 doi:10.20944/preprints202008.0554.v1

Social conflicts in *Dictyostelium discoideum* : a matter of scales

Mathieu Forget[1,2], Sandrine Adiba[1], and Silvia De Monte[1,2]

[1]Institut de Biologie de l'Ecole Normale Supérieure, Département de Biologie, Ecole Normale Supérieure, CNRS, INSERM, PSL Research University, Paris, France
[2]Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Plőn, Germany

August 21, 2020

 doi:10.20944/preprints202008.0554.v1

Abstract

The 'social amoeba' *Dictyostelium discoideum*, where aggregation of genetically heterogeneous cells produces functional collective structures, epitomizes social conflicts associated with multicellular organization. 'Cheater' populations that have a higher chance – quantified by a positive spore bias – of surviving to the next generation are selectively advantaged. Their spread is thus expected to undermine collective functions over evolutionary times. In this review, we discuss the two main approaches adopted to conceptualize social conflicts in *Dictyostelium discoideum*: describing spore bias as a property of cell populations (strains), or as a result of individual cell choices during the developmental process. These two points of view are often held equivalent and used interchangeably. While the population-level view allows for more direct evolutionary inference, however, the cell-level interpretation reveals that such evolutionary predictions may be modified if developmental mechanisms, such as dependence on the environment and intrinsic unpredictability of cell fate choices, are taken into account. We conclude by proposing a set of open questions that in our opinion lie at the core of a multi-scale description of aggregative life cycles, where the formulation of predictive evolutionary models would include cell-level mechanisms responsible for spore bias alongside population-level descriptors of multicellular organization.

 doi:10.20944/preprints202008.0554.v1

1 Introduction

Many unicellular organisms spend at least part of their lifetime within associations that have a functional role, as they allow their composing cells to resist stress, to be defended by predators, or to engage in collective behaviour. In some cases, multicellular organization has been integrated in life cycles that alternate periods when amoebae grow as single cells, and phases - typically triggered by nutrient depletion - where initially sparse cells gather in more or less complex multicellular aggregates (Grosberg & Strathmann, 2007; Du et al., 2015). The transition from a chiefly unicellular life style to aggregative life cycles occurred at least six times independently along the tree of life and in all major eukaryotic clades (Parfrey & Lahr, 2013; Du et al., 2015). Its repeated emergence suggests this form of multicellular organization is not the outcome of serendipity, but may reflect general organization principles (Grosberg & Strathmann, 2007; van Gestel & Tarnita, 2017).

The social amoeba *Dictyostelium discoideum* has been widely used to identify such principles and to explore the action of selection on cellular collective organization. The evolutionarily stability of its multicellular life cycle, despite conflicts among cells that adopt different social strategies, makes it a model organism for addressing both the maintenance of cooperative behaviour (Strassmann & Queller, 2011; Medina et al., 2019) and the evolutionary emergence of new levels of organization (van Gestel & Tarnita, 2017).

D. discoideum's life cycle comprises a vegetative phase, where cells grow in isolation, and a collective social phase induced by starvation (Kessin, 2001). The multicellular phase starts with aggregation, when cells converge towards aggregating centers by chemotaxis guided by the gradient of a signalling molecule, cAMP (Devreotes & Zigmond, 1988; Fisher et al., 1989). Eventually, most cells in the population belong to one multicellular aggregate, or mound, composed of tens of thousands individual cells. Later, mounds elongate into slugs, chemotactic and phototactic worm-like structures with the ability to sense and move towards bright and dry environments, like the soil surface (Raper, 1940; Bonner et al., 1950). Here, slugs produce fruiting bodies that can be picked up by insects and dispersed (Smith et al., 2014). Starting from the mound stage at least, cells proceed to differentiate into several tissues (Early et al., 1993; Kessin, 2001). Because of their prevalence and their ease of detection, most attention has been given to two cell types: spores, that seed the following generation, and stalk cells, that support the spore mass. Analogous to somatic cells in metazoans, stalk cells die.

Giving up one own's descent to favour spore dispersal is considered the most extreme degree of altruistic behavior, and raises the question of the evolutionary stability of such arrangement (Strassmann & Queller, 2011). In 'paradigmatic' multicellular organisms with single-cell bottleneck followed by clonal growth, conflicts between different cell types (e.g. between normal and cancer cells (Aktipis et al., 2015)) can get resolved by purging entire cell lineages (Godfrey-Smith, 2009). Their disruptive effect is instead enhanced when multicellular aggregates are genetically heterogeneous (Buss, 1982). In *Dictyostelium*, differ-

 doi:10.20944/preprints202008.0554.v1

ent genotypes can coexist within a same fruiting body both in the wild and in the lab, indicating that this organism has found solutions to curb the effects on fitness of such conflicts and their evolutionary impact.

The first fundamental issue when considering the action of selection on multicellular organization is how to measure differential fitness between co-aggregating genotypes. In *Dictyostelium*, reproductive success can be evaluated at the end of the life cycle, when cells are terminally differentiated into spores or stalk. Spore cells are indeed the only fraction of the population that is able to survive long periods of starvation, and reproductive success hinges upon their production. Moreover, cells that die forming the stalk provide a clear advantage to spores. Due to these features, shared also by other organisms such as *Myxobacteria* (Velicer et al., 2000), genotypes that tend to form an increased fraction of spores when mixed in chimerae are commonly called 'cheaters'. Strains that – being found in lesser proportion in the spores – get exploited by virtue of their disproportional contribution to the stalk, are called instead 'cooperators'. Practically, social strategies are assessed in chimerae obtained by mixing, prior to aggregation, cells belonging to two different strains. Spore bias is then typically quantified as the percentage of spores of one strain in the spore pool, relative to the percentage of cells of that strain in the initial mix (Kuzdzal-Fick et al., 2010, 2011; Gilbert et al., 2007) (this assumes that the spore-to-stalk ratio within fruiting bodies is constant, but see (Buttery et al., 2009, 2010) for generalizations). All else being equal, then, a cheater strain will see its frequency increased in the population of vegetative cells ensuing from spore germination, thus in the following generations. In the domain of evolutionary biology, most attention has been devoted to understanding why in *Dictyostelium* selection of cheater strains does not doom collective function altogether.

Here, we take a step back and examine the observational bases of different conceptual models for social interactions in *Dictyostelium* – as defined based on spore bias – and discuss their evolutionary implications. A fundamental distinction we make is the level of organization - the strain or the cell - at which social strategies such as cheating and cooperation are attached. Even though these two representations are often used interchangeably, we argue here that they are not necessarily equivalent. We first consider binary interactions between strains as genetically encoded strategies, where cheating is paralleled to the defective strategy in the Prisoner Dilemma's game. Then, we look at behavioural strategies at the cell level, where spore bias results from the developmental determination of cell fate. We first consider how phenotypic heterogeneity affects cell social behavior in clonal populations. Then, we discuss the interplay between phenotypic heterogeneity and genetic differences in determining social behaviors in chimeric aggregates. We finally focus on challenges that lay ahead for integration of cell- and population-level processes into formal frameworks, and for inclusion of mechanistic descriptions into predictive evolutionary models. Here, we take a step back and examine the observational bases of different conceptual models for social interactions in *Dictyostelium* – as defined based on spore bias – and discuss their evolutionary implications. A fundamental distinction we make

 doi:10.20944/preprints202008.0554.v1

is the level of organization - the strain or the cell - at which social strategies such as cheating and cooperation are attached. We first consider models depicting binary interactions between strains as the result of genetically encoded social strategies. Then, we look at behavioural strategies at the cell level, where spore bias results from the developmental determination of cell fate. We finally focus on challenges that lay ahead for integration of cell- and population-level processes into formal frameworks, and for inclusion of mechanistic descriptions into predictive evolutionary models.

2 Strategies of interacting strains

Spore bias is traditionally measured in chimerae where two different strains are mixed – often in equal proportions – at starvation, after which cells undergo only one more cell division. When strains are equivalent, thus, the fraction of spores belonging to one strain is expected to be equal to the proportion of that strain in the initial mix. Deviations from this 'neutral' composition of the spore mass quantify the degree of cheating of one with respect to another strain (Kuzdzal-Fick et al., 2010, 2011; Gilbert et al., 2007). Cheating behaviour is thus defined at the level of interacting populations of cells, connecting directly the genotype of the strain to the outcome of the social interactions. For instance, 'obligate cheaters' are genotypes, found in natural isolates or derived from lab strains, that have a positive spore bias when mixed with other strains, but that cannot develop alone (Buss, 1982; Kuzdzal-Fick et al., 2011; Ennis et al., 2000)). Such strains always have a reproductive advantage over different genotypes, however they cannot disperse if they meet a strain of their same kind.

Based on this population-level definition, the reproductive output in chimerae can be formalized as a game opposing individual strains: a cheater genotype exploits a cooperator genotype's contribution and therefore enhances its own representation in the following generation. Such a situation is represented by the Prisoner's Dilemma, a two-player game whose chief feature is that cheating is always better than cooperating, irrespective of the other player's strategy (Hofbauer & Sigmund, 1998). Evolutionary game theory predicts that, after many rounds of the game in which players meet at random, cooperators will be outnumbered by cheaters.

The problem of maintaining or evolving cooperation in two-players games has found several solutions in the general framework of game theory (Nowak, 2006). In the case of *Dictyostelium*, the most commonly invoked means of preventing the invasion of cheaters is kin selection, where high genetic relatedness is the key condition for cooperative behavior to be favoured by natural selection (W. E. Kerr, 1950; Strassmann & Queller, 2011). According to Hamilton's rule (Hamilton, 1964), in order for altruistic genes to increase in frequency, the level of genetic relatedness r between the cooperator and the recipient of the cooperative act must exceed c/b, where c is the cost paid by the cooperator and b is the benefit received by the recipient. Originally, the relatedness r in a population was defined, based on genetic identity by descent, as the probability that two

 doi:10.20944/preprints202008.0554.v1

random individuals share the same allele at one given social locus. Subsequently, other measures of social interaction bias towards individuals that carry the cooperative allele have been proposed as proxies for relatedness (Queller, 1994), most notably the frequency of cells of a given type in the population. In general, as long as cooperators have a sufficiently higher chance of interacting with cooperators than with cheaters, thus assort positively, the benefits of the cooperative act can outpace the individual costs (Fletcher & Doebeli, 2009). Consistently with the definition of strain strategies, relatedness or assortment are strain-level statistics, that describe the average behaviour of cells of a given genotype in the population. Based on this strain-level point of view formalized by the sociobiology framework, it is commonly considered that strong relatedness explains the maintenance of cooperative social behaviour against the spread of cheating in aggregative multicellular organisms (Strassmann et al., 2000; Medina et al., 2019).

Genetic assortment between strains

Evidence of genetic assortment in *D. discoideum* populations both in natural and artificial environments has been put forward in support of the importance of kin selection. In natural populations, assortment was quantified based on genetic identity. Relatedness between strains was estimated by polymorphism in microsatellite sequences, even though these were not strictly located in genes responsible for social behaviour. These molecular studies found higher levels of relatedness within fruiting bodies compared to soil samples (Fortunato et al., 2003; Gilbert et al., 2007). Further studies were conducted in the laboratory, where chimerae are obtained in standardized conditions and the relative frequency of the different strains controlled. Mixing natural clones, Strassman and co-workers observed that, in 15 pair-wise combinations, all formed chimeric fruiting bodies (Strassmann et al., 2000). In spite of such weak barriers to co-aggregation, the level of mixing between couples of strains was shown to quantitatively depend on their genetic distance. By looking on a finer scale at the composition of fruiting bodies, genetically distant strains were found to segregate more than closer ones (E. A. Ostrowski et al., 2008). Similar observations were also realized in different species, *D. purpureum* and *D. giganteum*. In *D. purpureum*, chimerae composed of up to nine natural clones were observed in the lab (Sathe et al., 2010). In individual fruiting bodies, strains of the two species were found to mix to varying degrees, with strains genetically farther apart segregating often in separate multicellular aggregates. All these studies support the idea that even though strains may be unable to completely exclude each other from groups, they can bias group composition so as to reduce genetic dissimilarity.

Assortment of related cells, measured at the population level by genetic relatedness and other proxies, can be achieved in different ways.
First, it can be the consequence of 'passive' mechanisms, that do not require any particular adaptation for strain-specific recognition. Passive sources of assortment are thus most relevant for explaining how multicellular organization

 doi:10.20944/preprints202008.0554.v1

emerged from unicellular ancestors, before more sophisticated means of cell-cell signalling was set in place. These mechanisms include limited dispersal in a spatially extended environment, whereby populations are structured in clusters of genetically identical individuals (Hamilton, 1964). Limited dispersal can for instance explain why a regional pool of species is not fully represented in single fruiting bodies that assemble locally aggregated cells. Non-specific differences in physical properties, such as adhesion or motility can moreover result in non-uniform mixing and sustain cooperative behaviour even when cells are initially uniformly distributed in space (Garcia et al., 2014, 2015; Joshi et al., 2017; van Gestel & Nowak, 2016).
Second, high assortment can be achieved through active sorting that makes cells group preferentially with cells of the same genotype, a mechanism also known as 'kin discrimination'. *D. discoideum* possesses a number of specific genes involved with cell-cell adhesion that are expressed during both aggregation and development, and that are central to multicellular organization (Glöckner et al., 2016). In particular, the family of Tiger genes coding for trans-membrane proteins provides a lock-and-key mechanism for adhesion between cells that carry a same allele (Benabentos et al., 2009). Analogous to self *versus* non-self-recognition mediated by major histone compatibility loci, Tiger genes display a 40-fold elevation in genetic diversity compared to the rest of the genome (E. Ostrowski et al., 2015; Flowers et al., 2010; Benabentos et al., 2009). Such a high degree of polymorphism is consistent with the idea that recognition with high genetic resolution is essential to achieve efficient segregation between co-aggregating strains (E. A. Ostrowski, 2019). It is moreover considered as a signature of the selective advantages conferred by novel genetic variants of 'green beards', that may compensate the loss of function expected when linkage between adhesion and cooperative behaviour is lost (Gruenheit et al., 2017).

Adhesion is certainly a source of assortment, but the timing of its deployment during *D. discoideum* life cycle is crucial to connecting assortment at the level of the population to the underlying genetically-determined cell features. Indeed, population-level biases are affected both by the way cells get distributed among multicellular aggregates and by their developmental path once the aggregates are established. One way to inquire whether adhesion affects one more than the other is to look at an intermediate level of organization, that of single aggregates or fruiting bodies. If cell sorting occurs already at the time of aggregation, cells of genetically distant strains will be chiefly found within genetically uniform aggregates of the same type. Measures of the variance in single-fruiting bodies composition suggest that segregation between genetically distant strains can be high relative to closer strains (Gruenheit et al., 2017; E. A. Ostrowski et al., 2008). Spore bias, measured as a population-level average over multiple fruiting bodies (as realized by washing whole dishes before spore count), may not be in such a case an appropriate measure of conflicts within multicellular fruiting bodies, which requires instead quantification of the composition of single spore heads (Buttery et al., 2009). On the other hand, in cases when cells do not segregate and aggregate composition reflects the initial mix, differential adhesion could still drive sorting during multicellular development. Tiger genes

 doi:10.20944/preprints202008.0554.v1

are involved both in segregation during aggregation (Benabentos et al., 2009) and in auto-organization within chimeric aggregates (Gruenheit et al., 2017), but the respective contribution of these processes to spore bias is still largely unexplored.

Evolutionary dynamics of genotypes

Observations both in natural and artificial settings thus establish that *Dictyostelium* strains do not assort as they would in the conditions assumed in null models that predict the evolutionary success of cheating. The resulting high degree of genetic similarity within multicellular aggregates is considered sufficient for supporting the evolution of costly cooperative behaviour (Strassmann & Queller, 2011). A quantitative application of Hamilton's rule is problematic though, given that all parameters involved in the theoretical relationship are hard to measure directly. An alternative empirical way to test the theoretical framework is then to observe the variation of strain frequencies on time scales longer than a single aggregation cycle.

Experimental evolution assays have been conducted by repeating cycles of aggregation and dispersal in conditions that are as close as possible to producing random cell encounters ('low relatedness' conditions) (Kuzdzal-Fick et al., 2011). Strains that increased in frequency in 30 cycles also produced a larger share of spores than the ancestral strain which was used to seed all the experimental lines. Estimation of the mutation rate from ('high relatedness') lines propagated clonally in a separate experiment moreover indicated that the change in frequency, estimated via a population genetics model, was not quantitatively compatible with random drift (Kuzdzal-Fick et al., 2011). It was therefore explained as a consequence of the selective advantage conferred by cheating. The realization of the tragedy of the commons, where only obligate cheater strains would persist, was however not observed, possibly because the experiment was not long enough. Other experimental evolution assays however did not support the hypothesis that selection always favours cheating strains. In an experiment involving mixtures of environmentally collected strains, 10 cycles of aggregation-dispersal were conducted starting in conditions of high and low cell density (Saxer et al., 2010). As expected, high density conditions associated to higher relatedness, and resulted in a smaller variability in the strains dominating the population. Unexpectedly, however, evolved strains at high relatedness were not cheated by the winner of the low relatedness treatment, as would have been predicted by the theory of kin selection, suggesting that evolution may be driven by factors that are independent of assortment.

Other than direct evolutionary experiments, methods from population genetics have been deployed in natural populations to reveal selection acting on cheating. The genomic signatures of 'social genes', *i.e.* genes preferentially expressed during the multicellular phase of the life cycle, display signs of rapid evolution (high rate of non-synonymous mutations) compared to the rest of the genome (Sucgang et al., 2011). This has been subsequently interpreted as the effect of diluted selection, occurring when the expression of social genes is tem-

 doi:10.20944/preprints202008.0554.v1

porally restricted to the multicellular phase of the life cycle (de Oliveira et al., 2019). When this effect is taken into account, previously reported differences in the level of polymorphism between pre-stalk and pre-spore genes (Noh et al., 2018) are no longer detected, making it impossible to conclude on the role of kin selection in shaping the evolution of social interactions in *D. discoideum*.

In conclusion, several observations are consistent with the expectations of the sociobiology framework, notably that maintenance of cooperation is facilitated by genetic homogeneity within aggregates. The evidence that this theoretical approach is able to describe evolutionary dynamics in aggregative multicellular species is however still contrasted. Unfortunately, kin selection formalism is not informative on what assortment-generating mechanisms or processes should be considered as the prominent cause of the evolution of higher-level organization. Moreover, the complexity of estimating population-level parameters as well as the fact that such parameters neglect intermediate-level population structure (such as groups) weaken the predictive power of this framework in forecasting the outcome of repeated cycles of co-aggregation. Models considering that cheating is purely the result of interactions between genetically distinguished strains have been therefore complemented by other approaches, where the focus is moved to a lower level of organization, that of single cells. As we will discuss in the next section, this opens the way to alternative conceptual perspectives, that include non-genetic effects of cell physiology, of the environment, and of the cell's social context.

3 Cell-level strategies

A central feature of aggregative multicellularity in *Dictyostelium* is that genetically identical cells differentiate into spores or stalk (see (Brown & Firtel, 1999) for a review of the underlying molecular processes). Viewed at the cellular level, cheating of one cell that is part of a binary chimera is then associated to a probability of becoming a spore higher than for cells of the other strain. This alternative point of view has implications in the way strategies are conceptualized. With the exception of few obligate cheater strains, that only form spores and can be thought of playing 'pure strategies', a cell-level strategy now reflects any single player's 'choice' between two alternative fates, one allowing survival, and the other leading to death. In game theory, this scenario corresponds to 'mixed strategies', defined by the probability of adopting one among a number of alternative pure strategies (Hofbauer & Sigmund, 1998). When the genetically-determined strategy is the probability of cheating by becoming spore, game-theoretical models predict that under many circumstances selection should not exclude cooperators, but rather lead to the coexistence of different social behaviours (Matsuda & Harada, 1990; Hudson et al., 2002; Uchinomiya & Iwasa, 2013).

Consideration of mixed instead of pure strategies is not the only change that has to be adopted in describing cell-level social conflicts in game theoretical terms. Another feature is that 'players' that interact in multicellular aggregates

 doi:10.20944/preprints202008.0554.v1

are no longer strains, but individual cells. The collective outcome of their individual behaviour is then more appropriately described by n-players games rather than as an instance of the Prisoner's Dilemma. Such games naturally introduce frequency-dependent payoffs and non-linearities (Gokhale & Traulsen, 2014). Even in the presence of social conflicts, the resulting evolutionary dynamics can thus depart qualitatively from the tragedy of the commons.

In this section, we consider the experimental evidence that supports different conceptual models of how cells make their strategic choices and determine their developmental fate. First, we discuss the effect of phenotypic heterogeneity on cell fate in isogenic populations, then we consider context-dependent cell behaviours in chimerae.

3.1 Phenotypic differences in isogenic cultures and spore bias

Genes are at the basis of cellular behaviour and dictate how external inputs are translated into specific phenotypic states. However, genotype alone does not entirely explain the probability that single *Dictyostelium* cells turn into a spore or contribute to the stalk. In isogenic populations, developmental fate is indeed also affected by phenotypic heterogeneity, and in particular by non-genetic differences among cells that were established before the beginning of the multicellular phase (Chattwood & Thompson, 2011). When, instead of belonging to different strains, the sub-populations that are mixed have the same genotype but distinct phenotypic states, one can still measure spore bias as discussed in section 1. The effects of non-genetic factors on social behaviour can thus be quantified by comparing the number of spores produced by each culture with the expectation from their proportions in the initial mix.

Physiological state

Already 50 years ago, it was reported that cultures grown on glucose have a positive spore bias when mixed with cells from a similar strain (carrying a marker mutation that does not affect development) grown in poorer medium (Leach et al., 1973). The quality of nutrients provided during vegetative growth has since then been confirmed to affect not only cell fate at the end of development (Takeuchi et al., 1986), but also the probability to join aggregates at all (Dubravcic et al., 2014).

Similarly, cells at varying degrees of starvation show a differential tendency to become spores. Cultures that have been starved for four hours before aggregation have a positive spore bias when mixed with freshly harvested cells of the same strain (Kuzdzal-Fick et al., 2010). Based on the aforementioned effects of food quality, starved cells should, on the opposite, be less represented in the spores. To solve this contradiction, it has been proposed that timing of aggregation prevails over nutritional state in determining cell fate (Kuzdzal-Fick et al., 2010). Indeed, pre-starved cells commit earlier to enter the multicellular

 doi:10.20944/preprints202008.0554.v1

phase and therefore have a head start in development. The advantages provided by anticipation are however bounded and relative to the history of the other sub-population. Cultures that have been starved at very discrepant times indeed segregate during aggregation and undergo distinct waves of multicellular development (Kuzdzal-Fick et al., 2010).

Differences in quality and duration of feeding result in heterogeneity of the physiological state of the cell, which can bias later developmental stages. Cells whose intracellular pH was artificially decreased, for instance, were found to be biased towards the stalk pathway (Kubohara & Okamoto, 1994). Similarly, concentration of Ca^{2+}, bimodally distributed in freshly starved amoebae, has been correlated with spore bias: lower intracellular calcium concentration is associated to a higher probability to become spores (Azhar et al., 1996). Finally, it was recently reported that cells with higher concentration of ATP before aggregation maintain such differential throughout development and eventually produce stalk cells (Hiraoka et al., 2020).

Intracellular calcium can reflect variations in the environmental concentration of folic acid, a chemo-attractant produced by bacteria (Yumura et al., 1996). Similarly, food quality, duration of starvation or intracellular concentrations in natural conditions are largely independent of the cell genotype. The effects of physiological heterogeneity on spore bias evidenced in laboratory conditions may thus have evolutionary implications also for wild populations.

Cell cycle phase

In addition to environmental variability, phenotypic heterogeneity may also arise as a consequence of intrinsically variable cellular processes. Previously mentioned physiological conditions affecting cell fate biases, indeed, appear to be linked to one other through their relation with cell cycle phase. Cytosolic Ca^{2+} concentration (Azhar et al., 2001; Jang & Gomer, 2011) and intra-cellular pH (Aerts et al., 1985) have been shown to vary during the cell cycle. This is also the case for two factors that play a central role in cellular organization within the multicellular slug, whose effects we discuss below in greater detail: sensitivity to a family of diffusive compounds responsible for differentiation into stalk cells (DIF) (Thompson & Kay, 2000) and cell motility (Walmod et al., 2004). The phase of advancement in the cell cycle could thus result in phenotypic heterogeneity within an isogenic population, and influence the ultimate developmental choice of any given cell.

Numerous studies support the notion that cell cycle phase at the onset of aggregation influences spore bias. The correlation between cell cycle phase in synchronized cultures and the frequency in the spore pool has been known for forty years (Zada-Hames & Ashworth, 1978). Experiments using cell cycle inhibitors (Gomer & Ammann, 1996) or release from stationary phase (Weijer et al., 1984) as means to synchronize cell cultures confirmed that cell cycle position at starvation reflects into developmental cell fate. By using single-cell RNA-seq Thompson and co-workers recently provided a molecular characterization of such observations (Gruenheit et al., 2018). They analyzed the transcriptome of

 doi:10.20944/preprints202008.0554.v1

an isogenic vegetative population of *D. discoideum* strain AX3 and identified more than 1600 genes that can be divided, based on their level of expression, in two clusters. One cluster is specifically expressed in cells that are in phase S/M, whereas the second is composed of genes expressed in late G2 phase cells. Then, using pre-spore and pre-stalk markers, they mapped cell cycle position to cell fate and showed that M/S phase cells mostly differentiate into stalk cells, whereas late G2 cells are enriched in spores. Consistently with a direct link between cell cycle phase and cell fate, the ratio of G2 to M/S phase cells in a population is around 4:1, which closely matches the ratio of spores/stalk cells within a fruiting body (Gruenheit et al., 2018).

Cell cycle phase effects on development lead Maeda and colleagues to propose the existence of a checkpoint in the late G2 phase, where cells bifurcate between growth and differentiation (Maeda, 2011). In cultures synchronized by a cold shock (Ohmori & Maeda, 1987), indeed, cells starved in mid-G2 phase (before the checkpoint) initiate aggregation more rapidly than cells starved in late G2 phase, and are more likely to become spores.

The correlation between cell cycle advancement and developmental timing was further supported through PCA analysis on single-cell transcriptomic data (Antolović et al., 2019). As early as at the mound stage, cells display heterogeneity in developmental advancement. The principal components of such variability also capture differences in cell cycle stages. Cell cycle phase is thus considered to be a determinant factor - though minor in amplitude compared to overall changes in the transcriptome throughout development - in determining eventual developmental choices (Antolović et al., 2019).

A consequence of the correlation between cell cycle phase and developmental fate is that cell-level strategy – the probability that a cell becomes a spore – is set based on phase positioning relative to the population, rather than being fixed once and for all by the strain's genotype. Consistent with this view is the capacity of cells to reprogram' their development when their local environment is perturbed. For instance, if one part of a slug is experimentally removed, cell fate decision are reassessed (Raper, 1940). Similarly, once extracted from their social context by dissolving a slug into fresh medium, cells de-differentiate and resume unicellular growth (Soll & Waddell, 1975) in a way that is highly robust to mutations in developmental genes (Nichols et al., 2020).

Before discussing the possible roles of cell cycle phase in chimerae, we address the question of how phenotypic differences established at the beginning of aggregation can determine, much later, cell social behaviors.

Cell phenotype through development

Several mechanisms are believed to be involved in transforming phenotypic differences at the beginning of multicellular development into divergent cell fates. Single-cell tracking (Houle et al., 1989; Araki & Maeda, 1995; Jang & Gomer, 2011; Gruenheit et al., 2018) and mathematical models (Maree & Hogeweg, 2001; Umeda & Inouye, 2004) indicated two main (non-exclusive) ways whereby cell fate gets established.

 doi:10.20944/preprints202008.0554.v1

First, cells could be primed to respond differently to differentiation signals that are equally available to all cells within an aggregate. Among the signals exchanged by co-developing cells, Differentiation Induction Factors (DIF) affect cell fate by inducing differentiation into stalk (Kay et al., 1983; Jang & Gomer, 2011). While extracellular concentrations in the mound gets readily homogenized by diffusion and cell mixing, cells differ in their responsiveness to DIF (Chattwood & Thompson, 2011). This parameter is correlated with cell physiology at the onset of aggregation. For instance, cells fed on a medium containing glucose, as well as those in a late phase of the cell cycle exhibit a lower DIF responsiveness with respect to cells grown without glucose and those in an early cell cycle phase (Thompson & Kay, 2000). Moreover, DIF responsiveness is also affected by heterogeneity in intracellular calcium established before the multicellular phase. Of the two subpopulations with low and high cytosolic calcium content observed in freshly starved cultures, only the latter increases the uptake of extracellular Ca^{2+} upon stimulation with one molecule of the DIF family, DIF-1 (Azhar et al., 1997).

Second, the geometry of the aggregate could impose or reinforce patterns through direct cell-cell contacts or morphogen gradients. Positional information within the mound and the slug is associated to the cell's eventual developmental fate. Phenotypic heterogeneity at the onset of aggregation could hence bias terminal differentiation by influencing where a cell is located within multicellular aggregates. The correlation between cell position and cell fate appears to get established as soon as cells organize into streams by attaching head-to-tail during their migration towards the mound (Fujimori et al., 2019). Maeda suggested that cell positioning during aggregation plays a central role in connecting cell cycle phase and developmental fate (Maeda, 2011). When facing starvation, cells that have passed the checkpoint between growth and differentiation would stop dividing and act as autonomously pulsing aggregation centres (Wang et al., 1988). By attracting cells at other stages of the cell cycle, they would gain a head start in establishing their position in the mound (Maeda, 2011), and subsequently gather at the center of the aggregates, a position linked with pre-spore fate (Huang et al., 1997).

As well as in the mound, position along the slug axis is associated to different cell fates in the future fruiting body: cells at the back of the slug tend to turn into spores, whereas most of those at the rear form the stalk. In a clonal population, cells may sort during slug migration on the basis of motility (Strandkvist et al., 2014) or adhesion (Houle et al., 1989; Garcia et al., 2015). Even though the exact role of differential motility and adhesion in establishing positional information is not yet completely worked out, their involvement in cell fate determination was confirmed by a recent single-cell transcriptomic study. Genes involved in cell motility and, to a lesser extent, in cell-cell adhesion were indeed found to be up-regulated in pre-stalk relative to pre-spore cells, indicating a likely role of cell sorting in establishing tissue organization (Antolović et al., 2019). Both calcium concentration (Azhar et al., 1996) and pH (Van Duijn & Inouye, 1991) differences, moreover, can result in heterogeneity in cell motility, which is also known to vary with the phase of the cell cycle (Walmod et al., 2004). Anal-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 August 2020 doi:10.20944/preprints202008.0554.v1

ysis of a handful of trajectories suggests that, corresponding to bimodality in calcium concentration at the onset of development (Azhar et al., 1996), also cell motility could be bimodally distributed (Goury-Sistla et al., 2012). It is appealing to think that initially faster cells would position themselves to the front of the slug, thus becoming stalk with a higher probability. However, this hypothesis would require a permanence of the population partition in faster and slower cells after starvation. Such permanence could be associated to differentials in concentration of ATP (Hiraoka et al., 2020). This compound indeed is involved both in cytoskeleton-mediated cell contraction (Clarke & Baron, 1987) and is consistently higher in pre-stalk cells, that show enhanced speed and cAMP chemotaxis (Hiraoka et al., 2020). Observations of vegetative cells, on the other hand, show that motility can change relatively rapidly in time, and reflect the rate of encounters with other cells (d'Alessandro et al., 2018). Moreover, recent observations of cells from disaggregated slugs observed two sub-populations moving at different speed, but these did not correspond to pre-stalk and pre-spore sub-populations (Nichols et al., 2020).

During development of a clonal population, a multiplicity of initially heterogeneous cellular phenotypic features can be translated into settled social roles. In this process, relative rather than absolute variability determines the social strategy of one cell. It is to be expected that whenever multicellular aggregates contain more than one genotype, contingency and local environment will keep affecting cell fate.

3.2 Cell-level strategies in genetic chimerae

Strain-level approaches, discussed in section 2, are based on averages over a whole population. Considering that such statistics are chiefly determined by the genotype is a reasonable approximation as long as phenotypic heterogeneity is small, or if it has negligible effects on the probability that cells develop into spores. However, we saw that non-genetic variability has a potentially important effect on establishing spore bias. We shall discuss now how cell behaviour in chimerae can result from the concurrence of genetic and environmental factors and what are their evolutionary implications.

Cellular 'lotteries'

As we just mentioned, it is well known that cell fate is influenced by several factors that are independent of the cell's genotype. We thus start by considering the extreme scenario where spore bias is chiefly determined by phenotypic variations. As a consequence, the genotype may not be the key observable to predict the evolutionary dynamics (Nanjundiah, 2019). Such scenario, represented by 'lottery' or 'musical chairs' conceptual models, has been invoked as the source of resistance to cheating: if one strain's contribution to the spores in chimera is not determined by its genotype, cheater strains cannot increase in frequency over multiple generations (Strassmann & Queller, 2011; Rainey, 2015).

 doi:10.20944/preprints202008.0554.v1

Several of the factors affecting cell fate in isogenic populations could contribute to loosening the link between the genotype of a cell and its probability of turning into a spore. A potential intrinsic source of unbiased phenotypic heterogeneity is rooted in the necessity of any cell to progress through the cell cycle. If, as discussed for monoclonal populations, the cell cycle phase is not synchronized in the population, and it sets the probability of forming a spore, then the fate of any focal cell will be essentially determined by the time when starvation occurs. Like in a "musical chair" game, the moment when aggregation starts is out of one cell's direct control, making cell fate choice a stochastic decision independent of the genes. As long as cell cycle phase is uniformly distributed in the overall population, a cell indeed cannot predict what its phase is relative to cells of its own or another strain. Population-level observations indicate that cells are generally desynchronized, as cultures can be synchronized by cold shock, release from stationary phase or treatment with drugs that block the cell cycle (Maeda, 1986; Araki & Maeda, 1995; Weijer & Duschl, 1984). Moreover, a quantitative mathematical model of phase drift along lineages indicates that cells loose rapidly synchronization in typical *D. discoideum* culture conditions (Gruenheit et al., 2018), even though they may not in other circumstances (Segota et al., 2014).

Unpredictability in cell-fate decision could moreover be the consequence of external rather than internal contingency: independent of the genotype, some cells may happen to be better fed than others after having encountered different amounts of food, or food of different quality. Such contingencies are expected to affect every cell in similar manner before aggregation starts. As a consequence, reproductive success would not be a heritable trait associated to any given genotype. Nanjundiah and co-workers proposed that the 'quality' of a cell when it faces starvation, established from a combination of genotype, environment and historical contingency, underpins the probability of developing into a spore (Zahavi et al., 2018). The stalk would be composed chiefly by cells that are anyways condemned by their poor nutritional status, while spores would comprise cells that have a higher chance of survival. In this perspective, cheating would not be expected to swipe through the population, and the conflicting nature of the interactions between spores and stalk would be also downsized.

Environmental fluctuations and bet-hedging

Pure lottery models seem to be unrealistic, as cell fate is certainly affected by genes. Evolutionary outcomes similar to lottery models can be nonetheless obtained when the probability of becoming a spore is genetically encoded, but it varies in different environments. Even though in any given situation spore bias can be predicted based on the genotype, its frequency in the long term depends on the sequence of conditions cells experience. Such contrast between short-term and evolutionary success is commonly encountered in microbial species, where multiple phenotypes – including those that appear maladapted to a specific environmental context – coexist within monoclonal populations (Ackermann, 2015; Grimbergen et al., 2015). Single-cell stochastic transitions between phenotypes

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 August 2020 doi:10.20944/preprints202008.0554.v1

with different adaptive value allow strains to cope with a varying environment by hedging their bets among several alternative behaviours (Kussell & Leibler, 2005). Effective evolutionary neutrality can then follow from temporal averaging over a series of unpredictable environmental changes, which evens up the performance in chimerae of different strains. Instead of supposing, as in lottery models, that cell fate is independent of the genotype, bet-hedging models assume that all strains face the same type of reproductive uncertainty. Let us consider the previously discussed case of different cell quality (Zahavi et al., 2018). Even if cells of a given strain have a higher quality in one specific environment, such relative advantage may not be conserved in other environments. Averaging over multiple aggregation-dispersion cycles in variable conditions, different strains may end up having the same overall success.

These concepts have been specifically applied to study the evolution of the so-called 'loner' strategy, adopted by *Dictyostelium* cells that do not join at all multicellular aggregates. In games traditionally opposing cheating to cooperation, addition of such a strategy is sufficient to avert the tragedy of the commons (Hauert, 2002). In *Dictyostelium*, the loner strategy has been proposed as a way to prevent the invasion of cheaters (Dubravcic et al., 2014; Tarnita et al., 2015). The potential relevance of non-aggregated cells has been supported by experimental observations both on lab and wild strains. Different studies reported that a sizeable fraction of cells is invariably found outside aggregates, and that these cells are able to start vegetative growth when nutrients become available (Dubravcic et al., 2014; Tarnita et al., 2015; Rossine et al., 2020). The partition of a population in loner and aggregated components was modelled as the consequence of a cell-level stochastic choice, where the genotype determines the probability of staying alone (Dubravcic et al., 2014; Tarnita et al., 2015; Martínez-García & Tarnita, 2016). Even if the genotype is supposed not to impact cell fate choice within aggregates, different strains still have different spore production because of their differential contribution to aggregates. Numerical simulations showed that frequent replenishment of nutrients favours genotypes that have a larger fraction of solitary cells, whereas more aggregative types that commit to social behaviour have an advantage in times of famine. On longer time scales, environmental unpredictability and limited dispersal therefore lead to coexistence of multiple genotypes in spite of differences in their social behaviour.

Cell-level response to social context

The variability experienced by cells does not reduce to extrinsic fluctuations that affect all cells equally. Even before multicellular groups can be clearly distinguished, the local environment of one cell is indeed dictated by other cells present within the same local neighbourhood. Similarly, in multicellular aggregates cells interact with each other through chemical signals (as reviewed in (Loomis, 2014)) and mechanical forces. Such local 'social' environment is particularly important to determine cell fate, thus strain dominance, in chimerae. When strategies are considered at the level of single cells, a manifestation of

 doi:10.20944/preprints202008.0554.v1

social context-dependence is that spore bias depends not only on the genotypes (as discussed in section 2), but also on the frequency of cells belonging to one or another of the co-aggregating strains.

Numerous studies indicate that frequency-dependent changes in spore bias is the rule rather than the exception in chimerae of both *D. discoideum* (Gilbert et al., 2007; Madgwick et al., 2018) and other dictyostelids (Sathe & Nanjundiah, 2018). Strains identified as cheaters by mixing equal amounts of cells of two genotypes thus have variable success against a cooperator counterpart when their relative proportions are changed. In particular, when they make up most of the population, the proportion of spores that a cheater strain produces may be upper bounded if the stalk/spore ratio is maintained. Moreover, in a chimera composed of a mutant that does not produce stalk cells (Buss, 1982) and a strain that develops normally, an increase in the proportion of cheater cells may cause a reduction of the size of the stalk relative to the volume of the spore head. Fruiting bodies with large heads may be more prone to collapse and would then reduce the potential to disperse of both strains, thus undercutting the reproductive success of the cheater itself.

Importantly, when frequency-dependence is taken into account in game-theoretical models for interacting strains, repeated rounds of co-aggregation can differ qualitatively from the tragedy of the commons in their evolutionary predictions. For instance, if spore bias is positive when cheaters are rare and negative when they are common, the evolutionary dynamics will lead to regimes of coexistence of opposite social strategies. Though context-dependent cell behaviour is often neglected when evolutionary projections are based on strain-level dominance of genotypes, a few mechanisms involving density or frequency of cells have been recently considered in their population-level effects on spore bias.

As discussed earlier in this section, a possible source of indirect effects on the proportion of spores produced by one strain in a chimera is the partition between aggregated and non-aggregated cells. When exploring the mechanistic bases of this partitioning, the probability of being a loner was found to depend, other than on the genotype, on cell density and environmental factors such as the hardness of the agar substrate (Rossine et al., 2020). Such dependence on both the biotic and abiotic context was explained by a mathematical model where the cell decision to aggregate is stochastic and conditional on a locally established quorum. In a genetic chimera, the probability that one cell of a given strain aggregates therefore depends on the nature and the proportion of other co-aggregating strains. For instance, strains that tend to aggregate less can still contribute to aggregation of another strain, and they do so more efficiently when they are more dense. The end result of cell self-organization in groups is then frequency dependence, which can sustain coexistence of multiple strains over evolutionary times (Rossine et al., 2020).

Cells can also modify their behaviour within multicellular aggregates, in response to proportions of co-aggregating strains. Within slugs, for instance, the concentration of diffusive compounds was suggested to be the key mediator of cell-level frequency-dependent fate determination (Parkinson et al., 2011). Re-

 doi:10.20944/preprints202008.0554.v1

sponsiveness to diffusible stalk-inducing factors (e.g. DIF) in particular, but also their production, indeed allows to predict the social linear hierarchy of strains that was previously established based on cheating ability (Buttery et al., 2010). When considering the mechanistic bases of cellular strategies, complex behavioural patterns – whereby strains would adjust their behaviour depending on the social partner – were therefore suggested to follow from simple principles of context-dependent decision-making, that naturally lead to frequency-dependent interactions (Matsuda & Harada, 1990; Hudson et al., 2002; Madgwick et al., 2018).

In conclusion, spore bias is a population-level manifestation of cell-level mechanisms that span ranges of genetic vs epigenetic determinism and that respond differently to the abiotic and biotic context. In pairwise interactions, the contribution of different factors with distinct and independent effects on strains fitness was quantified by an analysis of variance (Buttery et al., 2009). Variation in contribution to the spore head in binary chimerae of natural clones was partitioned in three components: indirect genetic effects of the social partner's genotype, direct effect of the strain's own genotype, and epistatic interactions between the genotypes of the two partners. The first component reflects the result of strain-level competition. The others connect to cell-level behaviour in a monoclonal population and in a chimera (other than the previously mentioned strain-level effects), respectively. The strain genotype (*i.e* the second component) was found to explain 57.6% of the variation in spore production, thus dominating the two terms linked to social interactions between strains. The importance of epistasis (23%) moreover suggests that the social context is as important as strain-level effects (Buttery et al., 2009). For such statistical analysis to be used in evolutionary studies involving other strains or conditions of aggregation, one would however need a mechanistic model able to explain how the partition in these three orthogonal components is realized, and what is the origin of the epistatic effects.

Discussion

In this review, we examined the experimental support to different formal descriptions of the relationship between cell genotype and collective phenotype, as quantified by spore bias. The different conceptual models lead to distinct evolutionary predictions on the effect of spore bias over multiple cycles of aggregation. When social behavior is defined at the strain level, sociobiology predicts that in the absence of positive assortment, natural selection leads to the tragedy of the commons: strains that are over-represented in the spores will spread in frequency as long as cycles of aggregation-dispersal continue (but they could be brought to an end by the cheater's own success). In a cell-level perspective, this scenario can be mitigated to varying degrees, depending on how much uncertainty single cells face. Indeed, spore bias is now the population-level consequence of cell decision-making, that is affected both by the genotype and by the environment (including other genotypes present in the chimera). The existence

 doi:10.20944/preprints202008.0554.v1

of cooperative behaviours, including self-sacrifice, becomes in this alternate perspective less, if at all, paradoxical. Indeed, stochastic cell fate determination can diminish the effects of selection in favour of 'cheaters'. Moreover, context-dependent cell choice can lead to frequency-dependent spore biases. Even in simple models, frequency-dependence can avert the invasion of unconditional cheaters, and pave the way to more complex evolutionary dynamics.

The dependence of the evolutionary predictions on the conceptual model chosen to describe the system highlights the need to identify the prominent processes determining spore bias (and other population-level statistics). In our opinion, the development of predictive evolutionary models hinges upon answering three main questions. First, is evolution of this biological system better described at the strain or at the single-cell level? Second, is it better described as a deterministic or a stochastic system? Third, on what time scales is context-dependence relevant? In the following, we discuss the contribution that different approaches can bring to solve these overarching unknowns.

Strain versus single-cell level strategies

Connecting cell-level to collective-level behavior is a classic undertaking not only for evolutionary biology (Okasha, 2006), but also for mechanistic bottom-up approaches to tissue organization (Ladoux & Mège, 2017).

The multi-level selection approach proposes that trade-offs between benefits and costs to the lower-level units can be scaled up to determine fitness at the collective level (Michod, 2007). Similarly to the sociobiological approach, that is also based on translating individual-level costs and benefits into inclusive fitness as a property of a whole population (Gardner & West, 2014; B. Kerr & Godfrey-Smith, 2009), the statistical description of the outcome of interactions does not inform on the underlying processes. If the relationship between different levels of description changed along an evolutionary trajectory, for instance due to environmental or demographic variations, then the predictive power of such descriptions would be curtailed.

Bottom-up approaches describing cell mechanics and movement, on the other hand, aim at classifying behaviours that emerge from interactions of units with differential physical properties. They yielded important insights, for instance, on how cells sort within tissues (Beatrici & Brunnet, 2011; Steinberg, 2007), and in particular on differentiation in *Dictyostelium* (Maree & Hogeweg, 2001). Although they remain simplified representations, mechanistic models are easier to interface with cell-level observations and can provide explicit descriptions of the origin of biases in aggregate composition and in spatial distribution of cells, as well as of collective functionality (Garcia et al., 2015; Guttal & Couzin, 2010; Joshi et al., 2017; van Gestel & Nowak, 2016; Staps et al., 2019; Colizzi et al., 2020). Their integration into evolutionary models is, however, less straightforward. It often relies on numerical simulation and poses the problem of how to estimate – let alone evolve – the large number of parameters involved in microscopic descriptions. Integration of cell-level self-organization processes into models for the evolution of multicellularity is a promising route to improve our

 doi:10.20944/preprints202008.0554.v1

understanding of different solutions to multicellularity beyond *Dictyostelium*, such as the multiplicity of existing life cycles, and to evaluate the role of selection acting at different levels of biological organization (Rainey & De Monte, 2014; De Monte & Rainey, 2014; van Gestel & Tarnita, 2017).

Finding meaningful ways to connect cell- and collective-level properties in assemblies that contain a collection of genotypes and phenotypes, and such that cell-level traits result in the functionality of the ensemble, is a central problem also in more general settings, like microbial communities (Tarnita, 2018; Liautaud et al., 2019; Doulcier et al., 2020). There, evolution of system-level properties through mutations in traits affecting species interactions, some of which of mutualistic or cooperative nature, is considered possible despite – and maybe thanks to – the high diversity among interacting cells. Viewing evolution of muticellularity in *Dictyostelium*, as well as in other microbes that form genetically heterogeneous aggregates, as an instance of community-level evolution may be useful for explaining the first emergence of higher levels of organization. Indeed, many solutions for social coordination that are available to extant species are likely adaptations to life within a multicellular structure. Central to such adaptations seems to be the capacity of cells to respond to the local context established by partaking a same aggregate.

Stochastic vs deterministic models

The second challenge for formalizing selective differences among *Dictyostelium* strains is to evaluate the importance of cell-level stochasticity and the extent to which this can be effectively captured by deterministic models. Advances in single-cell observation techniques revealed the ubiquity of cell-to-cell phenotypic variation, invisible to population-level measures (Altschuler & Wu, 2010). Intracellular fluctuations, for instance due to small numbers of transcription factors, combined with nonlinearities in gene regulation networks, are believed to be major determinants of phenotypic heterogeneity in microbes and beyond (Perkins & Swain, 2009; Balázsi et al., 2011; Norman et al., 2015) and are increasingly considered as key factors influencing their evolutionary dynamics (van Boxtel et al., 2017; Draghi, 2019). The presence, within a monoclonal cell population, of phenotypes that are maladapted to a given environment at any given time is explained by their long-term advantages. Indeed, in rapidly fluctuating environmental conditions, bet-hedging among alternative phenotypes confers an overall advantage (Kussell & Leibler, 2005; Grimbergen et al., 2015).

In *Dictyostelium*, genetically homogeneous populations produce both spores and stalk. Stochastic choices are thought to be involved at the onset of aggregation, in establishing aggregation centers (Gregor et al., 2010); during aggregation, in the decisions whether to follow the cAMP gradient (Rossine et al., 2020); and even during development, in mixing of pre-spore and pre-stalk cells within a slug (Weijer, 1999). On the other hand, phenotypic heterogeneity in isogenic populations can also result from deterministic sources, such as the distribution of cell cycle phase in asynchronously dividing cultures (Jang & Gomer, 2011; Gruenheit et al., 2018) or the spatial distribution of cell density

 doi:10.20944/preprints202008.0554.v1

(Vidal-Henriquez & Gholami, 2019).

The extent to which different sources of variability can be treated as equivalent, when one only considers their population-level collective effects, is an open question. Spiking gene expression, for instance, can produce regular oscillations if averaged over many cells responding to an external forcing, and an average stable signal if integrated over the timescale of aggregation (Corrigan & Chubb, 2014). It has moreover been proposed that heterogeneity in gene expression, with possibly long-term consequences on cell fate, results from modulation of spiking frequency, that happens on very fast time scales compared to the developmental process. Distributed individual cell choices, either driven by stochastic fluctuations or by asynchronicity, might indeed average out and be effectively represented by deterministic equations (Antolović et al., 2017).

In evolutionary game theory, for instance, mixed strategies describe cases when players have a fixed probability of adopting alternative fixed behaviours. For sufficiently simple games, the evolutionary predictions of the deterministic 'mean field' equations are identical to the case when a corresponding fraction of the population adopts one of the strategies (Hofbauer & Sigmund, 1998). Stochasticity is then encompassed by the same deterministic theoretical framework used for fixed strategies. Such equivalence is however not likely to hold when more realism is introduced in defining social interactions. For instance, multi-player cellular interactions, phenotype-dependent partitioning of cells into groups, or out-of-equilibrium phenotypic distributions can all limit the use of established theoretical frameworks, such as evolutionary game theory and population genetics, to derive effective macroscopic descriptions of cell-level stochastic processes.

Integrating context-dependence

Finally, a major obstacle to connecting individual-level stochastic behaviour and strain-level spore bias is that social and abiotic environments experienced by cells change on comparable time scales as the developmental process. In other words, there is a rapid feedback between the phenotypic state of one cell and that of the surrounding population. Such feedback potentially allows cells to evaluate the composition of the aggregate and decide consequently whether to turn into spores or stalk. Strain-level decisions would then be dictated by 'strategic' cell-level choices within one single generation rather than by long-term evolutionary processes (Madgwick et al., 2018). Just recently, molecular tools have been used to start examining how such decision-making is implemented during the process of aggregation and development (Gruenheit et al., 2018; Nichols et al., 2020).

The third major conceptual challenge in improving evolutionary models is hence to describe context-dependence in a mechanistic fashion. Predictions of different such models may then be compared to experimental data and with each other, so as to pinpoint what biological features are essential and what can be neglected with respect to their evolutionary consequences. It is generally accepted that when the conditions experienced by a cell do not vary too fast, the optimal strategy for coping with fluctuations is sensing the environment

 doi:10.20944/preprints202008.0554.v1

and switching phenotype accordingly (Kussell & Leibler, 2005). Such kind of response can occur on a rapid time scale – especially if it involves metabolic rather than regulation changes – and provides an important source of phenotypic heterogeneity (Schreiber & Ackermann, 2020).

Particularly important for *Dictyostelium* are variations in the social environment associated to its peculiar life cycle. The combination of short-term competition at the cell level within clonal aggregates and long-term organization has been addressed in relation to the evolution of multicellular life cycles (Rainey & Kerr, 2010; Hochberg et al., 2008; Wolinsky & Libby, 2016). Phenotypes that would be classified as cheats in the social phase were pointed out to have other functions, such as allowing reproduction of the higher-level structure and division of labour. More generally, feedbacks between ecology and the resulting evolutionary dynamics essentially influence the fate of cheating (Weitz et al., 2016; Lion, 2018; Tilman et al., 2019). Quantifying the importance of such feedbacks, however, requires to follow individual cells and their environment through their developmental cycle. Methodological advances in high-resolution single-cell microscopy (Sgro et al., 2015) and in the use of molecular markers (Muramoto & Chubb, 2008) allow nowadays to access the internal state of single cells at the same time as they undergo major rearrangements of their environmental context, paving the way to mechanistic models that integrate processes across spatial and temporal scales.

Considering cell-cell interactions in the course of evolution implies accounting for a number of spatial and organizational scales. These can be exploited to test adherence to reality of mechanistic models, beyond the ability to reproduce population-level statistics. For instance, the distribution of size and composition of single aggregates are further observables that are readily produced by individual-based models, where each cell is represented. Their use for quantitative comparison would be hugely empowered by the formulation of mesoscopic approximations for the aggregation process and their integration in evolutionary models.

Conclusions

The fascinating ecology of *D. discoideum* yields important insights into the establishment of social behavior in microbes. Our presentation of part of the extensive literature on the subject aims at pinpointing some conceptual hurdles involved with formalizing this process and their evolutionary consequences. In *Dictyostelium*, social behavior indeed results from complex interactions between cells and their environment. The experimental and theoretical models developed to describe such behaviours differ in the scale at which interactions are formalized and in the relative importance of intrinsic versus extrinsic factors in decision-making. The fact that different conceptualizations lead to different predictions on the evolution of aggregative multicellularity highlights the need to develop a unifying theory to understand social behaviors determination and evolution. This requires a mechanistic understanding of how cell-level proper-

ties translate into collective-level social behavior, of the role of stochastic and deterministic factors in cell fate determination, and for context-dependent decisions. In *Dictyostelium* and beyond, the combination of models and of single-cell observations now offer the opportunity to test different conceptual schemes and make testable predictions on their short-term and evolutionary consequences.

Acknowledgments

The authors thank Christophe Anjard for comments and discussions. This study was supported by the French Government under the program Investissements d'Avenir (ANR-10-LABX-54 MEMOLIFE and ANR-11-IDEX-0001-02PSL), and the ANR project ADHeC.

Competing interest The authors declare that they have no competing financial interests.

References

Ackermann, M. (2015). A functional perspective on phenotypic heterogeneity in microorganisms. *Nature Reviews Microbiology*, *13*(8), 497–508. doi: 10.1038/nrmicro3491

Aerts, R. J., Durston, A. J., & Moolenaar, W. H. (1985). Cytoplasmic pH and the regulation of the dictyostelium cell cycle. *Cell*, *43*(3), 653–657. doi: 10.1016/0092-8674(85)90237-5

Aktipis, C. A., Boddy, A. M., Jansen, G., Hibner, U., Hochberg, M. E., Maley, C. C., & Wilkinson, G. S. (2015). Cancer across the tree of life: cooperation and cheating in multicellularity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1673), 20140219–20140219. doi: 10.1098/rstb.2014.0219

Altschuler, S. J., & Wu, L. F. (2010). Cellular heterogeneity: do differences make a difference? *Cell*, *141*(4), 559–563. doi: 10.1016/j.cell.2010.04.033

Antolović, V., Lenn, T., Miermont, A., & Chubb, J. R. (2019). Transition state dynamics during a stochastic fate choice. *Development*, *146*(12), dev173740. doi: 10.1242/dev.173740

Antolović, V., Miermont, A., Corrigan, A. M., & Chubb, J. R. (2017). Generation of single-cell transcript variability by repression. *Current Biology*, *27*(12), 1811–1817.e3. doi: 10.1016/j.cub.2017.05.028

Araki, T., & Maeda, Y. (1995). Cell-cycle progression during the development of Dictyostelium discoideum and its relation to the subsequent cell-sorting in the multicellular structures. *Development, Growth and Differentiation*, *37*(5), 479–485. doi: 10.1046/j.1440-169X.1995.t01-4-00002.x

Azhar, M., Kennady, P. K., Pande, G., Espiritu, M., Holloman, W., Brazill, D., ... Nanjundiah, V. (2001). Cell cycle phase, cellular Ca2+ and development in Dictyostelium discoideum. *The International Journal of Developmental Biology*, *45*(2), 405–414. Retrieved from `http://www.ijdb.ehu.es/web/paper.php?doi=11330860`

Azhar, M., Kennady, P. K., Pande, G., & Nanjundiah, V. (1997). Stimulation by DIF causes an increase of intracellular Ca2+ in Dictyostelium discoideum. *Experimental Cell Research*, *230*(2), 403–406. doi: 10.1006/excr.1996.3420

Azhar, M., Manogaran, P., Kennady, P., Pande, G., & Nanjundiah, V. (1996). A Ca2+-Dependent Early Functional Heterogeneity in Amoebae of-Dictyostelium discoideum,Revealed by Flow Cytometry. *Experimental Cell Research*, *227*(2), 344–351. doi: 10.1006/excr.1996.0283

Balázsi, G., van Oudenaarden, A., & Collins, J. (2011). Cellular decision making and biological noise: From microbes to mammals. *Cell*, *144*(6), 910–925. doi: 10.1016/j.cell.2011.01.030

Beatrici, C. P., & Brunnet, L. G. (2011). Cell sorting based on motility differences. *Physical Review E*, *84*(3), 031927. doi: 10.1103/PhysRevE.84.031927

Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E. A., ... Kuspa, A. (2009). Polymorphic members of the lag gene family mediate kin discrimination in Dictyostelium. *Current Biology*, *19*(7), 567–572. doi: 10.1016/j.cub.2009.02.037

Bonner, J. T., Clarke, W. W., Neely, C. L., & Slifkin, M. K. (1950). The orientation to light and the extermely sensitive orientation to temperature gradients in the slime mold Dictyostelium discoideum. *Journal of Cellular and Comparative Physiology*, *36*(2), 149–158. doi: 10.1002/jcp.1030360203

Brown, J. M., & Firtel, R. A. (1999). Regulation of cell-fate determination in Dictyostelium. *Developmental Biology*, *216*(2), 426–441. doi: 10.1006/dbio.1999.9485

Buss, L. W. (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proceedings of the National Academy of Sciences of the United States of America*, *79*(17), 5337–5341. doi: 10.1073/pnas.79.17.5337

Buttery, N. J., Rozen, D. E., Wolf, J. B., & Thompson, C. R. (2009). Quantification of social behavior in D. discoideum reveals complex fixed and facultative strategies. *Current Biology*, *19*(16), 1373–1377. doi: 10.1016/j.cub.2009.06.058

Buttery, N. J., Thompson, C. R. L., & Wolf, J. B. (2010). Complex genotype interactions influence social fitness during the developmental phase of the social amoeba Dictyostelium discoideum. *Journal of Evolutionary Biology*, *23*(8), 1664–1671. doi: 10.1111/j.1420-9101.2010.02032.x

Chattwood, A., & Thompson, C. R. L. (2011). Non-genetic heterogeneity and cell fate choice in Dictyostelium discoideum. *Development, Growth & Differentiation*, *53*(4), 558–566. doi: 10.1111/j.1440-169X.2011.01270.x

Clarke, M., & Baron, A. (1987). Myosin filaments in cytoskeletons ofDictyostelium amoebae. *Cell Motility and the Cytoskeleton*, *7*(4), 293–303. doi: 10.1002/cm.970070402

Colizzi, E. S., Vroomans, R. M. A., & Merks, R. M. H. (2020). Evolution of multicellularity by collective integration of spatial information. *bioRxiv*, 2020.02.20.957647. (Publisher: Cold Spring Harbor Laboratory Section: New Results) doi: 10.1101/2020.02.20.957647

Corrigan, A., & Chubb, J. (2014). Regulation of transcriptional bursting by a naturally oscillating signal. *Current Biology*, *24*(2), 205–211. doi: 10.1016/j.cub.2013.12.011

d'Alessandro, J., Mas, L., Aubry, L., Rieu, J.-P., Rivière, C., & Anjard, C. (2018). Collective regulation of cell motility using an accurate density-sensing system. *Journal of the Royal Society, Interface*, *15*(140). doi: 10.1098/rsif.2018.0006

De Monte, S., & Rainey, P. B. (2014). Nascent multicellular life and the emergence of individuality. *Journal of Biosciences*. doi: 10.1007/s12038-014-9420-5

de Oliveira, J. L., Morales, A. C., Stewart, B., Gruenheit, N., Engelmoer, J., Brown, S. B., ... Wolf, J. B. (2019). Conditional expression explains molecular evolution of social genes in a microbe. *Nature Communications*, *10*(1), 3284. doi: 10.1038/s41467-019-11237-2

Devreotes, P. N., & Zigmond, S. H. (1988). Chemotaxis in eukaryotic cells: a focus on leukocytes and Dictyostelium. *Annual Review of Cell Biology*, *4*, 649–686. doi: 10.1146/annurev.cb.04.110188.003245

Doulcier, G., Lambert, A., De Monte, S., & Rainey, P. B. (2020). Eco-evolutionary dynamics of nested Darwinian populations and the emergence of community-level heredity. *eLife*, *9*, e53433. (Publisher: eLife Sciences Publications, Ltd) doi: 10.7554/eLife.53433

Draghi, J. (2019). Links between evolutionary processes and phenotypic robustness in microbes. *Seminars in Cell & Developmental Biology*, *88*, 46–53. doi: 10.1016/j.semcdb.2018.05.017

Du, Q., Kawabe, Y., Schilde, C., Chen, Z.-h., & Schaap, P. (2015). The Evolution of Aggregative Multicellularity and Cell–Cell Communication in the Dictyostelia. *Journal of Molecular Biology*, *427*(23), 3722–3733. doi: 10.1016/j.jmb.2015.08.008

Dubravcic, D., van Baalen, M., & Nizak, C. (2014). An evolutionarily significant unicellular strategy in response to starvation in Dictyostelium social amoebae. *F1000Research*, *3*, 133. doi: 10.12688/f1000research.4218.2

Early, A., Gaskell, M., Traynor, D., & Williams, J. (1993). Two distinct populations of prestalk cells within the tip of the migratory Dictyostelium slug with differing fates at culmination. *Trends in Genetics*, *9*(10), 341. doi: 10.1016/0168-9525(93)90027-F

Ennis, H. L., Dao, D. N., Pukatzki, S. U., & Kessin, R. H. (2000). Dictyostelium amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. , 6.

Fisher, P. R., Merkl, R., & Gerisch, G. (1989). Quantitative analysis of cell motility and chemotaxis in Dictyostelium discoideum by using an image processing system and a novel chemotaxis chamber providing stationary chemical gradients. *The Journal of Cell Biology*, *108*(3), 973–984. doi: 10.1083/jcb.108.3.973

Fletcher, J. A., & Doebeli, M. (2009). A simple and general explanation for the evolution of altruism. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1654), 13–19. doi: 10.1098/rspb.2008.0829

Flowers, J. M., Li, S. I., Stathos, A., Saxer, G., Ostrowski, E. A., Queller, D. C., ... Purugganan, M. D. (2010). Variation, sex, and social cooperation: molecular population genetics of the social amoeba Dictyostelium discoideum. *PLoS Genetics*, *6*(7), e1001013. doi: 10.1371/journal.pgen.1001013

Fortunato, A., Strassmann, J. E., Santorelli, L., & Queller, D. C. (2003). Co-occurrence in nature of different clones of the social amoeba, Dictyostelium discoideum. *Molecular Ecology*, *12*(4), 1031–1038. doi: 10.1046/j.1365-294X.2003.01792.x

Fujimori, T., Nakajima, A., Shimada, N., & Sawai, S. (2019). Tissue self-organization based on collective cell migration by contact activation of locomotion and chemotaxis. *Proceedings of the National Academy of Sciences*, *116*(10), 4291–4296. doi: 10.1073/pnas.1815063116

Garcia, T., Brunnet, L. G., & De Monte, S. (2014). Differential adhesion between moving particles as a mechanism for the evolution of social groups. *PLoS computational biology*, *10*(2), e1003482. doi: 10.1371/journal.pcbi.1003482

Garcia, T., Doulcier, G., & De Monte, S. (2015). The evolution of adhesiveness as a social adaptation. *eLife*, *4*, e08595. doi: 10.7554/eLife.08595

Gardner, A., & West, S. A. (2014). Inclusive fitness: 50 years on. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1642), 20130356. doi: 10.1098/rstb.2013.0356

Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E., & Queller, D. C. (2007). High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proceedings of the National Academy of Sciences*, *104*(21), 8913–8917. doi: 10.1073/pnas.0702723104

Glöckner, G., Lawal, H. M., Felder, M., Singh, R., Singer, G., Weijer, C. J., & Schaap, P. (2016). The multicellularity genes of dictyostelid social amoebas. *Nature Communications*, *7*(1), 12085. doi: 10.1038/ncomms12085

Godfrey-Smith, P. (2009). *Darwinian Populations and Natural Selection*. Oxford, New York: Oxford University Press. doi: 10.1093/acprof:osobl/9780199552047.001.0001

Gokhale, C., & Traulsen, A. (2014). Evolutionary Multiplayer Games. *Dynamic Games and Applications*, *4*. doi: 10.1007/s13235-014-0106-2

Gomer, R. H., & Ammann, R. R. (1996). A cell-cycle phase-associated cell-type choice mechanism monitors the cell cycle rather than using an independent timer. *Developmental Biology*, *174*(1), 82–91. doi: 10.1006/dbio.1996.0053

Goury-Sistla, P., Nanjundiah, V., & Pande, G. (2012). Bimodal distribution of motility and cell fate in Dictyostelium discoideum. *International Journal of Developmental Biology*, *56*(4), 263–272. doi: 10.1387/ijdb.113384ps

Gregor, T., Fujimoto, K., Masaki, N., & Sawai, S. (2010). The onset of collective behavior in social amoebae. *Science*, *328*(5981), 1021–1025. doi: 10.1126/science.1183415

Grimbergen, A. J., Siebring, J., Solopova, A., & Kuipers, O. P. (2015). Microbial bet-hedging: the power of being different. *Current Opinion in Microbiology*, *25*, 67–72. doi: 10.1016/j.mib.2015.04.008

Grosberg, R. K., & Strathmann, R. R. (2007). The evolution of multicellularity: a minor major transition? *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 621–654. doi: 10.1146/annurev.ecolsys.36.102403.114735

Gruenheit, N., Parkinson, K., Brimson, C. A., Kuwana, S., Johnson, E. J., Nagayama, K., ... Thompson, C. R. L. (2018). Cell cycle heterogeneity can generate robust cell type proportioning. *Developmental Cell*, *47*(4), 494–508.e4. doi: 10.1016/j.devcel.2018.09.023

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J. A., Wolf, J. B., & Thompson, C. R. L. (2017). A polychromatic 'greenbeard' locus determines patterns of cooperation in a social amoeba. *Nature Communications*, *8*(1), 1–9. (Number: 1 Publisher: Nature Publishing Group) doi: 10.1038/ncomms14171

Guttal, V., & Couzin, I. D. (2010). Social interactions, information use, and the evolution of collective migration. *Proceedings of the National Academy of Sciences*, *107*(37), 16172–16177. doi: 10.1073/pnas.1006874107

Hamilton, W. D. (1964). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, *7*(1), 1–16. doi: 10.1016/0022-5193(64)90038-4

Hauert, C. (2002). Volunteering as red queen mechanism for cooperation in public goods games. *Science*, *296*(5570), 1129–1132. doi: 10.1126/science.1070582

Hiraoka, H., Nakano, T., Kuwana, S., Fukuzawa, M., Hirano, Y., Ueda, M., ... Hiraoka, Y. (2020). Intracellular ATP levels influence cell fates in Dictyostelium discoideum differentiation. *Genes to Cells*, *25*(5), 312–326. doi: 10.1111/gtc.12763

Hochberg, M. E., Rankin, D. J., & Taborsky, M. (2008). The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. *BMC Evolutionary Biology*, *8*(1), 238. doi: 10.1186/1471-2148-8-238

Hofbauer, J., & Sigmund, K. (1998). *Evolutionary Games and Population Dynamics* (1st ed.). Cambridge University Press. doi: 10.1017/CBO9781139173179

Houle, J., Balthazar, J., & West, C. M. (1989). A glycosylation mutation affects cell fate in chimeras of Dictyostelium discoideum. *Proceedings of the National Academy of Sciences*, *86*(10), 3679–3683. doi: 10.1073/pnas.86.10.3679

Huang, H.-J., Takagawa, D., Weeks, G., & Pears, C. (1997). Cells at the center of Dictyostelium aggregates become spores. *Developmental Biology*, *192*(2), 564–571. doi: 10.1006/dbio.1997.8769

Hudson, R. E., Aukema, J. E., Rispe, C., & Roze, D. (2002). Altruism, cheating, and anticheater adaptations in cellular slime molds. , 13. doi: 10.1086/340613

Jang, W., & Gomer, R. H. (2011). Initial cell type choice in Dictyostelium. *Eukaryotic Cell*, *10*(2), 150. doi: 10.1128/EC.00219-10

Joshi, J., Couzin, I. D., Levin, S. A., & Guttal, V. (2017). Mobility can promote the evolution of cooperation via emergent self-assortment dynamics. *PLOS Computational Biology*, *13*(9), e1005732. doi: 10.1371/journal.pcbi.1005732

Kay, R. R., Dhokia, B., & Jermyn, K. A. (1983). Purification of stalk-cell-inducing morphogens from Dictyostelium discoideum. *European Journal of Biochemistry*, *136*(1), 51–56. doi: 10.1111/j.1432-1033.1983.tb07703.x

Kerr, B., & Godfrey-Smith, P. (2009). Generalization of the Price equation for evolutionary change. *Evolution; International Journal of Organic Evolution*, *63*(2), 531–536. doi: 10.1111/j.1558-5646.2008.00570.x

Kerr, W. E. (1950). Evolution of the mechanism of caste determination in the genus melipona. *Evolution*, *4*(1), 7. doi: 10.2307/2405530

Kessin, R. H. (2001). *Dictyostelium: evolution, cell biology, and the development of multicellularity* (1st ed.). Cambridge University Press. doi: 10.1017/CBO9780511525315

Kubohara, Y., & Okamoto, K. (1994). Cytoplasmic Ca2+ and H+ concentrations determine cell fate in Dictyostelium discoideum. *The FASEB Journal*, *8*(11), 869–874. doi: 10.1096/fasebj.8.11.8070636

Kussell, E., & Leibler, S. (2005). Phenotypic diversity, population growth, and information in fluctuating environments. *Science*, *309*(5743), 2075–2078. (Publisher: American Association for the Advancement of Science Section: Report) doi: 10.1126/science.1114383

Kuzdzal-Fick, J. J., Fox, S. A., Strassmann, J. E., & Queller, D. C. (2011). High relatedness is necessary and sufficient to maintain multicellularity in Dictyostelium. *Science (New York, N.Y.)*, *334*(6062), 1548–51. doi: 10.1126/science.1213272

Kuzdzal-Fick, J. J., Queller, D. C., & Strassmann, J. E. (2010). An invitation to die: initiators of sociality in a social amoeba become selfish spores. *Biology Letters*, *6*(6), 800–802. doi: 10.1098/rsbl.2010.0257

Ladoux, B., & Mège, R.-M. (2017). Mechanobiology of collective cell behaviours. *Nature Reviews. Molecular Cell Biology*, *18*(12), 743–757. doi: 10.1038/nrm.2017.98

Leach, C. K., Ashworth, J. M., & Garrod, D. R. (1973). Cell sorting out during the differentiation of mixtures of metabolically distinct populations of Dictyostelium discoideum. *Journal of Embryology and Experimental Morphology*, *29*(3), 647–661. Retrieved from `https://dev.biologists.org/content/29/3/647.long`

Liautaud, K., van Nes, E. H., Barbier, M., Scheffer, M., & Loreau, M. (2019). Superorganisms or loose collections of species? A unifying theory of community patterns along environmental gradients. *Ecology Letters*, *22*(8), 1243–1252. doi: 10.1111/ele.13289

Lion, S. (2018). Theoretical approaches in evolutionary ecology: environmental feedback as a unifying perspective. *The American Naturalist*, *191*(1), 21–44. doi: 10.1086/694865

Loomis, W. F. (2014). Cell signaling during development of Dictyostelium. *Developmental Biology*, *391*(1), 1–16. doi: 10.1016/j.ydbio.2014.04.001

Madgwick, P. G., Stewart, B., Belcher, L. J., Thompson, C. R. L., & Wolf, J. B. (2018). Strategic investment explains patterns of cooperation and cheating in a microbe. *Proceedings of the National Academy of Sciences*, *115*(21), E4823–E4832. doi: 10.1073/pnas.1716087115

Maeda, Y. (1986). A new method for inducing synchronous growth of Dictyostelium discoideum cells using temperature shifts. *Microbiology*, *132*(5), 1189–1196. doi: 10.1099/00221287-132-5-1189

Maeda, Y. (2011). Cell-cycle checkpoint for transition from cell division to differentiation. *Development, Growth & Differentiation*, *53*(4), 463–481. doi: 10.1111/j.1440-169X.2011.01264.x

Maree, A. F. M., & Hogeweg, P. (2001). How amoeboids self-organize into a fruiting body: Multicellular coordination in Dictyostelium discoideum. *Proceedings of the National Academy of Sciences*, *98*(7), 3879–3883. doi: 10.1073/pnas.061535198

Martínez-García, R., & Tarnita, C. E. (2016). Lack of ecological and life history context can create the illusion of social interactions in Dictyostelium discoideum. *PLOS Computational Biology*, *12*(12), e1005246. doi: 10.1371/journal.pcbi.1005246

Matsuda, H., & Harada, Y. (1990). Evolutionarily stable stalk to spore ratio in cellular slime molds and the law of equalization in net incomes. *Journal of Theoretical Biology*, *147*(3), 329–344. doi: 10.1016/S0022-5193(05)80491-6

Medina, J. M., Shreenidhi, P., Larsen, T. J., Queller, D. C., & Strassmann, J. E. (2019). Cooperation and conflict in the social amoeba Dictyostelium discoideum. *The International Journal of Developmental Biology*, *63*(8-9-10), 371–382. doi: 10.1387/ijdb.190158jm

Michod, R. E. (2007). Evolution of individuality during the transition from unicellular to multicellular life. *Proceedings of the National Academy of Sciences of the United States of America*, *104 Suppl 1*, 8613–8618. doi: 10.1073/pnas.0701489104

Muramoto, T., & Chubb, J. R. (2008). Live imaging of the Dictyostelium cell cycle reveals widespread S phase during development, a G2 bias in spore differentiation and a premitotic checkpoint. *Development*, *135*(9), 1647–1657. doi: 10.1242/dev.020115

Nanjundiah, V. (2019). Many roads lead to Rome: Neutral phenotypes in microorganisms. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *332*(8), 339–348. doi: 10.1002/jez.b.22909

Nichols, J. M., Antolovic, V., Reich, J. D., Brameyer, S., Paschke, P., & Chubb, J. R. (2020). Cell and molecular transitions during efficient dedifferentiation. *eLife*, *9*, e55435. (Publisher: eLife Sciences Publications, Ltd) doi: 10.7554/eLife.55435

Noh, S., Geist, K. S., Tian, X., Strassmann, J. E., & Queller, D. C. (2018). Genetic signatures of microbial altruism and cheating in social amoebas in the wild. *Proceedings of the National Academy of Sciences*, *115*(12), 3096–3101. doi: 10.1073/pnas.1720324115

Norman, T. M., Lord, N. D., Paulsson, J., & Losick, R. (2015). Stochastic switching of cell fate in microbes. *Annual Review of Microbiology*, *69*(1), 381–403. doi: 10.1146/annurev-micro-091213-112852

Nowak, M. A. (2006). Five rules for the evolution of cooperation. *Science*, *314*(5805), 1560–1563. doi: 10.1126/science.1133755

Ohmori, T., & Maeda, Y. (1987). The developmental fate of Dictyostelium discoideum cells depends greatly on the cell-cycle position at the onset of starvation. *Cell Differentiation*, *22*(1), 11–18. doi: 10.1016/0045-6039(87)90409-x

Okasha, S. (2006). *Evolution and the levels of selection*. Oxford : Oxford ; New York: Clarendon Press ; Oxford University Press. (OCLC: ocm70985413) doi: 10.1093/acprof:oso/9780199267972.001.0001

Ostrowski, E., Shen, Y., Tian, X., Sucgang, R., Jiang, H., Qu, J., ... Queller, D. (2015). Genomic signatures of cooperation and conflict in the social amoeba. *Current Biology*, *25*(12), 1661–1665. doi: 10.1016/j.cub.2015.04.059

Ostrowski, E. A. (2019). Enforcing cooperation in the social amoebae. *Current Biology*, *29*(11), R474–R484. doi: 10.1016/j.cub.2019.04.022

Ostrowski, E. A., Katoh, M., Shaulsky, G., Queller, D. C., & Strassmann, J. E. (2008). Kin discrimination increases with genetic distance in a social amoeba. *PLoS Biology*, *6*(11), e287. doi: 10.1371/journal.pbio.0060287

Parfrey, L. W., & Lahr, D. J. G. (2013). Multicellularity arose several times in the evolution of eukaryotes. *BioEssays*, *35*(4), 339–347. doi: 10.1002/bies.201200143

Parkinson, K., Buttery, N. J., Wolf, J. B., & Thompson, C. R. L. (2011). A simple mechanism for complex social behavior. *PLoS Biology*, *9*(3), e1001039. doi: 10.1371/journal.pbio.1001039

Perkins, T. J., & Swain, P. S. (2009). Strategies for cellular decision-making. *Molecular Systems Biology*, *5*(1), 326. doi: 10.1038/msb.2009.83

Queller, D. C. (1994). Genetic relatedness in viscous populations. *Evolutionary Ecology*, *8*(1), 70–73. doi: 10.1007/BF01237667

Rainey, P. B. (2015). Precarious development: The uncertain social life of cellular slime molds. *Proceedings of the National Academy of Sciences*, *112*(9), 2639–2640. doi: 10.1073/pnas.1500708112

Rainey, P. B., & De Monte, S. (2014). Resolving conflicts during the evolutionary transition to multicellular life. *Annual Review of Ecology, Evolution, and Systematics*, *45*(1), 599–620. doi: 10.1146/annurev-ecolsys-120213-091740

Rainey, P. B., & Kerr, B. (2010). Cheats as first propagules: A new hypothesis for the evolution of individuality during the transition from single cells to multicellularity. *BioEssays*, *32*(10), 872–880. doi: 10.1002/bies.201000039

Raper, K. B. (1940). Pseudoplasmodium formation and organization in Dictyostelium discoideum. *Journal of the Elisha Mitchell Scientific Society*, *56.2*, 241–282. Retrieved from https://www.jstor.org/stable/24333970?seq=1metadata$_i$nfo$_t$ab$_c$ontents

Rossine, F. W., Martinez-Garcia, R., Sgro, A. E., Gregor, T., & Tarnita, C. E. (2020). Eco-evolutionary significance of "loners". *PLOS Biology*, *18*(3), e3000642. doi: 10.1371/journal.pbio.3000642

Sathe, S., Kaushik, S., Lalremruata, A., Aggarwal, R. K., Cavender, J. C., & Nanjundiah, V. (2010). Genetic heterogeneity in wild isolates of cellular slime mold social groups. *Microbial Ecology*, *60*(1), 137–148. doi: 10.1007/s00248-010-9635-4

Sathe, S., & Nanjundiah, V. (2018). Complex interactions underpin social behaviour in Dictyostelium giganteum. *Behavioral Ecology and Sociobiology*, *72*(10), 167. doi: 10.1007/s00265-018-2572-9

Saxer, G., Brock, D. A., Queller, D. C., & Strassmann, J. E. (2010). Cheating does not explain selective differences at high and low relatedness in a social amoeba. *BMC Evolutionary Biology*, *10*, 76. doi: 10.1186/1471-2148-10-76

Schreiber, F., & Ackermann, M. (2020). Environmental drivers of metabolic heterogeneity in clonal microbial populations. *Current Opinion in Biotechnology*, *62*, 202–211. doi: 10.1016/j.copbio.2019.11.018

Segota, I., Boulet, L., Franck, D., & Franck, C. (2014). Spontaneous emergence of large-scale cell cycle synchronization in amoeba colonies. *Physical Biology*, *11*(3), 036001. doi: 10.1088/1478-3975/11/3/036001

Sgro, A. E., Schwab, D. J., Noorbakhsh, J., Mestler, T., Mehta, P., & Gregor, T. (2015). From intracellular signaling to population oscillations: bridging size- and time-scales in collective behavior. *Molecular Systems Biology*, *11*(1), 779. doi: 10.15252/msb.20145352

Smith, J., Queller, D. C., & Strassmann, J. E. (2014). Fruiting bodies of the social amoeba Dictyostelium discoideum increase spore transport by Drosophila. *BMC evolutionary biology*, *14*, 105. doi: 10.1186/1471-2148-14-105

Soll, D. R., & Waddell, D. R. (1975). Morphogenesis in the slime mold Dictyostelium discoideum. *Developmental Biology*, *47*(2), 292–302. doi: 10.1016/0012-1606(75)90283-3

Staps, M., van Gestel, J., & Tarnita, C. E. (2019). Emergence of diverse life cycles and life histories at the origin of multicellularity. *Nature Ecology & Evolution*, *3*(8), 1197–1205. doi: 10.1038/s41559-019-0940-0

Steinberg, M. S. (2007). Differential adhesion in morphogenesis: a modern view. *Current Opinion in Genetics & Development*, *17*(4), 281–286. doi: 10.1016/j.gde.2007.05.002

Strandkvist, C., Juul, J., Baum, B., Kabla, A. J., & Duke, T. (2014). A kinetic mechanism for cell sorting based on local variations in cell motility. *Interface Focus*, *4*(6), 20140013–20140013. doi: 10.1098/rsfs.2014.0013

Strassmann, J. E., & Queller, D. C. (2011). Evolution of cooperation and control of cheating in a social microbe. *Proceedings of the National Academy of Sciences*, *108*(Supplement 2), 10855–10862. doi: 10.1073/pnas.1102451108

Strassmann, J. E., Zhu, Y., & Queller, D. C. (2000). Altruism and social cheating in the social amoeba Dictyostelium discoideum. *Nature*, *408*(6815), 965–967. doi: 10.1038/35050087

Sucgang, R., Kuo, A., Tian, X., Salerno, W., Parikh, A., Feasley, C. L., ... Grigoriev, I. V. (2011). Comparative genomics of the social amoebae Dictyostelium discoideum and Dictyostelium purpureum. *Genome Biology*, *12*(2), R20. doi: 10.1186/gb-2011-12-2-r20

Takeuchi, I., Noce, T., & Tasaka, M. (1986). Prestalk and prespore differentiation during development of Dictyostelium discoideum. *Current Topics in Developmental Biology*, *20*, 243–256. doi: 10.1016/S0070-2153(08)60667-5

Tarnita, C. E. (2018). Fast evolution unlocks forbidden communities. *Nature Ecology & Evolution*, *2*(10), 1525–1526. doi: 10.1038/s41559-018-0688-y

Tarnita, C. E., Washburne, A., Martinez-Garcia, R., Sgro, A. E., & Levin, S. A. (2015). Fitness tradeoffs between spores and nonaggregating cells can explain the coexistence of diverse genotypes in cellular slime molds. *Proceedings of the National Academy of Sciences*, *112*(9), 2776–2781. doi: 10.1073/pnas.1424242112

Thompson, C. R., & Kay, R. R. (2000). Cell-fate choice in Dictyostelium: intrinsic biases modulate sensitivity to DIF signaling. *Developmental Biology*, *227*(1), 56–64. doi: 10.1006/dbio.2000.9877

Tilman, A. R., Plotkin, J., & Akcay, E. (2019). Evolutionary games with environmental feedbacks. *bioRxiv*. doi: 10.1101/493023

Uchinomiya, K., & Iwasa, Y. (2013). Evolution of stalk/spore ratio in a social amoeba: Cell-to-cell interaction via a signaling chemical shaped by cheating risk. *Journal of Theoretical Biology*, *336*, 110–118. doi: 10.1016/j.jtbi.2013.07.024

Umeda, T., & Inouye, K. (2004). Cell sorting by differential cell motility: a model for pattern formation in Dictyostelium. *Journal of Theoretical Biology*, *226*(2), 215–224. doi: 10.1016/j.jtbi.2003.08.016

van Boxtel, C., van Heerden, J. H., Nordholt, N., Schmidt, P., & Bruggeman, F. J. (2017). Taking chances and making mistakes: non-genetic phenotypic heterogeneity and its consequences for surviving in dynamic environments. *Journal of The Royal Society Interface*, *14*(132), 20170141. doi: 10.1098/rsif.2017.0141

Van Duijn, B., & Inouye, K. (1991). Regulation of movement speed by intracellular pH during Dictyostelium discoideum chemotaxis. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(11), 4951–4955. doi: 10.1073/pnas.88.11.4951

van Gestel, J., & Nowak, M. A. (2016). Phenotypic Heterogeneity and the Evolution of Bacterial Life Cycles. *PLoS computational biology*, *12*(2), e1004764. doi: 10.1371/journal.pcbi.1004764

van Gestel, J., & Tarnita, C. E. (2017). On the origin of biological construction, with a focus on multicellularity. *Proceedings of the National Academy of Sciences*, *114*(42), 11018–11026. doi: 10.1073/pnas.1704631114

Velicer, G. J., Kroos, L., & Lenski, R. E. (2000). Developmental cheating in the social bacterium Myxococcus xanthus. *Nature*, *404*(6778), 598–601. doi: 10.1038/35007066

Vidal-Henriquez, E., & Gholami, A. (2019). Spontaneous center formation in Dictyostelium discoideum. *Scientific Reports*, *9*(1). doi: 10.1038/s41598-019-40373-4

Walmod, P., Hartmann-Petersen, R., Prag, S., Lepekhin, E., Röpke, C., Berezin, V., & Bock, E. (2004). Cell-cycle-dependent regulation of cell motility and determination of the role of Rac1. *Experimental Cell Research*, *295*(2), 407–420. doi: 10.1016/j.yexcr.2004.01.011

Wang, M., Aerts, R. J., Spek, W., & Schaap, P. (1988). Cell cycle phase in Dictyostelium discoideum is correlated with the expression of cyclic AMP production, detection, and degradation. *Developmental Biology*, *125*(2), 410–416. doi: 10.1016/0012-1606(88)90221-7

Weijer, C. J. (1999). Morphogenetic cell movement in Dictyostelium. *Seminars in Cell & Developmental Biology*, *10*(6), 609–619. doi: 10.1006/scdb.1999.0344

Weijer, C. J., & Duschl, G. (1984). A revision of the Dyctiostelium discoideum cell cycle. , 22. Retrieved from `https://jcs.biologists.org/content/70/1/111`

Weijer, C. J., McDonald, S. A., & Durston, A. J. (1984). Separation of Dictyostelium discoideum cells into density classes throughout their development and their relationship to the later cell types. *Differentiation*, *28*(1), 13–23. doi: 10.1111/j.1432-0436.1984.tb00261.x

Weitz, J. S., Eksin, C., Paarporn, K., Brown, S. P., & Ratcliff, W. C. (2016). An oscillating tragedy of the commons in replicator dynamics with game-environment feedback. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(47), E7518–E7525. doi: 10.1073/pnas.1604096113

Wolinsky, E., & Libby, E. (2016). Evolution of regulated phenotypic expression during a transition to multicellularity. *Evolutionary Ecology*, *30*(2), 235–250. doi: 10.1007/s10682-015-9814-3

Yumura, S., Furuya, K., & Takeuchi, I. (1996). Intracellular free calcium responses during chemotaxis of Dictyostelium cells. *Journal of Cell Science*, *109 (Pt 11)*, 2673–2678. Retrieved from `https://jcs.biologists.org/content/109/11/2673.long`

Zada-Hames, I. M., & Ashworth, J. M. (1978). The cell cycle and its relationship to development in Dictyostelium discoideum. *Developmental Biology*, *63*(2), 307–320. doi: 10.1016/0012-1606(78)90136-7

Zahavi, A., Harris, K. D., & Nanjundiah, V. (2018). An individual-level selection model for the apparent altruism exhibited by cellular slime moulds. *Journal of Biosciences*, *43*(1), 49–58. doi: 10.1007/s12038-018-9734-9