

Social Selection in the Cellular Slime Moulds

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Abstract Starvation triggers a complex series of intercellular interactions in the cellular slime mould amoebae. As a result the amoebae aggregate, form a coherent multicellular structure with division of labour and, eventually, differentiate into a fruiting body made up of a stalk and a spore mass. Whether an amoeba dies and forms part of the stalk or becomes a stress-resistant spore depends both on pre-existing biases and on post-starvation signalling between amoebae. Mutual communication permits one amoeba to influence the phenotype, and therefore affect the fitness, of another. The implication is that social selection has been a major factor in the evolution of cooperative behaviour in these amoebae.

1 Introduction

This article discusses the potential for social selection during the cellular slime mould (CSM) life cycle. Social selection is natural selection in the context of social behaviour. With two exceptions the term ‘social behaviour’ is used as commonly understood: it is “the suite of interactions that occur between two or more individual[s]..., usually of the same species, when they form...aggregations, cooperate... or simply communicate across space”.¹ The exceptions pertain to

¹ <http://www.britannica.com/EBchecked/topic/550897/social-behaviour-animal>

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cell–cell repulsion during feeding, which may be considered anti-social behaviour, and inter-species interactions. Other than brief comments at the beginning (‘null hypothesis’) and the end (under ‘Summing up’) on possible evolutionary routes, we do not attempt to theorise on how social behaviour may have originated in the CSMs. The interested reader is referred to books and articles by (Bonner 1967, 1982, 2009) and speculations in our previous publications (Nanjundiah 1985; Kaushik and Nanjundiah 2003; Nanjundiah and Sathe 2011).

The characteristic feature of the Dictyostelid or CSMs is self-organisation of form and pattern. A large number of apparently identical amoebae come together via mutual attraction, work in coordination to build a motile multicellular structure, the slug and proceed to construct a terminally differentiated fruiting body made up of live spore cells and dead stalk cells.² Besides serving as the ‘hydrogen atom’ of eukaryotic multicellular development, the apparent altruism displayed by the amoebae that contribute to the stalk has made them an ideal system for studying the evolution of cooperation. The aim of this article is to point out that social selection must have played a major role in the process. In order to help the reader better to appreciate what this implies, and to illustrate how social selection differs from conventional natural selection, we begin by sketching a possible route for the evolution of post-starvation development in the CSMs that does *not* involve social selection.

What does multicellular development achieve? Starvation is the trigger for aggregation, the slug migrates to the soil surface, and the fruiting body contains amoebae that have differentiated into stress-resistant spores positioned along or at the tip of an erect stalk. Passing insects, worms and, indirectly, birds and mammals can all help to transport spores (Suthers 1985; Kessin et al. 1996; Sathe et al. 2010)—something that would be difficult in the case of a single spore on the soil surface. Therefore, by joining an aggregate, some amoebae improve their chances of (passive) dispersal from a nutrient-poor environment. More, on average, an amoeba that joins an aggregate must have a better chance of survival and reproduction than one that remains solitary and waits it out until food becomes available in the same place—or dies. Could a combination of standard physical and chemical processes and the properties of individual cells as they evolve via conventional natural selection be sufficient to ensure this?³

The first question we need to address is whether pre-existing traits of free-living amoebae might be sufficient to explain their coming together and building a complex differentiated structure? Motility and the ability to adhere to a surface (necessary in order to obtain traction) would be among the traits. That apart,

² The terms ‘Dictyostelid’ ‘CSM’ and ‘social amoeba’ are used interchangeably.

³ By conventional natural selection we mean a process of selection that makes an amoeba adapted to its physical and biotic environments, with the added implicit assumption that the environment created by conspecifics plays at best a minor role. The approach is analogous to considering a physical property as resulting from the behaviour of independent particles or a chemical property as ‘colligative’.

entirely chance factors⁴ could introduce an element of variation between amoebae, variation in more than one respect. For example, some amoebae will be more likely to withstand starvation than others. Even genetically identical cells will exhibit such variations, and if a group of amoebae contains many genotypes, the range of variation will increase further. Thus both stochastic and heritable factors affect the likelihood that a starved amoeba survives until food reappears in the same place.⁵

Now consider the following hypothetical but plausible sequence of events. The sequence can be thought of as a null hypothesis for how CSM social behaviour could have evolved with a weak role at most for social selection. An amoeba is present in an environment—say the soil—on which bacteria are distributed at a uniform density. The amoeba and its descendents feed, grow and divide. As they do so, local clusters of high density form entirely due to random movements (Houchmandzadeh 2009). Cells that come into loose contact adhere to each other (by the same means through which a cell adheres to a surface), and loose clusters go on to become tight aggregates. The phenotypes of cells vary on account of stochastic factors, and the variations are correlated with differences in the cells' ability to survive, reproduce or both (Nanjundiah 2003). Cell to cell differences in properties such as surface tension and viscosity cause the tight aggregates to change shape. At the same time, cellular heterogeneity leads to spatial segregation, and cells with similar physical properties sort out from others that differ in respect of those properties (Gierer 1977; Newman and Comper 1990; Forgacs and Newman 2005). Lastly, by virtue of being in a compact mass, some cells are elevated above the surface; this improves their chances of dispersal. Overall, the consequence is that an amoeba that forms part of a group has a better chance of survival than one that does not. This is one version of the null hypothesis of how aggregation and differentiation could have originated by a combination of natural selection (acting on an amoeba whose life cycle was spent as a solitary individual, with intercellular interactions playing at most a minimal role) and self-organisation (via physical forces), but without social selection.⁶ The null hypothesis that we have sketched is essentially 'a stochastic model of an elemental social system' (Cohen 1971).⁷ Going by a recent report of astonishingly rapid evolution of multicellularity with division of labour in unicellular yeast (Ratcliff et al. 2012), it

⁴ For example, minor differences in stored nutritional reserves.

⁵ Soil microorganisms can take active steps to defend themselves against stress. Even in the CSMs, a starved amoeba can encyst itself (see later). This does not affect the argument.

⁶ *Ipso facto*, morphological transition via self-organisation does away with the requirement that intermediate stages be adaptive (see Newman and Forgacs 2005). The evolution of fruiting bodies with an extracellular stalk bundle starting from single-celled fruiting bodies also could have been favoured by the purely physical consideration that it is harder to bend or break a bundle of cylinders than a single cylinder (Kaushik and Nanjundiah 2003).

⁷ Bonner (2013 and this book) advances the more extreme null hypothesis that morphological differences between CSMs are neutral—they are due to chance, not selection; also see Bonner and Lamont (2005).

may not be all that off the mark. Its main value is that it helps us to conjecture what features of CSM development were based on physical principles initially and in the course of time became reinforced—that is, were made more reliable—by natural selection, and what features may have arisen as *de novo* adaptations to social living. The extensive system of cell–cell interactions in the CSMs (to be discussed below) makes us confident that the form of natural selection that played a decisive role in this was social selection.

The rest of this article is organised as follows. We begin with a discussion of the conceptual issues that underlie social selection and point out that the CSMs are ideal for studying how it works. A brief sketch follows multicellular development in the CSMs, which can also be viewed as the development of division of labour and social behaviour. Next we list traits on which social selection can act. We go on to consider how social selection might work on a background of pre-existing differences of stochastic origin. The article ends with general remarks on the relevance of social selection for the evolution of CSM social behaviour. Most of the work on CSMs has been concentrated on a single species, *Dictyostelium discoideum*. It should be assumed that what we say comes from observations made on it; whenever another species is involved it is named explicitly. For a general background the reader is referred to an article and book by Bonner (1982, 2009) and earlier reviews (Kaushik and Nanjundiah 2003; Nanjundiah and Sathe 2011).

2 Social Selection

The simplest context in which natural selection can act is that of an asexual species of solitary individuals living in a spatially uniform and temporally unvarying environment.⁸ Under these conditions, given genetic variation and a genotype–phenotype correlation, natural selection leads to one genotype getting fixed. Mutation and genetic drift will generate and maintain genetic variation in the population. But, apart from stochastic differences (‘range variation’; Bonner 1965), every individual in the population will have the same phenotype.⁹ Spatial or temporal heterogeneity in the environment can change the picture and lead to stable polymorphisms.¹⁰ In all these situations fitness can be defined with respect to an environment that does not include conspecifics. Sexual reproduction makes the fitness of an individual depend on its ability to interact with another individual, but the interaction as such does not play an important role, at least in random-mating models.¹¹ Sexual selection brings in a qualitatively new feature: it requires

⁸ Or in an environment that at any given time is the same for all individuals.

⁹ Unless some phenotypes are neutral relative to one another (Bonner, 2013 and this book).

¹⁰ And can lead to non-intuitive outcomes, for example stable polymorphisms in asexual populations of non-interacting individuals (Rainey et al. 2000; Dean 2005).

¹¹ In particular, if the loci in question are not sex-linked.

the fitness of one individual to depend on traits in another. Inter-individual interactions in social groups share the feature. Darwin's explanations of sexual dimorphism and cooperative behaviour introduced social selection into evolutionary theory, and he recognised that the explanations required significant modifications to be made to ordinary natural selection (Darwin 1859, 1871).

Social selection refers to natural selection when the fitness of an individual depends on the social context (Crook 1972; West-Eberhard 1979). Social selection can take place when the phenotype of one individual depends on the phenotype (and therefore on the genotype) of a second individual. It involves a consideration of extended phenotypes in the sense of Dawkins (1981), but the extension applies to conspecifics within the same group rather than to artefacts (such as nests). In social selection an individual contributes to the environment of another and at the same time, selection acts on it. Each individual is therefore both an agent of selection and its object (Moore et al. 1997; Wolf et al. 1999). Social selection blurs the distinction between organism and environment, and that can have unexpected consequences for quantitative models of evolution. For example, it is no longer a straightforward matter to partition phenotypic variation between 'genetic' (=heritable) and 'environmental' (=non-heritable) components, because the environmental component too can be inherited. Also, a built-in tendency of positive feedback when conspecifics interact means that social selection can potentiate more rapid evolutionary change than conventional natural selection.¹² Indeed mutual communication and feedbacks are common to all groups in which sociality involves more than the mere coming together of units that benefit from a mere increase in numbers (Nanjundiah and Sathe 2011). For an external signal to be capable of influencing the phenotype of a cell, the phenotype must be flexible or plastic. Phenotypic plasticity at the level of the individual can enlarge the scope for natural selection—in this context, social selection—to shape the evolution of group behaviour in terms of both direction and pace. This is because the capacity to adopt different phenotypes acts as a multiplier¹³—it can enhance the range of potential group states and thereby amplify phenotypic variation at the level of the group (Fig. 1).

A number of reasons make the CSMs an excellent system for studying the working of social selection. An extensive system of intercellular communication (Kessin 2001) includes some signals that enhance the chances of survival and others that lessen it, indicating that social selection is acting (Nanjundiah and Sathe 2011). Social selection in the CSMs involves social behaviour without obligatory sexual reproduction. There is a sexual or macrocyst phase of development as well. It requires the co-aggregation of amoebae of different mating types, nuclear fusion and extensive cell death (Raper 1984). There is one strain of *Polysphondylium*

¹² A signal from A to B contributes to the fitness of B, and via reciprocal communication, feeds back on A. The Darwin–Fisher model of sexual selection driven by female choice is a classic example.

¹³ For example, if each individual in a group of three can exhibit any one of three phenotypes, the number of group phenotypes is at least 9.

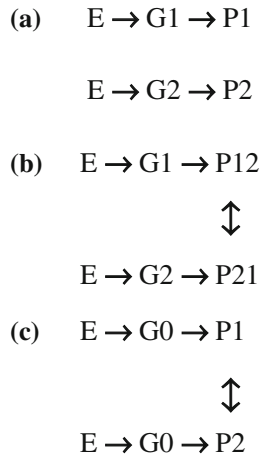


Fig. 1 A schematic depiction of how interactions and feedbacks form the background to social selection. E represents the common environment; G stands for genotype and P for phenotype. *Single arrows* indicate influences and *double-headed arrows* stand for interactions. In **a** P1 and P2 stand for phenotypes that result from genotypes G1 and G2; P1 and P2 do not interact, and their evolution is governed entirely by conventional natural selection. The genotypes of the individuals remain different. In **b**, because of interactions with P2, P1 is modified to P12 and similarly, P2 is modified to P21. In **c**, interactions between phenotypes can cause the same genotype G0 in the same environment E to give rise either to phenotype P1 or phenotype P2. One phenotype can modify another via direct signalling or indirectly. In the indirect route a phenotype in one individual can influence the genotype \rightarrow phenotype link in the other individual. This sketch illustrates what is required for social selection to act. For it actually to act, the interactions must have a bearing on the reproductive fitness of the relevant individuals. Even so, social selection may not have evolutionary consequences; it can do so only when the genotypes in question differ. Situations **a**, **b** and **c** are all found in the CSMs

pallidum that produces a ‘killer factor’ that is lethal to all strains except itself and its opposite mating type (Mizutani et al. 1990). It is obvious that the sexual cycle offers ample scope for social selection (Nanjundiah and Sathe 2011). However, it is rarely observed under standard laboratory conditions, we do not know how frequent it is in nature and studies on it are few. This article is restricted to making a case for the prevalence of social selection during the asexual life cycle.

Individual amoebae can go through asexual life cycles in a physical environment that, for all practical purposes, is both uniform and unchanging. But their social environment is extremely heterogeneous through space and time because of signalling from other cells, and that is associated with behavioural heterogeneity.¹⁴ Social groups can be generated easily in the laboratory, simply by throwing different cells together. This makes it possible for individuals of known genotypes to be allowed to interact and their fates followed over a large number of generations.

¹⁴ The signal-receptor systems that are responsible for intercellular communication can evolve too, by a different variant of natural selection known as “signal selection”: Zahavi 2006.

In other words traits that have consequences for fitness¹⁵ can easily be monitored and measured under controlled conditions and it is easy to measure fitness over one or more life cycles.¹⁶ The number of phenotypes exhibited by the CSMs depends on how one does the counting. If we restrict ourselves to gross cell morphology, there are four: amoeba, spore, stalk and basal disc (the last being absent in many species and the last two in some); if we take behaviour into account we need to add feeding cells, specifically chemotactic cells, ‘sentinel’ cells that phagocytose bacteria and clear toxins (Chen et al. 2007), and upper and lower cup cells that aid the spore mass to rise (Sternfeld 1998; Mujumdar et al. 2009); if we include gene expression patterns, the count goes up.¹⁷ Still, cellular phenotypes are few in number. Therefore one outcome of social selection, namely the origin and maintenance of phenotypic differences within the group, should be much easier to study in the CSMs than, for example, in insect societies with their many morphological and behavioural castes (Wilson 1971).

3 Development of Sociality

The cellular slime moulds are characterised by an unusual life cycle made up of distinct phases of unicellular growth and multicellular development, the latter being the social phase. Free-living amoebae feed on bacteria and other microorganisms that are present in the soil or on animal dung, grow and increase in number via serial mitotic divisions as long as the food supply remains.¹⁸ Once the food is exhausted a number of intracellular events including quorum sensing, the production and release of a chemical attractant, chemotaxis in response to an external gradient of the chemical, amplification and relay of the attractant and the development of intercellular adhesion systems make it possible for cells to communicate, attract each other via chemotaxis and form cohesive social groups consisting of anywhere from $\sim 10^2$ to $\sim 10^6$ amoebae depending on the species and food supply (Bonner 1967, 2009; Raper 1984; Kessin 2001). Division of labour appears soon after starvation sets in and one can detect presumptive cell types within the group, now a polarised mass called the slug. The slug moves

¹⁵ For example growth rate, the time required to complete development, migration and oriented movement (taxis), and, reproduction or cell death.

¹⁶ As of today most evolutionary experiments on the CSMs have been restricted to a single life cycle, but the situation is changing (Kuzdzal-Fick et al. 2011).

¹⁷ Spatial and temporal gene expression patterns have been studied in detail only in *Dictyostelium discoideum* (Bonner 1967; Olive 1975; Jermyn et al. 1989; Kessin 2001; Chen et al. 2007).

¹⁸ CSMs have also been found in water bodies and on trees (Olive 1975; O’Dell 2007; Sathe et al. 2010). In neither case have life cycles been properly studied. If they have an aquatic life cycle, it has not been studied. Recently a CSM was isolated from an infected human eye; the possibility that CSMs may be pathogens is new (Reddy et al. 2010).

towards the soil surface and undergoes a series of changes including tissue rearrangements. Some members of the group become encased in a stress-resistant polysaccharide coat and enter hibernation as spores; the others die and build a multicellular cellulosic stalk that provides a rigid support for the spore mass. The resulting structure, known as the fruiting body, is conventionally considered the terminal stage of the life cycle.¹⁹ The sequential stages that cells go through during development—feeding and growth, aggregation, multicellular migration and terminal differentiation—exhibit their own characteristic pattern of gene expression (VanDriesche et al. 2002).

The entire course of events from the start of aggregation to the construction of the fruiting body involves complex and coordinated movements of cells and cell groups. The next life cycle begins after dispersal, a process in which spores are passive participants. Water, insects and worms can all act as dispersers over short distances; wind, large animals and birds can spread spores over much larger distances (Cavender 1973; Suthers 1985; Huss 1989; Stephenson and Landolt 1992; Kessin et al. 1996; O'Dell, 2007; Sathe et al. 2010). Amoebae can be consumed and digested by nematode worms and presumably other animals but spores emerge unscathed (Suthers 1985; Huss 1989; Kessin et al. 1996). If a spore happens to land in a food-rich habitat, it germinates: the hibernating amoeba emerges and starts to feed, grow and divide once again until the next bout of starvation sets in. Within this broad similarity in life cycles, there are significant differences between species.

4 Traits on Which Social Selection can Act

Any trait that involves behavioural modification of one cell by another is a candidate for social selection. Candidates can be found throughout the CSM life cycle. However, very few have been monitored with regard to their consequences for reproductive fitness. In fact most of the data pertains to essentially one trait, namely spore formation. Consequently most of the evidence we have pertains to the influence of different social environments on the probability that an amoeba becomes a spore. All the same, it is of interest to list other traits on which social selection can act. (a) Amoebae of some species repel each other while feeding (Keating and Bonner 1977; Kakebeeke et al. 1979). Thus feeding cells can influence each other, albeit in an anti-social fashion: strictly speaking, they cannot be treated as non-interacting individuals. One can conjecture that the strength of

¹⁹ All Dictyostelids form fruiting bodies, but their forms are varied. The stalk can be branched or unbranched, cellular or extracellular, and when cellular, made up of live or dead cells. The arrangement of cells in the stalk can differ from species to species. See Bonner (1967) and Raper (1984) for details. In species where all cells form spores and each spore secretes an extracellular stalk, the stalk bundle may confer a group advantage via the collective behaviour of cells that act independently; see Kaushik and Nanjundiah (2003).

the repulsive signal is graded with the availability of food, repulsion being strong when food is abundant and weak when it is sparse (experimental evidence on the point is lacking). In the latter situation there would be a premium on getting together with other cells; indeed repulsion gives way to attraction after the food supply is exhausted. (b) Amoebae sense their numbers, or more correctly their density, in terms of the concentration of a released quorum sensing factor or factors (Clarke and Gomer 1995; Jain et al. 1992).²⁰ (c) Quorum sensing is a prelude to secreting and relaying the chemoattractant, which leads to long-range aggregation (Konijn et al. 1967; Shaffer 1975). Again, the amount and temporal profile of chemoattractant secreted and the sensitivity of the response would be subject to selection. (d) Aggregation depends on an elaborate coordination of sensory and motor behaviour among cells (Kessin 2001) besides the regulation of gene expression in anticipation of future requirements (VanDriessche et al. 2002). Aggregation territory sizes are tightly regulated (Bonner and Dodd 1962) and dependent on the production and degradation of the chemoattractant (Riedel et al. 1973; Nanjundiah and Malchow 1976); (e) Slug migration is a form of cooperative behaviour, and efficient and timely migration to the soil surface is necessary for efficient dispersal. In particular, the slug exhibits phototaxis and thermotaxis, and the sensory centres for both lie in the cells of the tip at the slug's anterior margin (Kessin 2001): this means that the polarised movement of the slug is coordinated by signals from the tip. Further evidence of the tip's guiding role comes from its resemblance to the classical embryonic organiser in the sense that a transplanted tip can direct the morphogenesis of the entire group (Bonner 1952; Rubin and Robertson 1975).²¹ (f) Culmination, a process that involves differentiation and the ascent of the spore mass, requires elaborate signalling between cells and tissues. The presumptive spore mass is helped to rise by two subsets of cells that cradle it above and below, the upper and lower cups; the lower cup offers passive support whereas the upper cup actively lifts the mass (Sternfeld and David 1982; Sternfeld 1998; Mujumdar et al. 2009). The cells belonging to both cups die but remain amoeboid in appearance (Sternfeld and David 1982). Terminal differentiation into viable spore cells requires the active involvement of a secreted peptide from presumptive stalk cells (Anjard et al. 1997, 1998a, b). (g) In some species spores contain quorum sensing compounds that inhibit their own germination (Russell and Bonner 1960; Bacon et al. 1973). At least in a mutant of *D. discoideum*, they make an auto-activator of germination (Dahlberg and Cotter 1977).²² Conceivably

²⁰ It has been suggested that quorum sensing may be a form of 'reproductive restraint', namely a prudent cessation of growth and cell division when the food supply becomes poor; see (Werfel and Bar-Yam 2004). However, there are sound arguments against this and similar models of group-level benefit; see Zahavi (2005).

²¹ Work with 2-dimensional slugs suggests that the tip may be a dynamic entity whose cellular composition keeps changing, not a fixed group of cells (Bonner 1998).

²² Curiously, the bacterium *Enterobacter (Aerobacter) aerogenes*, on which CSM amoebae feed, produces a substance that acts as an activator of spore germination (Hashimoto et al. 1976). Presumably the substance has been co-opted by amoebae to serve as an indicator of the

spore germination activators are also produced by spores of species that can germinate in the absence of food (for example *D. mucoroides* var. *stoloniferum*; Cavender and Raper 1968).

Under special environmental conditions that include starvation, high moisture, darkness, and low levels of phosphate, CSM amoebae belonging to opposite mating types aggregate and go through a series of events including cannibalistic feeding; the outcome is a dormant zygotic structure known as the macrocyst (Blaskovics and Raper 1957 Nickerson and Raper 1973; Erdős et al. 1975; Urushihara 1992). The sexual cycle offers special opportunities for social selection, because it requires the proximate co-occurrence of two cells that communicate and modify each other's phenotype, an extreme case of social selection. Mutzel (1991) has hypothesised that sexual predation and cannibalism may have been evolutionary forerunners of asexual multicellular development. In some strains of *P. pallidum* the 'sexes' also differ in morphology (Kawakami and Hagiwara 2002). It is tempting to think that as in many animals, here too sexual selection, if not in the classical Darwinian sense, in the form of signal selection, has been responsible for moulding the difference.²³ We have no evidence either way.

5 Stochastic Factors and Genetic or Environmental Differences can Make Pre-aggregation Cells Differ in Their Capacity to Withstand Starvation and Sporulate

There is ample evidence that differences in phenotypes can originate spontaneously among genetically identical amoebae—that is, on a stochastic basis and without cell–cell interactions.²⁴ This is true even when the amoebae have the same genotype and share the same environment. The evidence comes from different sorts of observations. (i) Certain differences between cells, e.g. in size, make them more likely to differentiate into one cell type than another (Takeuchi 1969; Bonner et al. 1971). (ii) Artificially provided pre-aggregation cues can bias the stalk-forming or spore-forming tendency of a cell. The cues can relate to nutrition

(Footnote 22 continued)

availability of food. This is an interaction between a predator and its prey and so does not form part of social behaviour within one species. It is mentioned here because of the unusual outcome, namely a 'closing of the loop' in the asexual life cycle: there is a smooth transition from the conventional end-point of the life cycle (terminal differentiation into spore and stalk cells) to its conventional beginning (feeding of bacteria by amoebae).

²³ See Zahavi (2006).

²⁴ Note that this is *not* the same as conventional phenotypic variation between the members of a species, which is usually thought to be based on genetic differences, environmental differences or genotype–environment interactions. Presumably something comparable could occur in other social organisms, e.g. the social insects, in which autonomous differences can be reinforced by inter-individual interactions.

(Leach et al. 1973), cell cycle phase at starvation (McDonald and Durston 1984; Weijer et al. 1984; Thompson and Kay 2000a) or cellular calcium content (Saran 1999; Azhar et al. 2001). (iii) When starved amoebae are plated at a density that is low enough to make intercellular communication unlikely and all of them exposed to an appropriate chemical stimulus, they can be induced to differentiate. However, not all cells respond in the same manner. The proportion that differentiates varies with the strength of the stimulus, indicating an element of randomness in cell to cell variability (Kay 1982).

What is finally perceived as an all-or-none distinction between two categories of cells, say spore and stalk, initially may be based on spontaneously occurring internal phenotypic differences (for example, differences in stored glycogen; Takeuchi et al. 1986. Heterogeneities in genotype or environment would be an additional source of variation that would amplify pre-existing phenotypic differences. The feature in question could vary continuously from cell to cell but be used to generate a qualitative difference between two categories—for example via a threshold mechanism or by some other means (Fig. 2).²⁵

Even after an apparently qualitative distinction is possible—for example, between stalk and spore cells—an element of residual variation will remain. It will ensure that spore cells (or for that matter stalk cells) are not identical; indeed they may be functionally non-equivalent too (Bonner 1965).²⁶ To sum up, the intercellular interactions that lead to social selection act on a background of phenotypic variation that is stochastic in origin or, depending on the circumstances, is influenced by different genotypes or different environments.

6 Intercellular Interactions Reinforce Spontaneously Occurring Differences and Lead to Social Selection

A characteristic feature of CSM development is that the two cell types in the terminal structure, the fruiting body, are present in constant proportions over a range of 10^3 – 10^4 when proportions are assessed in terms of dry weights, volumes

²⁵ Intercellular interactions can cause phenotypic differences to arise spontaneously among two or more cells. A well-studied case involves the combination of stochastic fluctuations and negative cross-feedbacks that leads to the distinction between anchor and ventral uterine (AC/VU) cells in *Caenorhabditis elegans* (Wilkinson et al. 1994). Analogous negative feedbacks seem to exist in *D. discoideum*; see Fig. 3. A bimodal distribution of cell motility may arise in *D. discoideum* and other systems because mutual inhibition between two signal transduction pathways can lead to bistability (Goury-Sistla et al. 2012).

²⁶ In his Ph.D. thesis (submitted to the Indian Institute of Science, 1996), Baskar reports that he was able to stain spores differentially using the dye neutral red. The spores were allowed to germinate and the resulting amoebae were compelled to aggregate (by being deprived of food). Following aggregation, highly stained and poorly stained amoebae sorted out to the slug anterior and posterior respectively; that is, they exhibited presumptive spore or stalk tendencies. The effect disappeared if feeding and cell division were allowed to intervene.

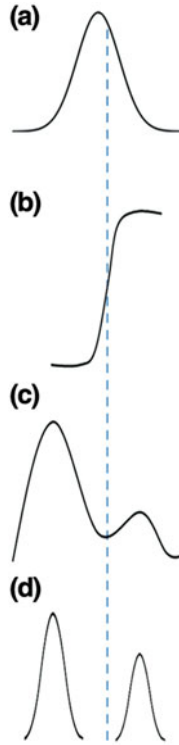


Fig. 2 Illustration of how a continuously varying unimodal phenotype can be transformed into a bimodally distributed phenotype or into two distinct phenotypes. The curve in (a) stands for the distribution of some quantity X that varies continuously from cell to cell. The distribution is symmetric around the mean and the value of X in most cells is close to the mean. The step function in (b) stands for a threshold filter (for example, a protein to which molecules of X bind cooperatively). One way in which the threshold filter could work is by the activation of a gene only in those cells in which the level of X is to the right of the vertical dotted line; the gene remains inactive in cells whose level of X is to the left of the line. Following this a second variable Y is distributed among cells either as a single bimodal distribution (c) or as two distinct distributions (d). Alternatively, d may reflect levels of a third variable Z . The distributions in c and d are supposed to reflect qualitative phenotypic differences relevant for reproductive fitness. In the CSM context, X would be a parameter that indicates a pre-aggregation cellular ‘quality’ related to fitness (e.g. nutritional state; see Atzmony et al. 1997) and Y and Z could stand for a parameter whose values are significantly different between prestalk and prespore cells

or total cell numbers (Bonner 1967; MacWilliams and Bonner 1979).²⁷ Therefore cells must be capable of actively adjusting their behaviour in response to the size

²⁷ Rafols et al. (2001) state that “the pattern of cell types is qualitatively the same for slugs of all different sizes, from 100 cells to more than $\sim 100,000$ cells”. The actual proportions vary from species to species. In *D. discoideum* $\sim 80\%$ of the amoebae form spores under standard laboratory conditions; in *D. giganteum* it is $\sim 50\%$; Raper (1940); Kaushik et al. (2006).

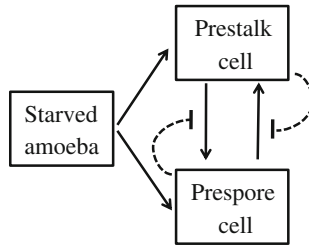


Fig. 3 An elementary scheme of intercellular interactions involved in cell-type differentiation in *D. discoideum* (adapted from Nanjundiah and Bhogle (1995); a scheme with some similar features was proposed by Loomis (1993) and Schaap et al. (1996)). Depending on the levels of one or more intracellular parameters, a starved amoeba has a certain probability of dying (forming a prestalk cell) and a certain probability of surviving (forming a prespore cell) even before the onset of aggregation. These probabilities vary from cell to cell (see Fig. 2). Prespore and prestalk cells can interconvert spontaneously (straight arrows), but interconversion is subject to inhibitory feedbacks (dotted lines). A prestalk cell differentiates terminally into a dead stalk cell and a prespore cell differentiates terminally into a live spore cell. A minimal model that accounts for the correct proportions of differentiated cell types requires, in addition to the processes shown here, a means whereby the strengths of the interactions are sensitive to overall size, i.e. to the total number of cells (presumptive stalk + presumptive spore). A complete developmental model would require the addition of a means for specifying other cell types (albeit within the broad categories indicated here), a consideration of cell and tissue movements and contacts

of the social group. Raper provided a direct demonstration when he showed that anterior or posterior fragments of *D. discoideum* slugs, i.e. fragments that contained a preponderance of one or the other cell type, could restore the minority component. This happened via trans-differentiation, that is, cell-type interconversion, on the part of some cells belonging to the majority (Raper 1940). A possible explanation would be that in the undisturbed condition any tendency to trans-differentiate from a presumptive stalk state to a presumptive spore state or vice versa is inhibited by negative feedbacks.²⁸ Inouye (1989) confirmed this in the case of trans-differentiation from the presumptive spore to the presumptive stalk state.

There is another reason for invoking negative feedbacks. When measured in groups of the same size, cell type proportions vary stochastically from group to group because of spontaneous fluctuations in the underlying physics and biochemistry—that is, because of an intrinsic lack of precision in the phenomenon. It so happens that even when different models predict the same mean proportions, the magnitude of the associated fluctuations that they predict depends on the precise model. When the experimentally observed fluctuations are compared with expectations based on various models, it turns out that one is required to invoke,

²⁸ Transdifferentiation, first indicated in Raper's observations on isolated prestalk and prespore fragments of the slug (Raper 1941), was shown explicitly later by Gregg (1965), and its kinetics was studied by Sakai (1973).

besides stochasticity, negative feedbacks and an ability to sense the size and the group (Nanjundiah and Bhogle 1995; see Fig. 3 below).

Direct evidence for the existence of intercellular interactions in social behaviour comes from a number of sources. First, identified diffusible chemical signals are known to pass from cell to cell and to lead to specific effects on the differentiation status of the recipient (Konijn et al. 1967; Bonner 1970; Shimomura et al. 1982; Van Haastert et al. 1982; Town et al. 1976; Morris et al. 1987; Thompson and Kay 2000b). Among the better-studied signals that mediate social behaviour, the lipid-soluble polyketides generically known as DIF (for differentiation-inducing factor) are made and released by *D. discoideum* cells (Kay 1997; for other social signals see Kaushik and Nanjundiah 2003). The DIFs can induce other cells to differentiate to a stalk-like state with varying degrees of efficiency. It was predicted (Atzmony et al. 1997) and subsequently verified (Kay and Thompson 2001) that the doubly chlorinated molecule DIF-1, the most potent known DIF, is made by amoebae that have a higher intrinsic predisposition to survive than the others. These are the presumptive spore-forming amoebae that are located in the slug's posterior; correspondingly, DIF-1 is broken down by presumptively stalk-forming amoebae in the slug's anterior (Kay et al. 1993). Second, the extracellular medium in which cells are shaken contains released factors that affect the development of other cells, of which only some of the active constituents have been identified (Oohata et al. 1997). At least two genes that are expressed in *D. discoideum* presumptive stalk tissue, *SDF-2* and *comD*, are required for spore differentiation from presumptive spore tissue (Anjard et al. 1998a, b; Kibler et al. 2003). The most compelling evidence for the existence of social selection comes from observations on synthetic social groups in which marked amoebae are combined soon after starvation and their fates monitored.²⁹ Generally, what is measured is the proportion of cells of a given type that contributes to forming spores, relative to the proportion in which the cells were mixed initially. Such experiments have been carried out with naturally occurring 'wild-type' or distinguishable variants and with mutants of known phenotype. In the most dramatic examples cells belonging to two strains that are unable to develop further after starvation do so when mixed and go on to form terminally differentiated fruiting bodies (Sussman 1952, 1954, 1955; Sussman and Lee 1955); in less dramatic cases an admixture of the wild-type rescues developmentally aberrant strains (Buss 1982; Filosa 1962).

When *D. giganteum* wild-type isolates are mixed in pairs, some strains aggregate freely and complete development together; others co-aggregate but sort out and form separate fruiting bodies; and yet others inhibit the development of the other component, which remains as unaggregated amoebae (Kaushik et al. 2006). When pairs of strains co-aggregate and go on to build chimaeric fruiting bodies, the most common outcome is that one strain forms a disproportionate number of

²⁹ As the topic has been reviewed extensively recently (Nanjundiah and Sathe 2011), we restrict ourselves to listing the main points.

spores relative to the other (Strassmann et al. 2000; Kaushik et al. 2006). Whatever be the intercellular interactions responsible, they influence reproductive fitness. But there are other puzzling features. For example, there are cases in which the asymmetries between pairs of strains are transitive ($A > B$ and $B > C$ implies $A > C$, where the $>$ sign means that a greater than expected proportion of the first member, and a smaller than expected number of the second number, forms spores). This hints at the existence of a dominance hierarchy. But the asymmetries weaken considerably or disappear when three strains are mixed ($A = B = C$; Kaushik, et al. 2006; Khare, et al. 2009). The implicit complexity or nonlinearity is suggestive of what is called the bystander effect by ethologists: the behaviour of one animal³⁰ towards another is affected by the presence of a third (Carlisle and Zahavi 1986). The specifics of how such an effect can work in a cellular context are a matter of much interest.³¹ Variations in developmental phenotypes seen after mixing cells of *triA⁻*, a morphological mutant, and its wild-type surrogate, Ax2, clearly show that phenotypes are partly autonomous and partly non-autonomous to the cells that exhibit them (Mujumdar et al. 2011)—again, a clear indication that intercellular signalling is involved.

7 Evolutionary Consequences of Social Selection

The manner in which the CSMs become multicellular (i.e., by aggregation of spatially separated amoebae) makes it possible, though not necessary, that the amoebae that come together belong to different genotypes. It turns out that fruiting bodies formed under natural conditions, or under laboratory conditions that may mimic what happens in nature, can be genetic chimaeras or clones (Gilbert et al. 2007; Sathe et al. 2010). Clonal as well as polyclonal groups of *D. discoideum* have been detected in nature (it is a straightforward matter to generate them by mixing in the laboratory); 77 % of the groups were clonal in one study (Gilbert et al. 2007). In contrast, among social groups of *D. giganteum* and *D. purpureum*, 15/17 sampled fruiting bodies were polyclonal and the estimated number of clones within a group ranged from 1 to 9 (Sathe et al. 2010). Social selection can lead to evolutionary change when social groups consist of more than one genotype (West-Eberhard 1989) and individuals belonging to the genotypes differ in fitness-related traits, as in fact they do (Strassmann et al. 2000; Fortunato et al. 2003; Kaushik et al. 2006). But if groups are genetically homogeneous, social selection can act but cannot affect genotype frequencies in the next generation. As mentioned earlier, genetically heterogeneous social groups are readily constituted in the laboratory. A number of observations have been carried out on such groups. Going

³⁰ In this case, of an ‘animal’ that, as Bonner (1994) has pointed out, is without nerves or muscles.

³¹ Mesnil et al. (1996) have reported a bystander effect in cancer tissue.

by the outcomes, one thing is abundantly clear: social selection mediated via intercellular signalling can lead to either an increase or a decrease in the probability that a cell survives relative to the same probability as measured in a clonal group; in short, social selection can have evolutionary consequences (Fortunato et al. 2003; Kaushik et al. 2006; Nanjundiah and Sathe 2011).³² However, past social selection in groups made up of related cells (i.e. kin selection) could have favoured the formation of genetically uniform social groups over heterogeneous groups. This could have been a means of guarding against the risk of being confronted by a cell belonging to a genotype that leads to its bearer deriving the benefits of group life without suffering the cost, i.e. without taking the risk of dying and forming part of the stalk (Mehdiabadi et al. 2006; Ostrowski et al. 2008).³³ On balance, it would appear that depending on the nature of their immediate neighbourhood at time starvation sets in, CSM amoebae can go through some life cycles entirely in the company of clone-mates and other life cycles as members of genetically heterogeneous groups (that conceivably include members of other species). Therefore, their traits must have evolved under social selection in social environments that were quite different from one generation to another (Kawli and Kaushik 2001). We see coordinated multicellular development in the CSMs as the evolutionary outcome of competition to sporulate. The competition is carried out between amoebae that differ in their intrinsic abilities to survive starvation and, on average, benefit by becoming part of a group along with other amoebae.

When aggregation involves cells of different mating types, it can lead to cell fusion, cannibalism and the formation of a stress-resistant zygote (the macrocyst). The sexual cycle is very different from the asexual cycle. But, considering the high level of mortality that accompanies macrocyst formation, it is evident that very strong social selection must be involved in it. Some CSMs mount a third response to starvation, which is to encyst themselves as single cells (the microcyst). According to Raper (1984) on the whole microcysts are found in species

³² In these experiments the genetically heterogeneous nature of the group, which is engineered by the experimenter, is merely a tool of convenience. Genetic heterogeneity makes it easier to distinguish between two classes of cells and compare the efficiency with which each forms spores with the corresponding efficiency when either is in a clonal group. The assumption is that the experiments are telling us something about social behaviour in a group of interacting CSM amoebae that belonged to different phenotypes originally or acquired different phenotypes following intercellular interactions. Once the group forms and is stable, whether it is clonal or polyclonal is unimportant.

³³ A 'kin effect' can be present without kin selection. When an amoeba dies as a stalk cell, and no spore cell has the same genotype, it is strongly selected against—its genotype disappears. On the other hand, if genotype is also present in one or more spores, the amoeba is subject to equally strong negative selection but its genotype survives. However, by itself this is not evidence of kin selection. Kin selection requires that the death of a stalk cell be selected *because*—whenever different genotypes are found in the same social group—it enhances the probability that another cell of the same genotype forms a spore relative to the probability of an unrelated cell forming a spore.

that—when they go through the asexual cycle—form delicate fruiting bodies. As far as is known, microcyst formation, which is likely to have been an ancestral trait carried over from an asocial amoeba, is independent of intercellular interactions. If so, the evolution of the microcyst may not have involved social selection. It would be interesting to know what factors (in addition to cell density) decide on the relative likelihood that an amoeba will form a microcyst on its own or aggregate with others.

There are scattered but intriguing reports of cell behaviour being modified by intercellular interactions with the effect being carried over into the next life cycle. In separate experiments, and with two different species, *D. discoideum* and *D. purpureum* respectively, Sussman and Kahn mixed cells of an aggregateless mutant with wild-type cells. From the chimaeric fruiting bodies that were formed, they recovered spores of supposedly mutant background and discovered that amoebae derived from them had regained the ability to aggregate—the mutant phenotype had mysteriously been ‘cured’ (Sussman 1952; Kahn 1964). Kaushik found that after cells of three distinct strains of *D. giganteum* had been carried through 24 asexual life cycles in combination with one another, they had lost the ability to develop by themselves (Kaushik 2002). It is impossible to explain these findings on the basis of our present understanding. The experiments must be repeated, their findings verified and the possibility of selection (following spontaneous mutation) excluded. If the findings are confirmed and no genotypic change has occurred, they would point to phenotypic change triggered by social interactions (which is not a surprise by itself) combined with what seems to stable inheritance of the new phenotype (which falls outside what we know about CSMs but, as a recent survey shows, is prevalent in many other systems; Jablonka and Raz 2009). A less drastic inference would be that this experiments point to the occurrence of what one could call (by analogy to the animal context) social learning with epigenetic inheritance. Takeuchi has provided an instance. He raised fruiting bodies from isolated anterior or posterior fragments of *D. discoideum* slugs³⁴ and allowed their spores to germinate. The amoebae that emerged were not provided nutrition and so were unable to divide; instead they were made to aggregate immediately with reference cells. The slugs that ensued showed a clear pattern of spatial sorting: amoebae that were derived from spores generated from slug anteriors tended to sort out to slug anteriors once again, and those derived from spores that had been generated from slug posteriors tended to sort out to slug posteriors; the effect disappeared when a phase of growth and cell division was allowed to intervene (Takeuchi 1969; also see footnote 24).

Finally, CSM amoebae participate in a number of cross-species interactions whose evolutionary implications remain unexplored. The interactions may or may not be significant in the natural ecology of the CSMs (of which we know very little). CSM species can co-exist in close proximity (Raper 1984), sometimes on

³⁴ Raper (1940) had shown that this was possible. Presumptive stalk and spore cells can interconvert when inhibitory influences from the complementary tissue are removed (see Fig. 3).

the same speck of soil (Sathe et al. 2010), and the extracellular signals that they use overlap; indeed they can form combined (inter-species) groups (Raper and Thom 1941). Interference with intra-species signals is possible and, in principle, could be significant for the evolution of social behaviour.³⁵ On the other hand as far as social behaviour within a species goes, inter-species interactions could be a form of ‘ecological noise’; at present we simply do not know.

Be that as it may, certain *P. pallidum* strains produce a killer factor that is lethal to other strains of the same species as well as to several *Dictyostelium* species (Mizutani et al. 1990). A different factor produced by *P. pallidum* causes *D. discoideum* amoebae to fuse and become multinucleated (Mizutani et al. 1991). Another instance of inter-species aggression in the CSMs is the behaviour of *D. caveatum*, which aggregates with amoebae of other species and proceeds to kill them and use them as food for increasing its own numbers (Waddell 1982; Nizak et al. 2007). We have already mentioned that *A. aerogenes* bacteria (for whom CSM amoebae are predators) produce a substance that activates spore germination in *D. discoideum* (Hashimoto et al. 1976; see footnote 20). Spores survive feeding by predatory soil nematodes (Kessin et al. 1996) and other soil invertebrates (Huss 1989), birds (Suthers 1985;) and mammals (Stephenson and Landolt 1992; Sathe et al. 2010), and in an environment containing them a CSM amoeba that can differentiate into a spore—which requires an intermediate social phase—would be at an advantage over an amoebal cell that cannot form a spore. Ellison and Buss (1983) found an intriguing case of cross-species communication and symbiosis (at least in one direction) involving a CSM. A soil isolate of *D. mucoroides* went through normal development only when exposed to a diffusible substance released by the fungus *Mucor hiemalis* or directly to the fungus itself. These cases point to selective pressures from the biotic environment that can affect social behaviour in the CSMs, but their prevalence and long-term consequences—if any—remain unknown.

8 Summing Up

Behavioural modification by means of intercellular communication occurs throughout the life cycle of the cellular slime moulds (CSMs). One cell can influence the likelihood that a second cell differentiates into a spore, or dies and forms part of the stalk. Thus the phenotype of a cell has both autonomous and non-autonomous aspects; both direct and indirect effects of natural selection have

³⁵ Cooperative communities of bacteria (Sachs and Hollowell 2012), nests founded by females of different species in social insects (Hunt 2009; especially the note there attributed to Snelling) and mixed-species foraging in bird flocks (Sridhar et al. 2009) all show that interactions between different species may be relevant for social evolution.

shaped traits in the cellular slime moulds. Indirect effects can work via communication among cells of a clonal or polyclonal social group of the same species. Communication can also take place between members of different species that happen to come together, or indeed between CSMs and other organisms in the environment. The possible impact of inter-organismal interactions on CSM development and behaviour is just beginning to be explored (Sonowal et al. 2013).

We offer three conjectures on what broad developments in understanding might lie ahead. Microarray analysis of temporal (Šášik et al. 2002) and spatial (Maeda et al. 2003) gene expression patterns during development in *D. discoideum* show a dynamic picture that—presumably—reflects constant interplay between cells. Temporal expression appears to occur in bursts within somewhat loosely defined time windows and later sequences of expression depend on earlier ones (Maeda et al. 2003). We know quite a bit about how the expression of individual genes is regulated and much of the knowledge has to do with gene products (or enzyme products) that are involved in intercellular communication. But a comprehensive view of the flow of information between cells and what it means for the coordination of multicellular behaviour is missing. Next, there is the prospect of insights from genome sequencing data. But, as with hopes raised by genome sequencing generally, expectations must be tempered with caution: Parikh et al. (2010) comment that the sequences of *D. discoideum* and *D. purpureum*, sister-species within the same clade, “are as divergent as those of man and jawed fish”.³⁶ Finally, there is the fundamental question of non-selectionist (but evolutionary) explanations for aspects of CSM social behaviour. The range of phenotypic plasticity in the CSMs is such that occasionally one species exhibits an aspect of development that resembles a feature found in another. Sometimes one finds a branched stalk (normally seen in a different genus, *Polysphondylium*) in a fruiting body of *D. discoideum*; Bonner (2003) has pointed out that a CSM with a cellular stalk, *D. lacteum*, makes fruiting bodies that are partly acellular (an acellular talk characterises the genus *Acytostelium*) when the number of cells in the aggregate falls below a threshold. Clearly these variations fall within the repertoire of *D. discoideum*. Such being the case, we need seriously to examine to what extent the origin of social behaviour in the CSMs requires a specifically adaptationist explanation and to what extent it can be accounted for more parsimoniously as the outcome of self-organisation among a group of cells that led solitary lives but were equipped with traits that could be co-opted for social living (Newman and Forgacs 2005; Ratcliff et al. 2012). The observed phenotypic differences in morphology and behaviour could then be largely neutral (Bonner and Lamont 2005; Bonner 2013).

³⁶ Because, based on their DNA sequences the last common ancestor of the two lived 400 million years ago (Sucgang et al. 2011). But *D. purpureum* and *D. discoideum* have a number of orthologous genes whose expression patterns overlap considerably (Parikh et al. 2010), i.e., in terms of gene expression patterns the species look very similar.

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