Slime mould and the transition to multicellularity: the role of the macrocyst stage

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Abstract. The transition from unicellular to multicellular organisms is one of the mysteries of evolutionary biology. Individual cells must give up their rights to reproduction and reproduce instead as part of a whole. I review and model the macrocyst stage in slime mould (*Dictyostelium*) evolution to investigate why an organism might have something to gain from joining a collective reproduction strategy. The macrocyst is a reproductive cartel where individual cells aggregate and form a large zygotic cell which then eats the other aggregating cells. The offspring all have the same genetic code. The model is a steady state genetic algorithm at an individual cellular level. An individual's genetic code determines a threshold above which it will reproduce and a threshold below which it will join a macrocyst. I find that cycles in food availability can play an important role in an organism's likelihood of joining the macrocyst. The results also demonstrate how the macrocyst may be an important precursor to other cooperative behaviours.

1 Introduction

The quest to synthesise hierarchical levels of organisation in artificial life is a significant open problem [3, 23]. To provide a deeper understanding into how we may be able to use evolutionary algorithms to generate and optimise hierarchical behaviour, we can study the major transitions in evolution [16]. This work focusses on the transition to multicellularity which appears to be one of the most difficult 'bridges' evolution has had to cross. It is unclear whether the transition only occurred once, or several times [4]. Phylogenetic evidence [2] suggests that multicellular organisms, especially metazoa, share a common ancestor. Furthermore, fossil evidence [16] indicates that multicellular life did not exist for 2,500 million years until the Cambrian period (approximately 540 million years ago) where all the multicellular phyla are represented.

Multicellular organisms essentially consist of clusters of individual cells with all cells expressing the same genotype. They therefore require gene-regulatory mechanisms for differentiating cells (with differentiations being passed from parent cell to offspring), cell adhesion and spatial patterning of cells [16]. One particularly crucial cell differentiation stands out: The organism must separate its reproductive (*germ-line*) cells from its body (*soma*) cells [7].

The requirement for isolation of the germ line from the soma was first argued to be necessary by August Weismann [7]. To identify why, we can distinguish the two types of reproduction that are present in metazoan multicellular life and look at the conflicts that arise between them. Firstly, intra-organism reproduction happens when cells replicate within the super-organism, for the good of the super-organism. Conflicts can occur with cells reproducing on their own behalf [17]: mutant cells can disrupt and compete with the super-organism. By generating a whole organism from one initial germ-line cell, it is clear that the vast majority of selfish mutations that disrupt super-organism-level processes will only survive one generation [7]. Therefore, secondly, to solve this problem super-organism reproduction involves the replication of the complete organism through the selection of a germ line cell to reproduce on behalf of the super-organism. However, there is still a conflict over which cell is to be the germ line since selfish mutations that disrupt the super-organism reproductive process will be passed onto the next generation. A stable, policed, germ-line/soma differentiation mechanism must have evolved at some point.

It is unclear where in the evolution of a multicellular lineage, stable, well policed, germ-line/soma differentiation and germ line isolation should occur. However, given the above problems faced with intra-organism conflicts[17], it seems likely that the germ-line/soma differentiation evolved early [7]. Thus, we consider evolutionary mechanisms that will explain a transition between unicellular organisms, which compete within their populations and compete with predators and prey, and early multicellular organisms which are clustered together and exhibit germ-line/soma differentiation. In other words, there is a transition from unicellular organisms which are optimised to maximise their own *direct* fitness to cells that must, on the other hand, maximise their *inclusive* fitness at the expense of their direct fitness (i.e., their ability to contribute their fitness to other cells that are highly related must be more important than their own replication chances). (See [10] for precise definitions of *direct* and *inclusive* fitness.)

Whether the evolutionary transition described above, of organisms clustering and differentiating a germ line, happened in one stage is unclear. Wolpert has presented a model where individual cells may split to produce a somatic body cell that sticks to its parent and is unable to reproduce [27]. What the benefits, through inclusive fitness, are to individual cells and their lineages from doing this is unclear. There is a debate on this subject with some arguing that size is an important reason for multicellularity [4] with undifferentiated population clustering, as modelled in [19] without a germ-line/soma differentiation, being an important first step. Others point out that local competition over food will negate the value of cooperation through relatedness [21, 26, 15]. For this reason Di Paolo warns against relatedness being used as an explanation for cooperative behaviour [9]. There therefore appears to be something of a paradox if we attempt to try to understand the transition to multicellularity with such models of clustering cells. Individuals that cluster compete with each other and may negate the benefits of cooperation through relatedness, yet both clustering and cooperation are needed for the transition to early multicellularity.

A different perspective considers multicellularity through aggregation [16]. Here cells either vegetate and reproduce individually, or aggregate to reproduce collectively. This presents a sort of half way house between the individual and early multicellular behaviour identified above. *Dictyostelium* (more commonly known as slime mould) is a model organism for multicellularity through aggregation [16, 20]. Individual cells can either vegetate and reproduce asexually on their own, or under different environmental conditions they also demonstrate collective reproduction behaviour, characterised by individual cells making sacrifices for the benefit of other cells' reproductive chances. This organism therefore demonstrates both the germ-line/soma differentiation [6] and clustering that is important for the transition. Biological evidence is now presented concerning *Dictyostelium discoideum*, one of the more studied species of the genus.

When there is a shortage of food and *D. discoideum* cells begin to starve, they aggregate and one of the two collective reproductive stages commences [22]. The more well known reproductive stage of *D. discoideum* sees the cells form a slug which collectively migrates. Once the cells find an advantageous location they form a *fruiting body*: cells at the front of the slug (20%) form a stalk and the rest form spore cells at the top of the stalk which are dispersed by the wind. Interestingly, the stalk cells die after the stalk is built. This differentiation between spore and stalk cells is arguably a germ-line/soma distinction [6]. Since cells that produce stalks do not pass on their genetic code, it is hard to see how this trait is selected for and maintained. Indeed there are examples of slime moulds strains that do not produce stalks [6]. Computer simulations addressing this question [1] have indicated that high dispersal of spores can lead to more stability in the stalk producing behaviour.

The second, less well known, collective reproduction stage in *D. discoideum* involves the formation of the *macrocyst* [22]. Again, when the cells are starving they aggregate. However instead of forming a slug, two cells merge to form a large *Zygote* cell which eats other aggregating cells. The resulting giant cell forms a hard cellulose outer wall and this macrocyst germinates after a few weeks. See Fig. 1 for a diagram.

The macrocyst stage is thought to be a precursor to the slug/stalk reproductive stage. Kessin [13] argues that evolution generally occurs in incremental stages. He notes that the previous stage to macrocyst development would be the microcyst stage (not observed in *D. discoideum*), where individuals form outer walls on their own. After the evolution of chemotaxis, aggregation could occur and the macrocyst evolved. With added cell adhesion and cell type differentiation into stalks and spores, fruiting body and slug behaviour would then become plausible.

The genetic makeup of the offspring of the macrocyst is an important question. The macrocyst is generally accepted to be the sexual phase of *D. discoideum's* development [22]. However experiments do demonstrate that Macrocysts can form from only one mating type [5]. The progeny of one macrocyst is observed to be of one genotype [25]. Only one nucleus remains in the zygote (or giant cell) after other ingested nucleii disappear [18].

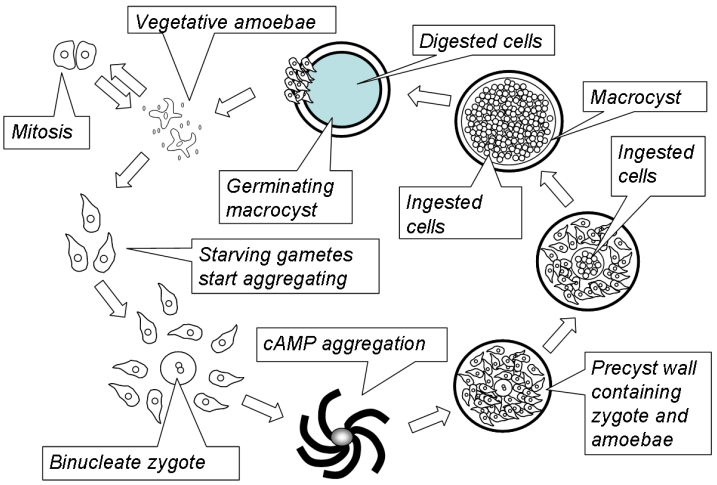

Fig. 1. The sexual and mitotic life cycles of *Dictyostelium* (based on [22]).

From Fig. 1 it is clear that differentiation in *D. discoideum* cells occurs when it starts to aggregate. Recent evidence implies that the cell may have genetic control over this event. Research [8, 11] suggests genes that can control or delay when or whether a cell will continue to grow or start aggregation. These findings indicate that the cell is capable of turning on or off aggregation to the macrocyst stage which can ultimately lead to cells being eaten by the zygote. This emphasises a need for an explanation as to why an individual might make the 'choice' to aggregate and almost certainly die.

I have produced a model of the *D. discoideum* macrocyst stage for several reasons: (i) to confirm that individuals that normally reproduce on their own are indeed prepared to gamble their own reproductive chances against the 'pot' of reproductive material contained in the macrocyst; (ii) to confirm my intuition that fluctuations in food availability are important to the viability of the macrocyst; (iii) to question the role individual mitotic split rates might play in the stability of the macrocyst; and (iv) to speculate on the role the macrocyst might play in the evolution of other altruistic behaviour (such as stalk/spore differentiation) and collective behaviour.

2 Methods

To investigate the questions in Section 1 I have built a computer simulation model of the macrocyst stage of *D. discoideum*. Assumptions in the model are based on the biological evidence presented. Notably I have assumed that all the offspring of a macrocyst are of the same genotype. Since sexual fusion does not seem to be necessary, I chose (on parsimonious as well as biological grounds) to model the macrocyst with no sexual recombination. Individual vegetative

behaviour was modelled with individuals having a genetically encoded energy threshold above which they mitotically reproduce.

D. discoideum cells are modelled as individuals in a non-spatial environment. At each time step, a number of individuals (N) are selected at random, each receives a 0.5 units of energy (representing food) with probability p. One cycle in the model contains two seasons. The amount and probability of food (N and p) changes value according to whether the season is 'high' ($N = 100, p = 0.6$) or 'low' ($N = 20, p = 0.3$). Each season lasts 200 turns. All individuals pay a daily energy cost ($E_{\mathrm{c}} = 1.0$) irrespective of season. If an individual's energy falls below zero ($x < 0$), it will die.

Each individual cell is modelled with two genes[1]. The genes model energy thresholds which determine the behaviour of the cell. Cells will join the macrocyst when their energy level is *below* the first gene, the *macrocyst join threshold* ($-2.0 < G_{\mathrm{join}} < 2.0$). When a cell's energy level is *above* the second gene, the *split threshold* ($5.0 < G_{\mathrm{split}} < 20.0$), the cell will pay an energy cost to split mitotically (see Fig. 1) and produce a new cell (sharing energy equally between itself and its offspring).

There is only one macrocyst in the model it is assumed to be immobile and therefore does not receive food from the environment. When cells join it, they contribute their own energy (x) plus a residual energy amount (equal to the cost of splitting) to the macrocyst's 'pot' (X). Before closing the macrocyst pays a cost E_{m} per individual joined every turn to reflect metabolisation and building of cellulose. If the macrocyst energy falls below zero ($X < 0$) then it (and all its joining cells) will die. When the macrocyst reaches a predetermined energy threshold (30.0), it closes and no other cells may join.

The macrocyst will germinate on the first turn of the high season. When it germinates, the energy is divided up into new cells with each cell receiving 2.5 energy units. All new cells will have the same genotype: a complete genotype (no recombination) is picked at random from all the cells that originally joined the macrocyst.

Simulations were run over 100,000 turns. Each simulation started with 100 individuals, each individual having a random genotype and a random energy between 0.0 and 5.0.

3 Results

To understand how the harshness of the low season can effect the viability of the macrocyst, simulations were run varying the probability of food in the low season. Interesting population dynamics, with macrocyst offspring outcompeting the non-joining population, were observed and these are presented in this section.

The average percentage of individuals which germinated from the macrocyst is plotted against the probability of food in the low season in Fig. 2. When

[1] Genes are represented as floating-point numbers in the simulation, point mutations occur at each time step over a gaussian distribution with standard deviation of 1% of the gene space

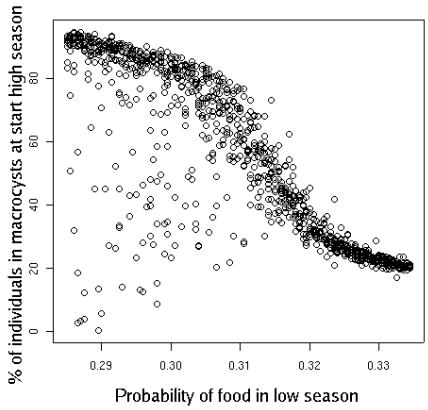

Fig. 2. Graph showing the percentage of individuals which germinated from a macrocyst at the start of the high season against the probability of food in the low season. Each data point (ten data points, each generated with different random seeds, per food-probability value) represents an average over a complete simulation run.

the probability of receiving energy is higher, few individuals ($\approx 20\%$) join the macrocyst. When there is a lower probability of food, more individuals join the macrocyst. However the rogue data points at the bottom left of the graph are of interest.

To investigate this disparity with some populations producing macrocysts and others not, the probability of food and seed value were selected from one of the rogue data points. The simulation was run over a longer (150,000) number of turns. A histogram was generated for the macrocyst join threshold at the start of each high season and the results are shown as a 3D mesh in Fig. 3A.

In the figure, the presence of macrocysts can be seen as spikes on the right hand side. An early tendency towards macrocyst joining is evident (far right of graph) but these genotypes die out after ≈ 25 cycles. A population which does not produce germinating macrocysts immediately flourishes. After ≈ 150 more cycles there are enough individuals to successfully produce a germinating macrocyst which survives to the end of the low season. Interestingly once this has happened the macrocyst very quickly wipes out the non joiners from the population. The offspring from the macrocyst must have some sort of competitive advantage over the non-joining population.

A closer look at Fig. 3A indicates that when there are not enough individuals joining the macrocysts to make them germinate, there is only a small tendency toward individuals that will not join the macrocyst when their energy is very low. Between cycle 25 and cycle 175, the histogram shows a larger proportion of individuals having a join threshold below zero, however some still remain with a threshold above zero. There is clearly little selection pressure against individuals sacrificing small amounts of energy when near to death.

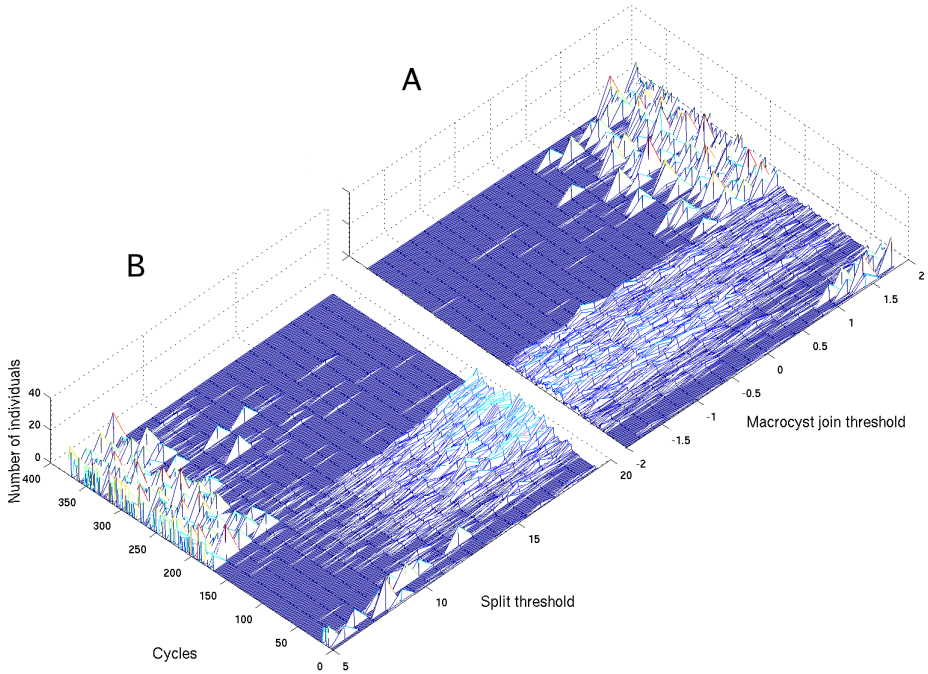

Fig. 3. 3D histograms of macrocyst join thresholds (A) and individual split thresholds (B) of the population at the start of each high season.

A second 3D histogram was generated for the split thresholds of the population at the start of the high season and can be seen in Fig. 3B. There is a clear disparity of the split thresholds between the macrocyst joining population and the non joiners. Again, in the first few cycles of the simulation (where the macrocyst joiners were predominant in Fig. 3A), the population has a low split threshold – individuals will split as quickly as possible. After ≈ 25 cycles the macrocysts die out. There is now a clear tendency for dominance in the population for individuals that split more slowly. Once the macrocysts return (after ≈ 160 cycles), the split thresholds of the population immediately return to lower values (< 7).

Simulations run with all individuals having the same, fixed, split threshold resulted in either the individuals all dieing, through starvation in the low season, or a small percentage joining the macrocyst when food is more plentiful (data not shown). The competitive advantage of the macrocyst joining population was no longer effective and macrocysts were only formed through enough individuals sacrificing their energy in a similar way to the non-joining population in Fig. 3A.

Other simulations have been run with variable split thesholds and the low season completely removed to see if parameters exist where a macrocyst can form and dominate the rest of the population. Simulations were run with vary-

ing parameters of N and p, both seasons having the same values. While some macrocyst production was observed it was only at the beginning of simulations where the random starting population allowed for enough individuals that joined the macrocyst and made it viable for a few cycles (data not shown).

4 Discussion

In Section 1 I have argued of the need for a model that demonstrates the transition between individual cells that ordinarily reproduce on their own to cells that become part of a super-organism, with only one genotype of the participating cells being passed on to future generations. For the macrocyst model to succesfully meet the requirements of this transition, it requires that all individual cells must be able to reproduce on their own. It also requires that individual cells must be clustered and that only one of the individual cells reproduces on behalf of the cluster. The model presented meets these requirements. Simulated cells that have the freedom to evolve a strategy in which they will not join macrocyst organisations (where their genes are highly likely to be destroyed) do not evolve this strategy under fluctuating environmental conditions.

The model does however stop short of demonstrating the type of germline/soma differentiation and clustering apparent in the metazoa where there is differentiation of the germ line early in development [17] and permanent clustering (as in other models, e.g., [27, 19]). The macrocyst's germ-line cell is the zygote which is not differentiated from any other cells in the super-organism. Also, the macrocyst cells are only clustered at one point of the life cycle. However, the fact that the macrocyst's offspring are of only one genotype and that they outcompete individuals that do not join the macrocyst is of some significance.

The fact that the macrocyst produces offspring of a single genotype is important in three ways. Firstly it has the effect of producing several homogenous offpsring which are all 'preprogrammed' to join the macrocyst at the start of the next low season. These offspring have a competitive advantage over individuals that do not join the macrocyst. The macrocyst therefore contributes to its future success. Since microbes can evolve many 'policing' mechanisms [24], it is not inconceivable that after several generations, the macrocyst way well have become established in the organism without the need for a harsh low season each cycle.

Secondly, the high relatedness of the offspring can be seen to promote other social behaviours. Relatedness is crucial for any traits that require many coordinated individuals or altruism to be successful. The aclonal nature of the macrocyst offspring means that it is highly likely that the next aggregation event will also be aclonal or at least highly related. If these individuals have the same mutation which means (perhaps under certain environmental conditions) they no longer fuse to form a zygote then other interesting collective behaviour may occur instead. These behaviours could include, but are not limited to, the slug behaviour of *D. discoideum* which requires many coordinated individuals [14], and the stalk behaviour of *D. discoideum* which requires altru-

ism from many cells [1]. The macrocyst has been argued to be a precursor of these behaviours [13]. The combination of the macrocyst model with one of the stalk/spore behaviour (based on [1]) will hopefully confirm how important the population homogenisation effects of the macrocyst were for the evolution and maintenance of stalk/spore behaviour in *D. discoideum*.

The homogeneous macrocyst offspring are important in a third way: By picking the genotype of its offpsring from one individual at random, the macrocyst stage eradicates the potential for cheating: If an individual were to evolve a 'cheating' trait so that its genes were most likely to be picked, the next population would all have that same trait - with no individual having any advantage.

To consider how split thresholds are important I analyse a complete cycle. In one cycle of the model presented here there are four phases for non macrocyst joining amoebae: (i) Early high season exponential growth; (ii) Population equilibrium at high season; (iii) Early low season exponential decimation of the population; (iv) Population equilibrium at low season. While it is easy to see that fast (low threshold) splitting amoebae would flourish during phase (i), these same amoebae will be closer to dying during phase (iii). The results suggest that a slow (high threshold) splitting strategy is more profitable, not only in phase (iii) but in phase (iv) as well. In phase (iv) individuals receive food with a low probability, those with a fast (low) split threshold are less adapted to survive fluctuations in food availability. The macrocyst allows individuals to avoid phases (iii) and (iv) and hence fast splitting individuals that germinate from it at the start of the high season are very well adapted to phase (i). This ability to perform well during circumstances of diminishing populations has already been observed as an important feature of early multicellular organisms [12].

While I have attempted to be faithful to biological evidence, the model presented here has made some assumptions and has some limitations. Further analysis and research is required into the biological plausibility of the split thresholds in the model. The question as to what might happen if individuals have a seasonally varying split threshold is also important. The model is undimensional and therefore lacks spatial effects (though the way the organisms are fed is set up to mimic a spatial environment): a spatial model would allow us to analyse what might happen if individuals could effect their chances of being the chosen genotype. The mutation rate in the model is unnaturally fast, however slower mutation rates provided similar results over longer periods. Finally there is only one macrocyst in the current model, future simulations will model more than one macrocyst.

The model and results presented in this paper demonstrate that, given the assumptions outlined, the *D. discoideum* macrocyst stage is plausible under the large fluctuations in food in the model. The results and analysis lead me to hypothesise that the model of the macrocyst presented in this paper, where individuals gamble their genes to become the germ line of a super-organism, may well have been a crucial stage in the transition to multicellularity. It must be noted that it is only a stage in the evolution of *D. discoideum* and may be relevant only to this organism. However, the facts that the slug behaviour of *D.*

discoideum is reminiscent of other metazoa and that their phylogeny implies a common ancestor imply that slime mould may give some important clues into the evolution of the metazoa and perhaps other multicellular organisms.

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