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SEXUALITY IN MUCORS[1]

THE keywords of the vice-president's address and the symposium which followed it last year were *Organization, Coordination* and *Cooperation* in botanical research. It is not my purpose at this time to discuss these topics further. A botanical committee of the National Research Council has been selected for this purpose. A second committee was chosen by the Botanical Society of America two years ago to help the first committee and last year a third committee was appointed to help the other two. The organization seems sufficiently complete unless I might suggest as a humble member of this last-named committee that a new committee be formed at this session to help us also in our deliberations which have not as yet taken place.

One of last year's speakers, in distinguishing types of true research worth while from investigations unworthy of the name, held up to ridicule a hypothetical investigation of a ham sandwich and the pseudo-scientist who would attempt a monographic treatment of such a subject. In defence of the maligned sandwich, a correspondent has offered the following lines:

> Sandwich perched by the lunchroom wall,
> I lift you down from the perches.
> Hold you here, ham and all, in my hand.
> Little sandwich! But if I could understand
> What you are, ham and all, and all in all,
> I should know what true research is.

It is not of so broad a subject as the sandwich in its entirety, neither of the ham nor of the bread between which it nestles that I wish to speak. Rather it is the mold that sometimes grows on the bread that encircles the ham, or more especially the less commonly

[1] Address of the vice-president and chairman of Section G, Botany, American Association for the Advancement of Science, St. Louis, December 30, 1919.

observed sexual reproduction of the bread mold and its relatives that I have chosen as my theme.

It will not be possible in the time available to enter into any detailed discussion of many questions that might suggest themselves in this connection. I shall instead give an outline merely of some of the investigations of the last fifteen years, both published and unpublished and shall attempt to show that the sexual relations of the mucors may have possible bearings upon our conceptions of sexuality in other diverse groups of the biological world.

The chart (9, Tafel VI.)[2] shows the typical vegetative condition of a mucor. A vegetative spore, usually multinucleate though sometimes uninucleate, sends out in germination a branching tube which forms the mycelium and rapidly covers the available substratum. This mycelium is multinucleate and, in the early stages at least, without cross walls—forming thus an enormous, much-branched, single cell—if a cell is defined in terms of the limiting cell walls. Multiplication is brought about chiefly by various types of nonsexual spores. The commonest are endogenous spores produced in sporangia, upwards of 70,000 individual spores being formed in a single sporangium. They may be apparently exogenous, formed singly or in chains on terminal swellings of fertile filaments and may be produced as chlamydospores by septation of the vegetative filaments. More than a single type of nonsexual multiplication may occur in a given species.

As regards their sexual reproduction, there are two groups of species. In the first group, represented by *Sporodinia,* a form common on fleshy fungi, the sexual spores known as zygospores are common and may be obtained from the sowing of a single vegetative spore. Such forms are therefore hermaphroditic or homothallic since their thalli or mycelia are alike sexually. In the second group, represented by *Rhizopus,* the bread mold, the zygospores are rarely observed and can never be obtained in pure cultures from the sowing of a single spore. In these diecious or heterothallic forms there are needed two plants of opposite sex growing in contact in order that sexual reproduction may take place. The two sexual groups mentioned are represented in the adjoining diagram (Fig. 4). Since in the three lower figures the two gametes (which later unite to form the mature zygospore) arise from branches of a single filament, these three forms are hermaphroditic. In the upper figure the gametes are represented arising from sexually different plants designated by the signs plus and minus which will be explained later. These therefore belong to the diecious group. The line of zygospores, which results when the opposite sexes of the diecious species *Mucor Mucedo* are grown in contact, is shown in the chart. The swollen heads produced on erect filaments from the plant on the right of the line are sporangia containing numerous nonsexual spores by which the plant may be propagated as distinct sexual races in much the same manner in which races of potatoes may be propagated by non-sexual tubers. The process of conjugation may be followed from the figures in the chart (9, Tafel VII.). Filaments of opposite sexual tendencies grow together and by the stimulus of contact produce swellings which push them apart. These swellings develop into the *progametes* from which by cross walls the sex cells, or gametes, are cut off. The dissolution of the intervening cross wall allows a fusion of the gametes and the zygote thus formed increases in size and becomes the mature zygospore. The gametes are typically equal in size in the diecious group and also in the hermaphroditic group except for certain forms to be discussed later. They are miltinucleate and hence have been called cœnogametes.

In the first lantern slide (1, Pl. IV.), we can see photographs of Petri dish cultures of certain of the mucors experimented with. The opposite sexes of the diecious species have been termed plus and minus for reasons

[2] Citations in parentheses throughout the text refer to the sources for the charts and lantern slides used in the original presentation of this paper.

to be explained shortly. By inoculating plus and minus spores in appropriate spots in the culture dish definite patterns may be obtained by the lines of zygospores formed where the opposite sexes meet as shown in Figs. 52–55.

IMPERFECT HYBRIDIZATION

It was soon established that in any given diecious species there are only two sexes present but at first there was no assurance that the two sexes of a given species were the same as those of any other. It has been discovered, however, that the opposite sexes of different species are capable of showing an imperfect sexual reaction when grown in contact. The photograph on the screen (Fig. 1,

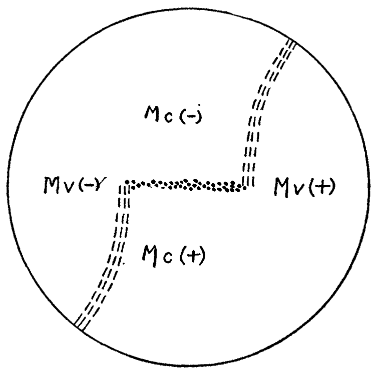

FIG. 1. Diagram of Petri dish culture showing zygospores (dots) between the (+) and (—) races of the same species, *Mucor* C and lines of "imperfect hybridization" (dashes) at contact between opposite sexes of different species, *Mucor* C and *Mucor* V.

which is taken from photograph in 7) represents a culture of the sexual races of two different species *Mucor* V and *Mucor* C.

Where the two sexes of *Mucor* C meet a line of zygospores is evident as might be expected. The sexual race of *Mucor* V on the right shows a reaction only with *Mucor* C minus as indicated by the white line where the two meet and must be considered the opposite sex from *Mucor* C minus, or plus. In like manner the other sex of *Mucor* V on the left shows a reaction only with the plus race of *Mucor* C and must therefore be considered minus. A microscopic investigation of the appearance of the white lines of sexual reactions shows the condition represented in Figs. 36–39 (1). The stimulus of contact leads to the formation of progametes. Sometimes the gamete is formed by one and more rarely by both the reacting filaments. In one strong reacter the gamete which is formed in this reaction transforms itself frequently into a thick-walled a-zygospore. The stimulus which leads to a dissolving away of the wall between the two gametes and their consequent fusion is constantly lacking. Since the sexual reaction between opposite sexes of different species is incomplete it has been termed *"imperfect hybridization."*

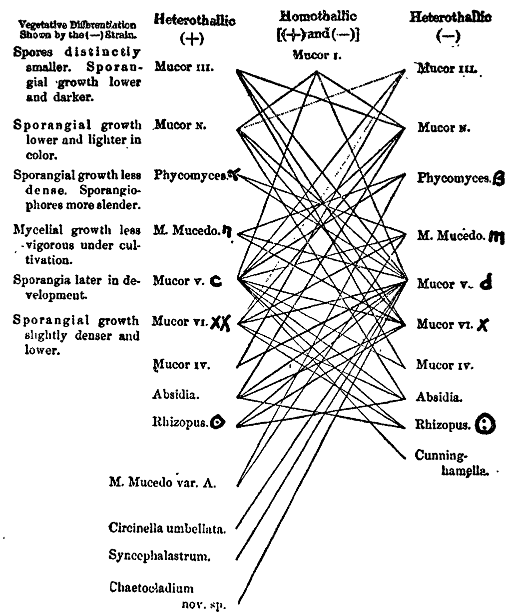

FIG. 2. "Imperfect hybridization" reactions shown by solid lines between sexual races of different species.

At first the paired races of different species were designated by letters and symbols. Lines in the diagram (Fig. 2) indicate some of the reactions that have been obtained between races of different species. All races that show a reaction with the "c" race of *Mucor* V used as a standard but not with its "d" race, are placed in the right column, while those that show a reaction with the "d" race of *Mucor* V and not with its "c" race are placed in the left column. In no case has the position of any of the races been determined by less than two positive reactions. Any race in the left column is theoretically capable of showing a reaction with any in the right column, while incapable of reacting with those in its own group and vice versa. Certain combinations, however, react with greater difficulty than others. It is obvious that in these two columns we have represented the two opposite sexes, male and female. There seemed, however, no way of determining which is to be considered male and which female since their gametes are typically equal in size.

In making the diagram, it was observed that of those species, in which there was evident a greater vegetative vigor of one sexual race over the other, the more vigorous race was always in the left-hand column. All those in the left-hand column were accordingly called plus and those in the other column minus despite the fact that in many species no vegetative difference between the sexes could be established. The most striking example of a difference in vegetative vigor is that of *Mucor* III shown in Fig 58 (1). In a considerable number of races in several different species, however, I have found that the plus race is not invariably more vigorous than the minus when a difference in vegetative vigor is observed, judging vigor by former criteria; but this fact does not detract in the least from the evidence that in the plus and minus races we have the two sexes represented.

The "imperfect hybridization" reaction is of convenience in determining the sex of unmated races. Thus when the diagram (Fig. 2) was made, a race of *Circinella umbellata* obtained from a curbstone in the shadow of Harvard University, by reacting with *Mucor* V plus and failing to react with *Mucor* V minus, was found to be a minus race and is so listed in the diagram. Later a race was obtained from a substratum sent by a missionary from China and was discovered to be its plus mate. It was a relatively easy matter then to obtain the zygospores by uniting these opposite races under suitable nutrient and temperature conditions. The last species in the minus column was found in 1903 in a culture of rat's whiskers gathered on an island in the Caribbean Sea. Perhaps somewhere, under some spreading palm, from India's coral strand, its mate is waiting; and another good missionary may help in spreading the gospel of a "form new to science."

I said a moment ago that it is theoretically possible to obtain an "imperfect hybridization" reaction between the sexual race of a given species and the opposite sexual race of any other species. In practise it has not always proved easy of accomplishment. Much depends upon the environmental factors such as the kind of nutrient—more, however, upon the sexual vigor of the reacting races. A race may react with the opposite sex of another species under temperature or nutrient conditions which will not allow it to form zygospores with its normal mate in its own species. Thus *Cunninghamella echinulata* will readily give "imperfect hybridization" reactions with species of the genus *Mucor* at temperatures below 20° C. but will not itself form zygospores at so low a temperature, while some species of the genus *Mucor* are weak in reaction when contrasted *inter se.* The vigor of the reaction, therefore, has no apparent connection with the taxonomic relationships of the forms involved. Cunninghamella it may be remembered is so distantly related to the genus *Mucor* that it was originally described as a Hyphomycete and assumed to belong to a group of fungi unrelated to the mucors.

Saito and Naganishi (13) report obtaining true hybrid zygospores between different *Mucor* species. They admit, however, that the species in question are very closely related. I have found, between what I have called the opposite sexes of a single species, differences sufficiently marked to be worthy of description as distinct species according to Bainier who has been one of the mycologists most prolific in fathering *Mucor* species. I, as well as others, have observed also considerable differences between the different strains of a single sex of a given species. It is possible that these Japanese investigators have been dealing with races with differences of the order just mentioned. The matter may be a question of what is a species. Burgeff, however, in his brilliant investigations of Phycomyces (10), has obtained a striking distinct mutant which he has been able to cross successfully with the normal stock.

It seems strange that the reaction initiated in the process of "imperfect hybridization" is, usually at least, unable to carry through to completion. We can assume something fundamental, common to all the plus races of the various mucor species, that causes a response when they are brought into contact with a minus race and something in addition that must be present peculiar to the same species in order to extend the reaction to a union of gametes and their development into normal zygospores.

These fundamental characteristics of plus and minus must be present also in the hermaphroditic or homothallic species since, as indicated at the top of Fig. 2, such hermaphroditic species may show sexual reactions with plus and minus races used as testers. The reaction is often strong enough to be indicated by white lines as shown in Fig. 56 (1) where the hermaphroditic *Mucor* I is reacting with both plus and minus races of *Mucor* V.

ZYGOSPORE GERMINATION

It will be of interest to note what occurs at the germination of zygospores formed by the sexual races. A zygospore at germination produces a short germ-tube, terminated by a germ sporangium. The condition is represented diagrammatically on the screen (4). In the hermaphroditic species investigated, all the spores in the germ sporangium are hermaphroditic and give rise to hermaphroditic plants as is to be expected. In the diecious species, however, there are two types of zygospore germination. In *Mucor Mucedo* the spores in a germ sporangium are all of the same sex—plus *or* minus, never mixed. In *Phycomyces,* on the other hand, the germ sporangium may contain spores of both sexes. The germ tube may be induced to grow out vegetatively before the formation of its germ sporangium. By this means its sexual condition can be tested. The germ tube of *Mucor Mucedo* has been found to be unisexual, of the same sign as its germ sporangium. Segregation or differentiation of sex in this species, therefore, must have taken place at or before the zygospore germinates. In *Phycomyces* sexual differentiation takes place in the germ sporangium and induced growth from a germ tube gives rise to a temporarily hermaphroditic condition. Such a hermaphroditic or homothallic mycelium is shown in the photograph (2). Its yellowish felted growth is strikingly different from the normal plus and minus races which are forming a line of zygospores where they meet at the upper right-hand corner of the culture. Occasionally spores in the germ sporangium of *Phycomyces* are hermaphroditic and produce such hermaphroditic mycelia. Burgeff has ingeniously produced them by mechanically mixing the protoplasm of plus and minus vegetative filaments and has given them the name of mixochimæras. He concludes that such mixochimæras are mixtures of plus and minus nuclei. That these sexual mixochimæras are bisexual is shown by their occasional production of hermaphroditic zygospores and by the fact that the scanty sporangia which they produce again divide up into plus and minus and occasionally hermaphroditic spores. Often they show a plus or a minus tendency by forming zygospores with the normal minus or plus test races of *Phycomyces.* If propagated by cuttings of the mycelium, they eventually revert to normal plus or minus races.

The diagram (4) has been shown in order to point out certain homologies between sexual differentiation in the mucors and that in other groups of plants. The mucor plant is the gametophyte, the flowering plant the sporophyte. The germ tube with its germ sporangium we have homologized with the sporophyte and Burgeff reports in *Phycomyces* a fusion of nuclei in pairs in the zygospore and a reduction division in the germ sporangium preceeding the formation of the spores. From the diagram it will be observed that the condition in the so-called hermaphroditic lily is homologous with that in the so-called diecious *Phycomyces* or *Marchantia* in which I have found a similar differentiation of sex in sporophytic sporangia. The forms just mentioned are of the same type of sexual differentiation and yet are termed hermaphroditic or diecious according to whether the sporophyte or the gametophyte is the more conspicuous. To insure greater precision, I have suggested the terms homo- and hetero-thallic as applied to the gametophyte and homo- and hetero-phytic as applied to the sporophyte. If in further discussion of the subject, I use the older terminology, it is only to avoid terms unfamiliar to the majority of my audience. The main point to be brought out is that diecious mucors are not to be homologized with diecious flowering plants and higher animals. More nearly are the sexual races of mucors to be compared with the gametes themselves of such higher plants and animals.

ENVIRONMENTAL FACTORS

Many investigators have succeeded in inhibiting the expression of one or both sexes on the gametophyte of ferns by varying the environmental factors to which they are exposed. The question arises as to the influence of environmental factors on sexuality in the mucors. Since gametes are formed only *after* the stimulus of contact between filaments with opposite sex tendencies and not independently, the question is reduced to the influence of external factors on zygospore formation and upon the sexual activity of the separate races. As a general rule, both for hermaphroditic and diecious forms it may be said that the limits within which zygospore formation is possible are narrower than those for non-sexual reproduction. Thus in *Cunninghamella echinulata,* non-sexual reproduction takes place in abundance at low and high temperatures while zygospores are formed only above 20° C. Certain other species will not form zygospores, although able to produce sporangia at a temperature as high as 26° C. Further examples of other factors than temperature might be given in support of the greater environmental requirements necessary for the sexual type of reproduction. So far as has been investigated, external factors have no influence in altering the inherent sex character in a given race, though they may change its power of showing a sexual response.

The plus and minus races of *Phycomyces* have been cultivated in separate test-tubes since 1903 and have now reached the 242d non-sexual generation of both plus and minus races. The plus and minus races of *Mucor Mucedo* have been cultivated for the same length of time. The minus race has gradually become weaker and has this year finally died out. There does not seem to have been any actual loss or change of sex in the process although the ability to respond sexually has weakened with the weakness of vegetative growth.

MUTATIONS

It is a question whether or not it will ever be possible to induce genetic changes in the mucors by changes in environmental factors. Such changes do occur in some forms, however, apparently spontaneously. In 1912–13, in an investigation as yet unpublished, I found numerous variants of various degrees of distinctness in the offspring of a single plant obtained by sowing non-sexual spores. Three forms from the hermaphroditic species *Mucor genevensis* will suffice in illustration. In the roll tube at the right is shown a number of mycelium colonies of a dwarf mutant. They are of about the same age as those in the tube at the left. The dwarf has no sporangia but is propagated by divisions of the thallus. Perhaps the weakness of its growth is responsible for the fact that it does not form zygospores as other races of this species do.

At the right of the Petri dish culture (Fig. 3) are shown two colonies of the normal parent race. The small dots scattered over the surface are zygospores formed by the hermaphrodite. (The large circles in the culture are the places where the races were inoculated.) Tests with plus and minus races show that this normal race is a hermaphrodite with a minus tendency. In the central vertical row, three colonies are growing of a mutant with a plus tendency. That it is also hermaphroditic is shown by the dark dots representing zygospores which it produces, larger and arranged

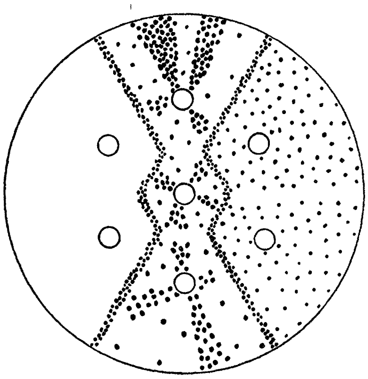

Fig. 3. Diagram of Petri dish culture of the hermaphroditic species *Mucor genevensis*. Circles represent points of inoculation, dots represent zygospores. Two colonies at right represent the normal parent stock; vertical row in center represents three colonies of a mutant with a (+) tendency; two colonies at left represent a mutant with (—) tendency.

in sectors more often than in the parent race. Since it has an opposite sexual tendency from the normal race, it forms a line of zygospores where it grows in contact with it. It also is forming a line of zygospores with the mutant on the left, two colonies of which are shown. This last mentioned mutant has, like its normal parent form, a minus tendency. Aside from its dense yellow growth, it is characterized by its well-nigh complete suppression of hermaphroditic zygospores on its own mycelium. If the suppression were complete and the race constant, we might be able to describe the origin of a diecious race from a hermaphroditic species. With the exception of the dwarf mutant which has been kept running since 1913, but which does not produce sporangia, and another possible exception, all the mutant variants found in this species eventually reverted to the normal type.

The tendency to reversion has been observed by Burgeff in his mutants of *Phycomyces* and attributed by him to a more rapid growth of the normal nuclei over the mutant nuclei in mixochimæras which he considers such variants to be. He was able to bring his mutants into a true-breeding condition by crossing them with the normal stock of opposite sex and obtaining the desired purity through segregation in the germ sporangia. Sex and mutant characters he found to segregate independently so that starting with a mutant in a plus race he was able eventually to obtain it in the minus condition.

DISTRIBUTION OF SEXUAL TYPES IN NATURE

A study has been made of the distribution in nature of the different sexual types. So far as the number of species is concerned, the diecious or heterothallic forms greatly predominate. If the table on the screen (5) were made to-day, we probably should have to more than double the list of species definitely known to have been mated. If unmated strains with sex determined by the "imperfect hybridization" reaction were added, the number would be still further increased. Of the homothallic (hermaphroditic) forms, very few would have to be added and the hermaphroditic forms it will be observed are those in which the sexual condition is readily determined by mere microscopic inspection and their zygospores therefore less likely to escape notice.

Table II. (3) above shows the distribution of races of *Rhizopus* obtained from different sources. As will be seen, the sexual strains are not at all local in their distribution. Those listed as neutral failed to give any reaction with the plus and minus test races. More extensive tests have recently been made with *Rhizopus* and other species as will appear in a later summary.

Collections of races of several different species have been made from diverse sources and the races within a given species tested for reactions *inter se*. Perhaps a form provisionally called "Dark" Absidia will serve as a convenient example. The collection of this species consists of 40 races. They have been contrasted with one another by twos in watch-glass cultures and all the possible combinations have been made as shown in the table. Grades A to D were assigned to the different strengths of sexual reaction measured by the number of zygospores produced in a given contrast. Each race was given a final numerical grade made up of the average of its reactions with all the other races and the races were arranged in the table according to their final grading. The plus and minus races were placed in series by themselves. There was no reaction when a plus was contrasted with another plus nor when a minus was contrasted with another minus. Whether they were of equal sexual vigor or one was weak and the other strong, the result of contrasting two races with the same sign was always negative. The collection of races therefore seemed dimorphic so far as sex is concerned. A race was either shown to be plus or minus or showed no reaction in a given combination and was provisionally classed as "neutral." There were no races evident that could be called sex intergrades.

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(*To be concluded*)

AN ANALYSIS OF AIM AND INCENTIVE IN A COURSE IN GENERAL ZOOLOGY

INCENTIVE AND AIM

That an incentive is necessary for the accomplishment of work is a postulate that needs no discussion. A review of my work as a teacher has led me to investigate the incentives that activate my students—mostly freshmen plunged into a course in general zoology. I have felt for some time that the aim of the course did not furnish an incentive for work.

Aim is confused with incentive because in some cases the two are equivalent. Aim is an aspiration for an ideal while incentive is an earthly motive. The statement of an aspiration may form an incentive for a few; but for most students the aim is soon forgotten.

INCENTIVE OF STUDENTS IN GENERAL ZOOLOGY

Since the motive that actually completes the work is not the aim, it is worth while to inquire what is the incentive. I recognize that the material with which I have to do shows a great deal of individual variation. In this "population" four classes can be discerned.

1. Those who work because the aim furnishes the incentive. In a so-called "general culture" course this is indeed a small class; in a technical school, however, the condition is reversed.

2. Those who work because of love for the subject, another small class. Although in some students this desire is inborn and probably hereditary, yet the proportion can be raised by an inspiring teacher.

3. Those who work for rewards. Our institutions, in their wisdom, through years of experience, have devised grades and honors. Some students have an inborn and probably hereditary ambition to seem better than their fellows and so react to this stimulus. Indeed competition can furnish a splendid incentive.

4. Those who work through fear. The same machinery erected to appeal to the ambitious reacts to prod the laggard. Under the threat of probation, condition, and exclusion, the victim struggles on. This is a large class and, in some ways, the most interesting. Although this group contains the dullards, yet the ranks are far from being homogeneous—the most brilliant member of the class may be buried in its ranks. How often have we seen a student, who, by constant threats, has just managed to scramble through our course, enter a technical school and not only lead his class but in time his profession. Lack of incentive is the key to his attitude toward the course in zoology. Being a more reasoning being than his fellows who work for love or rewards, and, feeling that the aim did not furnish an incentive, he gave his energies where, to his mind, results would be of more value.