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Applying home-based experiments on locally isolated *Dictyostelium discoideum* to qualitatively demonstrate taxis of social amoebae

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Abstract. *Claudio-Paragas CY, Balaoro-Banzuela RC, Dagamac NHA, Ocenar-Bautista CE. 2023. Applying home-based experiments on locally isolated* Dictyostelium discoideum *to qualitatively demonstrate taxis of social amoebae. Cell Biol Dev 7: 75-81.* Recent years have seen a growing interest in studies on slime molds based in the Philippines, but the inclusivity of Dictyostelids has been largely overlooked. The country has very few studies investigating this category of microbial predators over the past two decades despite their ecological importance in maintaining balance in the soil ecosystem. Thus, we consolidated a multifaceted assessment that examined the behavioral response of locally isolated dictyostelids to an array of external stimuli, particularly under light- and food-induced conditions. The tail-end movement of the motile cells of the clear-cut species, *Dictyostelium discoideum* Raper, was assessed through a proxy indicator based on the fructification of the species. This was done by setting up two simple home-based experimental setups that investigate the effect of light wavelengths and prey cell viability based on the differentiation rate and one choice experiment that looks into the designation of fructification of *D. discoideum*, whether it preferentially differentiates under light or in darkness. Our setups revealed the following: (i) fruiting bodies develop in any light wavelength, but fructification is fastest in white ambient light; (ii) dead microbial cells constitute a lag in fruiting body development; and (iii) the decision-making of *D. discoideum* does not prefer photo avoidance.

Keywords: Eumycetozoans, morphogenesis, protist, soil ecology, spores

INTRODUCTION

One of the most intriguing aspects in the study of slime molds is reflected in the unique life cycle of Dictyostelids, which conforms to a picture-perfect manifestation of social cooperation among microbial communities. This underlying behavioral trait is often linked with multicellularity, microbial sociality, and faunal societies. The cellular slime molds, dictyostelids, are diverse groups of single-celled eukaryotic organisms ubiquitously found in most soils (Liu et al. 2020). Having phagotrophic nutrition that engulfs microbial communities, these microbicidal predators are considered great bioindicators of soil microbial activity. They are vital to maintaining balance in the soil microhabitat (Coleman and Wall 2015). Dictyostelids are naturally used as model organisms in studies to answer concerns regarding multicellularity, social evolution, and cell biology (Müller-Taubenberger 2013). According to a study by Ostrowski et al. (2008), it has been found that affiliated genes among samples are connected to the organisms' behaviors, such as kin discrimination. In contrast, dictyostelids are observed to have a different interaction among aggregating with isolates genetically similar to them compared to ones being more geographically distant.

Arguably, the most well-studied dictyostelid species, *Dictyostelium discoideum* Raper, has a unique life cycle consisting of vegetative, social, and sexual phases (Li and Purugganan 2011). Its vegetative cycle is characterized by food and nutrition, where free-living haploid cells prey upon bacteria in their surroundings and divide mitotically at set intervals. Upon starvation when food becomes scarce, *D. discoideum* cells stop dividing and may enter its social or sexual cycle (Kin and Schaap 2021). On the one hand, the sexual phase of *D. discoideum* is initiated through the fusion of two haploid cells of different mating types, which is followed by the cannibalization of surrounding dictyostelids cells, giving rise to a specialized structure known as the macrocyst, where recombination and meiosis take place (Schaap 2011). On the other hand, the social cycle of *D. discoideum* commences with the aggregation of starving haploid cells, resulting in the formation of a multicellular motile slug and ultimately culminates in the production of spore-bearing fruiting bodies where the cycle can be reinitiated (Marée and Hogeweg 2001). One of the defining features of the social cycle of *D. discoideum* is marked early into the cycle, where the aggregation of individual amoebae is orchestrated by cascading cAMP-signaling pathways, which are generated by an interconnected network of adenylyl cyclases (Kawabe and Schaap 2023). The movement of haploid *D. discoideum* cells is owed largely in part to its section of cyclic AMP (cAMP), which induced a chemotactic gradient for the signal relay, kinesis, and the expression of genes

responsible for development and differentiation (Eidi et al. 2021). The multicellular slug contains differentiated cells with distinct localizations-prestalk cells predominate the anterior region, while prespore cells are otherwise localized in the posterior region (Inouye 1992).

Taxis in *D. discoideum* play an underlying role in their development and differentiation, particularly evident during the migratory slug phase, where its kinesis is mediated by light (phototaxis), temperature (thermotaxis), pH (acidotaxis), and wind (rheotaxis). The interplay among these environmental gradients orients the slug towards the soil surface, simulating an optimal environment for better spore dispersal following fructification (Fisher 1997; Marée and Hogeweg 1999). Behaviors of these cellular slime molds have received greater attention in temperate countries and have exhibited a shortfall in tropical regions since they were discovered by Brefeld in 1869. Recent experiments done in the Philippines are mostly with myxomycetes with their heavy metal biosorption and enzyme production (Macabago and dela Cruz 2014; Rea-Maminta et al. 2015), whereas the studies regarding dictyostelids have done isolation to discover their food preferences (Yulo and dela Cruz 2012b). Moreover, the recently published studies also concentrated on the slug phase of dictyostelids instead of studying the part of their fructification process (Kosugi and Inouye 1989; Marée and Hogeweg 1999). These bioassays conducted for the cognitive nature of social amoebae also utilized mostly expensive and not readily accessible equipment in the laboratory, as opposed to areas such as developing countries with a lack of funds and laboratory spaces.

Hence, this study aims to develop simple experimental assays that investigate the dynamics of food based on cell viability and phototaxis that also bear decision-making capabilities in lower eukaryotes, as exemplified by *D. discoideum.*

MATERIALS AND METHODS

Isolation of the *D. discoideum* employed in this study follows a modification of the protocol established by Cavender and Raper (1965). In this technique, 10 g of each collected soil sample from a montane habitat in Northwestern Philippines was diluted in 90 mL of distilled water to yield a 1:10 dilution. Then, 5 mL of the soil suspension was diluted in 7.5 mL of distilled water to yield a 1:25 dilution. Lastly, 5 mL from this suspension was transferred to Hay Infusion Agar (HIA, boil 10 g hay in 1 L distilled water for 20 minutes), and 15 g of agar was added to the mixture to reach a final dilution of 1:50. Subsequently, 0.4 mL 24-hour old suspension of *Escherichia coli* was added to the suspension to act as a food source which rendered the culture as two-membered (Yulo and dela Cruz 2012a; Guyer et al. 2017). Typical morphology of *D. discoideum* that includes stalk, spore characteristics, and branching pattern was observed to confirm identification for the specific species alongside the bases of various journals for further verification of their traits. They are then purified via isolation onto fresh new HIA plates with *E. coli* using a combination of agar blocking and spore touch technique.

Setup I: Fructification of *D. discoideum* in varying light wavelengths

The progression of the life cycle in *D. discoideum* cultures was investigated under different wavelengths of light based on 5 setups (Figure 1): (i) white light (380-780 nm); (ii) red light (620-780 nm); (iii) yellow light (570-585 nm); (iv) blue light (440-490 nm); and (v) dark setup. Commercially available lightbulbs were placed on top of the prepared light chambers in an undisturbed area. They ensured proper measurements for all agar plates and easily opened lids for observation. Moreover, the wavelengths of each are based on the standard measurement of each type of light. All these setups are repeated in 6 replicates per setup. The dark setup utilized aluminum foil, covering the whole plate; therefore, no light could seep through the samples. The *D. discoideum* colonies were isolated in HIA plates using agar block transfer by cutting a block of agar with the organism and transferring it to another blank agar plate. They followed by the spore touch technique, which is done by using a modified small glass pipette to become needle-like and rupturing the spores using it to introduce them onto the surface of the agar. Therefore, the spore touch technique ensured accurate and unbiased results by utilizing three spores for each plate. This was followed by introducing 0.4 mL 24-hour-old *E. coli* suspension as a food source. Cultures were incubated at room temperature for 24 hours under the above light. Life cycle structures (slug, aggregate, early fruiting body, mature fruiting body) were checked and counted every 6 hours. The data was then translated into binary information to see if fruiting bodies were present (1) or absent (0) at the time interval when it was checked. A logistic regression was then performed using the software JASP to account for their numerical equivalent regarding their fructification statistically.

Setup II: Fructification of *D. discoideum* in varying cell viabilities

Growth of dictyostelids using different viabilities of *E. coli* cultures (Figure 3) was assessed by first purifying *D. discoideum* colonies through agar block and spore touch technique onto fresh HIA plates. Upon introduction of spores, 0.4 mL of 24-hour old suspension of *E. coli* was inoculated in three different setups based on the viability of the *E. coli* cultures that were used as a food source: (i) live culture; (ii) dead culture (autoclaved); and (iii) mixture of dead (heat-killed) and live culture. Plates were then incubated under diffused white light or in the dark for 24 hours at room temperature. Observations were recorded at 6 hr., 12 hr., and 24 hr. time points based on the presence of mature fruiting bodies. Similar to the statistical analysis in Setup I, the data was initially transformed into binary information to see if the fruiting bodies were present (1) or absent (0) at the time interval when it was checked. A logistic regression was then performed using the software JASP to statistically account for which of those life stages dominates on the food source (cell viability) setup.

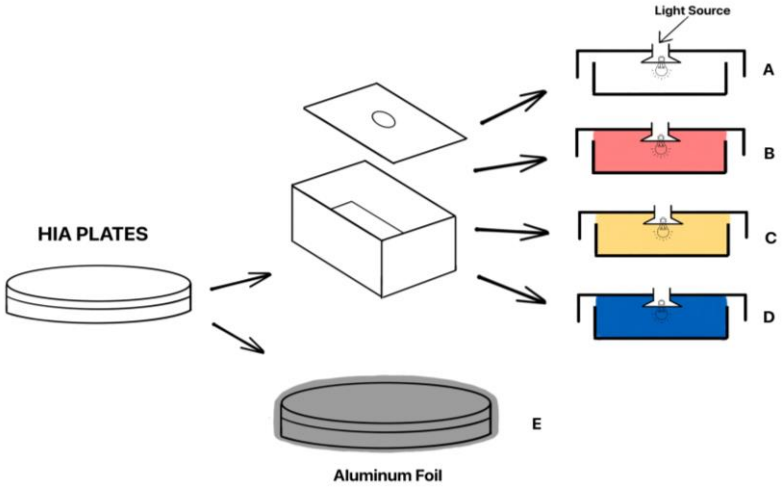

Figure 1. Experimental setup for *D. discoideum* fructification under different light wavelengths: A. White light, B. Red light, C. Yellow light, D. Blue light, and E. Dark setup

Figure 2. Sample experimental setup for observation of fructification under different light wavelengths

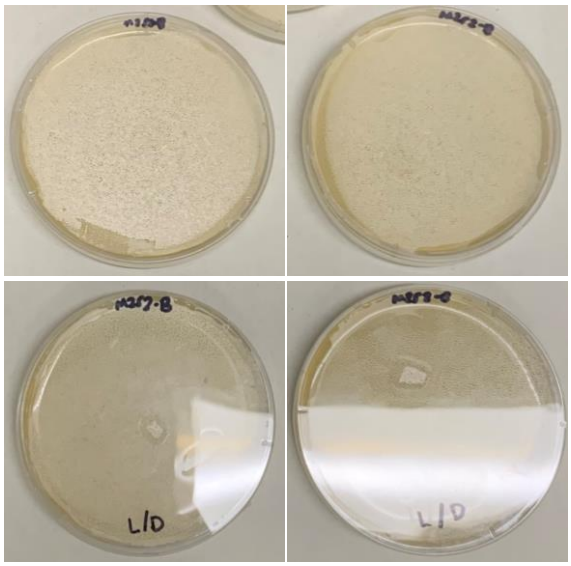

Figure 3. Sample experimental setup of agar plates in fructifying *D. discoideum* in varying cell viabilities

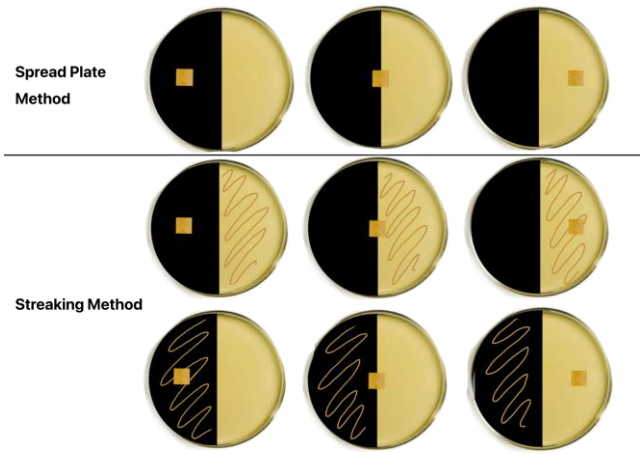

Figure 4. Experimental setup used for the phototaxis bioassay in which the plates differentiate in agar blocks and food source placement

Setup III: Light-based decision-making capabilities of *D. discoideum*

Given the wavelength of light preferred by *D. discoideum* in setup I and the preference for either live or heat-killed *E. coli* as a nutrition source in setup II. The directionality of *D. discoideum* toward or away from the light in the presence of food sources is assessed by producing HIA plates that are half-coated with black paint on both their top and bottom surfaces. This is then followed by the described isolation of *D. discoideum* from the purified plates employing the combination of agar blocking and spore-touch technique. The setup shown in Figure 2 consists of 9 plates with varying placements of the food source and the agar block relative to the light and dark halves of the plate to elucidate the preference of *D. discoideum* for either nutrition or light. Incubation is done under diffuse light which ran for 72 hours with observation points set at 24-hour intervals. The directionality is recorded in binary based on which side of the plate is

dominantly occupied by fruiting bodies-light side (1) or dark (0). This is then applied to the JASP software for logistic regression and data analysis.

RESULTS AND DISCUSSIONS

Response of *D. discoideum* morphogenesis to varying light wavelengths

In the response of *D. discoideum* isolates to different wavelengths of light, logistic regression was utilized to reveal the corresponding fructification patterns based on a 24-hour progression scale (Figure 5). Here, it can be observed that among the light wavelengths, the white light setup exhibited the earliest appearance of mature fruiting bodies (Figure 6). This can be seen as early as the 10th-hour mark. This is followed by the red light, which is evident between the 18th to 19th hour, and the yellow and blue light on the 21st hour. The dark light setup is the last setup to reveal any form of mature fruiting bodies, which can be seen between the 23rd and 24th hr mark.

Response of *D. discoideum* fructification to varying cell viabilities

Binary scores (based on presence/absence) of *D. discoideum* fruiting bodies designated in 6-hour intervals differed substantially among cell viabilities under diffused light, resulting in the earliest fructification of said species in live *E. coli* culture, followed by the mixed *E. coli* culture, and lastly, dead *E. coli* culture. The results of this experimental setup coincide with the previously discussed assay (see Setup I), which denotes a relatively delayed fructification of *D. discoideum* in the dark setup. Notwithstanding, this assay established that this phenomenon is regardless of the cell viability on which the species feeds as evaluated by how similar the fructification had appeared temporally.

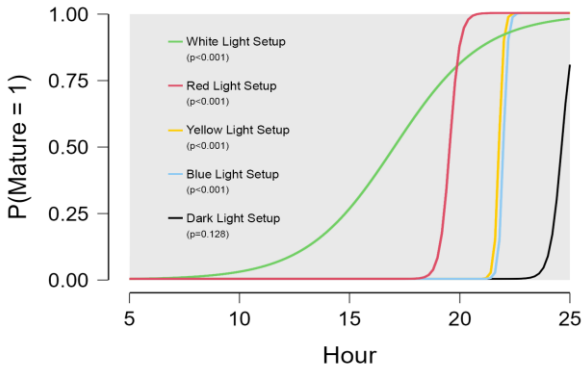

Figure 5. Fructification of dictyostelids in multiple wavelengths of light. Logistic regression curves show the probability of mature fructification bodies of *D. discoideum* based on 24-hour time progression and wavelengths of light

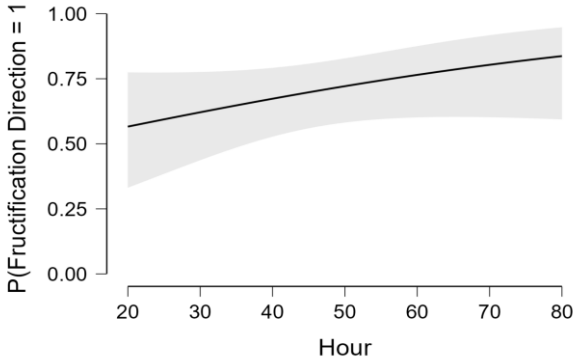

Figure 7. Fructification of *D. discoideum* under diffuse light using varying setups with plates that are half covered to portray darkness. Logistic regression plots illustrate the presence of the dictyostelids' mature fruiting bodies, contingent upon the presence of light

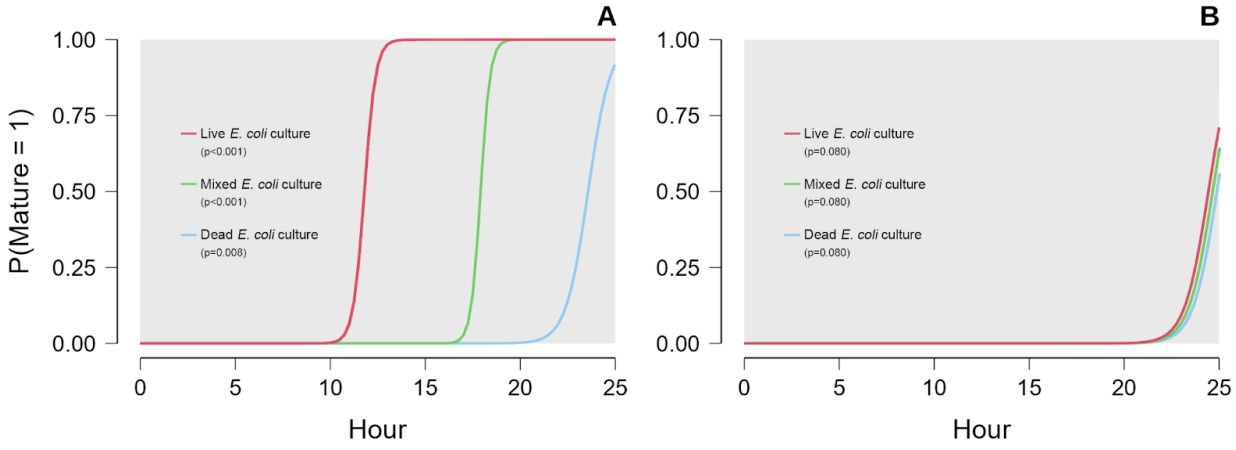

Figure 6. Fruiting body development of dictyostelids in different *E. coli* viabilities. Logistic regression curves show the rate of fructification of dictyostelids on 24-hour progression based on light setup: A. White, B. Dark

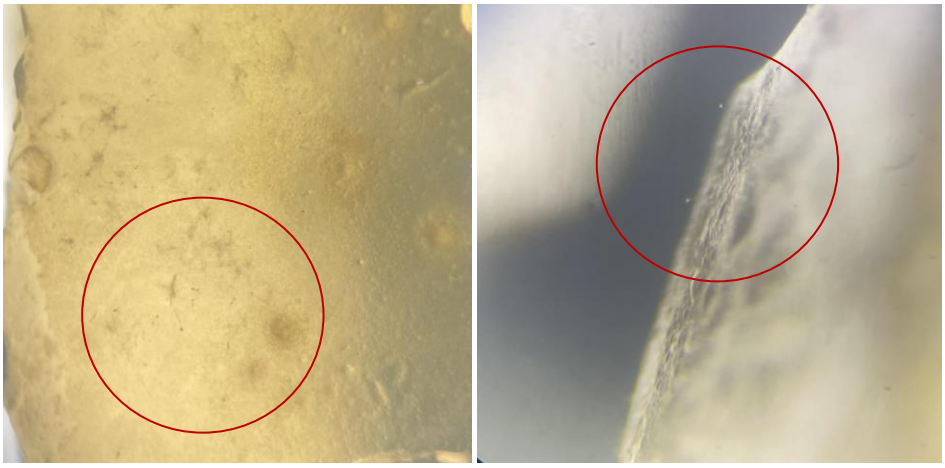

Figure 8. Two samples in different agar plates were observed to have positive phototaxis based on their placement

Light-based decision-making capabilities of *D. discoideum*

Upon subjecting the data to logistic regression analysis, binary scores were used (light=1, dark=0) based on the directionality of *D. discoideum* on the plate (Figure 7). Therefore, it demonstrates that the initial fructification of the dictyostelids, which were already expressing their preferred path, occurred at the 12th hour, exhibiting a progressive increase in abundance of positive plates until the 72nd hour. These fruiting bodies displayed that despite the method employed for food (streaking or spread plate) (Figure 4), a higher likelihood of occurrence will initially be on the side of the agar plate exposed to light, a trend that persisted consistently across the majority (70%) of the samples.

Discussion

This study showcases three simple home experiments that demonstrate the effects of certain conditions in the development of *D. discoideum.* Efforts to understand indigenous protists in the Philippines focus mainly on the taxonomy and diversity of isolates, and related studies on fruiting body development or food preferences are still limited in the tropics. Therefore, to address the basic cellular development among social amoebae using *D. discoideum* as a model, varying light intensity for growth and cell viability as a food source was tested with a simple setup that demonstrates cellular decision-making using phototaxis.

Effect of various light wavelengths on the morphogenesis of D. discoideum

Chemotactic aggregation in *D. discoideum* induces directed cell movement incorporating different forms, such as growing solitary cells and the more developed multicellular organism profile (Loomis 2015). As such, the spatial distribution of the selected cellular slime molds was subjected to different wavelengths of light and was observed for 24 hours. There has been reported evidence that suggests a photosensory transduction complex is present in *D. discoideum* involving at least five proteins: RasD, ErkB, filamin, PKB, and ErkB (Bandala-Sanchez et al. 2006). Therefore, the phototaxis behavior of the species can be attributed to the complex mentioned above, as evidenced by the morphogenic response observed in the bioassay. Throughout the lifecycle of *D. discoideum*, the motile stage, when they are slugs, becomes light-sensitive, allowing them to move towards locations with optimal light exposure to become mature fruiting bodies (Miura and Siegert 2000). With this, it was observed that the white light setup (380 to 780 nm) initiated the earliest fructification from *D. discoideum.* It contains every electromagnetic (EM) radiation in the visible light spectrum, thus cannot be limited to a specific wavelength. White light was also used in a study that displayed results for positive phototaxis using fluence-response (Hong et al. 1981). Red light (600-700 nm) consecutively showed species growth, followed by yellow light (570 nm) and blue light (450-495 nm). Hence, variation in light wavelength has successfully exhibited a significant difference in fructification.

Depletion of nutrients of *D. discoideum* follows different alternative pathways for survival – aggregation, which involves fructification, developing solitarily, or fusing and attracting cells, eventually forming a macrocyst (Schaap 2011). Such dictyostelids exhibit chemotaxis involving signaling complexes, attractant-induced cAMP synthesis, and signal relays. Present complexes in the species can be expected to make essential contributions in phototaxis, such as the protein RasD; a study involving gene disruption for RasD results in a near-total loss of phototaxis in mutant aggregates of Dictyostelium (Wilkins et al. 2000). The light deemed to influence the growth rate of the cultures, having a statistically significant difference between the multiple wavelengths (Chang et al. 1983). When exposed to dark conditions, dictyostelids form macrocysts, using ethylene as a certain trigger (Chang et al. 1983; Amagai 1984). This survival strategy, however, displays slow and inefficient germination, supporting the results of the morphogenesis assay under the dark setup. The mass movement of the cells in *D. discoideum* can suggest an oriented movement such as phototaxis

supported by different proteins but also triggers chemotactic aggregation primarily led by cAMP that completes the signaling pathways for morphogenesis.

Effect of various cell viabilities to the fructification of D. discoideum

The phagocytic nature of cellular slime molds has been extensively investigated in past literature, which designates a biomedical value to these organisms due to their generalist feeding behavior that predates diverse microbial species that include but not limited to gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Micrococcus luteus*, *Bacillus subtilis*, and *Staphylococcus aureus*) and yeasts (*Saccharomyces cerevisiae*, *Candida famata*, and *Rhodotorula* sp.) (Yulo and dela Cruz 2012b). In this experimental setup, *D. discoideum* isolates were inoculated in HIA plates with *E. coli* in varying viabilities (live culture, mixed culture, and dead culture) to assess their rate of fructification conforming to food supply pressure under light and dark setups. The assay's earliest recorded presence of *D. discoideum* was inducted 12 hours post-inoculation in the two-membered culture of live *E. coli* under diffused light. The time it took for the purified cellular slime molds to reach fructification in this setup surpasses that of related studies, which, under optimal conditions, took 24 hours on average to operate the completion of the said species' life cycle (Fisher 2001). Conversely, sporocarp formation in cultures with dead *E. coli* occurred most slowly. The underlying phenomena regarding the progression of cell aggregation in the isolates can be linked with the expression of an autocrine factor (pre-starvation factor, or PSF) by *D. discoideum* cells (relative to cell density), which triggers a series of gene expression pathways responsible for the cell differentiation and ultimately, fructification (Rathi and Clarke 1992) The availability of a bacterial food source poses an inhibitory effect on the onset of the pre-starvation response such that this response is induced following the progressive accumulation of PSF and consumption of bacteria, leading to a high PSF/bacteria ratio. Based on this conjecture, the lagged response in the setup with dead *E. coli* cells can be attributed to the increased ability of heat-killed bacteria to bind to the surface of *D. discoideum* cells more tightly than living *E. coli cells*, thus contributing to a much higher inhibitory activity thereby delaying the fructification of *D. discoideum* cells even further. This finding was denoted in a similar study conducted by Burdine and Clarke (1995) that reports greater fructification inhibitory activity of dead *K. aerogenes* than that of living bacterial cells. However, regardless of the delayed response shown by the dead *E. coli* and growth progression in all other setups, no significant difference is established in the fructification rate. This goes to show that predation of the *D. discoideum* and development of fruiting bodies is not a matter of preference towards cell viability of the food source but of the consequential mechanism that leads to the initiation of pre-starvation response brought about by various factors such as inhibition by the food source and PSF levels.

Light-based decision-making capabilities of D. discoideum

Phototaxis bears significance for multiple species as it assists in setting their course toward more favorable environments for their survivability, facilitated by their sensory and signaling mechanisms, thus enabling them to move towards conducive environments (Brodrick and Jékely 2023). This action is particularly evident for *D. discoideum*, as data suggests their response shows a preference for the lighted area, which may be due to the activated photoresponsive pigment protein and consequent intracellular signal transduction pathways (Poff et al. 1974; Poff and Butler 1974). The directional movement of dictyostelids may be influenced by light and nutrition, whereas in the particular experimental setup, despite the lack of *E. coli* on the illuminated side of the plate, the majority of the isolates still exhibited positive phototaxis eventually resulted in fruiting bodies (Figure 8). In their natural habitat, they commonly find their food beneath the soil and move towards the surface where their fructification takes place, optimizing the releasing of spores where different vectors are present for more proper dispersal (Yulo and dela Cruz 2012b).

Phototactic turning is triggered at the tip, with the slugs perceiving light exclusively within the anterior prestalk zone (Francis 1964; Poff and Loomis 1973; Fisher et al. 1984). Slugs are sensitive to light and subtle temperature gradients, enabling them to navigate toward an ideal site for fruiting (Khaire 2003). Most studies addressing the phototaxis of dictyostelids have predominantly concentrated on elucidating their response during the slug life cycle. However, extending this investigation to their fructification phase based on light stimuli is imperative to comprehend the phototactic mechanisms governing significant implications in their ecological adaptation and reproductive strategies. The presence of light was proved to be a crucial environmental cue used by *Heterostelium pallidum* (formerly known as *Polysphondylium pallidum*) to help with the transition of their slug stage to aggregation and fruiting body formation (Harper 1932). In a study by Fukuzawa (2018), *D. mucoroides* developed fruiting bodies under light conditions than in the dark, producing macrocysts (another response to food deprivation). Light can also influence the direction of the migration of *D. discoideum* by directly altering mitochondrial functions, along with responses to cAMP produced in discrete pulses synchronized in cells found at the anterior tip (Poff and Loomis 1973). Understanding the light-responsive behaviors of *D. discoideum* becomes a prerequisite to support further comprehension of the developmental biology of cellular slime molds and subsequent research regarding soil and microbial ecology.

The multifaceted behaviors of *D. discoideum* concerning light and its wavelength and cell viabilities remain contingent upon nutrient availability, phototaxis, chemotaxis, pre-starvation response, and such. This study has developed novelty by veering away from sophisticated laboratory bioassays that can be challenging among many laboratories in developing countries. As such, simple home experiments were created, which can even be employed in educational settings of the academia to demonstrate taxis as

a basic response among biological organisms. Interestingly, such simple bioassays can shed new light on many other unknown cellular ecology and adaptive significance of enigmatic groups of indigenous social amoebae.

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