

ped by the railroad and between Nogales and Imuris, one sees near the mountain bases low benches which have been eroded into overlapping oblique ridges of which the material seems to be all of morainal character. These overlapping ridges form prominent features in the landscape and are locally called by the Mexicans *cordones*, or 'chains.' They have not been observed by the writer south of Magdalena.

In traveling eastward from Benson toward El Paso, at a few points, mountain masses were observed, rising from the desert plains, around the bases of which were slightly elevated benches corresponding in some measure to the bench of morainal material along the eastern base of the Santa Rita Mountains. Occasionally, also, the railroad cut through low rolling ridges of material, morainal in structure and clearly not formed by sheet flood erosion.

On the Rock Island Railway, about a mile southwest of Santa Rosa, N. M., near the southeastern bank of the Pecos River, is a train of rolling hills which show morainal structure in the railroad cuts.

These observations lead to the conclusion that the quaternary history of some portion of this region is more complex than has been supposed. Clearly before the present period of arid climate and periodic sheet flood erosion was a time of low temperature and accumulated precipitation in the form of land ice which resulted in the formation of extensive deposits of rock debris around the bases of at least the higher mountain ranges. Climatic conditions limited the extent of these moraines so that the present desert basin areas, in many cases, remained unglaciated.

A further point of interest is the hypothesis, suggested by the facts observed, that some of the quaternary conglomerates which are abundant in northern Sonora and are so puzzling in respect to their origin may in part be the result of glaciation. The writer has, so far, been unable to formulate any other theory of origin which will account for the presence, in some of these beds, of boulders as large as two feet in diameter, in a matrix of angular

fragments, of many different kinds, mingled with fine material. In a bed of true volcanic breccia one does not ordinarily find several different kinds of eruptive rock varying greatly in composition.

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ZYGOSPORES AND SEXUAL STRAINS IN THE COMMON BREAD MOULD, *RHIZOPUS NIGRICANS*.<sup>1</sup>

FROM the preliminary communication of J. I. Hamaker in the May 4 number of SCIENCE, entitled 'A Culture Medium for the Zygospores of *Mucor stolonifer*' (*Rhizopus nigricans*), one would be led to suppose that the method of obtaining the zygospores of this species by admixture of strains from different sources was unnecessarily troublesome and uncertain. After having obtained the zygospores frequently for three months, he is brought to the conclusion, which is printed in italics, that 'with proper conditions of moisture and temperature, success is apparently dependent only on the nature of the substratum.' As a favorable substratum, a corn muffin bread is recommended and a detailed formula of the ingredients is given.

In a preliminary summary of a study of the sexual conditions in the Mucorineæ,<sup>2</sup> the present writer has used this same common bread mould *Rhizopus nigricans*, as a type of the heterothallic (*i. e.*, dioecious) group in which each species is to be considered an aggregate of two distinct sexual strains the interaction of which is requisite to zygospore formation. In contrast to homothallic (*i. e.*, hermaphroditic) species in which the mycelia are sexually all equivalent and which may produce zygospores from the sowing of a single sporangiospore, it is necessary in the heterothallic species, in order to obtain the zygospores in pure cultures, to sow spores together from both the sexually opposite strains which have been provisionally designated by the terms

<sup>1</sup>This paper was written while working under a grant as research assistant to the Carnegie Institution.

<sup>2</sup>'Zygospore Formation a Sexual Process,' SCIENCE, N. S., 19: 864-866. 1904.

(+) and (—). The rarity of the zygospores of heterothallic species therefore may be explained by assuming that when zygospores fail to form under conditions which of themselves are not unfavorable to zygospore formation, only one of the two sexual strains is present.

Ever since de Bary<sup>3</sup> discovered the zygospores of *Rhizopus* in 1865—now forty years ago—various and conflicting theories, based many of them upon the character of the substratum upon which the zygospores were accidentally found, have been brought forward to account for the rarity of their occurrence. The writer has attempted to show the insufficiency of the assumption that external conditions are of more than secondary importance, and has given at the same time a rather full account of cultural experiments with the strains of *Rhizopus*.<sup>4</sup> It is not the purpose of the present article to repeat the details there given. It seems not inappropriate on this occasion, however, to say a few words regarding the occurrence in nature of the strains of this species based upon recent investigations as well as upon those already published. For the sake of comparison, a list of those species the thallic condition of which has been determined, will be given arranged according to the type of their sexual reproduction, and something will be said concerning the cultivation of *Rhizopus* for class purposes.

In Table I. are listed the zygosporic cultures from which the sexual strains have been isolated by the writer. In making the separations, a large number of cultures were necessary as indicated in the table. Tests with both the standard (+) and (—) strains were not made for all the cultures listed, but no strains were found which alone in pure cultures could be brought to zygospore formation.

In the preparation of Table II., the individual strains obtained through the kindness of the writer's correspondents were tested in

<sup>3</sup> 'Beiträge zur Morphologie und Physiologie der Pilze,' II., 1866.

<sup>4</sup> 'Sexual Reproduction in Mucorinææ,' *Proc. Am. Acad.*, 40: 205-319, pls. 1-4. 1904.

separate cultures against standard (+) and (—) strains. Each strain therefore listed as (+) or (—) produced zygospores in contrast with the test strain of opposite sign and failed to produce them in cultures with the test strain of the same sign. Those strains which would form zygospores with neither the (+) nor the (—) test strain on the substratum used were provisionally listed as neutral. Judging from the possibility of inducing temporary neutrality in the strains of *Mucor Mucedo* by cultivation under unfavorable temperatures, one might suppose the neutrality in these strains of *Rhizopus* to be likewise but a temporary condition. With their exception, all of the 43 individual strains tested are either (+) or (—). To the 11(+) and 19(—) strains in Table II., should be added the 8(+) and 8(—) strains isolated from the zygosporic cultures of Table I. We shall then have from the strains tested 19(+), 27(—) and 13 apparently neutral. Out of this total of 58 individual strains, none have been found which will yield zygospores when grown alone in pure cultures. As may be seen by a glance at the tables, strains have been collected from various parts of the world, and the conclusion seems irresistible that the common bread mould, *Rhizopus nigricans*, is heterothallic and that the interaction of the two sexually opposite strains is necessary to the formation of their zygospores.

TABLE I. STRAINS IN SPONTANEOUS ZYGOSPORIC CULTURES.

Source of Zygosporic Cultures.	Substratum on Which the Zygospores Were First Discovered.	Number of Pure Cultures Tested.	
		(—)	(+)
Boston, Mass.....	Shells of almond nuts on sterilized paste.	1	29
Brookline, Mass.....	Shells of almond nuts on sterilized paste.	2	6
Newton, Mass. (a)..	Shells of almond nuts on sterilized paste.	3	18
Newton, Mass. (a)..	Shells of almond nuts on sterilized paste.	1	1
Newton, Mass. (b)..	Shells of almond nuts on bread.	3	18
Cambridge, Mass....	Decayed potato.	1	6
Chapel Hill, S. C....	Bread.	6	6
Stanford Univ., Cal.	Squash.	5	2
Raleigh, N. C.....	Sweet potato.	19	1
8 zygosporic cultures	Totals, 107 strains.	38	69

TABLE II. INDIVIDUAL STRAINS ARRANGED ACCORDING TO SEXUAL CHARACTER.

(-)	Neutral.	(+)
Boston, Mass.	Brookline, Mass. (2)	Brookline, Mass.
Jamaica Plain, Mass. (2)	Winthrop, Mass.	Worcester, Mass.
New Haven, Conn.	Middletown, Conn.	New Haven, Conn.
Cazenovia, N. Y.	Granville, Ohio.	Storrs, Conn.
Chicago, Ills.	Breslau, Germany.	Washington, D. C.
Lansing, Mich. (2)	Leipzig, Germany.	Ithaca, N. Y.
Austin, Texas.	Halle, Germany.	Delaware, Ohio.
Columbia, South Carolina.	Honolulu, H. I.	Madison, Wisconsin.
Nassau, Bahamas.	Margarita, Venezuela. (2)	St. Louis, Mo.
Progreso, Yucatan.	Caracas, Venezuela.	Paris, France.
Cambridge, England.	Port au Prince, Haiti.	Berlin, Germany.
Paris, France.		
Nancy, France.		
Bern, Switzerland.		
Berlin, Germany.		
Eisenach, Germany.		
Manila, P. I.		

TABLE III. LIST OF SPECIES, THE THALLIC CONDITION OF WHICH HAS BEEN DETERMINED, ARRANGED ACCORDING TO THE TYPE OF THEIR SEXUAL REPRODUCTION.

Homothallic.	Heterothallic.	(-)	Neutral.	(+)
1. <i>Sporodinia grandis</i> .	1. <i>Mucor Mucedo</i> .	2	—	1
2. <i>Spinellus fusiger</i> .	2-7. <i>Mucors</i> III.-VIII.			
3-4. <i>Mucors</i> I and II.	8. <i>Rhizopus nigricans</i> .	27	13	19
Heterogamic.	9. <i>Phycomyces nitens</i> .	1	3	6
5. <i>Zygorhynchus Moelleri</i> .	10. <i>Circinella umbellata</i> .	2	—	2
6. <i>Zygorhynchus heterogamus</i> .	11. <i>Cunninghamella echinulata</i> .	2	—	2
7. <i>Dicranophora</i> .	12. <i>Absidia caerulea</i> .	3	2	1
	13. <i>Absidia repens</i> .	1	—	1
	14. <i>Absidia</i> sp.	1	—	1
	15. <i>Helicostylum piriforme</i> .	1	—	3
	16. <i>Syncephalastrum</i> .	2	—	1
	17. <i>Mucor</i> N, n. gen.	1	—	1

In Table III., which contains the species investigated by the writer, arranged according to their thallic character, the figures opposite the heterothallic forms indicate the number of different (+), (-) and neutral strains which have been tested of these species. In addition the writer has a considerable number of cultures which the hybridization reaction would indicate are unmated strains of heterothallic species.

The sexual character of individual strains of all the heterothallic species investigated has remained unaffected when they are cultivated vegetatively. Thus the (+) and (-) strains of *Phycomyces* and *Mucor Mucedo* have been cultivated separately for 92 and 90 sporangiospore generations, respectively, and are as active sexually as when their zygospores were first discovered. Moreover, the two opposite strains of *Rhizopus* have by daily

transfers of mycelia been brought to the seventieth generation and by transfers of sporangiospores to the thirtieth generation without the production of zygospores; yet it is only necessary to make sowings together on the proper substratum of spores from the (+) and (-) tubes at the end of the series in order to obtain zygospores in abundance. The homothallic species have also been cultivated to many non-sexual generations, some of them for ten years and over, without change in their sexual behavior. Homothallism and heterothallism therefore seem to be fixed conditions in the forms in which the sexual character has been determined.

In view of the facts summarized, it would seem probable that Mr. Hamaker had been working with impure cultures rather than that he had discovered a new homothallic species of *Rhizopus*. This is further sug-

gested by experiments with zygosporic material which was part of a spontaneous growth on bread exposed in the laboratory of Leland Stanford University. Professor Campbell writes that he has almost always gotten zygospores in this way since 1892, yet an investigation showed that the culture contained but the two sexually opposite strains which were alone incapable of zygospore formation. *Rhizopus* shares with *Penicillium* the doubtful distinction of being the most common fungus weed in laboratory cultures, and it is almost impossible to keep a favorable substratum such as bread in a moist atmosphere for a week without obtaining an abundant growth of this characteristic 'bread mould.' When (+) and (—) spores are in the laboratory, one is very likely to get in spontaneous cultures a growth producing zygospores from the germination of sexually opposite spores which may chance to be together on the bread and, if a mixed sporangial transfer is made from a culture already producing zygospores, one is almost certain to obtain them. That spontaneous zygosporic cultures thus often occur has frequently been experimentally demonstrated by the writer in the Harvard laboratory.

The zygospore of the heterothallic species *Phycomyces*, in germinating, typically produces a sporangium containing both male and female spores.<sup>5</sup> If the germ tube be forced to grow into a mycelium before the differentiation of sex in the germ sporangium, a homothallic mycelium results. The condition in these homothallic mycelia is unstable and the delayed segregation of sex takes place at the formation of spores in the sporangia which they eventually produce. Until this homothallic character becomes fixed, one can not speak of a homothallic race of *Phycomyces*; but the facts already obtained are such that one would not be justified in saying that, in other heterothallic forms, homothallic races may not exist. The experiments with *Rhizopus*, however, render it extremely improbable that this species is other than strictly heterothallic. A few zygospore germinations of *Rhizopus* have been secured, but not as yet

in a condition to enable one to analyze the sexual character of the germination.

Although up to the time of the discovery of heterothallism in the mucors the zygospores of *Rhizopus* are found reported in the literature but five times, they can hardly be rare. They are generally covered by the sporangial growth and, to one unfamiliar with their appearance, are distinguishable only with the aid of a microscope. The writer has found them in spontaneous bread cultures in Eisenach and Halle, Germany, and Professor Lloyd and Miss Watterson have obtained them in the same manner in Teachers College, New York.

*Rhizopus* is not so sensitive to the influence of external conditions in regard to the formation of its zygospores as many other of the heterothallic mucors. The ordinary substrata used in the laboratory are few upon which zygospore formation can not be induced if proper regard be had to conditions of moisture. Carbohydrates are especially favorable and bread makes an admirable culture medium. The writer has in progress a series of experiments to determine the conditions under which zygospore formation is possible in the various species. The fact that in agar cultures with 1 per cent. peptone zygospores have been obtained by the addition of varying amounts of grape sugar from 0.1 per cent. up to 40 per cent., will be sufficient to show that zygospore production in this species is not limited to any great extent by the composition of our ordinary culture media. The temperature is not a very important factor in this instance. Up to at least 27° C., an increase in temperature accelerates the growth and increases the production of zygospores. More or less moisture in the surrounding air, on the other hand, is necessary to the formation of zygospores and, as the writer has already shown (*l. c.*), their production may be suppressed before that of sporangia by sufficient desiccation.

The writer<sup>6</sup> has already described the

<sup>5</sup>'Zygospore Germinations in the Mucorineæ,' *Annales Mycologici*, 4: 1-28, pl. 1. 1906.

<sup>6</sup>'Two Conidia-bearing Fungi, *Cunninghamella* and *Thamnocephalis*,' *Bot. Gazette*, 40: 161-170, pl. 6. 1905.

method of obtaining the zygospores of *Cunninghamella* by contrasting in cultures different strains of this species. By a sufficient accumulation of material from different sources, one might expect eventually to obtain the two sexual strains and, by their synthesis, the zygospores as well of any form in which the sexes are separated on different mycelia.

Undoubtedly a careful search below the sporangial growth would show that zygospores are more common in spontaneous cultures than is usually supposed. In searching for forms of the mucors, the writer had occasion to make cultures on sterilized paste and on bread of various substances among which the shells of different kinds of nuts were thus investigated. It may not have been a mere accident that in all the several instances in which shells of almond nuts were used, zygospores were produced, but failed to appear when shells from other kinds of nuts were employed. These almond nuts were obtained from different places in the neighborhood of Boston, though they all probably came from the same source originally. The writer would be glad to learn if others have a similar experience with the shells of this species from other localities.

In making cultures, the bread should be raised above the bottom of the culture dish by some convenient object, otherwise the bread is likely to become soggy and attacked by bacteria. Layers of moistened filter paper on the sides and bottom of the dish, which should be covered, ensure a proper moist condition in the surrounding air. Those desiring to have absolutely pure cultures may first sterilize the bread dry and then allow it to soak up a sufficient amount of sterilized water to give it a spongy consistency, after which it may be sterilized with steam for about five minutes. Prolonged sterilization as well as too much water in the bread makes it soggy and less advantageous for cultures. Sterilization is not necessary, however, for class work. Zygospores when present are likely to form in dense masses between the layers of moist filter paper lining the culture dish or in the folds

of crumpled pieces of filter paper placed in the culture as traps for their capture. Sporangia form where the air is dryer, and the habit of growth can be readily studied from the filter paper in the upper parts of the culture. The individual stolons can be more easily distinguished if darkened paper be employed. When zygospores are once obtained, mass transfers of the mycelium may be made to new cultures and thus the *Penicillium*, which is a usual weed in spontaneous cultures, may be eliminated. A culture producing zygospores may be dried with its substratum and used from time to time as 'seed' whenever zygospores are needed. Zygosporic cultures of the 'Harvard Strain' have thus been kept running for nearly ten years. The sporangiospores of *Rhizopus* are comparatively short lived, however, and generally do not retain their vitality for more than a year.

For methods of separating out the two sexual strains from a zygosporic culture, which is often a tedious process, one may refer to the writer's detailed experiments with this species already cited.

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#### RESULTS FROM MOORE'S METHOD OF SHIPPING BACTERIA ON COTTON.

IN SCIENCE of March 23, Messrs. Kellerman and Beckwith have called attention to the statement in Bulletin 270 of the New York Agricultural Experiment Station that [certain<sup>1</sup>] 'cultures of nodule-forming bacteria dried upon cotton were worthless for practical purposes and that the failure of such cultures was inherent in the method of their preparation.' At the same time they presented some excellent data upon the effect of drying legume bacteria under various conditions. Their most interesting experiment consisted in placing a culture "on cotton half of which was placed in a sterile Petri dish, to make drying very slow, half was dried rapidly and kept

<sup>1</sup> An important word omitted by Messrs. Kellerman and Beckwith in summarizing the statements contained in Bulletin 270.