

**Trail avoidance, spatial pattern
recognition, and tubule-crossing
efficiency in the true slime mold**
Physarum polycephalum

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When I was little I would get indignant when the distinction was made between "people" and "animals." I would pout and start talking taxonomy, informing some puzzled companion that actually, people are animals. When I read papers on intelligence and memory in slime molds last year, I was reminded of my childhood crusade to unite the animal kingdom. We share a common ancestry with slime molds as we do plants and animals. I began reading about emergence theory, and wondered if there wasn't something to be learned about human intelligence from slime mold intelligence. Research suggested that the intelligence of slime molds was emergent – arising from interactions among many simple and unintelligent components. I wondered, might studying the emergent mechanisms of memory and pattern recognition in slime molds elucidate the emergence of intelligence from repeated synapses in the human brain? Of course, I had a lot of studying to do. I discovered after initially designing my experiment that a whole field was dedicated to the sort of research I was performing. I took courses in computer science, graph theory, complex systems and network dynamics this year, and read many papers and books on my own in order to understand the complex nature of my seemingly simple system. I performed my wet lab work with very simple equipment on the biology floor of my high school. I designed my own assays, constructed operational definitions and parameters, and spent many 12-hour days taking data. Recently I have been working on a computational model of the slime mold in order to better understand how its behaviors emerge. A year and a half ago, when I began to consider my project, I knew I loved science but the realm of research was unknown to me. Nine months ago, when I was knee-deep in difficult data and theory, I was pretty sure I still loved science, but found research frustrating, exhausting, and confusing. Today, after refining and presenting my work for months without tiring of it, I know that science is my passion and research my vehicle of expression. I have taken amazing lecture classes, but research clarified my interest unlike any class has. I have learned in class about Heisenberg's uncertainty principle, the life cycles of stars, the hardly explicable formation of embryo from cell, and been awestruck. But research taught me about myself. My own fears, habits, abilities, and potential were thrown into relief in a new way. It astonished me that I could create new understand with an idea, some single-celled organisms, and a crate of petri dishes. There is nothing I would rather do.

Abstract: True slime molds exhibit memory and navigation that may resemble the precursors to functions performed by our complex cognition networks. *Physarum polycephalum* has been shown to use its trail as an external memory to aid navigation and to be able to anticipate temporal events. This study investigated the interactions of *P. polycephalum* with slime trail and its ability to respond to patterns. A series of experiments was conducted 1) Trail Avoidance: plasmodia were placed in Y-shaped traps with an empty arm and an arm covered in slime trail with a food incentive; 2) Spatial Pattern Recognition: monitored for directionality after following a trail of oats; 3) Tubule-crossing Efficiency: observed for efficiency behavior when it was necessary to cross slime trail. The results of these tests show that the slime trail avoidance in *P. polycephalum* overpowers positive chemotaxis towards food; that *P. polycephalum* detects and anticipates spatial patterns and that it utilizes existing tubule networks when it is necessary to cross its own trail. In addition to creating possibilities for more advanced soft computing, the complexity of the memory and navigational ability of true slime molds mirrors human centralized cognition and hints at a broader definition of intelligence.

Introduction

True slime molds exhibit memory and navigation that may resemble the precursors to our complex cognition networks. *Physarum polycephalum* has been shown to use its trail as an external memory to aid navigation (Reid et al. 2012) and to be able to anticipate temporal events (Saigusa et al. 2008). This yellow slime mold is a multi-nucleated, unicellular protist that spreads laterally by a shuttling back-and-forth of the cytoplasm called protoplasmic streaming, an oscillatory motion that has been implicated in the temporal memory of *P. polycephalum* (Miyake et al. 1994). Its search fronts are chemotropic, and when one reaches a food source the organism retracts all its other fronts and the transport tubules that extend to those fronts (Figure 1). This results in optimal foraging efficiency. When *P. polycephalum* withdraws its biomass, it leaves a network-like slime trail to which it is highly averse (Reid et al. 2012). As a result, former trails avert the slime mold from areas where it or another has already been, making slime trail effectively an external memory map that optimizes foraging (Reid et al. 2012). This chemical mapping is thought to be the evolutionary precursor to internal memory (Chung and Choe 2009). *P. polycephalum* has demonstrated many sophisticated behaviors typical of organisms with nervous systems (Bonner 2010): it recognizes and responds to temporal patterns (Saigusa et al. 2008), detects differences in length (Mori and Koaze 2013), designs optimally efficient transport networks (Tero et al. 2009), and by intracellular oscillation compares past environmental conditions to present ones (Miyake et al. 1994).

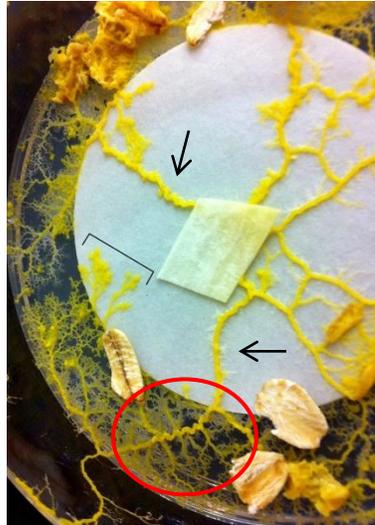


Figure 1: The structure of *P. polycephalum*. Arrows indicate tubules and bracket indicates a search front. Network structure is exemplified in red circle at bottom left. Photo by H. McShea.

P. polycephalum has been shown to have an innate temporal sensitivity that allows it to anticipate periodic events, indicating a capacity for memory of both period and phase of environmental changes, and for actions based on remembered information (Ball 2008, Saigusa et al. 2008). Saigusa et al. (2008) found that after experiencing an unfavorable stimulant three times at regular intervals, *P. polycephalum* would react at the fourth interval as it had previously although no stimulant was present then. *P. polycephalum* has also been found to maximize efficiency in its search for food. When placed on a medium that modeled the Tokyo metropolitan area with food in place of major cities and bright light (to which it is averse) in place of mountainous areas and bodies of water, plasmodia (individual *P. polycephalum* organisms) first spread their cytoplasm over the entire desirable area, and then withdrew to leave tubules that nearly exactly mirrored the Tokyo rail system (Tero et al. 2009). Similar studies have found that *P. polycephalum* demonstrates remarkable efficiency in navigating mazes and reconstructing other rail systems (Nakagaki et al. 2000; Adamatzky et al. 2012).

Recently *P. polycephalum* has found application in soft computing and biological robots. It can model efficiency in graph theory problems such as that of the travelling salesman (Zhu et al. 2013) and the creation of spanning trees (Adamatzky and Jones 2008). Electrical potentials caused by protoplasmic streaming in *P. polycephalum* have been used to produce biological Boolean gates (Tsuda et al. 2004) and to control robots (Aono and Gunji 2003, Tsuda et al. 2007). Conversely, mathematical and computational models of slime mold decision-making have been created in order to understand the mystery of their emergent intelligence (Jones and Adamatzky 2012, Traversa et al. 2013). True slime molds are ideal for forming biological circuits and for general application in emergent robotics because of the memristive quality – that is, memory of previous potentials or directions – of their tubules and the oscillatory nature of their protoplasmic streaming (Aono and Gunji 2003, Gale et al. 2013, Traversa et al. 2013). The understanding of emergence – that is, the way complexity arises from many simple components – is now thought to be central both to creating more efficient computers (Tsuda et al. 2004) and to comprehending the mechanisms of human cognition (Alivisatos et al. 2012).

This study further examined the potential for memory and navigation in *P. polycephalum* in three experiments: trail avoidance, spatial pattern recognition, and tubule-crossing efficiency. The trail avoidance experiment compared the behavior of *P. polycephalum* in a Y-shaped trap (similar to that used by Reid et al. in 2012) to that in a new “incentivized” trap. The control trap had a food source (sterile oat) at the end of each arm, and the incentivized trap had a food source at the end of only the slime-covered arm. It was predicted that plasmodia would avoid trail in the incentivized trap as they do in the unincentivized trap.

The spatial pattern recognition experiment tested the memory mechanism of *P. polycephalum*. Saigusa et al. (2008) showed that *P. polycephalum* remembers patterns in time. The present study extended that work to investigate whether it remembers patterns in space. A plasmodium that moves directionally forward after following a trail of oats may have detected the pattern of oats, and its forward directional movement after the end of the trail might indicate that it anticipates another oat. If directional movement is not significant, then trail-following might be only chemotactic, with no memory function involved.

The tubule-crossing efficiency experiment examined the small-scale interactions of slime-trail avoidance. It was known that *P. polycephalum* generally avoids its own trail, but not how it interacts with it when it has no choice. The slime trail is really a network of tubules, and so the interaction between it and a plasmodium is a series of small choices regarding individual tubules rather than a single choice regarding the whole mass of slime trail (Figure 2). It could be that the avoidance mechanism extends to these small interactions, or it could be that the plasmodium actually seeks out the tubules of old trail in order to take advantage of their efficiency. The second appeared true based on observation, and was tested more rigorously in this study.

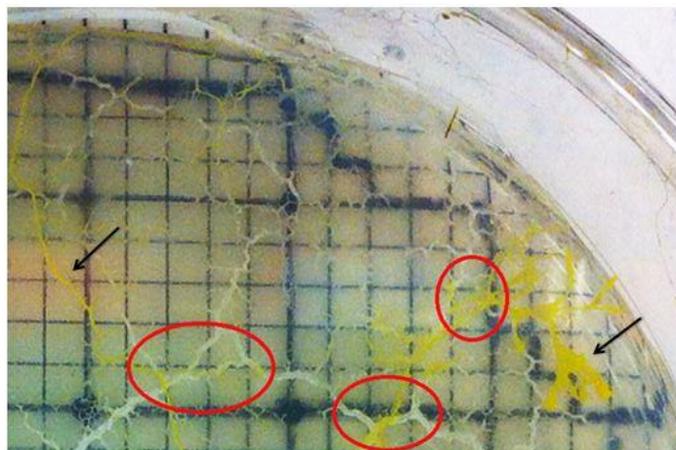


Figure 2: Interaction of *P. polycephalum* with slime trail. Red circles indicate areas of plasmodium-tubule interaction and tubule utilization; black arrows indicate areas where living plasmodium does not interact with slime trail. Grid units are 9 mm^2 in area. Photo by H. McShea.

Methods

Organisms were obtained from Carolina Biological, cultured from sclerotia (desiccated plasmodia), and maintained on 2% nonnutrient agar plates with sterile oats for food in a dark drawer at room temperature (23-25° Celsius).

Trail avoidance. A Y-shaped trap was constructed from 2% nonnutrient agar in a 100mm plate. One arm was covered in slime trail and one was left blank. Slime-covered arms were created by allowing a plasmodium to migrate across a plate for 72 hours and then cutting the arm from the agar of that plate, making sure it contained only slime trail and no living biomass.

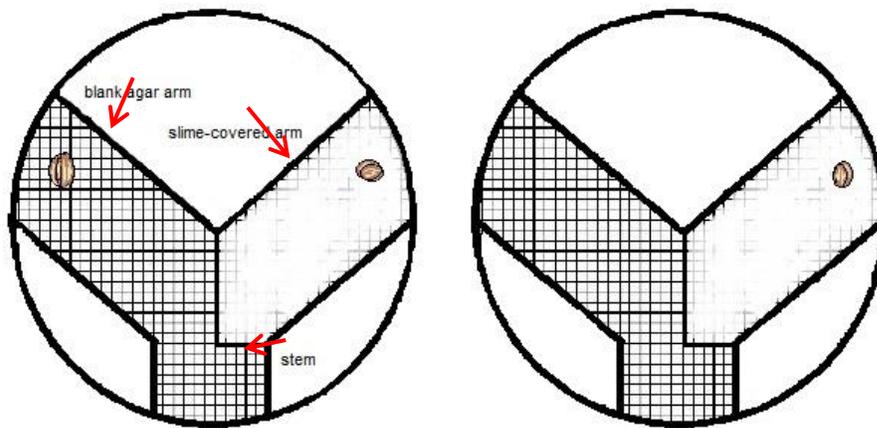


Figure 3: Unincentivized (left) and incentivized (right) Y-shaped traps. The stem of the trap is 32x15 mm, and each arm is 55 mm in length and 32 mm wide. The area within the plate which is not part of the Y-shaped trap is blank plastic plate, which is unappealing to *P. polycephalum*. The slime-covered arm was on the left in half of each group and on the right in half of each group (unincentivized n=10, incentivized n=25).

To control for left or right preference, half of traps had slime trail on the left arm and half had slime trail on the right arm. A *P. polycephalum* sclerotium was placed in the center of the trap's stem. A sterile oat (Carolina Biological) was placed at the end of each arm in control, or unincentivized, plates. In incentivized plates a sterile oat was placed at the end of the slime arm only (Figure 3). A scoring table was erected with a camera positioned 20 cm above a score card with a 3mm grid (this set-up was used in experiments II and III as well). Plates were placed on

this card and photographed every 6 hours, then photographs were analyzed and occupied grid squares were recorded. The area covered by the plasmodia in each arm was scored, as well as whether it had reached the oat. At 36 hours a final choice was recorded. If 75% of the plasmodium's area was in a region (left arm, right arm, or stem) of the trap, then that region was considered chosen.

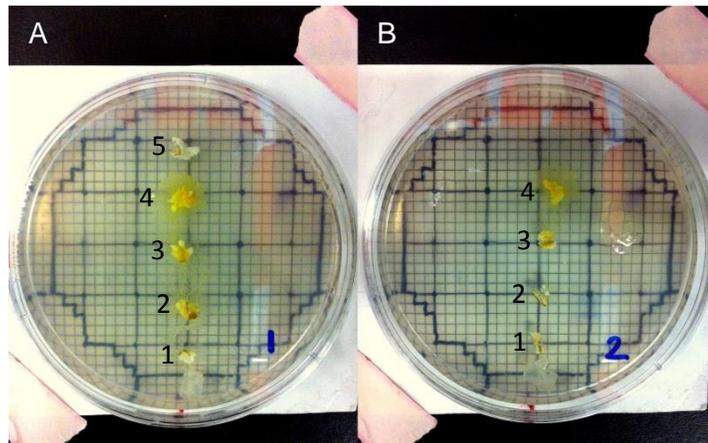


Figure 4: Set-up for experiment II. (A) Control group with a 5th oat after the trail of 4. (B) Group with 4 oats. Grid squares are 3mm^2 . Photos by H. McShea.

Spatial Pattern Recognition. Plates were prepared with 4 oats placed 1 cm apart in a line through the center of a 100mm plate with nonnutrient agar ($n=21$), and control plates were prepared with 5 oats ($n=22$) (Figure 4). A *P. polycephalum* sclerotium was placed 1 cm behind the 1st oat. Plates were monitored until plasmodia reached the 4th oat, at which point photographs were taken every hour until 3 hours after the 5th oat (or equivalent point) was reached. Lateral and longitudinal distance moved beyond the 4th oat was recorded as well as the location of the plasmodial search front in relation to the 4th oat. Directionality was defined as a ratio of movement forward over movement laterally; ratios greater than 1 indicate directional forward movement. Data analysis was performed in JMP® 10.0.2, 64-bit Edition by SAS Institute Inc.

Tubule-crossing efficiency. Sclerotia were placed on plates that had been completely covered in slime trail network by a plasmodium that had been removed (n=20). This procedure produced a plate with a patchy distribution of tubules and empty spaces among the tubules such that it was impossible for the migrating plasmodia to avoid old slime trail. After 24 hours of growth, each 9mm² grid square containing plasmodium was examined to see whether the organism was interacting (in contact with) with existing tubules or was on blank agar space. A photograph was taken, magnified, and analyzed every 6 hours from hour 24 to hour 60. Occupancy was defined as the presence of living plasmodium touching slime trail within a grid unit (figure 4). Variance in mass between plasmodia meant that occupancy ratios were most meaningfully expressed as a percentage.

Results

Trail avoidance. These tests yielded 100% slime trail avoidance in both incentivized and unincentivized groups (n=35) at the 36 hour decision point and throughout the duration of the experiment. Behavior did not differ between traps with slime trail on the left arm and those with slime trail on the right.

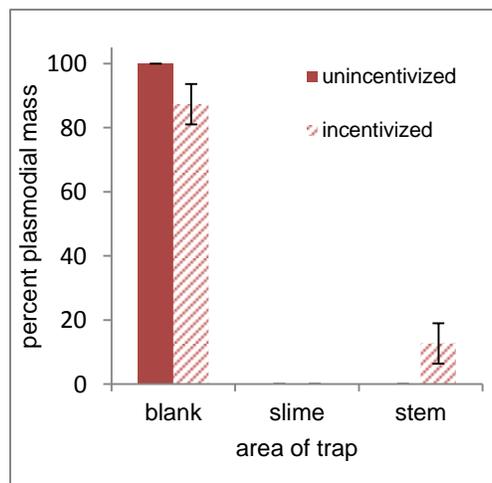


Figure 5: Distribution of slime mold mass at 36 hour decision point (unincentivized n=10, incentivized n=25). Error bars denote ± 1 S.E.M.

The plasmodia of the unincentivized group, which were given an oat at the end of both arms of the trap, had migrated entirely into the blank arm at 36 hours (Figure 5) and 9 of 10 had reached the oat in the blank arm. In the incentivized group, which was given an oat at the end of the slime-covered arm only, an average of 87.3% of plasmodial mass was found to be in the blank arm, while the rest of the organism remained in the stem of the trap (n=25). Again, no correlation was detected between slime arm side and plasmodial migration. No plasmodia migrated off the agar of the Y-shaped trap at any point in the experiment, and no plasmodia entered the slime-covered arm (n=35). Thus though movement into the blank arm was significantly different between the two, trail avoidance was completely pervasive in both unincentivized and incentivized groups.

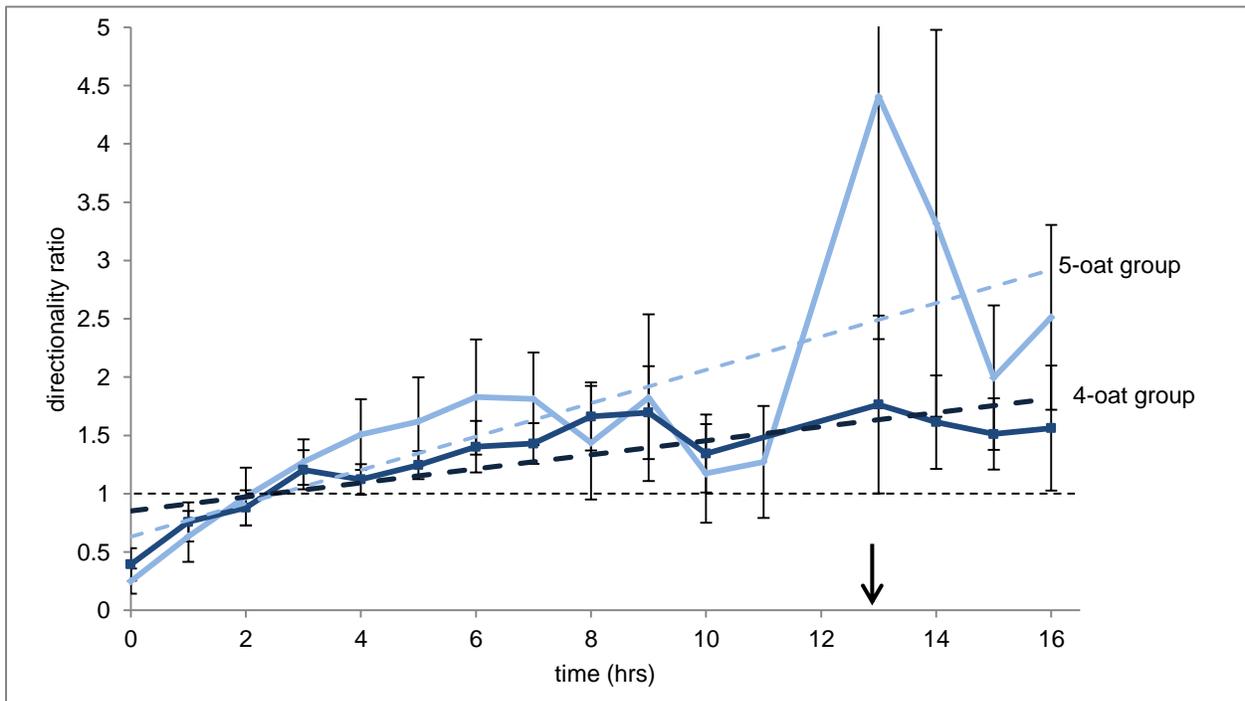


Figure 6: Directionality ratio of 4-oat (dark bottom function) and 5-oat (light top function) groups over time. Increase in directionality over time is significant for both, and directionality was not significantly different between the two groups. Dashed line indicates a directionality ratio of 1 and so a value above the line indicates forward movement and a value below the line indicates lateral movement; arrow indicates average time 5th oat or equivalent point was reached (12.92 hrs after reaching 4th oat).

Table 1: ANOVA table for the effect of oat number on linear slope of directionality ratio over time.

SOV	DF	Mean square	F ratio	Prob>F
Model	1	0.07400	3.2170	0.0811
Error	37	0.02300		

Pattern recognition. Slime molds on plates with 4 oats and plates with a 5th oat both showed a significant linear trend (table 1) in directionality ratio increase over time. Recall that directionality ratio is measured in units (3mm² grid squares) moved forward divided by an average of units moved laterally. Thus a directionality ratio >1 indicates directional movement forward, and a ratio <1 indicates directional lateral movement. Directionality-ratio trends were not significantly different between 4- and 5-oat groups (Figure 6).

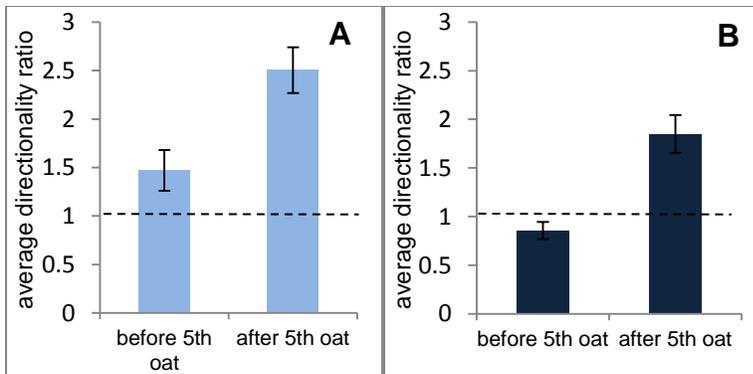


Figure 7: Average directionality ratio before reaching 5th oat or equivalent point and after reaching 5th oat or equivalent point. (A) Average directionality ratio of plasmodia in 5-oat plates before and after reaching the 5th oat (*t*-test *df*=104, *t*=2.6266, *p*=0.0099). (B) Average directionality ratio of plasmodia in 4-oat plates before and after reaching the point where a 5th oat would be if the trail continued (*t*-test *df*=74, *t*=5.3115, *p*<0.0001). Dashed line indicates a directionality ratio of 1 that indicates equal forward and lateral movement, error bars denote ±S.E.M.

Average directionality ratio in each group was near 1 before reaching the fifth oat or equivalent point (Figure 7), and greater after reaching the 5th oat.

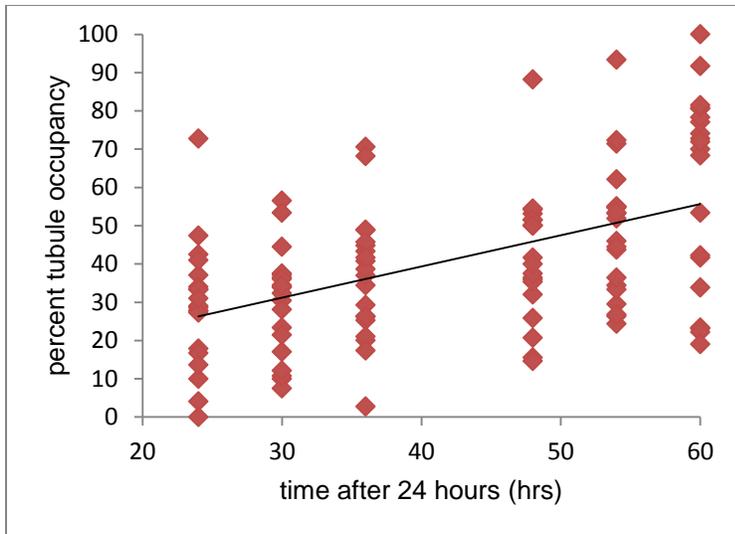


Figure 9: distribution of tubule occupancy over time and linear trend (n=20). Linear regression line $y=0.8156x+6.6947$ ($R^2=0.2582$, t -test on difference between initial and final mean $df=38$, $t=4.619$, $p<0.0001$).

Tubule-crossing efficiency. Tubule occupancy was observed to increase steadily over time.

Though tubule occupancy increase was not significant between time points, the fit of an increasing linear trend was significant (Figure 9).

Discussion

Trail avoidance. *P. polycephalum* strongly avoids slime trail in order to optimize foraging efficiency, however the results of this study indicate that plasmodia do not equate slime with lack of food (Reid et al. 2012). Three possibilities exist for why plasmodia avoided slime trail so completely. The first two are logical: plasmodia equate slime trail with depleted food and so avoid it, or plasmodia equate slime trail with unspecified undesirability and so avoid it. If the first were the case and plasmodia sensed the oat at the end of the slime arm then logically the lack-of-food avoidance mechanism would be overridden, since *P. polycephalum* has been shown to weigh variables in decision-making (Dussutour et al. 2010). However, if the connotation of the slime trail were simply undesirability, sensed presence of food would not logically override

it. Imagine a person waiting to cross a crosswalk. The signal across the street is the usual red hand, and the person takes this to mean there is the possibility of cars in the street. The person looks both ways and sees no cars; this information overrides that from the signal and so the person might cross anyway. Now suppose the signal flashes a skull-and-crossbones, or some other frightening symbol that isn't directly related to the street-crossing pathway in our person's mind. Now crossing the street is potentially unpleasant or dangerous regardless of whether any cars are coming. Lack of cars would not override this signal. While slime molds do not "think" or experience fear, they do correlate inputs as our person does (Dussutour et al. 2010).

A third option that may be analogous to the second is that the slime trail is chemically powerful and completely overwhelms or masks the "scent" of the oat. The first possible pathway for slime avoidance is unlikely because then plasmodia in incentivized traps would have migrated into the slime arm. Although it could not be determined whether *P. polycephalum* sensed the oat at the end of the slime-covered arm or not, it can be said that slime trail is an absolute logical or chemical repellent when the alternative of unexplored area exists.

Pattern recognition. Data show that after following a trail of 4 oats, *P. polycephalum* plasmodia without a 5th oat in front of them move just as directionally as those with a 5th oat. This suggests that some mechanism besides chemotaxis probably exists to guide the directionality of plasmodia in foraging behavior. As *P. polycephalum* has been shown to anticipate events in time (Saigusa et al. 2008), I propose that the guiding mechanism is a similar anticipation of "events" in space. It is possible that this is facilitated by the rhythmic oscillation described by Miyake et al. (1994). The tendencies of the 5- and 4-oat groups to move directionally towards and beyond the 5th oat

after following a 4-oat path indicate a sort of pattern-learning that could serve to optimize foraging in *P. polycephalum*.

Tubule-crossing efficiency. When plasmodia had no choice but to move across a slime-network-covered agar surface, they first explored pockets of blank agar and then consolidated their mass into the existing network of the slime trail to move across the plate. This study provides the first documentation of this characteristic of the navigating efficiency of true slime molds. Exploring blank spaces is adaptive because of their potential to contain undiscovered food, but consolidation into the larger tubules of the previous network is also adaptive, because it lends the plasmodium the efficiency of the network's previous occupant. Presumably that occupant's larger tubules had already connected desirable points in the most efficient way possible, and so by following its tracks a plasmodium could move quickly and without the process of extending large fronts and then condensing into tubules. True slime molds are widely regarded as model systems of efficiency and shortest paths (Bonifaci et al. 2012, Reid and Beekman 2013, Zhang et al. 2013, Zhu et al. 2013), and this appears to hold true for slime-trail crossing. The slime trail thus serves as an external memory map in two ways. When the alternative of unexplored area exists, it signals to plasmodia that the covered area is to be avoided because food is generally depleted in slime-trail covered areas. When avoidance is impossible, it provides a map of the most efficient path across the covered area.

Modeling

The model created was a modification of the Netlogo particle swarm optimization model. A background hill-and valley function with single optimum value was written as a simple representation of the glucose gradient exuded by a food source, and turtles sprout within a user-controlled radius centered at a random one of the function's local minima. Movement is initially random, then guided by personal best and patch availability: a turtle with less than 8 neighbors

will attempt to move forward in the direction of greatest increase of the food function, so long as that patch has not been covered by a slime trail. It was observed that these computational plasmodia move initially by extending large slow search fronts more than 20 turtles across. As these fronts approach higher function values (move up the food-diffusion gradient), they narrow and begin to divide and consolidate as those in the organism have been found to do. At low initial radii – that is, with few plasmodia or nuclei – splitting behavior is observed that is not discontinuous with biological observation.

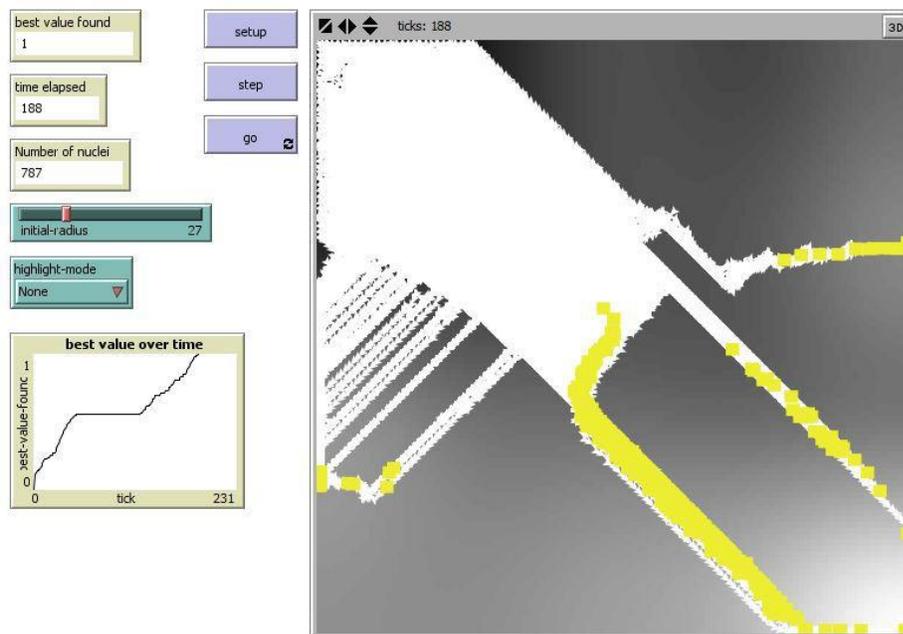


Figure 10: Initially, broad search fronts are formed. As the search front (yellow) slowly crosses the diffusion gradient (white and black) while leaving behind a chemical repellent (white), narrowed streams of plasmodia emerge.

It is exciting that such a simple rule set could be used to reproduce a hard-to-explain biological phenomenon. As the model is further refined, it can be used to determine the effects of various factors (plasmodial mass, chemical stimulus, etc.) on swarm behavior, and thereby provide insight into how these behaviors arise. At the time being, the model constructed displays some emergent behaviors comparable to those of *P. polycephalum*. An emergent swarm whose

movement is dictated by largely autonomous agents constrained only by their immediate neighborhood – like *P. polycephalum* – was successfully modeled.

Conclusion

The true slime mold *Physarum polycephalum* was shown in experiment I to have a slime-avoidance response strong enough to override chemotaxis towards food either logically or chemically. In experiment II it was found that *P. polycephalum* detects and anticipates patterns in space. Experiment III showed that when plasmodia must cross slime trail, they use existing tubules, presumably to maximize efficiency of navigation. Taken together, these tests contribute to the ongoing research goals of creating and understanding emergent systems, and elucidating the mechanisms of the memory and navigation capabilities of *P. polycephalum*. It could be conjectured based on the results of this study that the level of variance in an interaction in *P. polycephalum* indicates the cognitive complexity of the interaction. Slime trail avoidance was shown to be complete and unwavering, while there was more variance in pattern recognition and tubule-crossing results. Though this is due in part to difference in assay, it also could reflect degrees of complexity in interaction. Maybe slime avoidance is purely chemical, and pattern-recognition and tubule-crossing efficiency involve logical operations. This is where *P. polycephalum* becomes a prime model system for understanding emergence. Different levels of intelligence and complexity arise in the slime mold from simple oscillations and chemical signals. It might be that different interactions indicate different levels or types of emergent intelligence in *P. polycephalum*. By understanding how different complexities and behaviors arise in slime molds, we might be able to discover and apply general patterns of emergence in other biological systems, including the human mind. This understanding could also be applied in computation to create robotic systems that are more adaptable.

Intelligence has variously been said to be: the ability to solve problems and increase organismal fitness (philosophy of biology) (Trewavas and Baluska 2011), goal-directed adaptive behavior (psychology) (Sternberg and Salter 1982), and the achievement of behavioral sub-goals that support the system's ultimate goal in an uncertain environment (artificial intelligence) (Albus 1991). There is no widely agreed-upon single definition of intelligence, and so for these tests an operational definition will be proposed. What has been called increased fitness and the system's ultimate goal will here be interpreted as optimal foraging and maximal efficiency in resource acquisition. Problem-solving/adaptive behavior was interpreted as the capacity to make behavioral decisions based on environmental variables.

Though memory and navigation are not requisite for intelligence and do not by themselves suggest it, the mechanisms by which *P. polycephalum* remembers and navigates do suggest intelligence. In both the case of pattern recognition and tubule utilization, alternatives are presented and decisions made. Variance in response in experiments II and III actually supports this conjecture, because a true decision process would yield some bad decisions (whereas an entrained response like chemotaxis would not). Thus if intelligence is the capacity to make behavioral decisions based on environmental variables in order to acquire resources, the true slime mold *P. polycephalum* can be considered intelligent. This could be relevant to our own intelligence, especially if – as has been conjectured – our internal centralized neural networks likely evolved from an external memory similar to that of *P. polycephalum* (Chung and Choe 2009).

Further studies might aim to quantify the strength of slime-avoidance in order to compare it more effectively to other attractants and repellants, and to repeat a similar test on starved plasmodia to see if slime avoidance remains complete in starvation. The molecular mechanism of

slime avoidance has not been investigated either. In some cellular slime molds, slime trail proteins differ morphogenically from those present in the cellular membrane of plasmodia (Freeze and Loomis 1976, Sternfeld 1992). Similar differences in true slime molds could be signals for slime avoidance. Slime trail could be separated into its component macromolecules (different proteins, celluloses, lipids), and each could be spread on one arm of various Y-shaped traps to test avoidance. Protein interaction or a combination of cellulose or lipid and protein components might be responsible for the slime-avoidance response. Observation during this study suggested that *P. polycephalum* consolidates its mass into only the larger tubules of slime-trail networks, and as such further studies could examine the size threshold at which slime trail tubules provide viable guidance or efficiency. When *P. polycephalum* was shown to anticipate temporal events, it was found that pattern memory degraded over time (Saigusa et al. 2008), and so future tests could investigate whether spatial pattern memory degrades in the same way, or if there is an optimal frequency of events (oat spacing). All these proposed studies could help to synthesize a broader and more accurate understanding of emergent intelligence and could provide insight into the origins and evolution of memory as well as into the complex cognition of higher organisms.

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