MATING REACTIONS OF ENUCLEATE FRAGMENTS IN PARAMECIUM BURSARIA

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INTRODUCTION

The presence of distinct mating types in *Paramecium* has been demonstrated by several investigators (Sonneborn, 1937; Kimball, 1937; Jennings, 1938; Sonneborn, 1938; and Jennings, 1939). Under appropriate conditions individuals belonging to different mating types in the same "group" will, when they are placed together, immediately agglutinate and later form pairs. Such agglutination has been called the "mating reaction." The present study is designed to answer the question: Do enucleate fragments of *Paramecium* manifest mating reactions?

In this investigation we have studied, for comparison, the following phenomena: (1) mating reaction between whole animals, (2) mating reaction between nucleate [1] fragments and whole animals, (3) mating reaction between enucleate [1] fragments and whole animals, (4) mating reaction between enucleate fragments. The results of these comparative studies will be reported in the order given.

Preliminary work on this problem was originally begun at the Osborn Zoölogical Laboratory, Yale University, in collaboration with Dr. R. F. Kimball; but circumstances unfortunately prevented its completion there. The present study was carried on largely at the University of California at Los Angeles and completed at the University of Vermont. A preliminary report appeared in *Science*, **91**: 246 (1940).

MATERIAL AND METHODS

Paramecium bursaria—the green Paramecium—is especially favorable for this study for several reasons. (1) As far as we know, *bursaria* is the only species of *Paramecium* in which enucleate fragments are viable. (2) The tendency of animals of this species to creep slowly over the bottom of the container facilitates cutting with a glass needle to such an extent that large numbers of fragments can be obtained.

[1] In this paper the word "nucleate" is used to indicate the presence of both the macronucleus and the micronucleus; the word "enucleate" meaning the absence of both nuclei.

(3) The single micronucleus of this species (especially in the races used in the present investigation) is large and stains deeply with hematoxylin. (4) Stability of mating type permits one to obtain uniform and constant material for study.

For this study two races of *P. bursaria*—McD_3 and *Gr*14—were used.[2] They belong to two different mating types of Group II (Jennings, 1939). Under suitable conditions they give a marked mating reaction when placed together, and permanent pairs are later formed.

Race McD_3 was collected near Baltimore, Maryland; race *Gr*14 from south of Greensboro, North Carolina. McD_3 is a large race, while *Gr*14 is somewhat smaller. In both races the micronucleus is large and stains deeply with hematoxylin. After such staining the micronucleus or a piece of macronucleus could thus easily be detected if present in any fragment.

The animals were cultured in essentially the same manner as described by Jennings (1939). A number of cultures of each race were kept in the laboratory, and only those which gave the strongest mating reaction were used.

The animals to be operated upon were placed in a depression slide and cut with a fine glass needle under a dissecting microscope. When the needle passed directly through the mid-region of the animal, the nuclei were usually seen to be extruded from one of the fragments; while if the needle cut to one side of the mid-region one large nucleate and one smaller enucleate fragment resulted. Only fragments one-half the size of the original animal or smaller were isolated for testing since these were most likely to be enucleate.

When a definite mating reaction had been observed, the fragments were fixed in hot Schaudinn's fluid (95 cc. Schaudinn's fluid and 5 cc. glacial acetic acid) or in Bouin's fluid at room temperature. They were stained in iron-hematoxylin, destained in aqueous picric acid, and mounted in damar.

Observations

Mating Reaction between Whole Animals

Jennings (1939) has described in detail the mating reaction between whole animals in *P. bursaria*. We have, however, noted an additional feature. When the area of contact of one animal with another is small there appears a distinct flattening if not an appreciable indentation of that part of the cell (Fig. 1). At present an explanation of this phenomenon cannot be given, but at least the response indicates that the

[2] We are indebted to Professor H. S. Jennings for these two races of *P. bursaria*.

union between the animals involves more than a possible adhesion of the

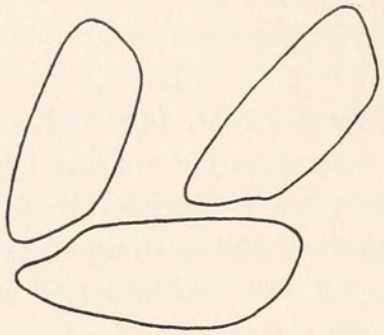

FIG. 1. Mating reaction of whole animals. Two individuals in this case have become attached to a third animal. In each animal there appears a flattening of the contour of the body at the region of contact. (This drawing and others to follow are free-hand sketches from the living material.)

cilia. It is significant that if members of a reacting pair (two whole animals, or a whole animal and a fragment) are gently separated with the glass needle, these flattenings or indentations (Fig. 3, *g–i*) do not disappear at once but only gradually round out to the normal contour.

Mating Reactions between Nucleate Fragments and Whole Animals

When *P. bursaria* is cut transversely with the needle there usually results one larger and one smaller fragment. It is the larger fragment which contains the nuclei, the nuclear complex being visible as a clear sphere in the living fragments. Shortly after cutting, such nucleate fragments of McD_3 were placed with whole animals of *Gr*14. The mating reaction took place at once, and on the following day intimate fusion of fragments with whole animals, similar to conjugation between whole animals, was observed. Conversely, nucleate fragments of *Gr*14 were placed with whole animals of McD_3, and such mixtures gave the same mating reaction and intimate fusion (Fig. 2) as described above.

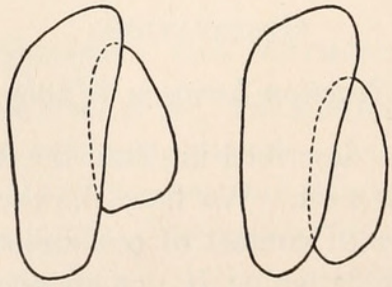

FIG. 2. Large nucleate fragments of *Gr*14 fused with McD_3 whole animals.

(Samples of such pairs were stained with aceto-carmine and a full nuclear complement was invariably found in the fragment.) It has not

yet been determined whether the intimate fusion between the nucleate fragment and the whole animal initiates nuclear changes in either or both partners.

It should also be noted that the nucleate fragments not only exhibited mating reactions immediately but also on the second and third days after cutting. After the mixture was made and mating reaction noted, it was placed aside undisturbed in a moist chamber. This mixture was again examined on the second and third days following. Such nucleate fragments as had not already fused in conjugation were again found to be exhibiting the mating reaction. Apparently the nucleate fragments which did not fuse with the whole animals gave repeated mating reactions.[3] Thus the nucleate fragments can react not only on the day when they are prepared, but also on subsequent days; and this is quite in contrast to enucleate fragments which give the mating reaction on the day of cutting but never subsequently, though they may be active two or more days later (see below).

Viability of Enucleate Fragments

Enucleate fragments of *P. bursaria* may remain alive for as long as four days, and during that time exhibit a surprisingly normal behavior, swimming actively in one direction, alternating the direction of movement, spiralling, and coming to rest adjacent to masses of food (Tartar, 1938). Observations on 100 enucleate fragments each of McD_3 and *Gr*14 showed that practically 100 per cent of such fragments were alive and active after one day, and 50 per cent after two days. Such hardiness of enucleate fragments made possible the investigation herein described. In the present study the enucleate fragments were tested within half an hour after cutting.

Mating Reaction between Enucleate Fragments and Whole Animals

Enucleate fragments of either race were found to give the mating reaction with whole animals of the other race (Fig. 3, *a–f*). Enucleate fragments never agglutinate with fragments or whole animals of the same race. The mating reaction was observed in 131 small enucleate fragments of McD_3 mated with whole animals of *Gr*14. A sample of fifteen of these fragments which reacted was fixed in Schaudinn's fluid and stained with iron-hematoxylin to test whether nuclei or parts thereof might be present in the fragments. In no case was a micronucleus or a piece of macronucleus found in a fragment.

[3] The races studied show a diurnal reactivity, discontinuing mating reactions in the late afternoon and not reacting again until the morning of the following day.

In the reciprocal cross enucleate fragments of *Gr*14 were placed with whole animals of McD_3. Such a mixture gave the same agglutination as described above. Twelve such fragments which reacted were stained and no trace of a nucleus or part of a nucleus was found in them.

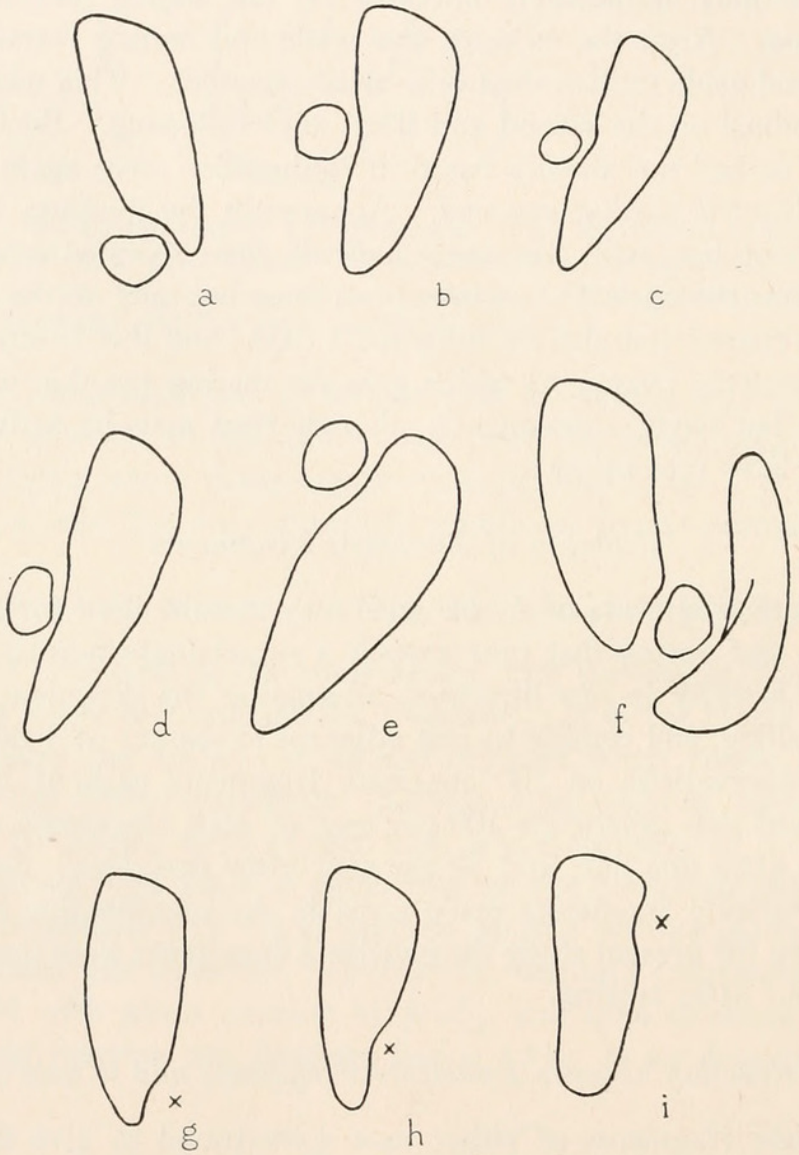

FIG. 3. Mating reactions between enucleate fragments and whole animals. Note the flattening or indentation of the body of the animal at the point of union with a fragment (Fig. 3, *a–f*). Whole animals which were separated from their attachment with enucleate fragments still retain typical indentation (X) at the points of union (Fig. 3, *g–i*).

Apparently in no respect does the mating reaction between enucleate fragments and whole animals differ from that between whole animals. Such similarity is shown by the following observations: (1) The reacting fragment agglutinates with the first animal of the other race with which it happens to collide. Subsequently, one, two, three or more ani-

mals of the other race may become attached to the fragment, thus forming the typical agglutinated clump (Fig. 3*f*). (2) The fragment remains securely attached to the whole animal, i.e., the two do not rotate upon one another. The direct medium of this union is not the surfaces of the fragment and the whole animal since the partners remain separated by the distance of their cilia. Yet the union is more intimate than might be suspected, for at whatever region the fragment attaches to the whole animal, a flattening or slight indentation of the normal contour of the whole animal is there produced (Fig. 3). (3) The pairs or clumps formed by the agglutination of fragments and whole animals break up at approximately the same time as that at which clumps of whole animals mated simultaneously break up into conjugating pairs and single animals. This latter point is the conclusion from a separate study of the duration of agglutination of enucleate fragments with whole animals in which two additional groups of McD_3 enucleate fragments (total number, 90) were mated with *Gr*14 whole animals. All enucleate fragments became separated from the whole animals 123 to 140 minutes after the beginning of agglutination and never again united with them. This was approximately the time required for simultaneously mixed whole animals, which formed large clumps, to break up into pairs and single animals (123 to 153 minutes).

The mating reaction between enucleate fragments and whole animals is thus in these respects altogether normal, *but it was never followed by true fusion as in conjugation.* It remains to be determined whether contact with the enucleate fragment is sufficient stimulus to initiate nuclear changes in the whole animals.

Although mating reaction is thus shown by enucleate fragments tested promptly after cutting, this is not the case if the enucleate fragments are kept for 24 hours before they are mixed with whole animals of the other race. Enucleate fragments from a McD_3 culture were prepared at the time when tests showed the animals of this culture to be most reactive with *Gr*14. These McD_3 enucleate fragments were placed with *Gr*14 whole animals 24 hours after they were cut. None of the 115 fragments so tested in delayed mixing showed any mating reaction although they were alive and active. At the time of mixing the enucleate fragments with the whole animals, control experiments showed that the McD_3 whole animals from the culture in question were again strongly reactive.

Mating Reaction between Enucleate Fragments

For a study of reaction between enucleate fragments, one fragment of each race was placed in a very small drop of culture fluid and the

pair observed. In approximately half of the cases the enucleate fragments agglutinated at once when, during their rapid movements, they first collided with one another (Fig. 4). Mating reaction between enucleate fragments was under these conditions apparently not so strong as between enucleate fragments and whole animals, for the pairs were easily separated by jarring, and even when not disturbed they remained attached for not longer than ten minutes. That the response is not a mere chance adherence, however, is shown by the fact that it never occurred between fragments of the same race even though these were observed to collide with one another. Although flattening at the point of contact was not observed in these small fragments, they swam together as one animal without rotating upon one another, a behavior typical of

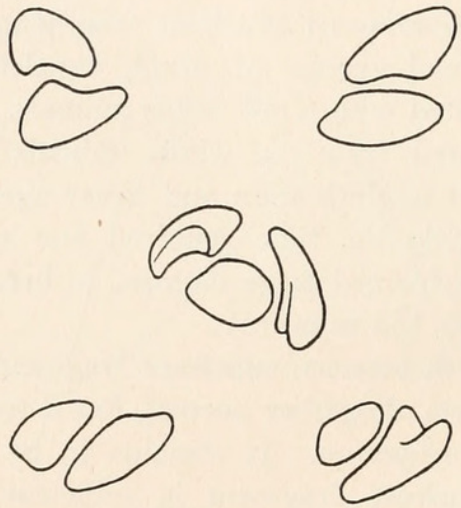

FIG. 4. Mating reaction between enucleate fragments. (These fragments were later fixed and stained and were found to be enucleate.)

the mating reaction. After the two reacting fragments separated, they frequently again agglutinated and became attached.

Thirty enucleate fragments (15 of each race) were tested as described; and of these 16 gave the mating reaction. Each pair of reacting fragments was separately fixed and stained in iron-hematoxylin. In no case was a nucleus or part of a nucleus found in any of the fragments.

Another method of observing the mating reactions between enucleate fragments consists of introducing under the dissecting microscope one fragment of one race into a drop containing several fragments of the other race and to watch the introduced fragment continuously. This procedure greatly increases the probability of the fragment finding a partner. Under these circumstances two more cases of mating reaction were observed: in one case the introduced McD_3 fragment remained

attached to two *Gr*14 fragments for five minutes; in the other case a McD_3 fragment paired with a *Gr*14 fragment for twelve minutes.

Still another method was to place three to six enucleate fragments of one race (*Gr*14) into a drop containing many enucleate fragments of the other race (McD_3). Out of a total of 22 *Gr*14 fragments so tested, 15 reacted. Typical mating reaction occurred; pairs of enucleate fragments and groups of three enucleate fragments were observed. When, as here, the drop of water in which the reaction is followed is of sufficient size that evaporation does not interfere and the reacting fragments need not be disturbed by replenishment of the drop, the conditions may be said to be most nearly normal; and under these circumstances pairs of enucleate fragments and groups of three enucleate fragments were found to remain continuously united in the mating reaction for as long as 34 minutes.

Thus a total of 25 cases of mating reaction between enucleate fragments has been recorded. Of these, 12 sample pairs of fragments were carefully stained and no nucleus found in either fragment. In every experiment, it is to be emphasized, the unmixed enucleate fragments of each race were conscientiously watched en masse, and in no instance did fragments of the same race react with one another though collisions between them were frequent.

Thus the cytoplasm alone (in the absence of the nuclei) exhibits the reactivity and diversity of mating type. Of course, this reactivity may be due to the retention of influence of the nuclei which have just been removed. This possibility is strongly suggested by the fact that enucleate fragments lose their reactivity within a day and do not regain it thereafter.

Summary

1. Comparative studies were made on the following phenomena in two races of *Paramecium bursaria* belonging to two different mating types: (*a*) the mating reaction between whole animals, (*b*) the mating reaction between nucleate fragments and whole animals, (*c*) the mating reaction between enucleate fragments and whole animals, and (*d*) the mating reaction between enucleate fragments.

2. In the mating reaction between whole animals a phenomenon hitherto unreported was observed: When two animals become attached there is a flattening or even an appreciable indentation at the point of union which is most striking when the area of contact is small.

3. Nucleate fragments of either race show mating reaction with whole animals belonging to the other race. The mating reaction may be followed by an intimate fusion, as in conjugation between whole animals, between the nucleate fragment and whole animal.

4. Enucleate fragments of either race give the mating reaction with whole animals of the other race. Mating reactions never occur between enucleate fragments and whole animals of the same race. Mating reaction between enucleate fragments and whole animals appears to be identical with that between whole animals, but it was never followed by intimate fusion as in conjugation between whole animals.

5. The mating reaction also occurs between enucleate fragments belonging to two different races. Controls showed that mating reactions never occur between enucleate fragments of the same race.

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