SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. I

BY H. S. JENNINGS

ZOÖLOGICAL LABORATORY, JOHNS HOPKINS UNIVERSITY

Communicated February 1, 1938

The discovery by Sonneborn[1] of two sex reaction types in a race of *Paramecium aurelia* opened a new chapter in the genetics of the Ciliata. The present study shows that similar reaction types occur in *Paramecium bursaria*, but that in number and interrelationships they differ greatly from the situation in the race of *Paramecium aurelia* studied by Sonneborn and by Kimball.[2]

In the work on the cytology of conjugation in *Paramecium bursaria*, in this laboratory, Dr. T. T. Chen observed that conjugating pairs could be obtained from mixtures of two of his cultures, but not from either culture separately. This formed the starting point of the present investigation. Two clones, designated *l* and *m* were obtained from the cultures of Chen, and the work was extended to many other stocks. The clones *l* and *m* had been collected from a pond at Alexandria, Virginia.

The mating reaction that occurs when two appropriate clones of *Paramecium bursaria* are mixed—such clones as *l* and *m*—is of the rapid agglutinative character described by Sonneborn[1] in *Paramecium aurelia*, and by Moewus[3] for various flagellates. Within a few seconds after mixture the individuals have clotted together in small groups containing two to a dozen or more individuals. In strongly marked reactions these groups quickly coalesce into masses that contain hundreds of individuals and are visible to the naked eye. The animals remain clotted for a half hour to two hours; in the latter part of that period the clots begin to break up, and it is now found that many of the component individuals have become united in pairs. After about two hours only united pairs and single individuals may be found in the mixture.

The pairs are formed from one member of each of the two clones that were mixed. This is readily demonstrated by removal of the green color from individuals of one of the two clones. The usual strong green color of *Paramecium bursaria* is due to the presence in the body of many cells of a green alga. The color can be almost completely removed by inducing the animals to multiply rapidly in a rich nutritive medium, such as the algal-bacterial medium described by Raffel.[4] The contained algae multiply much less rapidly than the Paramecia, so that the later generations contain but a few scattering alga cells which hardly color them at all. In this condition *Paramecium bursaria* is practically as colorless as *Paramecium aurelia*. When one of the two clones is thus made white, the pairs in the mixture are

found to consist always of one green individual and one white one. In the large clots also, green individuals are always in contact with white ones.

The reaction between two clones belonging to diverse reaction types occurs whenever the two clones are mixed, provided they are in the proper physiological condition. There is a period after conjugation, varying in length in different clones, in which the individuals will not mate. Further, mating does not occur when the individuals have recently been transferred to a rich nutritive medium and have grown very large and are rapidly dividing. The most favorable conditions for the mating reaction are furnished when, after such a period of rapid growth and division, the nutritive medium becomes poor and the animals are becoming thin and ceasing to divide. At such periods, if two clones of diverse type are mixed the clotting is strongly marked and many pairs are formed.

Different clones show marked differences in the readiness with which they are brought into the condition for clotting and conjugation. Some are refractory and can be induced to react only by bringing about the exact degree of nutritive decline that is most favorable to the mating reaction. Others react readily under all conditions except those of extreme plumpness and rapid fission resulting from rich nutritive conditions. The reaction occurs, however, only at certain periods of the day, diverse in different stocks, but excluding in all cases the evening hours, so far as observed. (These matters are to be dealt with separately.)

The clotting reaction above described occurs only when two clones of diverse sex reaction type are mixed. But in certain clones, perhaps in all, isolated pairing may occur between members of the same clone, if the clone is left for long periods in a condition of declining nutritive strength. If, for example, great numbers of individuals of a single clone are left for several days in a watch glass with a small amount of water, after some days isolated pairs may sometimes be found among them. The formation of such pairs is not accompanied by clotting, and is so rare that close study of a clone for months may yield no examples. Such exceptional pairs are left out of account here; they form no part of the usual picture of pairing. Their genetic consequences will be dealt with elsewhere.

In such clones as *l* and *m* we thus find two sex reaction types that are seemingly comparable to the plus and minus sex types of Chlamydomonas and other flagellates as described by Moewus,[3] and to types I and II of Paramecium aurelia (Sonneborn 1).

The matter was investigated further (1) by allowing *l* and *m* to conjugate together, producing many exconjugant clones, the sex reactions of which were examined, and (2) by collecting from natural habitats many stocks and studying their sex reactions. Results of the former line of work are presented in this paper.

Sex Reaction Types of Clones Descended from Exconjugants of Type l by Type m.—A large number of pairs were obtained by matings between the clones *l* and *m*, representing the two reaction types. From the exconjugants of these pairs clones were obtained, and the sex type of each was tested by examining its reactions against the two parental types. The design was to obtain if possible inheritance ratios for the two diverse types.

This purpose meets a serious obstacle in the great mortality among the exconjugant lines. Most of the exconjugants or their descendants die. This occurs even in the most favorable nutritive media and other conditions in which non-conjugants flourish, none dying. A large proportion of the exconjugants die without dividing. Others divide, but produce weak offspring, often small and abnormal, which die after a few generations. In the experiments, 142 pairs were isolated from $l \times m$; these should yield 284 exconjugants, and as two lines were to be cultivated from each exconjugant, there were potentially 568 lines of descent. But out of these, only 26 formed clones that continued to multiply and so could be tested; thus but 4.6 per cent. And these included representatives of but 11 of the 142 pairs.

With so high a mortality the determination of inheritance ratios for the sex reaction types is not possible, but study of the 26 descendant clones nevertheless furnishes important information as to the sex reaction types and their nature.

The exconjugant lines were designated as follows. To each pair was given a number, and the two members of a pair were called *a* and *b*. From each member two lines of descent, derived from the first division of the exconjugant, were isolated; these were designated 1 and 2. Thus the pair 67 furnished the four lines of descent 67*a*1 and *a*2, 67*b*1 and *b*2. The 26 exconjugant lines were tested by mixing samples of them respectively with *l* and with *m*, to determine the sex type. As was expected, some reacted only with *l*, others only with *m*.

But the clone 67*a*1 was found to form pairs with both *l* and *m*, and on examination this was found to be the case with all the 4 clones descended from pair 67—*a*1, *a*2, *b*1 and *b*2. They reacted strongly, forming immediately large clots, and numerous later pairs, equally with *l* and with *m*.

It was at first suspected that such clones must have become differentiated into the two sex types, as *Paramecium aurelia* differentiates into two sex types at endomixis, according to Kimball.[2] But examination showed that this is not the case. Any single individual of 67*a*1 (or the other clones of 67) reacts with equal readiness with either *l* or *m*.

This is discovered as follows. A single individual of 67*a*1 is introduced with capillary pipette into a drop containing individuals of clone *l* only. The first individual of *l* with which it comes in contact clings to it and initiates the pairing reaction. While in this conidtion, before firm union, the pair are removed to a drop containing only individuals of *m*. In the transfer

the two become separated, since they have not yet firmly united. Each then provokes a strong reaction with *m;* in a few seconds two small clots are formed. In one of these individuals of *m* are clinging to *l*, in the other to 67*a*1. It is demonstrated that the single individual of 67*a*1 reacts with equal readiness with type *l* or type *m*.

		1				2									3												4			
		m	C	23b1	23b2	l	B	3a1	5b1	15b1	28b1	107a1	107a2	88a1	5b2	67a1	67a2	67b1	67b2	88a2	88b1	88b2	97a1	97a2	97b1	97b2	169a2	1b1	169a1	169b1	169b2
1	m	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23b1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23b2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	l	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	28b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107a2	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	88a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	5b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67a1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97a1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	169a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
4	1b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169a1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169b2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−

TABLE 1

PARAMECIUM BURSARIA

Clones of the *l* − *m* group, including 26 clones derived from exconjugants of *l* × *m*. Classified table of matings, showing the four reaction types.

\+ Form clots and pairs when mixed.

− Do not clot and pair.

Also, if a single individual of 67*a*1 is allowed to multiply, and some of its descendants are tested with *l*, others with *m*, they are found to react immediately with both.

Thus we have, besides *l* and *m*, a third reaction type, which unites with both *l* and *m*. We are not dealing with simply two reaction types, as was at first assumed.

In the course of the tests a fourth reaction type was found, exemplified by the clone 1*b*1. This forms clots and pairs, not only with *l* and with *m*, but also with 67*a*1 and other members of its type.

To determine the exact situation, each of the 26 clones descended from *l* and *m* was tested with every other, as well as with the parental clones *l* and *m*. In addition, two other clones, *B* and *C*, were found to interact with members of this group; these were included in the tests. The testing of each of the 30 clones with each other one involves $\frac{1}{2}$ (30 × 29) or 435 tests. In each test, 50 to 100 or more individuals of each of the two clones to be tested are placed together in 2 or 3 drops of water and observed for clotting and pairing.

The results of these 435 tests are shown in table 1. As the table shows, the 30 clones (26 derived from *l* × *m*) fall into 4 diverse sex reaction types; there are indeed 4 types among the descendants of the two types *l* × *m*.

The first of these is the *m* type, containing but four clones, including two descended from *l* × *m*.

The second, the *l* type, includes nine clones; among them seven derived from the union of *l* and *m*.

The third, the 67*a*1 type, contains 13 clones, all derived from the union of *l* and *m*.

The fourth, the 1*b*1 type, contains 4 clones descended from *l* × *m*. Thus the 26 exconjugant clones derived from the union of *l* and *m* fall into four reaction types in the proportions 2:7:13:4.

The four types have the peculiar relation to each other that the members of each one clot and conjugate with the members of all the other three. The relations for the four types are shown in table 2, in which the plus sign indicates clotting and pairing in the mixture indicated.

TABLE 2

THE FOUR SEX REACTION TYPES AND THEIR INTERACTIONS, IN THE *l* – *m* GROUP

	l	*m*	67*a*1	1*b*1
l	−	+	+	+
m	+	−	+	+
67*a*1	+	+	−	+
1*b*1	+	+	+	−

Certain points with regard to inheritance may be gleaned from table 1. From the union of two parent types, *l* and *m*, we obtain 4 types; two of which (*m* and 1*b*1) are less abundant than the other two In some cases all four clones descended from a pair are of the same reaction type; this is the case in pairs 67 and 97. In other cases lines derived from the same pair,

and even from the same exconjugant of a pair, belong to different reaction types. This is the case for example with 5*b*1 and 5*b*2, with 88*a*1 and 88*a*2, with 169*a*1 and 169*a*2. A segregation of the sex types thus occurs in some cases at the first division after conjugation, as is true in *P. aurelia* (Sonneborn[1]).

The clones produced from the exconjugants of $l \times m$ differ in many ways besides the differences in their sex types. There are characteristic differences in size, in form, in vigor and particularly in their readiness to react sexually. Some, like the parental clones, react quickly under almost any condition. In others, specially favorable conditions must be supplied, of the nature already mentioned, in order to make possible the characteristic reactions when they are placed with individuals of another type.

A discussion of the relation of these facts to the problem of sex differentiation is reserved until the sex reaction types of numerous stocks collected in nature have been presented.

[1] Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **23**, 378–385 (1937).
[2] Kimball, R. F., *Ibid.*, **23**, 469–474 (1937).
[3] Moewus, F., *Arch. f. Protistenk*, **80**, 467–526 (1933).
[4] Raffel, D., *Biol. Bull.*, **58**, 293–312 (1931).

SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. II. CLONES COLLECTED FROM NATURAL HABITATS

BY. H. S. JENNINGS

ZOÖLOGICAL LABORATORY, JOHNS HOPKINS UNIVERSITY

Communicated February 1, 1938

Twenty-seven clones of *Paramecium bursaria* were collected from diverse localities: four from near Alexandria, Virginia, twenty-three from the neighborhood of Baltimore, Maryland. After allowing the single individuals to multiply in the laboratory, the sex type of each clone was tested, originally by mixing with individuals of the two first-discovered types *l* and *m*, described in the foregoing paper.

The four Virginia clones, *l* and *m*, *B* and *C*, which all came from the same pond, were found to form but two reaction types, *B* being of the same type as *l*, *C* of the same type as *m*.

But unexpectedly, the clones from the region about Baltimore did not react with any of the Virginia types. As shown in the foregoing paper, two additional reaction types were developed from the two that originally occurred in the Virginia group. None of the 23 Maryland clones reacted with