REVIEW ARTICLE

Collective behaviour and swarm intelligence in slime moulds

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One sentence summary: Slime moulds are powerful model systems for studying collective behaviour and swarm intelligence.
Editor: Karine Gibbs

ABSTRACT

The study of collective behaviour aims to understand how individual-level behaviours can lead to complex group-level patterns. Collective behaviour has primarily been studied in animal groups such as colonies of insects, flocks of birds and schools of fish. Although less studied, collective behaviour also occurs in microorganisms. Here, we argue that slime moulds are powerful model systems for solving several outstanding questions in collective behaviour. In particular, slime mould may hold the key to linking individual-level mechanisms to colony-level behaviours. Using well-established principles of collective animal behaviour as a framework, we discuss the extent to which slime mould collectives are comparable to animal groups, and we highlight some potentially fruitful areas for future research.

Keywords: *Physarum polycephalum*; *Dictyostelium discoideum*; unicellular communication; problem solving

COLLECTIVE BEHAVIOUR AND SWARM INTELLIGENCE

The field of collective behaviour aims to understand how simple behavioural interactions between group members lead to coherent, sophisticated behaviour at the collective level. Collective behaviour is apparent at all levels of biological organisation; bacteria act together to form rafts (Kearns 2010), plague-locusts march cohesively in bands (Bazazi *et al.* 2008), tiny termites build immense, sophisticated structures (Bonabeau *et al.* 1997; King, Ocko and Mahadevan 2015), swarms of honeybees 'vote' democratically for the location of their new home (Camazine *et al.* 1999; Seeley 2010; Diwold *et al.* 2011) and flocks of starlings collectively sense the direction of a predator's attack by compression waves propagating through the group (Procaccini *et al.* 2011). All of these groups are governed by the principles of self-organisation; control of the system is distributed, there are no leaders to command the group or

pre-designed blueprints to guide their actions and individuals possess only information from the local area around them (Camazine *et al.* 2001). Communication is the key that allows the group to transfer information in order to collectively sense its environment, navigate through space and build structures in ways that would be impossible for individuals to achieve on their own.

Swarm intelligence is the collective intelligence that emerges at the group level from the interactions between individuals, allowing the group to generate solutions to cognitive problems that are not available to individuals (Bonabeau, Dorigo and Theraulaz 1999; Krause *et al.* 2011). In swarm intelligent systems, problem solving and decision making are decentralised – there is no centralised information processing centre. Swarm intelligence has perhaps been best studied in the social insects where there are many examples of collective problem solving. The example of an ant colony solving a maze is a perfect illustration of swarm intelligence at work (see Box 1). The same

Box 1. Ant colonies solve mazes using swarm intelligence

Swarm intelligence allows groups of organisms to solve problems that exceed the cognitive capabilities of individuals. Consider the case of an ant colony solving a maze. Many ants begin to walk the maze at random, depositing a trail of volatile pheromones behind them. The ant traffic which happens to flow along the shortest path of the maze can make more return trips (and hence lay more pheromone) over the same time period than ants which happen to walk longer paths. Ants follow the strongest pheromone trail, depositing their own trail as they go, leading to a process of positive feedback that focuses the colony's traffic on the shortest path. At the same time, the volatile pheromone evaporates at a constant rate, allowing the colony to 'forget' the other paths in the maze. A single ant in a large maze is incapable of zeroing in on the shortest path—as it walks the maze randomly, its pheromone trail evaporates before it can be followed and reinforced by future visits (Sumpter 2006). Even small colonies of ants can be unable to establish an effective pheromone trail (Beekman, Sumpter and Ratnieks 2001). Only by coordination as a large group, mediated by communication through chemical signals, can the ants solve the problem, thereby exhibiting swarm intelligence.

Figure 1. Life cycle of a cellular slime mould such as *D. discoideum*. (**a**) Free-living, individual haploid amoebae. (**b**) Aggregation of amoebae. (**c**) Mound formation. (**d**) Emergence and elongation of the tip section. Elongation continues until the structure falls over to form the migrating slug in (**e**). (**f**) 'Mexican hat'. (**g**) Developing fruiting body. (**h**) Mature fruiting body with live spore cells atop the dead cells forming the stalk. (**i**) Dispersing spores. (Drawing courtesy of E.J.T. Middleton)

phenomenon is observed in slime moulds. The interest in slime moulds as model organisms for studies of collective behaviour and swarm intelligence initiated largely from work by Nakagaki, Yamada and Tóth (2000) and Nakagaki (2001) demonstrating that the slime mould *Physarum polycephalum* was capable of finding the shortest path through a labyrinth maze. When a large slime mould was allowed to build a network covering all surfaces of a maze, with food sources placed at the entrance and exit of the maze, the slime mould redistributed its biomass to allow it to efficiently colonise and exploit the resources. Via communication between all of its parts, the slime mould withdrew from dead ends and longer paths until a single tubule connected the food sources along the shortest path through the maze. This path was one of the four possible solutions, and since Nakagaki and colleagues pioneering work, subsequent studies have dramatically increased the complexity of the maze problem. Using a Towers of Hanoi-inspired maze of 32 678 unique solutions, only 2 of which were optimal paths, Reid and Beekman (2013) showed that *Physarum* could find the optimal solution to mazes of extreme difficulty. In these cases just as in ant colonies, the slime mould as a whole begins with global knowledge of all paths through the maze and settles on the maze solution by interactions between its constituent units. Though it has never been explicitly tested, it is unlikely that a small slime mould, in the absence of any navigational cues, could begin to explore the maze at random and eventually settle upon the shortest path. Thus, slime moulds too exhibit swarm intelligence.

Here we discuss collective behaviour and swarm intelligence in two slime mould taxa: cellular and plasmodial slime moulds. While both groups are formally classified as slime moulds (Mycetozoa), they differ substantially in their organisation, communication mechanisms and life history (each outlined in Figs 1 and 2). They also differ in the way in which collective behaviour manifests. In the cellular slime moulds, collective behaviour refers to cooperation between individual cells within a swarm, while in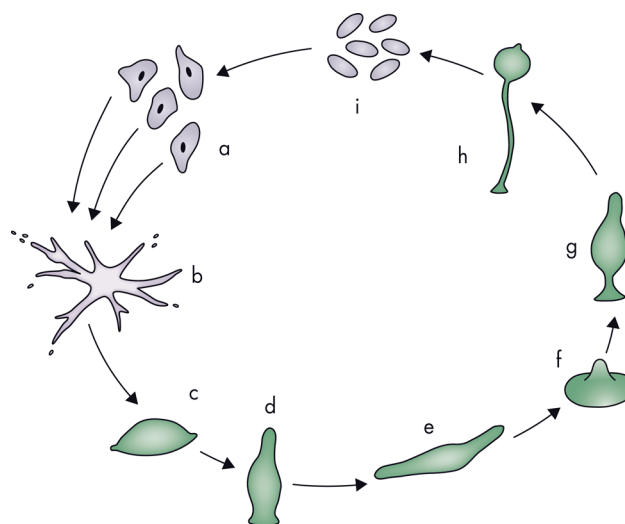 plasmodial slime moulds, collective behaviour refers to communication and cooperation between parts of the same multi-nucleate cell.

CELLULAR SLIME MOULDS (CLASS DICTYOSTELIA)

Cellular slime moulds spend the majority of their life cycle as independent, single entities capable of foraging, growing and dividing. If conditions deteriorate, individual cells aggregate to form a mobile multicellular 'slug' that can crawl to the soil surface before undergoing a remarkable transformation into a fruiting body containing many spores. Collective behaviour is a vital component in the migration and reproduction of cellular slime moulds. Formerly free-living individuals communicate with each other and coordinate towards a specific goal: to find and sporulate in conditions favourable to spore survival and dispersal. The process is coordinated primarily by cAMP signalling; although each cell is initially a free-living individual, waves of cAMP coordinate and synchronise amoeba behaviour.

PLASMODIAL SLIME MOULDS (CLASS MYXOGASTRIA)

Plasmodial slime moulds are multinucleate unicells capable of growing to over 900 cm^2 in size during their vegetative life stage, which is known as a plasmodium. These giant cells move in an amoeboid fashion, extending pseudopodia to explore the environment at speeds of up to 5 cm/hr (Kessler 1982). The general morphology of the most commonly studied plasmodial slime mould *Physarum polycephalum* is shown in Figs 2 and 3, where the exploratory 'search front' advances in a dense fan shape (Dove and Rusch 1980). Cell morphology behind the search front, and between multiple colonised food sources, is self-organised into a system of intersecting tubules where cytoplasm is rhythmically streamed back and forth to circulate chemical signals, nutrients and information (Collins and Haskins 1972). Plasmodial and cellular slime moulds offer insight into two
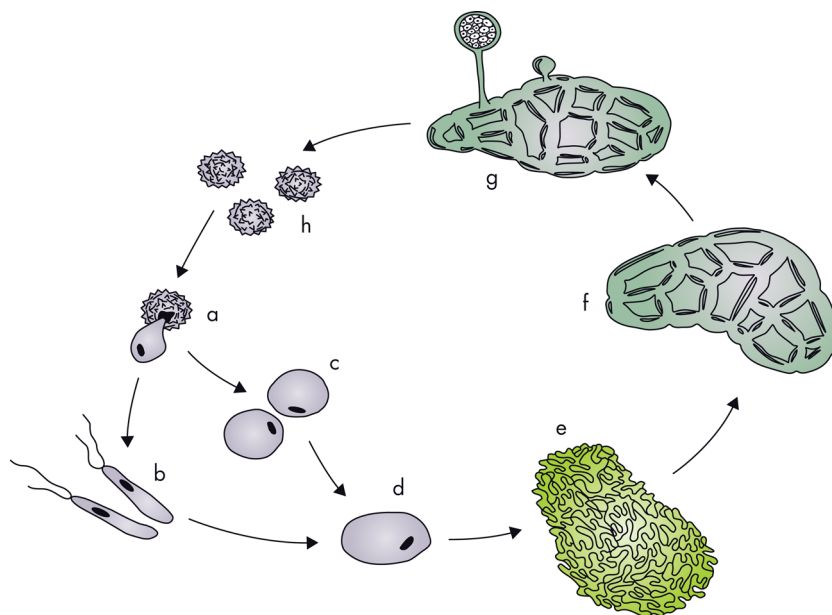
Figure 2. Life cycle of a plasmodial slime mould such as *P. polycephalum*. (**a**) Germinating spore. (**b**) Flagellated 'swarm cell'. (**c**) Amoeboid cell. Both (**b**) and (**c**) are free-living haploid stages. (**d**) Fusion of haploid cells to form diploid microplasmodium. (**e**) Microplasmodium grows via mitosis without cell fission to produce a macroscale, mature plasmodium in (**f**). (**g**) Sporangia fruiting from a starved plasmodium. (**h**) Spores for dispersal. (Drawing courtesy of E.J.T. Middleton)
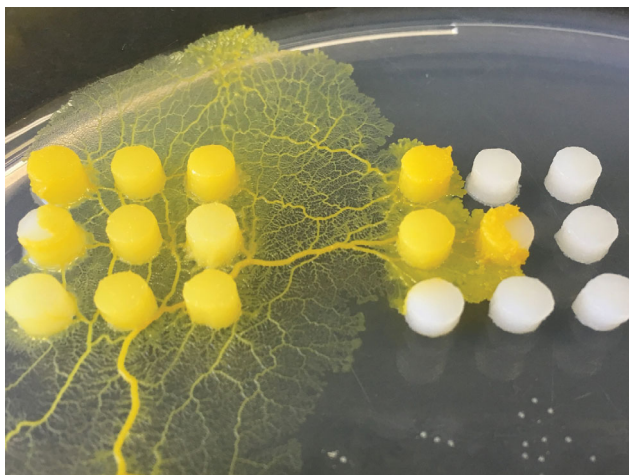

Figure 3. A *P. polycephalum* amoeba engulfing food sources as part of a problem-solving experiment. The white cylinders are food sources.

distinct strategies that have evolved to exploit the benefits of collective behaviour at the unicellular level.

SLIME MOULDS AS MODELS FOR COLLECTIVE BEHAVIOUR

Most of the theoretical and experimental work on collective behaviour has focused on animals. The conventional theory behind collective animal behaviour is based on the idea that individuals follow relatively simple behavioural rules that ultimately lead to patterns at a collective level. It is increasingly apparent, however, that even relatively small brained animals, such as ants, are capable of complex behaviours and have multiple, complex communication systems that are poorly understood. Ultimately, a complete understanding of collective behaviour would benefit tremendously from systems in which we could trace behaviour from intraindividual processes to in-

dividual behaviour to group-level behaviour. We are a long way from realising this in animals, where the neural basis of collective behaviours is scarcely studied. Slime moulds could bridge the divide between mechanisms (intercellular processes) and macroscopic collective behaviours. Both plasmodial and cellular slime moulds have been extensively used as model systems in microbiology; *Physarum* as a model for cellular locomotion and cytoplasmic streaming and *Dictyostelium* as a model for cellular signalling and development. As a consequence, there is a large body of available literature detailing the cellular and molecular processes that underlie cell behaviours.

Collective animal behaviour has conceptual roots in the study of self-organised physical systems where individual entities (particles, for example) are essentially interchangeable. In animals, however, individuals are shaped by a complex interplay of genetic and environmental factors. Here again, slime moulds have an advantage as a model system because it is possible to strictly control the influence of genetics and environment. In *Dictyostelium*, it is possible to create populations with precisely controlled genetic backgrounds. In *Physarum*, experiments are typically done using plasmodial fragments that share a common cell of origin and are thus genetically indistinguishable.

The experimental tractability of slime moulds combined with the relatively advanced understanding of the intercellular mechanisms driving cell behaviour makes slime mould a potentially powerful system for the study of collective behaviour. The aim of this manuscript is to develop cellular and plasmodial slime moulds as potential model systems for a holistic understanding of collective behaviour. To do so, we must first answer a crucial question: Are the collectives of slime moulds fundamentally different from animal collectives? Our opinion is that while they may differ in the specifics, slime moulds share many overarching features with animal groups. To illustrate this, we refer to the seminal work of Sumpter (2006), which outlines eight key 'principles' of collective animal behaviour. In the following section, we discuss the extent to which these principles are present in cellular and plasmodial slime moulds. It should be noted that these principles are neither exhaustive nor mandatory. Rather,

they provide a way to model and compare different types of collective systems.

PRINCIPLES OF COLLECTIVE BEHAVIOUR

Synchronisation

Synchronisation of behaviour is a key hallmark of many collective systems. In the cellular slime mould *Dictyostelium discoideum*, starvation causes cells to secrete the signal molecule cyclic adenosine monophosphate (cAMP). At the same time, they become sensitive to cAMP through the expression of cAMP receptors on the cell surface. Nearby cells respond to cAMP by migrating towards the area of highest cAMP concentration, and begin secreting cAMP themselves. These behaviours create a positive feedback loop, whereby the secretion of cAMP attracts more amoebae, who themselves react by amplifying the aggregation signal by secreting more cAMP. Whenever levels of cAMP increase, cells respond by temporarily producing more cAMP, followed by a brief refractory period during which they temporarily become insensitive to cAMP stimulation (Dormann, Vasiev and Weijer 2000; Gregor *et al*. 2010). The overall effect of these behaviours is the creation of unidirectional, outwardly propagating waves of cAMP that guide other cells. When cAMP levels are low, cells produce cAMP in random, non-synchronised pulses. Cells in close spatial proximity to one another eventually synchronise, resulting in multiple 'competing' pulse centres. As some oscillatory centres accumulate more cells by chance, cAMP concentrations in the surrounding areas increase, causing oscillatory centres to pulse at a faster rate, thereby releasing more cAMP into the environment. High pulse rate centres are able to entrain slower pulsing centres until, via a process of competition, a single oscillatory centre remains. Cells initially move towards the oscillatory centre as independent individuals, but as they encounter more cAMP waves they align and adhere to one another along their long axes resulting in distinct streams (Weijer 2004). Aggregation towards the main oscillatory centre continues until between 10^4 and 10^6 cells have joined the swarm. Cells entering the oscillatory centre begin to pile up forming a three-dimensional mount. Collective waves of movement still occur in the mound phase but the specific type and shape of waves (concentric rings, single and multi-armed spirals) depends on the strain (Dormann, Vasiev and Weijer 2000). At this stage, the individual amoebae have combined to create a multicellular 'slug' that is capable of directed movement.

The chemical waves of aggregating *Dictyostelium* are an excellent example of collective pattern formation. Waves induce chemotaxis towards their centres and can be either circular or spiral in shape; cells aggregating at the centre of each shape will ultimately form a spore body. In the early stages of aggregation, circular waves form as one or a group of synchronised cells (pacemakers) send out periodic pulses of cAMP; these waves tend to emerge and die out (Lauzeral, Halloy and Goldbeter 1997; Pálsson *et al*. 1997). Later in the aggregation process, circular waves can interact with one another, breaking up into spirals. Spirals do not require a pacemaker and are not extinguished once they arise. Circular waves compete with spiral waves; at low population densities, circular waves are favoured while high population densities favour spiral waves (Lee, Goldstein and Cox 2001). Spirals that ultimately win the competition go on to entrain the pacemakers responsible for the circular waves, thereby extinguishing them (Lee, Goldstein and Cox 2001). In contrast to the cellular slime moulds, which achieve collective behaviour through the aggregation and coordination

of multiple organisms, plasmodial slime moulds are single individuals, yet each is a collective of miniscule pulsing parts known as oscillators. Each tiny oscillator is a segment of tubule network or search front, which is actively expanding and contracting. The frequency of oscillation is determined by both local environmental conditions, such as the detection of food and interactions with adjacent oscillators (Durham and Ridgway 1976). When cell surface receptors recognise attractants such as food, the oscillation frequency in the cell area nearest to the attractant increases and cell surface tension decreases (Ueda, Hirose and Kobatake 1980). This response causes cytoplasm to flow in the direction of the attractant and leads to amoeboid movement in that direction. The opposite response is induced by repellents such as light and certain salts (Ueda, Hirose and Kobatake 1980). The physical coupling of adjacent oscillators means they become entrained to the frequencies of their neighbours, and information about the quality of local environments, encoded in oscillation frequencies, can be transferred to distant parts of the cell. Communication between, and recruitment by, individual oscillators is a form of distributed, collective behaviour that allows the slime mould to make complex decisions about how best to exploit its environment.

Positive feedback

When events in a system are amplified through reinforcement or recruitment, we call this phenomenon positive feedback (Bonabeau *et al*. 1997; Camazine *et al*. 2001). Once again, ants provide the classical example of positive feedback in collective behaviour (Box 1), as the isolated behaviour of a single ant (its pheromone trail) initiates a feedback loop that amplifies the signal and normalises the behaviour of the rest of the group. Positive feedback is evident in the oscillatory cAMP signalling observed in the aggregation of *Dictyostelium* amoebae, and also in the construction of efficient networks in the plasmodial slime mould *Physarum polycephalum*. In the latter case, attractants such as food increase the local oscillation frequency and initiate contraction waves propagating outwards. Tubes within the network that lie parallel to the direction of propagation are reinforced, as cytoplasm moves towards them, while perpendicular tubes decay. The reinforced tubes become thicker, the tubes that link the network via the shortest path accommodate the highest flow per unit time and the network length becomes optimised by positive feedback (Nakagaki, Yamada and Tóth 2000; Tero, Kobayashi and Nakagaki 2006). As emphasised by Sumpter (2006), strong positive feedback can be detrimental in environments where conditions change—swift and complete focus on the best option by the group can lead the group to be stuck with that option, even if it becomes the suboptimal choice at a later time. A comparison of the collective behaviour of slime mould and ants in the same maze problem by Reid and Beekman (2013) revealed that the positive feedback in *Physarum* was weaker than in the ant colonies, preventing absolute convergence on the single best solution, but allowing the slime mould to discover and utilise new solutions when they presented themselves.

Negative feedback and inhibition

Negative feedback mechanisms act to suppress positive feedback, and in doing so can help to stabilise behavioural responses, and prevent collective systems from converging on suboptimal solutions. Negative feedback is therefore vital to maintaining homeostasis in the face of fluctuating environments. Despite their key role in organising collective decision making,

negative feedback mechanisms have received far less attention than positive feedback mechanisms [a few examples include the 'no-entry' pheromone signal in Pharaoh's ants (Robinson *et al.* 2005) and the 'stop' vibratory signal in honeybees (Seeley *et al.* 2012)]. Negative feedback can also take the form of downregulation of a positive feedback signal (Czaczkes, Gruter and Ratnieks 2013). In *Physarum*, positive feedback generated through increased oscillation rate and tube size results in the engulfment of profitable food sources, while negative feedback likely generated by crowding at the food source causes engulfment to stop once the entire surface is covered (Latty and Reid, pers. obs.). Modelling studies strongly suggest that a negative feedback loop, driven by the production of the enzyme phosphodiesterase (PDE), 'turns off' intercellular cAMP production in aggregating *Dictyostelium* cells, ultimately giving rise to the characteristic cAMP oscillations observed during aggregation (Iglesias 2003; Sgro, Schwab and Noorbakhsh *et al.* 2015). Starving cells produce PDE that degrades extracellular cAMP and helps lower PDE concentrations in the interval between cAMP pulses (Jang and Gomer 2008).

In *Dictyostelium*, inhibition also plays a critical role in controlling the number of cells that ultimately make up the fruiting body. Fruiting body size is strongly shaped by natural selection; large fruiting bodies will collapse under their own weight, while in fruiting bodies that are too small, the spore mass will be too close to the ground, thus preventing optimal dispersal. During the initial aggregation phase, a large number of cells stream towards oscillation centres and in order for appropriately sized slugs to develop, this stream needs to break into smaller groups. The breakup of the stream is controlled by a protein complex called ' countin' which acts as a 'counting factor' such that cells can assess the number of cells in their immediate vicinity by secreting and measuring the concentration of 'countin' (Brock and Gomer 1999). 'Countin' works primarily by inhibiting cell-to-cell adhesion through the downregulation of cell adhesion proteins (Roisin-Bouffay *et al.* 2000) and by increasing cell motility. Fruiting body size is also modulated by PDE, which at high concentrations decreases the size of cAMP pulses, in turn decreasing the range of communication and ultimately leading to smaller aggregations (Brock and Gomer 1999).

Leadership

In animal groups, certain individuals can be leaders of the group through age, social status or possession of specific information. In the early stages of *Dictyostelium* aggregation, the waves of cAMP that give rise to the distinctive spiral and circular wave patterns are controlled by small numbers of 'pacemaker cells' that send out coordinated pulses of cAMP. These cells play a key role in organising collective behaviour in *Dictyostelium*. Growing evidence suggests that these leaders arise by chance, with no cells intrinsically destined to be leaders (Noorbakhsh *et al.* 2015).

As the individual cells aggregate, they begin to differentiate into those destined to form the stalk (prestalk cells) and those destined to form spores (prespore cells). The cells eventually form a mound with the prestalk cells self-assorting to form a nipple-shaped structure on top of the mound called the tip (Dormann and Weijer 2001)(see Fig. 1). The mound elongates until the entire structure falls over, forming the cylindrical slug with the prestalk cells in the anterior section. The prestalk cells act as leaders, inducing the rest of the cells to follow by releasing waves of cAMP that propagate backwards through the slug (Dormann and Weijer 2001). When the tip is transplanted into another slug, the prestalk cells maintain their role as leaders,

inducing a secondary axis to form as they commandeer some of the prespore cells from the host slug (Raper 1940; Rubin and Robertson 1975). Thus, leadership is a characteristic of prestalk cells that develops during their differentiation during the first stages of aggregation.

Integrity and variability

Unlike many self-organised physical systems, where components are thought to be functionally identical, the underlying components of self-organised biological systems tend to be variable. In an ant colony, for example, different individuals differ in their propensity to undertake certain kinds of work. Integrity refers to the fact that individuals will tend to 'stay true' to a particular behaviour. In *Dictyostelium*, individual cells differentiate into prestalk and prespore cells during the mound phase, and thereafter do not switch roles (except under special circumstances outlined in '*Redundancy*' below). Since 'individual' *Physarum* components are difficult to work with experimentally, the extent to which subunits display integrity or variability is unknown. However, several studies have found that equal sized fragments of the same source plasmodium display different food preferences, potentially indicating some degree of intraplasmodium variation (Latty and Beekman 2010, 2011a,b).

Stochasticity also allows a behaviourally homogeneous population to obtain behavioural variability. For example, in the early phases of *Dictyostelium* aggregation, when levels of cAMP are low, individual cells fire randomly, with no particular individuals acting as leaders (Gregor *et al.* 2010). By chance, some cells end up in areas with higher levels of cAMP; these become the pacemakers and drive subsequent aggregation dynamics (Gregor *et al.* 2010; Sgro *et al.* 2015). Thus, different behavioural roles arise from a behaviourally homogeneous population due to noise in the early aggregation period. The role of noise in determining behavioural 'roles' within *Physarum* subunits is unknown, but would be a potentially interesting area for future research.

Response thresholds

In animal collectives, individuals within the group can change their behaviour when the level of a stimulus exceeds some threshold, known as a 'response threshold'. In honey bee colonies, for example, individual bees will start fanning their wings to cool the nest when temperatures exceed their individual temperature thresholds. Having a population with a variety of individual response thresholds can promote graded (rather than precipitous) responses to changing conditions (Jones *et al.* 2004). In *Dictyostelium*, cells make several behavioural transitions. For example, increasing cell density leads to transitions from random oscillation by single cells to coordinated pulses when densities exceed a set threshold (Gregor *et al.* 2010; Sgro *et al.* 2015). While it is likely that these transitions are governed by response thresholds, to the best of our knowledge, such thresholds have not yet been characterised at an individual cell level. Individual *Dictyostelium* amoebae differ in their sensitivity to cAMP, and it has been suggested that it is a small population of hypersensitive cells that drive the self-organisation process (Wang *et al.* 2012, but see Gregor *et al.* 2010 and Sgro *et al.* 2015 for an alternative view).

Selfishness

By aggregating, individual *Dictyostelium* cells may gain the same benefits as 'selfish herds' of animals—individuals move towards

each other to lower their risk of predation (Hamilton 1971). A major protective component of the slug is the slime sheath—a mucopolysaccharide exudate, rich in protein and trilamellar cellulose that encloses the cellular core (Kessin *et al*. 1996). This slime sheath prevents nematode predators from eating the amoebae. The protective slime is exuded by the individual cells, but it is hypothesised that isolated cells cannot produce enough slime to provide a protective function (Kessin *et al*. 1996). As such, the individuals that aggregate to form slugs act selfishly but nevertheless gain the benefits of grouping.

The collective phase of *Dictyostelium*'s life cycle, the slug, acts to bring the group of cells to the soil surface to form the fruiting body and release spores for dispersal. This final stage in the collective process, known as culmination, produces a fruiting body consisting of around 80% spore cells and 20% stalk cells (Loomis 1982). The stalk cells elongate and form strong cellulose walls, eventually acting to hold the spore cells aloft, and dying in the process without producing spores of their own. Hence, altruism is a necessary aspect of collective behaviour in cellular slime moulds. Slugs often contain unrelated cells from multiple clones (Strassmann and Queller 2011), which allows for the prospect of selfish behaviour known as cheating. Cheating occurs when the frequency of a clone among the spores is greater than it was in the original mixture of aggregated cells. Cheating has been found to occur in mixed populations of wild clones (Strassmann, Zhu and Queller 2000; Fortunato, Queller and Strassmann 2003; Buttery *et al*. 2009), and slugs made from mixtures of clones do not move as far as slugs consisting of only one clone type, which may result from increased competition between the cells to remain in the posterior region of the slug (the only part to produce spores)(Foster *et al*. 2002). The fact that cheating genes have not completely outcompeted their non-cheating counterparts is most likely due to a suite of cheating control strategies (outlined in Strassmann and Queller 2011).

Redundancy

Physarum plasmodia are the epitome of redundancy; when severed from the main cell, fragments can become fully independent individuals within minutes. Although not tested, it appears that small cell fragments are capable of all the behavioural functions of a larger intact cell (Reid and Latty pers. obs.). This incredible redundancy is partially due to the syncytial nature of the plasmodium which can contain hundreds of thousands of independent nuclei (Martin and Alexopoulos 1969). *Physarum* plasmodia also display redundancy in the shape of their foraging networks. When foraging, *Physarum* can engulf and connect food sources to one another to form a foraging network. When given 36 food sources, *Physarum* built a network that included multiple links to each food source, thereby creating a network with built-in redundancy (Tero *et al*. 2010). Should any one tubule be severed, the plasmodium would not lose contact with the food source in the majority of instances.

Similarly, when slugs of *Dictyostelium* are cut into several sections of uniform cell types, the cells will reorganise to produce new slugs with both posterior and anterior characteristics, and each will contain both spore and stalk cells upon culmination (Raper 1940; MacWilliams and Bonner 1979). Thus, the individual amoebae that constitute slugs show a level of redundancy that allows them to maintain their collective behaviour, even when faced with the loss of a large part of their population.

BENEFITS OF COLLECTIVE BEHAVIOUR

The collective behaviour observed in plasmodial slime moulds is a result of communication and interaction between individual units that are spatially separated, yet genetically and physically interchangeable. Being a single organism, individual units do not have a 'choice' to behave selfishly, and individual units cannot exist on their own—collective behaviour is as much a part of the organism as its nuclei and mitochondria. As such, plasmodial slime moulds provide excellent models for mechanistic studies of how many simple, identical units can exhibit emergent, complex behaviour at the group level. The main benefit bestowed by collective behaviour to the plasmodial slime moulds (or at least to the focus species *Physarum polycephalum*) is the ability to solve complex problems (Fig. 3), such as making complicated trade-offs between speed and accuracy (Latty and Beekman 2011b), food quality and risk (Latty and Beekman 2010), exploration and exploitation (Reid *et al*. 2016) and risk and efficiency (Nakagaki *et al*. 2007). *Physarum* also anticipates periodic events (Saigusa *et al*. 2008), uses its slime trail as a spatial memory system (Reid *et al*. 2012) and constructs transport networks with similar efficiency to those designed by human engineers (Tero *et al*. 2010). The basal phylogeny of plasmodial slime moulds supports the hypothesis that ancient organisms, prior to the advent of multi-cellularity, used collective behaviour to solve complex environmental challenges, as a prelude to the evolution of nervous systems (Chung and Choe 2009; Chung, Kwon and Choe 2009). It is likely that the ancestors of today's slime moulds were capable of swarm intelligence before animals and fungi split from their common ancestor (Baldauf and Doolittle 1997), long before neurons even existed, let alone were concentrated to form anything that could be called a brain.

The case of collective behaviour in the cellular slime moulds provides a useful platform for further studies of 'selfish herd' behaviour, which has been studied exclusively in large metazoans. *Dictyostelium discoideum* cells live completely independent and successful lives during the solitary growth phase, so long as the environment provides them with enough bacterial prey. Food scarcity is the trigger that initiates their collective behaviour. In other swarming systems, such as zooplankton (Jakobsen and Johnsen 1988), food scarcity via conspecific competition is actually a cost imposed by aggregation, so the natural expectation would be for slime mould cells to show the *opposite* of collective behaviour when resources in their environment become depleted. Nor are the individuals coming together to reproduce, as sporulation is a form of encystment, with no genetic transfer between individuals or multiplication of individual units. Sporulation therefore serves primarily as a dispersal strategy to escape unfavourable conditions. Since individual cells are independently competent, and aggregation in resource-poor environments should only be detrimental, what benefits do cellular slime moulds gain from collective behaviour?

The main benefit is enhanced locomotory potential. Individual amoebae are capable of migrating short distances, travelling at most 1 cm when aggregating (Rifkin and Goldberg 2006). Migrating collectively as slugs allows the cells to travel up to twice as fast and cover distances of 10–20 cm within a few days (Kessin 2001). The massive size of slugs compared to their individual amoebae allows the group to traverse environmental obstacles such as air gaps in the soil, and detect gradients of light and heat that point towards the soil surface (Kuzdzal-Fick *et al*. 2006)—feats that would be impossible for individual amoebae to accomplish on their own. Amoebae can even disperse via slugs without following the remaining cells fate through

to sporulation, as slugs shed live cells as they move through the environment (Kuzdzal-Fick *et al.* 2006). These piggy-backing cells get a free ride via the collective navigation of the slug, arriving in new environments they could not have reached on their own, enabling them to acquire new prey, multiply and ultimately generate new slugs once the new environment has been exploited.

FUTURE WORK AND CONCLUSIONS

We have argued that slime moulds present a unique opportunity to link individual interaction mechanisms to emergent collective patterns. However, there is still much work to be done before we achieve this goal. For *Physarum*, the main area of future research will be in linking established cellular mechanisms to collective problem solving. We suspect that most, if not all, of the information needed to achieve this already exists, since *Physarum* has been a long-standing model organism for cytoplasmic streaming and cellular motility. At the same time, a growing body of knowledge details *Physarum*'s remarkable information processing abilities. A synthesis linking mechanisms to behaviour will likely require close collaboration between cellular and molecular biologists, who have an intimate understanding of the cellular mechanisms, behavioural ecologists familiar with the principles of collective behaviour and mathematicians familiar with modelling collective behaviour. It is our hope that this manuscript will stimulate collaboration and communication between these fields of research.

In cellular slime moulds, the picture is a little different. While we have a relatively good understanding of the mechanisms underlying collective behaviour in *Dictyostelium*, we know far less about whether or not the system is capable of the kind of complex decision making we observe in *Physarum*. Can *Dictyostelium*, for example, make trade-offs between conflicting cues? How do migrating slugs deal with noisy information? It may well be that the relatively limited function of slugs ('find an appropriate place to sporulate') limits the extent to which the system has evolved complex information processing. Nevertheless, future work would do well to probe the information processing abilities of migrating slugs by challenging them with more complex decision-making tasks.

Although the term 'swarm intelligence' is frequently used throughout the collective behaviour literature, there is no universally accepted definition. Here, we consider swarm intelligent systems to be those where the intelligence of the collective exceeds that of the individual. While ant colonies and beehives are often referred to as having 'swarm intelligence', the underlying assumption that the collective is better able to solve problems than the individual is rarely tested (but see Sasaki *et al.* 2013 for a notable exception). In practise, studying problem solving in individuals and collectives is often hampered by the fact that those systems that have highly organised collectives (such as social insects) are composed of individuals that evolved to function as part of a colony. When isolated, they often have difficulty performing individual tasks, similar to the way an individual neuron is not equipped to solve problems on its own. *Dictyostelium*'s transition from individuals to collectives offers an interesting way to study the differences between individual and group-level information processing. Unlike social insects, individual *Dictyostelium* amoebae have evolved the behavioural mechanisms necessary to function as individuals; as a result, meaningful comparisons between individuals and collectives are possible. Since individuals and collectives have differ-

ent 'goals' (individuals are concerned primarily with finding and consuming food, whereas collectives are searching for sporulation sites), the challenge will be to identify a problem-solving task that is appropriate for both individual cells and collectives. One possibility might be to devise situations where cells and collectives need to make choices between conflicting chemosensory information (food cues for individuals, sporulation site cues for collectives). Another option is to provide individuals and collectives with choices between resources that differ in quality and determine whether there are differences in either the speed or accuracy of decision making.

In *Physarum*, it should be relatively easy to study individual versus collective choice, as the organism can easily be fragmented into groups of varying size. The conceptual challenge is that it is unclear what would constitute an 'individual' *Physarum* (a single oscillating region? A single nucleus?). Nevertheless, it should be at least possible to examine the effect of group size on the speed and accuracy of decision making, even if we cannot directly assess individual versus collective problem solving. A battery of behavioural assays have already been devised for *Physarum* (Nakagaki, Yamada and Tóth 2000; Nakagaki *et al.* 2007; Latty and Beekman 2009, 2011a,b, 2015; Tero *et al.* 2010; Reid *et al.* 2012, 2013; Reid and Beekman 2013), so future work can take advantage of these protocols to test how group size influences problem solving.

Since plasmodial and cellular slime moulds belong to different taxonomic classes, it is perhaps unsurprising that researchers tend to limit their work to one or the other group. Both slime moulds have evolved different forms of collective behaviour, and much could be gained from a cross-fertilisation of ideas. For example, the slime trail of *Physarum* serves as a form of externalised spatial memory, allowing it to 'remember' areas it has already explored and avoid these in the future. This simple strategy not only increases the efficiency of its search behaviour, but also allows it to effectively navigate complex environments such as mazes (Reid *et al.* 2012). Migrating slugs of *Dictyostelium discoideum* leave a similar slime trail, but its function as a potential source of navigational information has not yet been investigated.

Almost all the research on plasmodial slime moulds has focused on *Physarum polycephalum*, while most cellular slime mould research has focused on *Dictyostelium discoideum*. There are many advantages to a single-species model system approach, not the least of which is the availability of species-specific tools and protocols. While we certainly think that targeted research should be aimed at these two well-known species, we also advocate that future researchers begin to investigate collective behaviour in other slime mould species. It will be very interesting to determine the extent to which different species have evolved different forms of collective behaviour, and to find out how differences in underlying mechanisms ultimately influence group-level behaviours.

CONCLUSION

Although they were initially developed for the study of animal collectives, plasmodial and cellular slime moulds embody many of the key principles of collective behaviour. At a macroscopic level, many of the behaviours we observe in slime moulds have analogues in animal groups. For example, locusts transition from a solitary phase to a gregarious phase that migrates collectively; as in *Dictyostelium*, the transition from solitary to gregarious is triggered by a lack of food (Guttal *et al.* 2012). *Physarum*

polycephalum exhibits the same kind of economically irrational behaviour as has been described in humans (Latty and Beekman 2011a). The strong similarities between disparate systems, representing different kingdoms of life, and different levels of biological organisation, suggest that collective biological systems may follow common design features.

ACKNOWLEDGEMENTS

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