Perspectives

The history of the cellular slime moulds as a "model system" for developmental biology

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1. Introduction

My main object here is to make some observations on the role played by slime moulds in the study of development. However, before I begin I would like to make a few broad generalizations about developmental biology in this century; generalizations that would probably cause a historian of science to faint.

Evolution, cell biology, biochemistry and developmental biology have made extraordinary progress in the last hundred years—much of it since I was weaned on schoolboy biology in the 1930's. Most striking of all is the sudden eruption of molecular biology starting in the 1950's. My reckless generalization is that each one of these surges of research activity in cell and developmental biology was due to their collision with genetics. Perhaps it would be more accurate to say that they fused rather than just collided because in each case an extraordinary fruitful symbiosis was the result. First, at the beginning of this century, genetics fused with nineteenth century cytology which gave us an understanding of how the genetic material was handled in the chromosomes in mitosis, and particularly in meiosis. Next, genetics fused with Darwinian evolution to give rise to population genetics, a signal advance at the time. Then with the revolution started by Watson and Crick on the molecular structure of the gene, it was possible, through molecular biology to (i) have a second fusion of genetics with cell biology making it possible to dissect out the biochemical or molecular events within a cell; (ii) to devise a new way of attacking phylogenetic problems in the study of evolution using molecular genetic techniques; and finally (iii) these new approaches made it possible to dissect out the sequence of molecular steps in development.

I am not done with my generalizations! During all the events I have described there has been a strong tendency to concentrate on "model" organisms. In this century there has been a tremendous emphasis on *Escherichia coli*, *Drosophila* and *Caenorhabditis*, but beginning back into the nineteenth century there are many others that have played a role. To mention a few, there is Mendel with his garden peas, followed by other organisms such as maize, amphibians, chick and sea urchin embryos, yeast, myxobacteria, zebra fish, *Arabidopsis*, and cellular slime moulds. One could add a few more and the list would still be incomplete: for instance, ciliate protozoa, *Hydra* and other hydroids, sponges, *Volvox* and other algae, myxomycetes, *Phycomyces* and other fungi, mice and other mammals. The degree to which these various examples have been directly affected by genetics and molecular biology varies, but even in those cases where the influence has been small (due to the lack of attention) this is beginning to change. In fact one can say that it is inconceivable to do developmental biology today on any organism without genetics and molecular biology. For completeness it should be added that there is now renewed interest in another collision: the realization that evolution and developmental biology are inseparable, something that was recognized by Darwin.

2. Cellular slime moulds: Early days

My reason for using cellular slime moulds as an example is that they (along with myself) went through the same evolution from a pre-genetic period to one deeply involved in molecular genetics. I have been there to watch every step, and I would like to follow that course here.

When, as an undergraduate, I began experiments on these slime moulds in 1940, there was only one other person, Kenneth Raper, working on them at that time. In fact he discovered the "model" species *Dictyostelium discoideum*, which is the species used in the majority of the experimental work today. His early experiments were in the classic mould of the embryology of that

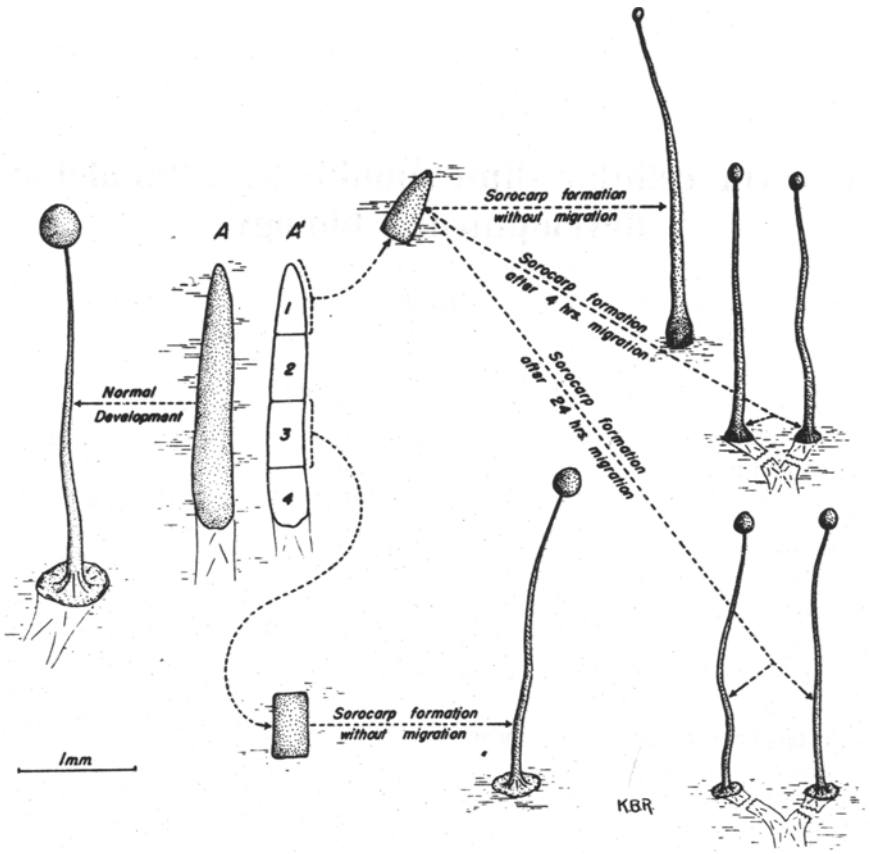

Figure 1. A comparison of the formation of fruiting bodies (sorocarps) from an entire slug (left) and from different fractions of one (right). If the apical fraction (which normally produces the stalk) migrates it slowly regulates and eventually produces a fruiting body of normal proportions. (From Raper 1940.)

time and are still recognized today as being at the root of all subsequent work.

As a young student at Harvard I developed two great interests. One was the fungi and other lower plants which were the province of my Professor, William H Weston. He was a charismatic teacher who exuded excitement for the possibility that cryptogamic plants made ideal subjects for experimental studies. He had many distinguished students, and when I started with him as an undergraduate, one of his finishing graduate students was John Raper who was making the pioneer discovery of sex hormones in the water mould, *Achlya*, and Ralph Emerson had made similar significant advances with another water mould, *Allomyces*. While surrounded by these older students and Weston himself, I knew I wanted to be a cryptogamic botanist. But then I took a course in animal embryology with Professor Leigh Hoadley, and was suddenly confronted with all the wonderful work of Hans Driesch, Wilhelm Roux, Hans Spemann, Edwin Grant Conklin, Ross Harrison and many others who had advanced experimental embryology in the nineteenth and early twentieth centuries. I became entrapped all over again—I wanted to become two people. Then one day it dawned on me: why not work on the embryology (or developmental biology as it became to be known later) of lower plants!

To do this I had to find the ideal organism. Because I was surrounded by water mould enthusiasts it was very tempting to choose them. However all that changed when one day I found the Ph.D. thesis of Kenneth Raper's (John's older brother), who did his graduate work with Professor Weston a few years earlier. The thesis described his discovery of the new species, *D. discoideum*, and his pioneer experiments on it. Here was exactly what I was looking for: the ideal non-animal embryo. I immediately wrote to Kenneth Raper and he sent me cultures with some gracious encouragement that has kept me going for the last sixty years.

Let me briefly describe *D. discoideum's* life cycle to show why I immediately saw the possibility of it being

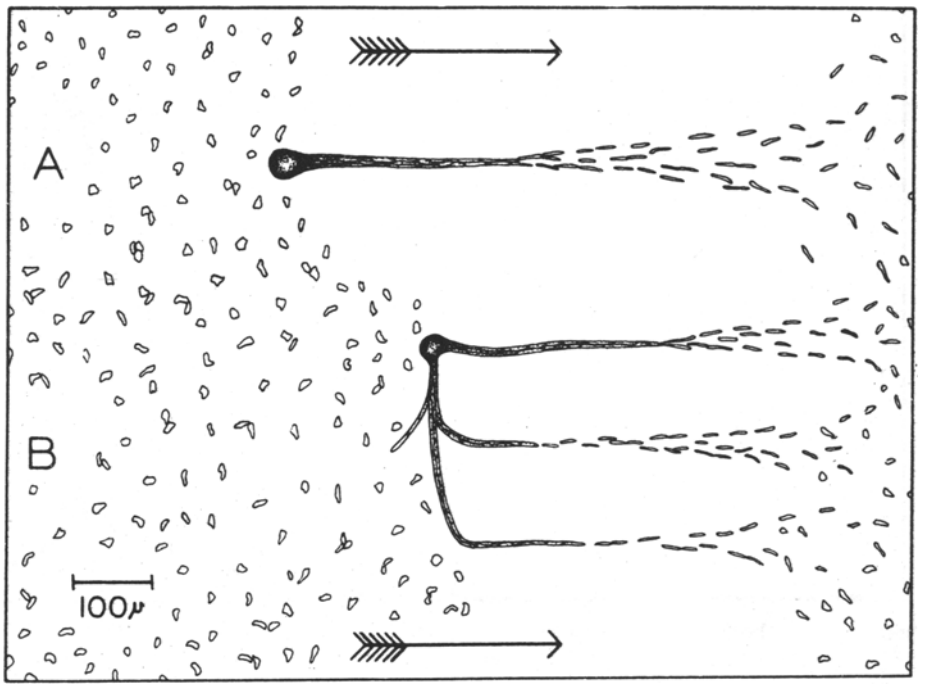

Figure 2. The effect of flowing water (arrows) on the aggregation pattern of *D. discoideum*. (**A**) The aggregation was initiated in moving water. (**B**) The aggregation started in still water and later subjected to moving water. (From Bonner 1947.)

ideal for experiments in developmental biology. Cellular slime moulds are common soil amoebae that feed as separate cells, and when they have depleted the food supply they stream together to central collection points to form a multicellular, sausage-shaped slug of many thousands of amoebae. This migrating slug is an organized multicellular individual that orients in a polar fashion towards light and in heat gradients. Eventually it will stop its forward motion and right itself. The anterior cells then form a stalk (the cells becoming vacuolate and dying in the process) and each of the posterior cells becomes encapsulated in a cellulose shell to become an asexual spore ready to start the next generation.

In part of this thesis (published in 1940) he described the experiments that had so inflamed my enthusiasm. By using a marker dye in the cells he showed what I just alluded to: the anterior amoebae made the stalk, and the posterior amoebae became spores. Furthermore, if a slug was cut into segments, each segment produced a normal, diminutive fruiting body (figure 1). In other words, cells destined to become spores could change and become stalk cells and vice versa. It was the same kind of "regulation" that Hans Driesch had discovered in his famous experiments on sea urchin embryos done in the previous century, where an embryo was cut in two and both halves regulated to form two perfect, diminutive larvae half the normal size. With sea urchins one had to be by the ocean at the right time of year in order to do Driesch's experiment, while Raper was able to do experiments all year around on an organism that took only three days to complete its asexual life cycle. This was clearly the way to go.

One of the early questions was the mechanism that brought the amoebae to the central collection points during aggregation. It now seems so obvious that the amoebae are drawn together by chemotaxis; it is hard to appreciate that this was not so in the early 1940's when I started my graduate work. At the time the distinguished animal embryologist, Paul Weiss, had discovered "contact guidance" in animal cells, especially nerve cells in tissue culture where the advancing cells follow the trails, or grooves in the substratum. He took an interest in my efforts with slime mould aggregation and warned me not to rush to any hasty conclusion that chemotaxis was involved. As I pursued my doctoral thesis I slowly accumulated evidence that aggregation did indeed involve chemotaxis. In a crucial experiment I had water slowly flowing over the top of an aggregate and—wonder of wonders—the amoebae upstream did not seem to know where the center was, but the amoebae downstream moved up long distances against the current

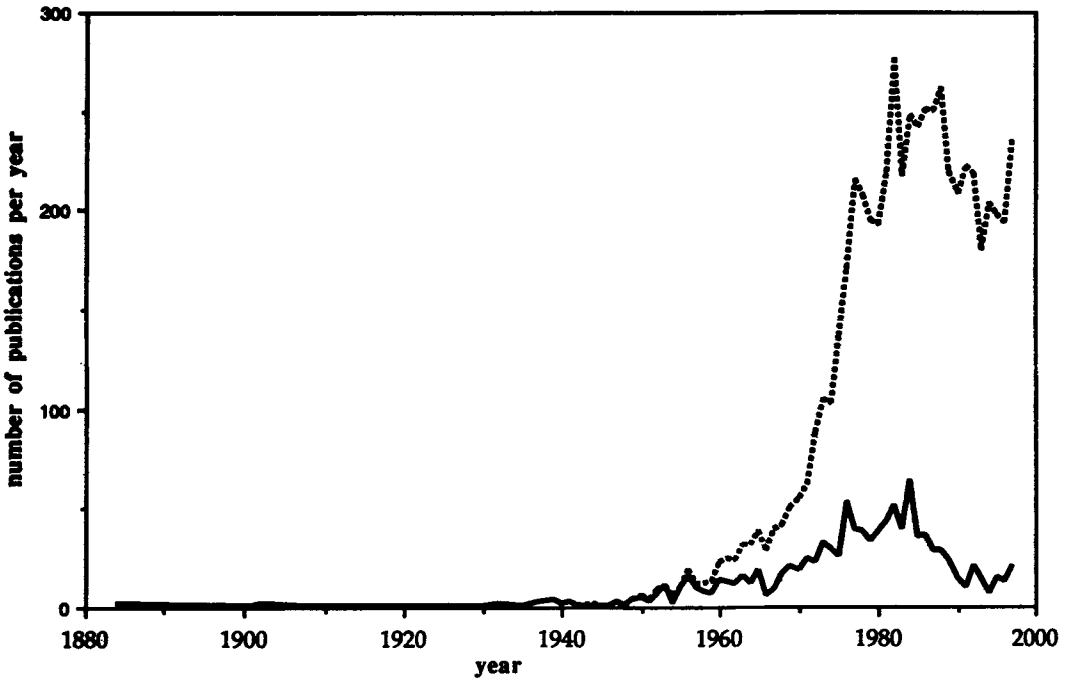

Figure 3. The number of cellular slime mould publications per year from 1869 to 1997. The total number of publications is indicated by the broken line, and the number of non-molecular publications by the solid line.

and towards the centre (figure 2). It was a glorious "eureka" moment; I instantly knew I had solved the problem for only a freely diffusing attractant would produce such a pattern.

The substance needed a name. I found in Edmund Spenser's 16th century poem, "The Fairy Queen" the witch Acrasia who, like Circe, attracted men and transformed them into beasts, and because *D. discoideum* was a member of the Acrasiales I decided in my youthful enthusiasm that it was most appropriate to call the attractant "acrasin". (In Spenser's poem Acrasia gathered her new lovers and "... through sorceree and witchcraft, she from far did thether bring".) I'm embarrassed to confess that in those early days I believed that it was not important to know the chemical nature of acrasin—the fact that it was a free diffusing molecule was sufficient in itself. How ridiculous that seems now!

3. The modern era

In fact it was not until 1967, some 25 years later that we were able to learn its identity. A few laboratories pursued the matter and for a while it was thought to be a steroid, but all we knew for certain was that it was a small dialyzeable molecule. Both Theo Konijn and I had found that *E. coli* attracted amoebae and that perhaps it might be giving off our acrasin. He decided to join me on a sabbatical leave from Holland to attack the problem. Shortly after I had left for Canada, he and a graduate student telephoned me in a tremendously excited state to say that they had found that cyclic AMP (a substance mentioned in a biochemistry course the student was taking) was active in a chemotaxis test at extremely low concentrations. Again an embarrassment for me—I had never heard of cAMP! It was the new discovery of Earl Sutherland that was to have such a big impact on the biochemistry of cell signaling. I went to visit an old friend who spent the summers in a cabin and had been, years earlier, a Professor at Johns Hopkins University, to explain my puzzlement, and to my amazement he said he knew all about cAMP because Earl Sutherland was a friend of his son and they had stayed in the cabin with him recently to do some fishing.

This was the beginning of a great surge of activity on slime mould biochemistry that continues to this day. But it is time for me to come back to my main theme of how developmental biology has changed since the 1940's and how the work on cellular slime moulds reflects those changes.

In recent years there have been a number of intertwined approaches to development in which biochemistry and genetics in conjunction with molecular biology have all combined. The progress of this phenomenon is easy to follow from the work on *Drosophila* and the nematode

Caenorhabditis, but cellular slime moulds have had a somewhat different history because their genetics is difficult. They have a sexual cycle, but for a number of reasons the system is an impossible one for genetic studies, the main one being that the zygotes are extremely difficult to germinate. This problem was largely circumvented in the 1970's by the clever use Pontecorvo's parasexual method in which diploid cells are induced and they return to haploidy by the loss of chromosomes. The disadvantage of the lack of conventional genetics was reduced in the 1980's when the new methods of gene cloning, protein identification, and the ability to introduce new genes by the targeted insertion of plasmids made it possible to do molecular developmental biology. *Drosophila*, *Caenorhabditis*, yeast, and other "model systems" had a big head start, but we were catching up.

I remember having dinner with Sydney Brenner some years ago and he told me that before he decided to begin his famous assault on the molecular development of nematodes he had seriously considered cellular slime moulds, but he felt what they lacked was a nervous system. I fully understand his worry, but given what we know now of the sensitivity and complex behaviour of slime moulds I wonder if a nervous system is all that important! However nematodes do have a straightforward sexual system which made genetics possible right from the beginning.

4. Tracking progress

Thanks to some community minded workers in the field, the cellular slime moulds are blessed with a computer library of all the papers published since their beginning, a number that is now approaching a total of 6000 publications. Last summer, at moments when I wanted to avoid serious work, I called up the publications for each year since 1869, the year Brefeld discovered them. Beyond recording the number I classified them as either molecular (which included biochemistry) or non-molecular (figure 3). The decision of where a particular borderline paper should be placed was in each case my own subjective decision determined by where the main emphasis of the research lay. The non-molecular contributions per annum are indicated by the solid line in figure 3, while all the slime mould publications are shown in the dashed line.

Of the total number of all the papers for the entire span of years (almost 6000) about 20% are biological and the rest molecular. Both categories rose to significant levels in the 1950's and by the 1980s they greatly increased, but clearly molecular research exceeded the more biological research by a wide margin. The latter has decreased during the 1980's, while the former has, in an up and down fashion, maintained a level of mostly over 200 publications per year.

There is a total of almost 1100 non-molecular papers which were further sorted into the following bins: those papers dealing with (i) systematics, phylogeny and ecology; (ii) those involving mathematical modeling; and a larger bin which I will call (iii) developmental physiology, a category in which I included any paper concerned with movement, taxes, timing, pure genetics, cell cycle and other aspects of cell sorting, and rather arbitrarily threw in the very few papers on descriptive cytology. As one might expect the 258 publications in the systematic-ecological category (i) are the most evenly spread over the years—they have been produced at a steady low level. There have been a total of 93 mathematical modeling papers (ii) which began in the 1970's, and since then have been produced at a fairly constant pace. The developmental physiology category (iii) is by far the largest and they began around 1940 and are mainly responsible for the peak of the solid line around 1980 which is shown clearly in figure 3.

Returning to the matter of the major trends in developmental biology during this century and how those trends are reflected in the work on cellular slime moulds, I will begin with a discussion of the category involving the largest amount of research and then proceed down the categories just discussed in the order of their decreasing number of publications (which is the reverse of the order above).

5. Molecular development

The surge of molecular publications – which can be seen as the widening gap between the two lines on figure 3 – begins in the late 1950's. This is to be expected because 1953 is the landmark year, the year of the Watson and Crick paper. After a rapid rise it begins level off, in a haphazard sort of way by 1980. I remember that in the 1950's when the work of Jacob and Monod and others on *E. coli* was in full bloom, the cry was that one needed a multicellular eukaryotic "model" to study gene regulation. Some thought the slime moulds were the answer, but different model organisms were chosen by others. It would be interesting to plot alongside the slime mould curve in figure 3 the curves for *Drosophila*, *Caenorhabditis*, *Arabidopsis* and others for comparison. My guess is that the shapes of the curves would be same, but they would vary as to when their surges begin, and of course in the magnitude of the surges. Molecular developmental biology has become a major industry, and what to me is especially interesting is that the cell types and the modes of development of these "model" organisms could not be more dissimilar, so now we can begin to compare these radically different developments on the molecular level, something that is bound to produce significant advances.

6. Developmental physiology

These studies, which include classical developmental biology, begin with the work of Raper around 1940, and they also increase to peak around 1980. It is notable that the group doing them is smaller, and is on the decline. The latter may be due in part because nowadays so often problems in developmental morphology are being carried out using molecular techniques and as a result I have thrown them in the molecular bin. There remain active groups of individuals addressing questions such as how cells and cell masses move and orient, especially the morphogenetic movements.

7. Mathematical modelling

The idea of using mathematics to understand slime mould development arose from the great interest on the part of applied mathematicians to understand biological pattern. In the case of slime mould pattern there are many possibilities that have been explored: from aggregation, to the mechanisms of movement of the migrating slug, to the final differentiation of stalk cells and spores, in which their proportion remains constant regardless of the size of the fruiting body. While this mathematical modelling meets with great resistance on the part of some whose interests lie primarily in the molecular aspects of development, it is clear to those who are willing to listen that these models have greatly strengthened our understanding, especially on how the more global properties of pattern might be governed. My prediction is that mathematical approaches will become increasingly important in the study of slime mould development—indeed in the developmental studies on all organisms—because the detailed molecular information is increasing at such a rate that its complexity will require mathematics to keep it in some sort of reasonable perspective.

8. Ecology, systematics, sociobiology and evolution

This bin is obviously an odd mixture. There have been a number of individuals who are constantly unearthing and describing new and interesting species. There have been a smattering of slime mould sociobiology studies which have attracted people from outside the field. I suspect that these two endeavours will continue at their present low-level pace. Where one might find an increase of interest is in the molecular phylogeny of the slime moulds; not only how they are related to one another, but their relation to the other major groups of organisms. Finally (and here I make no predictions but only express my hopes) one area that is virtually untapped and could be of exceptional interest is their soil ecology. It is so hard to see what is going on in a bit of opaque dirt, yet clearly all the forces of natural selection have been operating on the slime moulds, and the other minute soil organisms, for millions, if not billions of years—it is literally a black box of compelling interest.

Let me conclude by saying that slime moulds do not provide a better "model" than fruit flies or nematodes or any of the others, nor the reverse. Each one is equally important, and as they advance in new discoveries they all become more so. To me the exciting goal will not only be the success for each, but that ultimately we will have a better understanding of how development was able to be achieved in such different ways. Consider that *Drosophila* is an insect with complete metamorphosis and has a non-cellular stage in its early embryogenesis; *Caenorhabditis* is a nematode with development by orderly cell lineages and little regulation; *Arabidopsis* is a vascular plant, has no morphogenetic movements, for all the cells are encased in hard cell walls; slime moulds become multicellular, not by the growth of cells adhering to one another, but by the aggregation of starved cells that, once in the cell mass, show the fullest kind of regulation. If we can learn how in each case the genes and the proteins in these widely different organisms control the developmental pathways, we will be able to understand the differences and the similarities. This means that we will not only have some extraordinarily important answers in comparative developmental biology, but at the same time—for they are inseparable—some crucial evolutionary insights.

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