JOHN TYLER BONNER

The Cellular Slime Molds

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THE CELLULAR SLIME MOLDS

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SECOND EDITION

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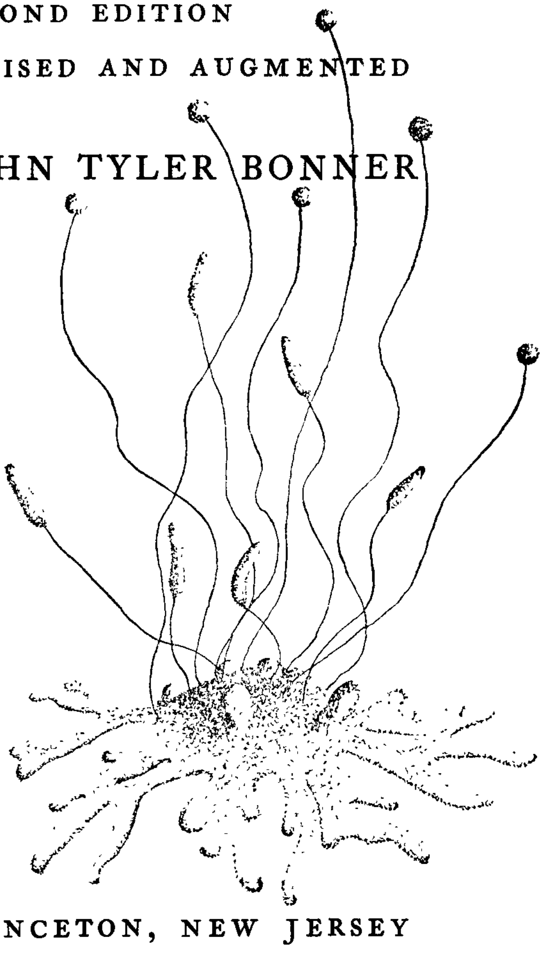
JOHN TYLER BONNER



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Preface to the First Edition

THE purpose of this monograph is twofold. First, an attempt has been made to give a comprehensive survey of all the different known aspects of the biology of the cellular slime molds. There has been a growing interest among experimental biologists working in widely different areas on these organisms, and therefore not only is the present status of the experimental knowledge stressed, but also life histories and relationships with other organisms, so that as complete a perspective as possible may be obtained. The field is yet young and the total amount of work done thus far is small, as the complete cellular slime mold bibliography in the back of the book will show. For this reason a thin volume is still possible, and it is hoped that in the years to come it will continue to serve as a useful summary of all the work done before 1959. But it must be admitted that this is not a mere summary of the field, for even though an effort has been made to include all the important facts, this is primarily an interpretation of these facts.

The cause of this may be found partly in human nature and partly in the second purpose of the book. The main reason that various workers have devoted so much of their energies to experimental studies of the cellular slime molds is that they are considered to be particularly useful organisms in the study of development. Therefore, inevitably, if one surveys and analyzes the literature, as has been done here, one hopes that by looking at all the known facts together, some new insights into the mechanism of development will appear. Writing this book has been of considerable value to me for just this reason, and I hope that it will serve as a useful stepping stone for the research of others in the future.

I should like to take this opportunity to thank Professor K. B. Raper, Dr. B. M. Shaffer and Professor C. H. Wad-

dington for their kindness in reading the manuscript and providing many helpful suggestions and criticisms. Also I should like to acknowledge my indebtedness to the National Science Foundation and the Eugene Higgins Trust of Princeton University for financial assistance provided during the preparation of this book and during the current experimental work from our laboratory presented in this volume.

Finally, I am grateful to the following individuals for permission to use their illustrations: Drs. E. H. Mercer, K. B. Raper, B. M. Shaffer, and M. Sussman. I also wish to thank Miss Kathleen Dodge for her original drawings.

Preface to the Second Edition

In the six years since the first edition was published the number of references on the cellular slime molds has jumped from 124 to 341. It has meant that in preparing this edition more than half of the book has been totally rewritten. Furthermore it is no longer possible to make a brief summary of the entire field and have it include all the facts. More than before this is a severe editing of the facts. It is primarily a discussion of the development of the cellular slime molds. The only aspect that is complete or encyclopedic in any sense is the bibliography.

From comments on the first edition it is clear that one of its uses was to introduce students and research workers to these organisms. It was a convenient survey of experimental problems in the cellular slime molds. If anything this aspect has been emphasized in this edition and, for instance, in the chapter on growth, practical information on laboratory methods are included. It is hoped that this book will serve as a useful stepping stone for any individual who wishes to engage in research on the slime molds.

As in the first edition I should again like to acknowledge my indebtedness to the National Science Foundation and the Eugene Higgins Trust of Princeton University for financial assistance provided during the preparation of this book and during the current experimental work from our laboratory presented in this volume.

I would also like to thank the following individuals for their most helpful critical reading of the manuscript: Mr. C. Ceccarini, Mr. E. G. Horn, Dr. L. S. Olive, and Dr. B. M. Shaffer. I am grateful to the following individuals for permission to use their illustrations: Dr. D. W. Francis, Dr. G. Gerisch, Dr. H. R. Hohl, Dr. T. M. Konijn, Dr. K. B. Raper, Dr. E. W. Samuel, and Dr. B. M. Shaffer.

Finally, I would like to express my deep appreciation to Mrs. Anne Tittler and Mrs. Rogene Gillmor, who combined running our full-time experimental program with helping on the bibliography of this book. I also thank Mrs. Gillmor for the new, original line drawings.

August 1966 J.T.B.
Margaree Harbour
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I. Aggregation Organisms

1. Slime molds

WHEN Brefeld made the first positive identification of a member of the Acrasiales in 1869, he mistakenly thought the cells fused to form a true plasmodium. This error was not corrected until 1880 when van Tieghem demonstrated that the cells remained uninucleate at all times; to underscore the fact that there was no plasmodial syncytium in the Acrasiales, the aggregated cellular mass was called a "pseudoplasmodium." As the term implies, there was originally a strong belief in the close affinity of Myxomycetes and Acrasiales, but it has become increasingly clear, as our knowledge has advanced in recent years, that there is probably no phylogenetic relation between the two at all. They differ in so many particulars that to associate them, except by remote analogy, seems at the moment out of the question. The only feature that they have in common, and this is a source of infinite confusion, is the fact that they both are called "slime molds." There are still a number of textbooks of elementary biology which, because of this general term, completely confuse the two, and it is not uncommon to find an illustration of a member of the Acrasiales with a corresponding text description of a Myxomycete. To the discerning eye of a student of these lower groups this is an unpardonable error, equal to confusing a nematode and an annelid because they both are "worms."

To separate the two unrelated slime molds one may simply use the terms Myxomycetes or Myxogastrales for one and Acrasiales for the other. If common names are desired, the former have been called "plasmodial" or "true slime molds," and the Acrasiales have been called "simple slime molds" by K. B. Raper and "amoeboid slime molds" by myself. However, I am inclined to think that the most useful term is "cellular slime molds," the name suggested by B. M. Shaffer.

To add to the confusion there are three other groups of organisms that are often associated with the slime molds already discussed, and all five groups may be conveniently classified in the following fashion:

Mycetozoa
- Labyrinthulales
- Plasmodiophorales
- Myxomycetales (or Myxomycetes or Myxogastrales)
- Acrasiales (or Acrasina)[1]
- Protosteliales (or Protostelida)

The logic of this grouping is that all five groups contain primitive colonial organisms that have both fungal and animal-like characters and all are to some extent slimy. Aside from these doubtful binding qualities, their relation to one another is uncertain. They are placed together because they represent five anomalous groups of unknown origin.

2. Labyrinthulales

This group is composed of one genus, *Labyrinthula,* and a mere handful of species. They also are parasites and are of considerable ecological importance, for as Renn[2] showed, they were responsible for the wasting disease of eel grass which did so much to change the whole aspect of our coasts some thirty years ago.

These marine parasites infect not only eel grass but many algae as well. The vegetative cell is spindle-shaped and produces a projection of material from each end, much as a developing nerve cell of an animal produces its axone. These

[1] Botanists and zoologists differ on the ending of names of orders, but since these organisms are neither plants nor animals the distinction is unimportant and the names are interchangeable.

[2] *Nature 135* (1935): 544.

projections of *Labyrinthula* form a network, and the uninucleate cells proceed to glide up and down it, travelling only on the tracks they have previously laid down. This pattern of growth has been given the unfortunate name of "net plasmodium." It does indeed form a net, but since the cells are at all times uninucleate, it is in no way a plasmodium. The spindle cells occasionally aggregate into a dense mass, and each cell becomes encapsulated into a spore, but again without any syncytium. Therefore, these now represent true aggregation organisms, in a sense, although the aggregation is of such a crude and primitive nature that it fails to provide the opportunities for experimental analysis found in the Acrasiales.

Until recently there had been no indication of any flagellated cells, nor any evidence of sexuality. Independently, Hollande and Enjumet[3] and Watson[4] discovered the presence of biflagellate swarmers, but there still has been no demonstration of sexuality in the life cycle of these organisms (Fig. 1).

The phylogeny of *Labyrinthula* represents a special problem, and G. M. Smith[5] excludes it from his general slime mold category, Myxomycophyta, on the basis that it shows similarities to the golden alga *Chlorarachnion*. The possibility that Smith is correct in the conjecture that *Labyrinthula* is an alga which has lost its photosynthetic pigments upon becoming parasitic, is certainly reasonable and not disputed. I would, however, not exclude it from the group of slime molds on this basis, for we are assuming that the heterogeneous Mycetozoa are very likely polyphyletic.

3. Plasmodiophorales

The Plasmodiophorales are a group of organisms which

[3] *Ann. Sci. Nat. Zool.* 11e Series, *17* (1955): 357-368.
[4] Ph.D. Thesis, University of Wisconsin (1957).
[5] *Cryptogamic Botany*, Vol. 1, 2nd edn. McGraw-Hill, New York, 1955.

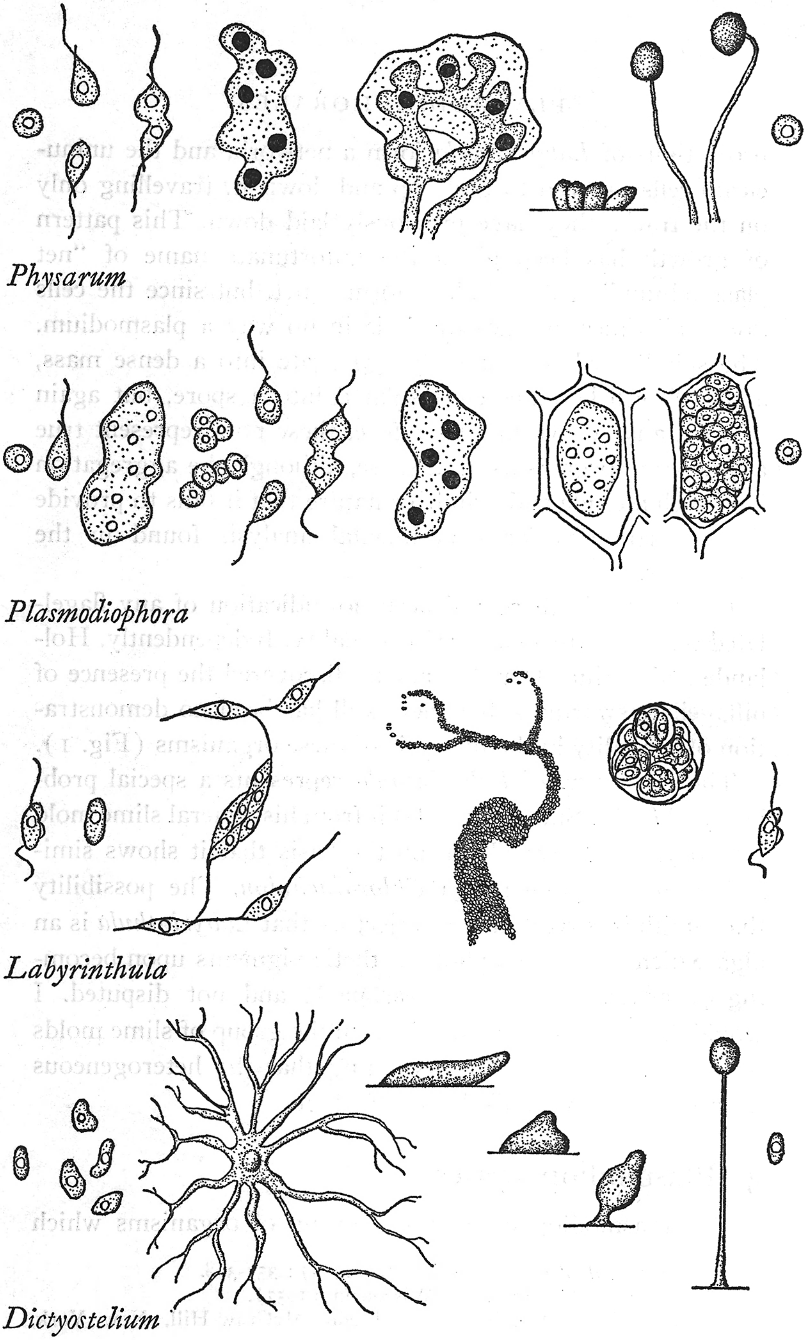

Fig. 1. The life cycles of representatives of the four groups of Mycetozoa. From top to bottom: Myxomycetales, Plasmodiophorales, Labyrinthulales, and Acrasiales. In the first two, where sexuality is well established, diploid nuclei are indicated by solid black dots while haploid nuclei are white. Also the first two are the only ones showing a plasmodial stage. In each the cycle begins (left) and ends (right) with a unicellular spore.

have been of interest principally to the plant pathologist, for all the known species, of which there are some thirty, are plant parasites. They are most noticeably destructive among cabbages, and the clubroot disease of cabbage was a great scourge some years back; now that effective methods of control are known, it is no longer of importance.

As will be seen from an examination of Karling's[6] volume on Plasmodiophorales, there is some variation in the cycle of different members of the group and even some variation in the interpretation of these cycles by different workers. Here I shall confine the discussion to Cook and Schwartz's[7] description of *Plasmodiophora brassicae,* which is the specific agent of the clubroot disease of cabbage (Fig. 1).

The spore of the mold germinates to produce a biflagellate swarmer, with one flagellum shorter than the other. As a matter of fact, the paired nature of the flagella was first discovered in the Plasmodiophorales in 1934 by Ledingham,[8] and only subsequently was the small second flagellum observed in the Myxomycetes. This swarmer penetrates the root of a cabbage seedling and becomes, inside a cell of the plant, a myxamoeba by reabsorbing its flagella. The swarmer (and also the myxamoeba) is haploid; therefore the plasmodium which results from mitotic divisions of the original nucleus of the myxamoeba is haploid and has no counterpart in the life cycle of Myxomycetes. The haploid plasmodium is minute, not exceeding thirty nuclei, and when it stops growing it cleaves, cutting off a number of haploid, uninucleate cells. Each of these is in fact a gametangium; by division each produces four or eight flagellated gametes. The gametes probably fuse to produce a diploid myxamoeba, and this myxamoeba, again by mitotic divisions, will produce a plasmodium which in this case will be diploid, although there is

[6] *The Plasmodiophorales.* New York, 1942.
[7] *Phil. Trans. Roy. Soc. London, Ser. B., 281* (1930) : 283-314.
[8] *Nature, 133* (1934) : 534.

still some doubt concerning this diploidy. Either at the uninucleate stage or after a few nuclear divisions the parasite becomes highly invasive and bores its way from one cell to another, in this way entering the cambium of the cabbage, passing to various parts of the root, and finally wandering out into the cortex. The plasmodium now becomes large and produces a corresponding effect in the cabbage root, which thickens into the club-like swellings. It is suspected that the plasmodium eventually undergoes meiosis and progressive cleavage to form numerous haploid spores.

Concerning the relationship of the Myxomycetes and the Plasmodiophorales, it should be noted that they are both of flagellate origin, but this is also true of the majority of colonial animals and plants. It was the old traditional view that the two groups were closely associated, one being parasitic and the other, free-living. However, with increased knowledge it became obvious that they differ in so many ways, such as the cytological details (e.g. the "cruciform" division charactertistic of the Plasmodiophorales mitosis) and the haploid plasmodium of the Plasmodiophorales, that the modern trend has been to consider the Myxomycetes a discrete group, with the Plasmodiophorales related to the lower filamentous fungi or Phycomycetes. This is based largely on similarities between Plasmodiophorales and Chytrids, the details of which would be superfluous in this discussion. There is a good possibility that each of these three groups, the Myxomycetes, the Plasmodiophorales, and the Chytrids, arose independently from flagellate ancestry. Perhaps all we can assert with confidence is that the origin and relationship of these groups is doubtful.

4. Myxomycetales

The Myxomycetes include some 400 species. Their fruiting bodies are macroscopic and easily recognizable on decaying wood or leaves. Most of the species bear their spores inside a sheath or peridium (Endosporeae), although individuals of the interesting genus *Ceratiomyxa* bear the spores singly on small papillae rising from the body of the mold (Exosporeae).

The life cycles of Myxomycetes were not completely understood until the work of Wilson and Cadman,[9] who were the first to clarify unequivocally the sexual nature of plasmodium formation. From their work and the work of others it is now possible to present a generalized life cycle that probably applies to a number of the members of the group (Fig. 1. See Alexopoulos[10] for a recent review).

The delicately sculptured spore germinates to liberate a haploid swarmer. In some cases the spore liberates four such swarmers, and in others a single swarmer may divide into four daughter swarmers. Either immediately after germination or after cell divisions, if they occur, the cells sprout one or two flagella. These flagella may subsequently be reabsorbed with the return of the amoeboid condition. The important point is that these cells, at either the flagellated or amoeboid stage, may serve as gametes and fuse in pairs. The resulting diploid cell is the fertilized "egg" that gives rise to the plasmodium, and now there follows a series of nuclear divisions without corresponding cell cleavages, and the protoplasmic mass begins a great period of expansion.

During this rapid growth the young plasmodium acts like a large amoeba, engulfing bacteria and other organic particles. Under the proper environment of nutriment, temperature, and moisture, the size of the plasmodium may increase to a few inches or more in diameter, and it is a common observation to see on a decayed stump in a wet forest a glistening viscous mass of slime, frequently a brilliant yellow. If adverse conditions should suddenly arise, the plasmodium can con-

[9] *Bull. Trans. Roy. Soc. Edinburgh* 55 (1928): 555-608.
[10] *Botan. Rev.* 29 (1963): 1-78.

tract into a thickened, hardened mass. This so-called sclerotium may be stored in a dry condition for some time, but it will soften and release a viable plasmodium after being replaced in a favorable environment.

Sporangium formation is a more organized affair. The protoplasm tends to concentrate in a limited region and there becomes cut up into a series of compartments; each of these rises into a small bleb which will develop into a sporangium. In stalked forms the blebs rise into the air. The protoplasm flows upward, depositing centrally a stalk of non-living material. After the bulk of the protoplasm has reached its apical position, an outside wall or peridium is secreted. A series of furrows forms, so that ultimately each nucleus is isolated in a block of protoplasm. Either before or during this process the nuclei undergo meiosis, so that the final isolated nuclei are haploid; each of these cells then secretes a hard wall to become a resistant spore. In some species a material is secreted in the larger cracks resulting from progressive cleavage and hardens to form a thread-like capillitium. Depending on the species, this capillitium may be delicately sculptured as well as hygroscopic, thereby helping the process of spore dispersal.

The variety of shapes of the fruiting bodies is great, and it is used as the basis of species classification. In some species the fruiting bodies have no stalk but are merely rounded or flattened masses projecting from the substratum. The majority are stalked, but the stalk may be single or branched. The great variation comes in the details of the structure of the stalk and the capillitium, the shape of the peridium, and the sculpturing of the spores.

It has been shown by numerous workers (see Alexopoulos[11]) that separate zygotes or plasmodia may fuse or that a whole cycle may be completed from a single zygote. In those

[11] *Ibid.*

cases where there is fusion of separate zygotes, one has a rudimentary kind of aggregation.

5. Acrasiales

My intention in this section is to give only a brief picture of the Acrasiales for purposes of comparison with the other slime molds. At the moment about two dozen species of the Acrasiales are known, all of which are free-living in the soil or are found on dung. The germinating spores liberate a single uninucleate amoeba; there are no flagellated cells. The amoebae feed on bacteria by phagocytosis and repeatedly divide by mitosis, each daughter cell remaining uninucleate and free and independent of the other cells. When a large number of such separate amoebae have accumulated, they will stream together to central collection points to form a cell mass or pseudoplasmodium. This concentration of cells involves no fusion of protoplasts, but the uninucleate character of the cells is essentially maintained following aggregation and during all the other phases of the life cycle (Fig. 1). The qualification necessary for this statement is the interesting observation of Huffman, Kahn, and Olive (1962) and Huffman and Olive (1964) that cells will form temporary anastamoses.

One important point here is that there is a natural separation between the feeding stages and the morphogenetic stages. Feeding will cease some time before aggregation, usually as the result of the depletion of the food supply, and from that moment on the energy for the morphogenetic stages comes entirely from the reserves stored up in the vegetative stage. In the Myxomycetes the feeding also comes first and is followed by fruiting. This separation cannot readily be demonstrated in the Plasmodiophorales because of their parasitic habit, and thus far there is no evidence for it in the Labyrinthulales.

The fate of the cell mass following aggregation depends to a great extent upon the species. In some there is a period of migration of the cell mass of variable duration. Generally there are signs of differentiation of two cell types. The anterior cells begin to form a stalk at the apex. The stalk consists of large vacuolate cells enclosed in a delicate, tapering cylinder of cellulose. It is formed by the cells at the periphery of the apex moving up to the top and becoming trapped in the stalk proper. Once there, the amoebae begin to swell in a gradual conversion to the large pith-like stalk cells. As this occurs, the cellulose of the stalk is continuously deposited around them.

The posterior cells are to become the sorus. Again there is variation among species, but usually the whole posterior portion is lifted as one mass into the air, so that it forms an apical glob of cells when the stalk formation is completed. Each amoeba in this mass of cells becomes encapsulated in a hard cellulose spore case, ready for germination and the next generation.

These cellular slime molds represent an excellent example of aggregation organisms, for during the aggregation process there is a coming together of cells, and these cells may or may not be of precisely the same genetic constitution. It is in every sense a gathering, and not merely a concentration of protoplasm in one spot, the latter being more the situation in the Myxomycetes. Furthermore, as a result of the aggregation one organism is made out of many; in the span of a few hours, without the help of growth, the separate cells come together to form a unified, coordinated, multicellular individual.

Concerning the phylogeny of the Acrasiales, the old tradition that they bear some relation to the Myxomycetes should undoubtedly be abandoned. The principal differences between the two are the total absence of a flagellated stage and the absence of the syncytial plasmodium in the Acrasiales.