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The evolution of communication and social behaviour in *Dictyostelium discoideum*

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Abstract. Exceptionally for a developing system, the pathways of intercellular communication are fairly well characterised in the cellular slime molds. This paper attempts to provide adaptive explanations for the origin of the following features and consequences of communication between cellular slime mold cells: the tendency to congregate, chemotaxis to a released signal, signal relay from cell to cell, oscillatory signal release and an invariant ratio of the terminally differentiated cell types. For the sake of specificity attention is directed at the species *Dictyostelium discoideum*. Central to the entire analysis is the assumption that contiguous groups of feeding cells are, and in the past were, genetically identical. It is suggested that, in respect of most of the features listed above, the critical event which started things off must have been the acquisition by the cell membrane of permeability for a substance normally produced intracellularly as a response to the stress of starvation. An argument is presented for treating social behaviour in these organisms, and in particular the suicide by cells which differentiate into stalk, as an example of group selection.

Keywords. *Dictyostelium*; slime mold; evolution; group selection.

1. Introduction

The aim of this paper is to advance adaptive explanations for some manifestations of organised behaviour in a primitive microorganism. A property will be said to be "adaptive" in the sense of being more useful for the survival and reproduction of the organism than the absence of the property and, sometimes, than other possible alternatives.

Almost by definition, communication or mutual signalling is a prerequisite for social behaviour (Wilson 1975). Excepting cases wherein sociality is only apparent and due to spatial proximity caused by other factors, the observation of social or cooperative behaviour indicates the existence of an underlying system of communication. Specifically, some form or forms of communication must be responsible for the integrated multicellular behaviour displayed by a developing organism. This leads one to the question of why any particular mode of communication, either identifiable directly or inferred from its effect on social behaviour, exists in development. In general there are two sorts of answer possible to such a question. One can think of a 'developmental' explanation, meaning basically an explanation in terms of whatever is known of the embryological process at a more basic level, ideally of its physics and chemistry. On the other hand one can aim for an 'evolutionary' explanation; that is, one can try to invoke natural selection. Seen in this light, the interesting questions which need to be answered are, in what manner is a particular form of communication adaptive? And how could it have evolved? Hardly anything is known about the details of intercellular communication in most developing systems for these questions to be considered seriously. The cellular slime molds, especially their best studied member, *Dictyostelium discoideum*, provide an exception to this general rule (Bonner 1967; Loomis 1975).

In *D. discoideum* multicellularity arises on account of aggregation of single amoebae because of the emission of, and attraction to, a known chemical signal (Bonner 1967). During aggregation the signal is released periodically and also relayed from cell to cell (Gerisch and Wick 1975: Shaffer 1975; Roos *et al* 1975). Strong circumstantial evidence indicates that the cells continue to communicate—albeit by other, not yet understood means—in the multicellular aggregate (Raper 1940; Lokeshwar and Nanjundiah 1983). Eventually, after a series of morphogenetic movements, essentially all the cells in the aggregate differentiate into one of two types whose relative numbers are constant (Bonner 1967).

The present work is the result of an effort to look at the following 4 features of communication from an evolutionary point of view: aggregation by chemotaxis, periodicity of signal release and signal relay, patterning within the multicellular aggregate and cell number proportioning in the terminally differentiated structure. In order to fix our frame of reference we restrict our attention to *D. discoideum*. A word about methodology: the approach—not necessarily in the same order—will be to (i) describe an existing property; (ii) suggest an adaptive value for it; (iii) conjecture an ancestral state (by which is meant, somewhat loosely, a state in which the property is missing); and (iv) point out that starting with the ancestral condition, the presently existing property could be acquired via plausible mutational steps.

In what follows I first sketch the development of *D. discoideum* in just sufficient detail for our purpose and then go on to suggest specific evolutionary hypotheses for the 4 features mentioned above. General issues related to the hypotheses are discussed at the end. Comparative development in the cellular slime molds has been recently discussed in a paper by Bonner (1982) which has strongly influenced this one.

2. The life cycle of *Dictyostelium discoideum*

As this description is highly condensed, the literature (Bonner 1967; Loomis 1982 and references therein) should be consulted for a fuller picture. *D. discoideum* is a free living soil amoeba. Controlled experiments performed in the laboratory enable one to build the following plausible account of its life in nature (figure 1). In their vegetative phase, individual cells feed on bacteria, grow and divide by mitosis. Under extreme environmental conditions cellular slime mold cells are capable of following one of two protective strategies, that of forming microcysts or macrocysts. Both are resistant structures. Microcysts are not known as yet in *D. discoideum*, and macrocysts are the first step in the sexual pathway. Since they are not formed under standard experimental conditions, we will not refer to them any further (Bonner 1982 has considered their possible significance). In the laboratory, amoebae starved of food go through a seemingly quiescent phase for a few hours prior to aggregating. Aggregation is by means of chemotaxis, usually accompanied by relay of the chemoattractant. The attractant is a chemical, cyclic AMP (cAMP), which is produced and periodically released by the cells. A cell which senses external cAMP moves towards its source and in turn itself releases a burst of cAMP. Consequently local variations in cell density get amplified. Ultimately

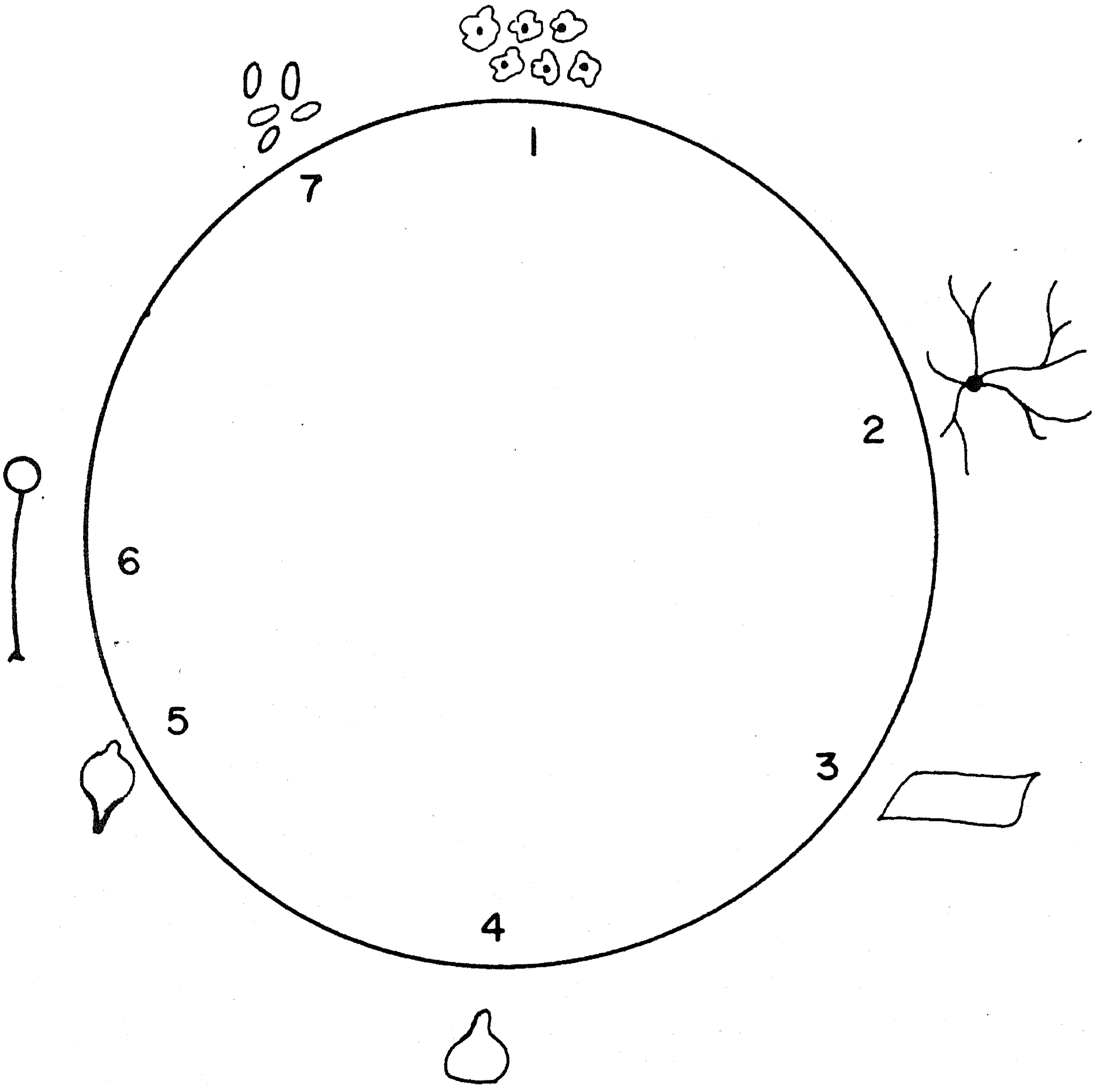

Figure 1. Schematic representation of the life cycle of *D. discoideum* (not to scale). (1), Free-living amoebae; (2), aggregation; (3), migrating slug, anterior to the right; (4), early culmination; (5), advanced culmination; (6), fruiting body with spore mass held aloft stalk; (7), spores.

all the cells in a neighbourhood end up piled on top of each other in a mound which is held together by specific adhesion molecules. The aggregate—now called the slug—often falls down and adopts a cylindrical form with a nipple-like protrusion, the tip, at its anterior end. After moving for a length of time which depends strongly on external conditions as well as the genotype, the slug stops, reverts to a mound-like shape, and the cells in it move in the manner of water in a reverse fountain and the entire mass rises upwards (the words 'up' and 'down' mean away from the two-dimensional substratum on which aggregation has occurred, or towards it). Irreversible differentiation sets in concurrently, most of the cells either turning into spores or, alternatively, dying and turning into the units of a cellulose-sheathed stalk. The final structure, called the fruiting body, has a disc-shaped base (also made of dead cells), an erect stalk and, above the stalk, a spheroidal mass of spores. Under suitable conditions each spore can germinate and give rise to an amoeba, setting off the life cycle anew.

3. Aggregation

If not the earliest, aggregation is certainly the most striking example of social behaviour in the cellular slime molds [mutual repulsion, observed in the vegetative phase of some slime molds, is conceivably a device for maximising the efficiency of feeding (Keating and Bonner 1977)]. Since starvation is the primary trigger, it seems intuitively reasonable to suppose that aggregation, like the coming together of individuals in more evolved social groups, must be a behavioural trait which has evolved to improve the chances of survival of the individual itself or of its genetic relatives. Therefore one must make it plausible that because of aggregation there is an increase, either in the probability that an amoeba turns into a viable spore or in the probability that a spore disperses to a favourable environment. A large mass of spores probably stands a better chance of dispersal than many isolated spores (Bonner 1982). In a fruiting body, just the fact that the spores are on an elevation should also help in dispersal, and we will take this up later. That apart, the assumption that aggregation might enhance sporulation seems justified, since cell-cell contact is normally a requirement for development, and so for sporulation (Gregg 1971; Newell *et al* 1971; Takeuchi and Sakai 1971). Precisely how contact aids development is not known; one possibility is that the binding or recognition of sets of cell-surface molecules is necessary. Alternatively, from observations on mutants which can sporulate in the absence of contact if simulated by exogenous cAMP (Kay and Trevan 1981), we might conjecture that 'contact' really means a degree of proximity sufficient for some substance emanating from one cell to reach a level at the site of a second cell high enough to induce the latter to turn into a spore (extracellular cAMP by itself is a non-specific differentiation signal in present-day *D. discoideum*; in conjunction with other substances it can influence either stalk or spore formation (Morrissey 1982)). The suggestion is, then, that in the ancestor of *D. discoideum* a single cell could sporulate in isolation but that the process could be helped along by a stimulus emanating from another cell in the same condition. What sort of substance would be appropriate to provide such a stimulus? A natural candidate would seem to be a chemical which was already being synthesised in the (ancestral) isolated cell during sporulation. Such a substance would reinforce a cell's response to starvation if, in addition to being produced intracellularly, it moved from one cell to another through a 'leaky' membrane. The leak itself would be selected for when it first arose by mutation. This would be so since sufficiently small clusters of post-feeding amoebae very likely consist—and consisted—of clones; the mutation would be simultaneously expressed in an entire group of cells. The possibility that the leak could be disadvantageous to a cell on account of its losing some of the signal substance must have been compensated by the benefit it conferred when it entered and stimulated other, genetically identical, cells. To sum up, a group of amoebae which stayed together after feeding was over would hold a selective advantage over one whose members continued to move randomly and so drifted apart. Thus the mechanism of 'aggregation', when it first arose, may have been nothing more than a decrease in cell motility once the density of food became too low to be reliably sensed; the effect may have been further strengthened by the acquisition of the ability by cells to adhere to one another.

4. Chemotactic attraction

Continuing with the line of argument we have used so far, it is not difficult to see how mutual attraction, by decreasing the spatial spread of post-vegetative cells, would also have been selected for. The agent of attraction could either be the same one that enhanced sporulation or, perhaps, a substance which worked inside the cell and guided cell movement by acting on contractile filaments, thereby causing local psuedopodial extrusion (the observed rapid accumulation of cytoskeletal actin in *D. discoideum* following an external cAMP stimulus (McRobbie and Newell 1984) could be a pointer to the original situation). The same substance entering from outside would have the same effect, but this time in a definite direction. A mutation which made the cell membrane porous for this substance would polarise cell movement centripetally towards wherever the cell density was slightly higher than in the neighbourhood, or wherever—if development were not perfectly synchronised in all cells—the first cell to leak out the substance was situated.

Until now I have tried to suggest how non-directed aggregation could be adaptive in itself, and how—by improving the efficiency of aggregation—a further beneficial adaptation could be conferred by either the simultaneous and mutual attraction of all cells in a neighbourhood or the attraction of most of the cells towards a center.

5. Relay

Consider now the possibility that a primary chemotactic stimulus might excite a sensitive cell to act as a secondary source of the stimulus. The advantage of having an attractant relayed outward from a centre, rather than having it released at about the same time by all cells, would seem to be the prospect of increasing the size of an aggregate. Since the signal should have the same range whether it is released by a cell on its own or whether a cell releases it in response to an external stimulus, it appears that the ability to relay would not have been favoured if all cells had—in the course of their development—started to signal simultaneously. In the absence of a means for synchronising development, simultaneous signalling does seem unlikely; the special care needed to achieve developmental synchrony in the laboratory suggests this. Therefore, in order to associate a selective advantage with the ability to relay, it needs to be assumed that under natural conditions some cells in a group are developmentally more advanced than others and, specifically, are capable—purely on statistical grounds—of releasing the chemoattractant earlier than most others. Given that relaying the chemotactic signal causes aggregates to increase in size, how might this be beneficial? It could be that large aggregates, with large volume-to-surface ratios, imply a reduction in the amount of the hypothetical substance which leaks to the outside world and is lost. For the same energetic investment, the yield in terms of effective signal strength would be higher. Another possibility, alluded to earlier (Bonner 1982), is that a large aggregate might improve the prospects for spore dispersal. Significantly, large slugs of *D. discoideum* move faster than small ones (Bonner *et al* 1953). Consequently if the time available for dispersal (before sporulation begins) is limited, the distance of dispersal would be greater for cells contained in the larger of two slugs. If this is to be used as a justification for increased aggregate size *via* relay, one will have to make the further supposition that aggregate motility existed earlier, probably having being selected as an aid to dispersal by itself.

There is another way of looking at the phenomenon of relay (Nanjundiah 1978). So far we have assumed that chemotaxis-mediated aggregation came first and that relay was a later refinement. Suppose things had been the other way round: consider a stage in evolution at which a clone of cells, lying close to one another, were faced with starvation. Just as we made a case for the original aggregation signal being a pre-existing component of the cell's internal response, we can argue that the cell-to-cell transfer of a substance, until then produced inside the cell in response to starvation and remaining there, would have enabled an entire group of cells to cope with a deteriorating environment faster than they would have in the absence of relay. Both clustering and chemotactic attraction could have arisen, in this way of looking at things, as later adaptations. Note that the first hypothesis (relay as a device for increasing the range of the chemotactic signal) makes it essential that the relay substance and the chemoattractant be the same (as is indeed the case in *D. discoideum*); on the second hypothesis, though it would probably be of advantage to have them the same, they could well be different.

An interesting point regarding the origin of relay is that on the second hypothesis the relay substance must have been able to activate its own synthesis. In other words, the reaction pathway responsible for forming the substance must have had in it an autocatalytic (positive feedback) step. The critical mutational event would then have been, as already suggested, a specific membrane 'leak'. If, on the other hand, chemotactic aggregation had arisen as an earlier adaptation (the first hypothesis), the important mutation would have been the one which made chemoattractant synthesis autocatalytic: that is, an early step in the synthetic pathway would need to be activated by a later step.

6. Oscillations

Given relay, and therefore an autocatalytic step; given that the living cell is thermodynamically an open system; and given that most biochemical reactions *in vivo* operate far from equilibrium, oscillatory reaction fluxes are almost a "natural" consequence (Higgins 1967), meaning that no special adaptive explanations are called for. Goldbeter and Segel (1977) have in fact shown that the observed cAMP oscillations in *D. discoideum* can be successfully modelled by assuming that the condition for relay exists, that is that an extracellular cAMP stimulus activates the intracellular synthesis of cAMP. Oscillations are on the other hand ubiquitous in biological systems (Winfree 1980); in particular, morphogenetic oscillations are found in myxobacteria (Kaiser *et al* 1979) as well as in other cellular slime molds, possibly with the involvement of cAMP (Schaap and Wang 1984). Even temporal patterning in chemical communication, though rare, is not unknown (Conner *et al* 1980). So it is worth considering whether the relatively rapid (period ca. 8 min) oscillations of cAMP in *D. discoideum* might be adaptive after all.

Under certain conditions, the course of glycolysis in yeast is oscillatory, also with a periodicity of some minutes (Hess and Boiteaux 1971). Since these oscillations involve the adenine nucleotide pool and therefore the adenylate charge (Atkinson 1968) of the system, Goldbeter (1974) has conjectured that the cell benefits by partitioning each oscillatory cycle into distinct energy-utilising and energy-yielding phases, something which is supposed to make for an improved metabolic efficiency. How far this argument can be carried over to cAMP oscillations in *D. discoideum*, where ATP levels do not vary significantly within a period (Gerisch *et al* 1977), is not certain. Richter and Ross (1981) have offered yet another adaptive explanation for the glycolytic oscillation. Using a quantitative model they have calculated that energy dissipation in the latter part of glycolysis (the pyruvate kinase step) is minimal precisely when an earlier step (the phosphofructokinase reaction) is periodic with a frequency within a defined range. Again, one does not know how good this explanation, depending as it does on the "tuning" of one reaction step by another, would be for the slime mold oscillations. The oscillations in this case occur—as far as is known, unlike glycolysis in yeast—both extracellularly and inside the cell, and it is easier to speculate on a possible adaptive role for extracellular oscillations. It has been shown (Nanjundiah 1973) that when the relevant parameters are assumed to have reasonable values, a pulsatile source of cAMP has a spatial range which is about an order of magnitude more than the range of an equivalent steady source which releases cAMP at the same average rate. The reasoning is based on the facts that (a) (in three dimensions) the concentration of a signal diffusing from a steady source falls off inversely with the distance, whereas the peak concentration due to an impulsive source decreases as the cube of the distance, and (b) the lower the frequency of successive pulses, the weaker the effect of the equivalent steady source. Then, given a plausible threshold for the sensitivity of a cell, either for the concentration of cAMP or for its spatial gradient, the threshold is reached at a farther distance from the source when the signal is released in a series of brief pulses than when it is released at a uniform rate. The conclusion remains valid even when the released signal profile is no longer a sharp pulse but is somewhat sinusoidal. However, in the latter case the range of the signal is enhanced by roughly a factor of two rather than by one order of magnitude. A periodic signal has two other advantages. One is that the receiver would not get adapted to it as readily as it might to a steady source. It is to be expected that a train of stimuli reiterated at an appropriately intermediate frequency would be much better at eliciting a response than the same train of stimuli applied either at a very low frequency (in which case each stimulus would—so to speak—be like the first stimulus, there being no reinforcement) or at a very high frequency (in which case there would effectively be one constant stimulus, to which the system could adapt). Just this has been observed in the case of *D. discoideum* cells with folic acid, a chemoattractant for feeding cells (Wurster and Schubiger 1977). Also, periodic pulses of cAMP are efficient at eliciting cell differentiation whereas steady levels are not (Gerisch *et al* 1975; Darmon *et al* 1975). Unfortunately, none of these experiments tested the effect of irregular pulses or different waveforms; one is still unsure as to precisely which stimuli constitute signals and which ones do not. The other advantage of a periodic signal is that at close distances there would be a lesser degree of ambiguity in locating a periodic source than a steady source (the amplitude of oscillations will, in any case, get damped with increasing distance from the source, the higher frequencies dropping out first).

7. Pattern formation and tissue proportioning

7.1 *Pattern in the slug*

The slug is a facultative migratory structure formed by an aggregate of *D. discoideum* before it differentiates into a fruiting body. It is in some ways a preparatory phase; the future stalk and spore cells are identifiable in its front and rear portions (Raper 1940). Depending on the type of experiment carried out, this separation into pre-stalk and pre-spore can be seen either as an expression of autonomous cellular predispositions (Takeuchi *et al* 1977) or as a consequence of the relative positioning of cells along the long axis of the slug (Raper 1940). Probably both factors are important and reinforce each other. Whatever be the cause, a spatial separation of presumptive cell types occurs within a few hours after aggregation is completed. It is difficult to avoid the conclusion that this spatial segregation must have co-evolved either with, or following, the evolution of the process of fruiting body formation and the geometry of the fruiting body. The chain of events could have been first, the transition of an aggregate into a fruiting body of a certain structure (a spore mass held aloft on erect stalk) and later, the appearance of an intermediate migratory phase in which the form of the fruiting body was anticipated. Migration would most likely be on account of an extension in time of the mechanism of amoeboid movement. However, morphogenesis in two other cellular slime molds displays features which indicate that this cannot be the whole story. In *Dictyostelium mucoroides*, stalk cells are formed and continuously released from a common pool consisting of all the cells in the migrating slug; in *Acytostelium* the stalk, which is acellular, is made up of material extruded from cells. Bonner (1982) treats this in some detail and goes on to speculate that patterning might be related to some developmental constraint imposed by the use of cAMP in chemoattraction.

It must be mentioned that the reasons for believing that cells in the slug communicate with one another go beyond the facts of integration (in a general sense) and pre-stalk—pre-spore patterning (in particular). The oldest and most convincing reason is that slugs belong to the class of regulative embryos; a fragmented slug gives rise to a diminutive but normally proportioned fruiting body (Raper 1940). Regulation was probably an adaptation in response to the selective pressure provided by physical fragmentation (Bonner 1982). Quantitative studies (Lokeshwar and Nanjundiah 1983) directed at a very early event in regulation—the regeneration of a new tip at the anterior margin of tipless fragments—indicate the involvement of long-range communication within the slug. How communication actually occurs is not known; the evidence from experiments on the rate of tip regeneration in genetically mosaic slugs is in agreement with, but does not prove, cell-to-cell relay (Lokeshwar and Nanjundiah 1985). If this result is confirmed, and if the same signal is responsible for both the rate of tip regeneration and long-range communication in the slug, one might conjecture that cell-to-cell relay during aggregation was a preadaptation which facilitated patterning in the slug.

7.2 *Tissue proportioning*

Under constant environmental conditions the number of spore cells in a fruiting body is in a more or less constant ratio to the number of stalk cells. The data of Stenhouse and Williams (1977), as analysed by Lokeshwar (1983), shows that 87·6 % ± 4·5 % of the cells in small fruiting bodies (630–3901 cells) form spores; the corresponding figure for large fruiting bodies (8404–18301 cells) is 87·1 % ± 4·8 %. Lokeshwar also makes a case for neglecting, in comparison with stalk and spore, the number of cells which form the basal disc (less than 1·5 % of the total); his own studies reveal no undifferentiated amoebae in the fruiting body. Two questions follow: (i) Why is the ratio of spores to stalk constant? (ii) Why do a certain fraction of cells—those that form the stalk—die and so sacrifice their genetic potential? Wilson (1975) and Bonner (1980) cite stalk formation in *Dictyostelium* as an example of altruistic behaviour and invoke kin selection in order to explain it. The following simple argument (V Nanjundiah, unpublished results) makes this explicit. However, I suggest (see later) that 'group selection' is a more appropriate term than 'kin selection' to describe the phenomenon.

The critical assumption will be that, at least under the conditions obtaining when the present form of the fruiting body first evolved, an aggregate consisted of genetically identical cells. The problem facing this aggregate would have been to transfer all or part of itself elsewhere because food was finished at the site of aggregation. Migration of the entire aggregate towards food (not known to occur in *D. discoideum* slugs), might have been a possibility. The alternative, given that some of the cells had committed themselves to form spores, would have been to ensure that as many spores as possible dispersed and helped to give rise to viable amoebae. With an erect stalk and a spore mass on top, the distance of dispersal would depend on the elevation of the fruiting body and so on the number of stalk cells, but too high a stalk would mean that there would not be many spores left to propagate. An optimal balance would be reached at some intermediate value for the proportion of cells turning into spores. The actual ratio of spore to stalk at this optimal balance will depend on the mode of spore propagation— which could be by means of water, insects or other small animals, or wind. Purely as an illustrative exercise let us imagine that the spore mass is detached by a gust of wind and free-falls on account of its own weight (turbulence being neglected). The distance of dispersal will be the horizontal distance covered by the mass in the time it takes to fall to the ground. For a fruiting body consisting of N cells of which N_1 are spores and $N_2 =$ $N - N_1$ make up the stalk, the height of the structure will be proportional to N_2. Then the time of fall, and so the distance covered, will be proportional to $\sqrt{N_2}$ or $\sqrt{N - N_1}$. Now, suppose that the availability of food, and so the probability of a spore germinating, is proportional to the distance of dispersion. A single spore, N of whose clonal descendants formed a fruiting body, would give rise to $N_1 \cdot \sqrt{N - N_1}$ (times some constant) spores. It is easily seen that the product $N_1 \cdot \sqrt{N - N_1}$ is a maximum when N_1 $= \frac{2}{3} N$, that is, at a fixed ratio of N_1 to N. Therefore according to this highly simplified model, $\frac{2}{3}$ of the cells in an aggregate should differentiate into spores and the rest into stalk if reproductive fitness is to be maximised. This conclusion still leaves us with the difficult problem of speculating on the possible mutational steps leading to proportioning. An intriguing but attractive possibility would be for proportioning to have evolved in parallel with patterning and spatial segregation of presumptive cell types, either in the late aggregate or in the early fruiting body.

8. Discussion

I have tried to provide adaptive explanations for some observed and inferred examples of communication between cells of *D. discoideum*. The sequence of evolutionary stages is suggested to have been as follows (table 1): sporulation of single spores → non-specific aggregation and sporulation in a group → aggregation by chemotaxis → relay of chemoattractant and oscillations → formation of the fruiting body with division of labour into stalk and spore → motility and patterning in the aggregate. Relay could have preceded chemotaxis instead of following it. The types of mutational events which could

Table 1. A hypothetical sequence in which the later mutational events enhance, but do not otherwise interfere with, the effects of the earlier ones. The initial situation is supposed to be one in which cells sporulate individually when starved; 'X' is a substance synthesised intracellularly in the course of sporulation. For simplicity X is assumed to be the same in each step; this would correspond to the roles played by cAMP in *D. discoideum.*

Effect of mutation	Consequence	Possible reason(s) for increase in fitness
Starvation-induced leak of substance ('X') through cell membrane	X can enter a cell from outside	(a) Improved ability to sporulate; (b) Cell responds to deteriorating environment more rapidly than it would have
Tendency of cells to stick to each other	Cells form clumps	Improved prospects for (post-feeding) dispersal, but probably also a decrease in feeding efficiency
Stickiness inducible by X	Cells clump only after feeding is over	As above, but no decrease in feeding efficiency
Chemotaxis to X	Cells actively congregate	Eventually, better dispersal
X relayed from cell to cell	Aggregate size increases	As above

have led to each new stage have been conjectured. In this discussion I confine myself to making a few general points regarding these explanations.

Two questions which occur at the outset are, how reasonable are adaptive explanations in this context? And how reasonable are the arguments that have been advanced suggesting a specific form of selective force for the appearance of a particular phenotypic trait? The answers to both of these questions will necessarily be indirect and partial, and so not conclusive.

Cellular slime molds differ quite a bit in their life cycles, but aggregation and the formation of fruiting bodies consisting of spore masses held aloft stalks are common to all species. The reason why the details of development differ from one species to another could have to do either with differing adaptations to different selection pressures or to the same selective pressures leading to more than one adaptive peak. The possibility that the more "primitive" species are in the process of evolving towards the *D. discoideum* state cannot be ruled out, but appears to be unlikely. More relevant, the adaptive explanation itself might be incorrect or, at any rate, no less likely than a non-adaptive one (Gould and Lewontin 1979). It has already been mentioned that the phenomenon of oscillations is a possible candidate for a selectively neutral trait, even though a plausible adaptive explanation exists. The extracellular release of a cyclic AMP phosphodiesterase might be a second such candidate; arguments for and against the viewpoint that this is a nonadaptive (or maladaptive) trait will be found in the literature (Nanjundiah and Malchow 1976; Gerisch 1976; Darmon *et al* 1978). So also the widespread occurrence of regulation—the constancy in relative proportions of adult cell types—

might well have a purely developmental, as opposed to evolutionary, explanation.

All the same, in the absence of a specific rival hypothesis—based on selective neutrality, developmental correlations, even maladaptation—presenting itself, experience indicates that it is a useful exercise to see whether an adaptive explanation can be constructed at all and if it can, whether the construction is natural or forced.

A necessary (though not sufficient) condition for a trait to have evolved by selection amongst differentially adapted phenotypes is that it be subject to genetic control; and a "natural" construction of an adaptive explanation is one that makes use of plausible mutational steps. In the case of intercellular communication in *D. discoideum* both these requirements are satisfied. Consider the following properties of known mutations in *D. discoideum*: (i) an ability to differentiate without cell-to-cell contact (Kay and Trevan 1981) or normal aggregation (Ishida 1980); (ii) a failure to produce cAMP (Bonner *et al* 1969); (iii) a failure to respond to cAMP by chemotaxis (Bonner *et al* 1969); (iv) an inability to amplify and so relay an external cAMP signal, with the consequent absence of oscillations (Wurster and Bumann 1981); (v) a failure of normal stalk-to-spore proportioning (Morrissey *et al* 1981); and (vi) the absence of a correlation between the spatial patterning of the presumptive cell types in the slug, which can be normal, and proportioning in the fruiting body, which can be highly aberrant (Morrissey *et al* 1981; Amagai *et al* 1983). The intention is not to suggest that these mutations must represent a reversion to an evolutionarily primitive situation, but rather to indicate the plausibility of the origin and evolution of (present-day) wild-type genes with the properties demanded. It must be admitted that in one respect this could be a misleading argument: because selection acts on the genotype and not on single genes, or in other words because the relation between genes and phenotypic traits is normally not one-to-one, the life cycle must have evolved as a whole—and not, as in a sense we have imagined, in bits and pieces. It might appear that caution is all the more warranted when discussing *D. discoideum*, given the manifold effects that extracellular cAMP has on its development (Loomis 1982). Nevertheless, if the effect of a mutation is such as to influence one aspect of the phenotype more than any other, and if the mutation arises early enough in evolution for phenotypic effects to be significant, one can assume, as a good first approximation, that the components of (in this case) the communication pathway have been independently selected (table 1).

Even if we accept that adaptive explanations for the various features of intercellular communication we have considered might be valid, could the same end result have been achieved by other selective forces? The forces we have taken into account have been (a) selection for an improved efficiency of sporulation and (b) selection for dispersal. The assumption which runs through our entire analysis is that spatially contiguous, genetically identical amoebae form the units on which selection can act. Bonner (1967) starts with the opposite assumption—that in nature cells of diverse genotypes commonly come together in an aggregate—and comes to quite different conclusions regarding the function of aggregation. One is that aggregation serves as a partial substitute for sexuality, because the nuclei of the many genotypes gathered together in one spore mass have the potential to be redistributed as new combinations. He also suggests that genetically diverse cells living in close proximity might have developed mutual deficiencies which could have been overcome by aggregation. There are hints from the literature that two other selective agencies, predation and the occurrence of symbiotic or commensal organisms, might favour aggregation and fruiting. Waddell (1982) has reported the isolation, from bat guano, of a species of predatory cellular slime mold. When tested in combination with a number of other species, the predator is able to delay the morphogenesis and (very probably) consume the amoebae of the prey species. Ellison and Buss (1983) have found a case of synergism between field specimens of *D. mucoroides* (a species similar in many respects to *D. discoideum*) and the fungus *Mucor hiemalis*: the presence of the fungus induces stalk formation in *D. mucoroides* and fungal extracts advance rate of fruiting in this and other species (though not in a laboratory strain of *D. discoideum*, which conceivably had already been selected for very rapid development). The stalkless variant of *D. mucoroides*, which forms in the absence of the fungus, aggregates normally but neither forms a slug nor migrates. The aggregating mass rounds up and differentiates straightaway into a mixture of spores and dead amoebae. While it is not possible at present to seriously consider which features of communication in *D. discoideum* might have been selected as adaptations to predation (to discuss commensal living is even harder), we can imagine that anything which lowered the risk of predation must have been strongly favoured. So, for instance, both the tendency to form fruiting bodies as well as an increase in the overall rate of development would have helped (rate, because in a communication network the same set of events can have different effects on fitness if the temporal relationships of the events differ (Bonner 1982)). Both environmental conditions (Schindler and Sussman 1977) and the genotype (Kessin 1977) can influence the life cycle of *D. discoideum* by affecting the speed of development. Similarly, using a substance which was harmful to a predator as an agent of communication or using a misleading signal (imagine that cAMP is an alarm pheromone for some predator) would clearly improve the prospects of survival.

It is important to note that the speculations in this paper refer only to the first steps in the evolution of pathways of communication in *D. discoideum*; what one finds today must be the result of many further steps of refinement. The most important refinement was probably the origin of receptors (Newell 1977). Receptors would enable signals to work their effect without having to enter a cell, allowing for increased efficiency and specificity. Also, the same substance used as a signal could then serve different ends, depending on which subset of receptors it happened to interact with at any given time. Other refinements could result in the signal becoming ritualised as has happened in higher animals (particularly in the case of courtship signals; see Wilson 1975). The signalling pathway would pick up successively arbitrary modifications and develop into a link between source and receiver which is highly reliable and specific but displays few clues as to how it could have evolved. Whatever be the extent to which the primitive signal has become ritualised, if the view adopted here is correct it must follow that all the conjectured "signal substances" must be components of the cell's internal response to a deteriorating environment. Haldane (1955) has asserted as a general principle that a signal is always a sign of a physiological or psychological condition within the sender; what I am suggesting is that in the present context the signal is so to speak the condition itself. To take a specific example, the choice of cAMP as a chemoattractant would not be totally arbitrary (in the sense that human language is said to be), but would be related to a role played by cAMP in the internal metabolism of a starved cell, even if the role were that of a waste product to be discarded. This is a testable statement.

Cellular slime molds are social organisms, and the origin and continued evolution of sociality all the way to the extreme altruism displayed by stalk cells must surely be a consequence of, in part, the high degree of genetic relatedness, or even genetic identity, of the amoebae in an aggregate (Wilson 1975; Bonner 1982). Nevertheless, I believe the term 'group selection' is better suited to describe the situation than 'kin selection'. Kin selection is usually invoked when an individual sacrifices part of its potential reproductive fitness for the benefit of another individual related to it by common descent (Hamilton 1964). In the slime mold case, leaving aside the suicide by presumptive stalk cells for the moment, each of the adaptations we have considered has the characteristic that it is adopted by every member of the group; therefore its cost or benefit is the same for every member. It is the group which must succeed or fail, in comparison with other groups, with these adaptations. Even if this is not true at any one time because an aggregate consists of more than one genotype, it must be a factor which comes into play once the less fit genotypes have been eliminated (we take it of course that the genotypes differ in respect of the adaptations referred to in this paper). An implicit assumption is that all pre-fruiting cells are functionally identical: if this were not so, 'kin selection' might well be an appropriate description. An important example of functional non-identity would be if, for example, only the future stalk cells were to release the chemoattractant.

What if a cell belonging to a cheater genotype fortuitously gets into an aggregate, a genotype which responds to external signals with the rest of the group but does not produce the signal itself? Armstrong (1984), in analysing the survival of "cheaters" which never form stalk cells, suggests that division of labour would not be an evolutionary stable strategy under conditions which favour such cheaters. I feel this side-steps the basic problem, which is to explain why division of labour is observed even though a "cheater" (of whichever sort) is always at an advantage whenever it arises. The correct answer, according to me, is that the frequency of the cheater genotype will increase from one generation to the next until an aggregate consists of so many cheaters that the strategy does not pay any more. If the cheater only responds to signals released by the wildtype but does not produce them itself, at some stage the signal strength will be so weak essentially everywhere except at the locations of the signalling cells that viable aggregation will simply not occur. If the cheater always differentiates into spores the final state in evolution will be an aggregate consisting only of mutant spores; in line with the assumption under which we have operated the spores will be unable to disperse and therefore to survive. The cheater genotype increases in fitness but eventually drives the group to extinction. Therefore the normal division of labour is best described as a case of group selection.

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