GENETICS OF PARAMECIUM BURSARIA. I. MATING TYPES AND GROUPS, THEIR INTERRELATIONS AND DISTRIBUTION; MATING BEHAVIOR AND SELF STERILITY*

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PARAMECIUM *bursaria* is one of the most widely distributed of the species of Paramecium, though it does not as a rule occur in such large numbers in a given region as does sometimes *Paramecium caudatum.* It occurs commonly amid growing vegetation in the quiet parts of pools, ponds or streams. It does not flourish in regions in which the water is foul or there is much decaying vegetation.

The species is commonly at once recognizable by the algal cells which it contains, which give it a green color. The algal cells are usually packed closely, but at times they are few and scattered or perhaps even nonexistent, so that the animals are nearly white. Such white individuals may readily be produced by allowing the animals to multiply rapidly in a rich culture medium. The ciliates then multiply more rapidly than do the algae, so that the animals are left with almost none of the algal cells.

Figures and descriptions of this species in comparison with others are given by WENRICH (1928), KALMUS (1931), and KAHL (1931). Many diverse biotypes occur, which may differ greatly in form, size and physiological characteristics. The typical form is more flattened, "slipper shaped," than in *Paramecium caudatum* or *aurelia.* The flattened body is slightly curved in a segment of a flat spiral, the broad oral groove extending on one surface from the anterior end to about the middle of the body. The anterior tip projects forward, to the right, and a little downward (toward the oral surface); in very pronounced cases this gives almost the appearance of a hook. Typical forms and sizes for certain biotypes are shown in outline in figure 7 on page 225. As there illustrated, the length ranges from about 100 microns (in the clone l figure 7, 2) to about 160 microns or a little more, in a clone of the type E from California (figure 7, 12).

Paramecium bursaria has been much employed in genetic and physiological investigations. The earlier studies on it are resumed by MAUPAS (1889, pp. 228–238), who himself studied the nuclear processes in conjuga-

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tion. The cytology of conjugation was again extensively studied by HAMBURGER (1904).

CULTURAL CONDITIONS

Paramecium bursaria flourishes and multiplies rapidly in the algal-bacterial culture medium of RAFFEL (1930). Powdered desiccated lettuce, 1.5 grams, is boiled for five minutes in a liter of distilled water; the fluid is filtered and distributed into pyrex flasks containing an excess of $CaCO_3$; the flasks are stoppered with cotton and autoclaved. This constitutes the stock fluid. The culture medium for actual use is prepared from this as follows. To 20 cc of the filtered fluid is added one 1 mm loop of *Flavobacterium brunneum* taken from a three- to five-day old agar slant; also three 2 mm loops of the alga *Stichococcus bacillaris* from an 18-day old agar slant. In this medium the rapidly dividing animals become large and plump. They flourish well in the same fluid diluted to various strengths with water; also in hay infusion. For keeping many clones for long periods, it is convenient to use a dilute hay infusion, made by boiling one gram of timothy hay (*Phleum pratense*) in 800 cc of water for ten minutes. This may advantageously be infected at the beginning with a small quantity of the algal-bacterial medium above described. On becoming established in a vial, finger bowl, or other small vessel in a small quantity of this dilute hay infusion, together with three or four bits of boiled hay an inch or less in length, cultures of the organism may flourish for months, without further attention, except that water lost by evaporation must be supplied at intervals. For the genetic experiments, sometimes more than a thousand such cultures were kept in progress for long periods.

PRINGSHEIM (1915), ERDMANN (1927) and LOEFER (1936) have cultured this species in bacteria-free mediums. Such culture was not attempted for the very numerous stocks required in the present investigation.

AGGLUTINATION AND MATING

The key to the genetics of the species is given by the peculiar relations in mating. Individuals belonging to the same clone usually do not mate ("self sterility"); exceptions to this are dealt with later. Individuals of diverse clones that are not of the same mating type (but yet belong to the same "group"), agglutinate and conjugate when mixed (provided they are mature and in the proper physiological condition) (figure 1).

The phenomena of agglutination or clumping, with later formation of pairs, when diverse mating types are mixed, are spectacular. In the typical case, when many individuals of the two types are placed together in a small amount of water, the individuals are seen to begin at once to become clumped into dense groups. The groups rapidly increase in size, so that in a few minutes clumps containing hundreds of individuals may be produced. Later the clumps begin to disintegrate, and many of the components as they separate from the mass are seen to be united in pairs by their oral surfaces. Such pairs continue union till the individuals have undergone the characteristic changes that accompany conjugation, including the exchange of the pronuclei. Other individuals that emerge from the agglutinated masses are single, and may remain so. The phenomena are similar in their gross features to the agglutination of diverse types of flagellates, as described by MOEWUS (1933) and others. They resemble also

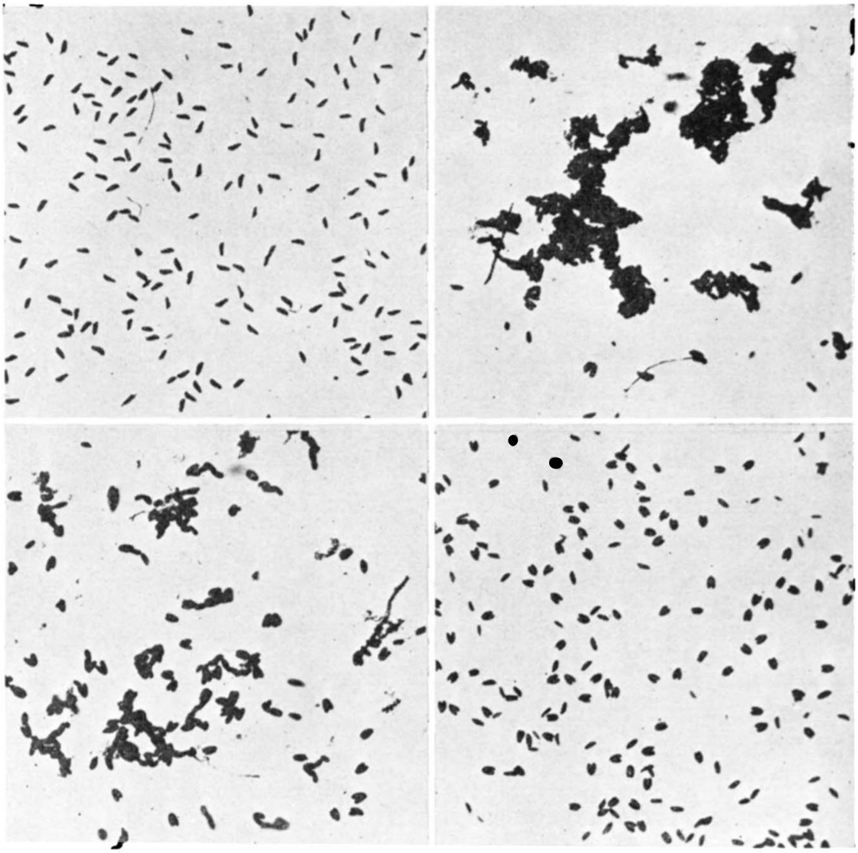

FIGURE 1.—Photographs of the mating behavior in *Paramecium bursaria*.

Upper left, single mating type (C); the individuals scattered singly.

Upper right, mixture of two types (P and Q), six minutes after the two were mixed. The individuals agglutinated in large masses.

Lower left, mixture of two types (P and Q) about five hours later. The large masses have broken down into small masses, chains (center above) and pairs.

Lower right, mixture of two types (P and Q) 24 hours later. Most of the individuals in pairs.

the agglutination of bacteria under certain conditions; and of blood corpuscles in blood mixtures of diverse types.

But in Paramecium the individuals are so large that they may be observed individually, and opportunity is presented for discovering the exact behavior in agglutination and pairing. The phenomena will therefore be described and illustrated in relation to the usual behavior.

BEHAVIOR IN AGGLUTINATION AND MATING

The behavior system in Paramecium bursaria. The individuals when swimming straightforwardly through the water rotate counterclockwise. That is, if the individual is swimming away from the observer, its upper surface rolls toward the observer's left. At times the animal swims without rotating; this is common in some biotypes, not in others. When not progressing steadily through the water, but feeding or exploring or otherwise under the influence of favorable conditions, the individuals may rotate in the opposite direction; that is, clockwise instead of counterclockwise. Such clockwise rotation is not usually continuous, but occurs only for brief periods. In some biotypes such clockwise rotation is common; in others it occurs very rarely.

But creeping along a surface is in this species much more common than swimming freely through the water. The oral surface is then against the substratum, and the animal moves forward without rotating. It commonly follows a course curving somewhat to the right, in the direction of the right anterior point above described.

To the stimuli commonly met in daily life *Paramecium bursaria* reacts in a manner that is essentially similar to the reactions of *Paramecium caudatum* or *aurelia,* as described in the writer's earlier works (JENNINGS 1906). The "negative" reaction consists in a quick turn to the left (when the oral surface is below); this may or may not be accompanied by a brief stoppage of the forward motion. In case of strong stimulation, the animal may swim some distance backward before giving the characteristic left turn, just as happens in other species.

Of special interest in connection with the mating behavior is the reaction to contact with loose and soft objects, such as fragments of bacterial zoogloea. As in other species, the individual brings itself into close contact with such objects, and may then remain quiet or move gently over the surface of the object. Several or many individuals may react in this way to a small shred of bacterial jelly, so that a dense aggregation is formed, superficially resembling the clumps formed in the mating reaction. But in those due to a small soft object, the animals do not adhere together, but move freely with relation to each other, so that such an aggregation is with a little practice quickly distinguishable from the mating agglutination, described below.

This contact reaction may occur upon contact with the bodies of other individuals. In such cases two or more individuals may remain for some time in contact, forming a close group. In such groups the bodies move readily upon each other, there being no adhesion of one to the others, as there is in the strong clumping reaction. This contact reaction is closely bound up with the active coordinated reactions of individuals to each other that form one of the two factors in the mating reaction; this is described in the next paragraphs.

BEHAVIOR IN CLUMPING AND PAIRING

Two diverse factors play roles in bringing the individuals together in conjugation. One is an active coordinated reaction of two (or more) individuals, in which they come in contact by some part of their bodies and thereafter move in a coordinated way. This will be called the "active reaction." The other is in typical cases of clumping much more conspicuous; it is an apparently physical adhesion which quickly produces the spectacular clumping mentioned in earlier paragraphs. This will be designated the "agglutination factor." These two will be described and illustrated separately.

1. *Active reaction of two individuals, resulting in coordinated behavior.* When two individuals come in contact by any part of the body, they may or may not react in a coordinated way. In cases in which they do react, the individuals begin to swim in coordinated motion, such as keeps their bodies (or at least their cilia) in contact. The bodies may shift on each other, changing the regions of contact; they may separate for a short distance and again come together; they may swim together for considerable distances, then separate. Or they may remain together, finally uniting in conjugation. Figure 2 shows some of the characteristic phases of this type of behavior. Frequently the individuals are in contact only by the anterior tip of the oral surface, as at A, figure 2. They then swim as a unit, in a spiral course. In other cases the two individuals are placed almost transversely or in the form of an X, the surfaces of the two oral grooves in contact at the region of crossing; the two then swim together in a spiral course (figure 2, C). Not infrequently the cilia of the entire oral surfaces of the two are in contact, so that the two individuals have the relative positions typical of conjugating pairs, save that there is an obvious space between the two bodies, bridged only by cilia. Individuals in this position may swim for a distance together in a spiral course, then partly or entirely separate (figure 2G, a to d), or the relative position may shift. There is great variation as to the regions of contact of the two individuals, though

in the commonest cases it is some portion of the oral surfaces that are in contact.

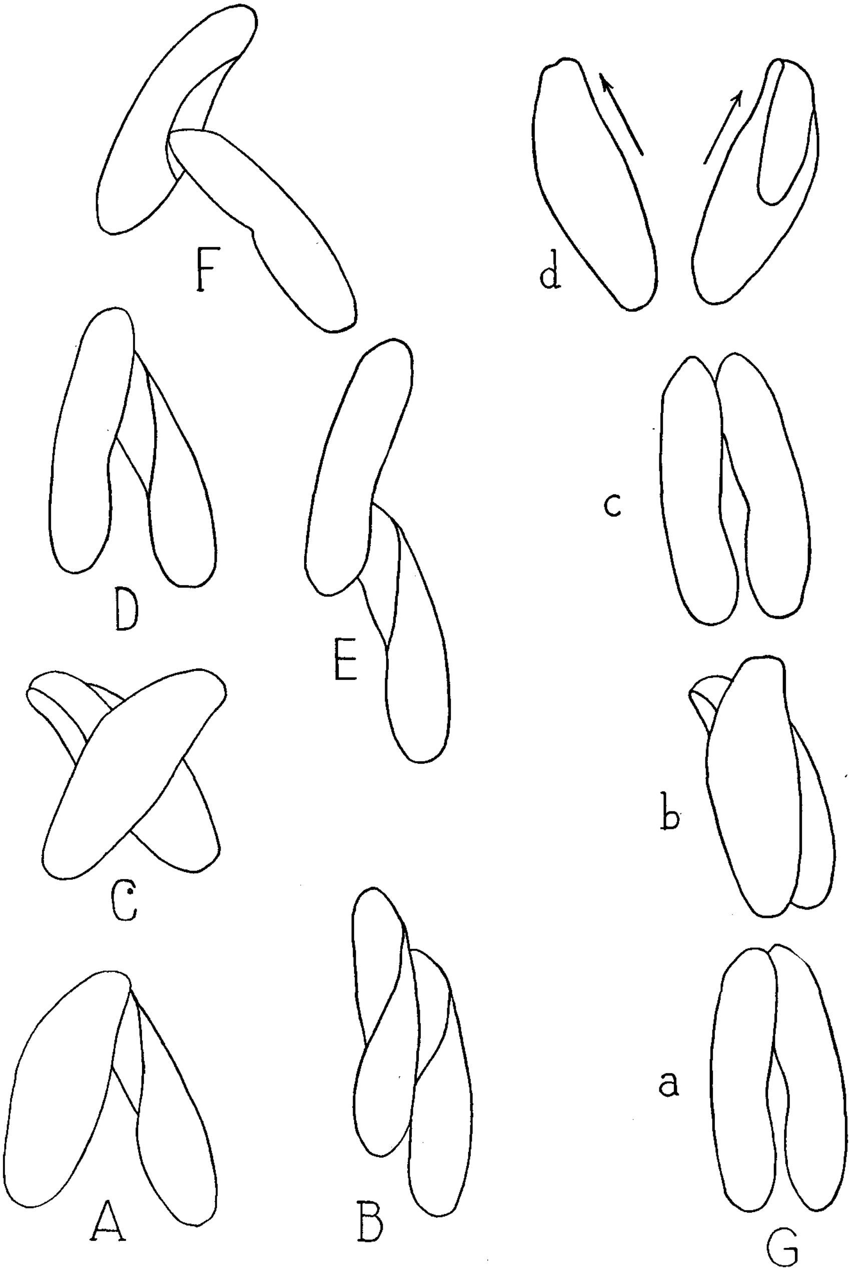

FIGURE 2.—Various positions in the active coordinated movements of two individuals in contact. At G, successive positions are shown (a, b, c, d) in the case of a pair that are in contact by the oral surfaces, swim for some distance in a spiral course, then separate.

In the coordinated spiral swimming of such pairs, it is a singular fact that the rotation is very frequently (but not always) clockwise, though in fully united pairs that are swimming about the rotation is almost always counterclockwise.

A position very commonly observed in such reactions is that shown at F, figure 2. The anterior tip of one individual is inserted into the oral groove at the region of the mouth of the other individual. The two may swim about for some time in these relative positions.

In a type of behavior not infrequently observed, the two bodies are at right angles and in contact at their middle; the two then revolve in such a way that one of the individuals rotates on its long axis, while the other circles about it, as in a spinning top.

In such behavior the relative positions of the two individuals may be varied in an indefinite number of ways; it is frequently shifted during the coordinated motion of the two. It is relatively infrequent that the region of contact for one or the other of the individuals is not a part of the oral surface, as illustrated at B, figure 2. But such cases occur, the motion of the two individuals being coordinated as usual.

Not very infrequently one sees two individuals swimming in a coordinated way, although the distance between them is much greater than can be bridged by the cilia: it may be as great as half the body length or more. Such individuals may swim in a common wide spiral, or one may follow the other, even in an irregular course. In such cases seemingly the water currents must serve as a guide to the movements of the two.

The coordinated behavior just described may occur between individuals of different mating types; it may then lead to closer contact and finally to union by the oral surfaces in typical conjugation. This is frequent when two clones of different type, but only partially mature, are mixed; it may result then in production of a few fully conjugating pairs. But it does not bring about the formation of dense clots, such as occur when mature clones of diverse type are mixed.

It is a remarkable fact that such coordinated reactions and movements are very common in cases in which all the individuals are of the same mating type, so that they do not finally form pairs and conjugate. It may occur when all the individuals are of the same clone, or when two clones belonging to the same (or different) mating types are mixed. It is very common also in immature clones, which do not form conjugating pairs. It must be considered an elemental form of social behavior, which may or may not be connected with the mating process.

Behavior of this general type was early observed and aroused much interest and discussion; see the summary in MAUPAS (1889, pp. 412–414). In the case of Onychodromus, MAUPAS observed that such behavior may occur between individuals that never fully unite in conjugation (1889, p. 300).

2. *Agglutination Factor*. The agglutination into large clots containing great numbers of individuals, which occurs when mature clones belonging to diverse types are mixed, is due to an additional factor; an actual physical adhesion of the bodies. This will best appear from a description of what occurs when such mixtures are made.

When mature individuals of different mating type (but of the same "group") are mixed together in large numbers, it is to be observed that the individuals are not pulled together or attracted from a distance. They continue to swim about in the same way as in unmixed groups. The swimming is no more rapid or "excited" than when there is no mixture. But the usual movements in varied directions of many individuals in a small quantity of fluid soon brings some of them into accidental contact. An individual of one of the types brushes against one belonging to the other type. Thereupon the two stick together exactly as if their surfaces were covered with some strong adhesive material. There is usually no definite reaction; each of the two individuals tries (as it were) to continue moving as before, but as they stick together, both are suddenly stopped, or one drags the other against the motion of the latter's cilia; or if the two anterior ends happen to lie in the same direction, the two swim forward together.

Any parts of the body that thus come in contact adhere. The two individuals may come in contact by their aboral surfaces, or their rear ends, or in any irregular way; thereupon they adhere, and begin to move in an irregular way resultant upon the divergent action of their free cilia. Figures 3 and 4 show a number of observed cases of such irregular adherence. Often one individual drags another backward or sidewise through the water.

It is the cilia that thus adhere together; at least this is the case at first. Often one may at the beginning see a definite open space between the two bodies, spanned only by the cilia, which are immobilized where in contact. Later the bodies may come into actual contact by their outer surfaces.

Two individuals thus stuck irregularly together flounder about in the crowded drop, and soon come into contact with other individuals of one or the other type. A third individual adheres irregularly to the two, then a fourth, and this continues until large masses are formed, containing twenty to a hundred individuals or more. In these the individuals are in irregular contact, by any parts of the body. In all this it is evident that the coming together of the individuals is the accidental result of their ordinary motions; they adhere when thus accidentally brought together.

Until the individuals come in contact there is no indication of stimulation or of a change in the behavior.

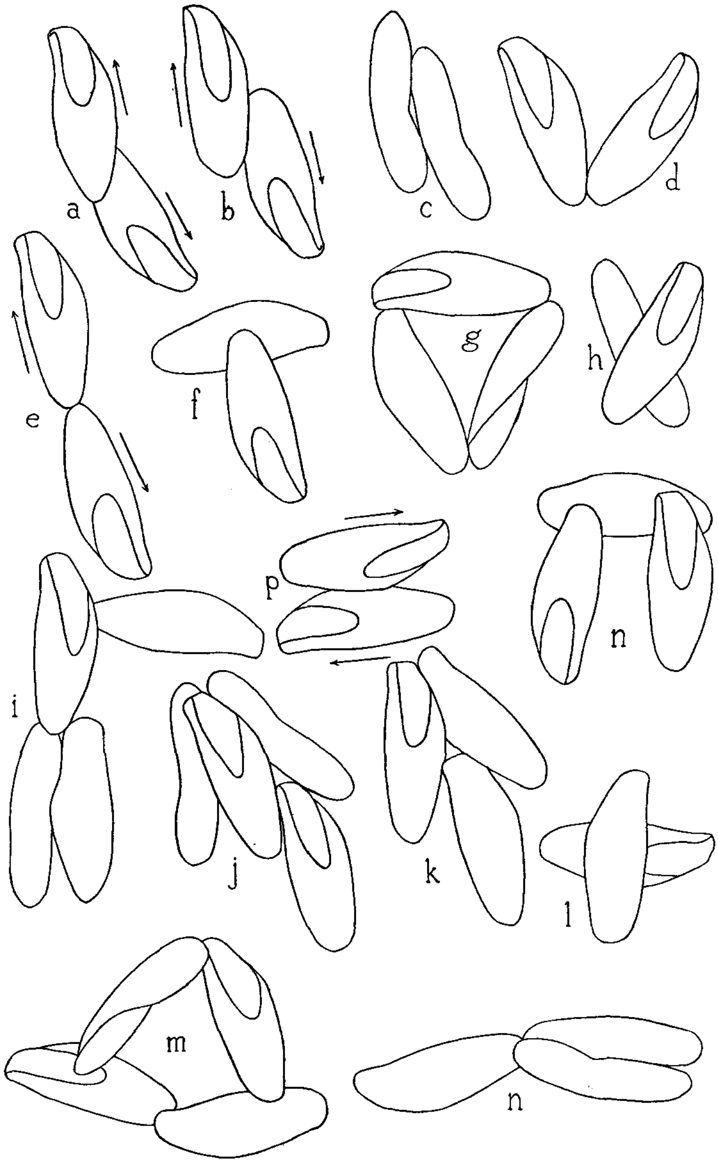

FIGURE 3.—Characteristic small groups formed by the adhesion of individuals in the clumping that precedes conjugation. The arrows present in certain figures show the direction in which the individual's cilia tend to carry it.

By bringing a single individual of one mating type into a collection of many individuals of another type, it may be seen that at times one such individual becomes the center of a group of five or six or more of the other type, each of the latter adhering to it by some small part of the body surface. In all cases the adhesion is between two individuals belonging to different types; members of the same type do not adhere together. Thus the large clumps contain approximately equal numbers of individuals of the two types. In the mixtures containing many individuals of both types, small groups are at first formed with all sorts of irregular attachments. Some sketches of small groups are shown in figure 3.

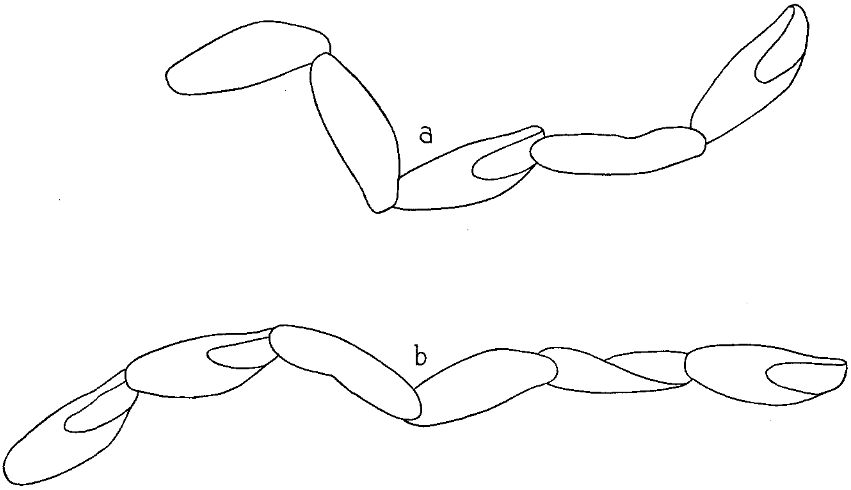

FIGURE 4.—Chains of individuals adhering together at their tips, in a late stage of the agglutination that leads to conjugation.

Within three or four minutes large masses of irregularly adhering individuals are formed. They commonly remain united for about half an hour, then begin gradually to break up into smaller masses. Yet (unless evening is coming on) masses of considerable size may thus remain united for two hours or more. As the masses break up many longitudinal chains are found to have been formed, the individuals united end to end (figure 4), individuals of the two types alternating. In such chains most (but not always all) of the individuals are oriented in the same direction, the anterior end of one individual overlapping slightly the posterior end of the one in front of it. Usually if not always it is now the anterior part of the oral groove that adheres to the side of the posterior part of the individual in front; it may adhere to either the oral or aboral surface or to either lateral surface of this posterior part. At times, such chains have one or more individuals reversed, so that these latter are dragged about with posterior end forward. In other cases two or three individuals are attached irregularly to the sides of the chain. Such chains of 5 to 20 or more individuals swim about in a snake-like manner, most or all of the individuals being oriented with anterior ends in the same direction, so that their motion is coordinated. At times the chains are zigzag in form.

In the clots above described the individuals adhere firmly together by whatever parts of the bodies are in contact. They do not move freely with relation to each other, as occurs in the groups described earlier, that result from the actively coordinated contact reactions. In all the clumping process the coordinated contact reaction plays little or no part, the main or exclusive factor being the rigid physical adhesion of individuals of diverse type that are in contact.

In immature or partly mature clones however this agglutination factor may be nearly or quite lacking; there is no physical adhesion of individuals that come in contact. In such cases scattered pairs may be formed seemingly as a consequence of the active coordinated contact reaction only (as before mentioned). In partially mature clones the physical adhesion becomes slightly developed, so that cases are seen in which two individuals react in the coordinated way earlier described, but if they try to swim apart they are found to adhere slightly. The physical adhesion factor increases as the clone grows older, so that at certain periods mating appears due to a nearly equal mixture of the coordinated contact reactions and the physical adhesion. When the clone becomes fully mature, in typical cases the clumping and mating appear due almost exclusively to the physical adhesion.

Yet even in the tightly adhering groups formed by agglutination the individuals may shift to some extent, moving slowly and with seeming difficulty. In this way the relative positions become slowly changed and some of the individuals come in contact by their oral surfaces; in time they come into the typical mating position. This is a slow process, requiring usually an hour or more, so that the exact method by which the final position is attained is difficult to detect. At first the pairs are united only by the oral cilia, so that there may be a space between the bodies. Later they come closer, until they appear to be fused; they then swim about as a single body, rotating counterclockwise.

Not all the individuals that are incorporated in the agglutinated masses finally become united in pairs. After some hours the masses are quite disintegrated, and it is found that many single individuals, as well as many pairs have separated from them. Nothing now remains but pairs and single individuals. As described below, toward evening the tendency to adhere weakens and then fades away completely, so that only those individuals that have become fused to pairs remain in union.

As set forth in my preliminary communication (JENNINGS 1938), it can readily be demonstrated that the two members of any pair belong to the two diverse types that have been mixed. Before mixture one of the types is induced to multiply rapidly, so that the algal cells which each individual contains are few, the individuals are therefore white, like *Paramecium aurelia*. The other type is allowed to remain of the typical deep green. When the two are mixed, the pairs formed always consist of one green individual, one white one.

The clotting and pairing take place in the commoner Groups I and II most strongly at about midday and for three or four hours after; but in Group III they may occur at any hour of the day or night. In the former two groups the tendency to clot when mixtures are made begins at about 8 a.m., becomes more marked as the day progresses, then at 4.30 or 5.00 p.m. begins to weaken; it may have completely disappeared by 6.30. In mixtures made in the evening from 7.00 on there is in these groups no clumping and pairing. A detailed investigation of the time relations and the relations to light and darkness is in progress.

In mixtures containing very large numbers of the two mating types, there is usually during the first day a strong clumping, and many pairs are formed. In some cases however there is hardly any clumping during the first day; this is probably the result of marked differences in the fluids in which the two sets were living. In such cases the first marked reaction comes the second day. In the evening the clotted masses have separated, many individuals being left single. The next day there is a renewed agglutination, with formation of additional pairs. On the third day there is again agglutination and pairing among the left-overs of the previous day. In some cases the reaction by this time has gone as far as it will, so that the next day there is no more clotting. But if the numbers are large, agglutination may be repeated on later days. In a certain case large numbers of two types of Group I were mixed in a Columbia dish on June 7. Agglutination and later dispersal occurred daily until June 17, and a few cases of adhesion of individuals occurred for two or three days longer.

The account of agglutination and the mating behavior just given is typical for mature stocks, including most clones derived from individuals collected under natural conditions. There are however some such clones that react in a much less pronounced manner. In some clones but a few individuals out of many show the tendency to agglutinate and pair. In these cases only small clots are formed, of two to five or six individuals; and later only a few pairs are found. In the strength of the tendency to agglutinate and pair many different grades are found among different clones collected in nature, from an entire absence to the typical strong tendency that results in the dense aggregations that are typical. In some clones, pairing occurs seemingly quite without agglutination, simply through the active contact reaction described in earlier paragraphs.

Some of the individuals collected in nature yield clones that for long periods do not agglutinate or pair, but later these mating reactions begin and progressively become stronger. Thus, five clones collected in July 1937, although frequently tested, gave no clotting or mating reactions until March 1938, when scattered unions occurred on mixture with typical clones. The clotting and pairing gradually increased until it became possible to assign the five clones to certain mating types of Group II. Other clones have been for months in the laboratory, but have as yet shown no sign of the clotting or mating reactions, when mixed with other clones, although they may, whether mixed with others or not, show the active coordinated contact reactions earlier described. It seems probable that such clones are in most or all cases immature (see the account of immaturity in a later section).

LENGTH OF TIME OF UNION

After the individuals have become united in pairs, union continues for from 24 to 48 hours. The time of union and separation was observed for 12 pairs belonging to Group I. The total time during which the two individuals remained united was as follows: one pair remained in union for 26 hours; six pairs for 28 to 31 hours; five pairs for 44 to 47 hours. These periods are in good agreement with the observations of MAUPAS (1889, p. 229), who found that at 20 degrees Centigrade the period of union was 36 hours. The fact that the union continues for so long a time is convenient for many purposes in the study of conjugation, since if a test mixture of types is inspected once in 24 hours all pairs formed may be observed.

CONDITIONS REQUIRED FOR CLOTTING AND CONJUGATION

Under usual conditions most clones derived from individuals collected in nature clot and pair in the typical manner described above, when mixed with others of a different mating type (but belonging to the same group). Certain conditions however may interfere with or prevent these mating reactions; some of these are intrinsic, others extrinsic. They may be classified as follows:

1. *Immaturity.*—For a varying period of considerable length after conjugation, the descendants of ex-conjugants neither agglutinate nor pair when mixed with other types. There is a period of immaturity during which the mating reaction does not occur. The tendency to react, when it first appears, is extremely weak and sporadic; so that in a mixture with a typical clone of a different type only a few scattered individuals react and pair. The first pairs are in some cases seemingly formed without any participation of the agglutinating factor, through the active contact reaction only. In other cases single individuals are seen to adhere in the agglutina-

tion reaction to single individuals of the other type. As the days or weeks or months pass a larger proportion of the individuals of the clone are seen to react, so that several or many pairs may be found in a mixture. Finally the typical agglutination and pairing phenomena are observed. Sometimes it is months from the appearance of isolated pairs till the time when all react strongly. During this period of partial immaturity the occurrence of agglutination and pairing depends in a high degree on the conditions; unless the most favorable conditions are supplied no pairing occurs.

The period of complete immaturity, when no mating reaction at all occurs, varies from two or three weeks up to many months. The shortest period thus far observed from conjugation to the beginning of the mating reaction was 12 days, in the case of a clone descended from a mating of the Types A and C of Group I; at this time but few individuals reacted, and these but faintly. In 25 other clones descended from diverse ex-conjugants of the same mating there was no mating reaction at this time; their reactions began later. Many clones descended from conjugations that occurred in Group II in April and May, 1938, are still without mating reactions in November of that year. The different clones descended from the ex-conjugants of the same epidemic of conjugation, between the same two parental clones, show very great diversities in the time when the mating reactions begin, and in the time when the reaction reaches its full strength.

The usual long period of immaturity, complete or partial, is inconvenient for genetic experimentation. Young clones produced by a given mating must be cultivated in isolation for long periods before the reaction type to which they belong can be determined.

On the other hand the definiteness of the reactions when mature clones are mixed presents a remarkably favorable opportunity for the study of the different periods of the life history, so much insisted on by MAUPAS (1889), CALKINS (1935) and others: youth, maturity, age. Detailed studies on these matters are in progress and will be presented later.

2. *Nutritive condition.*—Clotting and mating do not occur when individuals to be mixed are taken from a rich nutrient medium in which they are plump and rapidly dividing; nor, usually, when the mixture is made in such a medium. In general, plumpness of body tends to prevent the mating reaction. Starvation is not necessary; clotting and mating occur readily in well-fed well-grown individuals, provided their plumpness is not excessive.

Mature strong clones show little dependence on nutritive condition; they clot and mate whenever mixed, unless excessively over-fed and plump. But immature or refractory clones are extremely sensitive to the nutrient condition. The most favorable conditions for the mating reaction in such cases are found when the nutritive conditions are declining from a rich condition to a moderate or poor one. Such clones do not react either when plump, or when kept for some time without food. In practice, to induce reactions in immature or refractory clones, the following procedure is helpful. The individuals from the clone to be tested are mixed with mature individuals of known type in two or three drops of pure algal nutrient medium described in earlier paragraphs. This induces multiplication, but there is no pairing for several days. After three, four or five days however the nutrient medium is becoming exhausted, the individuals are declining in size, and now mating may occur. By this means mating may be often induced in clones that otherwise do not show it.

But very great differences in the effects of nutritive conditions on agglutination and mating are seen among the different young clones derived from ex-conjugants of a given mating. Some such clones have been observed in which addition of the strong nutrient medium to the mixture brought about the formation of pairs within 24 hours, while the animals were still plump from the increased food.

3. *Time of day.*—As before set forth, the mating reactions occur only at certain periods of the day; usually not at all in the evening after six or seven o'clock, nor in the morning from five to eight or nine o'clock. The most favorable time is for most types from about twelve noon to four p.m. The most favorable period for reaction differs in different groups and clones. A detailed study of this matter will be presented later.

4. *Shock of change from one fluid to another.*—It is of course easy to prevent the mating reactions by mixing the animals in fluids that are irritating or injurious. The common accidental differences between cultures of diverse clones often greatly delay the reactions when clones of different types are mixed. In such cases, when the two clones are mixed, clotting and mating may not occur until the next day, coming on in the favorable period of that day; it may then be as marked as when it occurs at once. In many cases however even marked differences of culture medium produce no interference with the reaction; the tendency to clot is so strong that it at once overcomes such diversities.

DIVERSE MATING TYPES; AND DIVERSE GROUPS OF TYPES

As mentioned earlier, the members of a single clone (all derived by fission from a single individual) do not clot nor mate together, under ordinary conditions. The single clone is self-sterile, in the same sense as certain plants are self-sterile (East 1929), and as the animal Ciona is self-sterile (Morgan 1938). The self-sterility is not complete, and as in other organisms, the usual term "self-sterility" does not accurately characterize the phenomena, since there is likewise cross-sterility of certain diverse clones.

If members of a single clone are mixed with members of another clone, clotting and conjugation may occur, in the manner described above. (For brevity, the complex of phenomena will be called the mating reaction.) But a given clone does not react with members of all other clones; with some other clones reaction is lacking. This selective reaction with certain clones and failure to react with others is found to include two diverse sets of phenomena; those leading on the one hand to the distinction of diverse mating types; and those leading on the other hand to the distinction of different groups of types. It will be convenient first to present the phenomena in the case of a single group.

If individuals are collected from a certain pond and by the isolated culture of single individuals a large number of clones are obtained, the following phenomena are observed. Members of the same clone do not react together. Members of a given clone react with those of certain other clones, while with still others they do not react. Two clones whose members do not react together may be said to belong to the same mating type, since their members have in this respect the same relation to each other as have the members of a single clone. On the other hand two clones whose members react together when mixed belong to diverse mating types.

By making all possible mixtures among the different clones, it is found that a number of diverse mating types exist. Clones of type A react with clones of type B, of type C, of type D, and so on. Similarly, clones of type B react not only with A, but also with C, D, and so on.

Extensive study shows that the number of diverse mating types is limited. In collections from a pond in the neighborhood of Baltimore, clones were found that belonged to five diverse mating types, which were designated as follows (from the designations of typical clones of each type): Fb, Fd, Fl, Fo and S. The relation between these types is this: members of any single type, as Fb, will not react with members of that same type, but will react with members of all the other types (with Fd, Fl, Fo, and S). Each type includes many different clones that do not react together. These five diverse types are members of what was called in my preliminary paper (1938) the Maryland Group, or Group II; the same designation is here used.

Since that paper was published, three more mating types belonging to this Group II have been discovered; these were designated from the typical clones first found as Gr6 and Gr14 (from collections made in North Carolina) and McD3, from a collection made near the site of the McDonough School near Baltimore. Thus this Group II ("Maryland Group") is now known to contain eight diverse types, of such relations that the individuals of any one type will react with those of any of the other seven types, but not with members of its own type. Tests of a very great number of clones of this group, collected in nature or bred in the laboratory have shown that all belong to one or another of these 8 mating types, so that there is some reason to believe that Group II consists of just 8 mating types and that no others will be found belonging to this group.

But the members of certain other clones do not react with any of the eight types of Group II, yet do react with certain clones. These clones thất react together constitute another group, with a number of different mating types, members of which react together, but not with any of the types (or clones) belonging to Group II. In my preliminary papers (1938) I described such a group, known as Group I, or the "Virginia Group." This group contained four diverse mating types, designated (from typical clones) as l, m, 67a1, and 1b1. When two types of this group are mated, they yield among their offspring the above four types and no more. Thus there is ground for believing that there are but these four types in Group I.

Since my preliminary papers were published another group, Group III, has been found, originally in material sent me by DR. COLEEN FOWLER, from collections made between Greensboro and Pineville, North Carolina. This Group III is known to contain (like Group I) four diverse types which were originally designated (from clones representing them) as Gr1, Gr2, Gr11, and Pi13. Any type of this group will react with any other type of the same group, but will not react with any of the types belonging to Group I or Group II. This Group III has thus far been studied less than the other two groups. Possibly other types belonging to it will be found, though it seems not improbable that, like Group I, it consists of just four types.

Any clone that is found to react (by agglutination and mating) with any type belonging to a particular group (as Group I) is invariably found to react with all the other types belonging to that Group, *except one*—this one constituting the type to which the clone under examination belongs. Furthermore, after a clone has been found to react with members of a given group (as Group I), it is found that it does not react with any of the clones of the other two groups.

After the three diverse groups, each with several types, had been found in the eastern part of the United States, the author anticipated that collections made in distant regions would show still other groups. But collections sent me from California (from a pond on the grounds of Stanford University) contained only individuals belonging to the commonest type E (Fd) of the large Group II, which is abundant in Maryland and other states of the Atlantic region. Again the four types of Group III were originally found in North Carolina, and for a long time were not known to occur in other regions. But in the summer of 1938 collections from Provincetown, at the tip of Cape Cod, Massachusetts, were found to consist entirely of one of the types (P) of Group III. Some of the groups are therefore certainly widely distributed. It is possible however that other groups in addition to the three now known will be discovered on fuller investigation.

So far as at present known, therefore, the species *Paramecium bursaria* consists of three groups, the members of any one of which do not mate with members of the other two. Groups I and III contain each four diverse mating types, while Group II contains eight mating types, so that there are in all sixteen mating types. The constitution of the species, so far as now known, may be represented as in the adjoining figure 5.

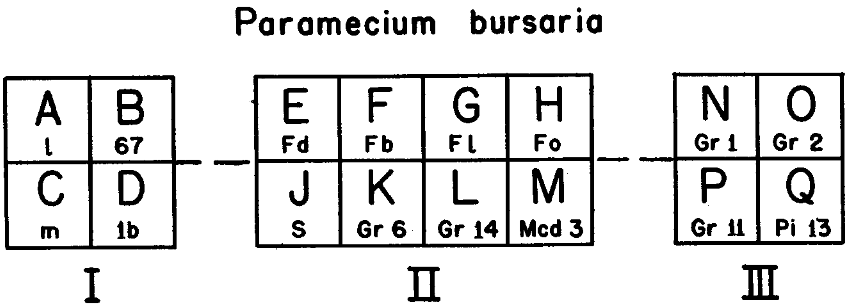

FIGURE 5.—Constitution of the species *Paramecium bursaria*. Three groups, I, II, III, that do not intercross; sixteen mating types A to Q; four in Group I, eight in Group II, four in Group III. The small letters or figures in the lower half of the squares are the designations employed in the investigation, from those of the clones that first exemplified each type.

The interactions of the 16 diverse types belonging to the three groups are shown in the table of figure 6. In this table the plus sign indicates that agglutination and conjugation occur, with formation of fertile ex-conjugants; the minus sign that they do not.

It will be convenient to designate (as in figure 5) the 16 different mating types of the three groups by the first 16 capital letters of the alphabet (excluding the letter I as ambiguous); that is, by the letters A to Q. The four types A to D constitute Group I; the eight types E to M, Group II; the four types N to Q form Group III. In figure 5 there are given also for convenience the more cumbersome designations that have been employed in the practice of the investigation; these are the designations of the clones that were first found to exemplify each type (as l, 67, Fd, Fb, etc.).

OCCURRENCE AND DISTRIBUTION OF THE TYPES AND GROUPS

When collections are brought into the laboratory, the following is the procedure in determining the groups and types represented. A considerable number of single individuals are isolated from the collections and cultivated in isolation till abundant clones are obtained from each. In the laboratory are kept cultures of the 16 known diverse reaction types—the four of Group I, the eight of Group II, the four of Group III; these may be known as testers.

The necessary tests are carried out on slides each of which carries two depressions. For the preliminary test three slides, with their six depressions, are required. From the clone to be tested a large number of individuals (50 to 100 or more) are placed in each of the six depressions.

Paramecium bursaria

		I				II								III			
		A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q
I	A	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	B	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	C	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
	D	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
II	E	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−
	F	−	−	−	−	+	−	+	+	+	+	+	+	−	−	−	−
	G	−	−	−	−	+	+	−	+	+	+	+	+	−	−	−	−
	H	−	−	−	−	+	+	+	−	+	+	+	+	−	−	−	−
	J	−	−	−	−	+	+	+	+	−	+	+	+	−	−	−	−
	K	−	−	−	−	+	+	+	+	+	−	+	+	−	−	−	−
	L	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	−
	M	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−
III	N	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	O	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	P	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
	Q	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−

FIGURE 6.—Table of the interactions of the 16 mating types A to Q of the three groups of *Paramecium bursaria*. The plus signs indicate that clumping and conjugation occur between the two types indicated; the minus signs that they do not.

Then to the first two depressions are added numerous individuals of two of the types of Group I (one of the types to each depression); to the third and fourth depressions are added individuals of two of the types of Group II; to the fifth and sixth depressions, two of the types of Group III. Thus each of the six depressions contains a mixture of individuals of the clone to be tested with individuals of one of the types.

The six mixtures are then examined under the binocular. The clone to be tested will as a rule be found to clot and pair with one or both of the types of one of the groups; it is thereby shown to belong to that group. If the new clone belongs to a certain group (as Group I), it is certain to react (if in proper condition) with at least one of the two tester clones of that group. For every clone belonging to a certain group reacts with all the types of that group except its own type. In this way it is discovered to which group the clone belongs. Suppose that it is thus found to belong to Group II. It is next necessary to test it, in the same manner, with each of the six remaining types of that group. It is invariably found that the new clone reacts with seven of the eight types of Group II, but not with the eighth. The one with which it does not react is the type to which it belongs. In a similar way, if it is found to belong to Group I or Group III, the type to which it belongs is discovered by testing with the remaining members of that group. The results of the tests are in typical cases sharply defined; either there is the spectacular clotting and pair formation described in earlier pages, or there is no reaction. The type to which the clone belongs is absolutely clear. In rare cases however, some of the clones are refractory; they do not form pairs with any of the 16 diverse types. If such clones are retested at intervals of weeks or months, some of them develop the capability of reacting, and the type and group to which they belong may be determined.

By the use of these methods the occurrence and distribution of the different types and groups were determined as shown in the following list. The collections were partly made by the author; for other collections from various parts of the United States I am greatly indebted to the following: DR. COLEEN FOWLER, DR. ZDENKA HURIANEK, DR. ELIZABETH S. KIRKWOOD, MISS AMICIA MELLAND, DR. KATHRYN STEIN, MR. N. FINKELSTEIN, DR. JOHN FRISCH, DR. A. C. GIESE, DR. N. HIGGINBOTHAM, DR. RICHARD KIMBALL, DR. AUSTIN PHELPS, DR. T. M. SONNEBORN, MR. M. A. WESSEL.

Group I. Four Types, A, B, C, D (or l, 67, m and 1b)

Types A and C were first collected from a pond at Alexandria, Virginia, as clones l and m, by DR. T. M. SONNEBORN. Later by the crossing of these two were produced types B and D (as clones 67 and 1b). Still later all four were collected from various natural habitats, as follows:

Type A. Two clones (l) from ponds at Alexandria, Va.; five from pools near Hebbville, Md.; three from a pond in Loudon Park Cemetery, Baltimore. Produced many times by crossing.

Type B. First produced (as clone 67) from a cross of Type A and Type C. One clone found in Loudon Park Cemetery, Baltimore; one from a pool near Texas, Md.; one from Jones' Falls at Dickeyville, Md.; one from pool at Hebbville, Md.

Type C. One clone (m) from a pond at Alexandria, Va.; one from

Staunton, Va.; two from pond at Loudon Park Cemetery, Baltimore. Produced many times by crossing other types.

Type D. First observed as a clone (1b) from a cross of types A and C. Seven clones collected from pool in Loudon Park Cemetery, Baltimore; six clones from pools near Hebbville, Md.; six from pool near Westminster, Md.

Thus the four types of Group I have been collected as 37 clones, from regions thus far limited to Maryland and Virginia. The 37 collected clones were distributed among the four types as follows: 10A, 4B, 4C, 19D.

The distribution of types in collections from particular localities is illustrated in the following:

Pool at Westminster, Md., six clones, all Type D.

Pool at Hebbville, Md., April 2, 1938; 12 clones: 5A, 1B, 6D. A later collection from the same pools gave clones that all belonged to Group II.

Pool in Loudon Park Cemetery, Baltimore; 13 clones: 3A, 1B, 2C, 7D.

Pool at Staunton, Va.; 23 clones, 1C; the other 22 belonging to Group II.

It appears probable that farther collections will show a much wider distribution of Group I.

Group II. Eight Types, E to M

Type E (or Fd). 45 clones from pools and ponds in the region about Baltimore; six from Hope Valley, Rhode Island; one from Compton, Rhode Island; four from Staunton, Virginia; 76 from a pool on the campus of Stanford University, California. In all, 132 clones collected from natural habitats.

Type F (or Fb). 14 clones from ponds and pools in the region of Baltimore; one from a stream in the southern part of North Carolina; two from Hope Valley, Rhode Island; 12 from ponds at East Aurora, New York; three from pond at New Haven, Conn. In all 32 clones.

Type G (or Fl). Five clones from about Baltimore; four from Staunton, Virginia; one from near Greensboro, North Carolina; one from Hope Valley, Rhode Island; two from Miller's Pond, Montville, Connecticut; 12 from East Aurora, New York. In all, 25.

Type H (or Fo). Seven clones from about Baltimore; three from Staunton, Virginia; three from pool in the plant house of Mt. Holyoke College, South Hadley, Mass.; three from Miller's Pond, Montville, Connecticut; seven from a pond at New Haven, Conn. In all, 23.

Type J (or S). 15 clones from about Baltimore; 11 from Staunton, Virginia. In all, 26.

Type K (or Gr6). 11 clones from about Baltimore; three from south of Greensboro, North Carolina; three from Miller's Pond, Montville, Rhode Island; one from the Cedar Swamp, Woods Hole, Mass. In all, 18.

Type L (or Gr14). Eight clones from about Baltimore; one from south of Greensboro, North Carolina; 12 from the Greenhouse at Columbia University, New York; one from Hope Valley, Rhode Island; one from Miller's Pond, Montville, Conn.; seven from New Haven, Conn. In all, 30.

Type M (or McD3). Two clones from about Baltimore; two from Hope Valley, Rhode Island; one from Compton, Rhode Island; four from New Haven, Conn. In all, 9.

As the above list shows, the eight types of Group II are very widely distributed, ranging from Cape Cod, Mass., in the east to California in the west, from East Aurora, New York, in the north to the southern part of North Carolina in the south. In the collections thus far made some of the types occur much more frequently than others. Of Type E (Fd) 132 clones from many localities have been found; of Type M (McD3) but nine. In a collection from a quarry hole at Texas, Maryland (near Baltimore), all the eight types were found to be present. Possibly fuller investigation would show that it is not uncommon for this to occur.

Group III. Four Types, N to Q

Type N (or Gr1). Three clones from a stream 50 miles south of Greensboro, North Carolina; four from a stream not far from Pineville, North Carolina.

Type O (or Gr2). Two clones from 50 miles south of Greensboro; four clones near Pineville—both in North Carolina.

Type P (or Gr11). One clone from 50 miles south of Greensboro, North Carolina; 14 from Provincetown, Cape Cod, Massachusetts.

Type Q (or Pi13). Three clones from near Pineville, North Carolina.

INDEPENDENCE OF THE THREE GROUPS; DISTINGUISHING CHARACTERISTICS

The three groups remain independent, not intercrossing, so far as my observations and experiments go. Clones that react strongly invariably clot and form pairs with members of one of the three groups, not with those from the others. If such strong clones belonging to two diverse groups are mixed, there is no clotting nor pair formation.

When, however, members of immature or refractory clones are tested with members of different groups, in very rare cases single pairs are formed in mixtures with members of more than one group; that is, isolated pairs are rarely formed with members of a group to which the immature or refractory clone does not belong. On repetition of the mixtures, no additional pairs are obtained; the pair found remains a unique example. Such pairs have been observed in but half a dozen out of hundreds of mixtures made. In two or three cases attempts were made to cultivate the ex-conjugants of such pairs, but without success. As set forth later, self-fertilization of clones occurs in very rare cases; it remains possible therefore that these isolated pairs were not crosses, but the result of self-fertilization in one of the two clones mixed. The matter requires thorough investigation, which will be difficult, owing to the extreme rarity of the occurrence of such pairs.

Are the three groups distinguishable by other characters than their mating reactions? As has already been mentioned, Group III differs from the other two groups in the fact that in Group III the mating reactions may occur at any time of day or night, while in Groups I and II they occur mainly or exclusively in the day time, particularly in the afternoon. There are indications also that the cultural conditions required in Group III differ slightly from those in Groups I and II. To determine whether there are structural and morphological differences between the groups will require more detailed studies than have yet been made. Such studies are in progress, the cytological comparisons being undertaken by DR. T. T. CHEN. All the groups, however, have the general form, structure and size that is characteristic of *Paramecium bursaria;* all have the type of micronucleus known for this species, and all contain the characteristic green algal cells.

When particular clones belonging to diverse groups are compared, often there are marked differences in size, form, or other respects, between them. But such differences are usually merely characteristic for the particular clones examined, and are not general for the two groups. This matter is taken up in connection with the question of diversity of the mating types, in the next section.

CLONAL DIVERSITY IN RELATION TO DIVERSITY OF MATING TYPES

Different clones in any of the groups differ greatly in size and form of the individuals as well as in physiological characteristics. The size, form, and physiological peculiarities of a clone remain characteristic of it for long periods; they are transmitted in multiplication by fission. Such characteristic differences in size and shape are shown in the outlines of typical individuals of certain clones, all shown to the same scale in figure 7. As the figure shows, clones very diverse in these ways may belong to the same mating type or to diverse mating types (the type as well as the designation of the clone is indicated in the description of figure 7). Observe the marked differences in size and form of numbers 1 and 2 of figure 7, both belonging to type A; of 11 and 12, belonging to type E, and in other cases. The situation observed is that which would exist if mating type, size, shape, and other peculiarities depend on diverse factors distributed independently. If there are any characteristics that go unvaryingly with

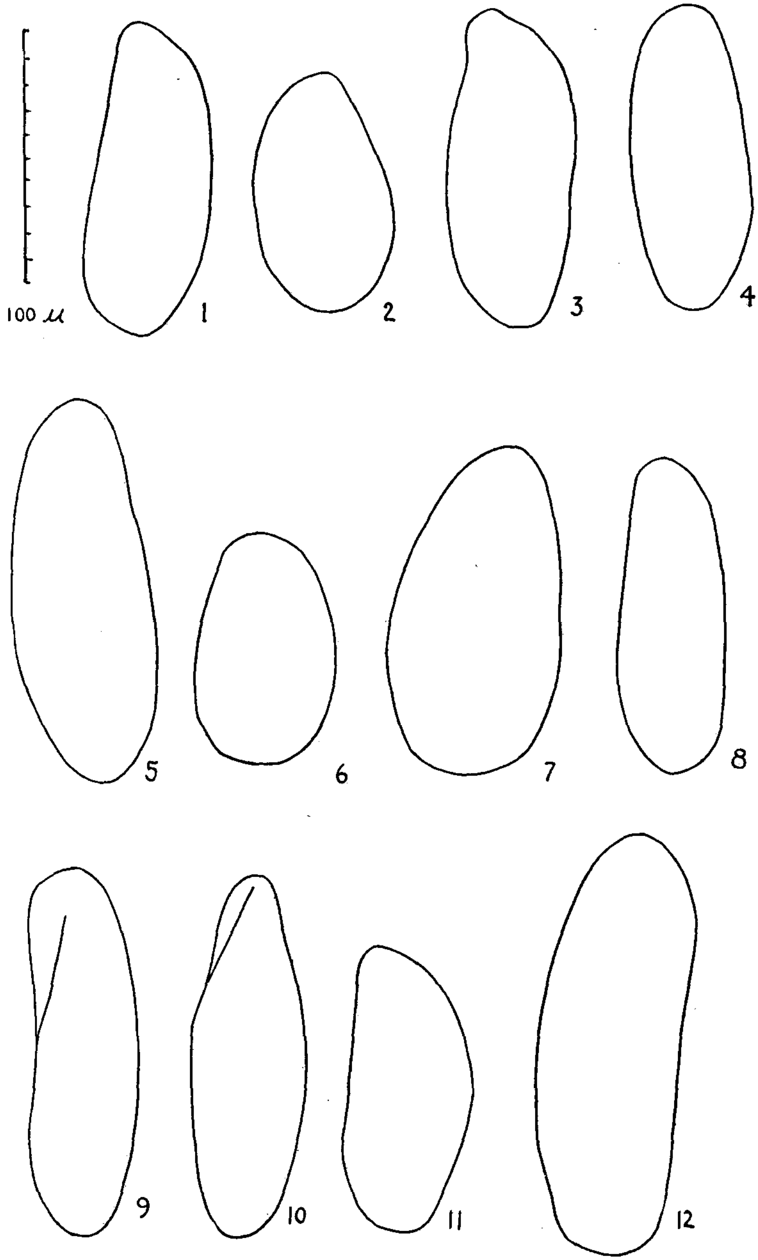

FIGURE 7.—Outlines of typical adult individuals of diverse biotypes of *Paramecium bursaria*, all under approximately the same conditions. Camera figures, all drawn to the same scale.

1, Type A, clone Lo2. 2, Type A, clone l. 3, Type C, clone m. 4 and 5, Type B, clone 67. 6 and 7, Type D, clone 1b. 8, Type D, clone Lo1. 9 and 10, Type M, clone McD3. 11, Type E, clone Fd. 12, Type E, clones from Stanford University, California.

a particular mating type or a particular group, these are so slightly marked as compared with great clonal differences that are independent of type and group, that they have not yet been detected. A great variety of combinations of morphological and physiological characters may be found in any mating type or group.

PERMANENCE OF THE MATING TYPES

Any single clone kept in mass culture in the laboratory and multiplying vegetatively, appears to retain indefinitely its characteristic mating type. Clones l and m, of types A and C, respectively, were brought into the laboratory in April 1937; they are now, in October 1938, of the same types A and C. Many other clones have been in mass culture in the laboratory for more than a year; all still react as the same mating type as at the beginning.

In *Paramecium aurelia*, according to SONNEBORN (1937) and KIMBALL (1937), there is commonly a change of type in a part of the individuals at endomixis, so that a clone that originally belongs to but one of the two mating types has after endomixis both mating types, which may then conjugate together. As endomixis occurs in *Paramecium aurelia* every two to four weeks, there are in that species frequent changes of mating type; a mass culture of a clone does not remain pure for a certain type. In *Paramecium bursaria* endomixis is seemingly infrequent. ERDMANN (1927), who examined into this, did not find endomixis to occur at all in isolation cultures, but did find all stages of the process in mass cultures. The clones l and m, above mentioned, have been kept in mass cultures in the laboratory for 18 months, so that if endomixis is a regular occurrence, it seems probable that the process must have been undergone several times; yet the original mating type is retained.

The retention of the mating type appears the more remarkable in view of the occasional occurrence of self-fertilization in a clone, as set forth in the next paragraphs.

Self-Fertilization. Any clone (derived by fission from a single individual) belongs, under usual conditions, to a definite mating type; it forms clumps and conjugating pairs only with members of other types of the same group.

But in very rare cases a pair, or more than one, is found in a mass culture of a single clone; or in a mixture of two clones that by all other tests belong to the same mating type. Conjugation has occurred between members of the same clone, or members of two clones that appear of the same type. This occurs so rarely that one may have clones under frequent examination for months without observing a single pair. But as months pass the number of such observations accumulates. In view of the fact that in *Paramecium aurelia* differentiation into two types is known to occur at endomixis, it would seem probable that this production of pairs within a single clone or single mating type is due to a differentiation, by endomixis or in some other way, into two or more diverse mating types, which then conjugate. The genetics of self-fertilization in *Paramecium bursaria* will be treated separately, in a paper to appear soon; there will be discussed also in the light of facts brought out, the seemingly permanent retention of the original mating type by clones kept for long periods.

Clones that do not conjugate. As before mentioned, when large numbers of individuals are collected from natural habitats and clones are obtained from them, some clones are found that do not react with any of the 16 mating types of the three groups. In some cases these are presumably immature clones; the mating reactions develop later, as before set forth. But some clones have never developed mating reactions. The clone Gr4 has been in the laboratory in mass culture since February 25, 1938; that is for more than eight months, yet has never reacted in any of the frequent tests given it. During that period many clones have been produced by conjugation, and have developed to maturity, so that they give strongly marked mating reactions. Other clones that have never reacted have been for long periods in the laboratory.

Such refractory clones may possibly be clones in which the period of immaturity is very long. Great genetic differences in the length of the period of immaturity are known to occur. Or such clones may be genetically defective, lacking entirely the tendency to conjugate. Or, finally, they may belong to other groups of mating types, in addition to the three above described. If no other members of such additional groups are present in the laboratory, there would be no opportunity for such clones to display the mating reactions.

GENETIC RELATIONS

In my preliminary paper (1938) certain general genetic relations were set forth. In Group I, by the conjugation of types A and C the two additional types B and D were produced; that is, conjugation of A and C produced all the four types of Group I. In descendants of ex-conjugants from mating of the members of two clones of diverse type, as A and C, usually but not always the descendants of the two ex-conjugants of a given pair are of the same mating type. But in some cases they are of diverse mating type. Further, in rare cases the two clones descended from the two individuals produced by the first division of an ex-conjugant are of diverse mating types, so that a segregation of the sex types may occur at the first division after conjugation, as in *Paramecium aurelia* (SONNEBORN 1937).

An extensive study of the genetic results of crossing and self-fertilization of clones in the various groups is in progress and will be reported in later contributions.

Historical

The fact that in the Vorticellidae the two members of a conjugating pair differ greatly has, of course, long been known; the early history of this matter is given by MAUPAS (1889, pp. 380–385). But with the free swimming ciliates the case is far otherwise.

In his great paper of 1889 MAUPAS records certain important observations and experiments which may now be interpreted as indicating the existence of diverse mating types in certain of the free ciliate infusoria. In four species, *Leucophrys patula*, *Onychodromus grandis*, *Stylonychia pustulata* and *Loxophyllum fasciola*, MAUPAS found that no conjugation occurs in a culture in which all the individuals are derived from the vegetative multiplication of a single parent (that is to say in a single clone), but commonly does occur if two such clones are mixed. This he says is based upon "several hundred" experiments of this type on the four species mentioned (see MAUPAS' general summary on this point, 1889, pp. 410–411). MAUPAS naturally concluded that the essential point here is the close relationship of the individuals that will not conjugate together. While this idea had a foundation of truth, it is now clear of course that it is not an accurate or adequate expression of the situation. On the one hand, as shown in the present paper, clones of entirely different descent, and coming from regions thousands of miles apart (California and Connecticut), may belong to the same mating type, so that they do not conjugate together. On the other hand, two clones so closely related as to have been derived from the same ex-conjugant may in some cases belong to diverse mating types, so that they readily conjugate and produce viable descendants.

The observations and conclusion of MAUPAS, just cited, appear not to have been taken up and developed for the ciliates until the appearance of the recent paper of SONNEBORN (1937). The present writer examined earlier into the matter, selecting for intensive study *Paramecium aurelia*. But in that species, as it turns out, conditions are exceptional and very diverse from those described by MAUPAS. In *Paramecium aurelia* conjugation frequently occurs between members of the same clone; it may occur even in cases in which the two individuals that conjugate are but four or five vegetative generations from the same ancestor; and such pairs produce viable offspring which again conjugate among themselves (JENNINGS 1910). This led to a turning aside from the study of the effects of relationship on conjugation.

But SONNEBORN in his studies of the results of endomixis, in this laboratory, found that in *Paramecium aurelia* endomixis may give origin, within a single clone, to individuals of two diverse mating types, which thereupon conjugate together. This opened up the entire subject.

Comparative

The relations found in *Paramecium bursaria* and set forth in the present paper differ from those in *Paramecium aurelia* (SONNEBORN 1937, 1938) in the fact that in any race or group of the latter species there are but two mating types, conjugation being limited to union between these two. The situation thus resembles that in the higher animals and plants, with their two sexes. In *Paramecium bursaria* on the other hand, as set forth in the foregoing, each group contains four or eight mating types, the individuals of any one type being able to conjugate with individuals of any other type of the group. This occurrence of but two mating types in the one species, while another species of the same genus has several, is of great interest. Study of the genetics of *Paramecium bursaria* promises to throw light on the nature of this difference. In both *Paramecium aurelia* and *P. bursaria* there exist a number of different groups—three thus far discovered in each species—the individuals of any one group not conjugating with those of other groups.

In the flagellates Chlamydomonas, Polytoma and Protosiphon, according to the magnificent investigations of MOEWUS (1933, 1935, 1935a) there are in each species or race just two mating types, which unite in copulation. The flagellates are haploid, so that the cells of the two mating types are closely comparable to the male and female sex cells or gametes of the more complex animals and plants. MOEWUS considers the phenomena as strictly sexual, speaking of the two types as the two sexes. In Paramecium on the other hand the organisms are diploid, so that the different types are not directly comparable to the male and female gametes of higher organisms but only to the two types of somatic individuals that constitute the two sexes. Yet the situation in Paramecium differs from that in the typical bisexual diploids, in the fact that in the infusorian both members of the pair produce descendants.

In the flagellates, according to MOEWUS (1933) and others, the two sex types give off into the water diverse "sex stuffs"; by filtering or centrifuging the water containing these may be made free of the flagellate cells. Such watery solutions of the plus sex stuff induce temporary clotting when added to cultures of the minus sex, and vice versa. According to PASCHER (1931) in Chlamydomonas the active gametes of one sex orient about a quiet gamete of the other sex and from a distance swim directly toward it, the reaction being without doubt mediated by the "sex stuff" present in the water.

The situation in *Paramecium bursaria*, in which as many as eight different mating types exist in a single group, is obviously more complex for the action of sex stuffs dissolved in the water, than in the flagellates. Further, the method of clotting and pairing described in the present paper does not seem to suggest the control of the reactions by sex stuffs dissolved in the water, as in the flagellates. No direct attempts to test the presence of sex stuffs and determine their possible role have as yet been made for *Paramecium bursaria.*

The many diverse mating types in *Paramecium bursaria* invite comparison with the facts of "multipolar sexuality" in certain of the fungi, notably in Ustilago and the Hymenomycetes. But in making such comparisons it must be held steadily in mind that the diverse mating types in Paramecium are diploid organisms, while the sexually reacting parts in the fungi are haploid. According to the account of BAUCH (1930), in Ustilago and presumably in Hymenomycetes a given local race has four diverse sexual types (haploid); these are seemingly determined by the combination of two pairs of factors, so that the four may be represented as AB, Ab, aB and ab. Mating among the four types occurs in accordance with definite rules depending on the similarity and diversity of the two factors present in each. The situation is such that usually a given type mates with only one other type out of the four that constitute the group—whereas in *Paramecium bursaria* each type mates with members of any of the other types of the group. In the fungi the four factors have in different races become modified in various ways, and these modifications alter the mating tendencies, so that there results a large number of diverse mating types. In principle and in details the situation in the fungi differs greatly from that in *Paramecium bursaria.*

The phenomena in *Paramecium bursaria* appear most naturally classifiable as "self-sterility," of the sort found in certain higher plants and animals. The single clone, like the single self-sterile plant, ordinarily does not fertilize itself: its cells do not unite in conjugation. But the single clone, like the single plant, may be fertilized by another; the cells of the single clone unite in conjugation with those of other clones, giving viable descendants.

But this is by no means the whole story, either in the infusorian or in the self-sterile plant. The farther phenomena in the two cases show many parallels in their details (compare the account of the general features of self-sterility in plants given by EAST 1929). In some cases the self-sterile plant becomes at times self-fertile, giving viable offspring; this is true also, as set forth earlier, for the single clones of the infusorian. Again, the phenomenon is not strictly one of "self" sterility merely, in either the plant or the infusorian. The single plant is sterile also with certain other plants, just as the single clone of *Paramecium bursaria* is sterile with certain other clones (those belonging to the same mating type). In most self-sterile plants the individuals can be divided into groups, such that those belonging to the same group are infertile together, while those belonging to diverse groups are fertile together. Such groups are comparable to the different mating types shown in this paper to exist in *Paramecium bursaria*. In the infusorian the number of such types is definite and limited; there are four in each of Groups I and III, eight in Group II.

The phenomena in the infusoria, while showing many points of resemblance to those in the self-sterile plants, of course differ from them in certain respects, particularly in the mechanism or physiology of self-sterility. In plants this appears to be a matter of relative rate of growth of the pollen tubes on the stigmas of individuals of different types; in the infusorian there is merely a failure to unite in conjugation.

For the division of the infusorian species into groups that do not cross conjugate, I have not found parallels in the accounts of self-sterility in the higher plants and animals. Its real parallel appears to be the actual differentiation of members of a genus into species that do not cross. In most organisms the groups so differentiated differ in certain morphological and physiological characters as well. This may turn out to be the case, in very slight degree, with the groups of *Paramecium bursaria*. In that case, or perhaps even without such additional differences, the groups might be considered slightly marked cases of differentiation into species.

SUMMARY

1. Ordinarily, members of a single clone of *Paramecium bursaria* do not conjugate together.

2. But when members of two diverse clones are mixed, in some of the mixtures the members of the two clones quickly clump into large masses containing many individuals. From these clumps many individuals later emerge united into conjugating pairs.

3. The behavior of the individuals in clumping and mating is described in detail. There are two main factors in the mating process: (1) an active coordinated reaction of two (or more) individuals that come in contact; (2) a physical adhesion between the bodies of individuals that touch each other. This physical adhesion results in irregular and uncoordinated movements; to it is due the rapid clumping into large masses. The two factors may be in operation separately or together.

4. Clumping and pairing depend upon a number of different conditions, the most important of which is the maturity of the clones. For several weeks or months the clones derived from ex-conjugant individuals are immature; they do not clump or pair. The tendency to clump and pair arises slowly, being weak in early periods, so that but few pairs are formed; later it gradually increases. Diverse ex-conjugant clones of the same parentage vary greatly in the length of the period of immaturity.

5. Other important conditions for clumping and pairing are the nutritive condition of the individuals, and the time of day. Clumping and pairing occur mainly in the afternoon; not at all in the evening and during night; little in the early forenoon.

6. When two diverse clones are mixed, in some mixtures clumping and pairing occur, in others not. Individuals of clones that do not clump and pair when mixed are considered to belong to the same mating type; individuals of clones that do clump and pair when mixed are considered to belong to diverse mating types.

7. When among many diverse clones all possible mixtures of two clones are made, a number of diverse mating types are found to exist, having the following relations: individuals of any single type may clump and conjugate with individuals of any of the other types, but not with individuals of their own type.

8. Three separate groups of such mating types exist, the types of any one group not clumping nor conjugating with any types from the other groups. Group I contains four mating types; Group II, eight mating types; Group III, four mating types. Thus the species as a whole is thus far found to consist of 16 diverse mating types.

9. The occurrence and distribution of the 16 mating types of the three groups are described. Group I (four types) has thus far been found only in Maryland and Virginia; Group II (eight types) ranges from Massachusetts to California and from New York to North Carolina; Group III (four types) has thus far been found in North Carolina and in Massachusetts. Some of the types of a given group appear to occur more frequently than others.

10. The three groups appear to remain independent, not intercrossing.

11. Different clones, whether belonging to the same or different mating type or group, differ greatly in size, form and physiological characteristics, and these features may remain nearly constant in vegetative reproduction. Differences observed between representatives of different mating types or groups usually turn out to be merely clonal differences, not characteristic of the entire type or group. It is as yet not certain that the three independent groups show any other constant diversities, morphological or physiological.

12. A clone belonging to a certain mating type remains of that type for many months of vegetative reproduction (observed up to 18 months).

13. Yet very rarely self-fertilization occurs; single pairs are found among the members of a single clone or single mating type.

14. Some clones collected from natural habitats have never paired with any of the 16 mating types. Others refuse for long periods to pair, but eventually yield a few pairs with certain of the types.

15. Certain genetic relations are stated, but in the main these are reserved for later treatment.

16. The phenomena in *Paramecium bursaria* are discussed in comparison with those in (1) *Paramecium aurelia*, (2) flagellates, (3) fungi, and (4) particularly with the phenomena of self-sterility in higher plants and animals.

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