar with at least one stalk each for each of the three clones on three different days ($n = 45$). We transferred each piece of agar to a separate plate and covered the surface of the agar with 20-25 μL of the *K. aerogenes* solution. We incubated each plate at 22˚ C for 6-10 days and then examined the plates for *D. giganteum* growth. As controls, we removed two sections of agar from areas of each

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plate where slugs had not migrated on the second and third days ($n = 12$) to ensure that cells were not spreading across the plates (Fig. 1e). We also tested the bacterial solution for contamination by *D. giganteum* by placing a drop on a separate plate each day ($n = 3$).

To test whether *D. giganteum* slugs can exploit bacterial resources following gap traversal, we took time-lapse videos of each *D. giganteum* clone traversing a gap with a bacterial strip on the opposite side (Video [2]). To capture the video, we placed a Sony DCR HC36 digital camcorder inside a cardboard box, sealed except for a 2-mm wide hole facing a 100 watt incandescent light bulb 45 cm from the box. The camcorder was set on night-vision mode, with the infrared light deactivated, supported by a miniature tripod. We took photographs once per minute by averaging 30 frames of interlaced video using BTV carbon pro (Ben Software). We edited one of the videos using iMovie HD 6.0.4 to highlight the passage of a single slug (Video [2]).

Results

Gap-crossing performance

We found a significant effect of species, placement, and species x placement interaction but no effect of clone within species ($P = 0.0001$, $P = 0.0002$ and $P = 0.51$, respectively, $R^2 = 0.81$, $n = 54$; Table 1). We found a significant number of *D. giganteum* slugs traversed the gap in all spatial configurations (Fig. 2, dark grey bars; $P = 0.003$, $P = 0.02$ and $P = 0.006$ respective to spatial configurations in Fig. 1 a - c; $n = 3$ each; one-

tailed *T*-test). In contrast, we found a significant number of *D. discoideum* slugs

traversed the gap only when *D. discoideum* was behind *D. giganteum* (Fig. 2, medium

grey bars; *P* = 0.15, *P* = 0.02 and *P* = 0.07, respective to spatial configurations in Fig. 1 a

- c; *n* = 3 each; one-tailed *T*-test). We also found a significant number of *D. discoideum*

fruiting bodies on *D. giganteum* stalks when *D. discoideum* was behind or in front of *D.*

giganteum (Fig. 2, light grey bars; *P* = 0.08, *P* = 0.01 and *P* = 0.01, respective to spatial

configurations Fig. 1 a - c; *n* = 3 each; one-tailed *T*-test). In the experiment with

monofilament line, we found a mean 7.5 ± 2.0 (s.e.) *D. discoideum* slugs traversed the

gap in the presence of line (*P* = 0.03, *n* = 3, one-tailed *T*-test), and no *D. discoideum* slugs

traversed the gap in the absence of the line.

Our time-lapse video confirmed that *D. discoideum* slugs use the *D. giganteum*

stalks to traverse the gap (Video [1]). In the videos, many of the *D. giganteum* slugs

differentiated to form spores as they were traversing the gap, probably because of the

lower humidity and more light in these plates (these plates were not covered with a lid

[see Methods] and were exposed to more diffusive light required to capture the video).

Nevertheless, *D. discoideum* was able to use *D. giganteum* stalks to traverse the gap

(Video [1] and Fig 3). In the experimental plates, *D. giganteum* slugs did not

differentiate while traversing the gap (Fig. 3a).

Exploitation of bacterial resources in *D. giganteum*

In the test for live cells left behind slugs, amoebae consumed bacteria in 15/15 sections of

agar (*n*= 45) cut from behind migrating *D. giganteum* slugs. The controls showed only

1/12 sections of agar from areas of the plate without stalks yielded growth of amoebae (Fig. 1e), suggesting cells were left behind particular slugs. The control bacterial solutions did not show contamination by *D. giganteum* amoebae (*n* = 3). The time–lapse videos showed each *D. giganteum* clone traversed the gap and exploited bacterial resource on the opposite side. Most videos showed many slugs traversing the gap, followed by clearing of the bacteria first in sections where slugs initially migrated over the bacteria. This pattern of local bacterial clearance suggests live cells are deposited into the bacteria by slugs. In one trial, only a single *D. giganteum* slug traversed the gap and bacterial strip, and continued migrating. The video showed bacteria being cleared in line with the path taken by a single slug (Video [2]).

Discussion

We found the stalk-migrating *D. giganteum* can traverse a 3-mm wide gap that the stalkless-migrating *D. discoideum* cannot traverse alone (Figs. 2 and 3). This suggests forming stalk during migration allows slugs to traverse gaps. This raises the question of why some species lack this behavior. The first hypothesis is that only stalkless-migrating species can gain an advantage in exploiting bacterial resources during migration. We tested this hypothesis by examining the ability of *D. giganteum* to exploit bacterial resources. We found *D. giganteum* slugs can exploit bacterial resources following by constantly shedding live cells, even after traversing a gap (Fig. 3b-e and Video [2]). This suggests the advantage of exploiting bacterial resources is not correlated with stalkless migration. An alternative hypothesis is that some species can use the stalks of other species. In support of this hypothesis, we found *D. discoideum* can use the stalks of *D.*

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giganteum to traverse gaps or fruit (Video [1], Fig. 3f-h). We also found *D. discoideum* can use small sections of 0.8-mm diameter line to traverse gaps.

The first hypothesis we tested here was that producing stalk during migration allows traversal of a gap. We used a simple obstacle cut in an agar substrate, and indeed under this condition we found superior gap-crossing performance of a stalk-migrating species (Fig. 3). However, for this benefit to be relevant to nature it must be found also on a natural substrate. On a natural substrate, other differences between species might affect the results. For example, *D. discoideum* might use its more dispersive pattern of migration (Bonner and Lamont 2005) or quicker migration speed (Dormann et al. 2007) to circumvent gaps. In our study, these other differences were unlikely to have affected the results, because the moat of mineral oil prevented *D. discoideum* slugs from circumventing the gap (Fig. 1d). On a natural substrate, it would be advisable to use intraspecific comparisons to isolate the effects of a stalked migration, as possible with mutants derived from mutagenesis (Ennis et al. 2000) or naturally-occurring variants (Raper 1984).

We also found live cells left behind *D. giganteum* can exploit bacterial resources following gap traversal on an agar plate (Video [2]). Whether this translates to an advantage on a natural substrate is an important question. For example, we do not know whether the number of cells left behind migrating *D. giganteum* slugs is sufficient to allow invasion of resources in soil. We also do not know if stalkless-migrating species can exploit bacterial resources on soil (Raper 1956; Kuzdzal-Fick 2007). Additionally, we do not know what advantage might accrue to *D. discoideum* slugs by fruiting on the stalks of other species in a gap (Fig. 3f and 3g). One possible advantage is in being

dispersed by small animals, such as earthworms and arthropods, that crawl through soil interstices (Huss 1989).

We here found that *D. discoideum* has a remarkable ability to use the stalks of other species as bridges and for fruiting (Video [1]), using its well-developed basal disc as a clamp (Fig. 3g). The basal disc of *D. discoideum* is the most well-developed of any species of social amoebae, and is the feature from which the species derives its name (Raper 1935). In nature, *D. discoideum* often occurs in animal feces with other species of social amoebae that respond to similar light, heat or gas gradients (Raper 1984; Suthers 1980; Stephenson and Landolt 1992; Bonner and Lamont 2005). If *D. discoideum* often uses the stalks of other species to traverse gaps or fruit, it might not require its own stalks to traverse gaps.

Conclusions

We found that *D. giganteum*, a species that produces a stalk during migration, can traverse small gaps on an agar plate. Given that such gaps are likely to occur commonly in soil and leaf litter, the ability to traverse gaps could be an important advantage to a stalked migration. In contrast, *D. discoideum*, a stalkless-migrating species, can traverse gaps or fruit in gaps by using the stalks of *D. giganteum*. This suggests between-species interactions could be important for allowing *D. discoideum* to migrate and disperse despite lacking a stalked migration.

Acknowledgments

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Tables

Source	d.f.	SS	*F* Ratio	*P*
Species	1	5121.9	147.4	<.0001
Placement	2	738.6	10.6	0.0002
Species x placement	2	612.0	8.8	0.0006
Clone (species)	4	114.2	0.8	0.5186
Error	44	1528.8		

Table 1. Degree of variation of number of fruiting bodies to traverse the gap explained

by species, placement of clones relative to other species, species x placement interaction,

and clone within species. These factors were fixed effects in the ANOVA model of Box-

Cox transformed data. Clone (species) was a nested effect and was not significant.

Figure Captions

Figure 1. Experimental treatments. Spatial configurations: (a) *D. giganteum* next to *D. discoideum* (b) *D. giganteum* in front of *D. discoideum* (c) *D. giganteum* behind *D. discoideum*. (d) The gap from a side view. (e) Test for live cells behind *D. giganteum* slugs. Sun emblem represents origin of light.

Figure 2. Results for gap-crossing experiment. Bars represent standard error for three replicate *D. giganteum* or *D. discoideum* clones. Treatments correspond to those in Fig. 1. Treatment (a) is when the species are next to each other, treatment (b) is when *D. giganteum* is in front of *D. discoideum*, and treatment (c) is when *D. discoideum* is in front of *D. giganteum*. Legend gives the results based on color-code, and the legend key gives a pictorial representation of each result. In the legend key, *D. giganteum* fruiting bodies are dark grey and *D. discoideum* fruiting bodies are light grey. The null hypothesis is mean no greater than 0 (one-tailed *T*-tests, $n = 3$ clones per species; * $P < 0.05$, ** $P < 0.01$). Sun emblem represents origin of light.

Figure 3. Photographs of results: **(a)** When the two species are next to each other (treatment (a)), *D. giganteum* can traverse the gap but *D. discoideum* cannot. **(b-e)** Still photographs from a video (available online) showing the ability of a single *D. giganteum* slug to exploit new resource from gap traversal. Note that bacteria are cleared in line with the path of the slug. **(f)** When positioned behind *D. giganteum* (treatment (b)), *D. discoideum* is able to traverse the gap and fruit on *D. giganteum stalks.* **(g)** *D. discoideum*

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fruiting body on a *D. giganteum* stalk (insert shows basal disc used as a clamp). **(h)** Still

photograph from a video showing *D. discoideum* slugs using the stalks of *D. giganteum*

to traverse a gap. Scale bar is (a) 1 cm (b-e) 4 mm (f) 8 mm for main photo and 2 mm for

insert (g) 1 mm (h) 2 mm. Sun emblem represents origin of light.
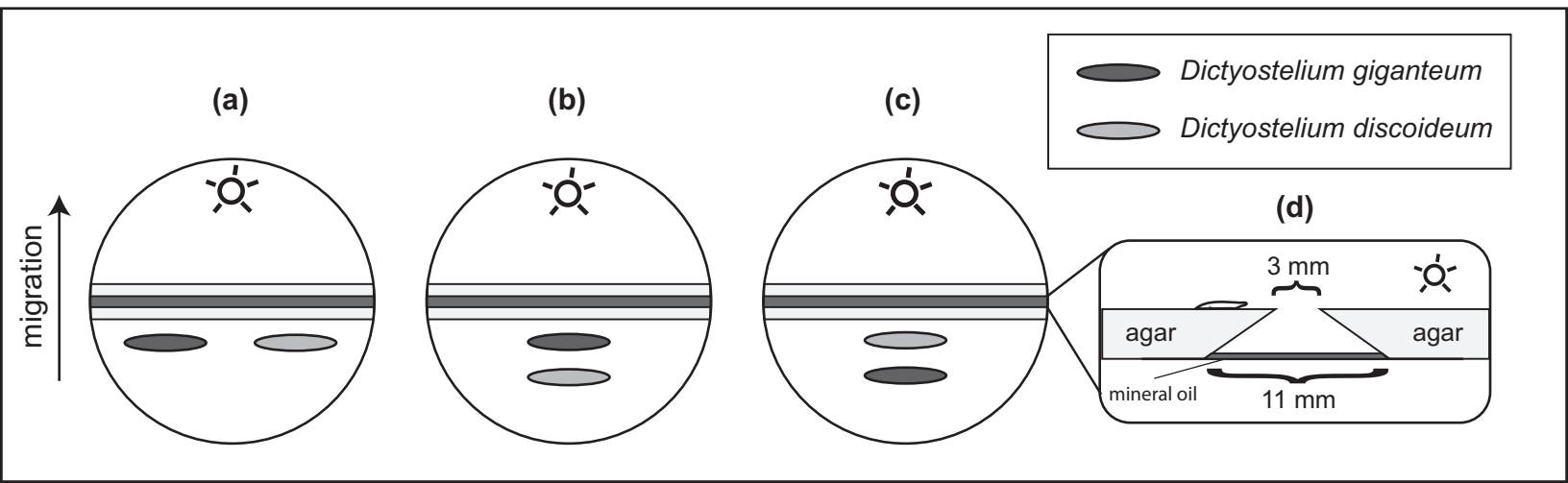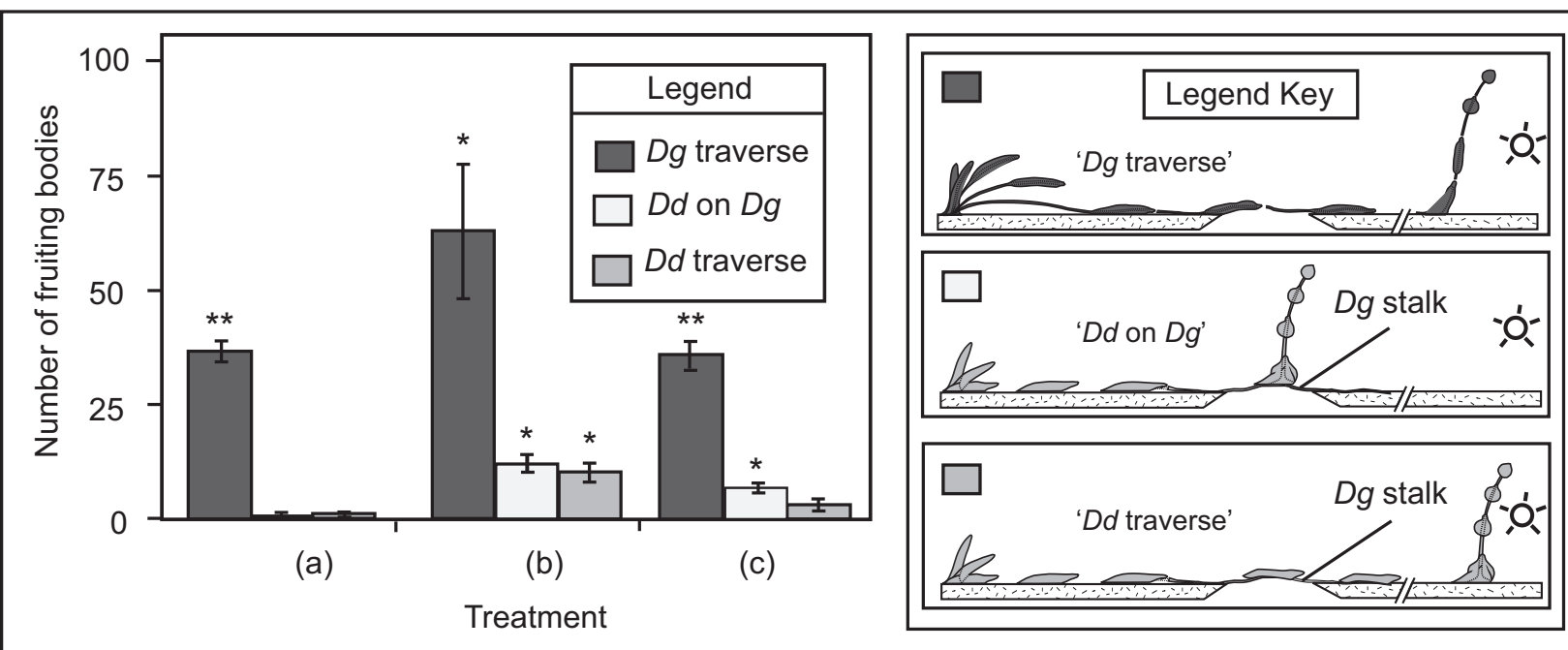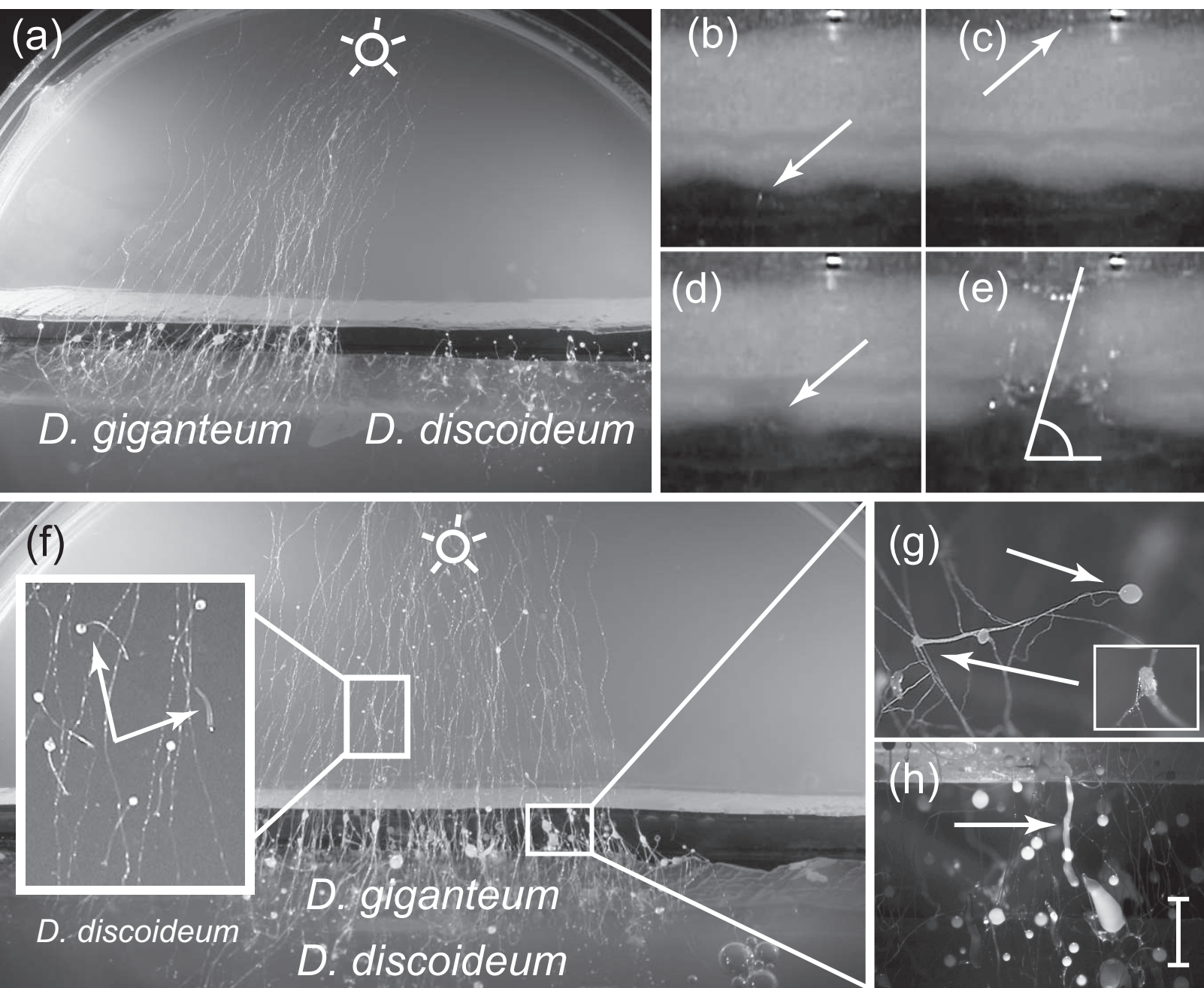
Figure 1

(a) (b) (c)

migration

Dictyostelium giganteum
Dictyostelium discoideum

(d)

3 mm

agar   agar

mineral oil

11 mm

Figure 2

Figure 3

(a)

D. giganteum   D. discoideum

(b)

(c)

(d)

(e)

(f)

D. discoideum

D. giganteum

D. discoideum

(g)

(h)

Video Captions

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