Experiments on the Genetics of a Slime Mold, *Didymium iridis*

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Introduction

During the past two years, I have had excellent success in using *Didymium iridis* as an experimental organism in the classroom, particularly in demonstrating its multiple allelic mating system (Collins, 1963; Collins and Ling, 1964). With additional genetic information rapidly accumulating for this organism (Collins, 1966; Collins and Clark, 1966), I feel that the time is now right for making available simplified methods, as well as a source of lyophilyzed material, for use in genetic experimentation in other classrooms. The lyophilyzed clones are available through the American Type Culture Collection, Washington, D. C.

The slime molds have great research potential, which derives chiefly from their unusual life history. In heterothallic species, such as *D. iridis*, both the haploid myxamoebal stage and the diploid plasmodial stage can be perpetuated indefinitely in separate cultures. The haploid stage typically exists as a large population of myxamoebae and swarm cells. Each one of the single-celled, uninucleate myxamoebae making up the population has the potential for dividing mitotically, thus increasing the size of the population, and for acting as a gamete. The plasmodial stage is obtained by mixing together myxamoebae from each of two single-spore-derived populations (= clones) of compatible mating types. Following a period of multiplication, some of the myxamoebae behave as gametes and fuse in pairs to give rise to zygotes. As a zygote grows, its nucleus undergoes a series of synchronous mitotic divisions, without accompanying cell wall formation, and gradually it becomes a large, multinucleate, diploid plasmodium. Meiosis occurs during differentiation from the plasmodial to the sporangial stage. The sporangia contain haploid spores which germinate by releasing haploid myxamoebae. The experiments to be described take advantage of some of the more interesting features of this life cycle.

Explanation of Experiments

Four lyophilyzed, single-spore-derived clones will serve as source material for all experiments. These were selected because among them they possess all of the necessary genetic characteristics for the experiments under consideration, while obviating the need for a large number of clones. For convenience, the clones are simply numbered 1 through 4, and the genetic markers which each possesses are as follows: #1 = A^1b; #2 = A^4b; #3 = A^4b; #4 = A^2B. The letter "A" refers to the mating locus, whereas "B" designates a locus which determines whether plasmodia will display the wild type brown color or the mutant cream color. By crossing these four clones in all possible pairwise combinations, six different crosses will be obtained. Because the mating system is a 1-locus, multiple allelic one, plasmodia should develop only in the five crosses heteroallelic for the A locus. Table I summarizes these results, and also indicates the expected color of each plasmodium. Insofar as plasmodial color is determined by alternative dominant (B) or recessive (b) alleles at a single locus, it is of course expected that only the crosses which bring together two recessive alleles (bb) will be cream colored, while those carrying one dominant and one recessive (Bb) should be brown. Fig. 1 illustrates the three possible genotypes regarding color.

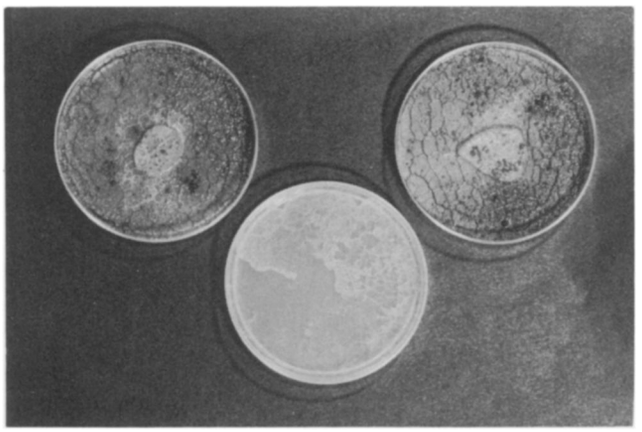

Fig. 1. A photographic representation of plasmodial color inheritance in *D. iridis*. BB or Bb produces brown plasmodia, whereas bb yields cream-colored plasmodia.

The ability of myxomycete plasmodia to migrate over surfaces is well known and can be taken advantage of in the laboratory. For example by allowing two different plasmodia to migrate toward each other until they come into intimate contact, it can be determined whether they are compatible or not. If they are compatible, in a matter of minutes the two plasmodia fuse and become a single entity with a common protoplasm. On the other hand, incompatible plasmodia do not fuse even when they remain in contact for extended periods of time. Using the five plasmodia referred to above in all possible paired combinations, ten different tests for compatibility can be made (Table II). It happens that only three of these combinations are expected to show fusion: 1.4 (A^1A^2Bb) X 2.4 (A^2A^4Bb); 2.4 (A^2A^4Bb) X 3.4 (A^2A^4Bb); and 1.4 (A^1A^2Bb) X 3.4 (A^2A^4Bb). The first and third combinations obviously will result in production of heterokaryons, because the nuclei of the two plasmodia differ at the mating type locus (i.e., A^1A^2 vs. A^2A^4). A closer look at Table II will reveal that 6 of the fusion tests will bring together a brown and a cream plasmodium. No fusions involving these two types is expected to occur, but in the author's laboratory we routinely create such color heterokaryons. For certain kinds of investigations heterokaryons provide exceptionally useful material.

Although significant information on what determines plasmodial compatibility in *D. iridis* is available, the genetic basis of the system is not yet fully understood. For this reason, no genetic symbols will be used to denote plasmodial compatibility genes at this time.

Procedures

All cultures described should be made in duplicate.

1. *Reviving lyophilyzed clones.* The entire contents of each tube, containing lyophilyzed myxamoebae and bacteria, should be placed on the surface of half-strength corn meal agar (12.5 gm agar, 8.5 gm Difco corn meal agar, 1000 ml dist. H_2O). Then place 3 ml of sterile dist. water in each petri dish. Incubate at 23°C or at room temperature. The bacteria will multiply rapidly and provide a source of food for the amoebae. These will be visible through the petri dish cover by transmitted light under a dissecting microscope with substage lighting and a magnification of X 60 or higher. Should difficulty be encountered in recognizing them with this microscope, a loopful of cell suspension may be transferred to a slide for observation under a compound microscope.

2. *Controls.* Plate out several loopfuls of cell suspension from each clone on separate dishes of of media. To every plate, add 3 ml of sterile dist. water and rotate the plate so as to distribute the amoebae evenly over the agar surface. These will serve as control cultures and are not expected to yield plasmodia. Should one want to maintain these clones in the vegetative condition for future use, repeat this transfer procedure every two weeks.

3. *Crosses.* In order to cross the clones in the six combinations as shown in Table I, take several loopfuls of cell suspension from 2 *different clones* which are about 1½ weeks old and put them together in a dish; add 3 ml of H_2O and a loopful of a dilute water suspension of the bacterium, *Aerobacter aerogenes*. Macroscopic plasmodia should be evident in all fertile crosses in 4-7 days. One set of plasmodia should be left in a well-lighted room (but not in direct sun light) without being fed. Sporangia probably will develop in these cultures when they reach an age of 10-12 days.

4. *Feeding plasmodia.* The second set of plasmodia should be fed sterile pulverized Quick Quaker Oats to insure their becoming large and vigorous. The oats should be sprinkled evenly over the surface of each plate but in sufficiently small quantities so that the agar surface is only about half covered with it. In 7-9 days following such a feeding, a plasmodium usually grows large enough to cover much of the agar surface area.

5. *Plasmodial color.* At this point it should be possible to distiguish between brown and cream colored plasmodia. However, if there is any doubt, by means of a spatula, cut out blocks of agar concainting a vigorous portion of plasmodium from each dish and transfer, plasmodial side up, onto plates of freshly poured half-strength corn meal agar (= CMA½). After they have migrated off the blocks (usually within a few hours), examine them for color again and record your results. The cream colored plasmodia are not entirely devoid of the brown pigment, so that under certain circumstances they may appear somewhat brownish.

6. *Maintenance of plasmodia.* To maintain plasmodia, transfer a vigorous portion of each every six days to fresh plates of CMA½. Repeat the feeding procedures. One feeding after each transfer is sufficient.

Table I
Four Clones Crossed Among Themselves in All Possible Pairwise Combinations

	1(A^1b)	2(A^4b)	3(4^4b)	4(A^2B)
1(A^1b)				
2(A^4b)	Cr			
3(A^4b)	Cr	O		
4(A^2B)	Br	Br	Br	

Legend: Br. = brown plasmodium; Cr. = cream colored plasmodium; O = no plasmodium. All figures in parentheses refer to genotype of clones.

Table II
Plasmodial Fusion Tests Using 5 Plasmodia in All Possible Pairwise Combinations

	(A^1A^4bb) 1.2	(A^1A^4bb) 1.3	(A^1A^2Bb) 1.4	(A^2A^4Bb) 2.4	(A^2A^4Bb) 3.4
(A^1A^4bb) 1.2		—	—	—	—
(A^1A^4bb) 1.3			—	—	—
(A^1A^2Bb) 1.4				+	+
(A^2A^4Bb) 2.4					+
(A^2A^4Bb) 3.4					

Legend: + = plasmodial fusion, whereas — = no fusion. The numbers in the chart refer to the parental clones which were used to obtain each plasmodium, i.e., 1.2 indicates clones 1 and 2 were crossed to make this plasmodium. The figures in parentheses denote plasmodial genotype.

7. *Plasmodial compatibility tests.* Tests for plasmodial compatibility may be carried out by placing agar blocks containing vigorous plasmodial fans together on CMA½. These should be positioned about 2 cm apart, and in such a way that the plasmodia will migrate toward each other. Ordinarily, it takes about five hours for the two plasmodia to collide, but length of time is extremely variable. (Should the two plasmodia fail to come into intimate contact in the amount of time available to the investigator, the procedures may be repeated at a more convenient time.) As soon as the two plasmodia touch, they should be examined at the point of contact by transmitted light with a dissecting microscope. If they are compatible, in a matter of a few minutes common veins will be established between them, and the protoplasm will be readily seen to flow from one to the other. On the other hand, incompatible plasmodia may become superimposed, and the inexperienced observer could mistake this for fusion. However, whatever doubt there may be is dispelled when the two plasmodia pull apart as they eventually do if they are incompatible.

8. *Fruiting.* Aside from the procedure mentioned in part 3, a convenient and fairly reliable method for obtaining large numbers of sporangia is as follows: When a fed plasmodium reaches 10 days old, transfer a piece of it to 4% water agar, but *do not* feed it. Incubate in a well-lighted room, preferably in indirect sunlight. Sporangia may develop in this culture overnight, but if none are produced, repeat the procedure 24 hours later using a second piece from the original plasmodium. Positive results are usually obtained from plasmodia which are from 10-13 days old. Age of plasmodium, as used here, refers to the amount of time elapsing after a plasmodium has been transferred to fresh agar and fed.

9. *Spore viability.* To test for viability, take a whole sporangium and smear its contents on the surface of CMA½ and add 3 ml sterile distilled water to the plate. *(Caution:* Spores of Myxomycetes are very small and readily airborne. Handle them with extreme care to avoid contamination.) If spores are viable, numerous myxamoebae and swarm cells will be present in 1-3 days. Incidentally, such mass-spore cultures which show viability will give rise to plasmodia which may in turn give rise to more sporangia.

10. *Single spore isolation.* For establishing new single-spore clones, carefully lift a sporangium by its stalk with a pair of fine pointed forceps and place it in a deep slit made in CMA½ in a petri dish. Add 3 ml sterile dist. water. Lift the cover just enough to allow entrance of your forceps, and then squeeze the sporangial head. Replace the cover and gently rotate the dish to distribute the spores. Now streak several loopfuls of this suspension onto the surface of a second CMA½ dish. By carefully adjusted transmitted light (I like a rather dark field) from a dissecting microscope at a magnification of X 60 or higher, the individual spores should be recognizable. Using an appropriately fine-pointed instrument, cut out a small block of agar containing only one spore and transfer it to CMA½. Add a drop of a very dilute water suspension of *Escherichia coli* to this plate; then flood the surface with 3 ml sterile dist. water. Incubate at 23°C or in a cool room. Viable spores should yield populations of amoebae in about a week.

Discussion

It is likely that it will not be feasible in every instance to carry out all experiments suggested in this paper. On the other hand, with the basic information provided, experimentation can be extended further than the formal procedures indicate. For example, one might want to make analyses of F_1 clones and plasmodia regarding segregation of mating types, color alleles, or plasmodial compatibility factors. Many more experiments are, of course, possible, and the reader is strongly urged to consult the pamphlet (Alexopoulos and Koevenig, 1964) entitled *Slime Molds and Research* for further suggestions and background information of a more general nature. It should be noted, too, that the pamphlet contains some striking photographs (many in color) of slime molds and some valuable pointers for collecting specimens from the field. A review of the literature on slime mold research is also available (Alexopoulos, 1963).

Worthy of special note, too, is a short article by Kerr (1965) which describes a simple and effective means for lyophilyzing spores and myxamobae which is suitable for use in the classroom.

Acknowledgments

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TOXICITY BIBLIOGRAPHY

Of special interest to health professionals working in toxicology and related disciplines is the National Library of Medicine's new quarterly publication, the *Toxicity Bibliography*. Drawing selectively upon current references in the Library's computer-based MEDLARS (Medical Literature Analysis and Retrieval System), the bibliography is designed to provide quick access to the world's relevant and significant journal literature in the field of toxicology. Coverage includes the adverse and toxic effects of drugs and chemicals reported in approximately 2,300 biomedical journals. Each quarterly issue contains references selected from the monthly issues of *Index Medicus* for the corresponding three months' period.

The bibliography, which began with Volume I, Number I (January-March 1968), 400 pages, is divided into two major sections. Section I, Drugs and Chemicals, contains references to articles indexed under subject headings for a chemical, drug, or similar substance for which the subheadings "adverse effects," "poisoning," or "toxicity" have also been applied. Section II, Adverse Reactions to Drugs and Chemicals with 18 subsections, contains references appearing under headings which, together with the subheading "chemically induced," denote signs, symptoms, disease states or congenital abnormalities caused by a drug or chemical.

The *Toxicity Bibliography* is a publication of the Library's Toxicology Information Program, which was established in 1967. This new quarterly bibliography is sold by the Superintendent of Documents (Government Printing Office, Washington, D. C. 20402) at an annual subscription rate of $9.00 ($11.25 foreign), or $2.25 per individual issue. Payments should be included with the order in the form of check, money order, or Superintendent of Documents Coupons.

WHO IS RESPONSIBLE FOR POLLUTION CONTROL

John W. Gardner, Secretary of Health, Education and Welfare, made it clear at the Water Pollution Control Conference held in New York City that he wants to give vigorous leadership to the campaign to clean up the Nation's dirty rivers.

"We like to think of ourselves as a civilized people," Secretary Gardner said. "Yet it took a major drought to dramatize the fact that we have made one of our loveliest rivers a torrent of filth." He pointed out that in the 10 minutes it took for him to make his speech, communities along the Hudson River poured more than 30 million gallons of raw or partially treated sewage into their river. "The cost of cleaning up this pollution both municipal and industrial is expected to run close to $400 million," he said. "That is a formidable figure but we can't afford not to pay it."

Secretary Gardner pointed out, however, that responsibility for pollution control and abatement "rests finally with the people with their State and local governments as it always has. The national government can help and we will help to every extent possible. But the larger task is yours. We are facing an enormous challenge . . . throughout this country, wherever people have assembled to build their homes and industries. We are going to have to readjust our thinking processes and look at the true magnitude of the problems of pollution of our environment. Then we can begin to design corrective measures that will really fit."

There are 107 peaks above 10,000 feet in the 411 square mile Rocky Mountain National Park, Colorado. Naturalists have identified 700 species of wild flowers there.