© Indian Academy of Sciences

CrossMark

# An individual-level selection model for the apparent altruism exhibited by cellular slime moulds

Amotz Zahavi[1], Keith D Harris[1]* and Vidyanand Nanjundiah[2]*

[1]*Department of Zoology, Tel Aviv University, 69978 Tel Aviv, Israel*

[2]*Centre for Human Genetics, BioTech Park, Electronic City (Phase I), Bengaluru 560 100, India*

*\*Corresponding author (Email, keithhar@post.tau.ac.il; vidyan@alumni.iisc.ac.in)*

†Amotz Zahavi: Deceased 12 May 2017.

In *Dictyostelium discoideum*, cells that become part of the stalk or basal disc display behaviour that can be interpreted as altruistic. Atzmony *et al.* (Curr Sci 72:142–145, 1997) had hypothesised that this behaviour could be the outcome of an adaptive strategy based on differing intrinsic quality as reflected by phenotypes that indicate differences in potential for survival and reproduction, followed by intercellular competition among amoebae of differing qualities. Low-quality amoebae would have a poor chance of succeeding in the competition to form spores; they could enhance their chances of survival by adopting a presumptive stalk strategy. Here we extend the hypothesis by making use of recent findings. Our approach is based on the view that an evolutionary explanation for the apparent altruism of stalk cells in D. discoideum must apply broadly to other cellular slime moulds (CSMs) that exhibit stalk cell death. Further, it must be capable of being modified to cover social behaviour in CSMs with an extracellular stalk, as well as in sorocarpic amoebae whose stalk cells are viable. With regard to *D. discoideum*, we suggest that (a) differentiation-inducing factor, thought of as a signal that inhibits amoebae from forming spores and induces them to differentiate into basal disc cells, is better viewed as a mediator of competition among post-aggregation amoebae and (b) the products of the 'recognition genes', *tgrB* and *tgrC*, allow an amoeba to assess its quality relative to that of its neighbours and move to a position within the aggregate that optimises its reproductive fitness. From this perspective, all cells behave in a manner that is 'selfish' rather than 'altruistic', albeit with different expectations of success.

**Keywords.** Altruism; Dictyostelium; recognition signals; reliable signalling

## 1. Introduction

Traits that appear to be socially beneficial and individually disadvantageous have long engaged evolutionary biologists, because on the face of it, individual-level natural selection should counteract their spread. The long-term persistence of such traits seems unlikely: the system seems to be intrinsically susceptible to destabilisation by a 'cheater' individual that benefits from sociality without paying the cost. Among eukaryotes, sorocarpic amoebae present the problem in its simplest form. These amoebae are common soil microorganisms that are found in many of the major eukaryotic groups of life. Their name comes from the remarkable example of convergent evolution that they exhibit: when they run out of food, spatially separated cells aggregate and construct a fruiting body made up of a mass of dormant spores above an upright stalk (Brown and Silberman 2013). In some groups the stalk—which plausibly is an aid to spore dispersal—is made up of viable cells; in some it consists of

an extracellular exudate; and in yet others it is made up of dead cells (Bonner 1967, 2009; Brown 2010; Brown *et al.* 2010). The last situation, which is common in the Dictyostelid or cellular slime moulds (CSMs), has attracted much interest among evolutionary biologists as an example of altruistic behaviour in a simple organism. Not unexpectedly, it has commonly been viewed through the lens of group or kin selection (Bonner 1967, 2009; Kaushik and Nanjundiah 2003; Gilbert *et al.* 2007).

In *Dictyostelium discoideum*, the best studied species, fruiting bodies consist of two main cell types, stalk and spore. Under standard conditions, some 20% of the amoebae build a stalk in the course of undergoing active cell death, while the other 80% form a mass of live spores (Raper 1940). After aggregation and before fruiting, there is an intervening motile stage known as the slug, which is a polarised cell mass. The anterior cells of the slug consist mainly of presumptive stalk (pre-stalk) cells; these pull the posterior, which consists mainly of presumptive spore (pre-spore) cells (Inouye and

Takeuchi 1979). If provided access to food, a pre-stalk or pre-spore cell can de-differentiate back into an amoeba and resume feeding and dividing; in some cellular slime moulds (though not in *D. discoideum*), this can happen also when the migrating slug encounters food (Shaffer 1961). The slug keeps moving for some hours on a laboratory plate or, in nature, until it reaches the soil surface. At this point the pre-stalk cells situated at the anterior come to a halt and undergo active cell death while forming an erect stalk. Meanwhile, pre-spore cells in the posterior 80% of the slug climb on top of the stalk and form spores. Some pre-stalk cells cup the spore mass above and below, and others form a basal disc at the bottom of the stalk (Sternfeld 1998; Mujumdar *et al.* 2009). By raising the spore mass above the surface, the stalk can facilitate spore dispersal via passing insects or worms; so it plays a major role in improving the chance that a newly germinated amoeba can find food elsewhere.

It is in this context that stalk cell death has been studied as altruistic behaviour displayed by pre-stalk cells, as it gives the impression of having evolved to increase the fitness of the pre-spores. There are several publications that try to explain pre-stalk cell behaviour in *D. discoideum* as an example of group selection acting via genetic relatedness (i.e. of kin selection). Findings of clonal wild-type populations appear to support a model whereby high relatedness stabilizes cooperation in fruiting body formation. Indeed, Gilbert *et al.* (2007) discovered wild-type natural populations in which fruiting bodies tended to be largely clonal, a state of affairs that would favour kin selection (without being essential for it). Kuzdzal-Fick *et al.* (2011) measured spore formation in chimeric fruiting bodies generated from 1:1 mixes of a laboratory strain of *D. discoideum* Ax4 (the ancestor), and putative genetic variants that had been cultured in the laboratory for several generations (the evolved lines) under either of two conditions. They found that when the evolved lines had gone through several life cycles under conditions that predisposed low relatedness (which meant, in their case, leading to good mixing), 'cheater' mutants had arisen in the lines: the 'cheater' contributed disproportionately to forming spores in the chimera. On the other hand, when the evolved lines came from a background of purely vegetative life cycles interspersed with single-cell bottlenecks (putatively 'high-relatedness' conditions), the proportion of cheaters that evolved was significantly lower.

While these studies identify relatedness as a possible factor that can stabilise social behaviour, they also show that stability depends on effects which are based on the group as a whole, i.e. effects that may not be attributable to relatedness per se. Indeed, as was found earlier in the case of the sister species *D. giganteum* (Kaushik *et al.* 2006), the 'cheater' phenotype in *D. discoideum* too depends on the social context, as Saxer *et al.* (2010) discovered when they carried out competition experiments similar to those described above on strains isolated from nature. They found that the propensity of a cell line to 'cheat'

was context-dependent. For instance, the strains that displayed cheater-like behaviour were not the same in low-relatedness and high-relatedness lines. Besides, a 'winner' that prevailed in pairwise mixes with each of five strains contributed no more than its fair share when pooled with all five. Laboratory studies on wild isolates showed that the tendency of different genotypes in a group to segregate correlates poorly with genetic relatedness (Gilbert *et al.* 2012). While not bearing on the significance of kin selection per se, or on the capacity for high relatedness to act as a barrier against 'cheating' in general (e.g. Bastiaans *et al.* 2016), this suggests that in *D. discoideum* and other CSMs, factors other than relatedness may be important for influencing a cell's propensity to be part of a social group and be capable of displaying 'altruistic' behaviour. Presumably, reasons such as these prompted Saxer *et al.* (2010) to draw attention to "the importance of new approaches to studying interactions in *D. discoideum*".

The 'new approach' we advocate for understanding the persistence of so-called altruistic behaviour in *D. discoideum* is very much the old approach of conventional Darwinian selection based on inter-individual competition. Atzmony *et al.* (1997) hypothesized that for a cell of relatively low quality, the chance of success (i.e. of forming a spore) would be nil, were it to attempt the pre-spore route. On the other hand, by surviving longer than it would have otherwise, such a cell could hope for some fitness advantage by following the pre-stalk route. In other words, the behaviour of pre-stalk cells could be 'selfish' rather than 'altruistic'. The aim of this paper is to extend the hypothesis that the pre-stalk strategy is likely to provide a fitness benefit to the very cells that adopt it, i.e. to the cells of lesser quality. Since the objection pointed out in the first sentence of the present paper—that individual-level natural selection should be expected to counteract their spread—seems to refute the above contention, our main aim is to make a case for taking the individual-level selection hypothesis seriously. The equally important task, of assessing critically how plausible the hypothesis of group (or kin) selection is relative to individual selection, will be taken up elsewhere.

Earlier discussions of the evolution of multicellularity via the aggregation of amoeboid cells have raised the possibility that the unicellular to multicellular transition may have originated via self-organisation among pre-adapted cellular units. In such a view, natural selection would have acted subsequently, and its effect would have been to stabilise the transition, in other words to make it a normal part of the life cycle (Nanjundiah 2016, 2017). Here the focus is rather on multicellular groups that have already come into being and exhibit developmental cell death. As pointed out by Atzmony *et al.* (1997), once group living has evolved, the action of selection on the cells that constitute a group cannot be ignored. The present article expands on that argument by taking a re-look at older results on *D. discoideum* with the help of information that has become available since then. Specifically, we use

recent findings on the genetic basis of individual recognition, details of the cell fate of pre-stalk cells and on improved understanding of the noxious properties of a metabolite, DIF-1, to reinforce the previous suggestion regarding how individual-level selection might operate in CSM aggregates. Our reasoning rests on the propositions that (a) some sub-types of pre-stalk cells are viable and only a portion of pre-stalk cells undergo cell death; (b) at every stage of the life cycle, even genetically identical cellular phenotypes differ with regard to fitness-related traits (e.g. nutritional status, calcium content, cell cycle phase); (c) therefore, competition for reproductive advantage always exists and may be facilitated by signalling; and (d) in the wild, CSM social groups are often genetically heterogeneous, with the component clones competing to become reproductives (Fortunato et al. 2003; Sathe et al. 2010; Strassmann et al. 2000). We conclude with an extensive discussion and propose experimental tests of the hypothesis. Where appropriate, we make use of findings on D. giganteum, other CSMs, or sorocarpic amoebae generally.

## 2. The pre-stalk population is composed of sub-types which differ in function and viability, and pre-spore cells may be heterogeneous with respect to the qualities required to become a spore

The term 'pre-stalk behaviour' implicitly assumes that the pre-stalk population is homogenous and contributes equally to the fruiting body, and as such, that all pre-stalk cells have the same fitness. However, gene expression patterns and functional studies show that there are at least four sub-types of pre-stalk cells. They display a distinct spatial organisation within the slug, with locations that are correlated with their final positions in the fruiting body. One sub-type forms the base of the stalk, another forms the stalk proper, and two others cradle the spore mass from below ('lower cup') and above ('upper cup') (Jermyn et al. 1989). The upper and lower cups are formed by anterior-like cells which are pre-stalk cells found in the posterior pre-spore region of the slug and are classified as pre-stalk based on cytology (Sternfeld and David 1981; Devine and Loomis 1985) and gene expression profiles (Gaskell et al. 1992). The upper cup lifts the ascending pre-spore mass and the lower cup supports it (Dormann et al. 1996; Sternfeld 1998; Mujumdar et al. 2009).

Crucially, upper and lower cup cells remain amoeboid and therefore potentially motile even after the fruiting body has formed (Hayashi and Takeuchi 1981; Sternfeld and David 1982). Also, these cells are viable; being amoeboid, they can resume feeding and divide if the spore mass is dispersed to a favourable location quickly. Dubravcic et al. (2014) found that ∼15% of starved D. discoideum amoebae continue to remain solitary when the rest have completed aggregation. Their response to starvation (apparently) is not to become part of an aggregate, but instead to take a chance on a fresh source of food appearing quickly. Modelling shows that given the right environmental conditions, this can work (Tarnita et al. 2015). Upper and lower cup cells seem to follow a strategy similar to that of cells that do not aggregate. Needless to say, in the long run the absence of food will lead to the death of both classes of non-sporulators, whereas differentiated spores will continue to remain viable.

It may be counter-intuitive to think that there is a fitness advantage associated with the strategy of pre-stalk cells undergoing cell death. However, one should consider the specific context and mechanisms underlying this process in CSM amoebae. Developmental cell death in D. discoideum shares some, but not all, features of metazoan apoptosis (Kawli et al. 2002). Crucially, in D. discoideum, cell death does not result in DNA fragmentation in the same manner as in 'classical' apoptosis (Cornillon et al. 1994). Apoptosis in multicellular organisms results in short DNA fragments, which precludes cells undergoing apoptosis from usefully transfecting other cells. Long segments of DNA in the extracellular space produced by the active cell death of pre-stalk cells could potentially transfect pre-spore cells or geminating spores. Horizontal gene transfer can serve as a means of ensuring the transmission of a portion of the genome through neighbouring cells, a phenomenon which is well-documented in many species of bacteria (Johnston et al. 2014). However, there is no evidence of horizontal gene transfer in CSM development, so at present we can only speculate on its contribution to the fitness of pre-stalk cells.

There are tantalising hints that pre-spore cells too constitute a heterogeneous class. In part, the hints come from the finding that the minimal promoters that are required for gene expression in anterior and posterior pre-spore cells are not the same (see Haberstroh and Firtel (1990), Kibler et al. (2003) and discussion in Nanjundaiah and Saran (1992)). Heterogeneities among pre-stalk and pre-spore cells raise an important question. Could there be a basic, which is to say pre-aggregation, functional heterogeneity among cells prior to the stage when they constitute an aggregate? We examine this possibility next.

## 3. Even before aggregation, amoebae differ with respect to their potential to form a spore or stalk cell

Pre-aggregation amoebae of D. discoideum differ in ways that correlate with whether they will become a spore cell or stalk cell after aggregation (reviewed in Nanjundaiah and Saran 1992). When mixed with amoebae that were not provided extra metabolisable sugar during growth, a larger than expected fraction of 'high sugar' amoebae end up in the spore mass (Leach et al. 1973; Noce and Takeuchi 1985). When mixed with amoebae that are in the early G2 phase of the cell cycle at starvation, amoebae that are in the mid-to-late G2 phase contribute preferentially to forming spores (McDonald 1984; Weijer et al. 1984). Finally, pre-

aggregation amoebae spontaneously develop differences in their sequestered and free calcium content; 'high calcium' cells show a tendency to die and form stalk, in contrast to 'low calcium' cells, which tend to form spores (Saran *et al.* 1994; Azhar *et al.* 1996). In short, cell cycle phase, response to DIF, cell calcium, sugar-related nutritional status and cell fate are correlated (Azhar *et al.* 1997, 2001; Thompson and Kay 2000a). We refer to a pre-aggregation cell whose tendency to form a spore is higher than its tendency to form stalk as a 'high quality' cell, and to one that has a higher tendency to form stalk than spore as a 'low-quality' cell. Importantly, the terms signify relative attributes. This means that when their companions are cells of still lower quality, cells that were of low quality in an earlier context, now qualify as high-quality cells.

Until the fruiting body is fully formed, the pre-stalk and pre-spore states remain labile, which reinforces the notion that 'quality' is a relative property. If a slug is cut perpendicular to its length, the anterior–posterior distinction in cell fates gets re-specified within each fragment (Raper 1940). Thus, anterior pre-stalk cells of the slug can trans-differentiate into pre-spore cells and vice versa, though the phenomenon is not equally efficient in both directions (Raper 1940; Sakai 1973; Shaulsky and Loomis 1993). Inouye (1989) showed that pre-stalk to pre-spore conversion was inhibited by a factor or factors released by pre-spore cells. Conceivably one of the factors could be DIF, to which we turn next.

## 4. Diffusible chemicals involved in differentiation can provide information regarding the relative quality of cells

A number of signals are involved in the coordination of development in *D. discoideum*. Among the most widely studied are two that have a role in intercellular communication: cyclic AMP, which affects the organisation of the aggregating amoebae (Bonner 1949; Shaffer 1957; Schaap and van Driel 1985), and the family of hexanones known generically as differentiation-inducing factor (DIF) (Morris *et al.* 1987). The DIFs are polyketides, planar oxygenated aromatic compounds, many of which are used by fungi and other organisms as antibiotics and anti-predation agents (Asahi *et al.* 1995). DIF-1 is a doubly chlorinated and methylated phenolic derivative synthesized by a malonyl-condensing polyketide synthase (Austin *et al.* 2006). In the following we focus on DIF-1, as it is the only signal that has been studied in detail with regard to spatial variation. For simplicity, we use the terms DIF and DIF-1, the form that is most potent in *D. discoideum,* interchangeably, leaving open the possibility that there are meaningful differences in the roles played by different isoforms of DIF—or for that matter, by hitherto unknown substances that play similar roles.

Initially DIF-1 was believed to be essential for the differentiation of pre-stalk cells, but this was later shown to be incorrect (Thompson and Kay 2000b). Rather, it induces cell death and is necessary for the differentiation of basal disc cells (Saito *et al.* 2008). Counter-intuitively, DIF-1 production is higher in pre-spore cells than in pre-stalk cells, and pre-stalk cells produce more of a dechlorinase enzyme that degrades it, compared to pre-spore cells (Brookman *et al.* 1987; Insall *et al.* 1992). This contributes to a concentration gradient of DIF that increases from the anterior to the posterior of the slug.

Besides its signalling role, DIF-1 is known to be a directly toxic chemical with inhibitory activity. DIF-1 functions similarly to an uncoupler, in the sense that by dissipating the membrane potential, it reduces the ability of *D. discoideum* mitochondria to produce energy (Shaulsky and Loomis 1995). Hence, the inhibitory activity of DIF-1 may be more effective on low-quality (presumptive pre-stalk) cells than high-quality (presumptive pre-spore) cells. Pre-spore cells replicate their mitochondrial DNA (Shaulsky and Loomis 1995); thus high-quality cells can compensate for the reduced efficiency of their mitochondria in the presence of DIF-1.

It is difficult to identify the benefit that a pre-stalk cell might gain from responding to DIF. What could be the benefit derived by synthesising a receptor that responds to DIF by activating a pathway of active cell death? And, assuming there is a benefit, why should such a cell also invest in producing an enzyme that degrades DIF? Considering the structure of DIF and its toxic effects on mitochondria, the response to DIF can be interpreted as a mechanism that defends the cell from its toxicity either by binding or degrading it. We suggest that the gradient of DIF (Brookman *et al.* 1987) along the slug reflects a relative quality which determines the role of a cell in the fruiting body. That would favour a distribution of cells along the anterior–posterior axis of the slug with, on the whole, a decreasing propensity to differentiate into stalk from anterior to posterior. While there is no evidence that such is the case, observed differences in properties along the length of the slug indicate that the possibility deserves to be explored (Durston 1976; Lokeshwar and Nanjundiah 1983; Haberstroh and Firtel 1990; Kibler *et al.* 2003).

## 5. Inter-individual recognition may serve as a means of quality-dependent assortment

The allelic constitution at two loci, *tgrB1* and *tgrC1*, affects the tendency of *D. discoideum* amoebae to cooperate with other amoebae (the two are members of large gene families, and not all members have been analysed; G Shaulsky, personal communication). These findings appear to favour an explanation of altruistic behaviour in amoebae based on kin

selection, as they revealed a mechanism which allows cells to associate preferentially with their kin (Hirose *et al.* 2011). Indeed, when two populations with different recognition alleles were mixed, they tended to form separate slugs. However, while allele identity may be correlated with genetic similarity, it cannot ensure it. In other words, a cell that makes use of one or two gene products for recognising kin also renders itself vulnerable to a 'cheater' that carries the relevant allele(s) but is genetically less similar overall. Further, recent information (Ho and Shaulsky 2015) shows that the tendency to segregate from cells of the 'non-self' type weakens after the formation of the slug and before the amoebae make the final decision that commits them to develop into stalk cells. A minority of cells with different alleles at the *tgr* loci is tolerated in slugs formed by cells with the majority allele, and later may also succeed in the competition to form spores. By itself, this minority may not contain enough cells to form a slug of a size sufficient to generate a motive force that can enable it to migrate successfully to the soil surface (Bonner *et al.* 1953; Inouye and Takeuchi 1979). Further, while monoclonal CSM populations are observed in the wild, this does not appear to be the rule. Filosa's (1962) pioneering study identified distinct phenotypes, and presumably distinct genotypes, among the clones that constituted a single fruiting body; some clones were incapable of aggregating on their own. Kaushik and Nanjundiah (2003) reported on a fruiting body (likely *D. giganteum*) that contained at least 10 clones and Sathe *et al.* (2010) found that 15 out of 17 samples of *D. giganteum* and *D. purpureum* collected in the wild existed as polyclonal groups, with up to 9 clones in a single fruiting body (Sathe *et al.* 2010).

These considerations do not rule out an inter-individual recognition role for tgrB/C, but they suggest that the role may not depend on the generation of monoclonal slugs. Instead, based on the following grounds, we suggest that the advantage of possessing the recognition genes *tgrB/C* does not rely on association with kin: (a) the factors which select for a trait need not be the same as those which stabilize it; (b) the expression of the recognition genes covaries with cellular quality (an assumption which can be tested); and (c) cells within the slug can benefit from sensing their relative quality.

Indeed, Wang and Shaulsky (2015) found that two functions, operating through distinct pathways, can be associated with the tgrB/C pair of gene products. The first is to favour the formation of a slug whose constituent amoebae have the same recognition allele. The other is to allow cells to participate in forming a common fruiting body. A recent study has shown that tgrC1 functions as a ligand and tgrB1 functions as its receptor (Hirose *et al.* 2017). We speculate that this 'secondary' function of the recognition genes allows individual amoebae to advertise their relative quality and assess the quality of neighbouring cells. In order to achieve this, information related to each other's quality is continually exchanged through the products of the recognition genes. There is a precedent for what we are suggesting: at least one other class of cell-surface recognition system is known that leads to the sorting of cells according to the level of expression of the relevant molecule (Duguay *et al.* 2003).

There are numerous observations from behavioural studies in animals which demonstrate that individuals compete within sets of phenotypically similar individuals, where the relevant set is defined by the sex, species or other classifying features of the individual (e.g. males compete against males for mates or territory). The most severe conflicts occur with individuals of similar quality (Møller 1987; Barrette and Vandal 1990). This suggests that individuals avoid conflicts with far superior or inferior individuals; in the former case the chance of success is low, while in the latter case the potential benefit may be of lesser value. To avoid wasteful conflicts, individuals advertise qualities which are of value within their set (such as the ability of individuals of a species to cope with its particular ecological challenges). Such signals, which are unique to the set, may also serve as signals that advertise belonging to the set (Zahavi 1993; Zahavi and Zahavi 1997). If the same principles of within-group competition are relevant to cell–cell interactions in developing CSM amoebae, it is possible that the tgrB/C gene products (or yet to be discovered equivalents) also serve as signals providing information on relative phenotypic quality.

## 6. Discussion

While models of indirect selection may explain the presence of a trait through the advantage conferred to the group vis-à-vis other groups, a model of direct selection must consider the adaptive significance of each trait at an individual level. Here we have considered the selective advantage of some of the phenotypes composing the fruiting body. We have suggested that the pathway that leads to the differentiation of pre-stalk cells may be interpreted as an alternative to adopting the pre-spore pathway. The alternative strategy gives lower-quality cells that are incapable of becoming spores a chance to survive, or, through active cell death, the possibility of transfecting neighbouring cells.

The consideration of alternative perspectives regarding the evolutionary stability of cooperation in CSM amoebae may also be crucial for integrating information on the genetic mechanisms underlying development of *D. discoideum* with observations and experiments on pure and chimeric groups of *D. giganteum* wild types (Kaushik and Nanjundiah 2003; Kaushik *et al.* 2006; Sathe *et al.* 2010, 2014; Nanjundiah and Sathe 2013). Besides the obvious fact that laboratory studies can mimic the spatial and temporal complexities of the natural environment (physical, chemical and biotic) only approximately, there is the awkward fact that only the

broadest features of the relevant natural conditions are known to us. As in other groups, social interactions in the CSMs can involve complex factors such as dependence on developmental background, the number of strains and their relative proportions and ecological factors; these are only beginning to be explored (Nanjundiah and Sathe 2011, 2013; Tarnita 2017). Hence the importance of being open to qualitatively different explanations for the evolution of social behaviour than what one might infer from laboratory-based experimental results. It is interesting that two of the leading advocates for a gene-centred view of evolution and a kinship-based view of the evolution of social behaviour, respectively, had doubts about the usefulness of those views as explanations of 'altruistic' behaviour in the CSMs (Williams 1966, pp 224–225; Hamilton 1972, p 225). In both cases, their uncertainty derived from Filosa's (1962) finding of significant phenotypic, and presumably genetic, variation among the cells that made up a social group in the wild and with the likely number of such variants within a group.

Our approach has been guided by a principle of parsimony that may be expressed as follows. An evolutionary explanation of the apparent altruism of stalk cells in *D. discoideum* must apply broadly to other CSMs that exhibit stalk cell death. Further, it must be capable of being modified to cover social behaviour in CSMs with an extracellular stalk, as well as in other sorocarpic amoebae that may have viable stalk cells. We justify the principle on the basis that for all practical purposes, sorocarpic amoebae occupy similar physical niches (Olive 1975) and are therefore likely to be subject to similar selective pressures.

Viewed thus, the phenomena that need to be explained belong to two categories. On the one hand, there is what one may call the 'engineering problem' of constructing a fruiting body. The engineering problem appears to have been solved first by *sporo*carpic amoebae. In them, a single amoeba forms a fruiting body by secreting an extracellular stalk; the amoeba itself differentiates into a spore cell at its top (which may cleave to form more spore cells; Olive 1975). On the other hand, there is the 'biological problem' of accounting for the evolution of a stalk by a multicellular aggregate, as happens in the *soro*carpic amoebae. In fact, depending on the species, there are two biological problems. The first is to explain the origin of a cellular stalk made up of *viable* cells; the second is to explain a cellular stalk in which the cells are dead. A recent molecular phylogeny raises the interesting possibility that within the Amoebozoa, the trait of sporocarpy could be ancestral to sorocarpy (Kang *et al.* 2017). This means that the engineering problem may have been solved by a sporocarpic ancestor and its operational basis (though not necessarily the trait itself) carried over in sorocarpic amoebae.

It remains to account for the evolution of aggregation and address the two biological problems. We suggest the following set of evolutionary events that could, but need not, occur sequentially. Because "*grades* of organizational complexity need not reflect *clades* of closest relatives" (Nanjundiah et al. 2018), it is pointless to speculate on whether the steps followed in sequence, or occurred independently in different lineages. (i) The aggregation of single sporocarpic amoebae may have been favoured given that for the same energetic input per cell, a bunch of stalks is mechanically more stable than a single stalk (Kaushik and Nanjundiah 2003). (ii) Following aggregation, inter-individual competition could have resulted in a subset of cells being compelled to form part of the stalk while remaining viable. The argument is similar to the one we have been making, and is based on differences in pre-aggregation quality, except that in this case the relatively low-quality amoebae would constitute a stalk made up of viable cells. On the reasonable ground that dispersal efficiency improves with height above the substrate, their chances of being dispersed would be lower than that of relatively high-quality cells, which would form spores and be positioned above the stalk. (iii) The actual death of stalk cells would be an independent evolutionary step. Its effect would be to maximise the difference in relative fitness between high and low-quality cells. *D. discoideum* happens to be a species that permits us to speculate on the molecular players behind the final step.

We will comment briefly on the two central issues that our approach emphasizes before concluding with a list of possible tests of the main hypothesis.

## 6.1  *Why do low-quality cells become pre-stalk cells?*

We suggest that amoebae compete for a chance to form spores, and that the outcome of the competition is influenced by DIF-like chemicals and perhaps other signals. The latter may include cyclic AMP, which continues to be secreted after aggregation is completed. The toxicity of DIF also explains why low-quality cells produce an enzyme to degrade it—they do so in order to protect themselves. Why do low-quality, presumptive pre-stalk cells respond to DIF-1 (and, in *D. discoideum*, also to its analogues, though less efficiently; Masento *et al.* 1988) by investing in producing the pre-stalk phenotype rather than by trying to become spores? The reason may be that a low-quality amoeba that finds it difficult to compete successfully to form a spore is better off investing its limited resources in becoming a pre-stalk cell.

Earlier we drew attention to a minority of pre-stalk cells that do not move to the front of the slug. We suggest that among cells of relatively low quality, these cells are of the highest quality; they are best capable of withstanding the level of DIF encountered in the posterior of the slug. Among them, the anterior-like cells form the upper and lower cups and remain alive as amoebae. In other words, in the case of the anterior-like cells, the pre-stalk strategy actually leads to survival (though, in the absence of food, presumably not for as long as a spore can

survive). If the anterior pre-stalk cells are removed from a slug, and as the slug proceeds to regenerate a new tip, what were anterior-like cells previously constitute the new anterior pre-stalk cells (Sternfeld and David 1982). We predict that in the newly regenerated slug, there will be a fresh subset of anterior-like cells generated from cells that come next in the hierarchy of quality. Another subset of pre-stalk cells consists of the rear guard cells that are found at the very posterior of the slug (Bonner 1967). They end up eventually as the basal disc, which is dead tissue. At present we are unable to satisfactorily correlate their spatial location and behaviour.

### 6.2 Cheaters

CSM amoebae whose ancestors lived in environments with high predation and high competition to form spores are likely to have evolved to produce more DIF and also tolerate high levels of DIF [for example, by producing more of its degrading enzyme, as was suggested in Atzmony *et al.* (1997) and confirmed by Thompson and Kay (2000a)]. When a clone of such amoebae is mixed with a clone that produces less DIF (for reasons to do with its own evolutionary background), the former is expected to contribute disproportionately more to spores than the latter. The expectation can be tested by experiments that study predation and identify the toxic effects of DIF-1. Clones that produce more spores than other clones that form part of the same composite fruiting body have been referred to as cheaters (Strassmann *et al.* 2000). Terming these clones cheaters suggests that they manipulate other clones to become pre-stalk cells. In the context of differentiation to pre-spore or pre-stalk cells, this would involve the release of higher levels of DIF. If, as we suggest, DIF was selected due to its toxicity, which ensures its reliable correlation with quality, these clones produce more spores due to their tolerance of higher levels of DIF.

Mathematical models based on intercellular signalling and competition between cells for spore formation successfully account for two features of *D. discoideum* development that are relevant to the present context. The first is the accuracy with which cell type proportions are determined (Nanjundiah and Bhogle 1995). The second is the fact that 'cheater'-like behaviour can be a simple consequence of differing production rates of, and sensitivities to signals among strains that are otherwise identical (especially, identical with regard to cell type proportions; Uchinomiya and Iwasa 2013).

### 6.3 Tests

According to our perspective, the apparent altruism of pre-stalk cells in *D. discoideum* should be viewed as a consequence of individual-level selection acting on amoebae of varying phenotypic quality that compete to form spores. It is evident that the details of the model rest on the experimental evidence currently available, and will need to be modified in two ways: first, as new facts become available, and second, in order to account for the diversity of developmental outcomes in the CSMs (Bonner 1967, 2009) and, more broadly, in the non-dictyostelid sorocarpic amoebae that are found in most of the major groups of life (Brown and Silberman 2013). Still, the verbal model sketched in this paper leads to testable predictions pertaining to the underlying assumptions regarding particulars that were not previously studied.

1. It is possible to test whether DIF serves as a means of defence by presenting amoebae with a potential predator and observing its release and potency.
2. It is possible to test the toxicity of DIF. We would expect DIF to be harmful to amoebae in the absence of the respective receptors and degrading enzymes.
3. The release of DIF should depend on phenotypic quality and also on the cell's position in the slug. Hence, we expect that the level of release of DIF would change after the slug is divided transversely. Pre-spore cells in both anterior and posterior fragments should compete to remain pre-spore cells by re-adjusting their production of DIF in accord with the quality of the cells with which they will be competing.
4. The expression of the recognition genes tgrB/C should vary with cell quality in addition to the stage of differentiation. Measuring their expression at different positions along the slug should reveal a differential profile, reflecting the gradient of phenotypes in the slug. Cells in the anterior, which will become pre-stalk cells, would have an expression profile that differs from those in the posterior.
5. If amoebae indeed use the differential expression of tgrB/C to navigate to their optimal position within the slug, we would expect to find that populations from different phenotypic backgrounds will migrate to different regions in a gradient of the recognition gene products.
6. The prediction that dying stalk cells transfect surviving cells and thus transfer some of their DNA to the next generation is also testable.

### Acknowledgements

colleague and co-author, the late Prof Amotz Zahavi. It is our deep regret that he could not take part in the revisions.

## References

Asahi K, Sakurai A, Takahashi N, Kubohara Y, Okamoto K and Tanaka Y 1995 DIF-1, morphogen of *Dictyostelium discoideum*, induces the erythroid differentiation in murine and human leukemia cells. *Biochem. Biophys. Res. Commun.* **208** 1036–1039

Atzmony D, Zahavi A and Nanjundiah V 1997 Altruistic behaviour in *Dictyostelium discoideum* explained on the basis of individual selection. *Curr. Sci.* **72** 142–145

Austin MB, Saito T, Bowman ME, Haydock S, Kato A, Moore BS, Kay RR and Noel JP 2006 Biosynthesis of *Dictyostelium discoideum* differentiation-inducing factor by a hybrid type I fatty acid–type III polyketide synthase. *Nat. Chem. Biol.* **2** 494–502

Azhar M, Manogaran PS, Kennady PK, Pande G and Nanjundiah V 1996 A Ca(2+)-dependent early functional heterogeneity in amoebae of *Dictyostelium discoideum*, revealed by flow cytometry. *Exp. Cell Res.* **227** 344–351

Azhar M, Kennady PK, Pande G and Nanjundiah V 1997 Stimulation by DIF causes an increase of intracellular Ca2+ in *Dictyostelium discoideum*. *Exp. Cell Res.* **230** 403–406

Azhar M, Kennady PK, Pande G, Espiritu M, Holloman W, Brazill D, Gomer RH and Nanjundiah V 2001 Cell cycle phase, cellular Ca2 + and development in *Dictyostelium discoideum*. *Int. J. Dev. Biol.* **45** 405–414

Barrette C and Vandal D 1990 Sparring, relative antler size, and assessment in male caribou. *Behav. Ecol. Sociobiol.* **26** 383–387

Bastiaans E, Debets AJM and Aanen DK 2016 Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. *Nat. Commun.* **7** 11435

Bonner JT 1949 The demonstration of acrasin in the later stages of the development of the slime mold *Dictyostelium discoideum*. *J. Exp. Zool.* **110** 259–271

Bonner JT 1967 *Cellular Slime Molds* (Princeton: Princeton University Press)

Bonner JT 2009 *The Social Amoebae: The Biology of Cellular Slime Molds*. (Princeton: Princeton University Press)

Bonner JT, Koontz Jr PG and Paton D 1953 Size in relation to the rate of migration in the slime mold *Dictyostelium discoideum*. *Mycologia* 235–240

Brookman JJ, Jermyn KA and Kay RR 1987 Nature and distribution of the morphogen DIF in the Dictyostelium slug. *Development* **100** 119–124

Brown MW 2010 *Placing the Forgotten Slime Molds (Sappinia, Copromyxa, Fonticula, Acrasis, and Pocheina), Using Molecular Phylogenetics* (Fayetteville: University of Arkansas)

Brown MW and Silberman JD 2013 The Non-dictyostelid Sorocarpic Amoebae in *Dictyostelids* (Berlin: Springer) pp 219–242

Brown MW, Silberman JD and Spiegel FW 2010 A morphologically simple species of Acrasis (Heterolobosea, Excavata), *Acrasis helenhemmesae* n. sp. *J. Eukaryot. Microbiol.* **57** 346–353

Cornillon S, Foa C, Davoust J, Buonavista N, Gross JD and Golstein P 1994 Programmed cell death in Dictyostelium. *J. Cell Sci.* **107** 2691–2704

Devine KM and Loomis WF 1985 Molecular characterization of anterior-like cells in *Dictyostelium discoideum*. *Dev. Biol.* **107** 364–372

Dormann D, Siegert F and Weijer CJ 1996 Analysis of cell movement during the culmination phase of Dictyostelium development. *Development* **122** 761–769

Dubravcic D, van Baalen M and Nizak C 2014 An evolutionarily significant unicellular strategy in response to starvation in Dictyostelium social amoebae. [version 2; referees: 2 approved]. F1000Research 2014, 3:133 (*https://doi.org/10.12688/f1000research.4218.2*)

Duguay D, Foty RA and Steinberg MS 2003 Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. *Dev. Biol.* **253** 309–323

Durston AJ 1976 Tip formation is regulated by an inhibitory gradient in the *Dictyostelium discoideum* slug. *Nature* **263** 126–129

Filosa MF 1962 Heterocytosis in cellular slime molds. *Am. Nat.* **96** 79–91

Fortunato A, Queller DC and Strassmann JE 2003 A linear dominance hierarchy among clones in chimeras of the social amoeba *Dictyostelium discoideum*. *J. Evol. Biol.* **16** 438–445

Gaskell MJ, Jermyn KA, Watts DJ, Treffry T and Williams JG 1992 Immuno-localization and separation of multiple prestalk cell types in Dictyostelium. *Differentiation* **51** 171–176

Gilbert OM, Foster KR, Mehdiabadi NJ, Strassmann JE and Queller DC 2007 High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl. Acad. Sci. USA* **104** 8913–8917

Gilbert OM, Strassmann JE and Queller DC 2012 High relatedness in a social amoeba: the role of kin-discriminatory segregation. *Proc. Biol. Sci.* **279** 2619–2624

Haberstroh L and Firtel RA 1990 A spatial gradient of expression of a cAMP-regulated prespore cell-type-specific gene in Dictyostelium. *Genes Dev.* **4** 596–612

Hamilton WD 1972 Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* **3** 193–232

Hayashi T and Takeuchi I 1981 Differentiation of various cell types during fruiting body formation of *Dictyostelium discoideum*. *Dev. Growth Differ.* **23** 533–542

Hirose S, Benabentos R, Ho H-I, Kuspa A and Shaulsky G 2011 Self-recognition in social amoebae is mediated by allelic pairs of tiger genes. *Science* **333** 467–470

Hirose S, Chen G, Kuspa A and Shaulsky G 2017 The polymorphic proteins TgrB1 and TgrC1 function as a ligand-receptor pair in Dictyostelium allorecognition. *J. Cell Sci.* **130** 4002–4012

Ho H-I and Shaulsky G 2015 Temporal regulation of kin recognition maintains recognition-cue diversity and suppresses cheating. *Nat. Commun.* **6** 7144

Inouye K 1989 Control of cell type proportions by a secreted factor in *Dictyostelium discoideum*. *Development* **107** 605–609

Inouye K and Takeuchi I 1979 Analytical studies on migrating movement of the pseudo-plasmodium of *Dictyostelium discoideum*. *Protoplasma* **99** 289–304

Insall R, Nayler O and Kay RR 1992 DIF-1 induces its own breakdown in Dictyostelium. *EMBO J.* **11** 2849–2854

Jermyn KA, Duffy KTI and Williams JG 1989 A new anatomy of the prestalk zone in Dictyostelium. *Nature* **340** 144–146

Johnston C, Martin B, Fichant G, Polard P and Claverys J-P 2014 Bacterial transformation:distribution, shared mechanisms and divergent control. *Nat. Rev. Microbiol.* **12** 181–196

Kang S, Tice AK, Spiegel FW, Silberman JD, Pánek T, Čepička I, Kostka M, Kosakyan A, Alcântara DMC, Roger AJ, et al. 2017 Between a pod and a hard test: the deep evolution of amoebae. *Mol. Biol. Evol.* **34** 2258–2270

Kaushik S and Nanjundiah V 2003 Evolutionary questions raised by cellular slime mould development. *Proc. Indian Natl. Sci. Acad. Part B* **69** 825–852

Kaushik S, Katoch B and Nanjundiah V 2006 Social behaviour in genetically heterogeneous groups of *Dictyostelium giganteum*. *Behav. Ecol. Sociobiol.* **59** 521–530

Kawli T, Venkatesh BR, Nanjundiah V, Kennady PK and Pande G 2002 Correlates of developmental cell death in *Dictyostelium discoideum*. *Differentiation* **70** 272–281

Kibler K, Nguyen T-L, Svetz J, Van Driessche N, Ibarra M, Thompson C, Shaw C and Shaulsky G 2003 A novel developmental mechanism in Dictyostelium revealed in a screen for communication mutants. *Dev. Biol.* **259** 193–208

Kuzdzal-Fick JJ, Fox SA, Strassmann JE and Queller DC 2011 High relatedness is necessary and sufficient to maintain multicellularity in dictyostelium. *Science (80-.).* **334** 1548–1551

Leach CK, Ashworth JM and Garrod DR 1973 Cell sorting out during the differentiation of mixtures of metabolically distinct populations of *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* **29** 647–661

Lokeshwar BL and Nanjundiah V 1983 Tip regeneration and positional information in the slug of *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* **73** 151–162

Masento MS, Morris HR, Taylor GW, Johnson SJ, Skapski AC and Kay RR 1988 Differentiation-inducing factor from the slime mould *Dictyostelium discoideum* and its analogues. Synthesis, structure and biological activity. *Biochem. J.* **256** 23–28

McDonald SA 1984 Developmental age-related cell sorting in *Dictyostelium discoideum*. *Wilhelm Roux's Arch. Dev. Biol.* **194** 50–52

Møller AP 1987 Variation in badge size in male house sparrows *Passer domesticus*: evidence for status signalling. *Anim. Behav.* **35** 1637–1644

Morris HR, Taylor GW, Masento MS, Jermyn KA and Kay RR 1987 Chemical structure of the morphogen differentiation inducing factor from *Dictyostelium discoideum*. *Nature* **328** 811–814

Mujumdar N, Inouye K and Nanjundiah V 2009 The *trishanku* gene and terminal morphogenesis in *Dictyostelium discoideum*. *Evol. Dev.* **11** 697–709

Nanjundiah V 2016 Cellular slime mold development as a paradigm for the transition from unicellular to multicellular life. *Multicellularity Orig. Evol.* 105

Nanjundiah V and Saran S 1992 The determination of spatial pattern in *Dictyostelium discoideum*. *J. Biosci.* **17** 353–394

Nanjundiah V and Bhogle AS 1995 The precision of regulation in *Dictyostelium discoideum*: implications for cell-type proportioning in the absence of spatial pattern. *Indian J. Biochem. Biophys.* **32** 404–416

Nanjundiah V and Sathe S 2011 Social selection and the evolution of cooperative groups: the example of the cellular slime moulds. *Integr. Biol.* **3** 329

Nanjundiah V and Sathe S 2013 Social Selection in the Cellular Slime Moulds in *Dictyostelids* (Berlin: Springer) pp 193–217

Nanjundiah V, Ruiz-Trillo I and Kirk D 2018, in press Protists and multiple routes to the evolution of multicellularity; in *History of Evolutionary Cell Biology* (eds) B Hall and S Moody (Boca Raton:CRC Press)

Noce T and Takeuchi I 1985 Prestalk/prespore differentiation tendency of *Dictyostelium discoideum* cells as detected by a stalk-specific monoclonal antibody. *Dev. Biol.* **109** 157–164

Olive LS 1975 *The Mycetozoans*. (Cambridge: Academic Press)

Raper KB 1940 Pseudoplasmodium formation and organization in *Dictyotelium discoideum*. *J. Elisha Mitchell Sci. Soc.* **56** 42

Saito T, Kato A and Kay RR 2008 DIF-1 induces the basal disc of the Dictyostelium fruiting body. *Dev. Biol.* **317** 444–453

Sakai Y 1973 Cell type conversion in isolated prestalk and prespore fragments of the cellular slime mold *Dictyostelium discoideum*. *Dev. Growth Differ.* **15** 11–19

Saran S, Azhar M, Manogaran PS, Pande G and Nanjundiah V 1994 The level of sequestered calcium in vegetative amoebae of *Dictyostelium discoideum* can predict post-aggregative cell fate. *Differentiation* **57** 163–169

Sathe S, Kaushik S, Lalremruata A, Aggarwal RK, Cavender JC and Nanjundiah V 2010 Genetic heterogeneity in wild isolates of cellular slime mold social groups. *Microb. Ecol.* **60** 137–148

Sathe S, Khetan N and Nanjundiah V 2014 Interspecies and intraspecies interactions in social amoebae. *J. Evol. Biol.* **27** 349–362

Saxer G, Brock DA, Queller DC and Strassmann JE 2010 Cheating does not explain selective differences at high and low relatedness in a social amoeba. *BMC Evol. Biol.* **10** 76

Schaap P and van Driel R 1985 Induction of post-aggregative differentiation in *Dictyostelium discoideum* by cAMP. *Exp. Cell Res.* **159** 388–396

Shaffer BM 1957 Aspects of aggregation in cellular slime moulds 1. Orientation and chemotaxis. *Am. Nat.* **91** 19–35

Shaffer BM 1961 The Cells founding aggregation centres in the slime mould *Polysphondylium violaceum*. *J. Exp. Biol.* **38** 833–849

Shaulsky G and Loomis WF 1993 Cell Type regulation in response to expression of ricin A in Dictyostelium. *Dev. Biol.* **160** 85–98

Shaulsky G and Loomis WF 1995 Mitochondrial DNA replication but no nuclear DNA replication during development of Dictyostelium. *Proc. Natl. Acad. Sci. USA* **92** 5660–5663

Sternfeld J 1998 The anterior-like cells in Dictyostelium are required for the elevation of the spores during culmination. *Dev. Genes Evol.* **208** 487–494

Sternfeld J and David CN 1981 Cell sorting during pattern formation in Dictyostelium. *Differentiation* **20** 10–21

Sternfeld J and David CN 1982 Fate and regulation of anterior-like cells in Dictyostelium slugs. *Dev. Biol.* **93** 111–118

Strassmann JE, Zhu Y and Queller DC 2000 Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408** 965–967

Tarnita CE 2017 The ecology and evolution of social behavior in microbes. *J. Exp. Biol.* **220** 18–24

Tarnita CE, Washburne A, Martinez-Garcia R, Sgro AE and Levin SA 2015 Fitness tradeoffs between spores and nonaggregating cells can explain the coexistence of diverse genotypes in

cellular slime molds. *Proc. Natl. Acad. Sci. USA* **112** 2776–2781

Thompson CR. and Kay RR 2000a Cell-fate choice in dictyostelium: intrinsic biases modulate sensitivity to DIF signaling. *Dev. Biol.* **227** 56–64

Thompson CR. and Kay RR 2000b The role of DIF-1 signaling in dictyostelium development. *Mol. Cell* **6** 1509–1514

Uchinomiya K and Iwasa Y 2013 Evolution of stalk/spore ratio in a social amoeba: cell-to-cell interaction via a signaling chemical shaped by cheating risk. *J. Theor. Biol.* **336** 110–118

Wang Y and Shaulsky G 2015 TgrC1 has distinct functions in dictyostelium development and allorecognition. *PLoS One* **10** e0124270

Weijer CJ, McDonald SA and Durston AJ 1984 Separation of *Dictyostelium discoideum* cells into density classes throughout their development and their relationship to the later cell types. *Differentiation* **28** 13–23

Williams GC 1966 *Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought* (Princeton: Princeton University Press)

Zahavi A 1993 The fallacy of conventional signalling. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **340** 227–230

Zahavi A and Zahavi A 1997 *The Handicap Principle: A Missing Piece of Darwin's Puzzle* (Oxford: Oxford University Press)

Corresponding editor: STUART A NEWMAN