
On the origin of differentiation

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Following the origin of multicellularity in many groups of primitive organisms there evolved more than one cell type. It has been assumed that this early differentiation is related to size – the larger the organism the more cell types. Here two very different kinds of organisms are considered: the volvocine algae that become multicellular by growth, and the cellular slime moulds that become multicellular by aggregation. In both cases there are species that have only one cell type and others that have two. It has been possible to show that there is a perfect correlation with size: the forms with two cell types are significantly larger than those with one. Also in both groups there are forms of intermediate size that will vary from one to two cell types depending on the size of the individuals, suggesting a form of quorum sensing. These observations reinforce the view that size plays a critical role in influencing the degree of differentiation.

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1. Introduction

In the past I have been concerned with two themes: one is the origins of multicellularity (Bonner 2000), and the other is the relation between the size of an organism and the degree of its differentiation (or complexity) as measured by the number of cell types (Bonner 1988; Bell and Mooers 1997). Here I want to bring these two themes together by considering the first beginnings of cell differentiation: the transition between organisms with one and two cell types. I will show that there is a close relation between size and complexity by describing two primitive groups of organisms where the jump from one cell type to two is tightly correlated with size. I present this to support my long held view that size increase is a prime mover of the increase in differentiation reflected by the number of cell types. Clearly cellular complexity will increase with or without a change in size: mutations involving cell differentiation that produce greater efficiency will be favoured by natural selection. Size increase produces a stimulus, an opportunity for finding new ways of providing that efficiency by increasing the number of cell types. For the two groups of organisms examined here, the effect of size in producing complexity is para-

mount: individuals with two cell types are consistently larger than those with one. These are two very different kinds of organisms, both with individuals that possess either one or two cell types. From this one might presume that they are the result of independent origins of differentiation, and in both cases they are directly affected by size.

This is a way of looking at the transition between clumps of uniform cells – the origin of multicellularity – and the beginning of the division of labour, or differentiation. Recently Pfeiffer and Bonhoeffer (2003) have proposed an interesting mechanism based on the advantages of metabolic cooperation between cells that might lead to giving a clump of cells a selective advantage, but, as they point out, their scheme only applies to conventional heterotrophs and therefore could not apply to the two cases examined here that involve phototrophs and organisms that becomes multicellular at a non-feeding stage of their life cycle. The organisms are the volvocine algae (*Volvox* and its close relatives) which are photosynthetic, and two lines of cellular slime moulds. As will be seen they are very similar in their size/differentiation relations, yet they differ in all other respects. Most important is the fact that the volvocine algae became

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multicellular by daughter cells arising through growth and adhering to one another, while social amoebae do so by the aggregation of cells.

2. Volvocine algae

All the information presented below is more fully developed in David Kirk's splendid book (1998). I urge the reader to look there for all the details and the references of the work mentioned here.

There is among the volvocine algae a hierarchy of sizes and complexities. There is good evidence that they all share a common ancestor closely related to the modern unicellular alga *Chlamydomonas*, but the old, conventional idea that they evolved by a simple, linear progression in size and complexity has been shown to be false. There is indeed an obvious span of sizes from the unicellular *Chlamydomonas* to the largest species of *Volvox* which may consist of 50,000 or more cells (figure 1). However, recent molecular phylogenetic studies indicate

that the history of the group is more complicated than was previously believed. *Volvox* itself is polyphyletic and has arisen independently more than once during the course of evolution. Even more relevant to this discussion, it appears that certain species of *Volvox* have been ancestral to smaller forms, such as *Pleodorina*, as well as the reverse. So there apparently have been evolutionary decreases as well as increases in size during volvocine evolution.

Here I want to focus on the two cell types that are present in the asexual life cycle of *Volvox*. They are the somatic cells which are biflagellate and responsible for the movement of the colony, and the reproductive cells, or gonidia, that are responsible for producing a daughter colony. The former are terminally differentiated and are incapable of dividing, while the latter become cell division specialists and are incapable of locomotion – it is a separation of the germ plasm from the soma.

There is another important bit of information concerning this dichotomy. Through the pioneer work of Richard Starr, followed by others including David Kirk, it is clear

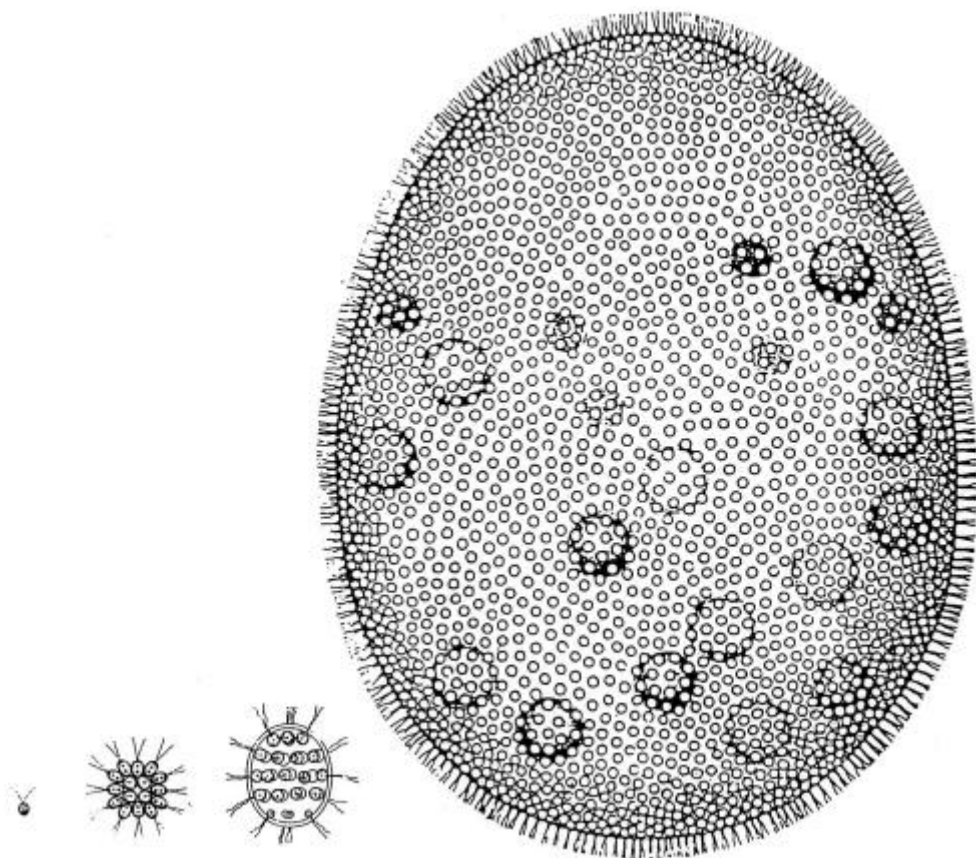


Figure 1. *Volvox* and its relatives showing their relative sizes. Left to right: *Chlamydomonas* is a single-cell flagellate, next is *Gonium*, a 16 cell colony; followed by *Pleodorina* with 32 cells, and finally *Volvox* with thousands of cells (From Bonner 2000 after W H Brown, *The Plant Kingdom*, Ginn, 1935).

that very few genes are involved in this transition from one to two cell types. The initial discovery was the mutation of one gene would transform a normal *Volvox* so that all the cells that would normally be somatic divided very rapidly and produced thousands of mini colonies in one large individual. Subsequently it was discovered that there were a few other genes involved, including ones that control the initial asymmetric cleavage that initiates the two differentiation pathways and genes that block somatic development in presumptive gonidia (Kirk 2001).

This two-cell-type state is correlated with size. At one end of the size range *Volvox*, which contains many hundred cells, always has both cell types. At the other end of the spectrum, *Gonium*, which never has more than 16 cells has no division of labour: all *Gonium* cells are initially biflagellate and involved in locomotion, but then later all of them shed their flagella and undergo reproduction by successive divisions to produce daughter colonies. However, the most instructive size-dependent behaviour occurs in genera of intermediate size.

Eudorina can have either 16 or 32 cells, depending on environmental conditions. In the 16-cell colonies all of the cells behave like those of *Gonium*, participating first in motility and then in reproduction. However, in 32-cell colonies the four cells at the anterior end of the colony often remain terminally differentiated somatic cells that continue beating their flagella, while the other 28 cells reproduce. In *Pleodorina*, which always has a germ-soma division of labour, the ratio of somatic cells to reproductive cells increases as the total cell number is increased. In colonies with 32 cells, 25% of the cells are sterile somatic cells, while 50% of the cells are somatic in colonies of 128 cells. Clearly within this critical intermediate size range the proportions of the two cell types varies depending on the size of the colony.

3. Cellular slime moulds: Dictyostelids

Cellular slime moulds provide a particularly interesting example of the relation of size to complexity. Most species of dictyostelids have two cell types: spores and stalk cells. The exceptions are the species of *Acytostelium* that only make spores; their stalk consists of a delicate strand of cellulose extruded by all the cells before they become encapsulated into spores (figure 2). We do not yet understand the phylogenetic relation between species of *Dictyostelium* and *Acytostelium*; we do not know which are ancestors and which are descendents as we did in the case of the volvocine algae; there remains the possibility that, as in *Volvox*, there might have been a mixture of the two and during evolution they could have gone backward and forward between the two states.

It is fortunate that Raper (1984) in his monograph on the dictyostelids gives enough information about the ave-

rage size of all the different species making it possible to do a comparative survey. In each case he gives the range of their heights, the range of the diameters of their spore masses (or sori), as well as the diameters of the spores. From this data I have averaged the size from the range for all three measurements. (In the case of elliptical spores I have averaged the mean lengths with the mean widths to give one figure for a diameter that is comparable to the diameter of spherical spores of some species.) I then divided the average sorus diameter by the average spore diameter which gives the sorus diameter in terms of the number of spores in a line across a sorus. Since the sorus approximates a sphere it is easy to calculate the number of spores in an average size sorus. There are no

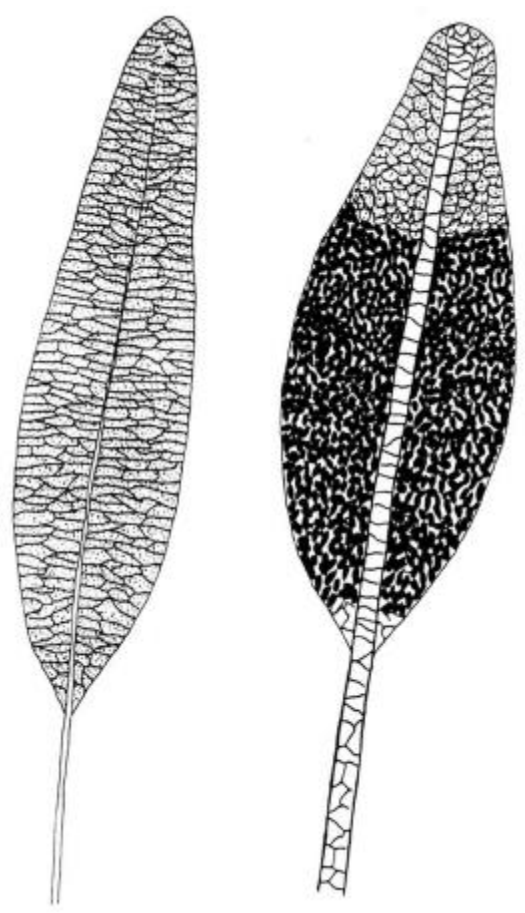


Figure 2. A diagrammatic comparison of the cellular structure of *Acytostelium* (left) and *Dictyostelium* (right). In *Acytostelium* all the amoebae exude a cell-free stalk and later become encapsulated into spores. In contrast, *Dictyostelium* has two cell types: the anterior prestalk cells enter the stalk tube at the tip and become vacuolated, dead stalk cells within the stalk cylinder, and the posterior prespore cells have already become spores. These have been drawn the same size to compare the cellular structure, but as discussed in the text, *Acytostelium* is very much smaller (from Bonner 1988).

doubt some systematic errors in such a procedure, but since all species are treated the same way it is reasonable to make comparisons between species.

I have plotted the average height of each species against the logarithm of the average number of spores for each and it can be seen at a glance that the shorter species have the fewest spores (figure 3). Furthermore, the first four points on the left with the smallest number of spores are all the species that have one cell type only, that is, species of *Acytostelium*. Next to them there is *Dictyostelium lacteum*, with the arrow pointing to it. This species is particularly interesting because it is known from some earlier work that if *D. lacteum* is grown on medium that provides little food, the smallest fruiting bodies will have a stalk that has a cell free zone at its upper end, similar to the stalk of *Acytostelium* (Bonner and Dodd 1962).

This is relevant to the concept that the division of labour and size are directly related: the smaller the fruiting body and its sorus, the greater the likelihood that it will have one cell type rather than two. *D. lacteum* is of special interest because this principle applies within one species: the smaller the aggregate, the greater the chances that it will have some acellular stalk, and *D. lacteum* lies between *Acytostelium* and all other species of *Dictyostelium* on the graph. Note also that the slope of the line rises for the larger dictyostelids which reflects the fact that the number of stalk cells to spores is roughly propor-

tional for different size fruiting bodies not only within a species (as is well known), but between species.

It is important to remind the reader that the cellular slime moulds are unusual in that size is not determined by multicellular growth, as it is in most organisms, including *Volvox*, but by the number of amoebae that enter an aggregate. Growth occurs at the unicellular stage by the separate amoebae in the soil which, upon starvation, aggregate to become a multicellular organism.

4. Cellular slime moulds: Acrasids

There are relatively few species of acrasids, another group of cellular slime moulds, but the same principle applies there also. They differ from dictyostelids in a number of ways. In the first place their amoebae have a different appearance and structure which sets them apart and is the basis for considering them as having arisen independently in their evolution. The other difference is that in some species not only can the cysts in the sorus germinate and produce a viable amoeba, but the cysts that make up the stalk as well. These acrasids have been examined in detail by Olive (1975) and each genus is represented by one species.

There are three forms in which the stalk cells are viable and therefore qualify as having one cell type, and one in which the stalk cells are dead (as in *Dictyostelium*) and

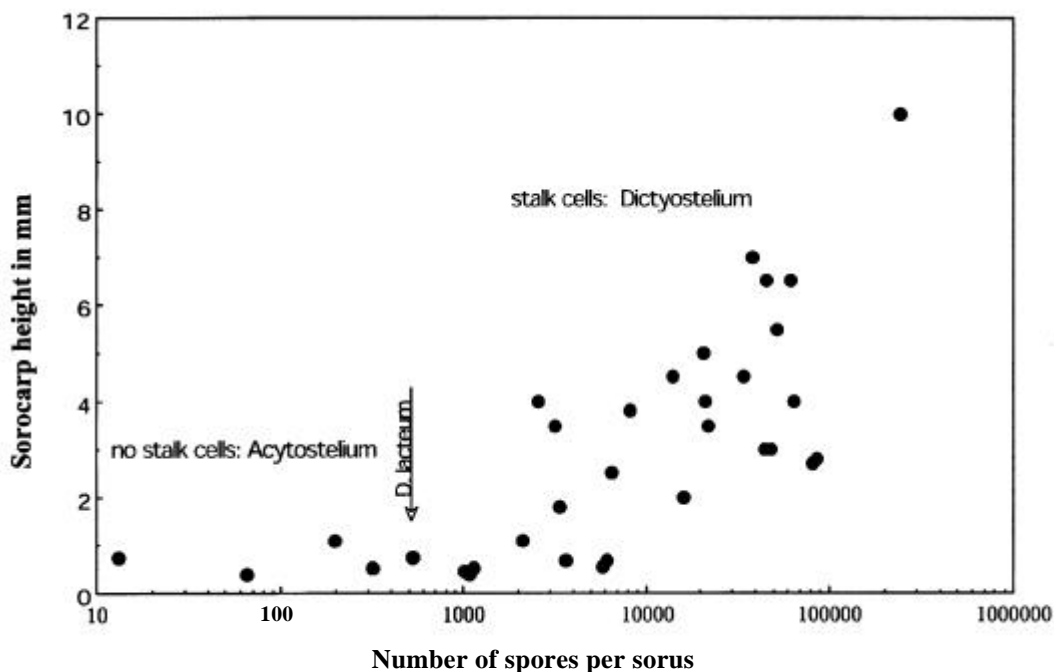


Figure 3. The log of the average number of spores for different species plotted against the height of the fruiting body. The species that have only one cell type have much fewer spores than those with two cell types. *Dictyostelium lacteum* (see arrow) is intermediate and has stalk cells except in very small individuals where the upper part of the stalk will be acellular.

therefore have two cell types (figure 4). In each case it is possible from Olive's description to estimate the average number of spores for each (table 1). It is obvious that again there is a clear correlation between size and the number of cell types. We know nothing of the phy-

logeny of the acrasids but again it would appear that size matters.

5. Conclusion

Through these two examples – volvocine algae and cellular slime moulds – it might be possible to gain some insight into the origin of differentiation, at least for these organisms. And indeed it might well lead to some general principles for the origin of the division of labour.

The first step must have been the invention of multicellularity itself. For the *Volvox* lineage it might simply have meant the sticking together of the dividing cells as a result of an adhesion mutation in a *Chlamydomonas*-like ancestor. For cellular slime moulds it could mean that gathering together cysts or spores in the soil is advantageous for dispersal and therefore encouraged by selection, and ultimately leading to aggregation by chemotaxis (Bonner 2003).

Once multicellularity has occurred, the constant selection pressure for efficiency in the new collective will have its effect. In the case of the volvocine algae, the larger the colony, the faster the locomotion. (There is an interesting study showing that clumps of spermatozoa of a wood mouse swim faster than separate individuals showing that for flagellated cells speed is gained by togetherness [Moore *et al* 2002].) G Bell and his colleagues have also argued that predators that feast on unicellular organisms cannot devour even the smallest volvocine colonies, and furthermore, the larger colonies compete more efficiently for nutrients, such as phosphorous (see Kirk 1998).

The central question here is what selective advantage might volvocine algae have gained by producing terminally differentiated somatic cells that are incapable of reproduction. It might be simply that they can reproduce while swimming and gaining energy through photosynthesis. *Gonium* will sink to the dark bottom of the pond in its non-motile reproductive phase where photosynthesis would be impossible. In *Eudorina* and *Pleodorina* we see an intermediate situation where in the larger forms there is an increase in ability to carry on photosynthesis while reproducing; this is the basis for suggesting a quorum sensing mechanism where a group of cells take into account their number so that they behave differently when they are large as compared to small. This means that the genetic mechanism for setting apart the two cell lineages must be one that is not rigid, but plastic enough to respond to size.

Very much the same argument can be applied to cellular slime moulds. Here a first step towards differentiation can be seen in the acrasids where in some species all the cells of the stalk are viable, while in others they are dead and only the terminal spores can propagate a new generation. As we have seen, the latter are bigger and one could argue that a larger sorus needs a stronger stalk for support, and dead cells provide the needed extra strength.

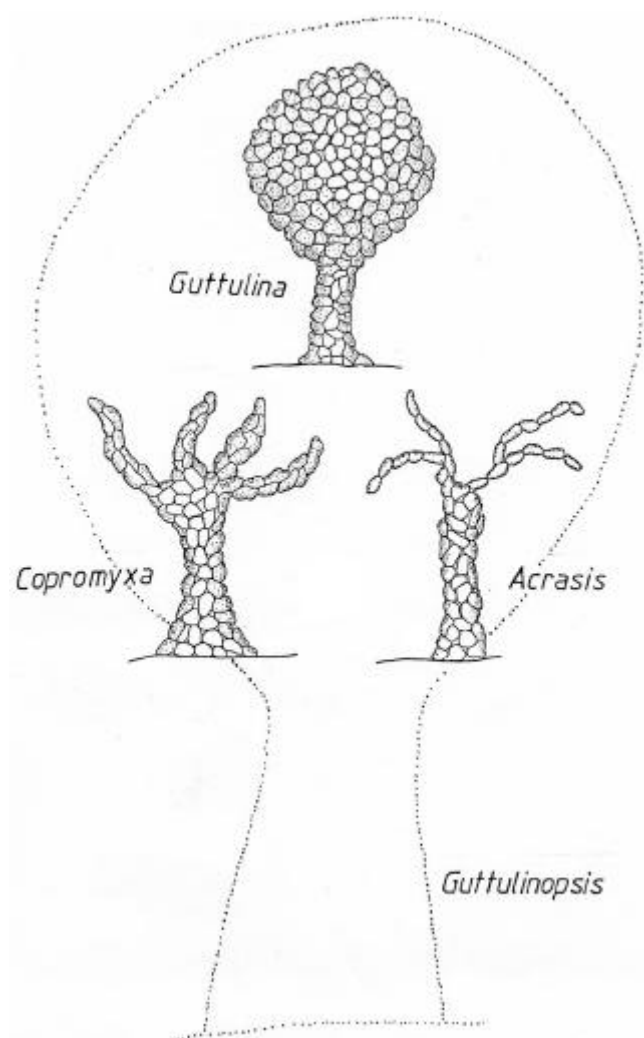


Figure 4. A diagrammatic representation of various acrasids. *Guttulinopsis* is the only one that has two cell types and it is much larger, as indicated in this drawing (modified from Gadagkar and Bonner 1994).

Table 1. The average number of spores in the different species of acrasids (see figure 4 for their comparative sizes).

	Average number of spores
One cell type	
<i>Guttulina</i>	~ 200
<i>Acrasis</i>	~ 200
<i>Copromyxa</i>	~ 2000
Two cell types	
<i>Guttulinopsis</i>	~ 24,000

In the case of the dictyostelids there can be a similar argument: the larger forms need more support, and the vacuolated, dead cells within the stalk provide the extra strength by creating a set of rigid internal cross struts. In *Acytostelium*, which is small with very few cells in each aggregate, a fragile acellular stalk is sufficient to support the tiny sorus.

There is another feature that is also correlated with large size in dictyostelids. Only the bigger forms have a migration stage; they can move to an area that is more favourable for fruiting; the smaller forms all fruit at the aggregation site. In other words with size increase there is not only the differentiation of a second cell type, but an additional stage of the life cycle – which is another kind of differentiation – providing an expansion of the opportunities for spore dispersal. Furthermore, the migration stage of *D. discoideum* shows an increase in efficiency for a slug moves over three times faster than isolated individual amoebae; another example of speed being increased by togetherness (solitary amoebae average 0.52 mm/h \pm 0.13 SD, $n = 59$; slugs of approximately 1 mm in length average 1.18 mm/h \pm 0.11 SD, $n = 30$).

The case of *D. lacteum* is particularly interesting, for it also shows some sort of quorum sensing. There, in very small fruiting bodies, they will have a partially acellular stalk as though there was a threshold concentration of a key substance, or substances, that stimulate a cellular stalk, but if there are very few cells, the concentration is insufficient to be wholly effective in producing a cellular stalk.

Why is there quorum sensing? It might be adaptive for it reduces the cost in those forms that are of intermediate size. By this I mean, in the case of *D. lacteum* if a cellular stalk is not needed for support, then by making part of the stalk acellular a few extra spores are produced, saved from being lost in the stalk. In larger species very small fruiting bodies can be produced in which the stalk is cellular; the smallest one is a fruiting body of *P. pallidum* made up of three stalk cells and four spores (Bonner and Dodd 1962). In other words quorum sensing is not universal and not found in larger species but only in a species at the borderline between large and small. In the volvocine algae it is only present in *Pleodorina* and *Eudorina* colonies, also of intermediate size. At some point in the evolution of both the volvocine algae and the cellular slime moulds a plastic mechanism involving quorum sensing arose to control the number of cell types. At the moment we cannot know if this is the initial step in the evolution towards the two cell type state, or whether it is a late refinement that arose only in species of intermediate size. Hopefully this will be revealed when we know

the genetic details of quorum sensing and the details of the genetic phylogeny of the forms of intermediate size.

In both the case of the volvocine algae and the cellular slime moulds the shift from one to two cell types involves a genetic change (which in the case of *Volvox* is known to involve very few genes). Size is the primary stimulus for change, and if there is selection for size increase, any genetic change that favours efficiency will follow. In the volvocine algae this means a separation of the germ plasm and the soma; in the cellular slime moulds it means making special cells that increase the strength of the support that helps the dispersal of the spores. The same argument would apply for the origin of the division of labour in other independent origins of multicellular organisms, from myxobacteria and cyanobacteria to higher animals and plants. The two examples described here are useful in that they allow us to see at high magnification (so to speak) how a new cell type has arisen during early evolution.

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