Sex in Microorganisms

SEX IN MICROORGANISMS

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PREFACE

In planning the symposium on which this volume is based, an attempt was made to present the evidence for "sex" in the principal groups of microorganisms, from the viruses through bacteria, fungi, and unicellular algae to the protozoa. Since the material which could be presented in the short time afforded by the two sessions devoted to the symposium was necessarily limited in scope, an effort has been made in this volume to expand to some extent the ground covered at the symposium itself.

Dr. Visconti discusses evidence for recombination of "genes" in viruses without claiming that the phenomena demonstrate sex for that group. Dr. Lederberg and Dr. Tatum review genetic evidence for "sex" in the bacteria, and Dr. Hutchinson and Dr. Stempen describe cell fusions in certain bacteria. These cell fusions could provide an opportunity for the recombinations of genes that have been indicated. Next, Dr. Raper offers a comprehensive coverage of sex in the fungi and the many variations in sex phenomena found in that group.

Passing on to the lower algae, Dr. Patrick describes syngamy in diatoms and Dr. Lewin reviews the sexuality of other unicellular algae, especially the flagellates.

Next is a review of sexual phenomena in the protozoa, with sections on the Mastigophora, the Sarcodina, the Sporozoa, and the Ciliophora. At the symposium, Dr. Cleveland reported on his studies of sexual reproduction among the flagellates living in the gut of the wood-feeding roach, *Cryptocercus punctulatus*, but he did not find it possible to provide a paper for the volume. Consequently, a review of his published papers on that subject follows the section devoted to the Mastigophora.

After the section on sex in the Ciliophora, Dr. Nanney summarizes the status of mating type phenomena in *Paramecium aurelia* and then presents certain newer interpretations of mating type behavior that have evolved in Dr. Sonneborn's laboratory at Indiana University. This paper is followed by Dr. Metz's discussion of mating type substances, with a comparison of the conditions found in *Paramecium* and other ciliates with those found in the Metazoa.

At the end of the volume is a short section devoted to comments

on the origin and evolution of sex, based primarily on conditions in the Protozoa, but including questions about all of the microorganisms.

It is hoped that this volume will reveal to readers not only that "sex" is widely distributed among microorganisms, but that many groups appear to get along without sexual reproduction, at least for indefinite periods of time, and that the details of sexual behavior vary widely among and within the different groups of microscopic organisms.

D. H. Wenrich

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Genetic Recombination in Bacterial Viruses

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At first sight the life cycle of a bacteriophage particle seems simple. It is adsorbed to a bacterium, and after a characteristic period of time called the latent period, which is 22 minutes for T2, the bacterium bursts open, releasing several hundreds of particles identical to the one adsorbed at the beginning. To the scientist concerned with the biological problem of self-duplication, this is interesting. In order to discover how and from what these particles are formed, we must see what is going on in the bacterium during the latent period. There has not been developed a method to look inside the bacterium without disturbing the process of phage formation. Suppose instead that the bacterium is broken before spontaneous lysis. What is found then?

In 1942 at Vanderbilt University, Delbrück and Luria started experiments along this line of thinking. The problem was to break the bacterium without damaging the phage or whatever was to be found inside. One effective agent was known: that agent was the phage itself. Suppose a bacterium is infected with two phages, T1 and T2. T1 has a latent period of 13 minutes, T2 of 22 minutes. The two phages will start to grow together, but after 13 minutes the progeny of T1 will supposedly break the bacterium open. What will happen to the progeny of T2? With this experiment began a series of unexpected results which led to the discovery of recombination. In the bacteria infected with both T1 and T2, nothing happened at 13 minutes; at 22 minutes the cells burst, but only T2 was found in the lysed culture. If, instead of a mixture of T1 and T2, only T2 had been used, the result would have been the same. The explanation is that T2 excludes T1. Both phages are adsorbed to the same bacterium, but only one can grow. If, instead of infecting simultaneously with T1 and T2, an advantage of 4 minutes is given to T1, many bacteria will yield T1 but not T2. The bacterial culture as a whole may yield

both phages, but each burst coming from one bacterium will consist entirely either of T1 or of T2. This effect was called mutual exclusion. At that time Delbrück and Luria made another peculiar observation. If a single bacterium was infected with many identical phage particles instead of one, the latent period and the yield did not change. This made it appear that actually only one particle took part in the growth. The principle of mutual exclusion could thus be extended to particles of the same strain. As soon as one particle begins to grow in a bacterium, a reaction is started that results in the exclusion of all other particles, even if they are already adsorbed. Quoting from Delbrück: "The mutual exclusion effect is so novel that its explanation calls for a bold hypothesis. We assume that the first virus which penetrates the cell wall will make the cell wall impermeable to other virus particles just as the fertilization of an egg by one spermatozoon makes the egg membrane impermeable to other spermatozoa."

In 1944 Luria found the first mutation in phage T2 (Luria, 1945). If a high concentration of bacteria is seeded on an agar plate, the bacteria will grow in a thin continuous film on the surface. If a bacteriophage is present on this surface, it will multiply by lysing the bacteria, producing a colony of phage called a plaque, which appears like a clear visible hole in the film of turbid bacteria covering the surface of the agar. Strain B of the colon bacillus, sensitive to T2, can mutate to B/2, resistant to T2. However, if a very large number of T2 phage particles are plated on B/2, some plaques are obtained. These plaques are due to T2*h* mutants which lyse B/2. Therefore we have two types of phage: T2 and T2*h*, and two types of bacteria: B and B/2. T2*h* lyses both bacterial strains, T2 only B and not B/2. Suppose we plate on B: T2 cannot be distinguished from T2*h*. If we plate on B/2, only T2*h* will give plaques. As a crucial test for the theory of mutual exclusion, Luria infected the same bacteria first with T2 and then with T2*h;* he found that T2*h* was excluded. He concluded that exclusion exists also among closely related phages like T2 and its mutant *h*. But the actual result of Luria's experiment was due to a different phenomenon discovered by Dulbecco several years later (1952) and called mutual exclusion between related phages. This exclusion is due to the interval of time allowed between the first and second infection, and not to exclusion in the previous sense. The longer this interval of time, the more complete is the exclusion.

In 1945 another mutant was found by Hershey at Washington University (Hershey, 1946). This mutant was called *r*. It gave much larger plaques than the wild type r^+, and it was directly recognizable on the plate. Skeptical of mutual exclusion between two particles of the same strain, Hershey tried mixed infections with the r^+ and *r* to analyze the burst of single bacteria. He used a technique already worked out by Delbrück. After infection, he plated the infected bacteria before the burst. Thus, each plaque on the plate represented the phage particles coming from one bacterium, because the burst takes place in the agar and remains concentrated at one point. If the principle of mutual exclusion was correct, all the plaques should have been either *r* or r^+. Hershey had no idea how mixed plaques would look. What he was planning to do was to sample some plaques at random and to analyze the population obtained from each plaque. But the plaques were neither of the *r* nor of the r^+ type. They were mottled, *r* and r^+ growing together to give a type of plaque very different from either one alone. The actual presence of the two types in the same plaque was confirmed.

Mutual exclusion had been demonstrated in a very clear way for morphologically and serologically unrelated strains like T1 and T2. But, what would happen if similar strains were used, for instance T2 and T4, which have many features in common? Following Hershey's lead, Delbrück found that T2 and T4 could grow together in the same bacterium.

In one experiment, Delbrück used, instead of T2, a strain of T2r. The original experiment of Hershey with mixed infected bacteria could be repeated, not using T2r and $T2r^+$, but using instead T2r and $T4r^+$. At this stage something unexpected occurred. The bacteria infected with T2r and $T4r^+$, besides yielding both infecting types, yielded also two new types, T4r and $T2r^+$. Recombination in phage had been discovered. Not only could two different phage particles grow in the same bacterium, but they could also recombine some of their characters. At the Cold Spring Harbor Symposium for Quantitative Biology in 1946 Delbrück and Bailey announced this result in their paper, "Induced Mutations in Bacterial Viruses." They used the following scheme to represent the new phenomenon:

$$T4r^+ \longrightarrow T4r \qquad \text{(under the influence of T2r)}$$

At the end of their paper Delbrück and Bailey made the follow-

ing statement: "Perhaps one might dispute the propriety of calling the observed changes induced mutations. In some respects they look more like transfers or even exchanges of genetical materials." At the same symposium, Hershey gave convincing evidence for the independent occurrence of *h* and *r* mutations in phage T2. The field was open for genetic investigation. A two-factor cross, *hr* by h^+r^+, could now be attempted.

In 1949 Hershey and Rotman published a paper in which they described the main features of phage genetics. The principal finding is the fact that in any two-factor cross, with equal multiplicity of the two parents, recombinants are obtained in different frequencies depending on the linkage relation between the two markers. Within the two parental types and within two recombinant types the frequencies are the same. What changes is the ratio of recombinants to the total. Shortly after the discovery of the *r* mutant, Hershey did some single-factor crosses to control the results of mixed infection.

The cross hr^+ x h^+r^+ (original experiment of Luria) is a one-factor cross, and obviously no recombinants can be found. Also *hr* x h^+r is a single-factor cross. From his strain *h* Hershey isolated an *r* mutant (the frequency of the *r* mutation is quite high), and he proceeded to cross *hr* by *r*. The amazing result of this simple experiment was the discovery in the yield of a high percentage of r^+. As mentioned before, the *hr* was isolated from an *r* mutant in the strain *h*. However, this strain can be prepared the other way round: an *h* mutant can be isolated from the *r* strain. Now we have again two strains, *hr* and *r*. We repeat the cross as before; the result is perfectly regular. No r^+ is found. Conclusion: Different mutations occurring at different loci give the same phenotype, *r*. Let us take *r*1 and *r*2. The following genotype will be obtained: $r1r2^+$, $r1^+r2$ (parental types); *r*1*r*2, $r1^+r2^+$ (recombinant types). Only $r1^+r2^+$ can be distinguished from the others. The existence of *r*1*r*2 can only be demonstrated by doing separate crosses with *r*1 and *r*2. In neither case is the normal type found in the yield. All the *r*'s that Hershey isolated proved to be at different loci.

THE INTRACELLULAR LIFE CYCLE OF BACTERIOPHAGE

The first step in the growth cycle of a phage is the adsorption of the phage particle to the bacterial surface. If the bacterial cell is

artificially broken up soon after infection, no phage will be found. For phage T2 this period, called the "eclipse period," lasts for about 10 minutes. From 10 minutes to 22 minutes mature phage particles can be recovered from artificially lysed bacteria. Doermann (1948) has shown that mature phage accumulates in the bacterium linearly with time.

Hershey and Chase (1952) have found that as soon as the phage is adsorbed it releases its nucleic acid into the bacterial cell while most of the protein part of the phage remains outside stuck to the bacterial surface. Only the nucleic acid part is necessary for the subsequent formation of new phage. At this point we must infer a mechanism by which a continuity is established between the infecting phage particle and the progeny phage which starts appearing in the bacterium 10 minutes after the infection. We assume that the nucleic acid moiety of the phage forms a new entity which we call vegetative phage. The vegetative phage grows in the bacterium, forming a population of particles called the pool of the vegetative phage. At a given moment vegetative phage particles are withdrawn from this pool and transformed into mature phage particles. The process of maturation which goes on linearly with time consists in the phage's attainment of a protein coat formed of an external membrane and a tail. When normal lysis occurs, a great many mature phage particles are released and all immature particles are lost. Whereas vegetative phage is the active phase of viral life in the sense of growth, mature phage is a dormant phase of the phage between two growth cycles.

Doermann (1951) has shown that among the first mature phage particles to appear in the cell, recombinants are already present. On the other hand, Levinthal and Visconti (1953) have shown that by delaying the lysis, the frequency of recombinants among the liberated phage particles increases considerably.

TECHNIQUE OF PHAGE CROSSES

The viral mutations used as markers are either host-range mutants or plaque type mutants. The first, indicated by h, are mutants which can lyse bacteria resistant to the normal h^+ phage strain. The plaque mutants indicated by r differ from the normal by the morphological character of the plaque.

The procedure of making a cross between two different strains consists in infecting a certain number of growing bacteria with two kinds of virus. These bacteria will lyse, yielding new phage. We refer to the infecting phage as the parent and to the phage which is liberated upon lysis as the progeny. Different ratios of parental phage types can be used to infect the bacteria, but the average procedure is to use equal multiplicity, let us say 5 particles of one type and 5 particles of the other per single bacterium.

A suspension of bacteria in buffered saline, containing 10^8 cells per milliliter, is mixed with an equal volume of a suspension of phage containing 10^9 of each parental type. Under these conditions, adsorption of the phage to the bacteria occurs without starting the growth cycle of the phage in the bacterial cell. After adsorption is completed, growth of the phage is started by adding nutrient broth. At the end of the latent period the bacteria will burst, yielding phage progeny. This progeny is plated and the different types of plaques scored. The ratios between the different types will give the result of the cross. As the mating occurs not between two particles but between groups of particles, a multiparental cross is possible if the bacterial cell is infected with more than two parental types. Hershey for the first time obtained results of triparental crosses, showing that some progeny particles contain markers derived from all three parental types.

HETEROZYGOTES

The phage particles recovered in the yield of a cross are considered haploids. This statement is made for the sake of simplicity, because Hershey and Chase (1951) have shown that 2 per cent of the phage particles originating from a cross are heterozygous for any character for which the two parents are different. Except for this 2 per cent, all the other particles give, on a subsequent infection, a pure yield of either one parental type or the other.

At first sight one is tempted to interpret this finding by assuming that a certain random fraction of the particles are diploids. In a two-factor cross this leads to the prediction that heterozygosis with respect to one of the two factors should be strongly correlated with heterozygosis with respect to the other factor. In contradiction to this, Hershey and Chase found zero correlation when the two factors are unlinked. Only when the factors are closely linked does some correla-

tion appear. One way of explaining this result would be to suppose that small frequent duplications of the linkage structures occur: small to account for the lack of correlation between distant factors, frequent to account for the 2 per cent heterozygous at any given locus. Practically every phage should carry some of these duplications.

THE LINKAGE SYSTEM

A linkage map in the usual sense of the term cannot be drawn directly on the basis of recombination data in phage crosses. The reason for this is that the phage cross is very different from a cross between two organisms in the classical sense of Mendelian genetics. An attempt can be made, nevertheless, to see if linkage exists, and to demonstrate linear arrangement of the loci. The first attempt of this kind was made by Hershey and Rotman with different mutants of T2. In two-factor crosses the frequency of recombinants in the total yield varied from 40 per cent downward. Three linkage groups were found, each of which showed 40 per cent recombination with the other two. As no higher value of recombination was obtained, these groups were considered independent or unlinked. Lower values of recombination between two loci indicate that they belong to the same linkage group. Inside the linkage groups all kinds of recombination values can be found. If these characters are chosen rather closely linked to each other by different combinations of three-factor crosses, which one is in the middle can be determined. If the order is A, B, C, the frequency of the ABC type will be much smaller in the cross AC x B than in the crosses AB x C or A x BC. More complete and elaborate data have been collected recently by Doermann and Hill (1952) working with phage T4. From these data full evidence can be obtained for the linearity of the markers on the different linkage groups.

THE MECHANISM OF GENETIC RECOMBINATION IN PHAGE

The interpretation of the data on phage recombination requires some generalization of the idea of a cross. That the mixed infection of a bacterium is not a straightforward analogue of a simple genetic cross can be easily demonstrated by the following facts:

1. In triparental crosses Hershey has shown that some progeny particles contain markers derived from all three parental particles.

2. In a two-factor cross with unequal multiplicity of infection (20 particles of parental type AB and one particle of parental type ab) the progeny contains more recombinants of one type, say aB, than of minority parents ab (Doermann, 1952).

As has already been mentioned, the progeny of a cross can be examined at different times, either by inducing premature lysis or by delaying the lysis. With closely linked markers, the fraction of recombinants increases linearly with time between the moment of appearance of the first mature phage and time 60 minutes after infection. These two lines of evidence derived from premature lysis and delayed lysis indicate that in the single growth cycle there is a drift in the course of time toward genetical equilibrium.

Two different kinds of hypothesis can be considered at this stage: (*a*) either the new phage is formed out of some kind of pool of the parental characters, or (*b*) matings occur between vegetative phage particles in such a way that the product of one segregation can mate again with some other particle.

What we are actually considering is a choice between some completely novel type of recombination occurring during reproduction of the phage and the classical mechanism of recombination by mating between pairs as it might take place in clonal reproduction of vegetative phage. The first hypothesis was strongly influenced by the phenomenon of multiplicity reactivation discovered by Luria in 1947. Bacteria infected with phage particles inactivated by ultraviolet light multiply and produce viable phage offspring in a large proportion of cases. Luria interpreted these results to mean that viable phage particles could be formed by recombination even when all the parental particles were unviable as a result of one or more lethal mutations. It is known now after more thorough investigation of this phenomenon that reactivation is not due to a genetic mechanism. The second hypothesis of the mating has been analyzed in great detail by Visconti and Delbrück (1953). Their theory is based on the following assumptions: (1) prophage particles multiply and mate pairwise at random inside the bacterial cell; (2) vegetative phage particles go on mating and multiplying up to the time of lysis; (3) mature phage particles which are accumulated in the bacterial cell do not mate. On the basis

of the theory, predictions about the result of some crosses have been made. Up to now these predictions have been verified.

The drift toward genetical equilibrium can now be easily explained by assuming that mating among vegetative phage particles goes on at a constant rate with time. The phage which matures at any moment inside the bacterium is withdrawn from a population of vegetative phage which has undergone a certain number of rounds of mating. The later the process of maturation is stopped by delaying the lysis, the higher will be the average number of matings and the greater will be the number of recombinants found. Two other important facts are explained by this theory. One is the lack of correlation between recombinants. In a cross AB x ab, if the yield of a single burst is examined, the number of recombinants Ab and aB should be the same. A lack of correlation between the two frequencies means that the process by which one recombinant is formed is independent of the formation of the other one. This fact is accounted for by this theory, as the maturation of a single vegetative phage into phage is an event independent of the maturation of any other particle. The other fact accounted for by this theory is an apparent negative interference, which is that recombinants between some two markers show a higher value of recombination between any other markers. It has already been mentioned that, if the crude recombination data of a cross are used to make a map, a linear arrangement of the markers belonging to the same linkage group will be obtained. Let us suppose that there are three unlinked factors, A, B, C. Considering any two of these markers, we will get in a cross 60 per cent parental types and 40 per cent recombinants. This fact can be explained as due to an incomplete genetical equilibrium reached by the population of vegetative phage at the moment of maturation. Suppose now that we compare a parental type to a recombinant, say AB to aB inside the class of recombinants Bc. Let the ratio be 1:1. Because of the random distribution of the number of matings per particle and because any mating with a similar type gives no possibility of recombination, we operate a selection in favor of recombination when we choose particles which have recombined for some other marker. When we apply the right calculation, this apparent negative interference disappears if we consider a single mating. Thus genetic maps can be made by calculating the average number of matings. For normal lysis in phage T2 a value

of five matings has been calculated. Recombination data obtained by Doermann in phage T4 led to the conclusion that if we refer to a single mating no interference can be demonstrated.

GENERAL CONCLUSIONS

Bacteriophage can be considered a haploid organism possessing nothing analogous to sex. A theory has been postulated which accounts for the recombination data known at present. This theory is supported also by some biological data on the intracellular growth of the bacteriophage. Some predictions of the theory have been verified. One important factor is not explained by the theory: heterozygosis. At the present moment phage looks rather unsuitable for genetic research, because the progeny of a single mating cannot be examined. We are in the situation of a geneticist who can examine his population once in a while, say once in every five generations. On the other hand, genetics of bacteriophage appears to be a powerful tool in the attempt to explain the main biological features of virus reproduction.

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Sex in Bacteria
Genetic Studies, 1945-1952

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FOR MANY YEARS bacteria were considered biologically exceptional organisms with no genes, nuclei, or sex, although the recognition of their biochemical similarities to other forms of life constituted one of the main foundations of comparative biochemistry. Over the last decade evidence has accumulated which has led to the satisfying conclusion that bacteria are not biologically unique but possess genetic and behavior systems more or less analogous to those of other forms, including nuclei, genes, and in certain instances even true sexual mechanisms for recombination of unit characters.

Historically, this change in our thinking in regard to bacteria stems from the pioneer concepts of Lwoff (1938) and Knight (1936) relating the nutritional requirements of mircooganisms to an evolutionary loss of synthetic abilities. If such losses in microorganisms were based on mutation and selection as required by modern concepts of evolution, the capacities for synthesis of essential nutrilites in microorganisms should be determined by genes, which should be subject to mutation, as are most genes in other organisms. Such considerations led Beadle and Tatum (1941) to the successful production by irradiation of nutritionally deficient or biochemical mutants in the heterothallic ascomycete *Neurospora*, and to the establishment of the genic basis of biochemical reactions leading to the synthesis of amino acids and vitamins. This relation has since been amply substantiated and extended by further work with *Neurospora* and other sexual microorganisms (cf. Tatum and Perkins, 1950).

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The next step in the evolution of our concepts of bacterial genetics was the experimental application of the *Neurospora* techniques to the production of biochemical mutants in these simpler organisms. The first nutritionally deficient (auxotrophic) mutants were produced in 1944 by x-ray treatment of *Escherichia coli* and *Acetobacter melanogenum* (Gray and Tatum, 1944; Roepke *et al.*, 1944). Subsequently, auxotrophic mutants have been obtained in almost every species of bacteria investigated (see Tatum, 1946; Tatum and Perkins, 1950). In addition to mutations to growth-factor dependence and reverse mutation to growth-factor independence, other types of mutant characters have been obtained, thoroughly investigated, and proved extremely valuable. These include such characters as virus resistance, antibiotic resistance, and capacity for sugar utilization.

The first auxotrophic mutants were obtained by the tedious and laborious process of plating out irradiated cells on fully supplemented medium, and then isolating individual colonies which were subsequently examined for failure to grow in the simple synthetic medium adequate for the original stock (minimal medium). The specific requirements of these deficient clones were then determined by systematic supplementation of the minimal medium with known growth-factors.

Later improvements in techniques have eliminated much of the labor involved in isolating and testing mutants of bacteria, particularly *E. coli.* These include (cf. Lederberg 1950, 1951a) the layer-plate technique in which only presumptive mutants are isolated for further testing, and the penicillin method in which non-mutants are actively eliminated, leaving mutant cells which form colonies after removal of the antibiotic and suitable supplementation of the medium. A further modification of this method, using solid medium and penicillinase, permits easy isolation of any desired type of auxotroph (Adelberg and Myers, 1952), and the isolation and testing of strains has been still further simplified by the replica plating method (Lederberg and Lederberg, 1952) which in a single operation permits transferring all colonies on a plate to a number of other plates with different supplements.

By all available criteria it is now generally accepted that most, if not all, characteristics of bacteria are controlled by hereditary units, and that these hereditary units in bacteria are analogous with genes in classically sexual organisms in the independence and randomness

of their mutation, the effect of physical and chemical agents on their mutation frequency, and qualitatively in the types of biochemical and enzymatic effects of their mutation (cf. Tatum and Perkins, 1950).

After establishment of this functional analogy between genes of bacteria and those of sexual forms, the next step logically was to ask if the analogy could be carried further, and if a mode of inheritance of bacterial characters similar to the Mendelian process in higher types could be detected. Although a number of workers had looked for character recombination in bacteria, and other microorganisms, even as early as 1908 (Browning, 1908; for other important references cf. Tatum and Lederberg, 1947), in most cases the biological materials available were not adequate for a definitive experimental test, although some suggestive evidence of a circumstantial nature has been obtained. The definitive nature of auxotrophic mutants and the relative ease of their isolation and diagnosis provided ideal material for testing the possibility of recombination of hereditary units in bacteria.

The independent occurrence and expression of auxotrophic mutations in *E. coli* permitted building up multiple mutant stocks of *E. coli* strain K-12 with several deficiencies by successive mutational treatment (Tatum, 1945). In this way, for example, cultures Y-10, requiring threonine, leucine, and thiamin, and 58-161, requiring biotin and methionine, were obtained. For simplicity in considering its capacity for synthesis of the factors concerned, strain 58-161 can be represented as biotin− methionine− threonine+ leucine+ thiamin+ ($B- M- T+ L+ B_1+$), while strain Y-10 would similarly be represented as $B+ M+ T- L- B_1-$. In this representation, in analogy with other organisms such as *Neurospora*, the genes determining alternative characters ($B+$ and $B-$ for example) are considered allelic.

Accordingly, a sexual process in a mixed culture of these two strains would involve reshuffling the indicated alleles at these five loci. If this were at random, any recombination might be expected, and might have been looked for. However, recombination to give a nutritionally independent type (prototroph) would be most easily detected, since it alone would grow on minimal medium, whereas any dependent types, including both parental strains, would not.

Experimental tests were carried out (Tatum and Lederberg,

1947) by growing the two strains either separately or together in complete media, then centrifuging out the cells, washing repeatedly to remove growth-factors, and plating mixtures of the washed cells into minimal agar. The results were striking in that about 100 colonies developed for each 10^9 cells examined, and on reisolation and purification these maintained their prototrophic character. Similarly treated single cultures of each strain gave no colonies on the minimal medium. This would be expected on the basis of the low frequency of mutation of each character to independence (*ca.* 1 in 10^7 cells) since the derivation of a prototroph from a triply deficient strain would then occur with a frequency of 1 in 10^{21} cells.

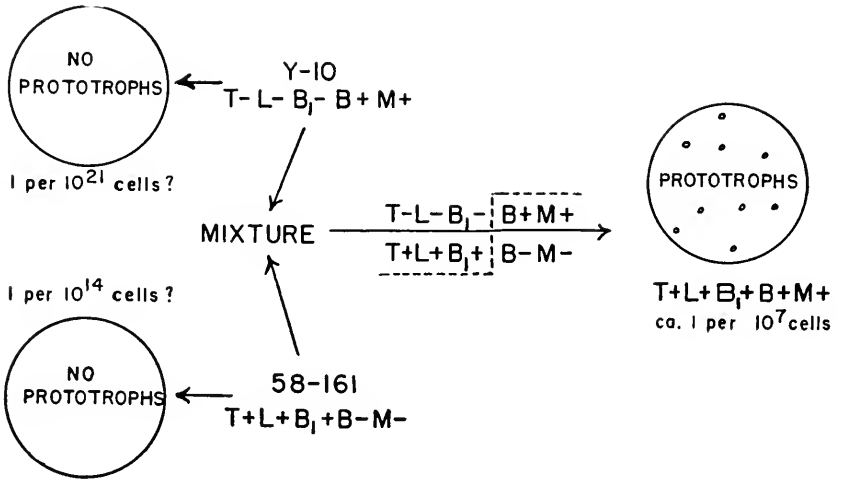

FIG. 1. Diagrammatic representation of recombination in prototrophs.

The simplest explanation for these results therefore appeared to be that gene recombination took place to give the prototroph $B+$ $M+$ $T+$ $L+$ B_1+, as shown in Fig. 1. Other possibilities, such as association of cells, or the formation of unsegregated diploid, or of heterocaryotic cells were made unlikely by various experimental tests which established the homogeneity and uniqueness of the derived prototrophs (cf. Tatum and Lederberg, 1947).

The only alternative to a sexual recombination seemed therefore a type of unilateral change by a non-cellular transforming principle similar to that involved in induced changes of type in the pneumococcus (Austrian, 1952). Two lines of evidence made this improbable.

First, cell-free filtrates gave no prototrophs and direct contact of the cells themselves seemed essential, as shown by growing the two types separated by an ultra-fine sintered glass bacterial filter (Davis, 1950). Second, the successful recovery of most of the possible recombination types in later crosses involving a much greater variety of characters, including resistance to antibiotics and viruses and sugar fermentation characters, would necessitate simultaneous transformation in different directions for different characters, and in both directions in different cells.

Thus the results of experiments of the type described above are satisfactorily explained only as resulting from a sexual mating process, followed by reassortment or segregation of genetic material. Repetition and extension of these experiments in a considerable number of laboratories during the past five years have amply confirmed the reality of the essential phenomenon and the validity of this conclusion.

These experiments have added considerable information about environmental factors affecting the recombination process, and support the concept that direct cell contact is necessary for the sexual process. Some of the strongest support comes from the demonstration by Nelson (1951) that recombination behaves as a bimolecular reaction, as if factors such as relative and absolute concentrations of the two types of cells, which would affect the frequency of contact of appropriate cells, similarly affect frequency of recombination. The experiments of Davis (1950), showing the need for cell contact, and in a more positive sense the production of a genetically diploid cell (Lederberg, 1949) likewise support the postulated occurrence of a cell to cell sexual process in *E. coli* K-12.

The intimate details of mating are still obscure. Owing to its infrequency we have been discouraged (until very recently) from any serious attempts to detect its morphological basis, and were obliged to be content with genetic inferences. E. Klieneberger-Nobel, of the Lister Institute, London, England, has made a most painstaking study of mating cultures of *E. coli* K-12 (unpublished work, quoted by her kind permission). Although she occasionally observed what appeared to be stages in the abortive development of L-forms (Klieneberger-Nobel, 1951), she was unable to correlate them in any way with recombination. The only conclusion that is warranted is that recombination in *E. coli* does not involve spectacular formations,

such as have been observed and speculated about in many bacteria. The possibility of a mating process involving a rapid conjugation and separation of the parent cells, without the intervention of special gamete or zygote structures, has not been excluded and is perhaps most likely.

The recently reported work of Hayes may throw further light on the conjugation process. Working with a single pair of K-12 stocks, Hayes (1952a) found that streptomycin treatment sterilized one (58-161) without affecting its recombination potency, but completely abolished recombination potency of the other (W677), and he postulated a unidirectional transfer of metabolically inert genic material from 58-161 to W677. The results of later studies (Hayes, 1952b) using ultra-violet irradiation for cell inactivation and stimulation of recombination (Clark *et al.*, 1950) were consistent with this hypothesis. He has suggested that the recombination may involve only a limited transfer of genic material, through a process which may not require the participation of two intact cells.

Granted the ability of *E. coli* K-12 to undergo a sexual recombination of genetic characters analogous to that found in other organisms, even if the morphological basis is still obscure, can the analogy be carried further? What evidence exists bearing on a chromosomal organization of the genes in *E. coli*? In all organisms that have been adequately studied, the genes are arranged in a linear order on chromosomes, whose distribution at cell division and during the formation of gametes follows very precise laws. It is difficult, in fact, to conceive of any other arrangement of the genes that would permit their regular and orderly distribution to the products of each cell division, without an uneconomical redundancy of the genetic factors for different traits. But aside from these speculations, there is considerable experimental evidence that the genes of *E. coli* are organized in linear order on one or more chromosomes (Lederberg, 1947; Rothfels, 1952; Lederberg *et al.*, 1951).

In the crosses mentioned so far, all the differences between the parents are directly involved in the selection of recombinants, so that we had no opportunity to investigate the segregation of factors whose expression is not enforced by the selective method. A number of mutant characters have been discovered, however, which are indifferent to plating on minimal medium. They include differences in the fermentation of various sugars, resistance to antibiotics, and resist-

ance to phages. Such characters will be called unselected, since their segregation is regulated by the internal mechanism of recombination rather than the exigencies of the technique.

The first unselected marker to be used in our experiments was resistance to phage T1 (Tatum and Lederberg, 1947). According to conventions, resistance and sensitivity are symbolized as V_1^r and V_1^s, respectively. V_1^r is a specially convenient marker, as it can be produced in any stock by the selection of spontaneous mutants with T1. Before they can be used in these experiments, such stocks must be carefully purified and, as for any marker, the stability and reproducible scoring of the mutation must be verified. A variety of crosses was carried out in which one parent was V_1^r, the other V_1^s. In each case we found a segregation for this marker, i.e., some of the prototrophs displayed the V_1^r trait, from one parent, and others V_1^s from the other. In control crosses, V_1^s x V_1^s gave only V_1^s, and V_1^r x V_1^r gave only V_1^r. Such a segregation in the first filial generation, the f-1, indicates a haplobiontic life cycle, similar to that of many unicellular organisms.

If an unselected marker were associated with one chromosome independent of others carrying the selected, nutritional mutations, the f-1 should show a mendelian ratio of 1:1. Different ratios were observed for each of the markers tested, the first evidence of linkage. The observed frequencies varied from one marker to another, and with a given marker, from one parental combination to another. In a given cross, however, the f-1 segregation ratios have been as reproducible as in any genetic material, which is to say they are subject mainly to sampling error.

A simple test of the significance of f-1 ratios can be made by reverse crosses, whereby a marker is introduced first in one, then in the other parent. For example, $BM-\ V_1^r$ x $TL-\ V_1^s$ is compared with $BM-\ V_1^s$ x $TL-\ V_1^r$. About 70 per cent of the prototrophs from the first cross are V_1^r. In the second cross, about 30 per cent are V_1^r, i.e., this ratio is inverted. The same result has been obtained in many reverse crosses involving different parental lines, and different markers and combinations of markers. It shows that the f-1 ratios have nothing to do with the physiological effects of the markers, but that they are due entirely to the mechanics of segregation. It also shows that dominance plays no role, and more generally that a genetic parti-

cle controlling each observed trait has been segregated, and is represented only once in the genotype of the recombinant cell.

By compounding elementary principles, genetic maps of *E. coli* can be constructed from segregation data involving numerous unselected markers (Cavalli, 1950; Newcombe and Nyholm, 1950; Rothfels, 1952; Lederberg *et al.*, 1951). By using other methods, the auxotroph mutations can be relieved of their burden of the selection of recombinants, and thus handled as unselected markers also. About half of the known markers of *E. coli* K-12 have been satisfactorily located in a single linear linkage group. Other markers have displayed a more confusing behavior which does not fit any scheme very satisfactorily, but is probably a result of rather complex chromosomal aberrations, for which there is independent evidence from the study of exceptional diploids (*v. infra*). It was long thought that *E. coli* had only one chromosome, but more recent evidence points to at least two, the segregation of which is not, however, entirely independent for secondary reasons (Fried and Lederberg, 1952). Cytological observations on haploid K-12 cultures have been interpreted by De Lamater (1952) as signifying three chromosomes, but further work is needed for the detailed concordance of genetic with cytological findings. Cytological study of *E. coli* has so far been confined to vegetative cells, whereas the genetic studies deal principally with segregation at meiosis.

The difficulties, briefly mentioned, in the segregation of certain misbehaving markers might appear to be fatal to a straightforward sexual interpretation of recombination except for the confirmatory evidence provided by exceptional diploid cultures. In ordinary crossings, the diploid condition has been inferred from its consequences of recombination and segregation, but is not directly observed. In 1946-47, many unsuccessful attempts were made to secure artificial diploids with agents such as camphor, acenaphthene, colchicine, and heat shocks, which have been used for other organisms (cf. Roper, 1952). More recently, however, a mutation, *Het*, occurred in one of our stocks which serves the same purpose (Lederberg *et al.*, 1951). Little is known of the action or genetic transmission of *Het*, but when it is present in one or both parents of a cross, several per cent of the prototrophs prove to be persistent heterozygotes. These heterozygous cultures continually segregate the alternative markers brought in

by the parents. Thus diploid cells heterozygous for lactose fermentation, *Lac*+/*Lac*−, produce mosaic colonies on an indicator medium, as shown in Fig. 2. The dark or "+" sectors consist of still heterozygous cells, *Lac*+/*Lac*−, and of *Lac*+ haploid segregants; the light or "−" sectors are *Lac*− haploid segregants. On complete medium, the faster-growing haploid cells soon outstrip the original diploids, but segregation can be effectively prevented on a minimal medium owing to the nutritional requirements of the haploid components. Single

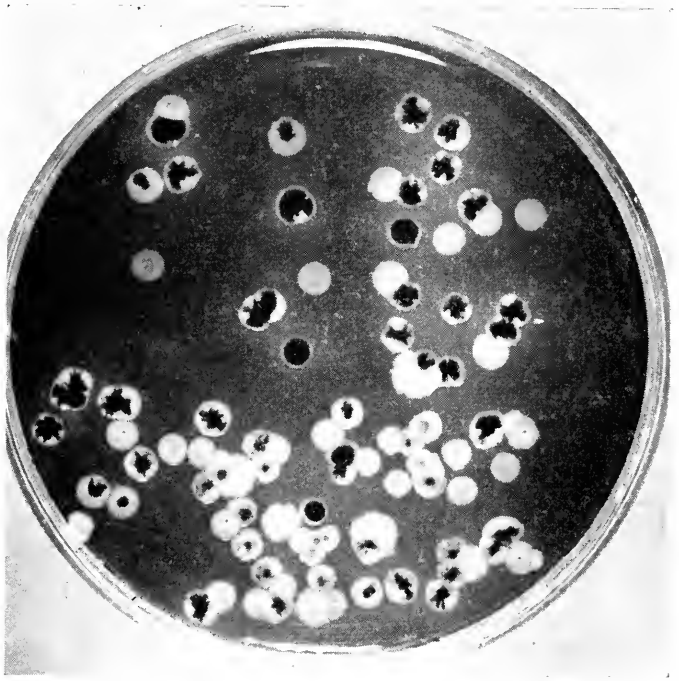

FIG. 2. Segregating diploid culture plated on indicator agar, showing variegated colonies.

cell studies (Zelle and Lederberg, 1951) have verified that genetic factors from two parents have converged to a single hybrid cell, the essence of sexuality.

Haploid and diploid cultures have been studied cytologically, especially for comparisons of their nuclear structure, by means of the Piekarski-Robinow technique (osmic fixation; HCl hydrolysis; Giemsa stain; mount in Abopon). This method gives brilliant nuclear preparations, but *E. coli* appears to be technically unsuitable for

unequivocal demonstration of mitotic figures, as claimed in the pioneering and provocative work of DeLamater and his associates (1951). Although nuclear aggregates that are very suggestive of mitotic metaphases and anaphases can be found with a brief search, definitive interpretations of *E. coli* cytology depend for the most part on the validity of the conclusions that have been drawn from technically superior material. It is difficult for a geneticist to imagine how bacteria could get along without some sort of mitotic process, but its details require critical and objective definition. The comparisons of haploid and diploid *E. coli* have revealed consistent and unequivocal differences, as shown in Figures 3 and 4 and elsewhere (Lederberg *et al.*, 1951). The determination whether the diploids show a doubling of the chromosome number is not yet subject to independent, objective verification.

The correlation of genetic heterozygosity with nuclear complexity is only a small step in the direction of a bacterial cytogenetics. It has been furthered by Witkin's studies, in which the segregation of mutant genes during fission has been correlated with the nuclear plurality of the bacterial cells at the time the mutations were induced (Witkin, 1951). These observations do accord, however, with a chromosomal theory of inheritance and sexuality in *E. coli.*

The work cited so far has been done with derivatives of a single strain, K-12, of *Escherichia coli.* A few early attempts to duplicate genetic recombination in other *E. coli* strains popular in genetic work were quite unsuccessful. Cavalli and Heslot (1949) discovered a culture in the British Type Culture Collection, NTCC 123, that was fertile with K-12, but a special screening method had to be developed before many new strains could be effectively studied (Lederberg, 1951a). Of nearly 2000 independent isolations of *E. coli* from various sources, over fifty have proven to be cross-fertile with K-12, and so far as has been tested, with each other. All of the new strains conform to the type *E. coli,* except for an occasional minor deviation, but are otherwise as heterogeneous as any sample of strains. They are serologically quite diverse: an immunogenetic study has been initiated which has so far put the antigens of *E. coli* on the same basis as the mammalian blood groups.

One important reason for undertaking this study of new strains was to investigate the sexual compatibility relations of *E. coli.* Until recently, several lines of evidence conspired to substantiate the idea

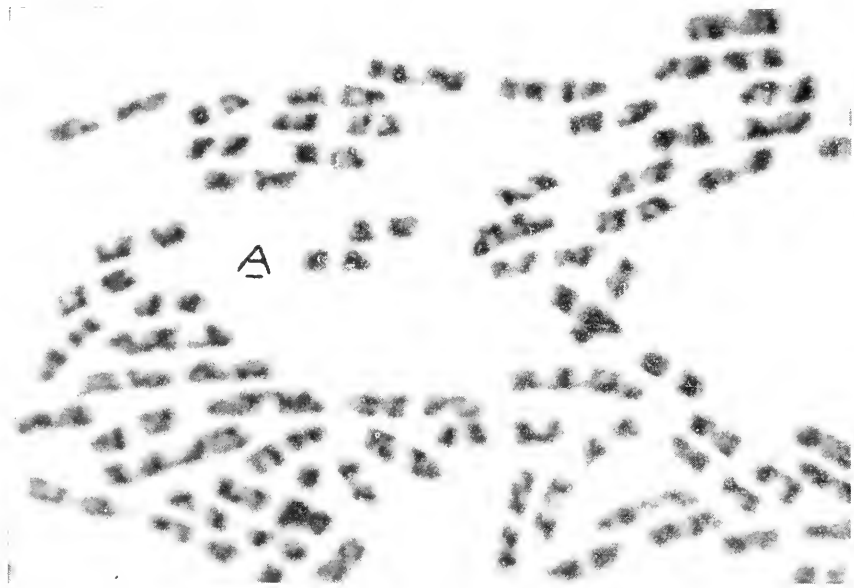

FIG. 3. *E. coli* K-12, haploid.

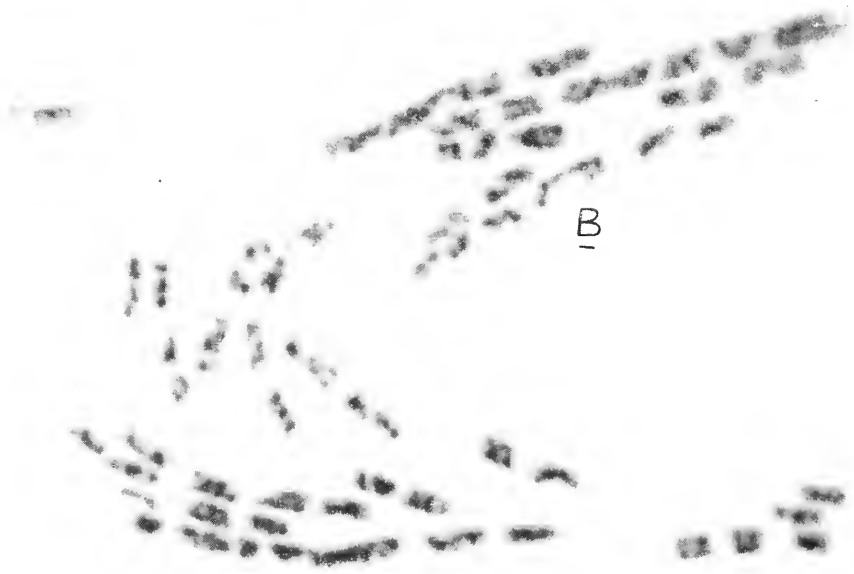

FIG. 4. *E. coli* K-12, diploid.

This photograph and Fig. 3 are taken from Osmic-HCL-Giemsa preparations by Miss E. R. Lively and are reproduced here at about 2,500 magnifications. Both include some cells that appear to display mitotic figures and others that do not.

that *E. coli* K-12 was homothallic. The crossable strains are all derived from a single pure culture. Several workers had suggested that a mating-type system might be obscured by mutations from one mating type to the other, as occurs in certain yeasts. However, this hypothesis was rejected because no segregation of mating preferences was observed from heterozygous diploids, as would have been expected from a heterothallic mating. It has since been discovered that a unique compatibility mechanism does operate in *Escherichia coli* (Lederberg, Cavalli, and Lederberg, 1952). The involved history of this discovery must be detailed elsewhere, and only the general conclusions can be given here.

Wild type K-12 carries a hereditary factor, *F*+, which is required for mating. Similarly, most of the auxotrophic mutants of K-12 are *F*+, and therefore mutually compatible, but the much used line descended from the threonine-leucine mutant, 679-680 (Tatum, 1945), is *F*—. Because most of the other tester cultures are *F*+, however, the *F*— "mutation" was not detected in earlier experiments. The empirical definition of *F*— is that crosses of two *F*— parents are completely sterile, although comparable crosses in which one or both parents is *F*+ are productive. The self-incompatibility of *F*— has been detected in two ways: sublines of 679-680 are mutually incompatible, and new occurrences of the *F*— "mutation" have been discovered which are incompatible with 679-680. The *F*— "mutation" is given in quotation marks because its inheritance and transmission set it apart from all of the other markers so far studied.

All the progeny of crosses within strain K-12 are *F*+, whether the parents were *F*— x *F*+ or *F*+ x *F*+ [*F*— x *F*— cannot, of course, be tested]. This was explained by the finding that *F*+ was contagious, that is that growing *F*— cells in mixture with *F*+ resulted in many of the former (identified by other genetic markers) becoming permanently *F*+. As many as 50 per cent of the originally *F*— cells may become *F*+ by this conditioning process within a few hours. "*F*+" is therefore tentatively regarded as an infective, virus-like agent, but this has not yet been confirmed by the isolation of "*F*+" in a cell-free preparation. The transmission of *F*+ is not accompanied by the transfer of any other marker, so far as is known.

The virus-like properties of the compatibility factor have led to some speculation on its relationship to a virus known to be present in *E. coli* K-12, the latent bacteriophage λ. This question has been

studied in some detail, and it can be asserted that λ is not related in any way to genetic recombination or to the sexual compatibility mechanism. Non-lysogenic, i.e., λ-free cultures, are fully compatible in sexual recombination, and the transfer of *F*+ is independent of the transfer of λ, which, unlike *F*+, can readily be obtained in cell-free filtrates. Genetic studies of lysogenicity (Lederberg and Lederberg, 1953) have, however, demonstrated a close relationship between this latent virus and the chromosomes of the bacterial host.

The details of the compatibility system are being studied at the present time. It has been noted that *F*+ x *F*+ crosses tend to be considerably less productive than comparable *F*+ x *F*—. Many of the results are consistent with the concept of relative sexuality as noted in many algae and fungi. That is to say, different cultures can be arranged in sequence of relative potencies, such that the productivity of a cross will be related to the difference in potency of the two parents. In *E. coli* K-12, the relative potency can be controlled both by environmental variations, and by genotypic effects. Within K-12, differences in the *F*+ agent itself have not been found. However, the *F*+ state as conditioned by some other wild-type strains appears to be unstable in K-12 cells, suggesting the possibility of genetic differences in the presumed agent itself.

The genetic basis of the observed "mutations" to *F*— in strain K-12 is not known, and these have not been experimentally reproducible. Many wild type strains are *F*— (i.e., non-infective) but retain their compatibility status, so that it is impossible to generalize on the causes of sterility or compatibility in the species *E. coli* taken as a whole.

In the absence of direct morphological evidence of sexual fusions, the principal alternative explanation for genetic recombination in *E. coli* has been "transformation" or "transduction." The biology of bacterial transductions is not very well understood (Ephrussi-Taylor, 1951; Austrian, 1952); in many ways it may be constructive to regard them as a limited form of hybridization. The chief distinction between transduction and sexual recombination is that the former seems to involve only a very small part of the whole genotype of the bacterium at each transfer (as in the capsular transformations in the pneumococcus), whereas sexual reproduction allows reassortment of the entire genotype of each parent, as in *E. coli*. The former seems to be correlated with an active unit that is morphologically and chemi-

cally much simpler than the intact cell; in *E. coli,* no unit other than the cell has been shown to be active in recombination.

A search for recombination in *Salmonella typhimurium,* a species distantly related to *E. coli,* has led to the discovery of another mode of transduction. In this system occasional bacteriophage particles appear to become fortuitously contaminated with genetic fragments from the host cells on which they are grown, and to be able to transduce these fragments to new cells which they may invade without killing them. The fragments retain their activity, and somehow enter the genetic organization of the new host. In this way, for example, flagellar antigenic traits from *S. typhimurium* may be introduced into cells of *S. typhi* to give a hybrid serotype or species not previously described (Zinder and Lederberg, 1952). The possibilities that sexual recombination may occur in *Salmonella* or that a genetic transduction may be found in *E. coli* are not unlikely, in view of the taxonomic relationship of these bacteria. It is from a superposition of these phenomena in a single species that we may expect to learn the most about each of them. At present, however, they appear to be quite distinct.

To sum up the present status of our knowledge of sexuality in *E. coli,* it has been shown that in mixed cultures under suitable conditions a small but significant number of cells of certain strains of this organism undergo a process of recombination of genes governing a wide variety of characters. This process apparently involves a cell-to-cell contact, and presumptively copulation, or conjugation, with zygote formation. Analysis of the recombination products indicates that the genes are present in linear order on one or more chromosomes. In essence, then, certain strains of *E. coli,* especially K-12, are capable of a sexual process, analogous in so far as it has yet been analyzed to that of other organisms.

It is to be hoped and expected that sexual phenomena will not be limited to *E. coli* among the bacteria. It is likewise to be hoped that the significance of suggestive cytological phenomena in bacteria such as apparent conjugation tubes (DeLamater, 1951), star-body formation (Braun and Elrod, 1946), filterable L-forms (Klieneberger-Nobel, 1951) and large bodies (Dienes, 1946; Stempen and Hutchinson, 1951) can be further evaluated by genetic analysis involving the recombination and tracing of suitable genetic markers.

The future also holds not only promise of correlation of genetic

and morphological aspects of conjugation and meiosis in *E. coli*, but the even more exciting prospects of discovering, elucidating, and correlating different modifications of sexuality and transfer of genetic material in microorganisms. With a clearer understanding of these relationships, the bacteria may be expected to occupy an increasingly important place in the study of the comparative biology of sex.

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Sex in Bacteria
Evidence from Morphology *

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THE ASSUMPTION that bacteria, together with blue-green algae, represent unique types of organisms lacking any mode of sexual reproduction has for many years challenged investigators in their search for such a phenomenon. Until recent years the methods of approach have been largely cytological with little apparent effort to correlate cytological and genetic evidence in the same organism. Genetic evidence for the existence of sexuality in bacteria is convincing (Lederberg, 1947; McElroy and Friedman, 1951), whereas the cytological evidence thus far presented is at most only suggestive.

By analogy with known sexual organisms, the demonstration of sexual reproduction in bacteria would require the fusion of gametes or of cells morphologically similar to vegetative cells. Such cell fusion could either be complete to form a single cell or incomplete as in conjugation. After fusion, nuclear material from the participants would be expected to fuse and later to segregate in preparation for the ultimate repetition of the process.

Sexuality in bacteria has also been claimed to occur by the fusion and segregation of nuclear material within single cells. Such a phenomenon, as well as cell fusion, has been reported to precede spore formation (as reviewed by Bisset, 1950, and Knaysi, 1951). On the other hand, DeLamater and Hunter (1952) and Lamanna (1952) discredit the occurrence of a sexual process in the formation of bacterial spores. Bisset (1950) has described "sexual vegetative reproduction" in which the nuclear material in vegetative cells fuses and is later thought to segregate. Microcyst formation is likewise thought

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to involve a sexual process. What appears to be similar fusion of nuclear material in several species of *Bacillus* and in *Clostridium perfringens* has been induced by exposure of the organisms to low concentrations of hydrogen peroxide (Cassel, 1951).

FUSION OF GAMETES

A sexual process in bacteria involving the fusion of male and female gametes, "spermit" and "oit" respectively, has been described by Enderlein (1925). It is claimed that aging bacteria give rise to small bodies called gonidia, each of which becomes transformed into a "gonit." These "gonits" do not reproduce as such on solid media, but in certain liquid media they may convert themselves into the male and female gametes. Enderlein maintains that the "spermit" morphologically resembles a vertebrate sperm with an oval to circular head and a connecting piece from which emerges a long, thin tail giving active motility to this cell. The "oit" is a larger spherical form with a flagellum inserted in a warty protuberance, although motility is indistinct or slow. Union of "spermit" and "oit" occurs directly after their formation and is supposedly followed by nuclear fusion.

These observations have apparently not been repeated nor more convincingly documented by others. On the contrary, Henrici (1928) has cast serious doubt on the validity of Enderlein's results.

FUSION OF BACTERIAL CELLS

Fusion of bacterial elements has been described as occurring by a unique process in *Azotobacter* and a number of other bacteria (Löhnis and Smith, 1916). Cells, spores, or gonidia in close proximity reportedly undergo dissolution with a subsequent mixing of their contents to form a "symplasm." Small granules later arise from the "symplasm" and enlarge into coccoid or oval cells. This series of events was postulated from the examination of fixed and stained films in which it is, of course, impossible to follow any development. Löhnis (1921) later attempted to study the formation of new bacterial cells from the "symplasm" in hanging drop cultures, but with unsatisfactory results.

In a number of instances spherical bodies found in bacterial cultures have been reported to result from cell fusion. In *Escherichia*

coli (Mellon, 1925) such spherical forms have been observed to one side of the junction of two rods. Strands of what was considered to be nuclear material were seen to extend into the round body from the two rods. Although the nature of this material was not definitely determined, the process was considered to be sexual in nature and the round forms were called zygospores.

Stoughton (1932) observed a similar phenomenon in *Bacterium malvacearum*. His attempts to study subsequent development of the zygospores in living preparations were only partially successful, because the multiplication of the ordinary rods was found to be so rapid as soon to overgrow the particular cell under observation. Thus most of the results were derived from the study of stained films. Other spherical forms in *B. malvacearum* (Stoughton, 1929) may arise as the result of budding from normal or slightly swollen or oval cells which occur in cultures aged for about 6 weeks.

The fusion of rods in pairs has also been described for a strain of *Bacteroides funduliformis* (Smith, 1944). Organisms removed from broth cultures at 3-hour intervals were stained with Giemsa stain without previous acid hydrolysis. During the first 6 hours the regular, single rods contained granules which stained deep blue. At 9 hours the rods had increased in length and were attached end to end in pairs with the apposed ends swollen and filled with the deep blue-staining material. Between 9 and 15 hours the swollen ends had apparently fused so that the organisms appeared as long rods with a central swelling. Between 18 and 21 hours the cultures consisted almost entirely of large round bodies which presumably resulted from the absorption of the rods into the central swelling. These round bodies containing the deep blue-staining material in clumped or discrete masses developed further by fractionating into ordinary rods, each rod receiving a few granules of the deeply stained material. This material was considered to represent a nuclear apparatus, although, unfortunately, its nature was not experimentally characterized further as, for instance, by the Feulgen reaction.

Utilizing a fixing and staining procedure of proved value in cytological investigations of higher organisms, Lindegren and Mellon (1933) postulated a possible sexual mechanism in the avian tubercle bacillus. From aceto-carmine-stained preparations they described the fusion of nuclei from adjoining coccoid cells with the subsequent growth and division of the zygote.

Using the technique so successfully employed by Robinow (1942) in the demonstration of bacterial nuclei, Klieneberger-Nobel (1949) has described cell and nuclear fusions in a number of different bacteria. The process described was essentially as follows. Rods or filaments break up into small cells designated primary cell units, each of which consists of a nuclear granule surrounded by a thin layer of cytoplasm. Fusion of two to many of the primary cell units occurs when the cytoplasm of adjacent units coalesces. This is followed by nuclear fusion which is said to involve the formation of "ramifications." Although the products of fusion, the L-bodies, are commonly round, oval, or spindle-shaped, their size and shape may vary with the number and arrangement of the cell units which fuse. These L-bodies can develop further in an appropriate environment to produce normal-appearing rods. This type of process was reported to occur in *Bacteroides funduliformis*, *Streptobacillus moniliformis*, and a strain of *Escherichia coli* under normal cultural conditions; in *Proteus* sp. under the influence of temperature changes; in *E. coli-mutabile* grown on nutrient agar containing lithium chloride; and in *E. coli-mutabile*, *Proteus* sp., and *Salmonella schottmülleri* grown on penicillin-containing medium.

Klieneberger-Nobel based her interpretations on this particular study on dead organisms. Certain of these interpretations are not in agreement with the results of later studies made directly on living cells. Stempen and Hutchinson (1951a) observed that when cell fusion occurred in *Proteus vulgaris* OX-19 only two cells were involved in each fusion observed. When *E. coli* is exposed to non-lethal concentrations of penicillin (Pulvertaft, 1952), aberrant forms resembling the L-forms of Klieneberger-Nobel are produced; however, all the aberrant forms noted developed as distortions of single rods which did not divide.

CELL FUSION IN *Proteus vulgaris* OX-19

Cell fusion has been demonstrated in *Proteus vulgaris* OX-19 by the direct observation of living cells in slide cultures (Stempen and Hutchinson, 1951a). The fusion process most commonly begins with the appearance of a budlike structure at the junction of a pair of rods attached end to end (Fig. 8). This bud becomes larger as the contents of the rods pass into it (Figs. 9 to 11). The rods are soon "absorbed"

completely by the growing bud, which now assumes the appearance of a round body, the so-called "large body" (Fig. 12). Figures 16 to 19 illustrate a fusion process in which the budlike structure appears on one end of the pair of rods. In some cases, after a rod has divided, the daughter cells fuse directly without bud formation to produce a large body. In such instances it is clear that sister cells fuse. In the other instances of fusion, the cells that fuse are already found touching end to end and it is impossible to determine whether these cells represent daughter cells of a previous cell division or cells of different origins. Cells that come in contact with each other as a result of elongation or crowding during growth have never been seen to fuse. It may be significant, however, that fusion through bud formation has not been observed in known sister cells.

Fusion of rods in *P. vulgaris* OX-19 occurs very infrequently. Numerous fields must usually be followed before a single case of cell fusion is observed. The exact frequency with which it does occur is unknown, because cell fusion can be detected only by direct microscopic observation and only a relatively few rods can be observed in each microscopic field examined.

Large bodies in cultures of *P. vulgaris* OX-19, however, are fairly numerous. The majority of these forms arise not by fusion but by the swelling or budding of single rods. The latter two methods of large body formation are illustrated in Figs. 1 to 3 and 4 to 7.

The behavior of nuclear material (chromatinic bodies) within the large bodies formed by cell fusion on the one hand and by budding or swelling of a single rod on the other hand would be interesting to determine with certainty. At the present time some information is available. In actively growing cells the areas corresponding in position to the chromatinic bodies appear lighter than the rest of the cell with the dark-phase contrast microscope, the reverse being true with bright-phase contrast (Tulasne, 1949a; Stempen, 1950). When this information is used in the examination of living organisms, the chromatinic material in large bodies arising by cell fusion appears to be in a compact mass immediately after fusion is complete. In other cases where the large bodies do not arise by fusion, the interior of these forms is composed of indistinct light and dark areas. This finding suggests that the chromatinic material does not occur in a compact mass but is more scattered throughout the interior of the large body. Actually, in impression smears of organisms which had been

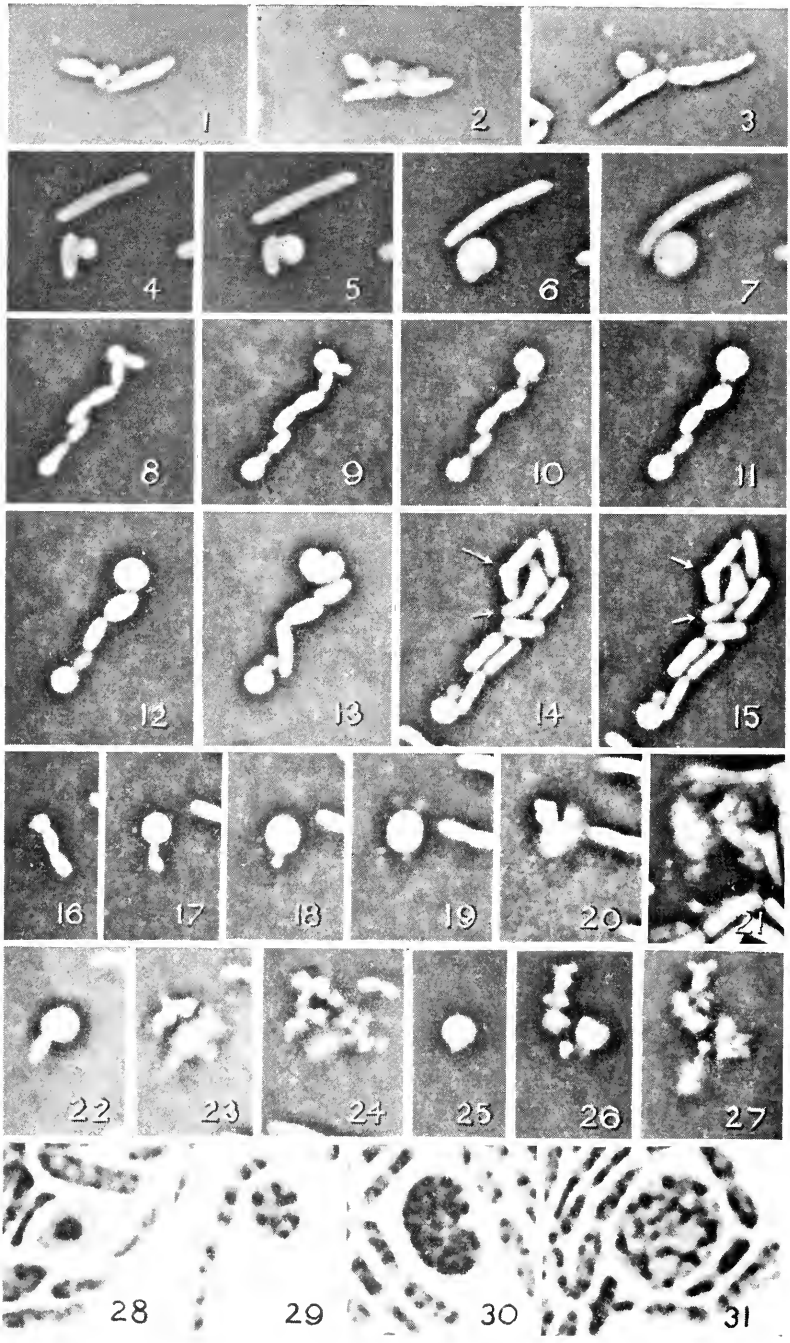

PLATE I. Figures 1 to 27 represent living cells in slide cultures (Stempen and Hutchinson, 1951a). Figures 28 to 31 represent cells from osmium-fixed and fuchsin-stained preparations made from 6-hour-old cultures (Stempen and Hutchinson, 1951b).

fixed, hydrolyzed, and stained by a method similar to that described by Robinow (1942), one can find large bodies in which the chromatinic material is in a compact mass (Fig. 28) and also those in which the chromatinic material is more distributed within the large body in the form of granules, rods, or filaments (Figs. 29 to 31) (Stempen and Hutchinson, 1951b). Any correlation between the results of the two sets of observations must be considered suggestive only, for there is no way of determining the mode of origin of the large bodies seen in such stained films.

Regardless of whether the large bodies arise by the fusion of two rods or from a single rod, they may undergo the same type of continued development. The large body may divide into two; each half then divides, and this process is continued until the normal rod form is restored (Figs. 12 to 15). In the restoration of the rod form from the large bodies of *Streptobacillus moniliformis* (Dienes, 1943) and *Bacteroides* strains (Dienes and Smith, 1944), the rods are reported to occur preformed in the large bodies; and Dienes (1946) states that the process in *Proteus* is similar to that in *Bacteroides*.

On the other hand, large bodies of *Proteus vulgaris* OX-19 may develop in another way. The body ruptures; the contents appear as a more or less homogeneous mass which is less refractile than the body from which it arose. In a short time thereafter one or more

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Figs. 1 to 3. Formation of a large body by the swelling of a single rod. The culture is 2¾, 3¾, and 4¾ hours old (× 2000).

Figs. 4 to 7. Formation of a large body by means of a lateral bud. The culture is 2, 2¼, 2½, and 2¾ hours hours old (× 2000).

Figs. 8 to 15. Fusion of two rods to form a large body with the subsequent division of the large body to produce rods. The culture is 1, 1½, 1¾, 2, 2¾, 4, 6¼, and 7¼ hours old (× 2000).

Figs. 16 to 21. Fusion of two rods with the formation of a large body on one of the pair of rods. The large body ruptures with the subsequent formation of a microcolony of granular forms. The culture is ¼, ¾, 1, 1½, 1¾, and 4 hours old (× 2000).

Figs. 22 to 24. Production of granular forms following the rupture of a large body and an adjacent rod (× 2000).

Figs. 25 to 27. Production of granular forms associated with the outgrowth of material from the large body. The large body later ruptures. The culture is 1¼, 2, and 2½ hours old (× 2000).

Fig. 28. Large body with chromatinic material concentrated in a single mass (× 3100).

Figs. 29 and 30. Large bodies containing different amounts of chromatinic material (× 3100).

Fig. 31. Large body with chromatinic material in a filamentous form (× 3100).

coccoid, refractile, granular forms ranging from 0.2 to 0.5 micron in diameter are suddenly evident at the periphery of the mass. The number of the granular forms increases quite rapidly until a microcolony is produced (Figs. 22 to 24). In a few instances masses of granular forms appear to extend out from the large body into the medium (Figs. 25 to 27). This process is similar to that described for *B. funduliformis* (Dienes and Smith, 1944) in which the granular forms (so-called L1 forms) grow out from the large body.

The granular forms of *Proteus* were not isolated in pure culture by Stempen and Hutchinson. The failure to do this may be due to the peculiarity of the strain employed, because Dienes (1949) and Tulasne (1949b) have reported success. These granules are reportedly capable of reverting to the rod form in subculture.

The similarity in behavior of large bodies regardless of how they are formed indicates that a basic similarity exists among them. Because large bodies have been reported to occur in old cultures (Henrici, 1925) or under the influence of injurious substances such as mercury salts, lithium chloride, or penicillin (Dienes, 1946), for instance, these forms have been considered by many to represent dead or dying cells referred to as involution forms. That some of the large bodies observed in cultures are degenerate forms may be true. That this does not apply to all large bodies is shown by two principal points. In *P. vulgaris* OX-19, at least, large bodies are often formed early during the growth cycle in a slide culture when conditions are favorable for growth and division of the normal-appearing rods. Also, the large bodies are capable of undergoing further development as indicated above.

Whether or not large body formation by cell fusion represents a sexual process cannot be stated with certainty. Determination of the behavior of the nuclear material presents the problems outlined above. A genetic approach to this problem is essential. If cell fusion does actually occur only between sister cells, it would be impossible to cross contrasting strains.

CONJUGATION

Processes resembling conjugation have been described for a number of different bacteria. For descriptions of some of the earlier claims, the reader is referred to the review by Löhnis (1921).

Agrobacterium tumefaciens, the causative agent of crown gall in plants, produces star-shaped aggregates of cells in which the cells appear to radiate from a common center. Löhnis (1921) interpreted such aggregates as conjunction. The term conjunction was used rather than conjugation because frequently more than two cells unite and there is no detectable sexual differentiation among them. The behavior of the nuclear material in such "stars" was studied with the aid of the Feulgen reaction by Stapp (1942) and later by Braun and Elrod (1946). These investigators suggest that nuclear material from the component organisms fuses in the center of the aggregate; although, as Braun and Elrod have pointed out, in many of the aggregates the Feulgen-positive material remained confined to the cell but concentrated in that part of the bacterium closest to the center of the star. They, therefore, were reluctant to interpret the phenomenon as conjugation because of the relatively few instances in which they observed what appeared to be an actual fusion of nuclear material.

Using the same method of approach, the study of fixed and stained cells, DeLamater (1951) described the process of conjugation in *Bacillus megaterium*. Vegetative cells, considered to be haploid, form conjugation tubes which are attached to only one cell or form a connection between the ends of two cells. At times a pair of tubes was observed connecting the ends of two cells or a loop-like structure formed on the side of a chain of rods connecting two cells of the chain. The extension of a conjugation tube from the end of one cell into the side of another in a different chain was taken as evidence that two cells of distinct origin could fuse. The simultaneous fusion of three rods was also observed on one occasion. The nuclei are described as migrating in both directions through these tubes so that both of the cells are usually "diploidized." Subsequently, it is claimed, the nuclei fuse and the cells enlarge and multiply vegetatively as diploids.

DeLamater has observed conjugation tubes on blood agar base plus 4 per cent human whole blood, on casein hydrolyzate medium, and in all the various sugars in which secondary colonies form. It was presumed that a relationship may exist between conjugation and secondary colony formation. The results from one series of experiments, however, show that, although secondary colonies can form on media containing blood or serum from different animals, conjugation tubes are formed only on media containing whole blood, plasma, or

serum from humans. It must be admitted that the time of sampling may influence these results. Secondary colonies were formed on media containing all of Cohn's blood fractions, but conjugation was observed only with the combined fractions (I, II, III-0, III-1, 2, IV, V, VI) and the fraction PGP (I, II, III), both of which contain fraction III.

The occurrence of conjugation tubes in *B. megaterium* has been challenged by Bisset (1952, 1953). It is pointed out that the rods of this organism occur in chains and that shrunken rods are frequently found joined at each end to their neighbors. These shrunken cells, Bisset claims, have been mistaken for conjugation tubes whose interior is presumed to be continuous with the two attached cells because DeLamater's preparations fail to show the cross walls that would be present.

As yet, serial photographs of the formation and behavior of conjugation tubes in living material have not been presented. If sufficient optical path differences exist between the migrating nuclei and the cytoplasm, it is theoretically possible to observe and photograph nuclear migration.

The genetic evidence of Hayes (1952) and of Lederberg and co-workers (1952) implies that cytoplasmic fusion is probably not involved when recombination occurs between certain mutants of the K-12 strain of *E. coli*. Hayes showed that cells "killed" by streptomycin were still capable of participating in recombination. He considered it probable that the viable cell extrudes genetic elements which adhere to the cell wall. The genetic elements, being unaffected by the streptomycin, can serve in recombination, whereas the killed cell acts as a passive carrier.

CONCLUSIONS

It has been demonstrated beyond question from continuous observation of living material that cells of *Proteus vulgaris* OX-19 may fuse together in pairs. There is no final demonstration as yet that this is a sexual process involving fusion and segregation of nuclear material.

Less convincing are the numerous reports of cell and nuclear fusion and of conjugation in several bacterial species, because the reports are based upon fixed and stained material in which any se-

quence of changes must be inferred by logic and analogy rather than demonstrated by direct observation.

When a film of fixed and stained cells is examined microscopically, one sees in each cell only one manifestation of the manifold morphological transformations of which the organism may be capable. All the different stages may or may not be present in the same film. Unless the behavior of living cells under as nearly the same environmental conditions as possible is also studied microscopically, the sequence of the stages is left to the imagination of the investigator.

Unfortunately, the bacteriologist with present-day methods is definitely limited in the amount of detail which he can resolve in the living cell. Therefore, fixed and stained cells must still be heavily relied upon particularly for the differentiation of minute internal detail. This, however, is no reason why one should confine all morphological studies to dead cells when the behavior of cells as units in such phenomena as cell fusion and conjugation could be examined in the living condition by the phase contrast microscope, an instrument well adapted to the study of gross morphology of bacteria. By what better way, techniques permitting, can one determine the behavior of individual cells in a culture than by observing and photographing in natural sequence the activities of these cells as they are actually performed?

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Life Cycles, Sexuality, and Sexual Mechanisms in the Fungi *

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THE FUNGI were once characterized, with considerable justification, as "a mutable and treacherous tribe." Probably no other characteristic or activity of the fungi contributed so prominently to this epithet as sex and the phenomena associated with sex. For the better part of a century the problem of sex in fungi has received a great deal of attention among students of the group, and a tremendous literature has accumulated through the years. The problem, however, seems to grow a trifle faster than does the solution, resulting in the interesting situation, like that described by Lewis Carroll, of losing only little ground by running very fast.

During the early decades of the century numerous scholarly publications summarized the existing information and integrated it into the more comprehensive problem of sexuality in plants and animals. The more notable of these works were Kniep's *Die Sexualität der niederen Pflanzen* of 1928, Gäumann's *Vergleichende Morphologie der Pilze* of 1926, and Dodge's translation and revision of this work in 1928, Link's highly intellectual review of reproduction published the following year, and, more recently, Hartmann's *Die Sexualität*, published in 1943. The implications of sexuality in fungi, however, remain largely unknown to biologists in general.

Several fungi, each carefully chosen to combine a number of specifically required characteristics, have recently been used as near-ideal research tools for the elucidation of basic phenomena of universal biological importance. More extended use along these lines, however, depends largely upon a greater awareness among biologists

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of the peculiar benefits offered by the fungi because of the variety of sexual and cultural characteristics which they possess. Equally important, of course, is an awareness of the limitations to their use.

No comprehensive, up-to-date source of such information is now available. This paper presents, in outline form, a comparative review of sex in fungi which might serve as a preliminary sketch for a source of this kind.

Essential sexual processes may be defined as those processes requisite to and including the juxtaposition and fusion of compatible nuclei and the subsequent sorting out of genetic factors in meiosis. These processes impose a cyclic progression of which plasmogamy, caryogamy, and meiosis are the irreducible cardinal events. The cycle, however, may be basically varied in three different ways: (1) by variations of the temporal relationships between the cardinal events by the intercalation, at different stages, of essential processes of growth; (2) by the imposition of genetic restrictions upon universal compatibility; and (3) by variations in the mechanical means of accomplishing the cardinal events.

These three modes of variation determine three distinct facets of sexuality, all separately definable but inextricably interrelated in the living plant: (1) life cycle, (2) basic pattern of sexuality, and (3) the sexual mechanism *per se*, respectively. Each facet is understandable only as a time-integrated and dynamic process. A detailed examination of each facet brings to light a number of facts which are little known but which are of considerable biological interest and are essential to an appreciation of the broad implications of sexuality in the fungi.

LIFE CYCLES

The fungi are commonly considered organisms which are essentially haploid—perhaps with nuclear fusion occurring now and again to give rise to a diploid phase which persists for a single nuclear generation. Although this is true of many species, particularly among the more primitive groups, the regular occurrence of exceptions to this simple pattern among the lower groups and the various complexities of the life cycle patterns characteristic of the more highly evolved forms make such a generalization meaningless. Life cycles among fungi run the gamut from completely haploid at the one extreme to completely diploid, minus the immediate products of meiosis, at the

other, and a unique nuclear association, known as the dicaryon, greatly increases the range of life cycle variability.

Since the dicaryon effects important changes in the life cycle and is peculiar to the fungi, it deserves a brief description and illustration at this point (Fig. 1.) The essential portion of the sexual process is initiated by the fusion of two sexual cells or organs, each containing one or more haploid nuclei (*N*). This fusion has been termed *plasmogamy* or *cytogamy*. The nuclei provided by the fusing elements may retain their individuality and become associated in one

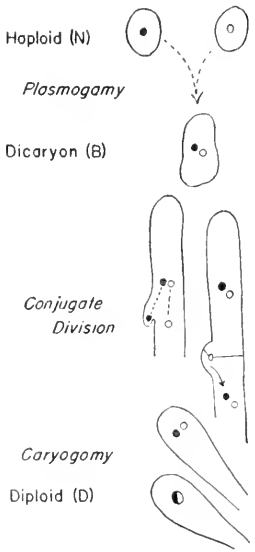

Fig. 1. Schematic representation of the initiation, multiplication, and termination of the dicaryotic association of compatible nuclei. The dicaryon occurs in the higher Ascomycetes in the ascogenous hyphae and universally in the Basidiomycetes in the "secondary" (dicaryotic) mycelium.

or more pairs, each pair known as a *dicaryon* (*B*). The dicaryon may be propagated for a short or for an indefinite period of time by repeated, simultaneous mitotic divisions of its members, the division figures of the two components commonly lying side by side. This process is termed *conjugate division*. Fusion of the two associated nuclei, or *caryogamy*, eventually occurs in terminal binucleate cells to establish the diploid phase (*D*). The dicaryotic phase thus serves, when present, to effect a temporal and spatial separation of plasmogamy and caryogamy. It also serves, because of the repeated divisions of its component nuclei, to increase greatly the productivity per sexual fusion both in numbers and in possible genetic recombinations.

Seven basic types of life cycles can be clearly distinguished; these are diagrammatically represented in Fig. 2 and are designated by the letters *A* to *G*. Changes in nuclear phase are here considered the cardinal events in the life cycle. These changes are indicated for each cycle, progressing clockwise, by differences in shading.

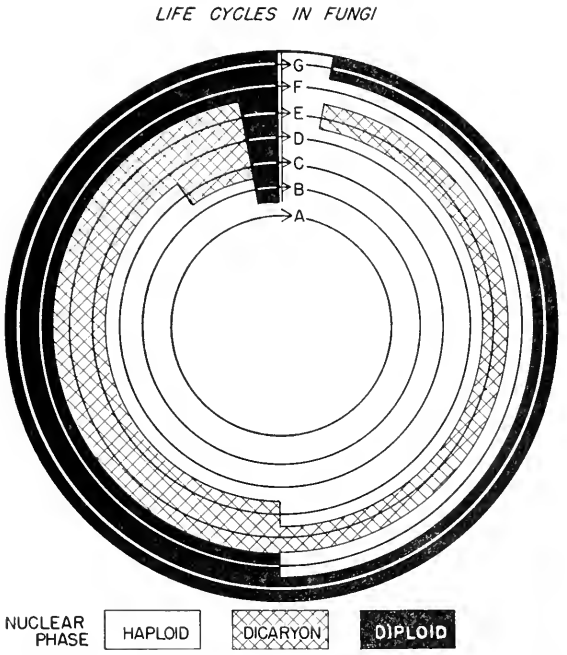

FIG. 2. Schematic comparison of life cycles in fungi.

In each cycle changes in nuclear phase are indicated, progressing clockwise, by changes in shading. The double vertical line at the top of the diagram represents meiosis, and each of the two narrow sectors adjacent to the line represents a single nuclear generation.

Asexual reproduction by spores or other specialized organs occurs at least in certain species belonging to each type and with few exceptions propagates the phase of the cycle from which the specialized reproductive cells are derived; the few exceptions will be mentioned later.

Asexual Cycle

Species apparently lacking any sexual expression or alternation of nuclear phase are fairly common throughout the fungi and con-

stitute a sizable proportion of all known species, approximately 20 per cent according to Bessey (1950). Because of the failure to observe rarely occurring sexual stages, the actual number of exclusively asexual species must be somewhat less than reported, but it must still be very large. The entire group known as the Fungi Imperfecti belongs here as well as numerous species which are clearly assignable by morphological characteristics to various groups throughout the perfect fungi, such as *Penicillium notatum*, the producer of the drug penicillin.

Certain of the benefits of sexuality are provided in many sexually sterile species by the association of nuclei of different origins in heterocaryotic mycelia, in which different genetic characters are expressed in much the same way as in dicaryotic mycelia or in diploid organisms (Pontecorvo, 1946). Of particular interest in this connection is the recent demonstration by Pontecorvo of recombination in low frequency of genetic factors in heterocaryotic fungal systems similar to that shown by Lederberg and Tatum (1946) in bacteria. The exact mechanism whereby such recombinations are achieved in fungi has not been fully elucidated. It has been shown, however, that there are formed occasional diploid nuclei, heterozygous for the characters carried by the heterocaryotic components, and that these, through a pseudo-meiotic rearrangement lacking reduction, produce diploids which are homozygous for one or more characters and which represent new genetic combinations (Pontecorvo and Roper, 1952; Roper, 1952).

Haploid Cycle

The predominant type of life cycle found in the Phycomycetes and the more primitive Ascomycetes is completely haploid with the exception of a single, diploid, nuclear generation, the fusion or zygote nucleus. This type of life cycle is the simplest possible one that allows for sexual fusion and the recombination of genetic factors and in all probability represents the primitive type from which the more complicated cycles have evolved. The general correlation between this type of cycle and the relative morphological simplicity of the forms exhibiting it, not only in the fungi but also in the algae, would tend to support this view.

Haploid Cycle with Restricted Dicaryon

A predominantly haploid cycle, which differs from the one discussed above by the separation in space and time of plasmogamy and caryogamy, is characteristic of members of the higher Ascomycetes such as *Neurospora*. At the time of the fusion of the sexual cells or organs one or more dicaryotic pairs of nuclei are formed, and these, by repeated mitotic divisions in the ascogenous hyphae, provide paired nuclei for a large number of ascal primordia within which caryogamy and meiosis occur. The multiplication of associated nuclei, though often extensive, is nevertheless restricted both in time and by the complete dependence of the ascogenous hyphae upon the haploid mycelium. The nature of the dicaryotic phase here would suggest for this type of cycle an evolutionary position intermediate between the exclusively haploid cycle and the more complex cycles to be found among the Basidiomycetes.

Haploid-Dicaryotic Cycle

The predominant life cycle in the Basidiomycetes, excluding many of the smuts, differs from the cycle just discussed by the unrestricted and independent growth of the dicaryotic phase. Both the haploid, or homocaryotic, phase and the dicaryotic phase are completely independent and capable of indefinite vegetative growth and are terminated by dicaryotization and fruit body formation respectively. The termination of each phase depends upon the achievement of certain requirements which is, in each case, largely a matter of chance. The cycle may therefore be considered to comprise two roughly equivalent phases and terminate in a single diploid nuclear generation, the fusion or definitive nucleus in the basidium.

A number of cases have been described among these forms in which differentiated spores produced by the dicaryotic mycelium re-establish the haploid phase (Brodie, 1931; Nobles, 1942). This occurs through the separation of the members of conjugate pairs of nuclei in the formation of uninucleate conidia or oidia. These specialized cells appear to attain their greatest effectiveness as fertilizing (dicaryotizing) agents, although germination in low percentage does

serve to sort out the original dicaryotic components into haploid, vegetative mycelia. Similar cells produced on haploid mycelia behave in an identical manner.

Dicaryotic Cycle

The extreme development of the dicaryotic phase is exemplified by the cycle in which the immediate products of meiosis, ascospores or basidiospores, fuse to initiate the dicaryotic phase. Both haploid and true diploid phases are thus reduced to single nuclear generations. This type of cycle is occasionally seen in the yeasts (Guillermond, 1940) and is of common occurrence among the smuts (Kniep, 1926).

The distinction made here between the haploid-dicaryotic and the dicaryotic cycles emphasizes the two extremes in what, in all probability, is a continuous series. Chance juxtaposition of compatible germinating spores of the haploid-dicaryotic type might result in the typical dicaryotic cycle; on the other hand, the experimental prolongation of the haploid phase, as sprout mycelia in the smuts for example, converts the typical dicaryotic cycle into the haploid-dicaryotic.

Of particular interest in this and the haploid-dicaryotic cycle is the failure of the dicaryon in many cases to constitute a physiological summation of its haploid components. This phenomenon is reflected in (1) the host specificity of the two phases in the heteroecious rusts (for example, the haploid phase of the "black stem rust" of wheat is an obligate parasite of the barberry, whereas the dicaryotic phase is an obligate parasite of grasses); (2) the saprophytic habit of the haploid phase versus the obligate parasitic habit of the dicaryotic phase of many smuts (Christensen and Rodenhiser, 1940); and (3) the complex pattern of fruiting requirements of the dicaryotic phase of certain Hymenomycetes as compared to the nutritional requirements of the component homocaryons (Schopfer and Blumer, 1940).

Haploid-Diploid Cycle

The alternation of haploid and true diploid generations, a common type of cycle in the algae and in the higher plants, occurs among fungi only in certain members of the aquatic phycomycetous order, the Blastocladiales, with *Allomyces* the best known example (Couch,

1942; Emerson, 1941; Harder and Sörgel, 1938; Kniep, 1929, 1930). The vegetative mycelia of the two generations are identical except for the specialized reproductive organs which they bear.

Diploid Cycle

The cycle that is typical for the animal kingdom, completely diploid except for the immediate products of meiosis, is found among the fungi in a number of yeasts (Guillermond, 1940; Winge, 1935) and perhaps in some members of the phycomycetous order, the Blastocladiales (Couch, 1942; McCranie, 1942). The latter case constitutes a slight variation of this cycle, as Wilson (1952) reports a single mitotic division of the meiotic products in *Allomyces cystogenus* prior to gamete differentiation. A regular haploid phase of two nuclear generations is unique among the fungi and, strictly, should be considered yet another type of life cycle.

BASIC PATTERNS OF SEXUALITY

Blakeslee, in 1904, in the course of an investigation of zygospore formation in the common "black bread mold," *Rhizopus nigricans*, first demonstrated "bisexuality" in the fungi. The term *heterothallism* was introduced to designate the occurrence, within a given species, of two kinds of individuals, each self-sterile and presumably differing in sexual sign, and the necessity of interaction between mycelia of the two kinds to accomplish sexual reproduction. The term *homothallism* was coined for the antithetical condition, the occurrence of only a single kind of individual, self-fertile and sexually self-sufficient.

The original definitions of homothallism and heterothallism, unfortunately, however, were somewhat ambiguous. The derivation of the term heterothallism implies differences of any sort between the individuals required for sexual interaction, whereas the original definition strongly implied differences in sexual sign. That Blakeslee was convinced of the sexual nature of the race differences, in spite of the slight and inconstant morphological differences, is strongly indicated by the work done by him and his associates during three decades toward the definite identification of (+) and (−) as ♀ and ♂ respectively (Satina and Blakeslee, 1928, 1929).

Blakeslee and other workers (1920) determined the pattern of

sexuality in most of the members of the Mucorales, the order of fungi to which "black bread mold" belongs. It is of interest in the present discussion that all species of this group having a sexual stage were unambiguously divisible into heterothallic and homothallic groups, and that in each heterothallic species no irregularities in respect to sexual sign were encountered, although individual isolates often varied widely in sexual potency.

In the half century that has elapsed since Blakeslee's first demonstration of obligatory intermycelial reaction for sexual reproduction, similar conditions have been reported for some members of every major group of fungi. The necessity for intermycelial reaction, however, is the only feature common to all cases: in some, sexual differences are clearly evident; in others, sexual differences equally clearly do not account for the pattern of self-sterility and cross-fertility. With the discovery of the several patterns of interactions, each differing in some important respect from that originally described in the Mucorales and termed heterothallism, a number of proposals have been made either to differentiate, by appropriate terms, these cases from heterothallism as originally defined or to redefine heterothallism in a more broadly inclusive manner. The chief result of these efforts has been to indicate the degree of prevailing confusion rather than to contribute to a unified scheme of categorization which was reasonably free of damning objections.

Whitehouse (1949), in a very comprehensive and excellent review, has recently advanced a logical system of differentiation which promises to clarify considerably the entire subject of sexuality in the fungi. He retains, on rational grounds and with historical justification, the term heterothallism to include all those cases in which intermycelial reaction is a requisite for sexual fusion. Two major types of heterothallism are distinguished: (1) *morphological heterothallism*, to include those cases in which the two interacting thalli differ by production of morphologically dissimilar sexual organs or gametes which are identifiable as ♂ and ♀, and (2) *physiological heterothallism*, to include those cases in which the interacting thalli differ in mating type, or compatibility, irrespective of the presence or absence of sexual organs or differentiated gametes *per se*. Homothallism is retained in the original sense: sexual fusion between elements of the same thallus or, in unicellular organisms, between individuals of the same

clone. A new term, *secondary homothallism*, is applied to self-fertile heterocaryons. These will be discussed in detail later.

Inevitably, there exist a number of forms which fit uneasily into a simple breakdown of this sort; in a group of organisms as varied as the fungi, this situation almost necessarily follows any attempt at categorization in respect to characteristics of the mature thalli. A somewhat less ambiguous system could be erected on the distinction between phenotypic and genotypic determination of sexual or mating behavior or both. The two major groupings here would be based upon the ability or inability of genetically identical nuclei (sister nuclei, daughters of a single primary meiotic product) to participate in sexual fusion. Such a distinction would roughly parallel that between homo- and heterothallism. Wide acceptance and common usage of the homo-heterothallism concept, however, dictate its perpetuation without radical change in spite of its intrinsic shortcomings. Recognition of the pattern of segregation at meiosis as the chief, and frequently the sole, factor in determining the ultimate sexual character or mating behavior, or both, of the thallus, however, results in a far clearer understanding of the homo-heterothallism concept.

Each mature thallus, at the stage in its development at which sexual fusions occur, commonly contains nuclei of only a single kind; that is, they are *homocaryotic* (a number of important exceptions to this generalization will be considered later). These sexually mature thalli thus represent the expressed potentialities determined at meiosis and imparted to the spores that constitute the immediate products of this process. Spores, and the thalli into which they develop, may be divided into four types in respect to the segregation of determining sexual or mating capacities; (1) segregation of sexual factors, (2) segregation of incompatibility factors, (3) segregation of sexual *and* incompatibility factors, and (4) segregation of neither sexual nor incompatibility factors. In the simple cases under consideration, spores of types 1, 2, and 3 give rise to thalli which are self-sterile but which are cross-fertile in those combinations bringing together complementary sexual or incompatibility factors. Such forms are clearly heterothallic. Spores of type 4, on the other hand, produce thalli of only a single kind, all of which are self-fertile; such forms are homothallic.

A number of complicating phenomena tend to mitigate somewhat the simplicity of this picture. Foremost among these is the reg-

ular association, initiated in spore formation in certain species, of two kinds of nuclei of dissimilar sexual or incompatibility types in a single thallus which is self-fertile. A form of this kind, in spite of its segregative pattern and the necessity of genetically dissimilar nuclei for sexual fusion, must be termed homothallic because of the self-fertile nature of its thallus.

A second complication is the possibility of final determination of sexual or mating behavior, in forms lacking this determination at meiosis, by environmental factors during the development of the thallus. A physiological differentiation of this sort between individual cells or groups of cells within a single thallus constitutes typical homothallic behavior; if the final differentiation involves different thalli, however, it must be termed heterothallic because of the self-sterile nature of the sexually mature thalli. Students of different groups of fungi have shown somewhat less than ideal accord in their integration of phenotypic determination with the homo-heterothallism concept. The general acceptance of the concept of the clone, now frequently ignored except in the study of unicellular forms, would resolve the more important discrepancies in interpretation of these phenomena.

A third mode of deviation from a strict dichotomy between homo- and heterothallism may arise through mutations of factors controlling mating behavior or modifying sexual expression.

These departures from strict homo- and heterothallism will be considered later in connection with the detailed accounts of the various patterns of mating behavior.

Homothallism

Of the several distinct patterns of sexuality to be found among the fungi, homothallism is the most common; it occurs in all major groups and, with very few exceptions, in a majority of species within each group. The critical differentiation of compatible elements is intramycelial and may involve single cells or relatively large groups of cells. The spatial relationship between differentiated elements of the fusing pair is also variable. This variability may best be illustrated by certain species in the aquatic phycomycetous order, the Saprolegniales: (1) ♂ and ♀ elements may together constitute a specialized

lateral hyphal branch, a stalked oögonium with an antheridial cell either differentiated in the stalk or arising from it; (2) ♂ and ♀ elements may arise from adjacent sections of main hyphae; and (3) ♂ and ♀ elements may arise from different main hyphae, each main hypha being differentiated in its entirety as ♂ or as ♀ (Coker, 1923).

Differentiation of sexual elements within a single thallus is usually reversible, either to the vegetative state or in some cases to sexual organs of the opposite sign. The vegetative development of unfused ♀ gametes of *Allomyces* (Emerson, 1941) and the ability of the differentiated sexual organs and even of isolated ♀ gametes of homothallic species of *Achlya* to regenerate normal hermaphroditic plants are typical examples of such reversibility (unpublished observations). A more extensive reversibility, from sexual organs of one sign to organs of the opposite sign, is fairly common in the homothallic water molds. The production of antheridial hyphae from oögonia and the occurrence of small oögonia intercalated in antheridial hyphae have been observed in various species (Coker, 1923; Humphrey, 1892; Maurizio, 1899). It has also been demonstrated in several homothallic species that sexual hormones from strongly sexed plants caused oögonial intials to redifferentiate and produce antheridial hyphae (Raper, 1950).

One further point in connection with true homothallism is of general biological interest. Sexual fusion normally occurs between elements carrying sister (genetically identical) nuclei. This would imply, a priori, that most fungi are deprived of the benefits occurring in the recombinations of genetic factors following sexual fusion between dissimilar elements. Two facts would tend to mitigate this deprivation: (1) the separate histories, often extended, of the two sister nuclei brought together in the sexual act allow considerable opportunity for the accumulation and recombination of minor differences due to induced or spontaneous mutations (Pontecorvo, 1947, 1950; Pontecorvo and Roper, 1952; Roper, 1952); and (2) juxtaposed thalli having totally different origins allow for extensive cross breeding and hybridization in forms with motile or non-motile differentiated gametes (Emerson, 1941, 1950) and for occasional cross breeding and even hybridization in forms lacking free gametes (Raper, 1950; Salvin, 1942). The extent to which either or both of these phenomena might duplicate in nature the benefits of enforced cross

breeding cannot be accurately assessed. The widespread occurrence of homothallism in fungi, however, is eloquent testimony of the evolutionary success of this pattern of sexuality among these forms.

Superficially it would appear that in homothallic species, typically uniting genetically identical nuclei, the usual sexual endeavor approaches a total sacrifice of quality for quantity; the exceptional cases which prevent the accomplishment of this biological absurdity appear to provide sufficient variability to allow for necessary adaptation and survival.

Heterothallism

Six basic patterns of sexuality have been called responsible for heterothallism among fungi. Beyond the single requirement for heterothallism, that the sexual act involve two individuals, these several patterns are quite distinct.

The distinctions between the various basic patterns imposing intermycelial mating reactions have been recognized by many authors, several of whom have proposed new terms for one or more of the seemingly coordinate patterns to distinguish them from heterothallism as originally defined. Some of these terms have been widely accepted and now constitute useful components of our working vocabulary; others have probably deserved the oblivion to which they have been relegated. It is certainly not the purpose here to add to this burden of awkward descriptive terms, but rather to differentiate as concisely as possible between a number of patterns which are based upon distinct genetic devices, are quite similar superficially, and which accomplish a common purpose.

The basic segregative mechanisms responsible for the six different heterothallic patterns are diagramed in Fig. 3. The order within the comparative listing here is not intended to convey any intimations of phylogenetic or evolutionary significance.

In typical heterothallic species the immediate products of meiosis, spores of one sort or another in most cases, differ among themselves in respect to either sexual sign, or incompatibility factors, or, in one known case, both sexual sign and incompatibility specificity.

The necessary use here of both sexual and incompatibility factors forces upon the reviewer the most unwelcome chore of attempting to distinguish concisely between the two; the onerous fact that a

clear distinction is impossible at the present time unfortunately does not excuse him from making the attempt. *Sexual factors* are those genetic determiners which characteristically result in morphologically distinguishable ♂ and ♀ plants, or ♂ and ♀ sexual organs or gametes or both. The criteria for the designation of ♂ and ♀ organs or cells or both are largely borrowed from mammalian reproductive processes and include relative size, inclusion of reserve food materials, motility,

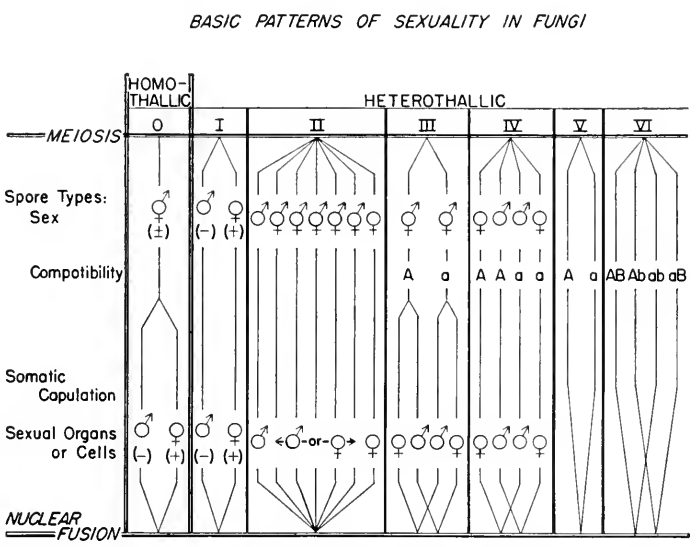

FIG. 3. The genetic devices that underlie the seven distinct patterns of sexuality in fungi.

All individuals of a species of type O are alike and functionally hermaphroditic; individuals of species of types I to VI are divided, by sexual or incompatibility differences, into two or more distinct mating strains among which cross-breeding is obligatory. Multiple allelic series at the incompatibility loci commonly occur in types V and VI, and the number of distinct mating strains of a single species of the latter type may be of the order of ten thousand.

and particularly the direction of nuclear migration (or transport) in fertilization. *Incompatibility* factors, by contrast, are those extra-sexual genetic determiners of mating capacity which operate either in addition to or in the absence of sexual factors. The difficulty of a sharp, universally applicable distinction between the two arises primarily from the occasionally known occurrence of heterothallic species in which demonstrable sexual differences exist in the absence of morphological differentiation. The best known example is the com-

plex of interbreeding species of the green alga, *Chlamydomonas*, which comprises heterogamous, anisogamous, and isogamous forms (Moewus, 1950). In other cases where sexual sign cannot be tested by cross-breeding with sexually differentiated forms, it is impossible to make a certain distinction between sexual and incompatibility control of mating behavior in the absence of clear morphological differences. This difficulty will be apparent in the following description of segregative patterns.

Single Alternate Sexual Factors. The simplest pattern of sexual differentiation yields two classes of progeny, each of which is either immediately distinguishable as ♂ or ♀ or bears differentiated ♂ or ♀ sexual organs or gametes respectively or both. Sexual dimorphism is typically rigid in plants belonging to this category.

Relatively few groups of fungi contain species which unquestionably show this type of differentiation. Among the more primitive monoflagellated aquatic fungi numerous species produce thalli which, at maturity, are differentiated into single gametangia with clear morphological distinction between ♂ and ♀ (Couch, 1942; Harder and Sörgel, 1938; Sparrow, 1943). Such forms are obviously heterothallic; whether the differentiation of the indvidual as a ♂ or as a ♀ is phenotypically or genotypically determined, however, remains uncertain although intensive efforts have been made to resolve the problem (Cantino and Hyatt, 1953; Emerson, 1950). Certain groups among these primitive fungi, particularly *Blastocladiella* of the Blastocladiales, constitute series grading from clear distinction between ♂ and ♀ thalli to forms that show no morphological difference between the two mating types (Stüben, 1939). The sure distinction here between sexual factors and incompatibility factors is not possible, but it would seem to the reviewer, in disagreement with the views of Whitehouse, that here as elsewhere a common pattern of sexuality most probably is shared by the members of a closely related group.

Sexual dimorphism is also known among the members of a few groups of Ascomycetes, in *Stromatinia narcissi* of the Discomycetes (Drayton and Groves, 1952), in many species of the Laboulbeniales (Benjamin and Shanor, 1950; Thaxter, 1908), and in *Pericystis* (Clausen, 1921).

The heterothallic members of the Mucorales, to which "black bread mold" belongs and in which heterothallism was first discovered, have long been cited as the classic examples of sexual segregation

among the fungi. Recent work and re-examination of earlier studies, however, leave such an interpretation in some doubt; current views rather favor the determination of mating type in these forms by incompatibility factors (Whitehouse, 1949).

Multiple Alternate Sexual Factors. A somewhat more complicated type of sexual segregation than that immediately preceding involves the determination of several sexual strains, each typically self-sterile but cross-fertile with all others. A heterothallic species of this type constitutes a linear series of sexual strains, each of which, with the exception of the two terminal strains, reacts as ♂ or as ♀ depending upon its position in the series relative to that of its mate; each of the two terminal strains reacts in a single sexual capacity, as ♂ or as ♀. This pattern of sexuality has been found in all heterothallic species of the biflagellate, phycomycetous orders, Saprolegniales, Leptomitales, and Peronosporales, which have been intensively investigated (Bishop, 1940; Bruyn, 1935, 1937; Couch, 1926; Leonian, 1931; Raper, 1940, 1947).

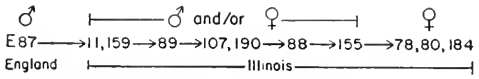

FIG. 4. In *Achlya ambisexualis* (a heterothallic species of type II) there are numerous self-sterile, intergrade strains in addition to pure-♂ and pure-♀ strains. The intergrade strains may be linearly arranged in respect to ♂ versus ♀ potentialities, and each strain can react as ♂ or ♀ or both. (From Raper, 1947.)

The mating pattern of a number of strains of *Achlya ambisexualis* best serves to illustrate this type of sexuality (Raper, 1947). Ten isolates of this species, collected from northern Illinois in 1946, when mated in all possible combinations, were found to belong to six strains. These strains, each self-sterile but cross-fertile in all combinations, could be linearly ordered, in respect to ♂ and ♀ potentialities, as shown in Fig. 4. In this series each isolate reacted as ♀ to those on its left and as ♂ to those on its right. A strong ♂ strain, E87, collected the following year in England, reacted as ♂ to all six strains from Illinois. Any intergrade mycelium is capable of reacting as ♂ and ♀ in different portions of its thallus when mated simultaneously with strong ♀ and ♂ plants.

Segregation at meiosis has been observed for plants exhibiting this type of sexuality in only a single species, *Dictyuchus monosporus*,

by Couch (1926), who found various intergrades in addition to ♂ and ♀ strains among the progeny of a ♂ by ♀ cross. These preliminary findings in conjunction with the many reports of multiple sexual strains collected from nature strongly indicate the random recombination of multiple sexual factors during the meiotic process. Further investigation of the segregative pattern, however, is needed to place this type of sexuality on the sound experimental basis shared by other patterns.

Hermaphroditism with Incompatibility Factors at a Single Locus. This type of heterothallic differentiation produces two self-sterile and cross-fertile strains, each morphologically and functionally hermaphroditic, and depends upon the equal segregation at meiosis of extrasexual determiners or incompatibility factors. Mycelia of each of the two strains characteristically produce both ♂ and ♀ sexual organs; sexual fusion is accomplished, however, only between the ♂ gametes or the ♂ gametangia of one strain with the ♀ elements of the opposite and compatible strain (Dodge, 1932; Drayton, 1932, 1934; Lindegren, 1932; Shear and Dodge, 1927; Wilcox, 1928).

The majority of the heterothallic species of the Ascomycetes, with the exception of the heterothallic yeasts, as well as many species of the rusts of the Basidiomycetes, exhibit this basic pattern of sexuality. In many Ascomycetes, *Neurospora* for example, the ♂, or fertilizing, element is characteristically the microconidium or spermatium, the ♀, an ascogonium. In other Ascomycetes differentiated gametes are not formed, and fusion occurs between morphologically distinct gametangia, antheridia and ascogonia, respectively. In rusts exhibiting this type of sexuality, spermatia bring about fertilization when brought into contact with receptive elements of the compatible mycelium, usually a specialized organ known as the "flexuous hypha" (Buller, 1950; Craigie, 1927, 1931, 1942).

The differentiation of sexual cells in plants having this type of heterothallism is phenotypic, and there are numerous cases in which differentiated sexual cells, spermatia or microconidia, of the Ascomycetes particularly (Dodge, 1932), have been shown to be capable of purely vegetative development. Conversely, in a number of forms, fertilization is accomplished with equal facility by microconidia, by asexual macroconidia, or, for that matter, by any cell of the vegetative thallus (Backus, 1939), and in some the ability to produce differentiated ♂ cells appears to have been lost, the function of fertiliza-

tion being completely assumed by asexual or vegetative elements (Dowding, 1933; Dowding and Buller, 1940).

Secondary homothallism, *sensu* Whitehouse, a phenomenon of fairly common occurrence in this group and shared to a greater or lesser degree by forms of other patterns in which incompatibility factors constitute the critical determination of mating behavior, results from the regular inclusion in the spore of two nuclei carrying opposed incompatibility factors (Ames, 1934; Dodge, 1927; Dowding, 1931; Dowding and Buller, 1934; Sass, 1929). Binucleate spores of this sort give rise to heterocaryotic mycelia which are self-fertile; in some species, sexual organs are present and appear to be essential, in other species sexual organs may be absent or greatly reduced and apparently non-essential. In several cases, occasional irregularities during spore production yield small, uninucleate spores, each of which develops into a self-sterile but cross-fertile mycelium which behaves exactly as do the individual mycelia of heterothallic species (Ames, 1934; Dowding, 1931). The initial binucleate condition thus predetermines a composite heterocaryon the net reaction of which is homothallic.

Alternate Sexual Factors with Incompatibility Factors at a Single Locus. A pattern of sexuality involving the independent assortment of sexual factors and incompatibility factors has been demonstrated to date in only a single species, *Hypomyces solani f. cucurbitae*, an ascomycetous fungus. The segregation of four distinct mating strains, ♂ of compatibility type *A*, ♀ of *A*, ♂ of *a*, and ♀ of *a*, constitutes the basic pattern of sexuality in this species (Hansen and Snyder, 1946). Four additional strains, however, appear among the progeny of certain crosses with a frequency of ca. 25 per cent; these are hermaphrodites and neuters of both incompatibility types. Cytological investigations (Hirsch, 1949) have revealed the mechanism that accounts for these anomalous strains. Frequent nondisjunction at meiosis of the two homologous chromosomes carrying the sexual factors yields two additional classes as regards sexual factors: one containing both, the other containing neither. The incompatibility alleles, segregating independently of the sexual factors and presumably located on different chromosomes, combine with these two classes at random to yield the four observed strains. Thus a total of eight different strains constitutes the array of distinct mating types in this species. Matings of ♀ x ♂, ♂♀ x ♂, and ♀ x ♂♀, of the proper

incompatibility types of course, yield the various classes of progeny predicted on the basis of the random assortment of the determining factors at the two loci and non-disjunctive doubling of the chromosomes carrying the sexual factors.

It is expected that this complex type of sexuality, with or without the non-disjunctive feature or other complications, will eventually be found in other species.

Incompatibility Factors at a Single Locus. This and the succeeding pattern of sexuality involved no sexual factors and no differentiated sexual organs. Mating is commonly reciprocal, and in multicellular organisms any cell of the thallus is potentially capable of donating a fertilizing nucleus and of accepting a fertilizing nucleus from the mate. The term *somatic copulation*—"Somatogamie" (Renner, 1916)—has been applied to this type of sexual fusion.

Species displaying sexuality of this type can be subdivided into two classes according to the number of alternate allelomorphs at the incompatibility locus.

In species of certain groups a single pair of alleles determines mating type; all individuals of each species therefore belong in one or the other of two mating categories, commonly designated *A* and *a*. The heterothallic yeasts are the best known examples of this pattern (Winge, 1935, 1944; Winge and Laustsen, 1939), and a recent review of the sexuality of the heterothallic smuts indicates basic control of mating behavior in this group by a one-locus, single-allelic-pair mechanism (Whitehouse, 1951).

Mutations at the incompatibility locus in certain yeasts (Lindegren and Lindegren, 1944; Winge, 1944) may be considered slight deviations of this pattern and may possibly indicate the mode of origin of the following pattern. Either *A* or *a* may occasionally mutate to altered states which permit fusion and ascus production within a single clone. The ascospores of such unions, however, have low viability. This possibly reflects either a lack of equivalence between the mutated alleles and the originals or the expression of deleterious, semisterility factors in the homozygous condition (Catcheside, 1951).

What would appear to be a much more highly evolved pattern of single locus control of mating behavior is characteristic of many Basidiomycetes, exclusive of the rusts and smuts. In these forms a very large number of completely equivalent alleles may be found in

various individuals at the single incompatibility locus, and mating occurs readily between any two haploid strains which carry different alleles (Brunswik, 1924; Buller, 1924; Vandendries, 1923; Whitehouse, 1949). Thus in lieu of the single pair, A and a, these forms each comprise an extended series of mating types which may best be designated by A^1, A^2, A^3, A^4, $\cdots$, A^n. This pattern of mating-type determination has been termed *bipolar sexuality*.

Incompatibility Factors at Two Loci. The final pattern of obligatory, interstrain mating behavior to be described is one found only among the Basidiomycetes, exclusive of the rusts and related groups. It involves mating-type determination by incompatibility factors at two loci; for example, the diploid condition may be designated $A^1A^2B^1B^2$, and independent assortment of these factors at meiosis yields progeny of four mating types, A^1B^1, A^1B^2, A^2B^1, and A^2B^2. Mating occurs only in those combinations having different alleles at both loci, for example, A^1B^2 x A^2B^1 and A^1B^1 x A^2B^2 (Brunswik, 1924; Hanna, 1925; Kniep, 1920; Mounce, 1922, 1926). This pattern of segregation was first described by Kniep about 1920 and was termed *tetrapolar sexuality*.

In tetrapolar species, as in the bipolar forms discussed earlier, the total number of mating types in the population is increased tremendously by the occurrence of multiple alleles at both loci (Kniep, 1922). The number of equivalent alleles at each locus, however, appears to be consistently different in two large groups of Basidiomycetes, the Gasteromycetes, which includes the "puffballs," and so on, and the Hymenomycetes, which includes the "mushrooms," "bracket fungi," and the like. In the former group about 10 alleles at each locus has been indicated as the extent of the series (Fries, 1940, 1943), whereas in the latter group as many as 27 alleles at each locus have been demonstrated (Brunswik, 1924; Fries and Janasson, 1941; Kniep, 1922) and the minimal total number of alleles at each locus in the population has been estimated to be of the order of 100 (Whitehouse, 1949). In all cases the alleles at each locus appear to be physiologically equivalent. The device of multiple incompatibility factors allows for almost complete outbreeding while maintaining inbreeding at 50 per cent in bipolar forms and at 25 per cent in tetrapolar forms.

Kniep, in his original work on tetrapolarity, observed that "mutations" occurred at the A and B loci in frequencies of about 2 per cent and slightly less than 1 per cent, respectively (Kniep, 1923). These

changes in the incompatibility alleles, repeatedly observed by various workers, however, seemed to occur only in the germling mycelia recently derived from basidiospores, never in well-established mycelia. It has recently been shown beyond any reasonable doubt, by Papazian (1951), that the *A* factor consists of a number of pseudo-alleles at closely linked but distinct loci, that these act together as a physiological unit, and that occasional recombination at meiosis yields new factors. These data are compatible with a composite factor comprising 4 to 10 distinct loci, the upper limits of which range (> 6) could account for the estimated factors in natural populations without necessary recourse to multiple allelomorphic series at any locus (Raper, 1953).

Practically all species of fungi which have sexual cycles may be definitely assigned to homothallism or heterothallism. There are, however, a few species that appear to occupy positions which are intermediate between these two opposed conditions. The yeasts, mentioned above, which are basically heterothallic but which produce frequent, low-viability mutants of the incompatibility factors may be considered to bridge, to some extent, the gap between true heterothallic and homothallic conditions. Mather (1940) has suggested the term *partial heterothallism* for cases of this type.

In a few cases, furthermore, indeterminate patterns of sexuality appear to be more closely allied with homothallism. Outstanding among such forms is the Ascomycete *Glomerella cingulata*, a sexually ambiguous species without peer. Edgerton (1914) described a strong sexual interaction in this species between weakly self-fertile strains. Subsequent and intensive work with *G. cingulata* (Andes, 1941; Edgerton, 1945; McGahen and Wheeler, 1951; Wheeler, 1950; Wheeler and McGahen, 1952) has revealed an extremely complicated pattern of sexuality which results from the interaction of numerous genetic factors, some exhibiting high mutation rates. As currently interpreted (Wheeler and McGahen, 1952), two loci, *A* and *B*, are considered primarily responsible for the basic sexual characteristics, with some twenty other loci modifying the sexual reaction. Two mutant states are known at each of the two primary sexual loci in addition to the two wild-type alleles. Thus all combinations between the three alleles at the two loci, A^+, A^1, and A^2 and B^+, B^1, and B^2, determine nine distinct strains, each having a characteristic pattern of self-sterility or self-fertility on the one hand and interstrain matings on

the other. The major characteristics of these several strains and the complex pattern of interstrain matings are diagrammatically represented in Fig. 5. The sexual characteristics of the various strains may be further modified by mutations at loci other than the primary sexual loci, *A* and *B*, and two of these, F^1 and st^1, have been described in detail (Wheeler and McGahen, 1952). Each of these mutants imposes self-sterility upon each of the normally self-fertile strains but

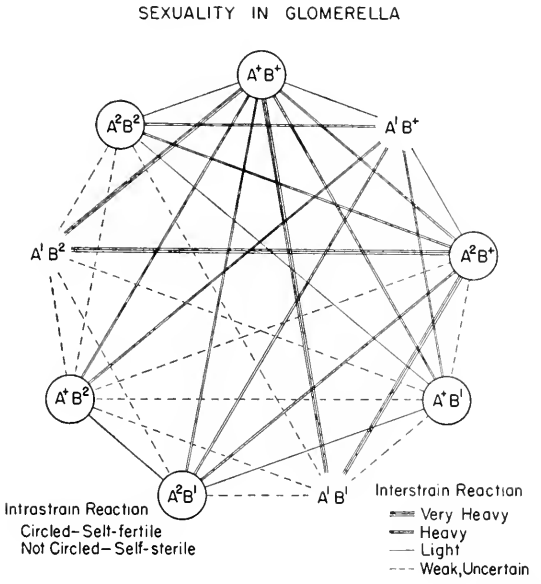

FIG. 5. Intrastrain fertility and interstrain reactions in *Glomerella cingulata*. Three allelomorphs at each of two loci, *A* and *B*, define nine strains, each having a distinct pattern of morphological and sexual characteristics. (Diagramed from data of Wheeler and McGahen, 1952.)

does not interfere with interstrain reactions provided that the two mates carry neither of the mutations in common.

According to the definitions of homothallism and heterothallism adopted here, *Glomerella cingulata* must be considered basically a homothallic species since the self-sterile strains are in each case derived through degenerative, mutative changes from self-fertile, wild-type strains. The enhancement of sexual productivity in interstrain contrasts between self-fertile strains and the occurrence of self-sterile but cross-fertile strains constitute a pattern that falls short of the totality of self-sterility and obligatory cross-breeding of true heterothallism.

Furthermore, the factors at the *A* and *B* loci appear to exert chiefly a quantitative control over intrastrain fertility and interstrain reaction rather than the qualitative control imposed by both sexual factors and incompatibility factors in truly heterothallic species. They differ from sexual factors in that they do not, in any case, determine unisexual strains, and they differ from incompatibility factors in that common factors do not in all cases prevent interstrain mating.

The pattern of sexuality in *Glomerella cingulata* is therefore basically different from any other known among the fungi. It is possible that here we have on display a species in the process of evolving from homothallism to heterothallism, or vice versa, and to accept Mather's concept of partial heterothallism may well be the best that can be done at the present time toward integrating this pattern into the general scheme of sexuality in the fungi.

SEXUAL MECHANISMS

The final aspect that must be considered to give a comprehensive understanding of sex in fungi is the sexual mechanism, the mechanical means by which compatible elements are brought together under the conditions imposed by the particular life cycle and pattern of sexuality involved.

The number of possible combinations of basic sexual mechanisms and developmental histories to be found among fungi precludes the consideration of all significant combinations. Let us rather list a few possible variants at certain critical stages and demonstrate by simple developmental histories the range of variety of specific overall patterns.

Sexual mechanisms may be differentiated on the basis of morphological differences at three critical points in the life cycle. These points are (1) meiosis, (2) the physical union of compatible sexual elements, and (3) the fusion of compatible nuclei.

(1) The immediate products of meiosis, with few possible exceptions among the fungi, are spores of various kinds, such as zoospores, ascospores, and basidiospores.

(2) In spite of the almost endless variety of sexual apparatuses among the fungi they may be considered to belong to four basic types, first recognized by Kniep (1928). Each type comprises definite

groups of plants, but such groupings have very little correlation with the major phylogenetic groupings. The four basic types are:

(a) *Gametic copulation*, in which the two elements brought together in the sexual act comprise uninucleate, free gametes of which both, one, or neither may be motile.

(b) *Gamete-gametangial copulation*, in which one fusing element is a differentiated uninucleate gamete and the other is a differentiated gametangium which produces no discrete, uninucleate gametes. The differentiated gametes may be either ♂ or ♀ depending upon the group.

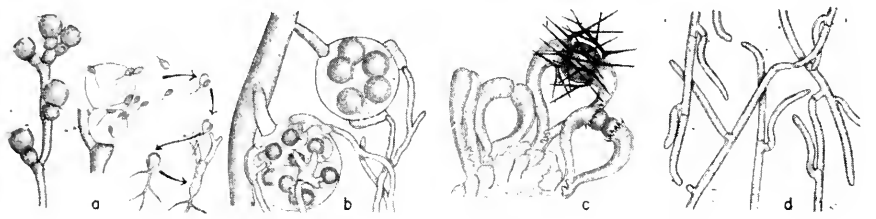

FIG. 6. The four basic modes of sexual fusion in fungi.

(a) Gametic copulation, *Allomyces arbuscula*. Motile gametes originating in ♀ gametangium (above) and ♂ gametangium fuse to form a zygote that germinates directly to produce a diploid plant (lower right). (b) Gamete-gametangial copulation, *Achlya ambisexualis*. Uninucleate gametes, or eggs, in spherical ♀ gametangium are fertilized by ♂ nuclei transferred through tiny tubes from ♂ gametangia; mature, fertilized eggs shown below. (c) Gametangial copulation, *Phycomyces blakesleeanus*. A pair of multinucleate gametangia, produced at the tips of large, arched processes, fuse to form a heavy-walled zygospore surrounded by spines. (d) Somatic copulation, *Schizophyllum commune* (schematic). Two types of hyphal fusion, tip-to-tip and tip-to-peg, are shown in two stages of development, in an early stage of mutual chemotropic attraction at the left and shortly after fusion at the right. The exchange of nuclei in somatic copulation is typically reciprocal, each mate fertilizing the other.

(c) *Gametangial copulation*, in which both fusing sexual elements are differentiated as gametangia; one or many pairs of nuclei may be involved, and the two gametangia may be differentiated as ♂ and ♀ or they may be morphologically indistinguishable.

(d) *Somatic copulation*, in which fusion occurs between undifferentiated vegetative cells; nuclear migration here is frequently reciprocal, each mate fertilizing the other, and the two compatible nuclei usually retain, once brought together, a dicaryotic association for an indefinite period prior to nuclear fusion.

The four basic types of sexual apparatuses, as exemplified in four well-known representative species, are shown, in surface view, in Fig. 6. Each of these types may be found in a wide array of morphological variations, but the basic aspects of the sexual progression, nuclear behavior, and so on are relatively constant in the variants of each type.

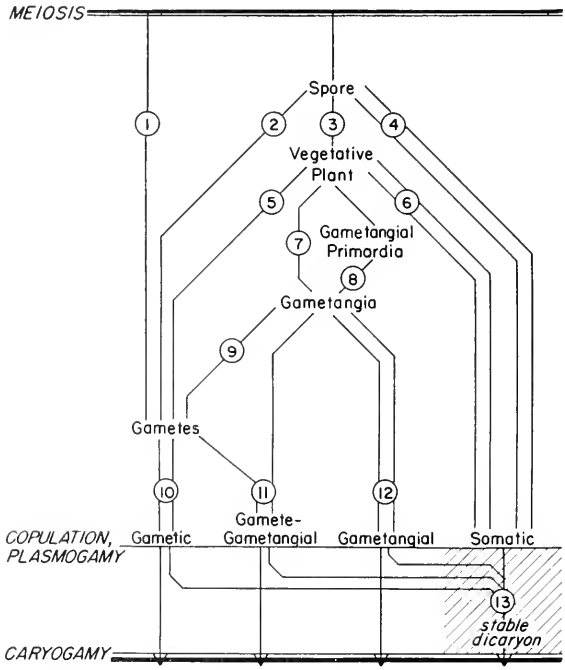

Fig. 7. Summary diagram of developmental sexual histories in fungi. The numbered lines trace developmental variations relating the critical events at meiosis, sexual fusion, and nuclear fusion.

(3) After the fusion of the two sexual elements there exist two possibilities with regard to the subsequent activity of the paired compatible nuclei: they may fuse immediately to establish the diploid phase, or they may become associated in one or more pairs and divide conjugately by mitosis to provide ultimately a large number of paired nuclei which finally fuse to form the definitive nuclei in the asci or basidia.

The developmental patterns relating these cardinal stages in the sexual cycle may be represented by the various pathways indicated in Fig. 7. The specific combination of events at the critical points in

the developmental cycle, meiosis, fusion of sexual elements, and nuclear fusion, defines about as well as is possible the sexual mechanism for any given species. A few well-known forms will be used here to illustrate the range of possibilities and also to demonstrate a shorthand system for designating the sexual mechanism and developmental sequence.

The production of gametes as the immediate products of meiosis may possibly occur in a very few species, members of the Blastocladiales of the aquatic Phycomycetes. A single mitotic division, however, has been reported as interposed between meiosis and the differentiation of gametes in the single species which has been cytologically investigated (Wilson, 1952).

Most species, if not all, produce spores immediately after meiosis, and the further developmental sequence is extremely variable. In a number of cases the fusion of these differentiated spores constitute the sexual act. Of common occurrence in the yeasts is the fusion of ascospores in pairs while still in the ascus to reestablish the diploid phase ((2—10)) (Winge and Laustsen, 1939) or, rarely, a dicaryon ((2—10—13)) (Guillermond, 1940); a similar sexual fusion is known in many smuts, in which sporidia, or basidiospores, fuse to establish a stable dicaryon ((4—13)) (Bauch, 1925; Kniep, 1926).

The spores give rise in other fungi to vegetative thalli or clones of vegetative cells prior to sexual activity. Vegetative cells may participate without any discernible sexual differentiation in either of two ways. In clonal, unicellular forms, such as many of the haploid yeasts, each individual cell is functionally a gamete, and fusion between such cells may be considered a gametic copulatory process ((3—5—10)) (Guillermond, 1940). In a large number of extensively developed mycelial forms, including the majority of the species of the Basidiomycetes, all vegetative cells of the thallus are capable of reciprocal somatic copulation to initiate the dicaryon ((3—6—13)) (Buller, 1924; Kniep, 1920, 1922).

Remaining fungi produce sexual organs or gametangia, and these are almost invariably essential for sexual activity.

The entire vegetative thallus may be differentiated at maturity into one or more gametangia, which may develop further in either of two different ways. The gametangia may undergo internal differentiation to produce uninucleate gametes which fuse in pairs, as in *Blastocladiella* ((3—7—9—10)) (Couch, 1942; Harder and Sörgel,

1938), or the gametangia may fuse without further differentiation, as in numerous monoflagellate Phycomycetes, such as *Siphonaria* ((3—7—12)) (Karling, 1945; Wager, 1913).

In yet other forms the sexual activity is relegated to gametangia which originate *de novo* as extra-vegetative structures. Three different patterns of further sexual development are found in these forms: gametangia may produce gametes which fuse in pairs, as in *Allomyces* ((3—8—9—10)) (Emerson, 1941; Kniep, 1929); gametangia of one sexual sign may produce differentiated gametes which react sexually with gametangia of the opposite sexual sign, as in *Achlya* (Bary, 1881; Raper, 1939) ((3—8—9—11)) or *Neurospora* (Backus, 1939; Shear and Dodge, 1927), and many rusts ((3—8—9—11—13)) (Buller, 1950; Craigie, 1942); the gametangia, morphologically differentiated in respect to sexual sign or not depending upon the species, may fuse directly with one another, as in *Mucor* and *Rhizopus* ((3—8—12)) (Blakeslee, 1904, 1920; Burgeff, 1924; Krafczyk, 1935) or *Pyronema* (Claussen, 1912) and *Ascobolus* (Dodge, 1920) ((3—8—12—13)). The developmental pattern of Phycomycetes and most of the lower Ascomycetes (Hemiascomycetes) differs from that of the higher Ascomycetes (Euascomycetes) and Basidiomycetes following plasmogamy in that their nuclei fuse immediately, whereas in the higher groups dicaryons are regularly formed.

The dozen or so developmental sexual histories and sexual mechanisms sketched here are the more common types encountered among the fungi. Most forms fit comfortably in one or the other of these patterns, but there are a number of cases that would be categorized variously according to the preferred interpretation of structural and behavioral characteristics.

Relatively little is known of the underlying physiological and biochemical aspects of sexual development and sexual activity. It has long been recognized that an intimate relationship exists between nutritional requirements and metabolic processes on the one hand and sexual differentiation and activity on the other (Coker, 1923; Dodge, 1920; Klebs, 1898, 1899, 1900; Molliard, 1903; Raper, 1952). The knowledge of such relationship, however, has commonly been arrived at quite empirically, and only in a few cases is there a glimmer of the underlying mechanism.

Intraspecific chemical regulators of sexual processes, sexual hor-

mones, were first demonstrated in the fungi, by Burgeff in 1924, in *Mucor mucedo*, a close relative of "black bread mold." Since that time sexual hormones have been demonstrated or postulated on good experimental evidence in various groups of fungi exclusive of Basidiomycetes (Backus, 1939; Bishop, 1940; Krafczyk, 1935; Raper, 1939, 1940, 1951, 1952; Zickler, 1952). In only a single case, however, has an understanding of the over-all role of hormones as the coordinating agents in the sexual process been approached.

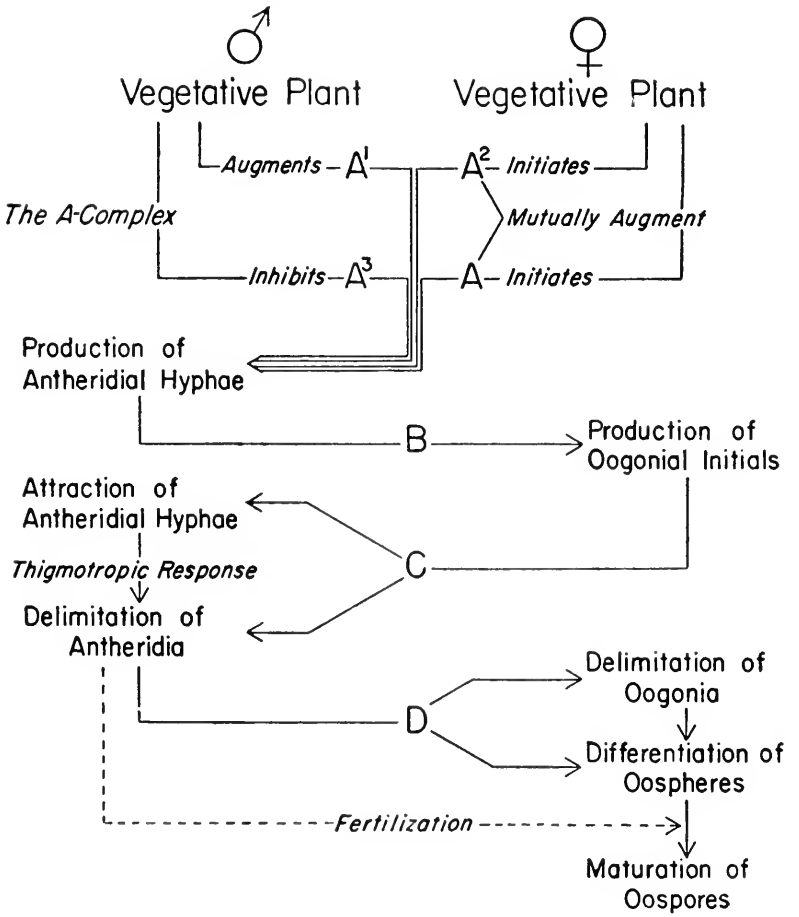

FIG. 8. The hormonal mechanism that coordinates the sexual interaction between male and female plants in heterothallic species of *Achlya*. Each line designated by a letter indicates a specific hormone, its origin, and its specific activity. (From Raper, 1951.)

In two heterothallic species of *Achlya*, a common genus of the aquatic Phycomycetes, it has been demonstrated that the initiation and coordination of each of a chain of interdependent reactions, which together constitute the sexual process, depend upon one or more specific chemical agents (Raper, 1940, 1951, 1952). The hormonal mechanism, as currently interpreted in these plants, is shown in Fig. 8. Essentially, the mechanism consists of a minimum of seven distinct hormones, four secreted by the ♂ and three by the ♀, which induce and regulate a series of reactions alternately in the ♂ and ♀; each reaction is chemically dependent upon and quantitatively regulated by the reaction immediately preceding. The entire sexual process, with the exception of the physical transfer of ♂ nuclei in the act of fertilization, has been shown to be coordinated in this manner. None of the sexual hormones has been either isolated in chemically pure form or identified.

CORRELATIONS OF LIFE CYCLES, SEXUALITY, AND SEXUAL MECHANISMS

The three principal facets of sex in fungi having been examined in some detail, it should now be possible to attempt some correlation between them and to approach some sort of integrated picture of the problem in its entirety. Such a correlation is attempted in Table I. In this table are shown the more frequent combinations of life cycle, sexuality, and developmental sexual history, as well as examples of these, chosen wherever possible, from those fungi which are relatively well known to biologists other than mycologists. Patterns of sexuality and developmental histories have been bracketed within each type of life cycle; indication of the actual combinations which are known to occur would serve only to obscure the important conclusions that may be drawn from this body of information.

The most striking fact that emerges here is also one of considerable significance, namely, no rigid and inclusive correlation exists between the various combinations of sexual features and the universally accepted phylogenetic groupings. To illustrate this: homothallism, possibly the most primitive of the various pattern of sexuality, occurs in conjunction with all types of life cycles, with practically all developmental histories, and in every major grouping from the most primitive Phycomycetes to the most highly evolved Basidiomycetes.

TABLE I

COMBINATIONS OF LIFE CYCLES, PATTERNS OF SEXUALITY, AND SEXUAL MECHANISM OCCURRING IN FUNGI

Life Cycle	Pattern of Sexuality	Sexual Mechanism	Example
A	None	None	
B	0	3—7—9—10	*Synchytrium*
		3—7—12	*Siphonaria*
	I	3—8—9—10	*Monoblepharis*
		3—8—9—11	*Achlya, Dictyuchus*
	II	3—8—12	*Mucor, Eremascus*
		3—5—10	*Zygosaccharomyces*
	V		
C	0		
	III	3—8—12—13	*Pyronema, Ascobolus*
		3—8—9—11—13	*Neurospora, Hypomyces*
	IV		
D	0		
	III	3—5—10—13	*Saccharomyces* sp.
		3—8—9—11—13	Rusts
	V	3—6—13	Gasteromycetes, rusts Hymenomycetes, smuts
	VI		
E	0		
	V	4—13	Smuts
F	0		
		3—8—9—10	*Allomyces*
	I	3—7—9—10	*Blastocladiella*
		3—5—10	*Saccharomyces* sp.
	V		
G	V	2—10	*Saccharomycodes*

In spite of the lack of any paralleled progression from simple to complex life cycles, patterns of sexuality, and sexual mechanisms and morphological characteristics, there are certain tendencies which are worthy of mention. There is a very loose correlation between morphological specialization and each of the three major facets of sexuality.

Life cycles, on the whole, become progressively more complex

proceeding from primitive to more highly specialized groups. The haploid cycle predominates in the Phycomycetes, the haploid with restricted dicaryon cycle in the Ascomycetes, and the haploid-dicaryotic and the dicaryotic cycles in the Basidiomycetes. The exceptions to this generalization, however, are numerous, and, when considered in respect to probable phylogenetic lines, they are more than a little puzzling. Haploid-diploid and diploid cycles, those cycles which would seem to be the most highly advanced of all, occur only in one group of aquatic Phycomycetes and in a number of yeasts.

The pattern of sexuality in heterothallic species shows a similar progression. The role of sexual factors as the critical determinants of mating behavior is for the most part limited to the more primitive forms, particularly the aquatic Phycomycetes, although there are several cases of strict sexual dimorphism among the Ascomycetes. A single pair of incompatibility factors at a single locus possibly occurs in the more complex Phycomycetes, the Mucorales, is very common among the Ascomycetes, and is frequently encountered in two large groups of Basidiomycetes, the rusts and smuts. The essentiality of differentiated sexual organs would seem to follow similar broad phylogenetic lines: they are present and functional in practically all Phycomycetes and most Ascomycetes, except the yeasts, and absent in the Basidiomycetes, except the rusts.

Multiple incompatibility allelism is known only among members of the most highly evolved fungi, the Basidiomycetes, and is unquestionably the most efficient of all means to insure for those species possessing it the maximal benefit to be derived from genetic recombination.

This might suggest a sort of coupling of the culmination of incompatibility control of mating behavior with a high degree of morphological development, particularly in the tetrapolar species, were it not for the fact that species which are obviously closely related to such tetrapolars are strictly homothallic and get along quite nicely with no restrictions imposed by incompatibility factors.

Sexual mechanisms and developmental histories are fairly constant within groups at the level of orders. There is also a tendency, in passing from primitive to highly evolved forms, to progress from gametic copulation through the loss of gametic differentiation in one sex or the other (gamete-gametangial copulation), to loss of gametic differentiation in both sexes (gametangial copulation), to the loss of

sexual organs competely and the ability of all vegetative cells to participate in sexual fusions (somatic copulation). The developmental histories of sexual aspects *per se*, furthermore, show a marked tendency toward simplification, probably through reduction, in most of the more highly evolved groups.

What, then, can rationally be said of the probable origin of the array of sexually different types that exist in the fungi at the present time? Any attempt to rationalize six different life cycles, homothallism, six distinct types of heterothallism, and four basic sexual apparatuses in the particular combinations in which they exist rapidly runs afoul of difficulties that appear to be insurmountable. This can be illustrated by testing two antithetical propositions. To start from the assumption, as many have, that homothallic forms having gametic copulation represent the primitive type from which all else has been derived must totally ignore the random distribution of homothallic and heterothallic species in every group of the fungi. The various patterns of heterothallism would necessarily have been independently evolved, at the appropriate levels, from the main stem of homothallic forms. How, then, is it possible to account for homothallic, bipolar, and tetrapolar species in a single genus, such as *Coprinus*, the members of which are obviously closely related phylogenetically, except that heterothallism, of two very precise types common throughout the much larger group to which it belongs, the Hymenomycetes, be independently evolved in this genus? Essentially the same situation obtains in every phylogenetic grouping and *in toto* constitutes a compelling argument against the derivation of heterothallism from homothallism. The alternative proposition, that the variously expressed forms of homothallism were derived from heterothallic ancestral forms, encounters equally serious difficulties. Most important of these difficulties are the twin necessities of (1) the origin of the various types of heterothallism from some one primitive heterothallic type and (2) the independent origin of homothallism in each homothallic species. The latter might conceivably occur in sufficient frequency to account for the large number of homothallic forms, but it would appear most unlikely. More serious is the difficulty of the evolution of the various types of heterothallism from other heterothallic types; the various types would appear to be much more closely related to the corresponding homothallic types within any phylogenetic grouping than to each other. Nor would espousal of

polyphyletic origin of the various groups make less difficult the rationalization of the existing sexuality of the fungi. There would remain the same difficulties mentioned above, only partially obscured by the introduction of additional uncertainties.

The nearest approach to a biologically feasible system which could account for the existing sexual complexity would reject both of the simple propositions stated above, but would constitute a partial synthesis of the two. The following suggestion would appear successfully to avoid the various objections to simpler derivation.

Homothallic forms, having gametic copulation, could well have given rise to a primitive homothallic group within which occurred the major evolutionary changes in life cycles and sexual mechanisms —the progressions from predominantly haploid to predominantly dicaryotic cycles and from gametic through gamete-gametangial and gametangial to somatic copulation. Each distinct type of heterothallism could have been independently evolved, at the appropriate level, from this primitive homothallic stem. Once the several types of heterothallism had evolved, existing homothallic species could well have been derived from them by relatively simple means. For example, several kinds of chromosomal aberrations causing a slight dislocation of the locus of a sexual or incompatibility factor could result, after transfer of the factor to the homologous chromosome through crossing-over, in genetically stable self-fertile individuals. Such self-fertile individuals, being assured the immediate benefits of sexual reproduction and being genetically isolated, might well prosper and constitute a significant factor in speciation in the fungi.

This scheme, unduly indirect at first glance, would account for numerous awkward facts implicit in any simpler hypothesis. The more important of these are: (1) the occurrence of homothallic and heterothallic species within groups having unique features which must have been evolved relatively recently, the genera *Achlya* and *Coprinus* for example; (2) the occurrence of the same precise types of heterothallism throughout large groups embracing widely divergent, morphological characters, the Hymenomycetes for example, with tetrapolar and bipolar species, the latter possibly a transitional stage between the former and homothallism, and the aquatic, biflagellate Phycomycetes with their peculiar multiple-sexual-strain type of heterothallism; (3) the lack of strictly heterothallic species that can be reasonably interpreted as intermediate between two distinct types

of heterothallism. Species of indeterminate sexuality can be cited in abundance, but they can be more easily rationalized as intermediate between the various types of heterothallism and homothallism.

The fungi, viewed from this particular bias, present many admittedly puzzling features that offer, however, no recognized outright contradiction to the essential idea of homo→hetero→homothallism. The lack of heterothallic species in a few large and long-established groups—the *Aspergillus-Penicillium*, etc., complex—is a case in point. Here, however, there is a majority of sexually sterile species, and the entire group may well represent a vestige of the primitive homothallic stem, well along its degenerative course toward unequivocal inclusion in the Fungi Imperfecti.

Further speculation along these lines for the present time, however intriguing, cannot produce a wholly satisfactory answer to the problem. There is as yet insufficient information to permit the postulation of a completely feasible system that would account for the complicated sexual situation now existing in the fungi. For the time being, we can only recognize the situation for what it is, and marvel.

SUMMARY

Sexual reproduction in fungi displays a tremendous range of variability. Recognition of three distinct features is necessary adequately to describe the role of sex in any single species. These facets are: (1) the life cycle, in which the critical events are synonymous with the initiation, the progression, and the termination of the essential sexual process; (2) the pattern of sexuality, which determines self-fertility or self-sterility, and in the latter case the exact pattern of inter-individual fertility; and (3) the sexual mechanism, the means by which sexual fusion is accomplished within the restrictions imposed by the life cycle and pattern of sexuality. Seven types of life cycles, seven distinct patterns of sexuality, and about a dozen or more basic kinds of sexual histories allow, in combination, a bewildering array of distinct sexual types.

Although there is a very loose correlation between morphological specialization and each of the three major facets of sexuality, no rigid correlation appears to exist between phylogenetic groupings and the various combinations of sexual features. A possible scheme to rationalize the sexual situation as now existing rejects the simple der-

ivation of heterothallism from homothallism, or vice versa, in favor of an indirect derivation of homothallism from the various types of heterothallism, each of which in turn was independently evolved from primitive homothallic forms. Present understanding is insufficient, however, to permit the postulation of a completely feasible system to account for the existing sexual complexity of the fungi. The multiplicity of sexual types and forms which contribute to this complexity, however, provides a wealth of material for those who would seek exact specifications in the tools for the elucidation of many basic phenomena of universal biological importance.

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Sexual Reproduction in Diatoms

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In diatoms reproduction is most frequently accomplished by vegetative division. Ordinary somatic mitosis occurs. This type of reproduction in a healthy culture of diatoms may occur several times during a twenty-four-hour period. The rate is dependent on the kind of diatom and the cultural conditions present.

Of much less frequent occurrence are auxospores. An auxospore is a resting cell which usually develops from a zygote. This process has been occasionally observed in many of the genera of diatoms but in only a comparatively few species.

To date there seems to be little correlation between the type of auxospore formation and the taxonomic relationship of the species. Indeed, several different types of auxospore formation have been observed in different varieties and forms of the same species.

Often auxospore formation seems to be the result of sexual processes. However, in many cases, as Sonneborn (Calkins and Summers, 1941) has said, perhaps we should "abandon the concepts of male and female in unicellular organisms and view sexual union as brought about by copulation-conditioning factors."

The cause of auxospore formation seems to be a combination of cell size and external environmental conditions. All species of diatoms are known to vary in length. According to Geitler (1932, 1935), auxospore formation occurs only when the cells of a taxon are of a certain length, characteristic for each taxon. Often this range in length is fairly wide, and again it is very narrow depending on the taxon. This range is very constant for each taxon. If auxospore formation does not occur when the cells are of the correct size, it never occurs. The cells often become smaller and morphologically quite changed, especially in culture.

Auxospore formation in diatoms has been a frequent subject of research during the nineteenth and twentieth centuries. Although the

complete process has been observed only in a relativly few species, it is partially known in a great many more. Because many species are very small and their chromosomes are numerous, detailed cytological studies are difficult. The best summaries of this work have been made by Geitler (1932) and Fritsch (1935).

The diatoms may be divided into two distinct taxonomic groups. Methods of auxospore formation characteristic of each taxonomic group will be treated under the appropriate heading.

CENTRALES

The Centrales are usually regarded as the more primitive group of diatoms because they are found in earlier geological strata than the Pennales.

The most common type of reproduction is fission after mitosis Microspores are also produced in this group, but as yet it has not been proved that they are reproductive cells. They are formed by the division of the protoplast. Each microspore has two flagella.

Other types of reproduction are those which result in auxospore formation. Little is as yet known concerning the nuclear reorganization that takes place in their formation.

The simplest type of auxospore formation is that which has been reported in the genus *Melosira*. The two halves of the wall of the cell are pushed apart by the protoplast. Over the protoplast is secreted a slightly silicified pectic membrane called the perizonium. After a lapse of time new valves and connecting bands are formed inside the perizonium, and a new individual results. In *Melosira nummuloides* the auxospore lies outside the theca of the parent. There is considerable difference of opinion about the nuclear phenomena that accompany auxospore formation in this genus (Karsten, 1897; Geitler, 1932).

In *Biddulphia mobiliensis* (Bergon, 1907) cell division immediately precedes auxospore formation. The two daughter protoplasts escape from the parent cell and form a pair of spores. Little is known about the nuclear behavior during this process.

In *Chaetoceras cochlea* (Fritsch, 1935) the auxospores arise laterally on the parent cell by budding. A similar lateral formation of auxospores also takes place in some species of Rhizosolenia (Schütt, 1893).

Iyengar and Subrahmanyan (1944) observed in *Cyclotella meneghiniana* zygote formation and subsequent auxospore formation. They considered this the result of autogamy and automixis.

Recently von Stosch (1950) has reported oögamy in *Melosira varians*. He has observed the filaments to be of two types, narrow male filaments and broader female filaments. In about 9 per cent of the cases where the size of the filaments overlap they are found to be monoecious.

The antheridial cells undergo reduction division. Two spermatozoid mother cells bud off the main cytoplasm in the second metaphase and then divide to form four spermatozoids. Flagella have not been seen, but it is presumed that the spermatozoids are flagellated.

The young oögonia resemble vegetative cells in shape, yet their plastids and chromatophores are larger. Meiosis takes place in the usual manner. In the first telophase one of the daughter nuclei gradually aborts. In the second telophase one of the nuclei becomes pycnotic. The two "polar bodies" thus formed are gradually absorbed.

The oögonium swells, and a strip of naked protoplasm is exposed between the margins of the epitheca and hypotheca. The spermatozoid may enter the oögonium as early as anaphase I or as late as the maturation of the egg nucleus. Later the zygote is released and swells to form a subglobose auxospore.

Geitler (1952e) has recently observed in *Cyclotella* sp. sexual reproduction similar to that described for *Melosira varians*. There seems to be no morphological difference in the filaments that form the eggs and sperms. In spermatogenesis there occurs a first meiotic and a second meiotic division which result in the formation of four sperms. The sperm enters the oögonium by the time diakinesis takes place. As a result of a first meiotic and second meiotic division of the nucleus, one egg nucleus and two pycnotic nuclei are produced. The sperm nucleus migrates during interkinesis from the peripheral region of the egg to the center, where fusion with the egg nucleus takes place after the second meiotic division is complete. A metagamic mitosis occurs between the formation of the first and second shells of the cell formed in the germination of the auxospore. Although metagamic mitosis has been observed many times in the Pennales, this is the first time it has been observed in the Centrales.

PENNALES

Auxospore formation is usually initiated by the coming together of the mother cells. Of course, in cases of apomixis and automixis, this may not occur. These cells are diplonts and usually considered not to be sexually differentiated. However, in *Navicula halophila* (Subrahmanyan, 1945), *Synedra ulna* (Geitler, 1939), and *Synedra rumpens* var. *fragilarioides* (Geitler, 1952f), it has been reported that one cell produces two passive gametes, and one produces two active gametes. This might indicate that the two mother cells are sexually differentiated.

These cells may be about the same size as in *Rhoicosphenia curvata* (Geitler, 1952a) or may be very unequal in length as in *Eunotia arcus* (Geitler, 1951b). Sometimes more than two cells come together for auxospore formation, as in *Gomphonema parvulum* var. *micropus*, *Achnanthes lanceolata*, and *Navicula seminulum* (Geitler, 1932). In *Anomoeoneis exilis* (Geitler, 1949b), *Navicula radiosa* (Geitler, 1952d), and *Synedra ulna* (Geitler, 1939), several cells often come together. Usually these cells are not sister cells, but in *Navicula seminulum* they may be (Geitler, 1932).

In most cases both cells are active and approach each other. In *Gomphonema parvulum* var. *micropus* one cell is attached by a gelatinous stalk and only one is mobile (Geitler, 1932). These cells assume various positions on contacting each other. The most common position is for them to lie opposite and parallel with their girdle faces in juxtaposition. The cells of *Gomphonema parvulum* var. *micropus* orient themselves so that the apical pole of one cell is opposite the basal pole of the other. Owing to the curvature of the frustule of *Rhoicosphenia curvata*, the cells may be in various positions (Geitler, 1952a).

Jelly is produced by both cells in varying quantities. Liebisch (1929) considered this jelly part of the hydrated pectin membrane of the cell. Other research indicates that it has a different origin. It is evident that more work needs to be done on this point.

This jelly is usually homogeneous and varies in thickness according to the kind of diatom. In *Achnanthes longipes*, *Navicula didyma*, and *Pleurosigma nubecula* it is fairly soft, whereas in *Frustulia rhomboides* var. *saxonica* and *Achnanthes lanceolata* it is relatively stiff.

Geitler (1932) thinks that tensions which develop in this jelly as a result of its viscosity determine to some extent the movement of the gametes and the position of the developing zygote and auxospore.

In many species a large quantity of jelly is produced and the copulating cells are embedded in it. However, in other species the jelly is only represented by the formation of a copulation tube or tubes. In *Eunotia arcus* and *Eunotia flexuosa* (Geitler, 1951c) the copulation tube is formed by papillae which are formed by each of the two copulating cells.

The number and shape of the tube or tubes may vary. Usually only one tube is formed; however, in *Frustulia rhomboides* var. *saxonica* (Geitler, 1949b) two tubes are present. The tube may be long and narrow as in *Eunotia arcus* (Geitler, 1951b) or short and narrow as in *Nitzschia subtilis* and *Amphipleura pellucida* (Geitler, 1932, 1952c). Usually the tube is formed at or near the middle of the longitudinal axis. In *Frustulia rhomboides* var. *saxonica* (Geitler, 1949b) one tube is found near each of the apices of the cell. In *Eunotia arcus* and *Eunotia flexuosa* (Geitler, 1951a,b) a tube may be formed at either end of the cell or on the girdle face of the dorsal or ventral side of the valve.

GAMETOGENESIS

So far as is known, meiosis occurs by means of two meiotic divisions. The spindle lies in the pervalvar axis of the cell. Sometimes it is tipped slightly to one side as in *Amphipleura pellucida* (Geitler, 1952c). The prophase of the first meiotic division appears to be normal, but, owing to the high numbers of chromosomes and the small size of the nucleus in diatoms, it is difficult to make out all the stages. The two nuclei resulting from the first meiotic division are usually normal. However, in Cocconeis one of the nuclei forms a polar body. It is believed to be an aborted gamete. In *Navicula seminulum* (Geitler, 1932), one nucleus becomes pycnotic and is ejected, whereas in *Navicula cryptocephala* var. *veneta* one nucleus is reabsorbed (Geitler, 1952f).

Cytokinesis usually follows the first meiotic division. Previous to this the chloroplasts usually have divided. The cell membrane is developed in a plane parallel to the valves of the cell.

The second meiotic division usually follows cytokinesis. One of

the two nuclei formed in this division usually degenerates. Sometimes it is reabsorbed in the protoplasm as in *Gomphonema parvulum* var. *micropus* (Geitler, 1932). In other instances it becomes pycnotic and remains in the gamete as in *Eunotia arcus* and *Eunotia flexuosa* (Geitler, 1951a, b) or is cut off as in *Amphipleura pellucida*. However, in *Navicula radiosa* (Geitler, 1952d) and *Navicula cryptocephala* var. *veneta* (Geitler, 1952f) both nuclei remain functional, so that each gamete has two functional nuclei.

One or two functional gametes may develop in each cell. If two gametes develop, they usually change position. Instead of lying parallel to the valves in the position in which they are formed, they come to lie one above the other when viewed from the apex of the cell. In *Navicula radiosa* (Geitler, 1952d) this change of position of the gametes does not occur.

One notable exception to this type of gamete formation is that found in *Eunotia arcus* and *Eunotia flexuosa* (Geitler, 1951a,b,c). In these species there is a transverse differentiation of protoplasts. In *Eunotia arcus* one chloroplast becomes very large, while in *Eunotia flexuosa* they both move to the same side of the cell. The spindle of the first meiotic division is formed so that one pole is close to the epitheca. Thus a very unequal cell division takes place. The larger cell develops into a gamete. The smaller cell forms what Geitler calls a "remaining cell." Geitler thinks that this remaining cell, by affecting the osmotic pressure of the cell, brings about the movement of the gamete. After cytokinesis occurs, the second meiotic division follows in both the gamete and the "remaining cell." One nucleus in each degenerates.

Another exception is, for example, in *Cymbella ventricosa* var. (Geitler, 1932) where parthenogenesis occurs. In such cases reductional division does not take place.

Sometimes it happens, as in *Eunotia arcus* (Geitler, 1951b), that the cells resulting from meiosis develop shells and become vegetative cells rather than gametes. This phenomenon has been observed several times in diatoms.

The sex differentiation of gametes, if it occurs, takes place during meiosis. Geitler (1932) thinks that the anisogamy recognized by the difference in size and movement is more apparent than real. He states that movement is due to tensions which develop in the cell. This is well described for *Amphipleura pellucida* (Geitler, 1952c). The first

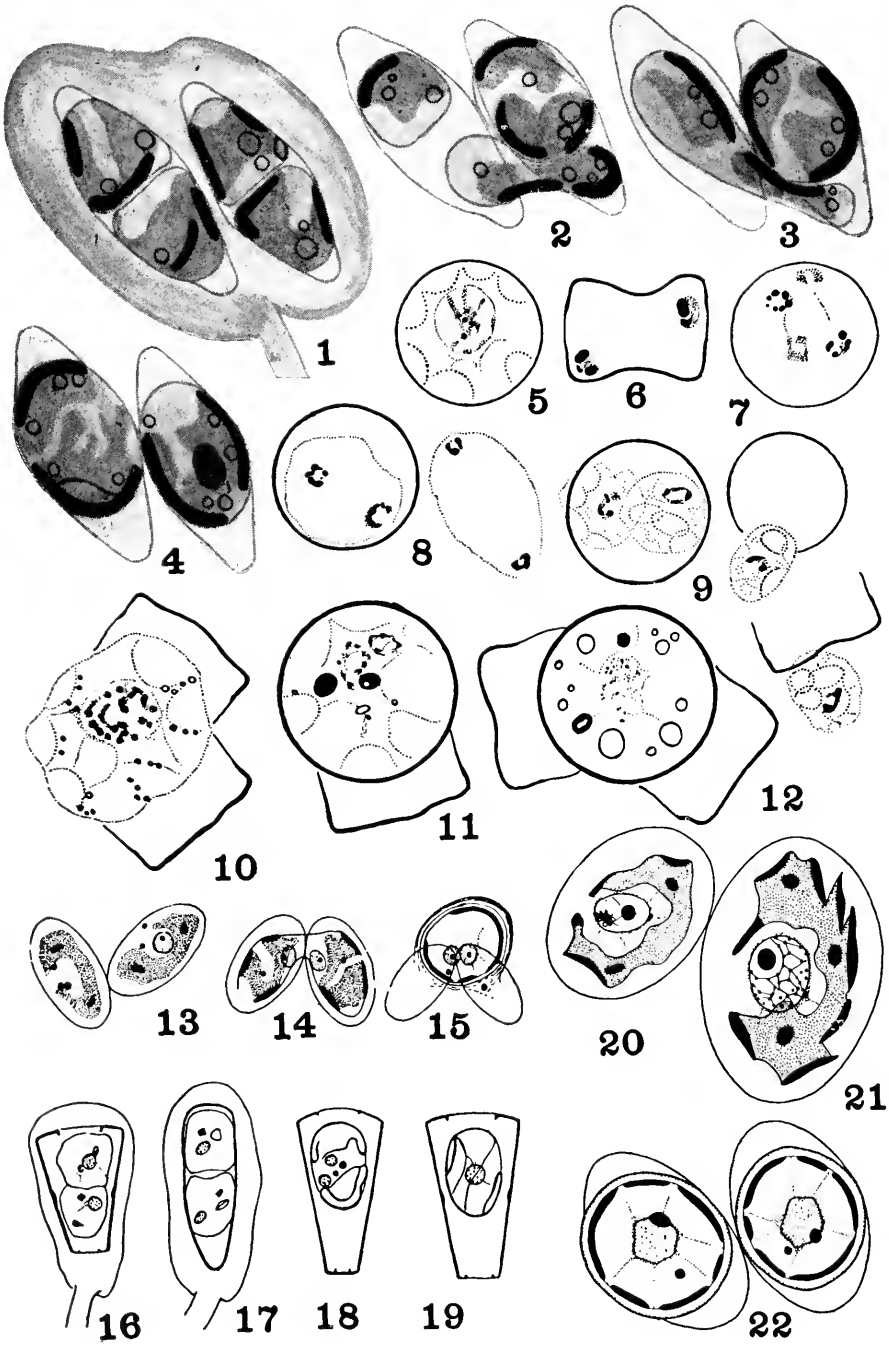

PLATE I

gamete to mature is the one that starts the movement. Carefully controlled experiments are necessary to determine the true condition.

FUSION OF GAMETES

The fusion of gametes in diatoms is of three types. Isogamous fusion is the result of equal movement of the gametes or of copulation of the gametes with both of them *in situ*. Anisogamous fusion is the result of unequal movement of the gametes. Autogamous fusion is the result of the fusion of two gametes in the same mother cell.

Isogamous fusion may be of three types. In one type, as in Amphora and Denticula (Geitler, 1932), the two gametes from each

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Figures 1 to 12 and 16 to 29 are taken from various papers by Lothar Geitler. Figures 13 to 15 and 20 to 22 after Fritsch.

Fig. 1. *Gomphonema parvulum* var. *micropus* (Kütz.) Cl., two mother cells in copulation jelly with two gametes in each cell (× 750, approx.).

Fig. 2, and 3. *Gomphonema parvulum* var. *micropus* (Kütz.) Cl., showing the fusion of gametes (× 750, approx.).

Fig. 4. *Gomphonema parvulum* var. *micropus* (Kütz.) Cl., one zygote in each mother cell (× 750, approx.).

Fig. 5. Spermatogenesis in Cyclotella sp., beginning of first meiotic division (× 900, approx.).

Fig. 6. *Cyclotella* sp., telophase of first meiotic division (× 900, approx.).

Fig. 7. *Cyclotella* sp., telophase of second meiotic division (× 900, approx.).

Fig. 8. *Cyclotella* sp., daughter protoplasts (× 900, approx.).

Fig. 9. *Cyclotella* sp., spermatozoa (× 900, approx.).

Fig. 10. *Cyclotella* sp., formation of oögonium (× 900, approx.).

Fig. 11. *Cyclotella* sp., mature egg cell with sperm nucleus (× 900, approx.).

Fig. 12. *Cyclotella* sp., zygote after fusion of sperm and egg nuclei (× 900, approx.).

Fig. 13. *Cocconeis placentula* var. *klinoraphis* Geitler, gamete formation (× 500, approx.).

Fig. 14. *Cocconeis placentula* var. *klinoraphis* Geitler, fusion of gametes (× 500, approx.).

Fig. 15. *Cocconeis placentula* var. *klinoraphis* Geitler, auxospore formation (× 500, approx.).

Figs. 16 and 17. *Gomphonema constrictum* var. *capitatum* (Ehr.) V. H., two gametes in each cell (× 400, approx.).

Fig. 18. *Gomphonema constrictum* var. *capitatum* (Ehr.) V. H., zygote resulting from automixis, nuclei not fused (× 400, approx.).

Fig. 19. *Gomphonema constrictum* var. *capitatum* (Ehr.) V. H., zygote with nucleus fused (× 400, approx.).

Figs. 20 and 21. *Cocconeis placentula* var. *lineata* (Ehr.) Cl., nuclear reorganization in two approximate cells (× 500, approx.).

Fig. 22. *Cocconeis placentula* var. *lineata* (Ehr.) Cl., auxospore resulting from automixis (× 500, approx.).

cell move into the jelly mass between the cells. In *Rhopalodia gibba* (Klebahn, 1896) the jelly is restricted and appears as a bridge between the two cells. Two zygotes are formed in this bridge. This is believed to be the most primitive type of auxospore formation.

Isogamous fusion may also take place within a copulation tube. In this type the zygote is formed within the tube. This type of fusion occurs in *Eunotia arcus* and *Eunotia flexuosa* (Geitler, 1951a,b). In *Navicula radiosa* each half of each mother cell rotates through an arc of 90 degrees and the two gametes copulate *in situ* (Geitler, 1952d).

Anisogamous fusion occurs if one gamete is active and the other is passive. As in isogamous fusion this may occur with or without a copulation tube. In *Gomphonema parvulum* var. *micropus* usually four gametes are involved. One gamete migrates into the other mother cell and fuses with the passive gamete. This stimulates the other gamete to move out and into the first mother cell. As a result two auxospores are formed. Of less frequent occurrence in *Gomphonema parvulum* var. *micropus* is the production of only one zygote from two gametes. Of rare occurrence is the production of one zygote from three gametes (Geitler, 1932).

Of common occurrence in anisogamous fusion is the production of a copulation tube. Depending on the species of diatom, one or two tubes may be produced.

Usually one tube is produced, as in many species of Nitzschia in which the gametes pass in succession through the tube. If two tubes are present, as in *Frustulia rhomboides* var. *saxonica*, the two fusions may take place at the same time (Geitler, 1949b).

An unusual type of anisogamous fusion is that reported for *Navicula halophila* (Subrahmanyan, 1945), *Synedra ulna* (Geitler, 1935), and *Synedra rumpens* var. *fragilarioides* (Geitler, 1952f). In these species two active gametes are formed in one mother cell and two passive gametes in the other mother cell. The resulting fusion produces two auxospores in the same mother cell. No copulation tube is formed.

Automixis is not common in diatoms. Several cases which need further investigation indicate that this is the means of reproduction. In no case is the nuclear behavior thoroughly understood. What seems to be a true case of autogamy is described for *Amphora normanii*

(Geitler, 1935), in which one auxospore is formed from a single cell. The protoplast contracts, and two nuclei, two nucleoli, and two chromatophores are formed. The valves of the cell are spread apart, and the protoplast is transformed into an auxospore. Later there is found only a single nucleus with a nucleolus.

A modified type of autogamy occurs in *Achnanthes subsessilis* and *Gomphonema constrictum* var. *capitata* (Karsten, 1897, Geitler, 1952b). Within a single cell two gametes are formed which later fuse to form a single protoplast which is transformed into an auxospore.

Parthenogenesis is a method of auxospore formation in *Cocconeis placentula* var. *lineata* (Geitler, 1932). The nucleus of the parent cell goes through two divisions, which correspond to the two meiotic divisions except that reduction in chromosome number does not occur. Polar bodies are formed. The protoplast then becomes transformed into an auxospore. Parthenogenesis is also known to occur in one of the varieties of *Cymbella ventricosa*.

Asexual auxospore formation has been reported for *Synedra affinis* (Karsten, 1897) and *Rhabdonema arcuatum*. In these species the mother cell divides by mitosis to form two daughter cells. These protoplasts, instead of developing normal vegetative walls, become auxospores. Further cytological investigation is needed to make sure that this is truly asexual formation of auxospores (Fritsch, 1935).

Sometimes two types of auxospore formation occur within a single mass of copulating cells. For instance, in *Gomphonema parvulum* var. *micropus* three cells come together. One cell forms an auxospore by automixis and the other two produce auxospores by heteromixis (Geitler, 1932).

The time interval for the fusion of gametes varies greatly. In *Navicula seminulum* the fusion of gametes takes 2 to 3 minutes, whereas in *Amphipleura pellucida* the process takes an hour (Geitler, 1932, 1952b).

DEVELOPMENT OF ZYGOTE AND AUXOSPORE FORMATION

On fusion of the gametes the zygote starts to develop. The fusion of the nuclei is often delayed until the auxospore is developed. In *Navicula radiosa* (Geitler, 1952d), *Navicula cryptocephala* var. *veneta* (Geitler, 1952f) there are two pairs or four functional nuclei.

During auxospore development one pair fuses and the other pair degenerates.

The zygote may be found in various positions. As a result of isogamy it is formed between the mother cells. The polar axis of the zygote is at right angles to that of the mother cells. As a result of anisogamous fusion the zygote is first formed in the mother cell. When two zygotes are formed, one is usually produced in each mother cell. However, in *Navicula halophila* (Subrahmanyan, 1945), *Synedra ulna* (Geitler, 1939), and *Synedra rumpens* var. *fragilarioides* (Geitler, 1952f) the two zygotes are produced in the same mother cell. Likewise in automixis and parthenogenesis the zygote is first formed in the mother cells. Later it migrates out of the mother cell. Usually in anisogamous fusion the long axis of the auxospore is parallel to that of the mother cell, whereas in isogamous reproduction the long axis of the auxospore is perpendicular to the long axis of the mother cells. However, if the jelly surrounding the copulating cells is relatively thin, the auxospores may vary somewhat in position. Geitler (1932) believes that this interesting correlation of the position of the auxospore with type of reproduction is a result of tensions developed within the jelly rather than a result of the type of gametes.

The zygote elongates in the formation of the auxospore. In this process the zygote membrane often breaks and appears as caps on the ends of the auxospore, as in *Frustulia rhomboides* var. *saxonica* (Geitler, 1949b). In *Anomoeoneis exilis* the zygote membrane persists as laminations over the poles of the auxospore (Geitler, 1949b). In *Nitzschia fonticola* (Geitler, 1932) the zygote membrane is elastic and does not break.

The perizonium, which is the auxospore membrane, develops under the membrane of the zygote. It becomes weakly silicified. The silicification starts at the center of the auxospore and develops out toward the poles. The perizonium may develop a distinctive pattern of markings or be smooth.

When the auxospore is mature, a nuclear division (metagamic division) occurs. One of the resulting nuclei is pycnotic. This phenomenon has been observed in various genera of the Pennales.

After a period of time the auxospore develops the shells typical of the vegetative cell. The first shell to develop is the epitheca. It is irregular in that it does not have a girdle band. Therefore the edges of the valve bend over and the valve has a curved appearance. The

hypotheca, however, is normal in that it possesses girdle bands. Thus the first vegetative cell is not symmetrical in appearance, and therefore it differs from subsequent vegetative cells.

SUMMARY OF TYPES OF AUXOSPORE FORMATION

Various authors have made classifications of the different types of auxospore formations. The classification given below is taken from Geitler (1932) but modified to include the results of more recent research.

Normal Type A

Two mother cells each produce two gametes, which copulate in pairs to produce two auxospores (Figs. 1 to 4).

(1) The gametes are isogamous; the apical axes of the auxospores are perpendicular to the apical axes of the mother cells. *Amphiprora alata* Kütz. (?), *Amphora coffaeformis* Ag., *A. cymbelloides* Grun., *A. ovalis* Kütz., *A. ovalis* var. *pediculus* Kütz., *A. pusio* Cl., *A. veneta* Kütz., *Auricula hyalina* Karst., *Denticula vanheurckii* Brun., *Epithemia argus* Kütz. (?), *E. sorex* Kütz., *E. turgida* (Ehr.) Kütz., *E. zebra* (Ehr.) Kütz., *E. zebra* var. *saxonica* (Kütz.) Grun., *Navicula radiosa* Kütz., *Rhopalodia gibba* (Ehr.) O. Müll., *R. gibba* var. *ventricosa* (Ehr.) Grun.

(2) Each mother cell produces a wandering and a resting gamete. The apical axes of the auxospores are parallel to those of the mother cells. *Achnanthes lanceolata* Bréb., *A. minutissima* Kütz., *Amphipleura pellucida* Kütz., *A. rutilans* (Trent.) Cl. (?), *Amphiprora alata* Kütz. (?), *Anomoeoneis sculpta* (Ehr.) Pfitz., *A. serians* (Bréb.) Cl., *Brebissonia boeckii* (Ehr.) Grun., *Cymbella affinis* Kütz., *C. caespitosa* var. *pediculus* (Ehr.) Brun. (?), *C. cistula* (Hemp.) Grun., *C. cymbiformis* (Kütz.) Bréb. (?), *C. gastroides* Kütz., *C. helvetica* Kütz., *C. lacustris* (Ag.) Cl., *C. lanceolata* (Ehr.) V. H., *C. parva* (W. Sm.) Cl. (?), *C. prostrata* (Berk.) Cl., *C. sumatrensis* Hust., *C. ventricosa* Ag., *C. ventricosa* Ag. var. I and II, *Frustulia rhomboides* var. *saxonica* (Rabh.) DeT., *Gomphonema constrictum* Ehr., *G. constrictum* var. *capitata* (Ehr.) Cl., *G. geminatum* (Lyngb.) Ag., *G. intricatum* Kütz. (?), *G. intricatum* var. *dichotomum* (Kütz.) Grun. (?), *G. longiceps* Ehr. (?), *G. olivaceum* (Lyngb.) Kütz., *G.*

parvulum var. *micropus* (Kütz.) Cl., *G. tenellum* Kütz. (?), *Libellus constrictus* (Ehr.) DeT. (?), *Navicula crucigera* (W. Sm.) Cl., *N. cuspidata* var. *ambigua* (Ehr.) Cl., *N. directa* Ralfs., *N. firma* Kütz. (?), *N. pygmaea* Kütz., *N. ramossissima* (Ag.) Cl., *N. scopulorum* Bréb., *N. subtilis* (Greg.) Ralfs., *N. viridula* Kütz., *Niedium affine* var. *amphirhynchus* (Ehr.) Cl. (?), *Nitzschia hybrida* Grun., *N. longissima* (Bréb.) Ralfs., *N. sigmoidea* (Ehr.) W. Sm., *N. subtilis* Kütz., *Pinnularia gibba* Ehr., *P. hemiptera* (Kütz.) Cl., *P. stauroptera* Grun. (?), *P. viridis* (Nitz.) Ehr., *Rhoicosphenia curvata* (Kütz.) Grun., *Schizonema lacustre* Ag., *Stauroneis phoenicenteron* (Nitz.) Ehr. (?).

(3) One mother cell produces two wandering gametes, and one mother cell produces two passive gametes. The apical axes of the auxospores are parallel to those of the mother cells. *Navicula halophila* (Grun.) Cl., *Synedra ulna* (Nitz.) Ehr., *S. rumpens* var. *fragilarioides* Grun.

(4) The gametes behave according to no rule; the auxospore position varies. *Achnanthes brevipes* Ag., *A. lanceolata* Bréb., *A. longiceps* Ag., *Navicula didyma* (Ehr.) Kütz., *N. fonticola* Grun., *N. hybrida* Herib. & Per., *Nitzschia longissima* (Bréb.) Ralfs., *Pleurosigma nubecula* W. Sm.

Normal Type B

Spermatozoa and an egg cell are formed (Figs. 5 to 12).

(1) An antheridial cell buds off two spermatozoid mother cells each of which produces two spermatozoids. One oögonium produces one egg cell. A spermatozoid enters the egg and a zygote is formed. *Melosira varians* Ag.

(2) Four spermatozoa are produced from an antheridial cell. One oögonium produces one egg cell. A spermatozoon enters the egg and a zygote is formed. *Cyclotella* sp.

Reduced Type A

Two mother cells each build one gamete; these fuse to form a single auxospore (Figs. 13 to 15).

(1) The gametes behave isogamously. *Cocconeis pediculus* Ehr., *C. placentula* Ehr., *C. placentula* var. *klinoraphis* Geitler, *C. placen-*

tula var. *tenuistriata* Geitler, *Cymatopleura solea* (Bréb.) W. Sm., *Eunotia arcus* Ehr., *E. flexuosa* Kütz., *E. formica* Ehr., *E. pectinalis* (Kütz.) Rabh., *Navicula crytocephala* var. *veneta* (Kütz.) Grun., *Rhoicosphenia curvata* (Kütz.) Grun., *Surirella capronii* Bréb., *S. splendida* Ehr., *S. striatula* Turp.

(2) The gametes behave anisogamously. *Navicula seminulum* Grun. *cocconeis pediculus* Ehr., *C. placentula* Ehr., *C. placentula* var. *pseudolineata* Geitler.

Reduced Type B

One mother cell develops an auxospore through automixis (Figs. 16 to 19).

(1) Two gametes of one mother cell copulate with each other. *Achnanthes subsessilis* Kütz., *Cyclotella meneghiniana* Kütz, *Gomphonema constrictum* var. *capitata* (Ehr.) V. H., Form I.

(2) The sexual nuclei of a mother cell copulate. *Amphora normanii* Rabh., *Bacillaria paradoxa* Gmelin (?), *Chaetoceras boreale* Baily, *C. densum* Cl., *Grammatophora marina* (Lyngb.) Kütz., *Libellus constrictus* (Ehr.) DeT. (?), *Navicula constricta* Grun. (?), *Nitzschia palea* (Kütz.) W. Sm.

Reduced Type C

The auxospore formation is apomictic (Figs. 20 to 22).

(1) From one mother cell there develop through vegetative division two auxospores. *Achnanthes longipes* Ag. (?), *Bacillaria paradoxa* Gmelin (?), *Cocconeis pediculus* Ehr., *Libellus constrictus* (Ehr.) DeT., *Navicula constricta* Grun. (?), *Rhabdonema arcuatum* (Lyngb.) Kütz., *Synedra affinis* Kütz., *Tabellaria* sp.

(2) From one mother cell (the mother cells may pair) there develops one auxospore.

(a) Parthenogenetically. *Bacillaria paradoxa* Gmelin (?), *Cocconeis pediculus* Ehr., *C. placentula* Ehr., *C. placentula* var. *klinoraphis* Geitler, *C. placentula* var. *lineata* (Ehr.) Cl., *C. placentula* var. *euglypta* (Ehr.) Cl., *Cymatopleura elliptica* (Bréb.) W. Sm., *C. solea* (Bréb.) W. Sm., *Cymbella cistula* (Hemphr.) Kirchn. (?), *C. sumatrensis* Hust. (?), *C. ventricosa* Ag. var. I, *Grammatophora marina* (Lyngb.) Kütz. (?), *Meridion circulare* (Grev.) Ag., *Navicula*

grevillii (Ag.) Heib., *Nitzschia palea* (Kütz.) W. Sm. (?), *Rhabdonema adriaticum* (Lyngb.) Kütz., *Surirella gemma* Ehr.

(b) Purely vegetatively. *Bacillaria paradoxa* Gmelin (?), *Melosira* and other Centrales.

DISCUSSION AND CONCLUSIONS

In diatoms the most common method of reproduction is vegetative division by mitosis. However, there do occur other types of reproduction which have been discussed in this paper. As in the protozoa, fungi, and other algae, it is hard to determine whether there is true sexual differentiation. It is reproduction which occurs as a result of meiosis and fusion. This type of reproduction has only rarely been found in the Centrales. In the Pennales it has been found in numerous species, but in most of them only rarely observed. This is particularly apparent when one considers that there are several thousand species of diatoms and that thousands of collections of diatoms have been made.

It is interesting to consider how this reproduction pattern, of commonly occurring asexual reproduction with infrequently occurring sexual reproduction, affects the structure of diatom species, their distribution, and the evolution of the group.

The common concept of the species is based upon populations in which sexual reproduction is obligate and each individual has a different genotype. Thus in a large population there is a great deal of intergrading variation. In diatoms there are species composed of many clones differing by sometimes small but disjunct variations. Each clone consists of many individuals with the same genotype. It is perhaps for this reason that we have commonly in diatom species so many named varieties and forms. This difference in species structure requires that any study of population structure based on random sampling must be carefully planned with these facts in mind.

This type of reproduction may also contribute to the distributional pattern of this group. Most species of diatoms have wider distributional patterns than are commonly found in higher plants. When colonization is attempted by an obligate sexually reproducing species, it is necessary for at least two individuals of opposite sex to be living successfully in a given environment. In an asexually reproducing form one individual may establish a colony. Thus, as Stebbins (1950) has pointed out, asexual reproduction may bring about more rapid coloni-

zation of a species. The fact that diatoms are diploid rather than haploid means that each individual may have genic flexibility for any given trait. This, coupled with asexual reproduction, no doubt is an important factor in establishing the wide distribution patterns of the species. Of course, many other factors contribute to producing the broad distributional pattern of diatom species (Patrick, 1948). This association of a wide distribution pattern of a species with asexual reproduction is also found in other algae, protozoa, and fungi.

The effect of this reproduction pattern on the evolution of diatoms is hard to evaluate, for it seems to produce effects some of which would favor evolution while others would slow it down. Asexual reproduction by its very nature greatly reduces chromosomal change, eliminates the accumulation of mutations which have occurred in various genotypes, and the recombination of genotypes. It is interesting to note that in the Centrales, in which reproduction by fusion has only rarely been observed, there are a fairly large percentage of species which have remained constant since Miocene times.

In the Pennales, however, the effect of asexual reproduction in reducing the rate of change in the species may not be so great. In this group "sexual reproduction" has been observed in a greater number of species. As Stebbins (1950) points out, in organisms with short generations the number of genic recombinations per generation can be reduced without affecting the flexibility in terms of the number of gene combinations available in a given unit of chronological time. Thus, if "sexual reproduction" occurs often enough, the genic flexibility will be preserved. The question then is how frequently does "sexual reproduction" occur.

The structure of the diatom species offers a favorable condition on which natural selection can operate. A species is composed of segregated population units in the form of clones between which gene exchange through sexual reproduction occasionally takes place. It must be remembered, however, that these segregated population units consist of a single genotype rather than several genotypes, as is usually the case in segregated population units of obligate sexual species.

The rate of evolution in diatoms undoubtedly is influenced by these various factors. Because of the varying frequency of sexual reproduction in different species, these algae are an interesting group for the study of some of these basic problems.

Note: Since this paper was written Geitler (1953a) has shown that *Denticula tenuis* Kütz. may produce auxospores by Normal

Type A-1 or by Reduced Type B-1. He has also shown (1953b) that in *Cocconeis placentula* var. *tenuistriata* Geitler two metagamic divisions occur. The first division is associated with the formation of the first shell in the auxospore and the second one with the second shell. Geitler and Mack (1953) point out that although in the genus Cymbella there is variation in the relation of the position of the axis of the auxospore and the position of the first shell to that of the mother cell, the arrangement for many species is very definite. Nipkow (1953) states that he has seen asexual auxospore formation by Reduced Type C-2-b in *Fragilaria crotonensis* Kitton but does not describe the various steps in the process.

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Sex in Unicellular Algae

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AT THE OUTSET it would be well to define the scope of this review. Waddington (1939) interprets "sex" in terms of sexual differentiation, Sinnott and Dunn (1939) extend it to cover gametogenesis and zygote formation, while Darlington (1937) considers sexual reproduction as embracing not only syngamy but also meiosis, as complementary processes essential for the completion of a sexual cycle. In many protists there is no differentiation between cells capable of acting as gametes; in only a few has the sexual cycle been followed through genetically; while in *Escherichia coli* knowledge of almost all aspects of sex but the purely genetic is for the most part inferential. For these reasons, in a field where information is so sparse and fragmentary, the author hopes that a certain latitude of interpretation will be allowed.

Smith (1951a) considers the class of algae to be divided into seven divisions. Among these the Cyanophyta are non-sexual, the Rhodophyta and Phaeophyta are virtually devoid of unicellular representatives, whereas in the Chrysophyta, the Pyrrophyta, and the Euglenophyta sexual reproduction is unknown in all but a few exceptional cases. These flagellates are further discussed by Wenrich (see "Sex in Protozoa: A Comparative Review" in this volume). A search for experimental material must be thus largely restricted to the diatoms and the green algae. It is perhaps regrettable that the ubiquitous species of *Chlorella*, *Scenedesmus*, and *Stichococcus*, so favorable in other ways for laboratory study, have not been known to exhibit sexual reproduction. The desmids offer promising material; but the success of Pringsheim (1919) in controlling zygote formation and germination in *Cylindrocystis* has apparently not been followed up.

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Drs. S. H. Hutner, J. C. Lewin, and L. Provasoli have offered many critical suggestions which have been considered in the preparation of this review.

It is therefore with the Volvocales that this review will be chiefly concerned.

No attempt has been made to discuss the literature on this subject exhaustively here. The field has already been well surveyed by Fritsch (1935), Moewus (1941), and Smith (1951a); see also Luyet (1950). Particular attention will be given here to studies of cultures and experiments under controlled conditions, where a measure of reproducibility can be expected in the results. The most critical investigations are those which involve material of genetic uniformity and known physiological background, in a constant physical and chemical environment and in the absence of any other living organisms.

SEXUAL DIFFERENTIATION

Vegetative Cells and Gametes

In a unicellular organism sexual differentiation may be taken to indicate a difference between the two sexes or mating types; it may also be considered to refer to the differentiation often found between vegetative cells and gametes (see Moewus, 1933). In the simplest case, as in many species of *Chlamydomonas* (Klebs, 1896; Smith, 1950b), *Polytoma* (Pringsheim and Ondratschek, 1939), and *Dunaliella* (Lerche, 1937), all haploid cells may be capable of sexual fusion. In *Chlamydomonas gymnogyne* (Pascher, 1943) the sexually active cells are morphologically identical with vegetative cells but appear to differ in their more trembling mode of progression. However, in most *Chlamydomonas* species and in related genera, for example, *Chloromonas* (Korschikoff, 1926), *Chlorogonium* and *Haematococcus* (Schulze, 1927), *Phyllocardium* (Korschikoff, 1927), and *Brachiomonas* (Moewus, 1944), it may be observed that only the smaller cells, those most recently liberated after cell division, take part in mating. Each young cell may be said to pass through a gametic stage and then to lose its sexual activity as it enlarges prior to asexual division. In some such cases the cells may be naked during the gametic phase and only develop cell walls later. Nayal (1933) has described in *Protosiphon* the formation of two types of swarmers, rounded zoospores and elongate facultative gametes, both capable of direct development into vegetative cells.

In *Chlamydomonas paupera* (Pascher, 1931-32) differentiation is carried a stage further. The species is homothallic and isogamous; the gametes after liberation develop first a male sexual potentiality and later, if not involved in sexual fusion, become female. Unmated gametes in culture ultimately die without growing into a form capable of asexual reproduction (that is, they appear incapable of parthenogenesis), so that here one finds a dichotomy between obligate gametes and asexual cells. A similar condition has been described in *Tetraspora* (Geitler, 1931), in *Stephanosphaera* (Moewus, 1933), and in the microgametes of *C. praecox* (Pascher, 1943). (According to Moewus, 1941, in some algae the ability of gametes to reproduce asexually is controlled by a single, partially sex-linked gene. In *C. eugametos*, in which all motile cells are potentially gametes, he claimed to have obtained by x-irradiation numerous mutants in which the gametes lacked this ability to divide vegetatively. This paradoxical situation awaits explanation.) Since both gametes and vegetative cells are produced after haploid mitosis, it is unlikely that they could differ genetically; and in a case of this sort it would be of great theoretical interest to determine what synthetic faculty or cytoplasmic moiety may be lost in gametogenesis, thereby rendering the sex cells incapable of further multiplication in the haploid condition.

Outbreeding Mechanisms

In sexually reproducing organisms the frequency of sibling matings may be reduced (1) by sexual dimorphism between the gametes (by anisogamy or oögamy) or (2) by genetically inherent factors of self-sterility, or intraclonal sexual incompatibility. These two outbreeding mechanisms are not mutually exclusive, and homothallic as well as heterothallic species may be found to exhibit isogamy, anisogamy, or oögamy. Multipolar heterothallism has not been demonstrated in any alga. Subdioecism or subheteroecism has been described in *Chlamydomonas* and other genera by Moewus (1934 *et seq.*).

Sexual Dimorphism. In some species (*Chlamydomonas elongata* for example) the gametes may be of various sizes but capable of pairing in all combinations—a condition referred to by Korschikoff (1923) as ataktogamy. In others the gametes are morphologically similar but exhibit what may be described as physiological anisogamy; for example, *C. gymnogyne*, in which only one gamete in each pair

regularly sheds its cell wall before cytogamy (Pascher, 1943), and *C. moewusii*, in which only one gamete remains motile after pairing (Lewin, 1950a). (See page 119.)

The condition in which the two types of gametes are morphologically distinguishable is generally known as anisogamy or heterogamy; where one gamete is non-motile at the time of fertilization (and is thereby designated as female), the species is said to be oögamous. Sexual differentiation of this sort has been discussed by Fritsch (1935) and Smith (1950a, 1951a), who have pointed out that more than one condition may exist within a single genus. Thus sexual reproduction has been observed in only about 10 to 20 per cent of the described species of *Chlamydomonas;* of these over forty are isogamous species, about eleven are anisogamous, and three show fairly well-marked oögamy (Skuja, 1949). *Phyllomonas striata* exhibits marked anisogamy (Korschikoff, 1926); *Chlorogonium oogamum* (Pascher, 1931) and *Carteria iyengarii* (Ramanathan, 1942) are oögamous. If we are to consider heterothallic species exhibiting anisogamy or oögamy as derived from heterothallic isogamous forms, we may envisage the evolution of the larger, or female, gamete from either the *plus* or the *minus* mating type, and we need not expect that in all evolutionary lines the same mating type would have become the female. This consideration can hardly be pursued, however, until some of the physiological bases for heterothallism have been elucidated.

Heterothallism. There are few diploid unicellular algae other than the pennate diatoms, and of the latter almost all species which have been critically examined appear to be homothallic or monoecious (Geitler, 1949). In the case of *Navicula halophila*, however, Subrahmanyan (1946) has presented circumstantial evidence for genetically controlled dioecism, a condition found in *Fucus vesiculosus*, for instance, but almost unknown in the lower Protophyta. Sex in diatoms is more fully discussed (in this volume) by Patrick.

Among haploid organisms, homothallism (monoecism or synoecism) may be defined as the condition in which a complete sexual cycle can take place within a single clone, and heterothallism (dioecism or heteroecism) as that state in which two haploid clones of different genotype—different genetic mating type—are required for sexual reproduction.

A discussion of homothallism, specifically in combination with

isogamy, would hardly be complete without some reference to the heated controversy which raged between biologists at Berlin and Prague during the 1920's and 1930's. Hartmann (for example, 1932, 1943) adopted the theoretical anthropomorphic concept, borrowed perhaps from Aristotle (for example, 1910), that there can be no sexual union without sexual differentiation. If compatible gametes are morphologically and, as in homothallic species, genetically identical, then, he postulated, there must nevertheless exist some invisible physiological difference between them, and the strain is said to possess "bisexual potency." On the other hand, Mainx (1933) and Czurda (1933a) saw no reason for adopting this hypothesis, for which they found no corroboration in their experimental observations on algae and other organisms, and they freely accepted the fusion of identical cells in syngamy, just as it occurs in the hyphal anastomoses of fungi, or the formation of plasmodia in Myxomycetes.

And then, in the early 1930's, Franz Moewus, a student of Kniep and Hartmann at the Kaiser Wilhelm Institute, reported experimental support for the theory of bisexual potency from his investigations of unicellular algae such as *Protosiphon* (1933, 1935a), *Polytoma* (1937), and *Chlamydomonas eugametos synoica* (1938a). In a clonal culture of such homothallic, isogamous forms, both "male" and "female" gametes are said to be produced; and, when pairing takes place, unless precisely similar numbers of the two mating types are present, there will of necessity remain a few residual gametes (*Restgameten*) of the supernumerary sex. Suspensions of residual gametes from several cultures, freed in some way from already paired cells, may be tested for mating type by mixing them in combinations of two or with heterothallic tester stocks and observing in which mixtures mating is reinitiated. Lerche (1937) reported preliminary observations on *Haematococcus*, in which, using morphologically distinguishable red and green clones, she was able to demonstrate the regularity of residual gamete fusions; and Moewus (1940a) carried out a similar experiment, using the marker "eyeless," in *Botrydium granulatum*. But Pringsheim and Ondratschek (1939) in Prague sought in vain to confirm Moewus' assertions about residual gamete behavior in *Polytoma* and *Protosiphon*, thereby lending further weight to the initial objections of Czurda (1933a). Pringsheim and Ondratschek pointed out that residual gametes could hardly be expected to behave in the manner described by Moewus in organisms where sexuality is claimed to

be largely controlled by the presence of soluble "sex substances" in the medium (Moewus, 1938b, etc.) (see page 115). From the evolutionary standpoint, it would seem that a homothallic isogamous species, with non-genetic mating-type differentiation within a single clone, would be at a disadvantage, since such a mechanism would presumably reduce the potential sexual fertility of the species, while in no wise promoting outcrossing or bringing other compensatory advantages.

Another concept inherent in Hartmann's theory of sexual differentiation is that of relative sexuality. Several examples of such behavior in different genera of algae, such as *Chlamydomonas, Polytoma,* and *Protosiphon*), have been reported by Moewus, and in certain cases viable zygotes have been obtained by mating "strong" gametes with "weak" gametes of the same sex. Extensive physiological and genetic investigations have been described in many such cases, and these have been reviewed in some detail by Moewus (1939a, 1941, 1950a), Chodat (1941), Smith (1951a), and others. Relative sexuality has apparently not been established in any unicellular alga investigated by other workers. No report of sex reversal in algae has come to the notice of the present author.

ISOLATION OF SEXUAL STRAINS

The isolation of unicellular algae capable of sexual reproduction under laboratory conditions has always presented difficulties. These are attributable partly to the fact that the diplophase is rarely identifiable in nature, and therefore, in the heterothallic species, two haploid clones of complementary mating type must first be isolated. It is reasonable to assume that, in many species, reproduction is entirely asexual; whereas in others the conditions favorable for eliciting a sexual response, whether in homo- or heterothallic forms, are unknown except for fragmentary and largely empirical data for a few species. Schreiber (1925) had no success in finding mating strains among seventy *Chlamydomonas* clones tested.

Moewus (1931) succeeded in isolating mating types of a species of *Chlamydomonas* which he accordingly named *C. eugametos*, later retaining the name despite its prior description as *C. sphagnophila* by Pascher in 1930 (see Moewus 1934, 1935b). A number of sexually compatible clones of *Chlamydomonas,* probably identical with *C.*

eugametos, were isolated from various soil samples by Gerloff (1940), who described the species as *C. moewusii*. Apart from a single homothallic strain from the Cameroons, all these isolates conformed to regular heterothallism. In 1948 Provasoli isolated complementary mating types of a *Chlamydomonas*, the description of which closely accorded with that of *C. moewusii* Gerloff. It has been found (see Hutner and Provasoli, 1951, and Smith, *in litt.*, November 1951) that these isolates mate in reciprocal combinations with strains of *C. eugametos* obtained directly or indirectly from Moewus, and it therefore seems very probable that the two names are synonymous, as Gerloff (1940) had originally suggested.

Smith recognized the difficulty of finding sexually active strains among haploid clones isolated at random from soil and accordingly devised a procedure (1946, 1947) which yielded considerably better results. Essentially, this consisted of the examination of freshly flooded agar cultures for the formation of mating pairs, which would indicate either homothallism or the presence of both mating types of a heterothallic species, originating from a single diploid zygote. He reported (1950b) the isolation by this means of fifteen heterothallic, not interfertile, species of *Chlamydomonas*, and (1951b) a number of homothallic species. Using a modification of this method involving a prior enrichment for zygospore-forming species, Lewin (1951) isolated from ten soil samples three heterothallic and two homothallic species.

HYBRIDIZATION

In 1916 Pascher reported the hybridization of two species of *Chlamydomonas* and described the progeny emerging from the hybrid zygotes. Strehlow (1928-29) obtained zygotes by crossing *C. paradoxa* (*plus*) with *C. botryoides* (*minus*), though he could not effect the reciprocal cross, nor could he induce the zygotes to germinate. Moewus (1935b) reported success in crossing *C. eugametos* with *C. paupera*, obtaining zygospores which showed a greater sensitivity to chilling than did those of the parent types, and haploid progeny exhibiting segregation of six morphological character pairs.

He went on to describe extensive experiments in hybridization of *Polytoma* spp. (1937), and of other species of *Chlamydomonas*. In 1940 he reported that he had been able to isolate from 500 soil

samples a number of clones of five species including anisogamous and oögamous forms capable of interspecific mating. If we are to accept Pascher's classification (1927) of this heterogeneous genus, then these interfertile species of Moewus' include representatives of three different subgenera (Smith, 1946).

Since doubt has been cast on Pascher's results by Hartmann (1934), and on those of Moewus by a number of other workers (see page 126), the information available in this field can hardly be described as illuminating.

INDUCTION OF SEXUAL ACTIVITY

A considerable amount of attention has been focused on the physiological conditions which may induce cells to become sexually active or may promote gametogenesis. As yet, no clear picture has emerged from the many investigations carried out on various organisms by a number of workers, each employing a different approach (see reviews by Czurda, 1933b, and Bold, 1942). In the case of heterothallic species, it is of course essential at the outset to have isolates of both mating types; and this difficulty has undoubtedly contributed to the failures reported in the past (for example, by Reichenow, 1909, for *Haematococcus;* cf. Schulze, 1927). A few of the factors which have been found to influence sexuality are discussed below.

Light

Preliminary Observations. Klebs (1896) found that, though dim light or darkness favored the formation of gametes in *Protosiphon*, light tended to promote the process of copulation. He observed that swarmers, formed in a mineral nutrient medium, could be reversibly stimulated to sexual activity either by dilution of the medium or by illumination, and he postulated that the action of light might be through the photosynthetic formation of organic compounds, which combined with and neutralized some constituent(s) of the medium inhibitory to copulation. However, he was not able to identify this inhibitor with the nitrate, phosphate, potassium, or calcium salt in the medium he used.

In *Chlamydomonas media* (Klebs, 1896) and *C. eugametos* (Moewus, 1933), light appeared essential for sexual activity, which ceased

completely in darkness, and Moewus attributed these effects to the formation and destruction of "sex substances" (see page 115). In an attempt to confirm some of Moewus' results, though unable at the time to obtain cultures of *C. eugametos,* Smith (1946) examined the sexual behavior of a number of *Chlamydomonas* species isolated from Californian soils. He found that all three heterothallic species tested were capable of mating in darkness, though the activity was appreciably stimulated by light. Lerche (1937) reported that light was not essential for the initiation of mating in *Dunaliella,* and Maher (1946-47) showed that *Protosiphon* was capable of completing its sexual cycle in complete darkness.

Quality of Light. The experiments reported by Smith in 1946 were made on suspensions of cells which had been initially grown in light and were subsequently darkened prior to being tested for mating ability. In 1948, having isolated the facultatively heterotrophic *Chlamydomonas reinhardi,* he was able to extend his observations to cultures which had been grown in darkness on nutrient media containing sodium acetate. He found that such cells, although motile, were incapable of sexual activity unless they had been subjected to a period of illumination. Red light (6150 to 5900 A) or blue light (4357 A) was tested in place of white light, and in all cases the sexual clumping of cells could be induced. In this respect *C. reinhardi* apparently differs from *C. eugametos,* which, according to Moewus (1939b), can only receive sexual stimulation from light at the blue end of the spectrum (4300 to 5000 A).

In his description of *C. chlamydogama,* Bold (1949) stated that copulation occurred only in illuminated cultures. Using the strains isolated by Bold, Cadoret (1949) was able to confirm the observation and to investigate the character of light responsible for the physiological effect. His experiments indicated that for *C. chlamydogama,* unlike *C. reinhardi,* white light could not be replaced by either red or blue light, but that only a combination of both qualities of light would induce sexual clumping and pairing. Cadoret suggested that, for the manifestation of sexual activity, light must be absorbed by two distinct substances, perhaps green and orange in color respectively, and that chlorophyll and a carotenoid may both be involved. The role of carotenoids in the sexual life of *C. eugametos* has been given some weight in the publications of Moewus and co-workers

(see Moewus, 1939c, 1940c, 1950b, for example); the possibility that chlorophyll may be involved is discussed briefly below.

Provasoli and Pintner (see Hutner and Provasoli, 1951) found that light was required for the mating of *C. moewusii*. Preliminary experiments indicated that any wavelengths within the visible range were active in promoting sexual clumping and pairing, and they concluded that at a sufficient intensity all qualities of light were stimu-

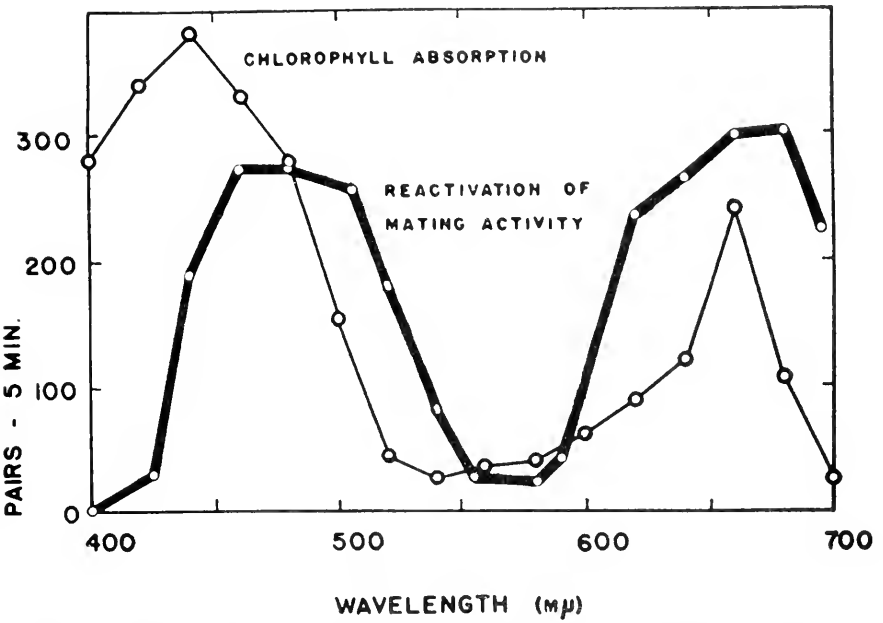

FIG. 1. Action spectrum for sexual induction in *C. moewusii* Gerloff.

latory. It has since been shown (Lewin, unpublished) that in this species light is required for the sexual activation of both mating types, and unless both gametes have received illumination no mating takes place. Not all wavelengths are equally effective: an action spectrum for sexual induction in *C. moewusii* is presented in Fig. 1. It will be observed that the form of this curve is not dissimilar to the absorption spectrum of chlorophyll. The suggestion is therefore made that the action of light on mating in *Chlamydomonas* is mediated by the chloroplast, and that sexual activity is promoted by some product or

state resulting directly or indirectly from photosynthesis. It is significant that sexual activation and photosynthesis are inhibited to the same extent by 7×10^{-4} *M* phenylurethan. The effect of light cannot be replaced by raised oxygen tension in the medium. However, some indications of the nature of the active product have been obtained and are discussed below.

Period of Illumination. Klebs (1896) observed that cells of *Chlamydomonas media* capable of active mating in light lost their sexual activity in darkness, under which condition he considered that they returned to a vegetative phase. Since gametes only reappeared in such cultures after a week of reillumination, it seemed likely that "rejuvenation" of the culture by vegetative reproduction was needed to restore sexual activity. Moewus (1933), on the other hand, noted that cell suspensions of *C. eugametos* which had been kept in darkness could regain their sexual activity after only 30 to 45 minutes in light, indicating that the same cells were concerned in the recovery.

Smith (1946) found that as short a period as 5 minutes of illumination markedly stimulated sexual activity in *C. minutissima.* In his later investigations with *C. reinhardi,* which can be grown *ab initio* in the complete absence of light, he found that sexual activity began to be demonstrable after 90 minutes of illumination in bright light. Using the same species, Wendlandt (see Smith, 1951b) showed that, at lower light intensities, longer periods of illumination were required before the first appearance of sexuality; whereas, for a constant period of illumination, lower light intensities induced a less intense sexual response (as measured by the number of clumps of mating cells per unit volume). Once illuminated, cultures of this species retained their sexual activity for at least a week in darkness (Smith, 1948).

Experiments with *C. moewusii* (Lewin, unpublished) have shown that sexually active cell suspensions reversibly lose their activity in darkness and regain it in light, these changes taking place in a matter of minutes, so that considerations of cell division do not arise. The species is peculiarly well suited to studies of this mechanism, since cell pairs, once united, remain active without further fusion for some hours (see page 121), and their numbers provide a convenient direct assay of mating activity. By using this fact it has been shown that cells, sexually inactivated by a period of darkness, may completely regain activity after 10 to 30 minutes of illumination in

white light. The reactivation curve does not commence to rise at the origin, but a minimum of ½ to 10 minutes is necessary before any restoration of sexual activity can be demonstrated. The length of the lag period, the slope of the curve, and the height of the asymptote are affected by a variety of factors including intensity and quality of light, temperature, and the length of the preceding period of darkness to which the cells have been subjected.

Medium

Attempts have been made by many workers, using different organisms, to evolve media which would specifically elicit or promote sexual activity. Various degrees of success have been achieved, and it is not proposed to review the literature on the subject here. In general, it has been found that dilution of the medium, or transfer of the cells from agar to liquid culture, has a marked effect in promoting gamete formation in a number of diverse algae. Lerche (1937) concluded that in *Dunaliella* the effect was attributable to depletion of nutrients (cf. Klebs, 1896; Schreiber, 1925; Strehlow, 1928-29), since she found sexual activity to increase in aging cultures. In *Cylindrocystis*, an apparently homothallic desmid, copulation was found to be regularly induced by the depletion of nitrogen in the medium (Pringsheim, 1919). This does not appear to be the case, however, in other algae; for example, *Chlamydomonas pseudoparadoxa* (Moewus, 1933), *Chlamydobotrys* (Behlau, 1935), *Polytoma* (Pringsheim and Ondratschek, 1939). In *Chlamydomonas* spp. (Klebs, 1896; Moewus, 1933; Lewin, 1949a), *Brachiomonas* (Moewus, 1944), *Protosiphon* (Bold, 1933; Maher, 1946-47) and other genera, the empirical device of flooding agar cultures with water or dilute mineral media has generally been found to be the simplest and most effective method for obtaining suspensions of sexually active cells.

Schulze (1927) found that gametogenesis in *Chlorogonium* could be induced in 16 to 20 hours at pH values of 7.9 to 10.4. Maher (1946-47) obtained good gamete formation in *Protosiphon* between pH 4.0 and 9.0 but noted that at the lower values gamete motility was reduced. In *Chlamydomonas moewusii* gamete formation is more active in media of higher pH; pairing takes place within a pH range of 6.0 to 10.0, being most active between 7.0 and 9.0. In *Polytoma*, too, pairing was found to be most active at pH 8.0 (Pringsheim and

Ondratschek, 1939). According to Moewus (1935c, 1950a), the sex ratios among gametes formed by certain homothallic *Protosiphon* strains and by *C. eugametos alpina* are markedly affected by pH.

Warming for short periods tends to stimulate gametogenesis in *Protosiphon* (Klebs, 1896; Maher, 1946-47). It was found that aeration promotes sexual activity in the heterotrophic flagellate *Polytoma* (Moewus, 1933; Pringsheim and Ondratschek, 1939), but its action in illuminated suspensions of photosynthetic algae is probably less marked.

It has been found (Lewin, unpublished) that sexual clumping and pairing in *Chlamydomonas moewusii* are dependent on the concentration of the Ca^{++} ion in the medium. Sexually active cells lose their ability to mate after washing in distilled water but regain it when $CaCl_2$ is added. The optimum range of Ca^{++} for mating lies around 3 to 30 ppm, being higher in media containing much phosphate or amino acids, which combine with a proportion of the free cations. Mating is also inhibited when the Ca^{++} is removed by such agents as citrate or oxalate. Inhibition by 0.02 *M* citrate may be reversed by washing the cells in mineral media, or by the addition of 0.001 *M* $CaCl_2$, and may be slightly alleviated by higher concentrations of $MgCl_2$ or $SrCl_2$. Since, once the cells have paired, citrate does not reverse the mating reaction, it appears that the Ca^{++} is concerned primarily in the specific agglutination which occurs between the flagella of mating cells (see page 117). Loeb (1915) and Vasseur (1949) showed that the presence of Ca^{++} was required for the agglutination of spermatozoa and for fertilization in certain echinoderms. Possibly this requirement is related to the free superficial amino groups, which, as indicated by Metz and Donovan (1951), take an active part in this process. The phenomenon may also be in some ways compared with that of the adsorption of bacteriophage by host bacteria (Puck and co-workers, 1950).

"Genetyllin"

To provide a material basis for discussion of the experiments described in this section (Lewin, unpublished), it has been postulated that in *Chlamydomonas moewusii* there is a specific agent or hormone responsible for sexual activity. We shall refer to this hypothetical agent as *genetyllin*. Genetyllin is formed within the cells under the

action of light, the energy probably being absorbed by chlorophyll (see page 109); and as a result some stimulus passes into the flagella, rendering them temporarily susceptible to intersexual agglutination. When cells are kept in darkness, they rapidly lose their ability to mate, and we may attribute this to disappearance or decomposition of genetyllin. This reaction follows a course exhibiting two phases, both affected by temperature. In the first 0 to 15 minutes after darkening there is no loss of sexual activity; the duration of this phase is controlled largely by the period of pre-illumination. There then follows the second phase, during which the activity falls off exponentially to zero; its duration ranges from 15 to 60 minutes or more, depending on temperature. If we further postulate that this exponential loss of activity results from a unimolecular breakdown of genetyllin, it can be deduced from such data that at 30° its half-life is less than 1.5 minutes; at 10° it is 9 minutes; and at 5° it is about 22 minutes.

A problem immediately presents itself, namely, can this hypothetical agent be replaced by any compound supplied in the medium? A number of known substances (glutathione, cysteine, acetate, succinate, glucose, hexose diphosphate, phosphoglycerate, and adenosine triphosphate) were subjected to a reasonably sensitive test (the displacement of the genetyllin breakdown curve in darkness) without any positive result, perhaps as a consequence of permeability barriers. Unsuccessful attempts were also made by Provasoli (personal communication) and by the present author to demonstrate the presence of a solute, in filtrates from sexually active cells, capable of evoking a sexual response in non-sexual cell suspensions. Smith (1946) reported a similar lack of success in experiments conducted with other species of *Chlamydomonas*, notwithstanding the positive results claimed for *C. eugametos* by Moewus (1933) (see below).

It thus seemed possible that, at least in *C. moewusii*, lack of success in attempts to demonstrate the activity of solutes in the medium might be attributable to the non-diffusible nature of genetyllin, and this possibility was tested indirectly in the following way. It had been found that cells subjected to more than 30 minutes of illumination retain full sexual activity for some minutes after transfer to darkness. The longer the period of pre-illumination, the longer the period of darkness during which full activity is retained (see page 110). This suggested that, though genetyllin disappears at a constant rate

in all cases, the cells may have built up, during an extended period of illumination, a supply of the agent exceeding the threshold for 100 per cent gamete mating; and that it is only when the level of genetyllin falls below this threshold that mating activity declines. Suspensions of cells containing various amounts of reserve genetyllin were therefore prepared (different pre-illumination periods being used) and were mixed with suspensions of sexually inactive cells. In no case was there evidence that activity could be transmitted to inactive cells by this means; and one may conclude that genetyllin can probably not be transmitted from cell to cell through the medium.

Genetyllin may prove to be as mythical as the Genetyllides themselves. The concept has been introduced, with some misgivings, in an attempt to indicate a less orthodox approach to the subject of sex substances than is provided by a search for soluble hormones.

Sex Substances

A few authors have claimed to have demonstrated the presence of "sex substances" in culture media from sexually active cells. A clear distinction should be drawn here between at least four different actions attributed to such agents.

1. Positive chemotaxis, drawing gametes of one mating type or sex toward their prospective partners (see page 116).

2. In heterothallic species, the induction of clumping in a clonal suspension by treatment with filtrate from cells of *opposite* mating type.

3. The sexual activation of non-sexual cells by treatment with filtrate from sexually active gametes of the *same* mating type.

4. The induction of the formation of gametes which behave temporarily as a single mating type by treating cells of a homothallic strain ("phenotypically dioecious") with filtrate from the appropriate mating type of a heterothallic species.

Sex substances of type (2) were reported in the supernatant medium after centrifuging gamete suspensions of *Tetraspora* (Geitler, 1931). In *T. lubrica*, which is regularly heterothallic and appropriately lubricous, Geitler found that a certain amount of intraclonal clumping could be induced in cultures by the addition of concentrated "extracts," practically free from cells, prepared from suspen-

sions of opposite mating type. Unlike the normal sexual reaction, such clumping was not followed by the formation of gamete pairs. Though he considered the possibility that this effect might be due to cell breakdown products, he decided that this was unlikely. In 1933 and 1934, Moewus reported a similar phenomenon in contaminated cultures of *Chlamydomonas eugametos* and in *Protosiphon* and *Stephanosphaera*. He found that the active agent did not diffuse into cotton or agar, and evidence for its insoluble nature lay in the fact that activity could be removed by filtration through membranes with 0.01 μ mean pore size, though not by coarser membranes with 0.75-μ pores. When such intraclonal mating responses were induced, Moewus observed that the cells in each clump appeared to have aggregated around a central point, which he suspected might be a bacterium, though he was unable to confirm this. It may be noted that Pringsheim and Ondratschek (1939) were able to induce aggregation of various flagellates by the addition of bacteria alone. (Moewus also reported that solutions containing active sex substance did not freeze when cooled to $-10°$, but that, when the agent was removed by filtration, the filtrate froze normally—a phenomenon inexplicable on simple physicochemical grounds.) Lerche (1937) was able to demonstrate sex substance activity in centrifugates from *Dunaliella;* but in this case she found that filtration through a membrane with 0.75-μ pores would remove the active agent, again suggesting that large particles, rather than a solute, were responsible for the effect.

Jollos (1926) reported that filtrates from "strong" *plus* gametes of *Dasycladus* tended to augment the sexual response of "weak" *plus* gametes, and to reverse that of weak *minus* gametes (*mutatis mutandis*, strong *minus* gametes behaved similarly); but he did not exclude the possibility that such changes could be attributed to non-specific alterations in the composition of the medium.

On the other hand, Smith (1946) and Hutner and Provasoli (1951) were unable to demonstrate the presence of any soluble sex substances in three species of *Chlamydomonas* and in *C. moewusii* respectively. E. Hinreiner (M. Starr, *in litt.*, July and September 1952) found no flavones in culture filtrates from *C. moewusii* or *C. eugametos*, despite the activity claimed for certain compounds of this sort in *C. eugametos* (Moewus, 1950a,b). Attempts to demonstrate the activity of specific water-soluble sex substances in *Parame-*

cium, in which clumping is likewise associated with agglutination of the cilia, have been equally unsuccessful (Metz, 1948).

In 1927 Schulze reported some type (3) activity in *Chlorogonium* filtrates, possibly attributable to their depleted nutrients. Apart from this uncertain case, sex substances of types (3) (gamones*) and (4) (termones) have apparently been reported in unicellular algae only by Moewus, whose published results contain a number of discrepant features. They have been reviewed extensively by Thimann (1940), Chodat and de Siebenthal (1941), Murneek (1941), Sonneborn (1941, 1942), Hartmann (1943), Lang (1944), Cook (1945), Smith (1946, 1951b), Raper (1952), and by Moewus himself (1941, 1950a,b). For various reasons it is considered unprofitable to discuss them further here.

THE COURSE OF THE MATING PROCESS

Chemotaxis

Except in such aplanogametic algae as diatoms and desmids, and rare exceptions like the strange *Chlamydomonas* sp. described by Pascher (1918a), fertilization is accomplished in unicellular algae as a result of the motility of flagellated gametes of one or both sexes.

In oögamous organisms, a common device promoting fertilization is chemotaxis. The immobile eggs secrete soluble substances which diffuse into the surrounding medium, and, by swimming up the diffusion gradients, the antherozoids tend to approach the female gametes. The sperm attrahent in *Sphaeroplea* eggs can be adsorbed on cotton fibers (Pascher, 1931-32), but its chemical nature has remained unknown. The discovery that the chemotactic agent in certain fucoids is volatile and may be akin to hexane (Cook and Elvidge, 1951) is one of the most remarkable of recent contributions to this field.

According to Pascher (1931-32), chemotaxis may also be involved in the mating process of *Chlamydomonas paupera*, which is homothallic and isogamous. In this species gametes which come to rest early act as females, and it is only after their swimming has been arrested that other gametes, acting as males, begin to cluster around

* Moewus (1941) refers to the agents responsible for type (2) reactions as gamones.

them; Pringsheim and Ondratschek (1939) consider their arrest of motility an important corollary of chemotaxis.

In species where both gametes remain actively motile, however, a chemotactic mechanism would seem to be virtually useless. None has been observed in *Chlamydomonas moewusii*, for example (Hutner and Provasoli, 1951). In *C. eugametos* Moewus (1933) found no evidence for chemotaxis when gametes of one mating type were allowed to swim near the mouth of a capillary tube filled with filtrate from cells of the opposite mating type, or near cotton or agar soaked in such a filtrate. Some years later (1939b) the same author reported in this species positive chemotactic responses to gentiobiose and identified the naturally occurring tactic agents with the gamones, viz., proportional mixtures of *cis*- and *trans*-dimethyl crocetin. His earlier failure to demonstrate such agents he explained (1941) by the fact that he had not at first appreciated the photolability of the substances concerned.

Clumping

When active gamete suspensions of *C. moewusii* are mixed, the cells seem to show no appreciable change in behavior until, by random contact, flagella attached to cells of complementary mating type make contact and adhere (Gerloff, 1940; Lewin, 1952b). As in *C. eugametos* (Moewus, 1933), large clumps of 100 or more cells may be quickly built up in dense suspensions, whereas in sparser cultures the cells associate in smaller groups of as few as two or three cells. Such clumps were first described by Berthold (1881) in gametes of *Ectocarpus*, and have been since observed in a wide variety of other algae with motile gametes. Non-swimming (ultraviolet induced) mutants of *C. moewusii* (Lewin, 1952a) mate comparatively poorly with normal swimming cells, as might be expected. In paralyzed stocks of the homothallic *C. dysosmos*, or in mixtures of *C. moewusii* in which cells of both mating types are unable to swim, virtually no mating takes place unless the suspensions are mechanically agitated to bring compatible pairs into contact.

In *Tetraspora* (Geitler, 1931) and *C. moewusii* (Hutner and Provasoli, 1951), clumping has been observed between cells killed by gentle heating, ultraviolet irradiation or other treatments, and living cells of the complementary mating type.

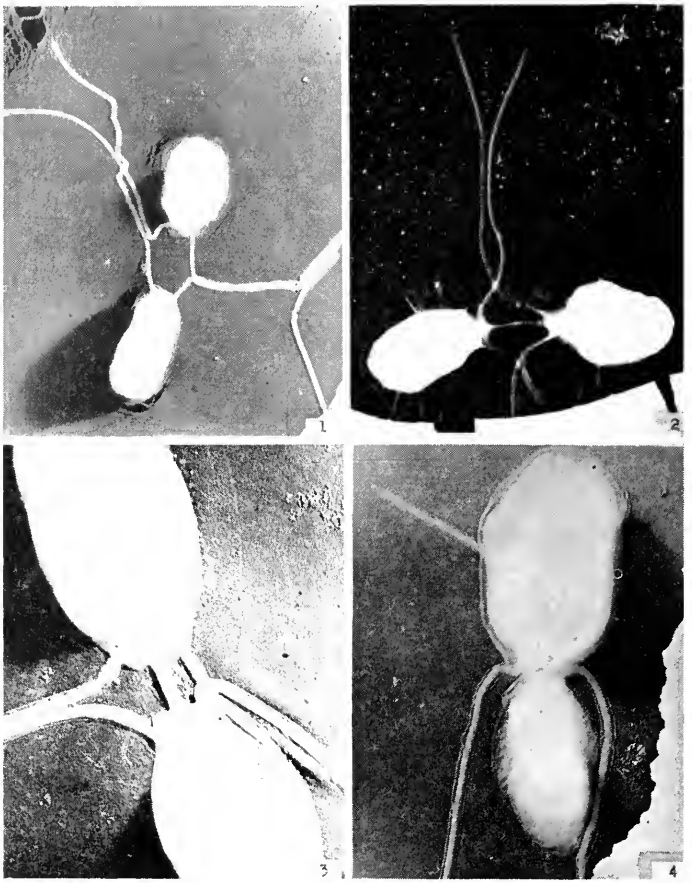

PLATE I. Stages in copulation of *Chlamydomonas moewusii* Gerloff. (Electron micrographs: Pd-shadowed, 7:1)

1, Agglutination of flagella (× 1500); 2, formation of protoplasmic bridge and dissociation of flagella (× 2500); 3, protoplasmic bridge (× 4000); 4, vis-à-vis pair (× 3000).

The electron microscope was made available by kind permission of Dr. J. L. Melnick, of the Section of Preventive Medicine, Yale University; and much valuable assistance was received in its operation from Mrs. J. O. Meinhart and Dr. W. Gaylord.

Pairing

In the formation of mating clumps and in the subsequent pairing of gametes, the flagella of *C. moewusii* play an important role (Lewin, 1952b). Mutant strains in which the flagella are lacking (Lewin, 1952a) are completely asexual. It is probable that these organelles are equally essential in the sexual processes of other flagellated algae, as are the cilia of *Paramecium* and other ciliates (Jennings, 1939). By their aid the mating cells are brought together and held until they pair and cytogamy can be initiated. As soon as pairing has been accomplished, the agglutination of the flagella ceases, perhaps as a result of a stimulus transmitted through the papillae and basal granules, and the flagella extend free in the medium once more. The whole process occupies less than 10 minutes (at 23°); typical stages are illustrated in Plate I.

In most unicellular algae, perhaps all, the process of fusion commences between the anterior ends of the mating cells. In those in which the gametes are not naked cells, the wall appears to dissolve first in this region, and a minute process may emerge, as in *Polytoma* (Pringsheim and Ondratschek, 1939), *Chlamydomonas proboscigera* (Korschikoff, in Pascher, 1927), and *C. engametos* (Moewus, 1933). A thin connecting strand of material, probably protoplasm, has been observed in *Chlamydomonas* and *Phyllocardium* (Korschikoff, 1927), *C. praecox* (Pascher, 1943), *C. chlamydogama* (Bold, 1949), *C. moewusii* (Lewin, 1950a; see Plate I, 2-3), and others, connecting the two cells; and ultimately this widens until the protoplasts fuse.

Although there are several records in the literature of gametes which have begun to fuse at their posterior ends—for example, in *Protosiphon* (Klebs, 1896), *Dunaliella* (Cavara, 1906), *Tetraspora* (Geitler, 1931), and in *Carteria* and *Chlamydomonas* spp. (Mitra, 1950)—there is good reason to believe that in most, if not all, cases the observed cell pairs were not indulging in syngamy but had resulted from incomplete cell division, as pointed out by Lerche (1937). Such twinned cells have been described or figured in *Chlamydomonas braunii* (Goroschankin, 1890), *C. stellata* (Gerloff, 1940), *C. variabilis* (Behlau, 1939), *C. upsaliensis* (Skuja, 1949), *C. moewusii* (Lewin, 1952a), *Haematococcus* (Reichenow, 1909), *Chlorococcum* (Bold, 1930), *Protosiphon* (Nayal, 1933), and so on.

Planozygotes

Once pairing has been initiated, the course of events leading to zygote formation differs according to species. In some the cells come to rest almost at once, whereas in others there is a further period of motility, possibly with the biological advantage of locating the zygospore in a suitable resting place. In most cases this motile stage occurs after cytogamy and perhaps also caryogamy, so that we are justified in referring to it as a planozygote. The axes of the cells usually swing round until the gametes are laterally appressed, and then not only do the cells fuse, but also the two pairs of flagella become coordinated into a single integrated motor apparatus, so that a quadriflagellate planozygote is formed, resembling a cell of *Carteria*. In fact, Behlau (1939) has shown that planozygotes of *Chlamydomonas variabilis* were apparently described by Jacobsen as "*Carteria ovata*" and, following Korschikoff (1926), Behlau considers that "*Chlorobotrys gracillima*" and "*Tetradonta variabilis*" are likewise diplophase cells of *Chlamydobotrys gracilis* and *Chloromonas paradoxa* respectively.

According to Teodoresco (1905) the planozygotes of *Dunaliella* are biflagellate, one pair of flagella being shed after copulation. In *Carteria iyengarii*, the planozygote, formed by the fusion of a naked, non-motile egg cell with a quadriflagellate microgamete, is propelled from some time by the flagella of the male cell (Ramanathan, 1942) like that recorded in the phycomycete *Monoblepharella* (Sparrow, 1939). Such planozygotes may remain actively swimming for some hours in *Protosiphon* (Klebs, 1896), *Phyllocardium* (Korschikoff, 1927), and *Tetraspora* (Klyver, 1929), or, in dim light, for as long as 1 to 3 weeks in *Chlamydomonas paradoxa* and *Chlamydobotrys stellata* (Gerloff, 1940; Strehlow, 1928-29) and *Phyllomonas striata* (Korschikoff, 1926). In *Raciborskiella*, according to Wislouch (1924-25), the planozygotes are even capable of diploid mitosis!

Vis-à-vis Pairs

An unusual exception to the general pattern of planozygote formation is furnished by *Chlamydomonas engametos* (Moewus, 1933; Mitra, 1951) and *C. moewusii* (Gerloff, 1940). In these forms, after gamete pairing has been initiated, cytogamy is arrested for a period, the cell pairs continuing to swim actively for some hours

(see Plate I, 4). (Moewus and Gerloff erroneously refer to this stage as a planozygote.) Since the cells in each pair are attached by their anterior ends, along a common axis, the two pairs of flagella would operate in conflict if both remained active; but here, at least in *C. moewusii*, another coordinating mechanism comes into play. One of the cells, though morphologically indistinguishable from its partner, ceases to beat its flagella, and the propulsion of the pair is thus left entirely in the hands of its mate. Such pairs regularly progress in one direction, always propelled by the same partner; they do not move forward and backward, as described for *C. eugametos indica* by Mitra (1951). It has been shown (Lewin, 1950a) that this difference in behavior is genetically inherent in the two mating types and can be considered to be controlled by a gene inseparably linked with the "sex" locus. A homothallic species, recently isolated, exhibits similar behavior of the vis-à-vis pairs, presenting a special problem of intraclonal differentiation which is now being studied. Like the planozygotes of *Protosiphon* (Bold, 1933; Maher, 1946-47), of *C. variabilis* (Behlau, 1939), and of several other *Chlamydomonas* spp. (Smith, 1950b), the gamete pairs of *C. moewusii* resemble free haploid cells in being positively phototactic, though those of *C. eugametos* (Moewus, 1940c) are said to show a reversal of taxis following copulation.

Recent evidence indicates that, in *C. moewusii*, there may be exchange of intracellular metabolites through the protoplasmic bridge joining the two copulants, so that one might be justified in referring to such pairs as a special case of heterocaryosis.

Cytogamy

In darkness, the gametes of *C. moewusii* remain in vis-à-vis pairs indefinitely, until death overtakes them. In dim light, a day or more may pass before fusion takes place (cf. *C. eugametos;* Moewus, 1933); while in bright light (300 to 500 foot-candles) cytogamy is consummated 4 to 8 hours after pairing, and at least 10 hours of additional illumination are required if a viable zygote is to result (Lewin, 1949b). In this respect *C. moewusii* differs from some organisms, in which light energy has been shown not to be essential during the process of fusion (for example, *Protosiphon;* Maher, 1946-47), and from others (for example, *Navicula halophila;* Subrahmanyan, 1946) in which light has been found actually to inhibit the process. There

is some evidence that in *Chlamydomonas* the wavelengths of light concerned are those absorbed by the chloroplast, so that the energy for cell fusion may be provided indirectly by photosynthesis. The flagella are not normally shed (as stated by Gerloff, 1940: cf. *C. upsaliensis;* Skuja, 1949) but can be seen to be withdrawn or resorbed, as in *Tetraspora* (Klyver, 1929), in *Protosiphon* (Bold, 1933), and in *C. eugametos indica* (Mitra, 1951) and *C. chlamydogama* (Bold, 1949), in the course of about 30 minutes. Half an hour later the cells finally fuse, the actual fusion taking place quite rapidly in this as in other species of *Chlamydomonas.*

The fate of the chloroplasts is usually unknown, though in *Phyllomonas* (Korschikoff, 1926) they are said to fuse. A study of the genetics of plastogene mutants in such algae would undoubtedly provide valuable information on this point.

In *Chlorogonium oogamum* (Pascher, 1931) and *Carteria iyengarii* (Ramanathan, 1942) the egg cells shed their walls before fertilization. In those species where the gametic cells are furnished with a cell wall, the statement has occasionally been made that the walls themselves coalesce in cytogamy (for example, *Chlamydomonas braunii,* Goroschankin, 1890; *C. eugametos,* Moewus, 1933; *C. eugametos indica,* Mitra, 1951; *C. moewusii,* Gerloff, 1940; *C. upsaliensis,* Skuja, 1949); but it seems most likely that in all species with walled gametes—as described in *C. media* (Klebs, 1896), *C. elongata,* (Korschikoff, 1923), *C. paupera* (Pascher, 1931-32), *C. gymnogyne* (Pascher, 1943), *C. chlamydogama* (Bold, 1949), and *C. moewusii* (Lewin, 1952c)—the walls are actually shed before (Smith, 1950b) or during cytogamy. In *C. moewusii* and other species of *Chlamydomonas* the fusing gamete protoplasts become invested in a primary zygote membrane of characteristic staining properties (Lewin, 1952c), and the formation of such a membrane appears to be a common phenomenon among related algae.

The Diploid Zygospore

Fusion of the nuclei is usually difficult to follow: in *Chlamydobotrys* and *Protosiphon* caryogamy may take place about 4 hours after copulation (Strehlow, 1928-29; Bold, 1933). Meanwhile, within the primary membrane, the zygote secretes a thick, highly impermeable wall, often characteristically patterned in relief (in *Brachio-*

monas this wall may appear mauve), and a number of other metabolic changes become apparent in the young zygospore. In most species starch is first accumulated and later replaced by oil, the pyrenoids fragment or become invisible, and in many cases the cells accumulate haematochrome and become bright orange or red. In others they remain green, as, for example, in *Phyllocardium* (Korschikoff, 1927), *Chlamydomonas media* (Klebs, 1896), *C. monoica*, (Strehlow, 1928-29), *Chlorococcum* (Starr, 1949), *C. eugametos* (Moewus, 1933) and *C. moewusii* (Lewin, 1949b). Although in the haplophase *Polytoma fusiformis* is competely apochlorotic, the zygospores have been described as chlorophyllose (Korschikoff, 1926; Strehlow, 1928-29), a unique feature that would present a number of interesting problems to the experimental phycologist.

Smith (1950b) has pointed out that one may distinguish two types of zygotes, those in which no growth takes place after cytogamy, and those wherein photosynthesis and growth continue in the diplophase, in the planozygote (for example, *Phyllocardium*, Korschikoff, 1927) or in the zygospore stage (for example, *Tetraspora*, Klyver, 1929, *Chlamydomonas chlamydogama*, Bold, 1949, and *Chlorococcum*, Starr, 1949). The volume of each gamete in *C. moewusii* is about 200 cubic microns, while that of the mature zygospore approaches 12,000 cubic microns, indicating a thirty-fold increase in bulk in approximately 60 hours. This rate of growth is comparable with that of the haplophase, which is capable of five generations (doublings) in a similar period (Lewin, 1952a). Though there is no vegetative division in the diplophase (except perhaps in *Raciborskiella*, Wislouch, 1924-25), and we can hardly speak of an alternation of sexual generations (cf. Behlau, 1939), the line dividing *Chlamydomonas* from such an alga as *Chlorochytrium* (Chlorococcales) is thin. According to Kurssanow and Schemakhanowa (1927), the vegetative cell of *Chlorochytrium lemnae* is typically diploid and, like the zygote of *Chlamydomonas moewusii*, is capable of enlargement but not of direct mitotic division. By meiosis and succeeding mitoses, 256 swarmers are produced which are capable of copulation to form quadriflagellate planozygotes; and these in turn settle down to an endophytic existence as a new diplophase. Thus the sexual cycles of *Chlamydomonas* and of *Chlorochytrium* are seen to differ in no essential respect, but merely in their relative emphasis on the haploid or diploid phase.

Zygospore Germination

The red, stellate zygospores of *Protosiphon* can be induced to germinate only after a period of some weeks of dormancy (Klebs, 1896). According to Nayal (1933), young green zygospores of one strain of *Protosiphon*, when formed under moist conditions, may germinate soon after being transferred to fresh media, while older red spores, especially if they have been subjected to heat or desiccation, remain dormant for some days before being capable of germination. The zygospores of *Polytoma* germinate readily under favorable conditions (Pringsheim and Ondratschek, 1939). However, in a depressingly large number of studies on the sexual cycles of unicellular algae, the obstacle of zygospore germination has proved insurmountable. The walls of such spores are usually relatively thick, and in some species are said to consist of three layers of different composition (Gerloff, 1940). Though actually only 1 to 2 μ thick, they may achieve a remarkable degree of impermeability to water and chemical agents. Thus zygospores of *Chlamydomonas moewusii* have been known to survive after several days of immersion in absolute acetone or after 4 years in air, though the zygotes of the related *C. eugametos* and those of *C. media* are killed by drying (Moewus, 1933, 1950c; Klebs, 1896). Zygospores of a strain of *Protosiphon*, isolated from desert silt, were shown to remain viable after heating at 75° to 91°C for 18 hours, or at 50° to 60° for 15 to 18 days (Nayal, 1933): while spores of *Polytoma* were found to be capable of germinating after contact with solid CO_2 for 10 minutes, or after four weeks' desiccation over $CaCl_2$ followed by 10 minutes at 75° (Pringsheim and Ondratschek, 1939). In *Chlamydomonas eugametos*, Moewus has reported (1946, 1950c) genetic variation in the resistance of zygospores to high temperatures and osmotic pressures.

Within such impenetrable walls, nuclear changes are extremely difficult to follow (Kater, 1929; Zimmermann, 1921) while we know nothing of the underlying metabolic changes associated with the dormant state. In our present profound ignorance, we have to resort to empirical methods for inducing germination, and a number of such studies have been carried out (see Bold, 1942). In *Cylindrocystis*, Pringsheim (1919) found that germination of zygospores—even before maturation was complete—could be induced by transferring them to fresh nutrient media. Lerche (1937) achieved a fair degree of

success with zygospores of *Dunaliella* by subjecting them to a controlled program of maturation, desiccation, and resuspension in media of augmented salinity. Starr (1949) effected zygospore germination in *Chlorococcum* and *Chlamydomonas chlamydogama* by incubating the spores for 48 hours at 37°. In many species of *Chlamydomonas* Smith (1950b) found that about 20 days were required for the ripening of the zygospores, which would then germinate regularly 1 to 2 days after being transferred to fresh media. On the other hand, Lewin (1949b) was able to induce only sporadic germination of ripe *C. moewusii* zygospores in a fraction considerably lower than the 10 per cent achieved at room temperatures by Gerloff (1940), but he found that spores in which full maturation had been arrested by starvation in darkness would germinate regularly. Moewus and Banerjee (1951) suggested that germination may be naturally arrested by the accumulation of some inhibitor such as *cis*-cinnamic acid, which can be removed from some zygotes by exhaustive washing in water.

The New Haplophase

In most unicellular algae, meiosis precedes zygote germination, and the sexual cycle is completed by the liberation of haploid zoospores. In desmids there is a tendency for two or three of the four post-meiotic nuclei to degenerate, so that only one or two haploid cells emerge from a zygospore on germination (Pothoff, 1928). It appears likely that in *Protosiphon*, too, meiosis precedes zygospore germination. Possibly only one post-meiotic nucleus survives and multiplies in the emerging thallus, which would otherwise be potentially heterocaryotic. But the zygotes of this alga are not very favorable material for cytological study, and despite several investigations of its life cycle (for example, Bold, 1933; Maher, 1946-47) this aspect remains obscure.

In species of Volvocales in which the zygote exhibits no growth after syngamy (see page 123), four zoospores are produced as a rule (Smith, 1950b), often within an extruded vesicle; whereas in cases where the zygotes enlarge in the days immediately following their formation a larger number may be formed as a result of subsequent mitoses. In *Dunaliella* (Lerche, 1937) and in *Chlamydomonas moewusii*, 4, 8, 16 (Gerloff, 1940), or 32 zoospores may be formed, the number depending on the size of the zygote, and hence on the condi-

tions of its formation. In *C. eugametos*, such zoospores are said to be immediately capable of acting as gametes (Moewus, 1933).

A few attempts have been made to follow meiotic segregation among the products of zygote germination. Pascher (1916) succeeded in crossing two 10-chromosome species of *Chlamydomonas* and in effecting some analysis of genetic characters among the progeny: it is claimed (Pascher, 1918b) that these early experiments were the first to establish *directly* the fact of Mendelian segregation in any plant.

Clones may be grown from zoospores produced after zygotic meiosis, and in such cultures sex (or mating type) has been shown to segregate equally in heterothallic species of *Gonium* and *Eudorina* (Schreiber, 1925), *Chlorogonium* (Schulze, 1927), *Dunaliella* (Lerche, 1937), *Chlamydomonas eugametos* (Moewus, 1933), *C. variabilis* (Behlau, 1939), *C. reinhardi* (Smith and Regnery 1950), and *C. moewusii* (Lewin, 1950b). However, the occasional formation of homothallic and neuter strains, variously attributed to cytologically demonstrable non-disjunction of sex chromosomes (Hartmann, 1934), to crossing-over between sex loci, and to mutations affecting sex determination and behavior, have been reported in a number of algae investigated by Moewus (see reviews by Jennings, 1941; Beadle, 1945; and Sonneborn, 1951; also Moewus, 1944). Philip and Haldane (1939) have criticized some of these results: Moewus' replies to these criticisms (1941, 1943) are far from satisfactory.

In *Dunaliella*, Lerche (1937) observed that zygotes would occasionally give rise on germination to only two zoospores, cytokinesis apparently coinciding with the first meiotic division. By taking advantage of such exceptional cases, she was able to distinguish first from second division segregations and thus to estimate the map distances of two unlinked loci, for mating type and for a morphological marker from *forma "oblonga,"* from their centromeres. Moewus (1940b) claimed to have mapped forty-two characters on the ten chromosomes which he observed in *Chlamydomonas eugametos* and its allies, in which crossing-over invariably took place between whole chromosomes, as he had found also to be the case in *Protosiphon*, *Polytoma*, and *Brachiomonas*. However, he illustrated (1936) chiasmata which appear similar to those of other organisms and stated (1944) that, in nature, crossing-over occurs in the four-strand stage. Several serious criticisms of these reports can be put forward, as has

been pointed out by Pätau (1941) and Harte (1948). In *C. moewusii* the genetic behavior of eight ultraviolet-induced mutant characters and of mating type has been investigated (Lewin, 1953); and evidence for linkage was obtained between *t* and *l*, two mutant characters respectively affecting cell division and flagellar activity, and between "*p*-aminobenzoic-less" and the mating-type locus. J. Hartshorne (personal communication) has studied the genetics of an ultraviolet-induced mutant "eyeless," and of two suppressed semi-lethals found in wild stocks of *C. reinhardi*. In this species, as in *C. moewusii* and in virtually all other organisms which have been studied, crossing-over takes place between chromatids, and two or four different haploid genotypes can be obtained from a single zygote.

CONCLUSION

Some of the greatest strides in the field of microbiology have been made when a number of workers, using different approaches and techniques, have concentrated on a single organism, such as *Neurospora* or *Escherichia coli*. It might not be rash to predict that similar progress will result from a concerted attack on the control of sexuality in a suitable selected unicellular alga. Many problems should be solved in this way, and others will inevitably arise; for the simplicity of microorganisms is more apparent than real.

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Sex in Protozoa
A Comparative Review

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In planning the symposium, it seemed especially important to include the highly interesting sex phenomena in the flagellates living in the intestine of the wood-feeding roach, *Cryptocercus punctulatus*, being worked out by Cleveland, and the latest developments in our knowledge of mating-type behavior in *Paramecium*. Since these papers could cover only a small fraction of the subject of sexual reproduction in the Protozoa, it seemed desirable to offer a review of sexuality in the group as a whole. In preparing the material for publication, the coverage presented at the symposium has been expanded. Unfortunately, Dr. Cleveland did not find it possible to provide a discussion of his results for the volume; hence I have added a condensed review of his published papers on this subject to the discussion of the Mastigophora.

In addition to such general works on Protozoa as those of Kent (1880-82), Bütschli (1882-89), Minchin (1912), Wenyon (1926), Calkins (1933), Kudo (1946), Grassé (1952-53), and Hall (1953), the reviews of Jennings (1920), Bělař (1926), Calkins and Summers (1941), Hartmann (1943), Doflein and Reichenow (1949-53), and Luyet (1950) are useful as references. For many of the major groups of Protozoa our knowledge of sexuality is very scanty, but for others there is an extensive literature. References to the literature in this review are therefore selective rather than comprehensive. In general, the sequences of classes and orders as given in Kudo's *Protozoology* (1946) will be followed. Kudo divides the Phylum Protozoa into two subphyla, the Plasmodroma and the Ciliophora. The Subphylum Plasmodroma is separated into the classes Mastigophora, Sarcodina, and Sporozoa, which will be discussed in that sequence.

CLASS MASTIGOPHORA

Kudo divides the Mastigophora into two subclasses, the Phytomastigina, or plant flagellates, and the Zoomastigina, or animal flagellates. The Phytomastigina include the orders Chrysomonadina, Cryptomonadina, Phytomonadina, Euglenoidina, Chloromonadina, and Dinoflagellata. The books by Fritsch (1935) and Smith (1950, 1951a), are useful in connection with this group.

Order Chrysomonadina

Evidence for sexual reproduction in this group is scanty and incomplete. Schiller (1925) saw sixteen small cells inside a mother shell of *Calyptrosphaera sphaeroidea* (Coccolithophoridae) and thought they might be gametes, but recorded no cell fusions. The same author (1926) reported fusion of isogametes produced by division of the protoplast of *Dinobryon sertularia*. The zygotes lost their flagella and sank to the bottom, but no further development was observed. Krieger (1930) noted that cysts of *Dinobryon cylindricum* and of *D. divergens* were binucleate. This was confirmed by Geitler (1935), who also observed cyst formation. The nucleus divided (without reduction) before the cyst walls were developed. Germination of the cysts was not seen, but Geitler supposed that the two sister nuclei united before germination, thus accomplishing autogamy. Schwarz (1932) described gamete formation, syngamy, postzygotic meiosis, and development of four new cells from the zygote in *Ochrosphaera neapolitana* (Fig. A, 1 to 12). Lackey (1938) occasionally found a globule of protoplasm between two loricae of *Chrysococcus spiralis* (13) and less often for *C. hemisphaerica*, which he thought might possibly indicate "conjugation." Skuja (1950) reported isogamy with encystment of the zygote in *Dinobryon borgei*, and Mack (1951) found encysted zygotes with adhering loricae of the uniting individuals in *Chrysolykos planktonicus* (15).

While there are thus strong indications of isogamy in this group of flagellates, full details of the life histories and chromosome conditions are not provided in any of the descriptions except that for *Ochrosphaera neapolitana*.

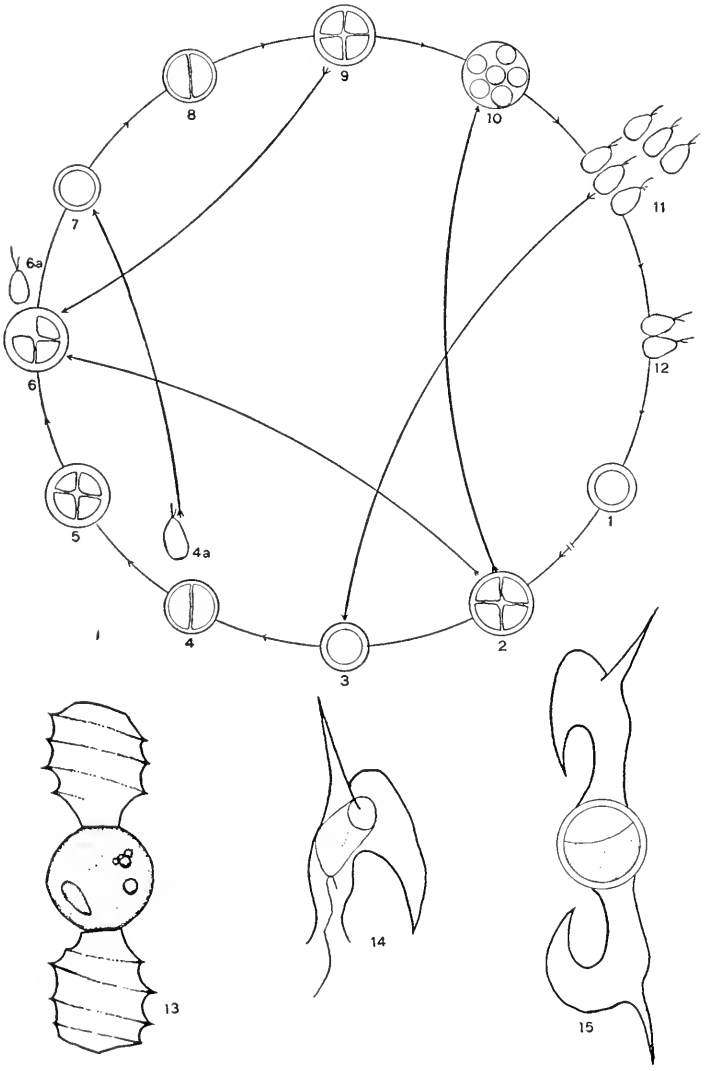


FIG. A.

1-12, diagrammatic life cycle of *Ochrosphaera neopolitana* (Coccolithophoridae) from Schwarz (1932), redrawn. Non-motile stages predominate. 1, zygote; 2, four cells resulting from meiotic divisions; 3, vegetative cell, derivable from a gamete or zygote; 4-6, division of vegetative cell; 4a, 6a, escape of "zoospores"; 7-10, development of gametocytes and gametes; 11, escape of gametes; 12, fusion of gametes. 13, apparent fusion of protoplasts of two individuals of *Chrysococcus spiralis*, from Lackey (1938), redrawn. 14, vegetative stage; 15, zygote of *Chrysolykos planktonicus* with adhering loricae, from Mack (1951), redrawn.

Order Cryptomonadina

No instances of sexual reproduction in this group have been found.

Order Phytomonadina

This group includes the chlamydomonads and volvocids that are discussed by Dr. Lewin in this volume. They will not be considered here except to state that sexual reproduction is extensively represented in the group.

Order Euglenoidina

Perhaps the most frequently referred to account of syngamy in the euglenoids is that of Dobell (1908) for the colorless *Copromonas subtilis* (Fig. B, 1 to 8.) Cytoplasmic fusion of ordinary individuals is followed by two meiotic nuclear divisions after each of which one daughter nucleus degenerates. The haploid pronuclei fuse to form a diploid nucleus. The zygotes usually encyst but may not before resuming normal vegetative activity. This life cycle was confirmed by Berliner (1909) for *C. major* and by Woodcock (1916) for *C. ruminantium*, and I have seen such cell fusions in my own cultures in which *C. subtilis* had developed. However, the absence of any chromosome determinations or other mitotic details leaves the accounts incomplete and therefore unsatisfactory.

Haase (1910) described syngamy between amoeboid gametes of reduced size in *Euglena sanguinea*, but the descriptions are not convincing, and later students of this species including Mainx (1928) and Günther (1928) could not confirm the findings of Haase and discounted her interpretations. Biecheler (1937) in France reported fusion of vegetative cells of an undetermined species of *Euglena*.

Krichenbauer (1937) found individuals of *Phacus pyrum* with two nuclei of different size, the smaller one being more heavily stained (Fig. B, 9, 10). He also found stages indicating nuclear fusion (11, 12). In a cell stained with Feulgen, a large nucleus seemed to show diakinesis (13). Two nuclear divisions (14, 15) were followed by division of the cell into four daughters. Krichenbauer saw four-part divisions many times among living specimens of *Phacus caudata*. He

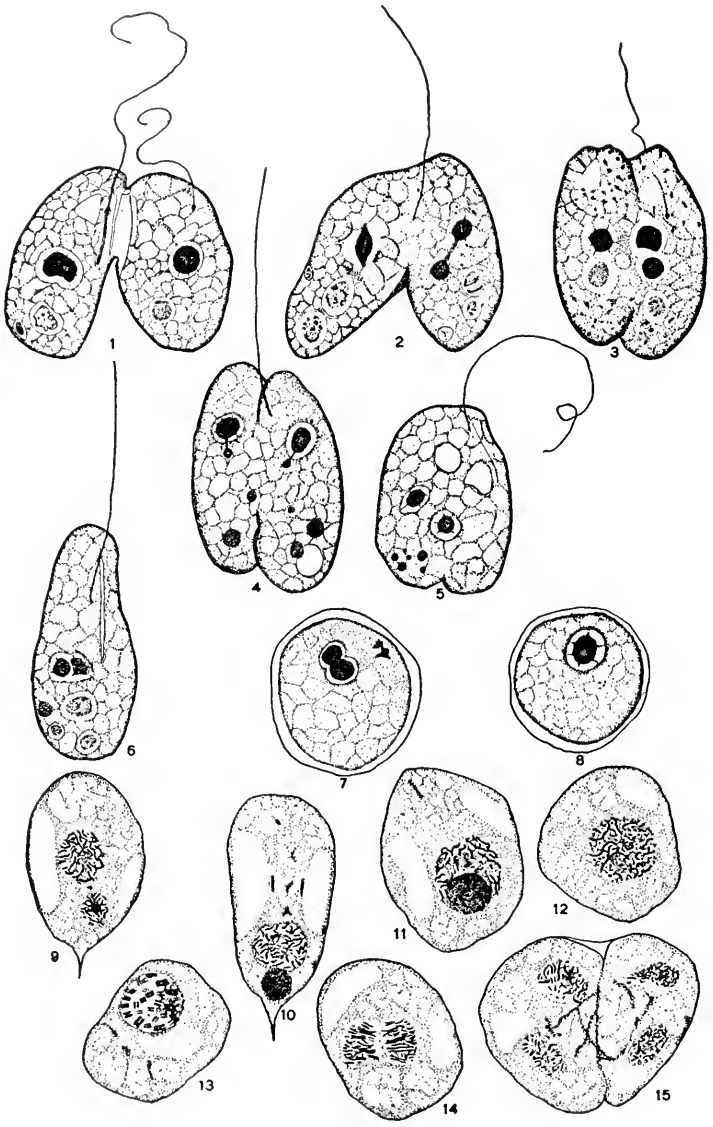


FIG. B.

1-8, syngamy of *Copromonas subtilis,* from Dobell (1908), redrawn. 1, early stage of cell fusion; 2-5, formation of "reduction" nuclei, further progress of fusion; 6, nuclear fushion in zygote; 7, 8, encystment accompanied by completion of nuclear fusion. 9-15, "sexual" stages in *Phacus pyrum,* from Krichenbauer (1937), redrawn. 9-12, fusion of a larger with a smaller nucleus; 12, enlarged nucleus with two endosomes; 13, "zygote" with "diakinesis" stage of nucleus; 14, first "meiotic" division; 15, after second "meiotic" division.

interpreted the evidence as indicating autogamy followed by a two-division meiosis and completed by four-part cytoplasmic divisions. Although suggestive, the evidence lacks completeness and alternative interpretations can be made. None of the accounts of syngamy in the euglenoids is complete and satisfactory.

Order Chloromonadina

No instances of syngamy in this group have been found.

Order Dinoflagellata

There is some evidence for syngamy in the dinoflagellates, drawn mostly from studies on *Noctiluca* (Fig. C, 1-5), certain free-living peridinians, and a few parasitic species.

Cienkowski (1871a) described fusion of adult specimens of *Noctiluca*, but the evidence can readily be interpreted otherwise, as can also that presented by Ishikawa (1891). Both cases appear to be examples of fusion following incomplete binary fission. On the other hand, evidence that minute swarmers (3 to 5) may undergo syngamic fusion is somewhat more convincing. Pratje (1921) saw swarmers adhering in pairs and complete fusion of isolated pairs, but did not see nuclear fusion. Hofker (1930) observed that swarmers derived from a single individual did not fuse. When he isolated together a number of individuals undergoing swarmer formation (Fig. C, 3), he found many active young specimens of *Noctiluca* the next morning and these developed into typical adults. He believed that the young specimens had resulted from fusion of swarmers. Gross (1934) also found that swarmers from a single parent did not unite, but those from different parents did, accompanied by nuclear fusion. However, he did not follow the development of the "zygotes" thus formed. Thus we have a highly suggestive series of observations indicating syngamy of swarmers in *Noctiluca*, which, however, lack completion.

Apparently Joseph (1879) was the first to report cell fusion in the Peridiniidae, having seen united pairs of *Peridinium stygium*. Stein (1883) showed a number of illustrations which were interpreted as indicating stages of "conjugation" for *Glenodinium putrisculus* (Stein), *Heterocapsa triqueta* Stein, and *Amphidinium lacustre* Stein,

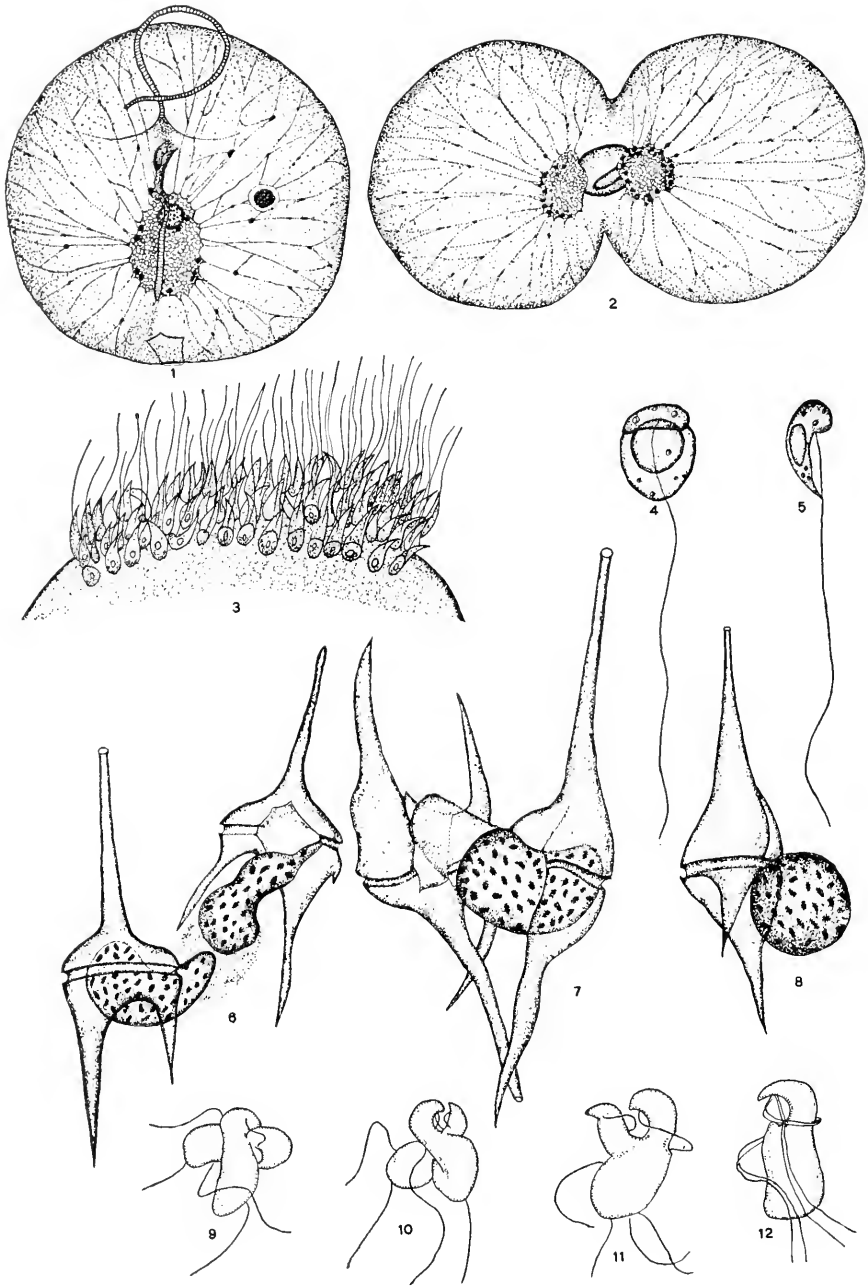


FIG. C.

1-5, *Noctiluca miliaris,* from Kuhn (1921), after Pratje (1921), redrawn. 1, vegetative individual; 2, binary fission; 3, multiple fission producing "swarmers"; 4, 5, enlarged face and edge views of "swarmers." 6-8, *Ceratium hirundinella,* formation of "zygote," from Kuhn (1921), after Zederbauer (1904), redrawn. 9-12, four stages in the fusion of gametes of *Coccidinium mesnili,* a parasitic dinoflagellate, from Chatton and Biecheler (1936), redrawn.

but in each case a rearrangement of sequences seems to indicate cell division rather than cell fusion. Zederbauer (1904) showed association of individuals of *Ceratium hirundinella* in pairs with the extrusion and fusion of protoplasts between the "parents" to produce a rounded "zygospore" which encysted (Fig. C, 6 to 8). Development of the

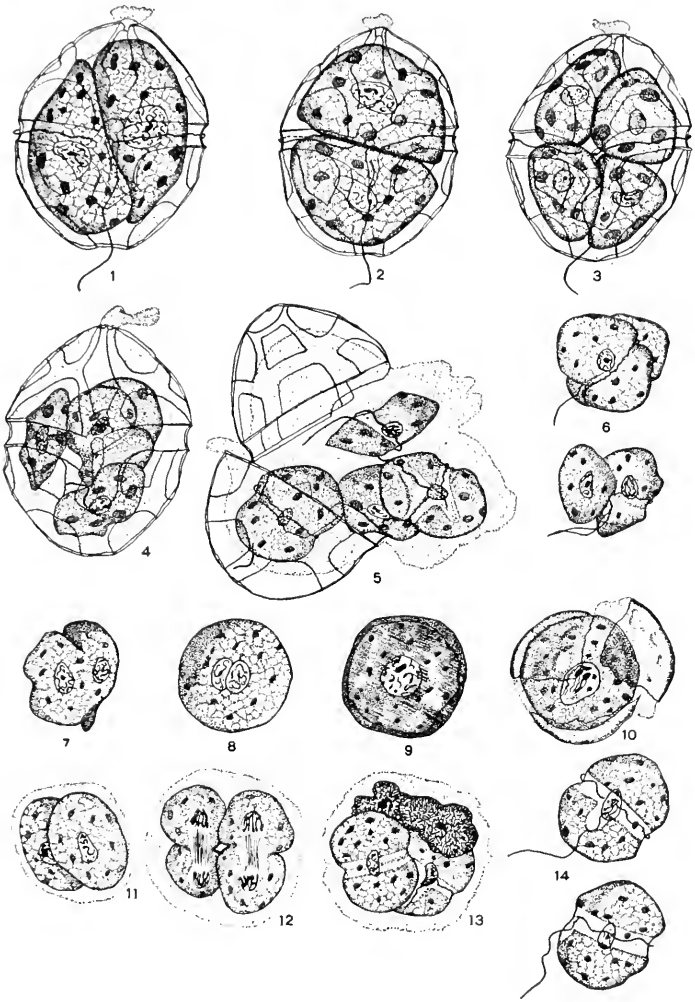


FIG. D.

1-14, stages in sexual reproduction of *Glenodinium lubiniensiforme*, from Diwald (1938), redrawn. 1-4, development of gametes; 5, escape of gametes; 6, 7, fusion of gametes; 8, nuclear fusion; 9, encystment of zygote; 10, excystment of zygote; 11-12, development of "swarmers"; 13, degeneration of two "swarmers"; 14, excysted "swarmers."

"zygospore" was not followed. He called the process "copulation." Entz (1907) found similar stages of this species in preserved material from "Balaton-Sees." Hall (1925) interpreted binucleate cysts of this species as possibly indicating previous cell fusion.

Perhaps the most interesting reports for this group are those of Diwald (1938) for *Glenodinium lubiniensiforme*. The life history includes the following: binary fission, with parent shells separating along the girdle; formation of two vegetative "swarmers" inside the parent shell; encystment followed by excystment and binary fission; sexual reproduction with the following stages: (a) two successive divisions inside the parent shell forming four gametes (Fig. D, 1 to 4) which are released from the shell (5); (b) fusion of isogametes to produce zygotes (6 to 8); (c) encystment of the zygotes (9); (d) excystment of zygotes after at least 10 days' rest (10); (e) while still immobile, and surrounded by a jelly-like covering, two successive divisions to produce "swarmers" (11 to 13); (f) development of flagella and resumption of vegetative life (14). Diwald believed that meiosis took place during the two divisions following excystment of the zygotes, but chromosomes counts were not given. He found that gametes did not form for a certain time after excystment. Further-

TABLE I

RESULTS OF MIXING GAMETES OF 16 CLONES OF *Glenodinium lubiniensiforme* IN ALL COMBINATIONS

"Copulation" indicated by +; no union indicated by −. (From Diwald, 1938)

	2	4	12	3	5	6	7	8	9	10	11	13	14	15	16
2	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
4	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
12	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
6	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
8	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
9	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
10	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
11	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
13	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
14	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
15	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
16	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−

more, plus and minus clones could be established. Filtrates from plus clones induced gamete formation in minus clones and vice versa. Gametes from any one clone did not fuse with each other. When mixtures of plus and minus clones were made, gametes from any plus clone would unite with those of any minus clone and vice versa (Table I). This demonstration of the formation of chemical attractants is a matter of great interest; it recalls the claims of Moewus and others for chemical attractants in *Chlamydomonas* (see paper by Lewin).

Duboscq and Collin (1910) described phenomena interpreted as sexual reproduction in parasitic dinoflagellates living in some marine tintinnoid ciliates. In his monograph on parasitic dinoflagellates, Chatton (1920) called this parasite *Duboscquella tintinnicola* and stated that "gametocytes" were produced from larger, vegetative stages by successive divisions. Two divisions, probably meiotic, then produced flagellated gametes which had something of the appearance of *Oxyrrhis marina*. These fused in pairs, producing zygotes which rounded up and lost their flagella. Further development was not followed. Later, Chatton (1927) described meiotic gametogenesis in *Paradinium poucheti*, a parasite of the body cavity of larger copepods of the genus *Acostia*. The flagellispores which developed were considered to be haploid gametes, but fertilization was not observed. Chatton and Biecheler (1936) described fusion of flagellispores (Fig. C, 9 to 12) formed by the parasitic dinoflagellate *Coccidinium mesnili* which parasitizes another dinoflagellate, *Crypotperidinium*. Thus there seems to be very strong evidence for syngamy in this group.

Order Rhizomastigina

Kudo recognizes four orders in the Zoomastigina: Rhizomastigina, Protomonadina, Polymastigina, and Hypermastigina.

In the Rhizomastigina we have the accounts of sexual reproduction in *Mastigella vitrea* and *Mastigina setosa* by Goldschmidt (1907). "Chromidia" were said to give rise to "secondary nuclei," each of which with a bit of cytoplasm became a "gamete." The gametes were said to emerge from parents and fuse to form zygotes which grew into adults. Bělař (1926) stated that these accounts were based on misinterpretations involving small fungoid parasites. However, Ivanic (1936) has described "copulation" in *Mastigina hylae*, an amoebo-

flagellate inhabiting the rectum of tadpoles. Fusion of cells was supposed to be followed by nuclear fusion, but further development was not reported. The evidence in this case is not convincing and is too incomplete to establish syngamy.

Order Protomonadina

In this group one of the most important families is the Trypanosomatidae, which consists of the so-called haemoflagellates. They are uniflagellate and have a characteristic kinetoplast (sometimes erroneously called a parabasal body) in addition to the nucleus. An enormous amount of research has been conducted on this group, especially the trypanosomes, yet no complete account of syngamy has been produced.

The early interpretations of sexuality in haemoflagellates by Schaudinn (1904), Prowazek (1904a, 1905), Moore and Breinl (1908), Moore, Breinl, and Hindle (1908), Baldrey (1909), Schilling (1910), and Lebedeff (1910), based largely on polymorphism, have mostly been discredited. Doflein (1910) saw the wide variety of morphological types in cultures of *Trypanosoma rotatorium* but cautioned against calling these "male," "female," and "indifferent" without convincing evidence. In 1926, Wenyon reviewed the descriptions of sexuality in Trypanosomatidae as reported in the literature up to that time and concluded that in no case had authors submitted sufficient evidence to support the interpretations of sexuality. Since then, additional descriptions of phenomena interpreted as sexual have appeared from time to time, especially during the past decade.

Elkeles (1944) reported possible sexual phenomena for *Trypanosoma cruzi* as had Chagas (1927) and Muniz (1927) some years earlier. Vanderplank (1944) described two types of individuals for *T. congolense* and *T. rhodesience*. In the latter species, one type had two paired and two unpaired chromosomes ($n=6$), and the other had two paired and one unpaired chromosome ($n=5$). In *T. congolense* one type had three pairs of chromosomes and the other had three paired and one unpaired chromosome. It was supposed that meiosis took place, the unpaired chromosomes acting like the sex chromosomes of other animals, and that gametes so formed would fuse with other haploid cells. Later (1947) the same author exhibited demonstrations showing nuclear divisions with three chromosomes

supposed to be gamonts or gametes of *T. congolense*. Also, "latent bodies" were said to form after sexual union, and these contained one or two nuclei and six chromosomes.

Roskin and Schischliaiewa (1928) described mitoses with only three chromosomes, not only in *T. congolense* but also in *T. equiperdum, T. brucei* and *T. "surra,"* and Wolcott (1952) found only three chromosomes in *T. lewisi*. In many protozoan nuclei the prophase chromatids often become rather widely separated so that the chromosome number appears to be double that shown in the metaphase and anaphase. Thus the condition of two paired and two unpaired chromosomes reported by Vanderplank may well be a prophase stage showing three pairs of chromatids, two of which are somewhat more separated than the others, and the combination of two pairs and one unpaired might well be a case where the chromatids were visible for two of the chromosomes and not well separated for the third.

Fiennes (1945) also reported sexual phenomena for *Trypanosoma congolense*. Developmental forms were found in sections and smears from the skin of infected cattle. *T. congolense* was stimulated to "conjugate" by adding a drop of 0.3% sodium chloride to a drop of mouse blood containing the flagellates; micro- and macrogametes were said to develop from sexually mature forms and fuse within 10 minutes. A "microgamete" penetrated the translucent posterior region of a "magrogamete," the posterior end entering first. The "zygote" thus formed was about 20 μ long and motile, with trypanosome shape, but contraction of the body produced an "oöcyst" about 10 μ in diameter. The "oöcyst" appeared to form "sporoblasts," probably eight in number, which divided into many "sporozoites." The fate of these "sporozoites" was uncertain. Trypanosomes in mouse blood treated with weak salt solution showed three different forms: (1) immature sexual or asexual stages which showed no changes except swelling; (2) mature sexual stages which united as described; (3) infective (?) forms which were changed into a variety of developmental stages, round, stumpy, elongate, and so on, usually found in the tsetse fly. The predicted confirmatory papers have not been seen.

Fairbairn, Culwick, and Gee (1946) reported syngamy in *Trypanosoma rhodesience* and *T. simiae*. Metacyclic forms of *T. rhodesiense* occurred in two distinct types with significantly different mean lengths. These two types produced three blood types, long,

short and intermediate (cf. three types mentioned by Fiennes for *T. congolense*). All forms were said to have six chromosomes (possibly three pairs of chromatids). In a suspension in Ringer's-glucose solution, the following events usually took place within 20 minutes: two trypanosomes approached and intertwined, their centers making contact but their ends free. After a short time the anterior end of each fused with the posterior end of its mate, producing a fusiform body with two nuclei and a flagellum at each end. After a time, these fusiform bodies divided at the middle, producing two trypanosomes. Sometimes before the fusiform body divided one partner appeared to degenerate and the other might or might not free itself. Instances were found of more than two flagellates fused together. Similar conditions were observed on stained slides. Sometimes head-to-head fusions also took place. In *T. simiae* identical processes were seen. The authors stated that in blood trypanosomes syngamy could be induced by almost any marked and sudden change of environment. Each of the three forms, long, intermediate, or short, could fuse with itself or with either of the other two. That is, sex in *T. rhodesiense* was not obviously related to morphology. (This statement is interesting since most other authors have made differences in morphology the basis of sex differentiation.) The authors considered that syngamy was an adaptive process in which two flagellates associated to exchange three chromosomes. (Such an exchange of a haploid set of chromosomes would correspond to conjugation in the ciliates.) The authors noted that Hoare (1936) had reported somewhat similar phenomena in *T. congolense* in blood films and interpreted the appearances as autoagglutination or agglomeration. Agglutination is also suggested by the lack of distinctions between uniting forms noted above. Cytological evidence for the "exchange of chromosomes" was not presented.

In 1951, Culwick, Fairbairn, and Culwick reported that when they inoculated a mixture of *T. rhodesiense* and *T. brucei* into rats the resulting infections were different from the parent strains. Cyclical transmission of this infection by *Glossina morsitans* did not restore the morphology seen in the parental types. Hybridization was considered an explanation, and similar results were obtained when *T. gambiense* and *T. brucei* were the "parent" species. However, the notorious polymorphism of all three of these species might well cast doubt on the interpretation given.

Other members of the Trypanosomatidae have been credited with sexuality. Flu (1908) described "male" and "female" stages in a leptomonad flagellate from the gut of *Melophagus ovinus*. Although he saw no "copulation," certain stages were interpreted as being "oökinetes."

Adie (1921) reported "conjugation" between free flagellates of *Leishmania donovani* in gut cells of the bedbug, *Cimex lectularius*, which had been fed on spleen juice from a case of kala azar. Patton (1922) stated that he had confirmed the intracellular stages reported by Adie, but Wenyon (1926) remarked that the bugs died after feeding on the spleen juice and were then placed in an incubator at 27°C. The developmental stages were found about 36 hours afterwards. He thought that the observed development was more like that in a culture than that in a vector and was skeptical of the interpretations given.

Although there appears to be a considerable amount of evidence for cell fusions in members of the Trypanosomatidae, it must be confessed that the observations reported are usually capable of interpretations other than "sexual," and in no case are the so-called "sexual" phenomena complete with satisfactory chromosome counts and meiosis.

In the somewhat related Cryptobiidae there are several accounts of sexual reproduction. The most convincing is that of Bělař (1916), who repudiated his interpretation in 1926.

Possibly the earliest accounts of cell fusion in free-living members of the Protomonadina were those of Dallinger and Drysdale for several small unnamed flagellates (1873a,b; 1874a,b), in addition to one with the characters of *Polytoma* (1874c), and the "calycine monad" (1875), which apparently was *Tetramitus rostratus*. Cell fusion was followed by encystment. At excystment, large numbers of small granules were released which, according to the authors, grew more or less rapidly to the adult condition. While evidence for cell fusion might be accepted, the formation of large numbers of minute granules which grow into adult flagellates has not been confirmed.

Additional accounts of fusion of ordinary cells followed by encystment have been given for a number of protomonads, for example, for *Monas vivipara* (Prowazek, 1903); *Bodo lacertae* (Prowazek, 1904b); *Monas termo* (Martin, 1912); *Helkesimastix faecicola* (Fig. E, 1 to 6) (Woodcock and Lapage, 1915); *Cercomonas longicauda*

(7 to 12); and *Spiromonas angustata;* with some evidence for *Heteromita globosa* (Woodcock, 1916); and *Prowazekella lacertae* (24-28) (Wenyon, 1920). Woodcock (1916) also described fusion of individuals of *Spiromonas angustata* within a cyst (13 to 18), and Alexeieff (1925) described fusion of active flagellates of *Alphamonas* sp.

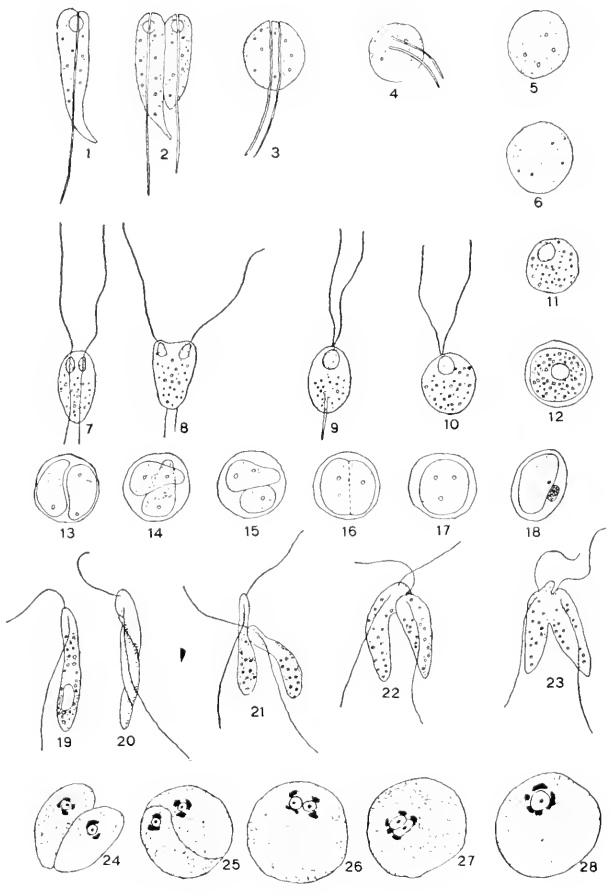


Fig. E. Cell fusion in protomonad flagellates, all redrawn.

1-4, fusion of vegetative cells. 5, 6, encystment of zygote, of *Helkesimastix faecicola*, from Woodcock and Lapage (1915). 7-12, cell fusion followed by encystment of zygote of *Cercomonas longicauda*, from Woodcock (1916). 13-18, fusion of cells after encystment of *Spiromonas angustata*, from Woodcock (1916). 19, 20, vegetative cells. 21-23, fusion of cells of *Alphamonas sp.* (? = *Spiromonas angustata*) from Alexeieff (1925). 24-28, cell fusion and encystment of *Prowazekella* (= *Proteromonas*) *lacertae*, from Wenyon (1920).

(19 to 23). Prowazek's "*Bodo*" *lacertae* is probably identical with Wenyon's *Prowazekella lacertae*, now called *Proteromonas lacertae*, and *Alexeieff's Alphamonas* sp. has been identified by Woodcock (1921) as the *Spiromonas augustata* described by the latter in 1916. Prowazek interpreted his evidence as indicating reduction divisions followed by fusion of nuclei, but Wenyon (1920) does not support that interpretation, although he does show cell and nuclear fusion (Fig. E, 24 to 28). It is probable that the reduction nuclei of Prowazek were the paranuclear bodies (formerly called parabasal bodies) characteristic of this flagellate. Prowazek (1904b) also described "autogamy" in cysts of "*Bodo*" *lacertae*, but this is probably a misinterpretation, as indicated by Wenyon (1920).

Dangeard (1910) described "autogamy" for *Anthophysa vegetans*. He believed that a nuclear division took place in weakened cells which were unable to complete cell division. These binucleate animals encysted, a clear area appeared in the middle, later to disappear and to be followed by nuclear fusion. Dangeard was not able to follow the process of excystation.

Order Polymastigina

A half century ago some protozoologists were ready to accept incomplete evidence as indicating sexuality; for example, for *Trichomonas intestinalis* (=*T. hominis*) and *Lamblia intestinalis* (=*Giardia lamblia*) by Schaudinn (1903), and for *Trichomastix lacertae*, *Trichomonas* "*intestinalis*" from the rat, and *Hexamitus* "*intestinalis*" from *Testudo graeca* by Prowazek (1904b). In some cases developmental stages of *Blastocystis* were confused with stages in the life history of the flagellates.

The account of cell fusion followed by encystment and the later emergence of minute sporules for *Tetramitus rostratus* by Dallinger and Drysdale has already been mentioned. Bunting (1926) worked out the life history of this flagellate, which showed that it could transform completely into an amoeboid stage that could encyst. Only amoebae encysted, and only amoebae came out of the cysts. Amoeboid stages readily transformed into flagellate stages and vice versa. Under appropriate conditions either the amoeboid or the flagellate stage could persist indefinitely. Bunting watched fusion of living flagellates under the microscope (Fig. F, 1 to 7), and stained prepara-

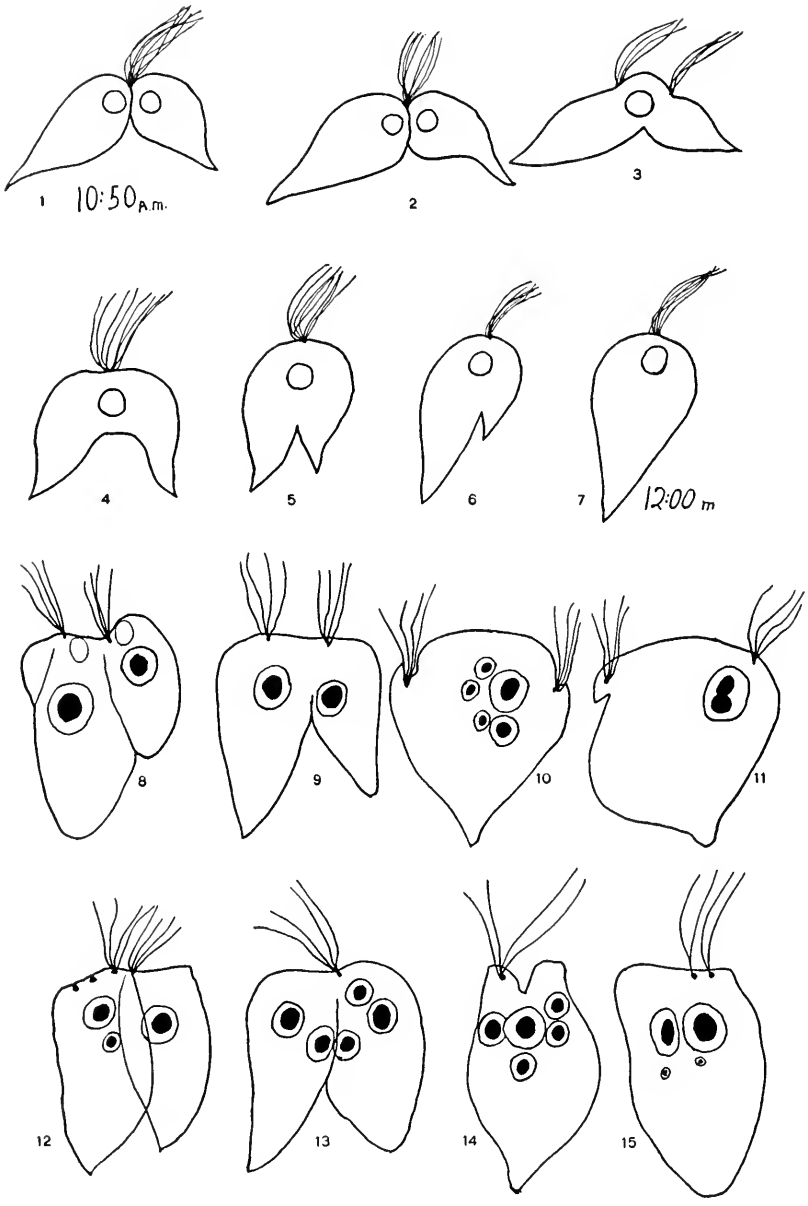


FIG. F.

1-15, *Tetramitus rostratus* from Bunting (1926); 1-7, fusion of living flagellates; 8-11, fusion of animals of unequal size, stained slide; 12-15, fusion of flagellates of equal size, stained slide. Note multiple nuclei suggesting meiotic phenomena.

tions of such fusing individuals showed nuclear conditions somewhat like those reported by Dobell (1908) for *Copromonas subtilis* (8 to 15). However, Bunting did not claim that a process of syngamy had taken place. These processes of cell fusion probably are significant, and further studies are needed with that possibility in mind.

The only satisfactory accounts of syngamy in members of the Polymastigina are those of Cleveland for the flagellates living in the gut of the wood-eating roach, *Cryptocercus punctulatus*. The same may be said for the Hypermastigina. A review of Cleveland's papers follows.

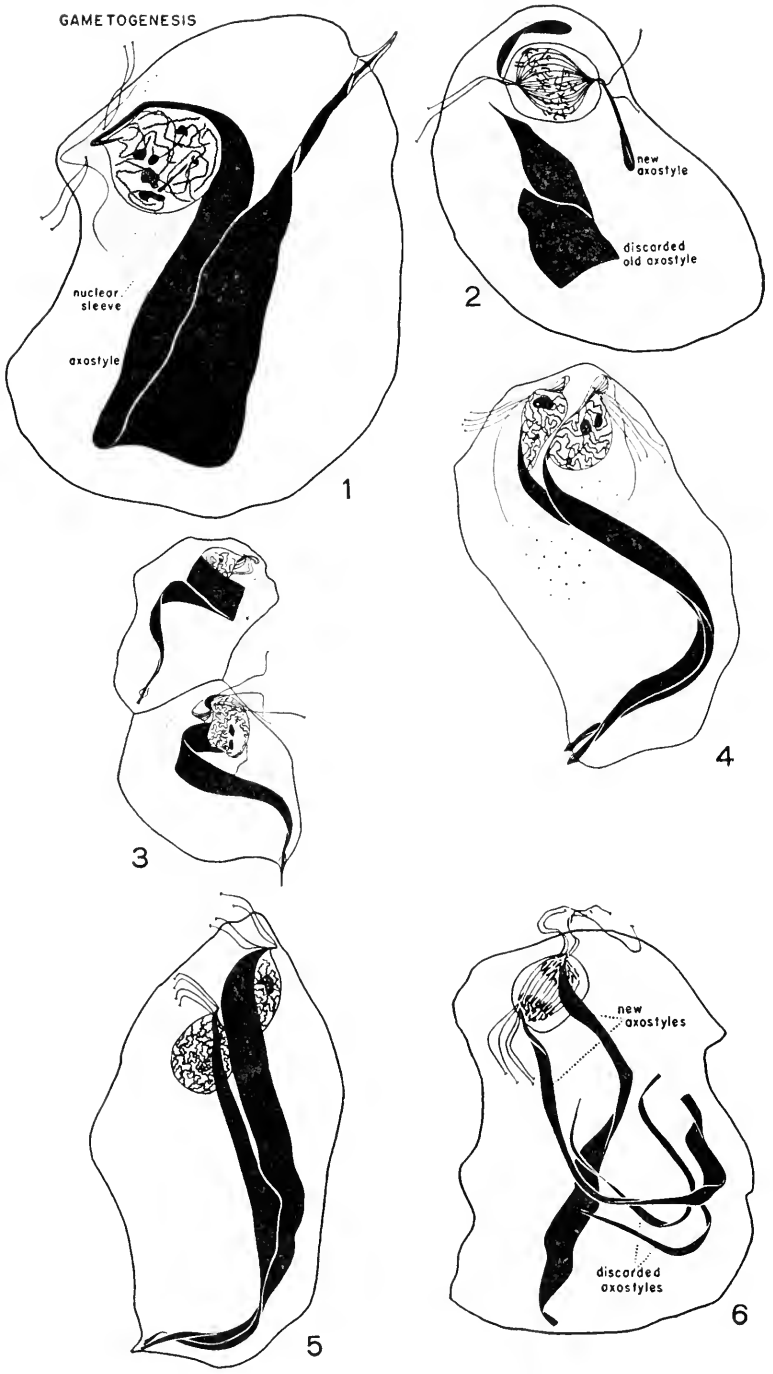
HORMONE-INDUCED SEXUALITY IN ANIMAL FLAGELLATES

One of the most interesting developments in the field of sexual reproduction in Protozoa is the occurrence of syngamy in the flagellates that inhabit the gut of the wood-eating roach, *Cryptocercus punctulatus*. These conditions have been brought to our attention by Cleveland who, with his associates, published an extensive paper on the morphology, taxonomy and host-parasite relations of this group of flagellates in 1934.

More recently Cleveland has begun a series of papers revealing the details of "sex" in these flagellates. In two preliminary papers (1947a,b) this author noted that the flagellate fauna of the roach consists of some twenty-five species distributed into two orders, eight families, and twelve genera, ranging from relatively simple polymastigotes to extremely complex hypermastigotes, all of which exhibit some form of sexual behavior at the molting period of the host. Between molts, no sexuality occurs. In termites, closely related flagellates do not exhibit any sexual activity. At each molt of termites, the flagellates are lost and must be regained from other members of the termite colony by proctodaeal feeding.

Cleveland believes that the sexual activities of the flagellates in *Cryptocercus* are induced by some direct effect of the molting hormone of the host, rather than by an indirect effect through such agencies as a decrease in food supply, increase in carbon dioxide, or decrease in oxygen, all of which occur during molting.

One remarkable feature of this behavior is that different flagellates follow different patterns of sexual activity, both in relation to the process of molting itself and in relation to the various ways in


GAMETOGENESIS
nuclear sleeve
axostyle
1
new axostyle
discarded old axostyle
2
3
4
5
new axostyles
discarded axostyles
6

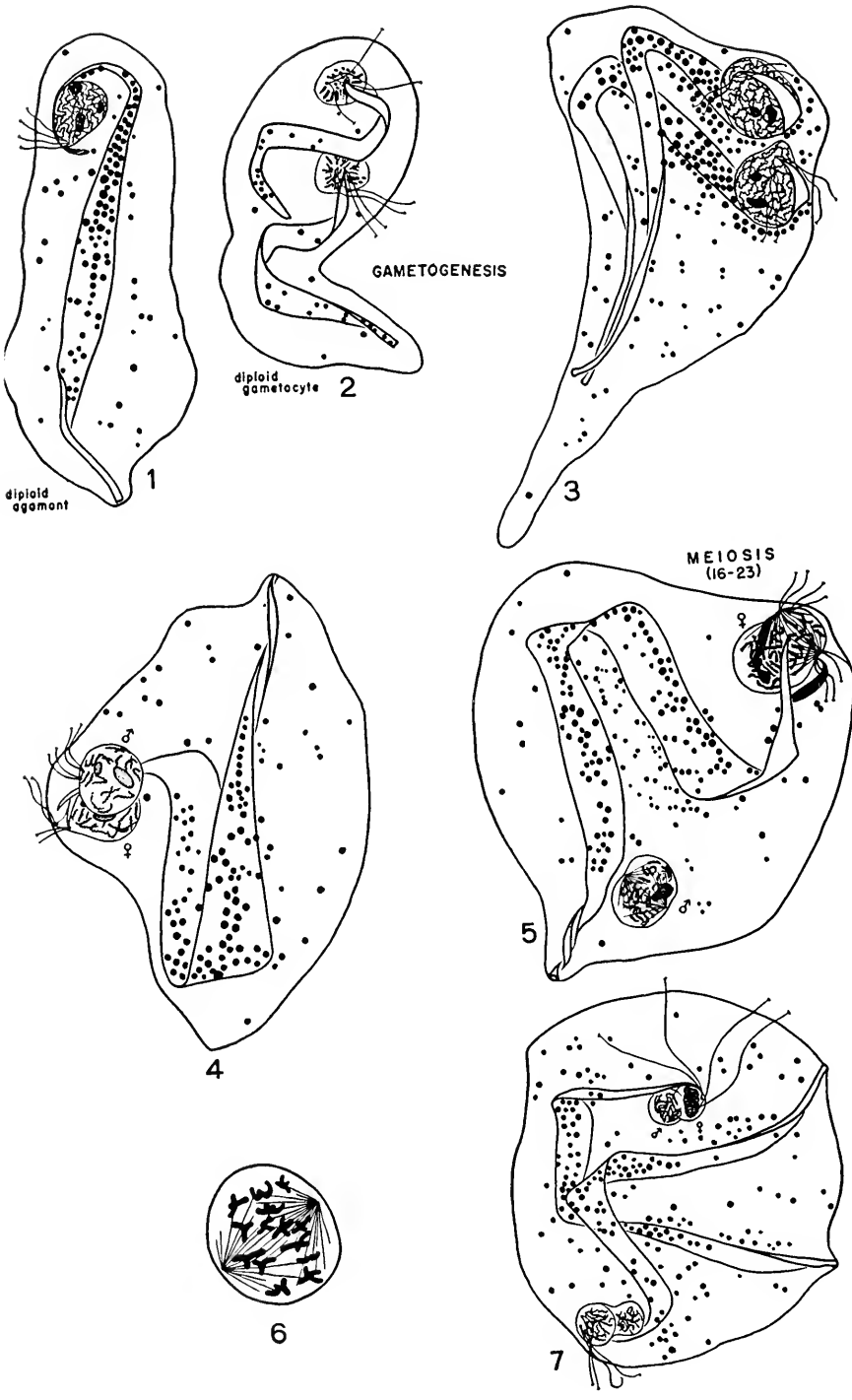
which meiosis, gamete formation, and syngamy can be accomplished. These variations allow for an analysis of the process of meiosis and an evaluation of the differences between meiosis and vegetative mitosis. Diploidy and polyploidy may arise through chromosome duplication without centriole duplication as well as through syngamy. The series of papers on individual genera and species gives the important details.

In preparing these reviews of Cleveland's published papers, I have, to a large extent, adopted my own phraseology. In doing so there has been the risk of failing to convey the exact meanings that Cleveland had in mind. It is to be understood, therefore, that I am assuming full responsibility for the statements made in these reviews. Naturally, limitations of space have made it necessary to omit a great many important aspects of Cleveland's work; hence the reader is urged to read the original papers in order to appreciate their full importance.

Polymastigotes. In three separate papers Cleveland described the sexual behavior of members of the genera *Oxymonas* (1950a), *Saccinobaculus* (1950b), and *Notila* (1950c), all belonging to the Polymastigina. These three genera are closely related, each having four anterior flagella, intranuclear centrioles, flat, rather broad axostyles, and a relatively large number of chromosomes. In *Oxymonas* (Fig. G, 1 to 4) and *Saccinobaculus* (5 to 6) the sexual cycles are very similar. In each the animals are haploid, and gametogenesis consists of the division of a gametocyte (1) (which before the division cannot be distinguished from an ordinary agamont) into two gametes which are only slightly dissimilar in size. During this division in *Oxymonas doroaxostylus* the nucleus leaves the nuclear sleeve, and this sleeve with the old flagella and axostyle degenerates, new flagella and new axostyles being developed by the daughter centrioles for

FIG. G.

1-4, *Oxymonas doroaxostylus*, from Cleveland (1950a). 1, Gametocyte, prophase, nucleus still associated with extranuclear organelles, axostyle (heavy, black body), flagella, and nuclear sleeve shown; 2, gametocyte, metaphase, old flagella, and nuclear sleeve have disappeared, old axostyle disintegrating, each centriole producing a new axostyle; 3, gametes fusing, anterior end of one joining posterior end of the other; 4, axostyles are fused for half or more of their length and nuclei are touching. 5, 6, *Saccinobaculus ambloaxostylus*, from Cleveland (1950b). 5, axostyles of gametes beginning to fuse bringing the pronuclei close together; 6, anaphase of zygotic meiosis, old axostyles nearly disintegrated, new axostyles about half grown.


diploid
agamont
1
GAMETOGENESIS
diploid
gametocyte
2
3
MEIOSIS
(16-23)
♀
♂
4
5
6
7

the daughter cells which are gametes (2). In *Saccinobaculus ambloaxostylus* the parental axostyle is not discarded until the metaphase of nuclear division, and the parental flagella are retained, two going to each daughter, two new ones being developed from new centrioles arising by division of parental centrioles, a new axostyle growing out from each new centriole.

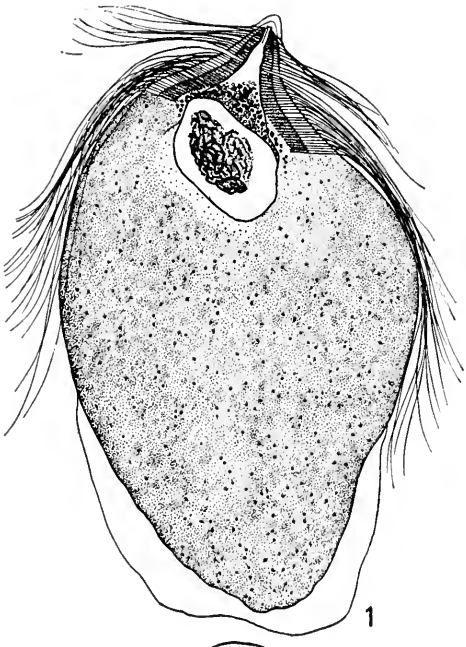
In *Oxymonas*, two gametes meet and fuse (3), first the cytoplasm, then the axostyles (4), and finally the nuclei. Since gametogenesis begins 6 to 7 days before molting of the host, and meiosis, which is zygotic, does not begin until approximately 1 day after molting, the zygote stage lasts at least 6 days with no loss of extranuclear organelles. In *Saccinobaculus*, after cytoplasmic fusion (5), nuclear fusion is delayed 2 or 3 days during which the cells become spherical and remain rather inactive until the host molts several days later. Within 8 to 12 hours after molting of the roach, the zygote becomes more active, and, some 20 to 24 hours after molting, meiosis (6) begins, to be completed in about 24 hours.

In the usual two-division meiosis the chromosomes are duplicated during the first division, whereas the centromeres are not. In the second division the centromeres are duplicated but not the chromosomes. In both these flagellates meiosis is accomplished by a single nuclear division during which neither chromosomes nor centromeres are duplicated. Only slight pairing takes place, and the chromosomes move to the poles as monads, as seen in the second meiotic division of most other animals. Random segregation takes place. Cytoplasmic division of the zygote completes the formation of two haploid agametes.

In *Oxymonas nana* essentially similar phenomena take place, but in this species the zygote encysts. Later, excystation and division into haploid agametes occur.

FIG. H. *Notila proteus*, from Cleveland (1950c).

1, agamont with nucleus, nucleoli, four flagella, axostyle, and axostylar granules; 2, gametocyte shortly before cytoplasmic division, each nucleus with four flagella and a half-grown axostyle; 3, axostyles of fused gametes beginning to fuse at their posterior ends; 4, chromosomes of male and female nuclei have regained their major coils preparatory to meiosis, male nucleus has become dissociated from fused axostyles and is moving away; 5, male nucleus has moved to the posterior end of the cell continuing its development, old fused axostyles still connected with female nucleus which has two new axostyles and eight flagella; 6, early anaphase of male nucleus, the female nucleus at this stage presents the same picture; 7, each male nucleus is fusing with a female nucleus.



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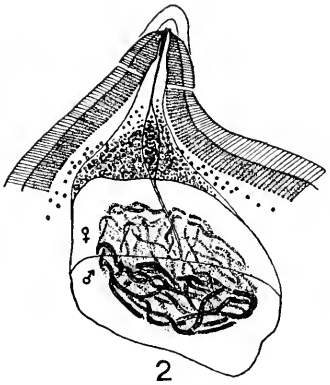

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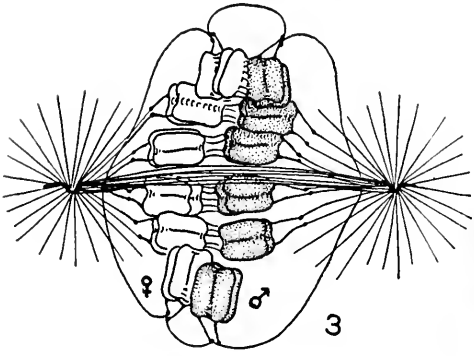

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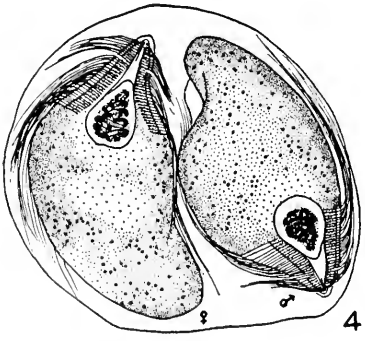

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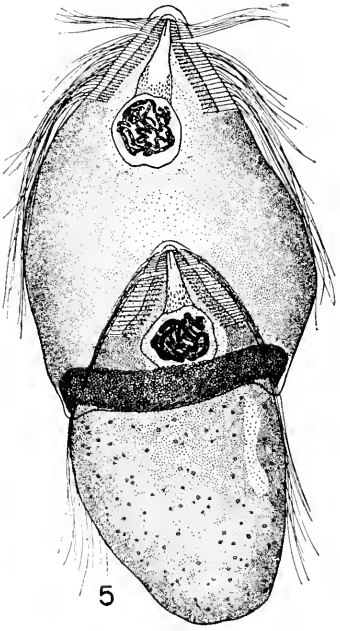

5

Between molts of the host the diploid *Notila* (Fig. H, 1) (Cleveland, 1950c) divides by mitosis with twenty-eight chromosomes. About 7 days before molting of the roach occurs, this flagellate becomes a gametocyte without any obvious morphological changes. In a single division somewhat more rapid than usual (2), this gametocyte produces a male and a female diploid "gamete," the latter being somewhat larger. Each "gamete" possesses four flagella, an axostyle, and two intranuclear centrioles. These "gametes" increase in size, then fuse, first the cytoplasm (3), then the axostyles (4), but not the nuclei. In this condition the organisms remain for 7 to 8 days. Then the male nucleus becomes detached from its axostyle, moving to another part of the cytoplasm but retaining for a time its four flagella and two centrioles.

Next meiosis occurs. This is nearly synchronous in the two nuclei (5). There is no duplication of chromosomes in connection with this division; hence reduction is accomplished by one division. Two male and two female haploid nuclei, each with fourteen chromosomes, are produced. Soon after meiosis is completed, fusion of the pronuclei begins. A male pronucleus always fuses with a female pronucleus, thus forming two diploid nuclei (7). Cytoplasmic division of this "double zygote" produces two diploid asexual cells. There are, of course, occasional deviations from the account just given.

Hypermastigotes. The first hypermastigote flagellate for which a sexual cycle was described by Cleveland (1949) was *Trichonympha*. All the species of this genus go through the same cycle simultaneously, and all are haploid with twenty-four chromosomes during periods between molts of the host.

Gametogenesis begins about 3 days before the host molts. At molting time gametocytes are encysted, and nuclear division has taken place but not cytoplasmic division. After molting, development of

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FIG. 1. *Trichonympha*, from Cleveland (1949).

1, a gametocyte in early stage of encystation; 2, detail of anterior end, duplication of chromosomes has occurred, note difference in staining in nucleus; 3, vertical view of metaphase-anaphase, most of male chromatids going to one pole and most of female chromatids to the other, interconnections between light and dark groups result from homologous pairing; 4, gametocyst of smaller species, gametes have more space in which to move, are about ready to excyst; 5, male gamete is almost halfway in the female gamete and has already become slightly smaller owing to some of its cytoplasm having been passed to female.

gametes proceeds and excystation takes place within 15 to 20 hours; within 4 to 6 hours, after excystation, fertilization begins. Excystation, maturing of gametes, and fertilization may continue until 35 to 40 hours, but in most cases are completed in 24 to 30 hours, after molting. Some of the cysts are egested just before or just after the host molts. The same development takes place in cysts remaining in the original host as in those egested and taken up by a newly hatched nymphal host.

After a haploid gametocyte encysts (Fig. I, 1), it produces two unlike gametes. The first step is the production of two unlike daughter chromosomes by each chromosome in the nucleus. These daughter chromosomes differ in staining capacity in Heidenhain's haematoxylin, the male chromosomes staining somewhat more darkly than the female (2). Separation begins during prophase of division, and a special type of union insures that the male group of chromosomes separates from the female group on the mitotic spindle (3). After cytoplasmic division, which produces two gametes (4), only slightly, if at all, different in size, cytoplasmic differentiation of gametes takes place. This may become initiated before excystation but usually begins afterwards.

At the posterior end of the female gamete, a ring of deeply stainable granules is gradually formed. The clear area within this ring may be everted as a cone, or retracted. It is apparently attractive to the male gametes, in which dark granules are rather uniformly distributed. A male gamete becomes attached to the fertilization cone and follows this as it is withdrawn into the body of the female gamete (5). Penetration by the male gamete is rapid, after which the fertilization ring disappears and the cytoplasmic organelles of the male gradually degenerate. The male pronucleus then approaches and fuses with the female pronucleus, thus producing a zygote. By two typical meiotic divisions, each accompanied by cytoplasmic divisions, haploid agamonts are produced.

There are a good many variations from the typical series of events just described. A small percentage of individuals that remain in the roach do not encyst or go through any sexual development whatever. During gametogenesis the two resulting cells may be incompletely sexed, becoming gynandromorphs. Such cells attempt fertilization but usually fail to complete it. In a few cases two male gametes may succeed in penetrating a female gamete, and their nuclei

may even fuse with the female nucleus. Sometimes chains of gametes result from incomplete fusion of gynandromorphs. A female gamete may be found at the anterior end of such a chain and a male gamete at the posterior end. Rarely autogamy may take place within a cyst.

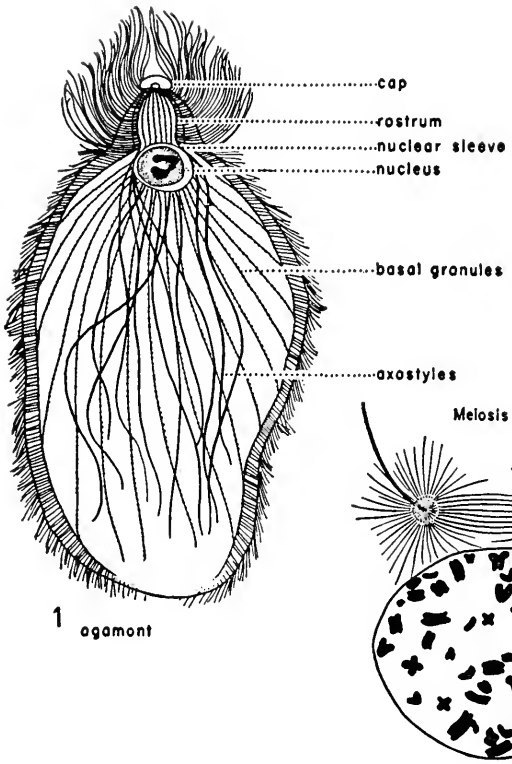
Eucomonympha shows some morphological similarities to *Trichonympha*, although placed in a separate family by Cleveland. The sexual cycle of *E. imla* (Cleveland, 1950d) is similar to that of *Trichonympha* in many respects, but there are characteristic differences.

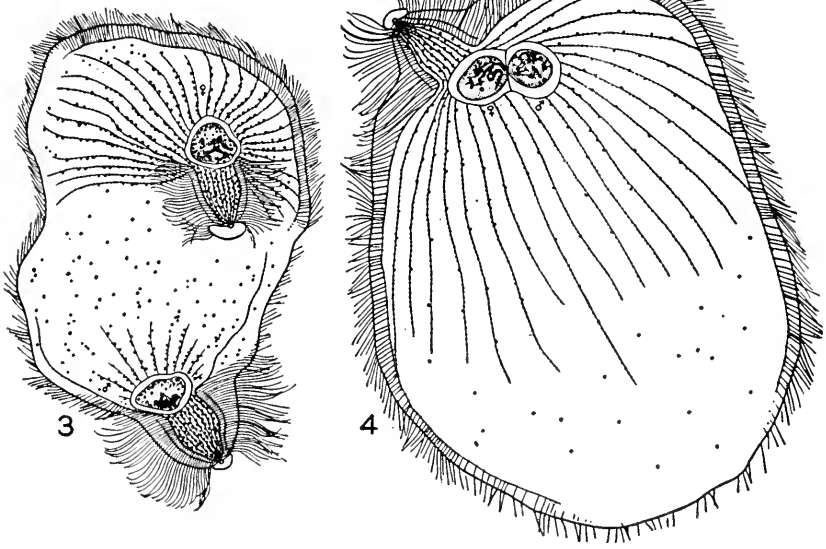
The asexual animal (Fig. J, 1) is haploid with about fifty chromosomes. It begins its sexual activities about 4 or 5 days before the host molts by becoming a gametocyte without obvious morphological change. This divides into a male and a female gamete by a single nuclear and cytoplasmic division. As a rule, the male gamete is considerably smaller than the female and its cytoplasm stains more darkly because it contains many stainable granules. There is no special fertilization area as in *Trichonympha*, males becoming attached to and entering almost any part of the female gamete. At the point of contact, the cytoplasm of the female begins to soften to permit the entrance of the male (2). However, the body of the male gamete does not enter completely into that of the female. When half or more of the male gamete has entered, its anterior end turns posteriorly and the male organelles begin to degenerate. Most of the flagella and associated structures are dissolved, but the rostrum, sometimes with the axostyles and rostral flagella, projects posteriorly (3) and is pinched off and discarded.

The freed male pronucleus moves to and fuses with that of the female, thus producing a zygote (4). Further development is suspended for about 4 days, then chromosome duplication occurs, but not the centromeres. As the achromatic figure is developed by the centrioles (those of the female gamete), pairing of chromosomes occurs and tetrads are formed and divided (5), the daughter dyads going to opposite poles. Cytoplasmic division completes the first meiotic division. The second meiotic division, of the usual sort, produces haploid vegetative individuals, which multiply by asexual mitoses as do other similar flagellates.

Sometimes two male gametes will fuse with the same female. There is no encystment at any part of the cycle.

Leptospironympha, the next hypermastigote to be considered by


cap
rostrum
nuclear sleeve
nucleus
basal granules
axostyles
Meiosis I
1
agamont
2
5


3
4

Cleveland (1951a), is placed in the Spirotrichonymphidae. It has a rostral area at the anterior end, and two long spiral flagellar bands reaching nearly to the posterior end of the cell. *L. wachula* (Fig. K, 1) has an axostyle, but *L. eupora* lacks this organelle.

A vegetative individual of *L. wachula* becomes a gametocyte by loss of the axostyle and the long flagellar bands and a rearrangement of the flagella in the rostral region (2). Also, the cytoplasm becomes denser, becoming filled with many small dark granules. Thus the gametocyte presents a decidedly different appearance from that of vegetative cells.

Gametogenesis is initiated by duplication of the ten chromosomes, each of which produces sister chromatids. This occurs between the sixth and fifth days before the host molts. Some of the chromatids are darker than others, but the contrast is not so marked as in *Trichonympha*. However, as in the latter genus, the chromosomes become arranged into two groups which separate from each other in the ensuing gametogenic mitosis. That is, a male set separates from a female set (3). Cytoplasmic division follows very quickly and may take place before nuclear separation is complete, and even sometimes so early that an anucleate cell is produced. An anucleate male was seen to attach itself to a female gamete.

The organization of the gametes is like that of the gametocytes except that the female has more cytoplasmic granules which tend to congregate near the posterior end of the cell, and this area alone is attractive to the male gamete, as in *Trichonympha*. By the time a male gamete has pushed half way into the cytoplasm of the female (4), the two cytoplasms begin to fuse and the male gamete progresses no farther. The cytoplasmic organelles of the male gradually disintegrate while the male pronucleus migrates to and fuses with that of the female. During fusion of the gamete nuclei, the chromosomes are tightly coiled (5, 6) and remain so during the ensuing meiotic division. Thus the chromosomes are not duplicated and pairing is slight. The undivided homologues are segregated on the spindle that is

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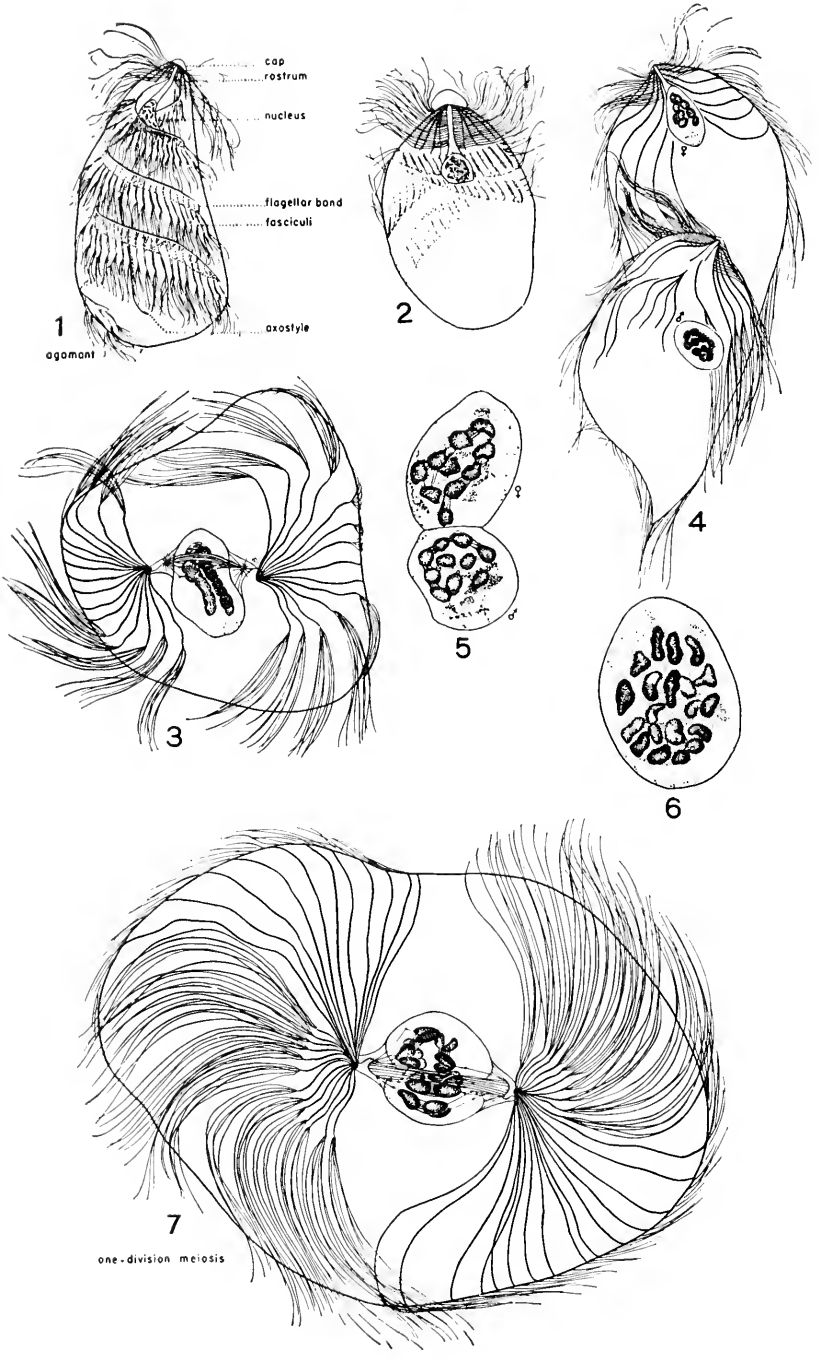
FIG. J. *Eucomonympha imla*, from Cleveland (1950d).

1, an agamont seen partially in optical section; 2, stage in union of gametes; 3, rostrum of male gamete has rotated 180 degrees, rostrum of female gamete should extend anteriorly but has been bent in fixation; 4, male pronucleus in contact with female pronucleus, male rostrum has been discarded, axostyles omitted; 5, metaphase of first meiotic division, with about 50 tetrads, organelles other than centrioles, achromatic figure, and nucleus, omitted.


cap
rostrum
nucleus
flagellar band
fasciculi
axostyle
1
agamont
2
3
4
5
6
7
one-division meiosis

formed, and thus reduction is accomplished by a single nuclear division (7). The new asexual cells begin to develop flagellar bands, but division is rapid for 2 or 3 days, so that the flagellar bands may not attain full length until after this period of rapid division has ended.

Urinympha is a hypermastigote with two anterior groups of flagella symmetrically placed opposite each other. Besides the flagella there are numerous parabasal bodies and very slender axostyles on each side (Fig. L, 1). It has a diploid nucleus. Its sexual cycle has recently been described by Cleveland (1951b).

In ordinary mitosis no organelles are lost. The two centrioles elongate and produce a spindle at their distal ends. The two flagellar areas separate and go to the daughter cells, a new group of anterior flagella and other organelles developing to match the group passed on from the parent. The sixteen chromosomes divide much as in other hypermastigotes.

Meiosis, which is pregametic, begins 5 to 6 days before the roach molts and takes less than a day for completion, in most cases only a few hours. The achromatic figure forms and functions much as it does in ordinary mitosis, but the other organelles behave differently. The flagellated areas with most of the parabasals and axostyles move away from the nucleus with which the nuclear sleeve and achromatic figure remain (Fig. L, 2). The old flagellar apparatus remains for some time, undergoing various forms of disruption, and does not disappear until after the new flagellated areas are formed in the zygote. Meanwhile the nucleus undergoes a single meiotic division, the chromosomes pairing and segregating without dividing, producing haploid pronuclei (3). The cell body does not divide, but autogamous pronuclear fusion takes place very soon after the haploid nuclei are formed.

During autogamous nuclear fusion, one centriole, with a remnant of the nuclear sleeve, moves away and degenerates. After nuclear

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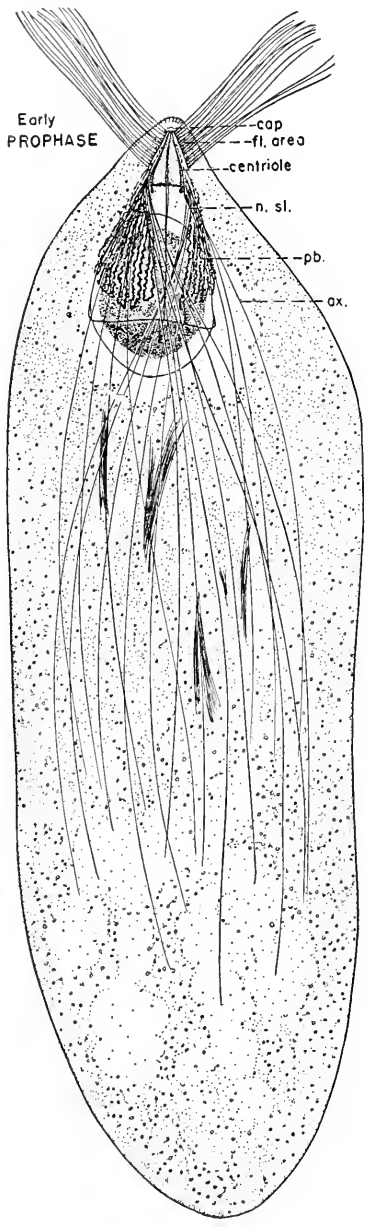
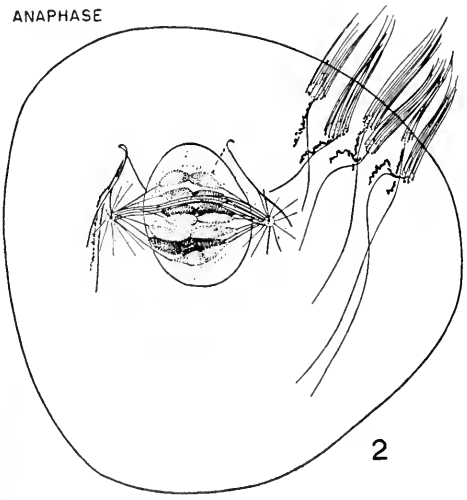
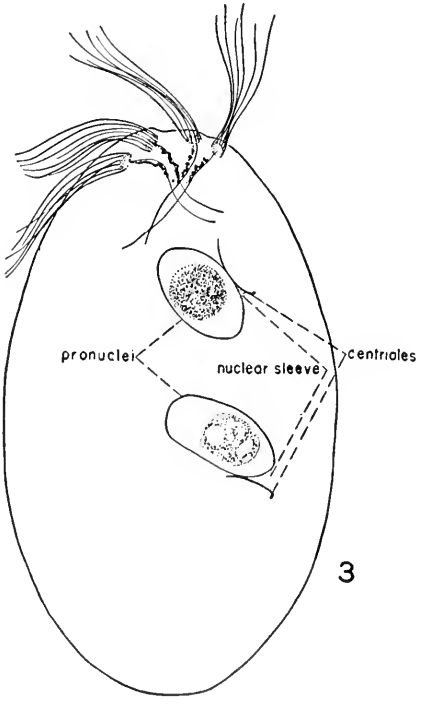
Fig. K. *Leptospironympha wachula*, from Cleveland (1951a).

1, agamont; 2, late stage in formation of gametocyte, spiraling portion of flagellar bands in process of disintegration; 3, early anaphase of gametocyte, each group of sister chromatids forms a partial ring and rings lie close together, those of one ring slightly darker than those of the other; 4, male gamete entering female gamete, the male is losing its extranuclear organelles; 5, flattening of juxtaposed membranes of fusing pronuclei; 6, nuclear fusion completed; 7, anaphase of one-division zygotic meiosis, cell has increased greatly in size.


Early
PROPHASE
cap
fl. area
centriole
n. sl.
pb.
ax.


ANAPHASE
2


pronuclei
nuclear sleeve
centrioles
3

fusion the remaining centriole divides, and from each of the daughter centrioles a new half of the flagellar apparatus, along with axostyles, parabasals, and nuclear sleeve, develops. Even after the new flagellar apparatus develops, the old flagellar areas are still persisting. This condition lasts for about 2 days, and then "pseudoencystation" takes place with a rounding up of the cell but without the formation of a cyst membrane. At this time the old flagellar apparatus completely disappears. This rounded-up condition lasts for about 2 days, then, shortly after the host molts, the organisms resume normal activity.

During these events a good many irregularities have been noted. For example, when one of the centrioles fails to degenerate during autogamic nuclear fusion, two complete sets of flagella and associated structures develop. These tend to pull the cell into two parts, with or without pulling the nucleus into two parts also. Anuclear and partially nucleate cells die.

Sometimes, instead of meiosis and autogamy, endomitosis may take place producing a $4N$ or even an $8N$ nucleus. Usually centrioles are also duplicated, such duplication leading to the production of extra sets of flagella. These cells do not survive. Occasionally, instead of a meiotic nuclear division, an ordinary mitosis takes place without cell division. The two diploid nuclei so produced fuse to produce a $4N$ nucleus. This raises the important question why pronuclei fuse. Perhaps the gametogenic nuclear divisions produce physiological differences in the nuclei which result in their fusion. Sometimes pronuclei are cut into two parts by the spindle. In such cases the two parts of the same nucleus do not fuse with each other but with the other pronucleus, or with parts of it, if it has also been cleaved into parts. Another type of anomaly is division of the centrioles, in which case new sets of extranuclear organelles are produced but the nucleus fails to divide.

The very simple type of sexual cycle in *Urinympha* is considered by Cleveland in relation to other cycles from an evolutionary

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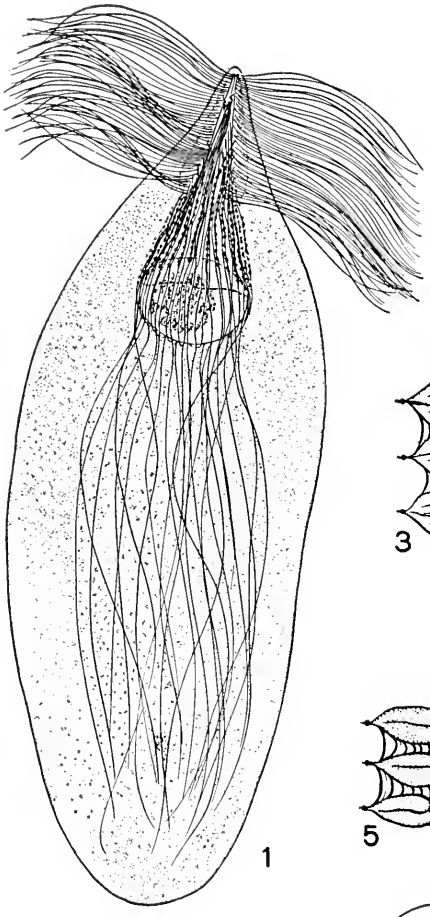
FIG. L. *Urinympha talea*, from Cleveland (1951b).

1, diploid agamont in early mitotic prophase, showing two groups of flagella, parabasals (pb), axostyles (ax), etc.; 2, entire anaphase of meiotic cell; eight chromosomes are going to each pole; nucleus in center, with greatly disrupted flagellar areas, parabasals and axostyles to the right; halves of nuclear sleeve almost separated; 3, entire meiotic cell, discarded flagella, parabasals, and axostyles above the centrally placed pronuclei.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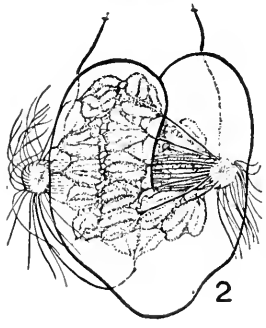
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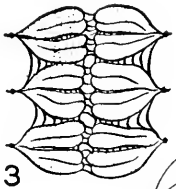
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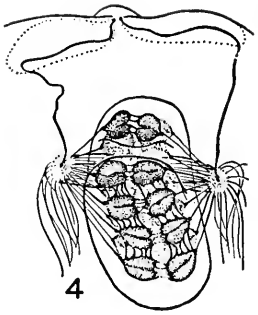
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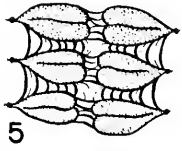
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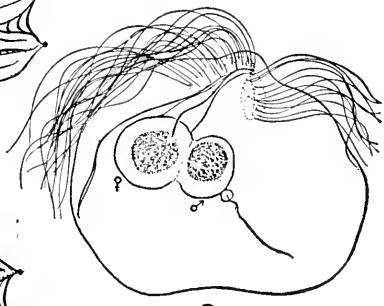
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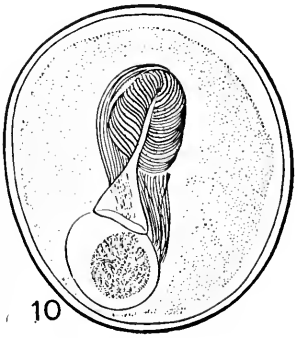
9


10

point of view. This aspect of the subject is discussed in the paper "Comments on the Origin and Evolution of Sex."

In its morphology *Rhynchonympha* (Cleveland, 1952) is intermediate between *Urinympha* and *Barbulanympha*. Its sexual cycle shows similarities to that of both these genera but has features peculiar to itself.

Sexual activities of diploid *Rhynchonympha tarda* (Fig. M, 1) begin about 9 days before the host molts and end 10 to 12 hours after molting. First there is a meiotic nuclear division (2, 3) accompanied by cytoplasmic division. In the cells thus produced a second meiotic nuclear division (4, 5) takes place without cell division. The two haploid pronuclei thus formed fuse in a process of autogamy (6 to 9). This series of events takes about 2 days. The resulting cell, which Cleveland hesitates to call a zygote, increases considerably in size for about 2 days, then undergoes a reorganization during which the old set of extranuclear organelles gradually degenerates while a new set develops from two centrioles. Pseudoencystation, which lasts about 3 days, follows. During this period the flagellate may be passed along to newly hatched and uninfected hosts. After some 12 to 15 hours the flagellates resume their activity, and a period of rapid cell divisions ensues.

In *Rhynchonympha* the size of the chromosomes ($2N = 20$) varies considerably, but all have median or submedian centromeres, in contrast to those of *Urinympha*, which are similar in size and have terminal centromeres. In the first meiotic division, homologous chromosomes pair only at their distal ends, so that no tetrads in the ordinary sense are produced and crossing-over is thus practically impossible. Lateral joinings of non-homologous chromosomes produce groupings on each side of the equatorial region, as described for *Trichonympha* and *Leptospironympha* (2, 3). In the second meiotic division the behavior of the chromosomes is very similar to that in the first, except that there is no duplication, there being a minimum

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FIG. M. *Rhynchonympha tarda*, from Cleveland (1952).

1, entire animal, showing organization; 2, metaphase or early anaphase of first meiotic division, non-homologous chromosomes joined into two rings, homologous chromosomes connected at the equatorial plane; 3, details of stage shown in 2; 4, metaphase of second meiotic division, two rings of non-homologous chromosomes, as in 2; 5, details of stage shown in 4; 6, stage in fusion of pronuclei, organelles attached to female nucleus; 7-9, successive stages of pronuclear fusion; 10, encysted animal.

of contact between homologous chromosomes, while the non-homologues join to form into two rings which pass intact to opposite poles of the spindle (4, 5).

During mitosis, after separation of the daughter groups of chromosomes, in late anaphase, the middle portion of the elongated nuclear membrane dissolves and releases the contained granules into the cytoplasm. Cleveland states his belief that such a partial breakdown of the nuclear membrane may be much more prevalent in Protozoa than previous statements would indicate.

The first meiotic division is like an ordinary cell division in that the parental group of extranuclear organelles is separated into its constituent halves, one of which passes to each daughter cell. A new half set or group develops from each daughter centriole as the parent centrioles function in nuclear division. After the second meiotic division, the half set of extra nuclear organelles associated with the male pronucleus degenerates, leaving the other set to function in the usual manner (6). About 4 days after fusion of the pronuclei the older complement of organelles gradually degenerates while an entirely new group develops from the two centrioles which have remained in contact with the nucleus. This is the process of reorganization, mentioned above; it involves neither nuclear nor cytoplasmic division.

There are a good many variations from the series of events described above. For example, instead of pseudoencystation, sometimes true cysts are formed (10) in which there is no development.

Certain of the more important aspects of the sexual cycles of these flagellates living in the gut of *Cryptocercus* are shown in Table II. There it will be seen that, of the three polymastigote flagellates, *Oxymonas* and *Saccinobaculus* are haploid and that postzygotic meiosis is accomplished in one division. *Notila* is diploid, but again the pregamic meiosis involves only one division.

In the hypermastigote group, *Leptospironympha*, *Trichonympha*, and *Eucomonympha* are haploid. The postzygotic meiosis of *Leptospironympha* is accomplished by a single division, whereas in *Trichonympha* and *Eucomonympha* there are two meiotic divisions. The diploid *Urinympha* achieves pregametic meiosis in a single division and autogamy takes place, whereas the other diploid, *Rhynchonympha*, requires two pregametic meiotic divisions to produce haploid gametes which fuse in autogamy. Not only are these diversities in

TABLE II

SUMMARY, ESPECIALLY OF MEIOTIC PHENOMENA, IN THE FLAGELLATES IN THE GUT OF THE WOOD-FEEDING ROACH, *Cryptocercus punctulatus*, AS REVEALED THUS FAR BY CLEVELAND

Numbers in parentheses in column 2 refer to chromosomes; those in column 3 refer to numbers of meiotic divisions. Further explanations in the text.

Genera	Adults	Meiosis	Autogamy
Polymastigina			
Oxymonas	Haploid (25)	Postzygotic (1)	—
Saccinobaculus	Haploid (30)	Postzygotic (1)	—
Notila	Diploid (28)	Pregametic (1)	—
Hypermastigina			
Leptospironympha	Haploid (10)	Postzygotic (1)	—
Trichonympha	Haploid (24)	Postzygotic (2)	—
Eucomonympha	Haploid (50)	Postzygotic (2)	—
Urinympha	Diploid (16)	Pregametic (1)	+
Rhynchonympha	Diploid (20)	Pregametic (2)	+

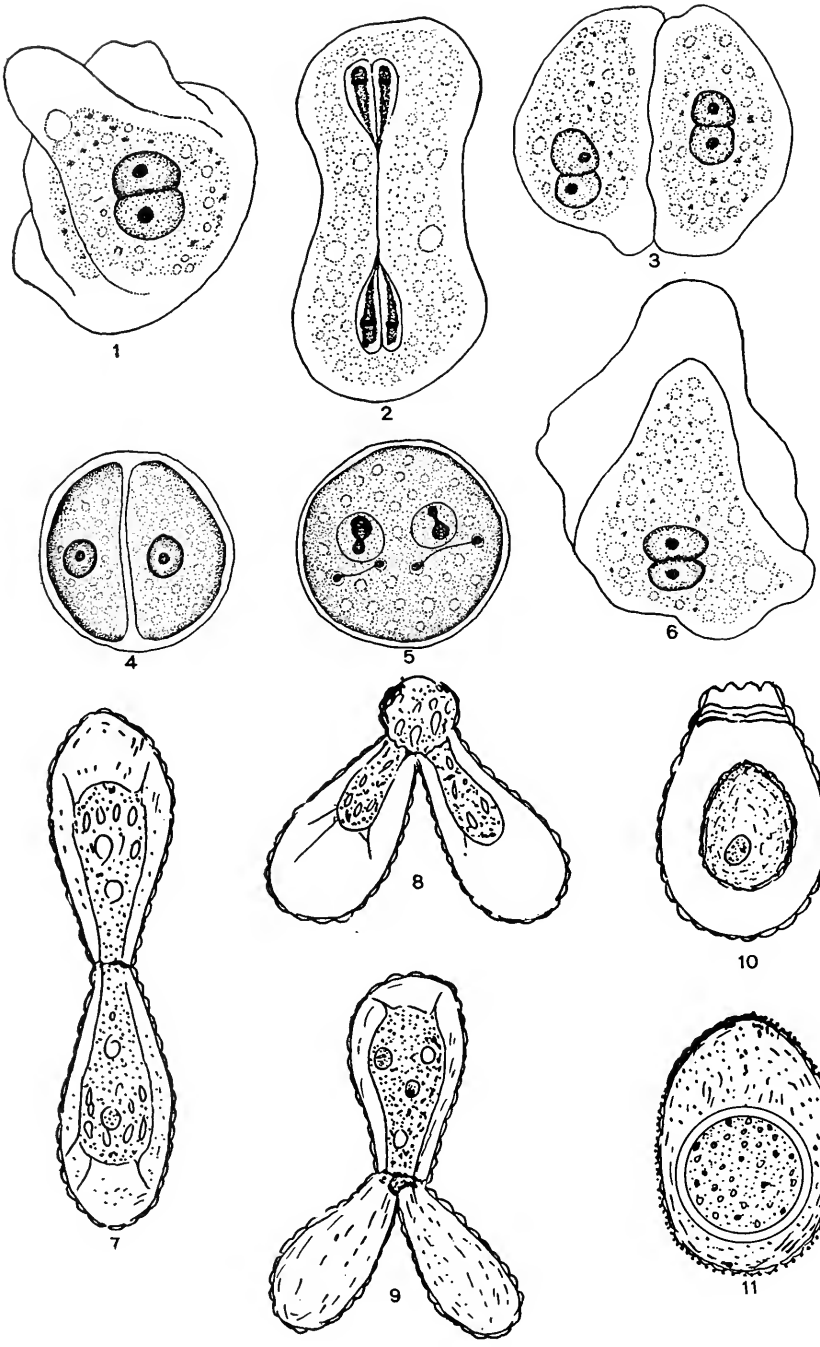
sexual behavior very striking, but they are also interesting because they occur only under the influence of the molting hormone of the host and the time relations to the molting event are varied in the different flagellates.

CLASS SARCODINA

Syngamy has been reported for a good many representatives of this class, especially for the more highly evolved Mycetozoa, Foraminifera, Heliozoa, and Radiolaria. The Mycetozoa, on the border line between the Protozoa and the Fungi, have been considered by Dr. Raper in his paper on the fungi.

Order Proteomyxa

Much diversity occurs among the organisms in this group, but generally they have slender, branching, and anastomosing pseudopodia. Cell fusion has been reported for a few members such as *Nuclearia simplex* by Dangeard (1886) and Aratari (1889), for *Vampyrella* spp. by Dangeard (1886), and for *Chlamydomyxa montana* by Penard (1904a), but nuclear details were not given.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Order Amoebina

Among the accounts of sexual reproduction in amoeboid forms, that for *Sappinea* (*Amoeba*) *diploidea* (Fig. N, 1 to 6) appears to be the most acceptable. First described by Hartmann and Nägler (1908) and Nägler (1909), the life cycle is about as follows. This species is normally binucleate, the nuclei lying close together and dividing simultaneously at each binary fission (1, 2). Under certain conditions, two of these binucleate animals come together and develop a common cyst membrane about them (3, 4). In each amoeba the two nuclei fuse in a long-delayed fertilization karyogamy. The cytosomes next fuse, then each diploid nucleus undergoes a "reduction" division, one daughter nucleus degenerating. A second "reduction" division produces a haploid nucleus and a second reduction body (5). These two nuclei remain as those of the vegetative animal which emerges from the cyst (6).

The general features of this life cycle were confirmed by Deschiens (1933), who added a process of schizogony, and partially confirmed by Kropp (1939). In none of the accounts are there clear indications of chromosome numbers.

In addition to his study of *Sappinea diploidea*, Nägler (1909) also reported evidence for autogamy for *Amoeba froschi* and *Amoeba alba*, but the accounts are not convincing. Other descriptions of sexual reproduction in smaller amoeba are those for *Amoeba minuta* (Popoff, 1911), in which the formation of "gamete" nuclei out of chromidia was reported, and for *Amoeba mira* (Gläser, 1912), in which two maturation divisions were described without syngamy having been seen.

For *Amoeba proteus* (*Chaos diffluens*) and its close relatives there are numerous accounts indicating complex life cycles, often

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Fig. N.

1-6, *Sappinea* (*Amoeba*) *diploidea*, from Kuhn (1926), after Hartmann and Nägler (1908), redrawn. 1, binucleate vegetative amoeba; 2, binary fission; 3, association of two vegetative amoebae; 4, encystment of such a pair; 5, fusion of cytoplasms and meiotic nuclear division; 6, binucleate vegetative amoeba emerged from the cyst. 7-11, *Euglypha scutigera*, from Penard (1938), redrawn. 7, two normal individuals attached at oral areas; 8, new fusion body beginning to emerge; 9, new larger individual derived from fusion of the two original animals; 10, encystment of "zygote"; 11, older cyst.

including formation of secondary nuclei from chromidia, and the development of amoeboid or flagellated "gametes," and the like. Some of the more recent accounts are those of Hausmann (1920), Hulpieu and Hopkins (1927), and Jones (1928). But Johnson (1930), who reviewed previous work and repeated some of the earlier experiments, concluded that *Amoeba proteus* reproduces exclusively by binary fission, and that internal parasites, introduction of small amoebae which pass through ordinary filter paper, and mycetozoa derivable from airborne spores could readily explain the great variety of life cycles reported.

The formation of amoeboid or flagellated "swarmers" has been described for the large multinucleated amoeba, *Pelomyxa palustris*, by Greef (1874), Korotneff (1879), Veley (1905), and Bott (1907), (Bott reported fusions of gametes), but Schirch (1914) declared that this amoeba reproduces only by binary fission accompanied by division of all the nuclei, or by a process of budding, and that previous accounts of sexual reproduction involved parasites. Wilber (1947) stated that "*Pelomyxa*" *carolinensis* (*Chaos chaos*) and similar multinucleated amoebae reproduce only by plasmotomy. Parasites may have been involved in the account of the formation and fusion of flagellated "gametes" from a large multinucleated marine amoeba reported by Schepatieff (1910).

Older accounts of sexuality in endozoic amoebae, such as that for *Entamoeba coli* by Schaudinn (1903), which was "confirmed" by numerous other authors, are no longer credited. Sexuality in *Endamoeba blattae* has been described or assumed by several authors (for example, Mercier, 1909, 1910; Elmassian, 1909; and Morris, 1936) but has not been confirmed. Lucas (1927) separated the amoebae of different sizes in the roaches (previously fitted into one life cycle) into three different kinds: the largest was *Endamoeba blattae* (Bütschli, 1878); a smaller one was named *Entamoeba thomsoni;* and a still smaller one was called *Endolimax blattae*. Meglitsch (1940) made an extensive study of the nucleus and of nuclear division in *Endamoeba blattae* but found no evidence of sexuality.

Thus we have no convincing evidence of sexuality in amoebae except that for *Sappinea diploidea*, and even that is incomplete.

Order Testacea

The tendency for testate rhizopods to display plasmogamy, or the fusion of the cytoplasms of two or more individuals, has been noted by many investigators, from the earlier observers, such as Bütschli (1875) for *Arcella vulgaris*, Gabriel (1876) for "*Troglodytes*" (=*Chlamydophrys*), and Leidy (1879) for *Euglypha alveolata*, *Arcella vulgaris*, *Difflugia lobostoma*, and other species, down to recent times.

Bütschli (1875) saw small amoebae in the shells of two of three specimens of *Arcella* which had their cytoplasms fused. He believed that these small amoebae were offspring rather than parasites, as did Hertwig (1899) and his followers, Elpatiewsky (1907), Swarczewsky (1908), and others. Swarczewsky, for example, described a life cycle for *Arcella vulgaris* which included, besides the usual binary fission, the formation of two types of amoebulae (in different individuals) with nuclei derived from the chromidia of the parents. These were supposed to be macro- and microgametes which fused to form spherical cells that developed into adults. In addition, chromidiogamy was described in which the cytoplasm of two parents fused and their nuclei degenerated. The chromidia were supposed to fuse or mingle and then give rise to secondary nuclei which became enclosed in parental protoplasm to produce a brood of amoeboid stages; these developed through a *Nuclearia*-like stage to the adult condition. In still other cases, after the formation of secondary nuclei out of the chromidia, a process of schizogony followed which produced small amoeboid individuals either without the parent emerging or after the emergence of the parent. Each such "offspring" developed through a *Nuclearia*-like stage to the adult.

Dangeard (1910) reported that he had seen no other form of reproduction in *Arcella* besides binary fission. He stated that there is only one kind of nucleus, denying that secondary nuclei formed from chromidia, but he thought that uninuclearity might result from nuclear fusion. Like others, he saw numerous examples of plasmogamy involving various numbers of individuals. He declared that the pseudopodiospores of other authors were parasitic *Nuclearia* and that the previously reported gamogony was the union of gametes of *Nuclearia* that he had previously described for *Nuclearia simplex*.

The idea that nuclei can arise from the so-called chromidia has

largely been abandoned in recent times. The presence of small amoebae in the shells of *Arcella* is not too uncommon (I have observed them myself), but there seems to be no real evidence that they have any part in the life cycle of *Arcella*. Definite evidence of syngamy in this genus appears to be lacking.

Plasmogamy has been reported for certain other members of the Arcellidae. Although Gabriel (1876) and Schaudinn (1903) reported cell fusion in *Chlamydophrys stercorea*, the well-known process of budding and colony formation in this species might be misinterpreted. Martin (1912) cultivated this species for a year without seeing any signs of sexuality. However, Bělař (1921) reported plasmogamy for *Chlamydophrys minor* and *C. schaudinni*, which included nuclear fusion, but he stated that karyogamy resulted in death of the cells.

Penard (1902) merely mentions finding a pair of individuals of *Pyxidicula cymbalum* in "conjugation," as did also Cash and Hopkinson (1909) for *Cryptodifflugia oviformis*. However, Ivanic (1935a) reported complete "copulation," including nuclear fusion, for *Cochliopodium digitatum*. "Copulae" with food reserves were able to undergo further development, but those without such reserves could not. After a rest period, during which the food reserves were used up, each "copula" divided into two cells in the parent shell. These emerged in amoeboid form and each produced a new shell.

Plasmogamy, or the fusion of cytoplasms, has frequently been reported for species of Difflugia, for example by Leidy (1879), Jickeli (1884), Verworn (1888, 1890), Rhumbler (1898), Penard (1902), Zuelzer (1904), Cash and Hopkinson (1905), Edmondson (1906), Goette (1916), Pateff (1926) and Dangeard (1937). In some cases the contents of one shell migrated into the other one, after which nuclear fusion might occur (Dangeard), but usually was not observed (Rhumbler).

Rhumbler (1898) stated that he had seen hundreds of cases of plasmogamy in *Difflugia lobostoma*, sometimes involving three individuals and occasionally four. During the previous several years he had stained many pairs but found no unusual nuclear conditions. On the other hand, Zuelzer (1904) reported not only plasmogamy in the multinucleate *D. urceolata*, which had nothing to do with reproduction, but also "copulation" and "conjugation." Plasmogamy lasted for as short a time as 2 hours, but more often for 2 or 3 days, and exceptionally for 8 to 14 days. Plasmogamy seemed to be more fre-

quent under poor cultural conditions and in warmer weather. In "copulation," two individuals first underwent plasmogamy; then the contents of one shell flowed into the other. The chromidial material of the two then mingled, but karyogamy was not observed. Encystment followed, but before encystment "chromidia" emerged from the nuclei, most of which degenerated. This process occurred regularly in late winter. "Conjugation" was similar to "copulation" except that three individuals were involved. In time, new secondary nuclei were said to form from the chromidia in the cyst.

A few other genera of the Difflugiidae are represented in the records of "copulation" and "conjugation" for example, *Centropyxis aculeata* for which Rhumbler (1895) reported "conjugation" without karyogamy, and Schaudinn (1903) reported "copulation" followed by encystment.

"Copulation" and "conjugation" have also been recorded for species of *Euglypha* and its relatives. Blochmann (1887) isolated a pair of *Euglypha alveolata*. Two days later the contents of these two had combined to form a third and larger shell. Reukauf (1912) made a similar observation for the same species, but encystment followed, as it did after "copulation" in an unnamed species described by Awerinzew (1906) and in *Euglypha scutigera* by Penard (1938) (Fig. N, 7 to 11). These independent observations agree so well as to details that complete cell fusion seems to be indicated.

Other species in the Euglyphidae for which "copulation" or "conjugation" has been reported are *Cyphoderia* (Rhumbler, 1895; Cash, Wailes, and Hopkinson, 1915); *Trinema lineare* (Penard, 1902); *Nebela collaris* (Cash and Hopkinson, 1905); *Nebela* and *Assulina* (Awerinzew, 1906); *Trinema enchelys* (Cash, Wailes, and Hopkinson, 1915).

Although the above-cited records show that cytoplasmic fusion is a common phenomenon among the Testacea and that complete fusion of two cells including karyogamy may take place, in no case has there been a demonstration of haploid-diploid sequences.

Order Foraminifera

Early students of the Foraminifera noted that in many of the polythalamous species there were two types of shells: one with a larger first chamber, or proloculum, designated megalospheric, and

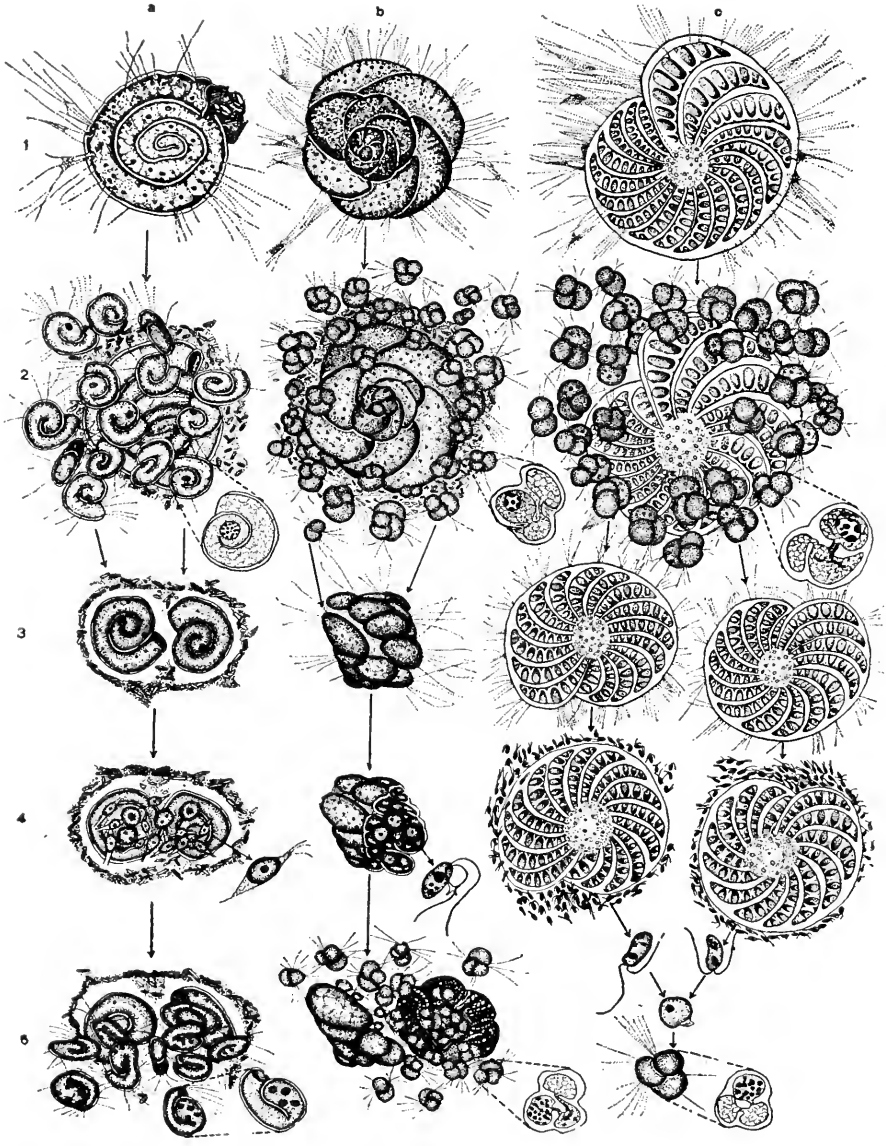


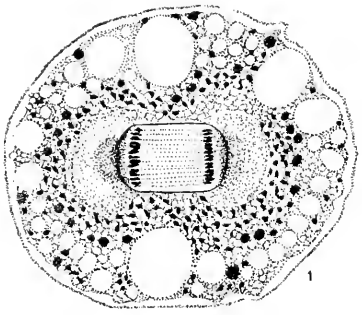
FIG. O. Selected stages of the life cycles of (a) *Spirillina vivipara*, (b) *Discorbis patelliformis*, and (c) *Polystomella crispa*, from Myers (1938), redrawn.

Stages: 1, microsphaeric adult; 2, formation of megalosphaeric offspring; 3, association of megalosphaeric gamonts (a, b), no association (c); 4, production of gametes which may be amoeboid (a) or flagellated (b, c); 5, new generation of microsphaeric young produced by syngamy.

the other with a smaller proloculum, called microspheric. It was also noted that microspheric individuals were commonly multinucleate and that megalospheric ones were uninucleate. Many of the general features of the life cycles including alternation of sexual with asexual generations were worked out by such authors as Lister (1895), Schaudinn (1903) and Winter (1907), but apparently Myers (1935, 1936) was the first to describe a complete life cycle with cytological details. He found in *Patellina corrugata* and *Spirillina vivipara* (Fig. O, a, 1 to 5): that all nuclei result from mitotic division of other nuclei, instead of being formed from chromidia as postulated by Schaudinn (1903); that gametes are amoeboid in these species (a, 4); that gamete formation is preceded by the association of two or more gamonts inside a cyst or temporary brood chamber (a, 3); that gamete formation is accompanied by a reduction in chromosome number; and that microspheric agamonts also surround themselves with a temporary cyst before producing young megalospheric gamonts by multiple fission (a, 2). Later Myers went to England and was able to corroborate the earlier accounts of life histories involving flagellated gametes.

As shown by Myers (1938), *Dicorbis patelliformis* (Fig. O, b, 1 to 5) has a life cycle somewhat intermediate between that of *Spirillina vivipara* (a) and *Polystomella crispa* (c). The microspheric adult (b, 1) gives rise to megalospheric offspring by a process of multiple fission as shown by the other two species (b, 2). When these megalospheric gamonts are mature, they associate in pairs with their ventral surfaces in contact (b, 3). Between these parents a brood chamber is formed in which flagellated gametes are produced (b, 4). These unite in pairs, thus producing zygotes which develop into microspheric young. These new individuals undergo growth and development to a stage with several chambers before being released from the brood chamber (b, 5). In *Polystomella crispa* there is no association of gamonts before the formation of large numbers of flagellated gametes (c, 4), so that fertilization is more a matter of chance than in the other species illustrated.

Föyn (1937) studied nuclear conditions in the microspheric stages of *Discorbina vilardeboana*. The adult shells had from seventeen to twenty-one chambers, and there was much variation in the numbers of nuclei, their sizes, and their distribution among the chambers. Schizogony was preceded by two mitotic divisions. In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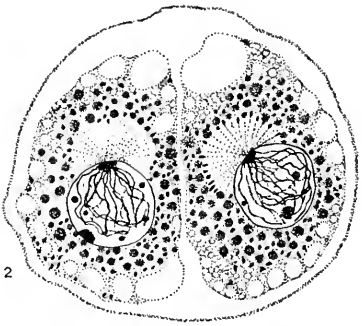
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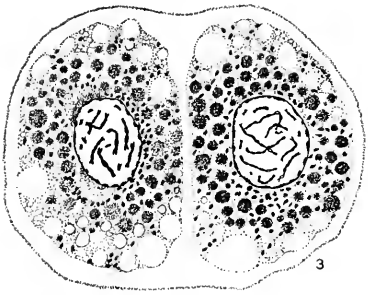
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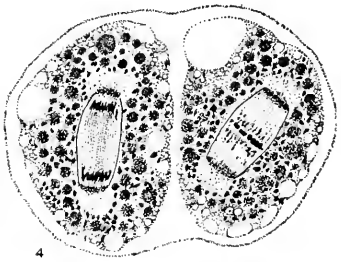
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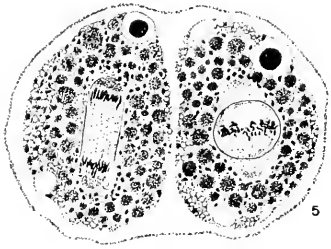
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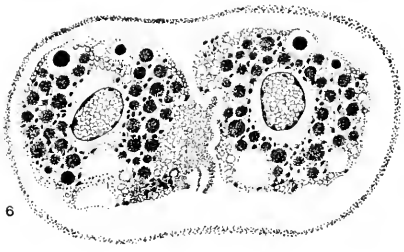
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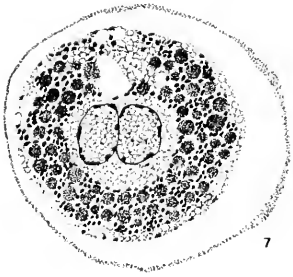
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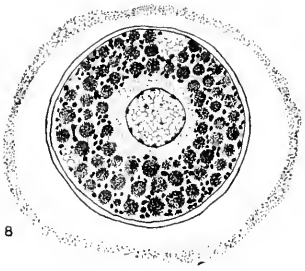
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some examples of the first of these divisions the prophase and metaphase chromosomes had the appearance of tetrads. Föyn stated that the number of chromosomes varied between ten and twenty, but the drawings showing the tetrads have the smaller numbers. Although Föyn suggests that these appearances, which resemble meiotic divisions, may be the result of fixation, it is possible that a true meiosis is indicated as claimed by Le Calvez (1946, 1950).

Le Calvez (1946), in new studies of *Discorbis vilardeboana* with flagellated gametes and of *Patellina corrugata* with amoeboid gametes, declared that chromosome reduction takes place during the last two nuclear divisions preceding the formation of gamonts by multiple fission (schizogony), the entire gamont generation, therefore, being haploid. In a still more recent paper, Le Calvez (1950) reiterated his conclusions about *Patellina corrugata* and *Discorbis vilardeboana* and added a study of association of gamonts of *Discorbis mediterranensis*. In the last species association takes place among gamonts derived from a single microspheric "schizont" (agamont). Two sexual tendencies were evenly distributed among the gamonts, and Le Calvez believed that chromosome reduction played a decisive role in determining this sexual segregation. He believed that gamonts produce chemical attractants which bring together the gamonts of opposite sex which are morphologically indistinguishable. Some kinds of Foraminifera, he stated, like *Discorbis orbicularis* and *Entosolenus marginata* show only a succession of mononucleate generations which may be regarded as haploid parthenogamonts. If attractants are produced by these animals, they appear at two stages, one to bring gamonts together, and the other to attract the gametes together.

Order Heliozoa

Sexual reproduction seems to be well established for a few species of Heliozoa. Cell fusion, or plastogamy, is a fairly common phenome-

FIG. P. Syngamy in *Actinophrys sol*, from Kuhn (1926) after Bělař (1923), redrawn.

1, mitotic division of an individual with temporary cyst membrane; 2, two gametocytes resulting from this division, "bouquet" stage of nuclei; 3, early tetrad stage, left (male) individual slightly more advanced than right (female); 4, first meiotic division; 5, second meiotic division, first "polar bodies" above; 6, initiation of fertilization by pseudopodial formation by male (left); 7, fusion of nuclei in zygote; 8, encystment of zygote.

non for *Actinophrys sol* and certain other Heliozoa, as stated, for example, by Penard (1904b). According to Schaudinn (1896), after plastogamy, individuals of *Actinophrys sol* separate again without nuclear fusion and without loss of pseudopodia. In addition, Schaudinn described a process of syngamy, involving first the fusion of two individuals followed by withdrawal of pseudopodia and encystment in a common gelatinous membrane; then a second folded membrane formed about each individual. After this a "reduction" division took place in each cyst, one daughter nucleus migrating to the periphery and condensing into a "polar body." Then fusion of the two haploid cells, including karyogamy, took place. Later the zygote nucleus divided mitotically and two daughter cysts were produced, from each of which a new vegetative individual emerged.

Schaudinn's account, while similar in general features, varied in detail from the later accounts of Distaso (1908) and Bělař (1923). Distaso described encystment of a single individual followed by division within the cyst producing gametocytes, then two maturation divisions, each producing a "polar body." Fusion of the haploid isogametes followed. He called the process autogamy but failed to give details about the chromosomes. Bělař's more complete account (Fig. P) agreed in all general features with that of Distaso, but he counted forty-four chromosomes in vegetative divisions, twenty-two tetrads in the first maturation, and twenty-two dyads in the second maturation division. After maturation short pseudopodia appeared at one end of one of the gametes to initiate the fusion into a diploid zygote (Fig. P, 6 to 8). The zygote formed a more permanent cyst wall, and after a rest period emerged and assumed the active state.

It may be noted that in one gametocyte the progress of maturation was slightly more rapid than in the other (3 to 5). Since the gamete so produced took the initiative in bringing about fertilization, it is regarded as the male.

Sexual reproduction in *Actinophrys sol* is essentially similar to that described for *Actinosphaerium eichhorni* by Hertwig (1898). Under certain conditions, such as starvation after being well-fed, multinucleate adults withdrew their pseudopodia and encysted, producing the "mother cyst." About 95 per cent of the contained nuclei degenerated. The cytoplasm within the "mother cyst" then divided into a series of "primary cysts," each containing one of the surviving nuclei. (This primary cyst is comparable to the initial cyst of *Ac-*

tinophrys sol.) Each "primary cyst" divided into two "secondary cysts" within which each nucleus gave off two "polar bodies," one after the other. The pairs of secondary cysts then fused inside the primary cyst membrane, thus producing a "conjugation cyst" which included the zygote. The zygote eventually emerged as an active individual.

In addition to asexual fission and budding, Zuelzer (1909) included "gamogonie" in the life history of the marine heliozoon, *Wagnerella borealis*. "Gamogonie" was of rare occurrence and involved the formation of "flagellispores" which were presumed to be gametes. There has apparently been no confirmation of this account, so that it remains questionable. Sexuality has thus been fully demonstrated only for *Actinophrys sol* and *Actinosphaerium eichhorni* among the Heliozoa.

Order Radiolaria

This group consists of marine members of the Sarcodina, which have radiating pseudopodia like those of the Heliozoa but differ from the latter group by the possession of a membrane, the central capsule, between the inner medullary region (endoplasm) and the outer cortical region (ectoplasm).

Most of the Radiolaria have rigid siliceous skeletons, but one group has skeletons of strontium sulfate. Some kinds have no skeletons, and others have loose spicules or crystals. Some are colonial in organization. Kudo recognizes four suborders: Actipylea, Peripylea, Monopylea, and Tripylea.

Some Radiolaria reproduce by binary fission or by budding or even by multiple fission. These processes occur among skeletonless and colonial forms. Some of the Actipylea and Tripylea also reproduce by binary fission. However, the most characteristic method of reproduction is supposed to be the formation of large numbers of flagellated swarmspores. These are of two types: isospores, usually round, oval, pear- or spindle-shaped, each with a "crystal," which Hertwig stated was organic rather than inorganic, and with one, two, or three flagella (Fig. Q, 2, 3); and anisospores, usually without crystals, often bean- or kidney-shaped, with two flagella and appearing in two different sizes in the same species, or even in the same individual (4, 5). The isospores are believed to represent asexual off-

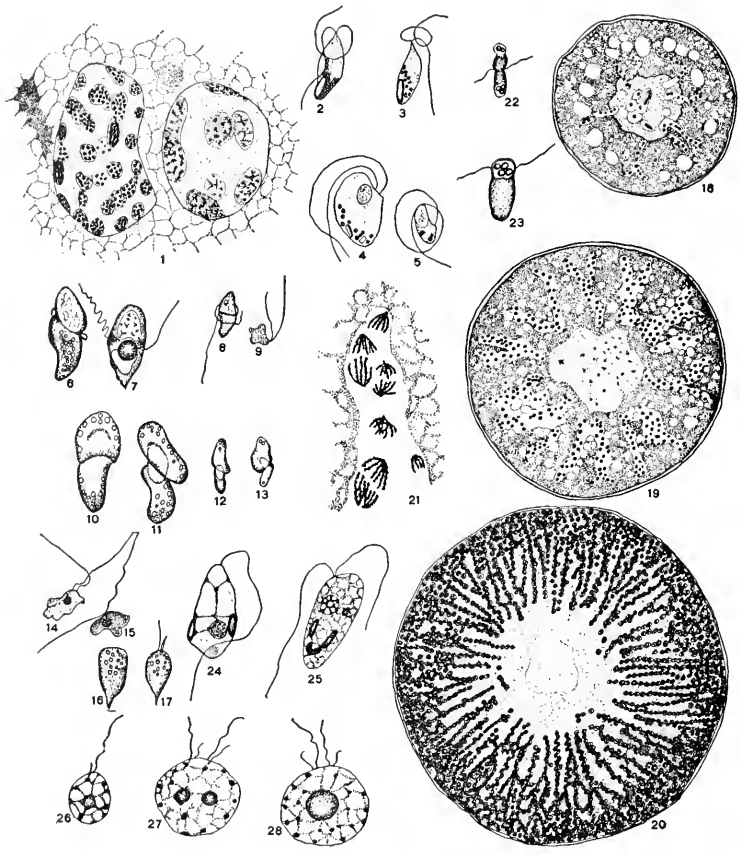


FIG. Q.

Life-cycle stages of various Radiolaria: 1-5 from Brandt (1885); 6-17 from Brandt (1905), 18-23 from Kuhn (1926), after Huth (1913); 24-28 from Schewiakoff (1926), all redrawn. 1, developing microspores (left) and macrospores (right) in *Sphaerozoum punctatum;* 2, 3, isospores of *Collozoum fulvum;* 4, 5, macrospore and microspore of *Collozoum inerme* (2-5 from living material); 6, 7, macrospores, 8, 9, microspores of *Sphaerozoum punctatum* (from living material); 10-23, *Thalassicolla nucleatum:* 10, 11, macrospores; 12, 13, microspores; 14, 15, isospores killed with iodine; 16, 17, from living material; 18-20, early to late stages in *Schlauchkernserie*, 21, higher magnification showing mitoses in stage shown in 20, note increase in size with growth of *Schlauchkernserie;* 22, microspore from *Schlauchkernserie;* 23, macrospore from *Spindelkernserie;* 24, "gamete" of *Phyllostaurus cuspidatus;* 25, "gamete" of *Pleuraspis costatus;* 26, "gamete," 27, 28, "zygotes" of *Acanthostaurus purpurascens.*

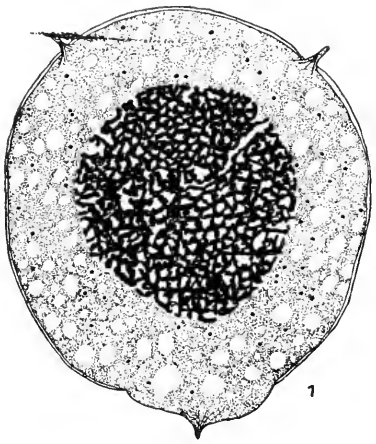
spring, while the anisospores are thought to be gametes. However, there is much confusion in the literature on the subject.

Although the Radiolaria had been the objects of study from the first half of the nineteenth century onward, Cienkowski (1871b) and Hertwig (1876) were among the first to report the two kinds of swarmers and Haeckel (1887), in his Challenger Report, indicated his belief that sporulation was the common method of reproduction in Radiolaria.

Brandt (1885) studied the Sphaerozoon group (colonial, Peripylea) at Naples and recognized, besides the swarmers, young vegetative, young reproductive, old vegetative, and old reproductive stages. The last stage produced the isospores and anisospores. Somewhat diverse conditions were found even within the same genus. For example, for *Collozoum* he found both isospores (Fig. Q, 2, 3) and anisospores in *C. inerme* (4, 5) and *C. pulvulum*, but only isospores in *C. pelagicum* and only anisospores in *C. hertwigi*. Similar differences occurred in the genus *Sphaerozoum* (6 to 9). Different conditions prevailed in different groups. For example, in the Collosphaerida microspores and macrospores developed in different individuals, the "spores" being spindle-shaped and sometimes having a crystal, while in the Sphaeroida macro- and microspores developed in a single individual (1), the "spores" being bean-shaped and with or without a crystal; isospores generally were spindle-shaped and had crystals. In all cases except the acanthometrids (which belong to the Actipylea) the "spores" had two flagella. Like his predecessors, Brandt was unable to follow the development of swarmers to the young vegetative stages.

Enriques (1919) also studied the colonial Radiolaria, finding "zoospores" in three species of *Collozoum*, two species of *Sphaerozoum*, and one species of *Collosphaera*. He also reported macro- and microspores (anisospores) in two species *Collozoum* and one species of *Sphaerozoum*, but saw no fusions among the flagellispores.

In 1905 Brandt found in *Thalassicolla nucleata* (Peripylea) that some individuals developed only isospores and others only anisospores. The isospores (Fig. Q, 14 to 17) in life were rather wedge-shaped or spindle-shaped, but always with two flagella, not one, as stated by Hertwig. The anisospores differed considerably in size, the macrospores being 16 to 17 μ long and the microspores only 8 to 10 μ long. Both kinds had rounded ends and slanting furrows near the middle,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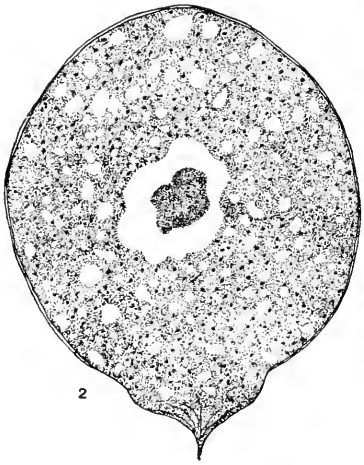
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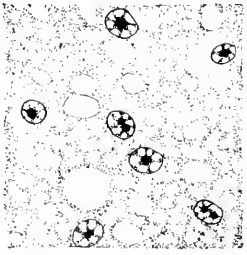
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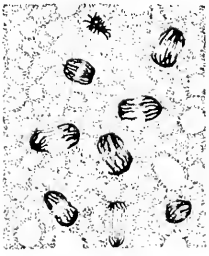
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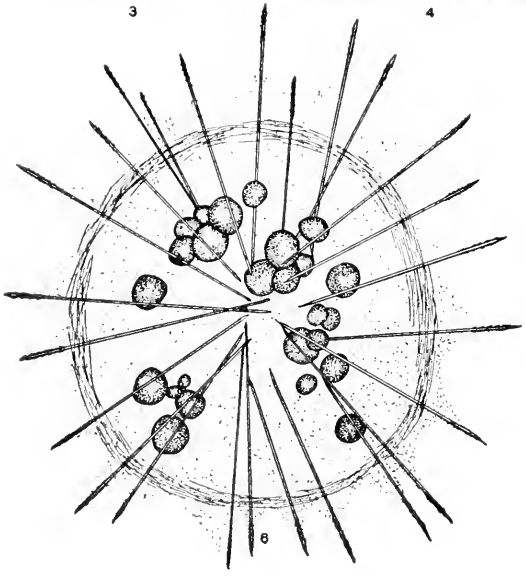
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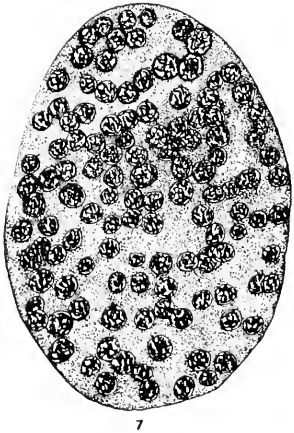
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giving them the appearance of certain dinoflagellates, like *Gymnodinium* (10 to 13). In 1885 he had seen similar spores in *Sphaerozoum punctatum* (6 to 9). The nuclei of the two kinds were also different. He believed that the two kinds of anisospores were produced by the same parent. They emerged separately, then mingled together. He never saw any pairing, but he did not put flagellispores from different parents together.

Huth (1913) made an extensive study of *Thalassicolla*, mostly *T. spumida* and *T. nucleata*. He described two different series of developments, the *Schlauchkernserie*, which led to the formation of microspores (Fig. Q, 18 to 22), and the *Spindelkernserie*, which resulted in macrospores (23). During development of the spindle series there was a great increase in the size of the animals and the size of the central capsule. The latter increased approximately a hundredfold in volume and the nucleus grew proportionately. During the later stages the nucleus became lobulated, then broke up into spindle-shaped areas, each with one or more nuclei. Further nuclear divisions resulted in large numbers of nuclei, but the spindle-shaped areas disappeared, as did the old nucleus itself. The very numerous small nuclei became rather uniformly distributed throughout the capsule and eventually, each with a small amount of cytoplasm, became a macrospore (23).

In the *Schlauchkernserie* a similar growth of the animals took place (Fig. Q, 18 to 20). Groups of small nucleus-like bodies formed in the large nucleus, these groups being surrounded by a membrane, giving them a tubular appearance. These tubes grew through the nuclear membrane out into the surrounding endoplasm (18). As they grew, they split up (19) and eventually occupied most of the space in the central capsule (20). In these "tubes" mitoses showing a relatively small number of chromosomes seemed to be continually taking place (21). Eventually microspores (22) were formed.

Fig. R. Stages of "sporulation" in the radiolarian, *Aulacantha scolymantha*, from Kuhn (1926), after Borgert (1909), redrawn.

1, early stage of dissolution of "primary" nucleus, small chromatin bodies ("secondary nuclei") appearing in endoplasm; 2, endoplasm full of "secondary" nuclei, mere remnant of "primary" nucleus; 3, "secondary" nuclei when first seen in endoplasm; 4, "resting stage" of "secondary" nuclei; 5, mitoses of "secondary" nuclei; 6, break-up of cytoplasm into "spheres" or various sizes; 7, one of the "spheres" enlarged to show many nuclei.

Borgert (1900) made a study of the division stages of *Aulacantha scolymantha* (Tripylea). In the mitoses he estimated about 1000 chromosomes. (Haecker, later, in 1906, estimated 1500 to 1600 chromosomes in *Castanidium variable*). In 1909 Borgert extended his study of nuclear division and described a quite different type of development. In this, chromatin migrated out of the nucleus as small units (Fig. R, 1, 2); these later became small nuclei which divided mitotically with about 10 to 12 chromosomes (3 to 5). The central capsule disappeared, and the cytoplasm became separated into many "spheres" (6) of various sizes each containing many small nuclei (7). Development of these nuclei was not followed, but Borgert believed that the macro- and microspores were gametes and fused to produce zygotes. No fusions were observed.

It will be recalled that Brandt (1905) described anisospores that resembled small dinoflagellates. Chatton (1920), in his monograph on parasitic dinoflagellates, reviewed the literature dealing with sporulation in Radiolaria and concluded that the anisospores were, in most cases, parasitic dinoflagellates. He noted that anisospores were bean- or kidney-shaped with a constriction or furrow at the equator from which two flagella arose, and they had no "crystals." He believed that the anisospores described by Brandt and the *Schlauchkernserie* flagellispores described by Huth were parasites. The facts that Huth's *Schlauchkernserie* began development in the nucleus, then pushed out into the surrounding endoplasm, and that they were at all times surrounded by their own membrane, and that the contained nuclei appeared to be in continuous division (Fig. Q, 18 to 21) indicated characters corresponding to those of species of *Syndinium* that Chatton had found parasitic in copepods. Although Chatton was unable to observe any fusions among the "anisospores" of *Syndinium*, he and Biecheler (1936) later observed fusion between dinospores of slightly different size in *Coccidinium mesnili*, another parasitic dinoflagellate (Fig. C, 9 to 12). Chatton was quite willing to admit that isospore development and that in Huth's *Spindelkernserie* were part of the life history of the Radiolaria.

If Chatton's contentions are correct, we are left without any sexual phenomena in the Radiolaria in the accounts reviewed up to this point, since isospores were not thought to be gametes. However, when we come to the study of Actipylea (Acantharia), the group with skeletons of strontium sulfate, the story is somewhat different.

Schewiakoff (1926), in his monographic study of "Acantharia" collected in the Gulf of Naples, described the development and emission of large numbers of flagellispores from various species. In one case he estimated the number produced at one swarming to be 17,000. These "swarmers" resembled in most respects the isospores described for other groups by previous authors (Fig. Q, 24, 25). They ranged in size from 2 μ to 10 μ. Certain species produced swarmers with only one flagellum, others with two unequal flagella, and still others with two equal flagella. Schewiakoff believed these to be gametes but did not actually observe fusion. He did, however, find among ordinary biflagellated swarmers some of double size with four flagella and two nuclei and others with four flagella and only one large nucleus. This series (Fig. Q, 26 to 28) was thought to show gamete fusion to form zygotes. He was unable to observe any development of these "zygotes."

Although I have not examined all the papers dealing with the life history of the Radiolaria, of those reviewed Schewiakoff's observation of "double" flagellispores appears to be our only direct evidence for sexuality in the Radiolaria. However, if this interpretation is correct, it may be that the isospores of other Radiolaria may be gametes and that fusion fails to take place between gametes produced by the same parent, as seems to be true in the dinoflagellates. Surely this whole subject of sexuality in the Radiolaria is in need of extensive reinvestigation.

CLASS SPOROZOA

The literature on reproduction in this parasitic and spore-bearing group of Protozoa up to about 1930 has been monographically treated by Naville (1931), who provides a much more complete review than can be attempted here. Older reviews are those of Bütschli (1882-89), of Balbiani (1884), and of Labbé (1899).

Kudo segregates the Sporozoa into the subclasses Telosporidia, Cnidosporidia, and Acnidosporidia. In the Telosporidia and Cnidosporidia sexual reproduction is well-known; it usually alternates with various forms of asexual reproduction. The Subclass Telosporidia consists of three orders, Gregarinida, Coccidia, and Haemosporidia, with intermediate groups linking them together.

In the Schizogregarinaria, Coccidia, and Haemosporidia, asexual

reproduction is commonly by multiple fission (binary fission in *Babesia*). In the Eugregarinina, increase in numbers does not take place during the trophic period but is provided for by the production of large numbers of gametes by gamonts encysted in pairs, and also by multiplication within the oöcyst or spore membranes.

Order Gregarinida

In this group, both isogamy and anisogamy have been described, but in anisogamy there is usually not the extreme divergence between the gametes found in the Coccidia and Haemosporidia. However, the male gamete may be relatively slender (Fig. T, 54; Léger and Duboscq, 1903), and it sometimes has a flagellum (Léger and Duboscq, 1909; Goodrich, 1949).

The life history of a gregarine begins when a host swallows one or more spores, each typically containing eight minute sporozoites. When released in the host's digestive tract, the sporozoites usually penetrate into the epithelium; there they may come to rest and begin to grow, or continue on into the coelome or into some coelomic organ. If the early stages of development are intracellular, a trophozoite soon outgrows its cellular environment and emerges into the digestive tract (or other cavity), retaining an anchorage to the epithelium by means of the epimerite or mucron.

In many of the cephaline gregarines, some, at least, of the trophozoites early become detached and join with another one which may or may not remain attached to the host's tissues (Fig. S, 7, 8). In this association in pairs, or syzygy, the anterior end of the satellite (male) is usually attached to the posterior end of the primite (female) (7).

Sometimes two or more satellites may be attached to one primite, or there may be a chain of individuals attached one behind the other (Fig. S, 4). In many cases (the so-called solitary species) pairing does not take place until just before encystment. In some gregarines attachment is at the anterior end of both members. This may occur in cephaline species, for example *Stylorhynchus oblongatus* (Fig. T, 49; Léger, 1904), or in acephalines, for example *Kalpidorhynchus arenicolae* (50; Cunningham, 1907). Pairing may also be lateral, either with "heads" together as in *Cystobia chiridotae* (51; Dogiel,

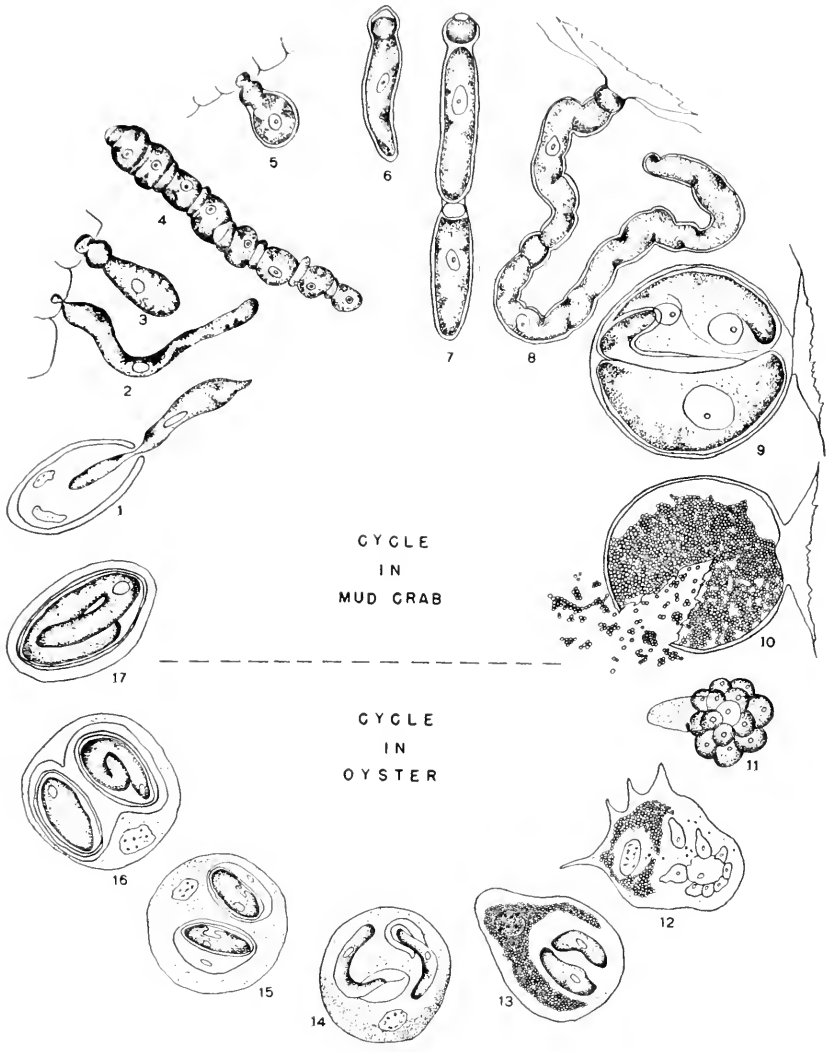


FIG. S. Life cycle of *Nematopsis ostrearum* from Prytherch (1940), redrawn.

1, escape of sporozoite in intestine of crab; 2, attachment to intestinal epithelium; 3, development of epimerite and septum; 4, precocious syzygy of young trophozoites; 5, reattachment to intestinal wall; 6, more mature trophozoite; 7, syzygy of a pair of trophozoites; 8, full-grown pair of trophozoites (gamonts), attachment to intestinal wall; 9, encystment of gamonts forming gametocyst; 10, rupture of gametocyst releasing clusters of sporozoites (gymnospores); 11, single gymnospore discharged into sea water, ready to enter an oyster gill; 12, engulfment of gymnospore by oyster phagocyte, separation of sporozoites; 13-16, growth of sporozoites, each becoming enclosed in a sporocyst; 17, fully developed spore.

1906) or "heads" in opposite directions as in *Lecythium thalassemae* (52; Mackinnon and Ray, 1931).

When mature, each pair rounds up and becomes encysted (Fig. S, 9). In this gametocyst each gamont produces a relatively large number of gametes after a series of nuclear divisions. In anisogamy the uniting gametes are from the different parents, and it is assumed that the same is true in isogamy. Each zygote secretes a sporocyst or oocyst membrane within which eight sporozoites are commonly formed.

Both pregametic and postzygotic meiosis have been recorded for

TABLE III

MEIOSIS IN EUGREGARINES

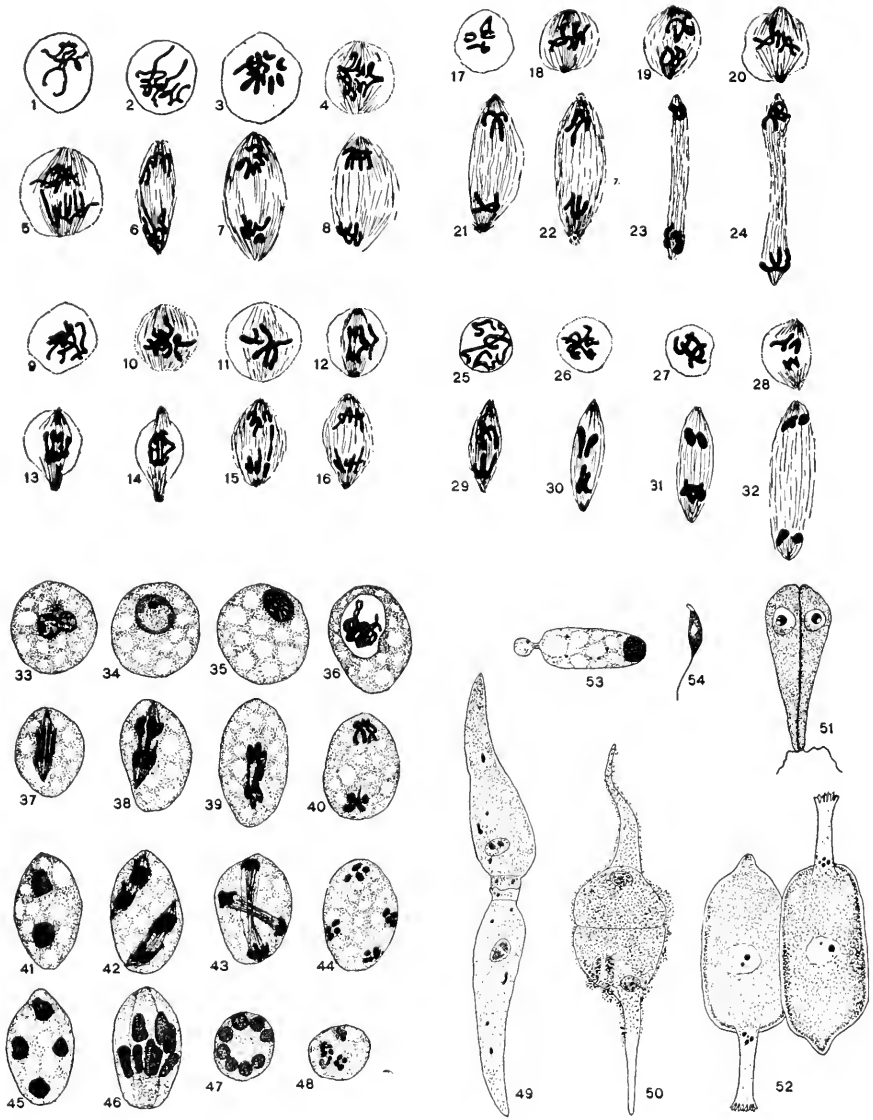
(Sequences in each group are chronological.)

Genus and Species	Meiosis	Diploid-haploid Number	Author
A. Acephalina			
Monocystis rostrata	Gametic	8–4	Mulsow, 1911
" *agilis*	"	8–4	Bastin, 1919
Diplocystis schneideri	Zygotic	6–3	Jameson, 1920
Monocystis sp.	Gametic	10–5	Calkins and Bowling, 1926
Urospora lagidis	"	4–2	Naville, 1927a
Monocystis sp. A	"	8–4	" 1927c
" " B	"	8–4	" 1927c
" " C	"	4–2	" 1927c
" *mrazeki*	Zygotic	8–4	Hahn, 1929
Monocystella arndti	"	22–11	Valkanov, 1935
Apolocystis elongata	"	8–4	Phillips and Mackinnon, 1946
B. Cephalina			
Gregarina ovata	Gametic	8–4	Paehler, 1904
" "	"	8–4	Schnitzler, 1905
Echinomera hispida	"	10–5	Schellack, 1907
Gregarina ovata	Zygotic		Schellack, 1912
Stenophora juli	Gametic		Trégouboff, 1914
Monoductus lunatus	Zygotic	4–2	Ray and Chakravarty, 1933
Hyalosporina cambalopsisae	"	4–2	Chakravarty, 1935
Zygosoma globosum	"	12–6	Noble, 1938
Actinocephalus parvus	"	8–4	Weschenfelder, 1938
Stylocephalus longicollis	"	8–4	Grell, 1940
Gregarina blattarum	"	6–3	Sprague, 1941

gregarines. Most cases of pregametic meiosis have been reported for members of the Acephalina (Table III), while most members of the Cephalina show postzygotic meiosis, especially in more recent reports (Table III).

For the acephaline gregarines various authors including Mulsow (1911), Calkins and Bowling (1926), and Naville (1927a, c) have reported pregametic meiosis, while Jameson (1920) and others described postzygotic meiosis. Figure T (1 to 48) shows selected illustrations from Naville's (1927a) paper on *Urospora lagidis*. Naville illustrated mitoses for the last four divisions in gametogenesis. Figure T shows mitoses for both female (1 to 8) and male (9 to 16) gamonts for the second stage where four chromosomes divide and separate. In the third stage (17 to 32), only two chromosomes go to each pole in the anaphases. The fourth stage (not illustrated here) also shows two chromosomes going to each pole. Thus there are two meiotic divisions according to Naville. Zygote formation and development of the eight sporozoites within it are also illustrated (33 to 48). Four chromosomes appear in the mitoses. The evidence for postzygotic meiosis offered by Jameson (1920) seems equally clear. Grell (1940) after a critical review of the literature thought that probably all gregarines had postzygotic meiosis.

If it is true that some gregarines exhibit pregametic and others postzygotic meiosis, then it may make little difference whether the trophic existence is under the control of a haploid or a diploid set of chromosomes. Since, however, the more highly evolved cephaline gregarines (Table III) and Coccidia (Table IV) generally have postzygotic meiosis, these groups are apparently under no handicap by living a haploid existence, the zygote being the only diploid stage in the life cycle. Why should the more primitive (?) acephaline gregarines show both types of cycle? If the gregarines and Coccidia have evolved from the flagellates, as has been suggested by a number of authors, for example Bütschli (1882-89), Minchin (1912), Alexeieff (1912), and Cleveland (1949), one wonders if the diploid and haploid condition in some gregarines represents a carry-over from diploid and haploid flagellated ancestors. In the group of flagellates living in *Cryptocercus*, both haploid and diploid cycles are found. This may indicate that neither type of cycle has become predominant in the flagellates, although such evidence as we have indicates that free-living flagellates are usually haploid. Since diploidy could arise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by the simple process of endomitosis, or the failure of a zygote to undergo meiosis, perhaps the difference between these two types of cycle is not of outstanding importance in the evolution of these groups.

Order Coccidia

In typical Coccidia there is pronounced anisogamy, but in the Suborder Eimeridea the macro- and microgametes are generally formed independently of each other, the small flagellated microgametes swimming to and fertilizing the large non-motile macrogametes. In the Suborder Adeleidea the gamonts become associated before the gametes are produced. Although some of the older accounts of gametogenesis in the Coccidia indicated pregametic meiosis, nearly all recent accounts show postzygotic meiosis, so that the vegetative stages are haplonts (Table IV).

Figure U shows diagrammatically the life cycle of *Adelina deronis*, a typical member of the Adeleidea, as described by Hauschka (1943). The zygote (1) nucleus undergoes a meiotic division (2, 3), reducing the chromosome number from twenty to ten. A second division produces four nuclei, two of which proceed to the next division in advance of the other two (4). Other nuclear divisions result in eight to fourteen, usually twelve, nuclei. The cytoplasm then divides into uninucleated sporoblasts which surround themselves with sporocysts to produce spores, each with two sporozoites (5, 6).

FIG. T.

1-48, stages in gametogenesis and sporogenesis of *Urospora lagidis* from Naville (1927a). 1-8, stages in mitosis of female and 9-16 of male gamonts, second period of development (note four chromosomes dividing and passing to each pole); in 3, the eight chromosomes are explained as resulting from a precocious prophasic separation of chromatids; 17-24, female, 25-32, male, stages in mitosis of third period of development (two chromosomes pass to each pole); 33-48, development of zygote and sporozoites; 33, fusion of gamete nuclei in zygote; 34, resting stage of zygote nucleus; 35-40, first mitosis of zygote; 41, binucleate stage; 42-44, second mitosis in zygote; 45, tetranucleate condition; 46, 47, eight nuclei following third mitosis; 48, small abortive zygote. 49, "head" to "head" pairing of *Stylorhynchus oblongatus*, from Léger (1904). 50, anterior end pairing of *Kalpidorhynchus arenicolae*, from Cunningham (1907). 51, lateral pairing, both "heads" together, in *Cystobia chiridotae*, from Dogiel (1906). 52, lateral pairing, "heads" toward "tails" of *Lecythium thalassemae*, from Mackinnon and Ray (1931). 53, macrogamete, 54, microgamete, of *Pterocephalus nobilis*, from Léger and Duboscq (1903). (All illustrations redrawn.)

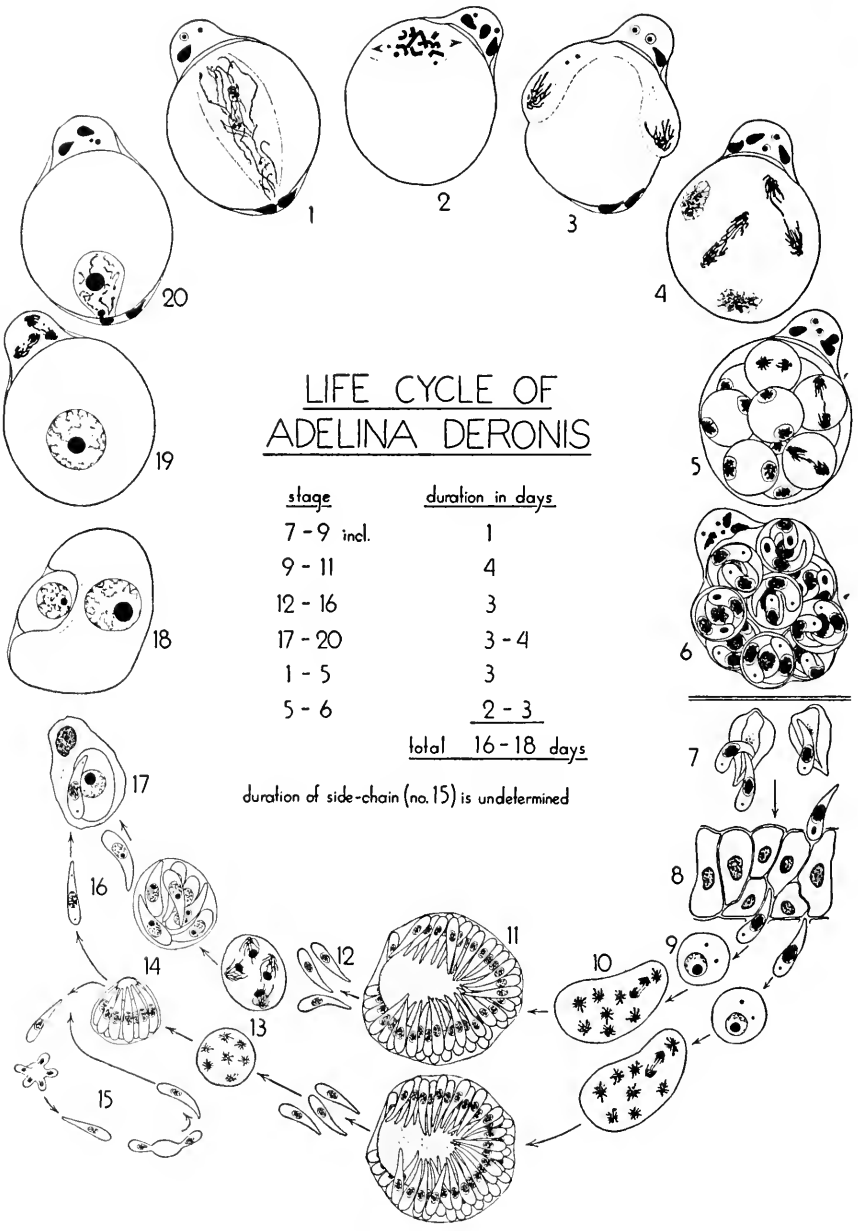
LIFE CYCLE OF
ADELINA DERONIS
stage
duration in days
7 - 9 incl.
1
9 - 11
4
12 - 16
3
17 - 20
3 - 4
1 - 5
3
5 - 6
2 - 3
total 16 - 18 days
duration of side-chain (no. 15) is undetermined
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TABLE IV

MEIOSIS IN COCCIDIA

(All these reports show zygotic meiosis.)

Species	Meiosis	Diploid-haploid Number	Author
Karyolysis sp., sp. Haemogregarinidae	Zygotic		Reichenow, 1921
Adelea ovata Adeleidae	"	8–10—4–5	Greiner, 1921
Aggregata eberthi Aggregatidae	"	12—6	Dobell, 1925
Klossia helicis Adeleidae	"	8—4	Naville, 1927b
Barrouxia sp. Eimeriidae	"	10—5	Wedekind, 1927
Adelina cryptocerci Adeleidae	"	16—8	Yarwood, 1937
Ovivora thalessemae Aggregatidae	"	14—7	Mackinnon and Ray, 1937
Klossia loosei Adeleidae	"	8—4	Nabih, 1938
Adelina deronis Adeleidae	"	20—10	Hauschka, 1943

On being taken into the digestive tract of a host, the sporozoites are released (7) and they penetrate the gut wall (8) into the coelome where they enter coelomic cells. The first generation of merozoites (10 to 12) greatly increases the infection. The schizonts of the second generation of merozoites (13, 14) are already differentiated as to sex, about twice as many smaller merozoites being produced from a male schizont than larger merozoites formed from female schizonts. Young macrogamonts penetrate host cells and are joined by micro-

FIG. U. Diagrammatic outline of life cycle of *Adelina deronis*, from Hauschka (1943).

1, zygote, chromosomes undergoing synapsis; 2, metaphase, meiotic division of zygote; 3, telophase, first zygotic division; 4, nuclear divisions in zygote, two dividing faster than the other two; 5, sporogony, young spores; 6, fully developed spores; 7, release of sporozoites in host gut lumen; 8, penetration of gut wall by sporozoites; 9, 10, young schizonts; 11, 12, merozoites, first generation; 13, schizonts, second generation; 14, 16, merozoites, second generation; 15, aberrant form of schizogony; 17, young gamonts in host cell; 18, nearly full-grown gamonts; 19, nuclear division in microgamont; 20, one microgamete penetrating macrogamete.

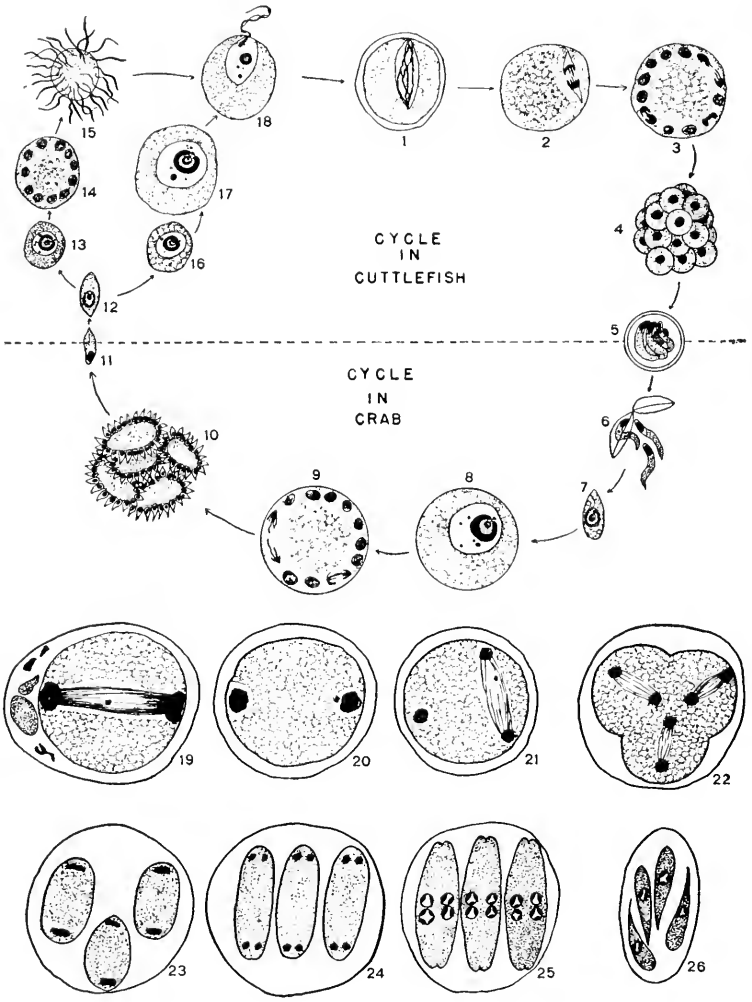


FIG. V.

1-18, outline of life cycle of *Aggregata eberthi,* from Dobell (1925). 1, fertilized zygote; 2, first nuclear division in zygote; 3, later nuclear divisions in zygote; 4, formation of sporoblasts; 5, mature spore; 6, release of sporozoites in digestive tract of crab; 7-10, growth of schizont and formation of merozoites; 11, 12, passage of merozoites to cuttlefish; 13-15, development of microgametes; 16-18, development of macrogamete; 18, fertilization. 19-26, sporulation of oöcyst of *Chagasella* sp., from Gibbs (1944). 19, first nuclear division in young oöcyst; 20, binucleate stage in oöcyst; 21, one nucleus divides; 22, the three nuclei divide as cytoplasm divides into three parts; 23, three young spores; 24, 25, further development of spores; 26, mature spore with four sporozoites. (All illustrations redrawn.)

gamonts (17). Both increase in size (18) until they are mature; then four microgametes are produced by the microgamont (19). One of these fertilizes the macrogamete (20) to produce the zygote (1). In the Eimeridea, gamonts develop independently, as in *Aggregata eberthi* (Fig. V, 12 to 18).

Order Haemosporidia

Typical members of this group, as found in the Plasmodiidae and Haemoproteidae, have life cycles similar to those of the Eimeridea among the Coccidia, except that two hosts are involved and sporocysts are not formed. In vertebrate hosts asexual reproduction by schizogony takes place, and gametocytes (gamonts) are formed which do not proceed further in their development until taken into the digestive tract of the proper blood-sucking invertebrate host, in which sexual reproduction and sporozoite formation take place. In the Babesiidae, or at least in the genus *Babesia*, asexual reproduction may be by binary fission instead of multiple fission, and the sexual stages occur in ticks. There does not seem to be any satisfactory account of meiosis or chromosome cycles in the Haemosporidia.

Subclass Cnidosporidia

In this subclass the sporozoites (sporoplasms) are amoeboid, as are often the trophic stages; thus the group shows affinities with the Sarcodina. The spores contain polar capsules in each of which there is a coiled filament which is discharged upon appropriate stimulation. In some of the Microsporidia and in the Helicosporidia the coiled filament is not enclosed in a capsule. Kudo divides the Cnidosporidia into the orders Myxosporidia, Actinomyxidia, Microsporidia, and Helicosporidia.

Order Myxosporidia

Figure W provides a diagrammatic representation of the life cycle of *Ceratomyxa blennius* (Noble, 1941). A binucleate sporoplasm (1) emerges from the spore which has entered the digestive tract of a new individual host (*Hypsoblennius gilberti*). Its haploid nuclei fuse and the zygote (2) migrates to the gall bladder of the

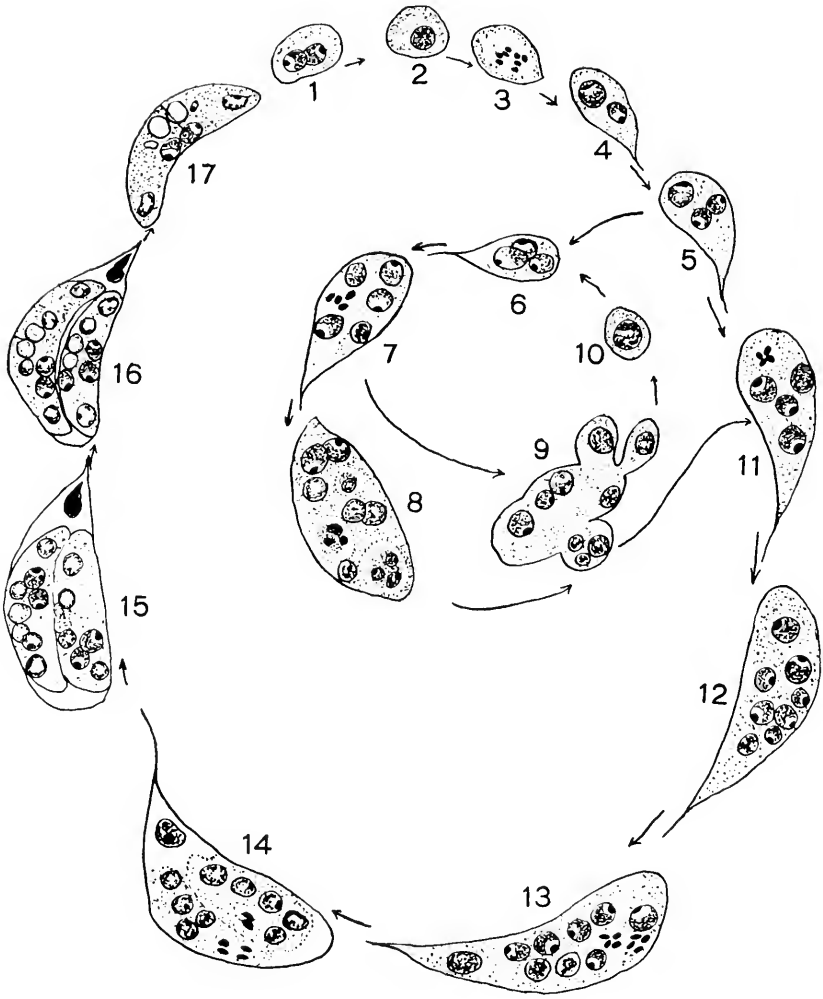


FIG. W. Life cycle of *Ceratomyxa blennius*, from Noble (1941), redrawn.

1, zygote, released from sporocyst in gut of host (*Hypsoblennius gilberti*); 2-5, growth accompanied by nuclear divisions with four chromosomes; 6-10, further growth and asexual reproduction; 11-17, sporogony, each pansporoblast (11-13) producing two spores (14-16); 14, meiosis, producing two nuclei each with two chromosomes.

host, where the rest of the development takes place. Growth (2 to 5) is accompanied by nuclear divisions showing four chromosomes (3). Further growth may be accompanied by asexual reproduction (6 to 10) or the production of spores (11 to 17). Each trophic individual becomes a pansporoblast (11 to 14) and produces two spores (15, 16). All nuclear divisions show four chromosomes except that (14), in which meiosis takes place, producing the gamete nuclei, each with two chromosomes. The two haploid nuclei, shown with marginal endosomes, remain in the sporoplasm (15 to 17) while the other nuclei, belonging to the "somatic" cells which produce the valves of the sporocyst and the polar capules, eventually disappear.

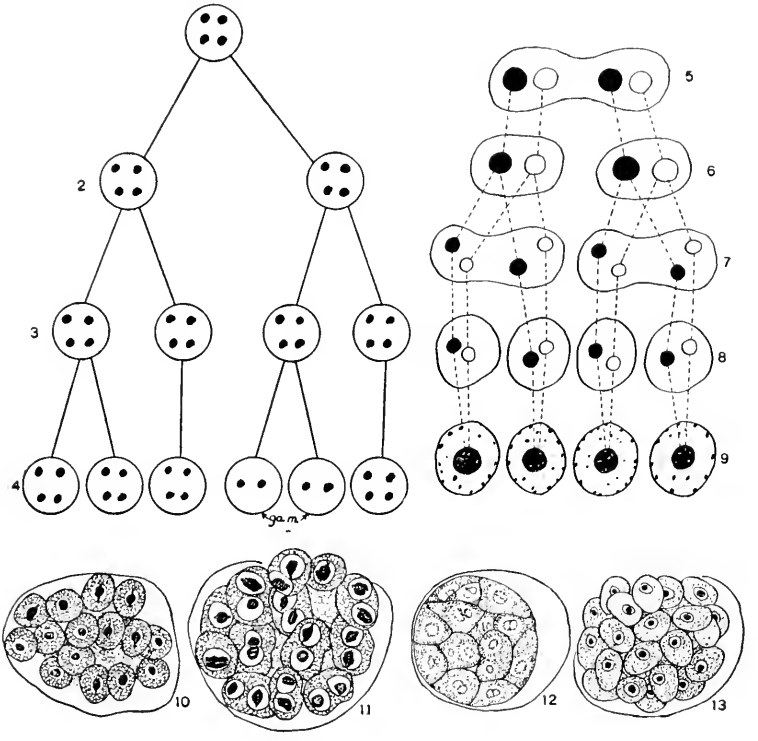


FIG. X.

1-4, diagrammatic sequences of mitoses within a single sporoblast of a myxosporidian (cf. Fig. W), from Noble (1944). Meiosis in one nucleus at third division. 5-9, nuclear history in sporogony of *Thelohania legeri*, from Kudo (1924a), including nuclear fusion (9). 10-13, stages in sporogony of *Haplosporidium limnodrili*, from Granata (1914); 10, gametes; 11, 12, fusion of gametes; 13, resulting spores. (All illustrations redrawn.)

Figure X (1 to 4) shows diagrammatically the sequence of mitoses within a single sporoblast, according to Noble (1944). At the end of nuclear division in each sporoblast there are six nuclei, two of which are sister haploid nuclei, the other four belonging to the somatic cells as explained above. Georgevitch (1935, 1936) reached a similar conclusion in regard to the life cycles of Myxosporidia in general, with the exception that he believed that meiosis takes place in two nuclei during the final nuclear divisions, one haploid product of each division degenerating like a polar body. If the interpretations of Georgevitch and Noble truly represent the life cycles of the Myxosporidia, then the cycles are relatively simple. However, there is much disagreement in the extensive literature on the group, but only one more example will be cited.

Naville has contributed a series of reports on this group besides providing a general review in his monograph (1931). In his study of *Sphaeromyxa sabrazesi* (1930a) along with other Myxosporidia, he described meiosis in cells segregated from the general protoplasm of the trophozoite. There are two kinds, larger (female) and smaller (male). By two meiotic divisions, the larger cell produces two "polar bodies" and the smaller gives rise to four equal small haploid cells. A smaller haploid cell unites with a larger one without nuclear fusion to constitute a pansporoblast which produces two spores. Thus all nuclei in the sporoblast are haploid according to this interpretation. In other respects the process of spore formation is similar to that of *Ceratomyxa blennius* (Fig. W.) More detailed studies are needed to reevaluate these more complicated life cycles, described by many authors, in the light of the interpretations of Georgevitch (1935, 1936) and of Noble (1944).

Order Actinomyxidia

The spores of members of this order are somewhat like those of the Myxosporidia but have ternary symmetry with three sporocyst valves and three polar capsules. There are a number of descriptions of life cycles, but only one will be mentioned. According to Naville (1930b), in *Guyenotia sphaerulosa* early trophic stages are always binucleate but the origin of the nuclei is obscure. After growth and nuclear division, two large rounded cells are surrounded by a protoplasmic membrane containing two nuclei, which may increase to

four. The two larger cells are male and female gamonts each of which is destined to give rise to eight gametes, the male line developing faster than the female. The first division of the gamont is "gonial," showing the diploid number of four chromosomes. During the second division, which is heterotypic, reduction to two chromosomes takes place. The third division is homeotypic, again, with but two chromosomes. Thus eight macrogametes and eight slightly smaller microgametes are formed. These fuse in pairs to produce eight zygotes, each of which becomes a sporont and develops into a spore.

Order Microsporidia

In this group the spores are small, and in the majority there is only one polar capsule or polar filament. The small size of many of these organisms makes the study of their development difficult. Most accounts in the literature are incomplete.

In *Thelohania legeri* (Kudo, 1924a) young sporonts develop as shown in Fig. X (5 to 9). A quadrinucleate stage (5), formed after schizogony, divides into two binucleate cells (6). These undergo division with both nuclei dividing at the same time (7, 8). In the four binucleate cells so produced (8), nuclear fusion takes place (9). The uninucleate sporonts thus formed proceed to the formation of spores. In several other accounts Kudo has described autogamy in connection with the development of the sporont, for example in *Thelohania opacita* (1924b), *Stempella* (*Thelohania*) *magna* (1925), and *Nosema aedis* (1930), all parasitic in mosquitoes. Meiosis is not clearly indicated.

Debaiseaux (1928) postulated two somewhat different patterns of development, one for the polysporous and the other for the monosporous Microsporidia. For the former, vegetative development may be by binary fission or by plasmodium formation, but eventually a special binucleate stage is reached. This divides by a peculiar type of division to produce two binucleate cells within each of which nuclear fusion takes place to produce a zygote. This becomes a sporont and forms a spore (see Fig. X, 5 to 9). For the monosporous forms a similar vegetative development ends likewise in a special binucleate stage which undergoes the special type of division producing two binucleate cells. In these cells nuclear fusion does not take place until after spore formation. In *Plistophora chironomi*, Weiser

(1943) found a diploid number of about twelve chromosomes with a meiotic division preceding sporogony. Just when fusion of gametes occurred was not determined. Much more study is needed to elucidate the problems of syngamy in the Microsporidia.

Order Helicosporidia

This order consists of one species, *Helicosporidium parasiticum*, the life cycle of which has not been fully worked out.

Subclass Acnidosporidia

In this subclass, as presented by Kudo, there are two orders, the Sarcosporidia and the Haplosporidia. No sexual stages have been determined for the Sarcosporidia.

Order Haplosporidia

This group shows some similarities to certain members of the Microsporidia, but the spores are simple, not having any polar capsules or polar filaments. Accounts of the life cycles are usually incomplete. For *Ichthyosporidium giganteum*, Swarczewsky (1914) described a life cycle which included meiosis and fertilization within the sporoblast during development of the spores. This would presumably involve pregametic meiosis. A similar life cycle is claimed by the same author for *Ichthyosporidium hertwigi* and for *Pleistophora periplanetae*. Granata (1914) has described gametogenesis in *Haplosporidium limnodrili*. The gamete fusion stages are shown in Fig. X (10 to 13). Gametes are formed (10) and fuse (11, 12), producing zygotes which become spores (13). Although these life histories are not so complete as desired, at least they show evidence for the formation and fusion of gametes.

Comments on Sexuality in the Sporozoa

Certain topics in connection with syngamy in the Sporozoa merit separate discussion. Two of these are (1) sex determination and sex differentiation, and (2) the effect of the host on life cycles.

Sex Determination and Sex Differentiation

Telosporidia. There is a considerable body of literature which tends to show that sex determination in the Telosporidia takes place soon after zygote formation, possibly during the first zygotic nuclear division. Joyet-Lavergne's extensive publications show cytoplasmic differences between male and female trophozoites and gamonts among the Eugregarinida, and especially among the Cephalina. It will be recalled that in this group each zygote commonly gives rise to eight sporozoites, each of which may grow into a gamont without further division.

Using neutral red, Mühl (1921) showed that, for *Gregarina cuneata* and *G. polymorpha* from the larvae of *Tenebrio molitor*, the two members of a pair in syzygy stained differently, although there was much variation when pairs were compared to each other. Joyet-Lavergne (1926) obtained similar results with the same species and with *Steinina ovalis*. Other vital dyes, which color the cytoplasm directly, such as methyl blue, cresyl blue, Nile blue, and dahlia violet were used. With these the anterior member of the pair, the primite (female), stained more intensely than the posterior member, the satellite (male). Tests for pH showed little or no differences between the sexes, a point confirmed by Göhre (1943). Tests for golgi apparatus and for fats also showed differences in the cytoplasm of the two sexes. Sex differences in the gamonts either before or after encystment were shown by these various techniques when the gamonts themselves were morphologically indistinguishable and when similar gametes (isogametes) were produced by the gamonts. As Göhre (1943) remarked, sex differences in gregarines become apparent very early, and are probably genotypic in origin. Literature in this field up to 1930 is reviewed by Joyet-Lavergne (1931) in his book.

Certain conditions in the Coccidia are suggestive. In *Adelina deronis* Hauschka (1943) found evidence of differential behavior between the four nuclei derived from the zygote nucleus (Fig. U, 4), two nuclei proceeding to the next division faster than the other two. This difference in division rate resulted in about twelve sporoblasts and twelve spores (6). It is possible that eight of these are male and four female. In addition, Hauschka could recognize sex differences in the second generation schizonts and their merozoites (14).

There was also evidence that a single parasite, passed along from parent to daughter during fission of the parent, was unable to carry on the life cycle; that is, any individual parasite is male or female, but not both. However, if a single spore containing two sporozoites can give rise to both sexes, then the two sporozoites should be of different sex. Otherwise a male spore and a female spore would have to be taken into a new host to produce male and female gametes.

In the case of *Chagasella* sp., Gibbs (1944) showed that after the first division of the zygote nucleus in the oöcyst (Fig. V, 19, 20) only one of the two daughter nuclei divided (21). This differential division, by analogy with *Adelina deronis*, could indicate maleness for the nucleus that divided and femaleness for the one that did not. Thus sex determination would be accomplished at the first nuclear division in the zygote. During further development the cytoplasm of the oöcyst divided into three parts while the nuclei also divided, producing three sporoblasts which became spores (22-23). Each spore produced four sporozoites (24-26). According to the suggested interpretation, two of the spores would be male and one female. However, Gibbs stated that the oöcyst membrane gradually disappeared so that the salivary glands of the host (a hemipteran insect) often contained many single spores. This suggests that the spore is the infective stage.

The so-called spore of a gregarine, derived as it is directly from a zygote, is equivalent to the oöcyst of the Coccidia. It is interesting that in gregarines generally, and in many of the Coccidia, the oöcyst is the infective stage. This should contain both sexes. When spores develop in the oöcyst, as in so many Coccidia, they might well be male or female, but not both. Becker (1934) was able to maintain infections of *Eimeria* in rats by inoculating a new host with a single oöcyst. Experiments are needed to see if a single spore of *Eimeria* (there are four in each oöcyst) would be capable of maintaining the life cycle if transferred alone to a new host. Why should the spore not be an infective stage?

Perhaps nature offers such an experiment in the case of *Aggregata eberthi*, a coccidian that requires two hosts to complete the life cycle (Fig. V, 1 to 18). According to Dobell (1925) this species undergoes gametogony, fertilization, and sporogony in the cuttlefish, *Sepia officinalis*, while schizogony takes place in the crab, *Portunus depurator*. The zygote (1), formed in the cuttle fish, does not pro-

duce a resistant oöcyst membrane as does that of most Coccidia. By numerous divisions many nuclei are formed from the zygote nucleus, the number varying with the size of the zygote. The cytoplasm then divides into as many sporoblasts as there are nuclei (4). Sporocysts next form about the sporoblasts which produce spores. Within the spore cytoplasm the single nucleus divides, then only one of the daughter nuclei divides, just as occurs in the oöcyst of *Chagasella* (21). Therefore, only three sporozoites are formed in each spore (5). Since the spores are not confined by an oöcyst membrane, they are free to separate from each other. One would suppose that a single spore would be able to continue the life cycle. When taken into a crab, the schizogonic part of the cycle develops. When a cuttlefish eats a crab, the merozoites from the crab continue the cycle in the cuttlefish by developing into gamonts and gametes, which unite to produce zygotes again (12 to 18). By analogy to *Chagasella* (19 to 26), we might suppose that sex determination takes place in the spore in this case, rather than at the first postzygotic nuclear division. Possibly two of the three sporozoites in a spore are male and the other female. If so, any spore could continue the life cycle in a new host.

If we could assume that any spore with two or more sporozoites contains both male and female elements, then most of the spores of gregarines and Coccidia would contain both sexes. However, in the life cycle of *Nematopsis ostrearum* (Prytherch, 1940), each spore contains only one sporozoite (Fig. S, 17). To produce an infection with new gamonts, such a sporozoite would have to be able to give rise to both male and female elements; otherwise male spores and female spores would both have to enter a new host in order to continue the life cycle. Perhaps the fact that sporozoites enter a new host oyster in clusters (Fig. S, 11) would help to insure that both sexes would be present in an infected oyster. We are thus faced with the interesting problem of the mechanism of sex determination in this group of parasites.

In typical Haemosporidia, as a rule, no sex differences are visible until the gametocyte (gamont) stage is reached. The gametocytes, however, are distinguishable as to sex, and the gametes are strikingly different, as in the parasite of malarial fever for example. Presumably a mosquito must take up both kinds of gametocytes in order for fertilization to take place in its digestive tract. After sporozoites are formed and migrate to the salivary gland of the mosquito, they are

ready to infect a new host. One wonders if a single sporozoite could give rise to both kinds of gametocytes in a new vertebrate host. In the case of *Babesia* (Dennis, 1932) the gametocytes are not morphologically differentiated in the vertebrate host. Just where and how sex determination takes place in the Haemosporidia can only be guessed at, but one might suppose that the mechanism would be like that of the Coccidia.

Cnidosporidia. In the Myxosporidia the available evidence indicates that the trophic stages are commonly diploid and that meiosis takes place at some stage during sporogony. However, according to Naville (1930a), meiosis takes place at a very early stage in sporogony with evident differences between the sexes, whereas according to Georgevitch (1935, 1936) and Noble (1944) all nuclei of a myxosporidian are diploid except the gamete nuclei, which are formed at the last nuclear division during spore formation. Since these nuclei seem to be exactly alike (Fig. W, 15 to 17), there is no sex difference, and therefore no sex determination. Furthermore, the interpretations of Noble indicate that the two gamete nuclei, as sister nuclei, unite to produce a diploid zygote in a process of autogamy. Autogamy is likewise indicated for the Actinomyxidia, Microsporidia, and Haplosporidia as previously indicated. Just what biological advantages are derived from such a process is difficult to imagine.

The Effect of the Host on Life Cycles

It is interesting to speculate about what determines the shift from one stage of a life cycle to the next. In gregarines, for instance, what induces gametocyst formation? In Coccidia, what causes the cessation of schizogony and the onset of gametogony? In the Haemosporidia, what determines that certain merozoites will become gametocytes (gamonts) instead of schizonts? Are such changes of activity due to some inherent cyclic tendencies, or do they occur in response to conditions or stimuli provided by the host?

Cleveland's remarkable discovery of the inducement of sexuality in the intestinal flagellates of the wood-feeding roach, *Cryptocercus punctulatus*, by the molting hormone of the host naturally raises the question whether parallels can be found elsewhere. Possibly similar conditions have been described where different stages in the life cycle are correlated with developmental stages of the host. For example,

Nowlin (1922) reported that a gregarine (*Schneideria* sp.), found in the fly *Sciara coprophila* goes no further in its development in the larval stage of the host than the fully grown trophic stage (gamont); in the pupa, the gamonts unite in pairs, become encysted, and begin sporulation. In the adult, sporulation is completed. Whether any of these changes in the life cycle are influenced by molting or other hormones of the host is, of course, unknown at the present time, but the correlation may have some well-defined host-relationship basis. Shortt and Swaminath (1927) reported a somewhat similar relationship between *Monocystis mackiei* and another dipteran host, *Phlebotomus argentipes*. In this case sporulation did not begin until the host became adult.

As shown by Wenyon (1911) and later by Ganapati and Tate (1949), the development of the gregarine *Lankesteria culicis* in mosquitoes shows similarities to that of the species cited above. Trophic development takes place in the gut of the larva, and union of gamonts, cyst formation, and sporogony take place in the malpighian tubules of the pupa. Only spores are found in the adult.

Hentschel (1926) reported a correlation between the development of the acephaline gregarine *Gonospora varia*, with the sexual development of its host, the polychete worm, *Audouinia* (*Terratulus*) *tentaculata*. The coelomic parasites live among the developing genital products of the host and complete their life cycle at the time of sexual maturity of the worm. Occasionally gregarines are present in segments which do not contain gonads. In these segments the parasites are unable to complete their full life cycle, remaining small and apparently unable to produce spores. Hentschel suggested that the host gonads may produce a hormone or other substance which is essential to the growth of the gregarines. The same author (1930) reported a somewhat similar relationship between *Gonospora arenicolae* and its host, *Arenicola ecaudata*, except that the correlation was not so close as for *G. varia* and *Audouinia tentaculata*.

For those gregarines which have two hosts, as do members of the family Porosporidae (including *Nematopsis*, Fig. S), there is a correlation between stages in the life cycle and the change of hosts; this is true in other cases of two-host development, as in the coccidian Families Aggregatidae and Haemogregarinidae and in the Order Haemosporidia. It is fair to assume that, when any transmissible stage in the cycle leaves one of the alternative hosts, transfer to the other

host constitutes a necessary stimulus to further development. Yet the well-known development of micro- and macrogametes of Haemosporidia from warm-blooded hosts when blood containing the gametocytes is drawn and reduced to "room" temperature suggests that mere reduction in temperature induces gamete formation. However, we have the definite circumstance, in these two-host parasites, that transfer to an alternate host is required for the life cycle to continue. Whether the host influences are in the nature of "hormones" or other specific substances is yet to be determined. It is possible that the relation between the molting hormone of the wood-feeding roach and the sexual activities of its contained flagellates is unique.

SUBPHYLUM CILIOPHORA

The Subphylum Ciliophora is divided into two classes, the Ciliata and the Suctoria. Class Ciliata contains Subclass Protociliata, in which there are two or more similar nuclei in each animal, and Subclass Euciliata, in which each animal has one or more macronuclei and one or more micronuclei. All the Protociliata are endozoic, mostly in the large intestine of Amphibia. Sexual phenomena in ciliates have been reviewed by a number of writers, more recently by Diller (1940a), Turner (1941), and Finley (1946).

Subclass Protociliata

According to Neresheimer (1907), the life cycle of *Opalina ranarum* and *O. dimidiata* includes binary fission through most of the year. In the spring, cysts are formed which pass into the water of breeding pools to be taken up by a new generation of tadpoles, in which sexual reproduction takes place. Similar uninucleate gametes fuse in pairs to produce zygotes which become encysted. When the zygotes excyst, they develop into adults in the new host. Metcalf (1908) confirmed most of this life cycle for *Opalina* (*Protoopalina*) *caudata*, *O.* (*P.*) *intestinalis* and *O. dimidiata*, except that he found the gametes to be different in size and failed to confirm the encystment of the zygote. Valkanov (1934) also found anisogamy. Figure Y shows diagrammatically the life cycle of *Opalina ranarum* as illustrated by Prenant (1935) and based on the work of Zeller (1877) and Konsuloff (1921). None of the authors mentioned above gave

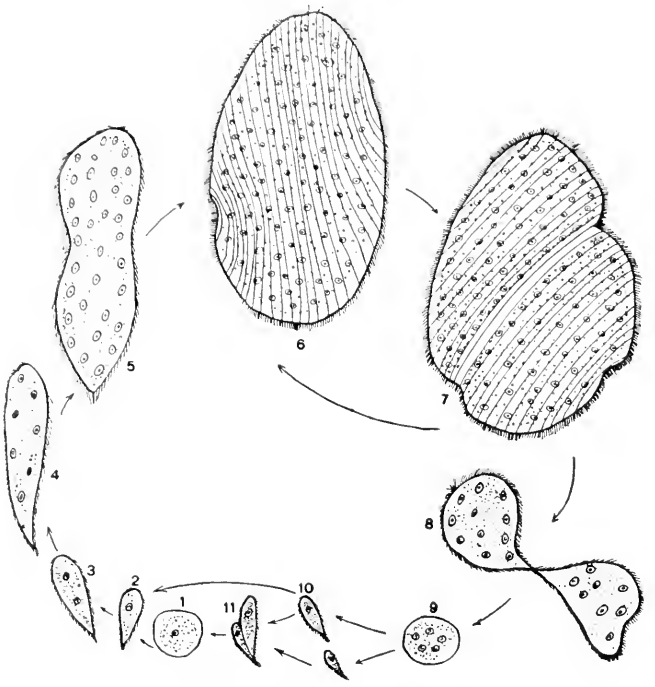


FIG. Y. Life cycle of *Opalina ranarum*, from Prenant (1935), after Zeller (1877) and Konsuloff (1921), redrawn.

1, encysted zygote in gut of tadpole; 2-6, development of zygote into multinucleate adult; 7, binary fission of adult; 8, rapid fission leading to precystic stages; 9, cyst eliminated into water to be ingested by tadpoles; 10, mononucleate gametes from excysted individuals; 11, fusion of gametes to produce zygote.

satisfactory accounts of chromosome numbers, but Chen (1936, 1948) showed that adults have a diploid set of paired chromosomes. Meiosis has not been described.

Subclass Euciliata

Kudo divides this subclass into the Orders Holotricha, Spirotricha, Chonotricha, and Peritricha. In these groups, as well as in the Suctoria, sexual reproduction, with rare exceptions, takes the form of conjugation, or some modification of that process. While there are many variations in conjugation, these are not generally associated with any particular order, except for the Chonotricha and Peritricha. Consequently it seems best to depart from the strictly taxonomic

sequences as previously followed. One may find about as many variations within the orders Holotricha and Spirotricha as there are between them.

In the process of conjugation, two individuals become attached to each other and exchange gamete nuclei, after which they separate and a new nuclear apparatus arises from the products of the fusion nucleus, or syncaryon, in each exconjugant, while the old macronuleus gradually disappears.

Conjugation in *Paramecium caudatum*

Conjugation as it occurs in *Paramecium caudatum* is usually considered typical. Accounts have been published by a number of authors, for example by Gruber (1887) (under the name *P. aurelia*), Maupas (1889), Calkins and Cull (1907), Dehorne (1920), Müller (1932), Penn (1937), Diller (1940b, 1950a) and Wichterman (1953). The principal steps are shown diagrammatically in Fig. Z, which is based on the accounts of Maupas and later authors.

Two individuals become attached to each other along their oral surfaces (1); then the micronucleus in each, after a long period of preparation involving the "crescent" stage (not illustrated), undergoes the first pregametic (meiotic) division (2). The two nuclei thus formed quickly go through the second pregametic (meiotic) division to produce four haploid nuclei (3). Three of these nuclei begin to degenerate while the fourth divides to form the pronuclei (4). The pronuclei usually take a position near the oral region, where a cone-shaped protrusion, the paroral cone (Diller, 1936), extends from each conjugant toward the other (4, 5). Through these cones the migratory pronucleus (male) of each conjugant passes into the mate (5), where it fuses with the stationary pronucleus (female) to form the fusion nucleus or syncaryon (6). The conjugants then separate (7), and the further changes take place in the exconjugants. In each exconjugant the macronucleus gradually becomes transformed into a skein (7 to 9) which breaks up into small fragments (10, 11). Meanwhile, by three successive mitoses the syncaryon gives rise to eight small nuclei (7 to 9). Four of these begin to grow and are known as macronuclear anlagen. As growth of the anlagen proceeds, three of the other nuclei grow slightly, then begin to degenerate and eventually disappear, while the eighth nucleus becomes the functional mi-

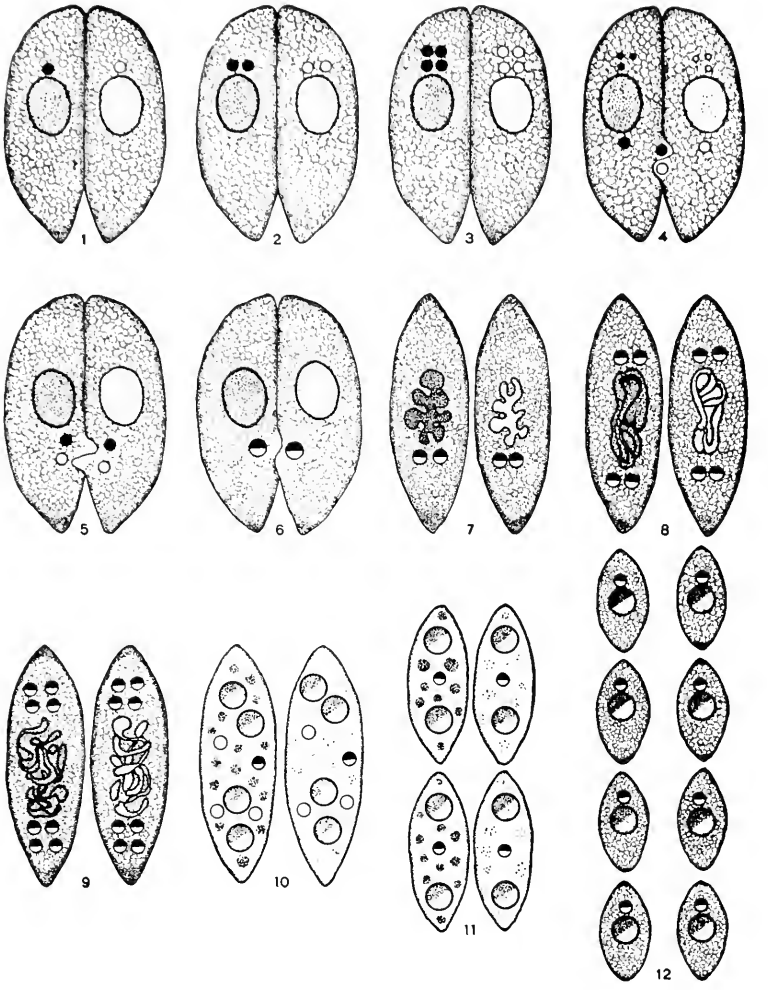
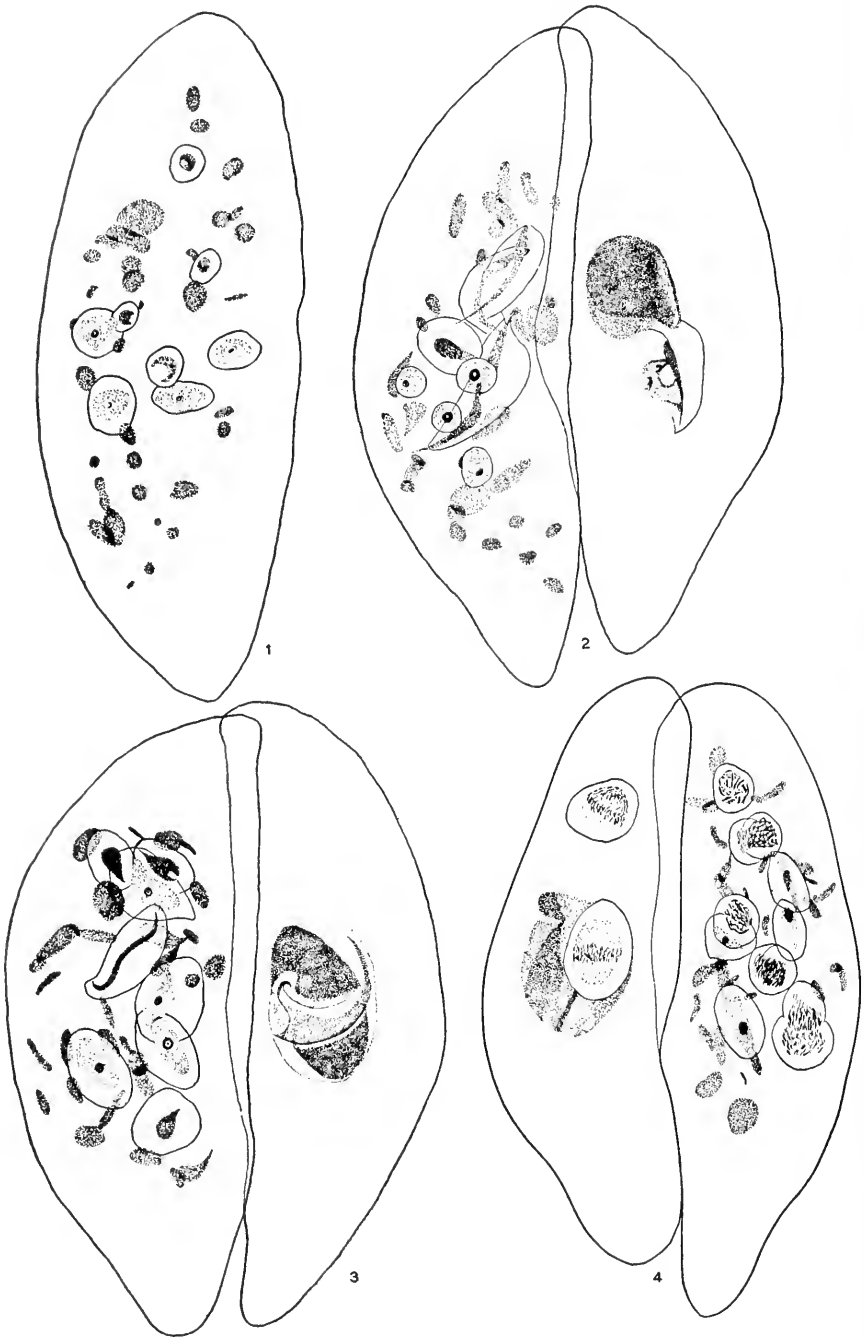


FIG. Z. Diagrammatic outline of conjugation in *Paramecium caudatum*, original.

1, pair of conjugants become attached along oral region; 2, first pregametic micronuclear division; 3, second pregametic division; 4, degeneration of three products of second division, fourth product dividing to form pronuclei, migratory pronuclei in paroral cone; 5, passage of migratory pronuclei to other conjugant; 6, fusion of pronuclei to form syncarya; 7, separation of conjugants, division of syncarya, beginning of resolution of macronuclei; 8 second postzygotic nuclear division, early skein stage of macronuclei; 9, third postzygotic nuclear division, late skein stage of macronuclei; 10, in each exconjugant four products of syncaryon become macronuclear anlagen, degeneration of three products, persistence of eighth product as functional micronucleus; 11, first postconjugation cell division, two anlagen going to each daughter; 12, second postconjugation division, each cell has a new macronucleus and a new micronucleus derived from the syncaryon.


1
2
3
4

cronucleus (10). Two cell divisions, each accompanied by a division of the micronucleus, serve to segregate the four macronuclear anlagen of each exconjugant into four descendants (11, 12). During these changes the fragments of the old macronucleus gradually become absorbed while the anlagen attain full size. At the end of these two divisions, there are four new cells derived from each conjugant. Each of these cells has a new macronucleus and micronucleus derived from the syncaryon (12).

Beginning with Bütschli (1876) and Maupas (1889), students of conjugation have noted deviations from the typical series of stages. Diller (1940b) has called attention to a number of the variations for *P. caudatum.* One nucleus may degenerate at the end of the first pregametic division, as happens regularly in *P. bursaria.* Instead of three products of the second pregametic division degenerating, two, three, or four may begin the third pregametic division, although only one normally completes it to form pronuclei. Among the products of the syncaryon there may be various numbers of nuclei, up to sixteen, with varying fates for the supernumerary nuclei. When the usual eight products are produced, as a rule only four become macronuclear anlagen, but as many as seven may do so. More recently Diller (1950a) has reported an extra postzygotic division in the exconjugant of *P. caudatum.* After the first division one of the daughter nuclei degenerates, the other proceeding to the second division. Two more divisions are necessary to produce the normal eight products derived from the syncaryon.

Reconjugation between normal individuals and exconjugants has been noted by many observers; the subject is reviewed by Diller (1942), who showed many examples for *P. caudatum.* Figure AA (2 to 4) shows three such reconjugating pairs. As pointed out by Diller, other observers have reported this phenomenon, for example Bütschli (1876) for *P. putrinum* (?), Doflein (1907) and Klitzke (1914) for *P. caudatum,* Müller (1932) for *P. multimicronucleatum,* and Sonneborn (1936) for *P. aurelia.* This process has also been described for other ciliates, for example by Enriques (1908) for

Fig. AA. Reconjugation in *P. caudatum,* after Diller (1942), redrawn.

1, normal exconjugant with four anlagen and four smaller nuclei, three of which are degenerating; 2, 3, exconjugants united with normal animals, all showing crescent stages indicating recent association; 4, normal with two dividing micronuclei united with exconjugant having eight small dividing nuclei, four anlagen present.

Chilodonella uncinatus, and by Collin (1912) for various suctorians.

Wichterman (1939, 1940) placed conjugating pairs of *P. caudatum* under a precision microcompressor which enabled him to observe nuclear phenomena in living animals. Under these conditions he discovered that in each conjugant self-fertilization (autogamy) took place instead of cross fertilization. He called this process "cytogamy." Cross fertilization probably occurs in most instances of conjugating pairs, as shown by Diller (1950b).

Conjugation in Other Species of *Paramecium*

Different species of *Paramecium* show varying degrees of divergence from the account just given for *P. caudatum*. *P. bursaria* and *P. trichium* are like *P. caudatum* in that each has a single, rather large micronucleus, whereas *P. aurelia* and *P. calkinsi* usually have two smaller, more vesicular micronuclei, and *P. multimicronucleatum*, *P. woodruffi*, and *P. polycaryum* commonly have four micronuclei of the *P. aurelia* type.

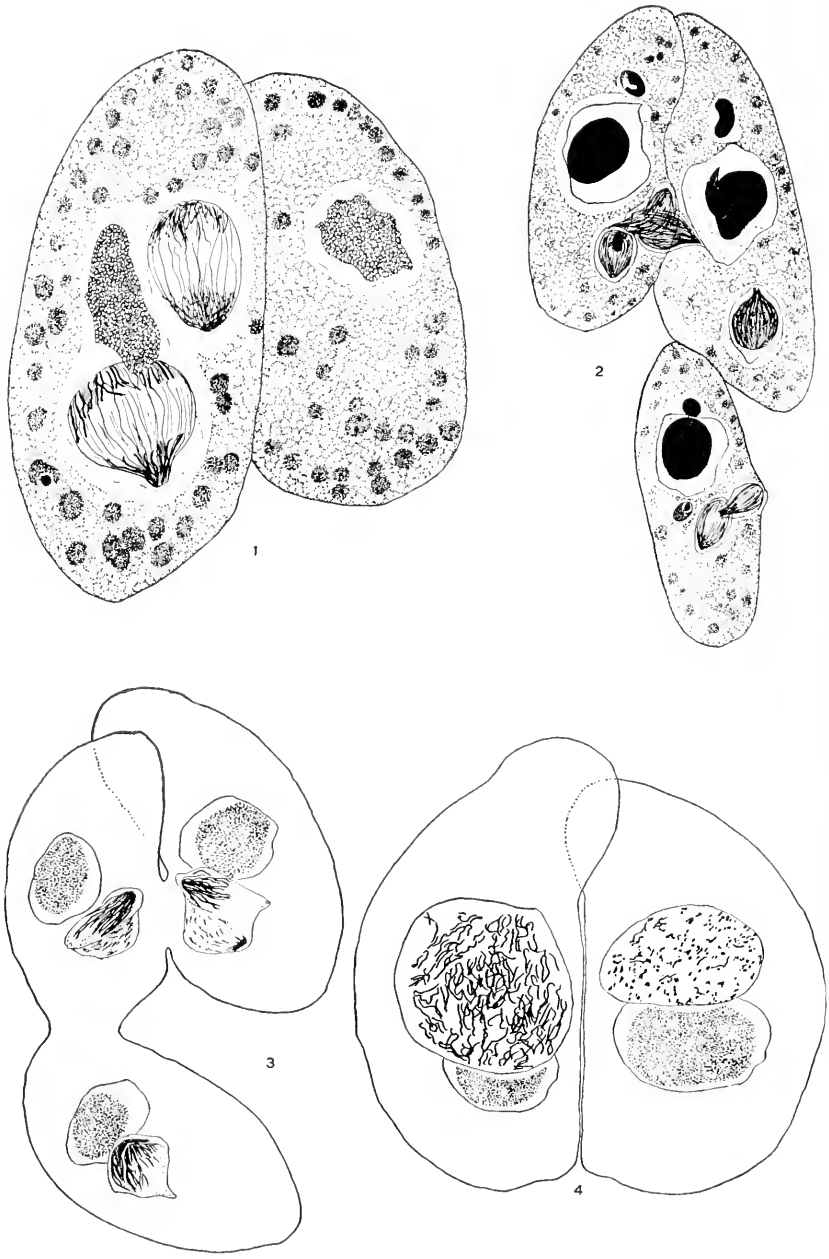
Although *P. bursaria* has a micronucleus much like that of *P. caudatum*, the stages of conjugation are somewhat different. For accounts of conjugation in this species we may refer to those of Maupas (1889), Hamburger (1904), Chen (1940a, b, c, d; 1946a, b; 1951a, b) and Wichterman (1948a, 1953). According to these authors each of the first two pregametic divisions are followed by degeneration of one of the products. The remaining haploid nucleus divides to provide the two pronuclei. According to Maupas and Hamburger, after exchange of pronuclei and syncaryon formation, two divisions provide four nuclei, two of which become macronuclei and two micronuclei. One cell division segregates these into two animals each with one nucleus of each kind. However, Chen (1946b, 1951a,b) and Wichterman (1948a) found that after the first postzygotic nuclear division one nucleus degenerates, so that it takes two more divisions to produce the four products which develop into two macro- and two micronuclei. In this species the old macronucleus does not fragment but undergoes gradual absorption in the exconjugant.

All these authors have recorded variations in the conjugation of this species. Hamburger (1904) recorded passage of a pronucleus from one conjugant to the other without receiving a pronucleus from the partner, leading to one "haploid" and one "triploid" indi-

vidual, a condition later reported by Chen (1940a, b, d) and Wichterman (1946). Hamburger also found one conjugant with no micronucleus, as did Chen (1940a, b, d) later (Fig. AB, 1). Chen showed that the amicronucleate animal could receive a pronucleus (hemicaryon) from its partner which would retain a "haploid" nucleus. Development of such nuclei could be called parthenogenesis. Maupas found an exconjugant with three macronuclear anlagen and three micronuclei, and another with four anlagen and four micronuclei, possibly the result of the failure of one nucleus to degenerate after the first postzygotic division, and Hamburger found ten micronuclei and six macronuclear anlagen in an exconjugant, indicating sixteen products from the syncaryon.

Chen (1940a, b, d; 1951b) called attention to the great variation in nuclear size, chromatin content, and chromosome numbers in *P. bursaria*, which indicates a high degree of polyploidy; and he showed that animals with such different nuclei apparently conjugate successfully. He (1940c; 1946a) also described cases of conjugation between three animals. Usually, two assume the common parallel position with oral areas together, while the third individual has its anterior end attached to the posterior end of one member of the pair. The two in the normal position undergo normal conjugation while the other individual undergoes autogamy (Fig. AB, 2). Chen (1951a) also reported that, when a double monster consisting of incompletely separated daughter animals conjugated with normal specimens, many kinds of association occurred but the most typical was for a normal animal to conjugate with the anterior member of such a tandem pair (Fig. AB, 3). In this case the sequence of events was essentially the same as when three animals conjugated. The anterior pair conjugated normally while the posterior member of the tandem pair underwent autogamy. In a study of conjugation between members belonging to two different varieties, Chen (1946b) found that during the first 16 hours of association nuclear phenomena were normal, but after that time various abnormalities appeared and the participants failed to survive.

In another study Chen (1951b) determined the results of conjugation between an "old" strain which had been maintained in the laboratory for about 20 years and a "young" strain which had been cultured for about a year. In Fig. AB (4), it will be seen that the numbers, sizes, and shapes of chromosomes are different in the two


1
2
3
4

conjugants. Conjugation appeared to be normal, but about 50 per cent showed variations in nuclear number in the exconjugants, the range being from 2 to 20. In many cases the "haploid" nuclei either with or without exchange developed parthenogenetically. Jennings (1944) had shown that conjugation between an "old" and a "young" clone or between two "old" clones resulted in high mortality among exconjugants. Chen's study reveals some of the cytological conditions in such crosses.

It may be noted in passing that conjugation of more than two animals has frequently been reported in the literature. For example, Maupas (1889) figured three individuals of *Loxophyllum fasciola* and three of *L. obtusum* in conjugation, and four individuals of *Spirostomum teres*. Recently Weisz (1950) reported multiple conjugation in *Blepharisma undulans*, involving often three, occasionally four, and rarely five individuals. Nuclear changes took place in all associates, and autogamy was considered probable for some of the individuals. Elliott and Nanney (1952) also reported frequent conjugation of three animals in *Tetrahymena* sp. The three animals were all attached together in normal positions, and the evidence indicated that tripolar fertilization took place.

Paramecium trichium is the smallest species in the genus and normally has a single micronucleus of the *P. caudatum* type. Diller (1948) reported many variations in the conjugation of this species (Fig. AC). Most commonly the progress of events followed the scheme already indicated for *P. caudatum*, except for the absence of a crescent stage preceding the first pregametic division (Fig. AC, column a). As shown in (a, 2), one nucleus may degenerate after the first division, as regularly occurs in *P. bursaria*. Autogamy (cytogamy, Wichterman) may take place instead of cross fertilization (column b). After degeneration of one of the two products of the first pregametic division, either cross fertilization (column c) or

FIG. AB. Stages in conjugation of *P. bursaria*, redrawn.

1, conjugation between normal and amicronucleate individual, from Chen (1940b); 2, conjugation of three animals, posterior animal undergoing autogamy, from Chen (1946a); 3, conjugation between a double monster and a normal animal, all in syncaryon stage, note differences in chromosome picture of nuclei in the anterior pair, indicating exchange, attached animal at left undergoing autogamy, from Chen (1951a); 4, conjugation between old and young clones, prophase of first micronuclear division showing differences in chromosomes, from Chen (1951b).

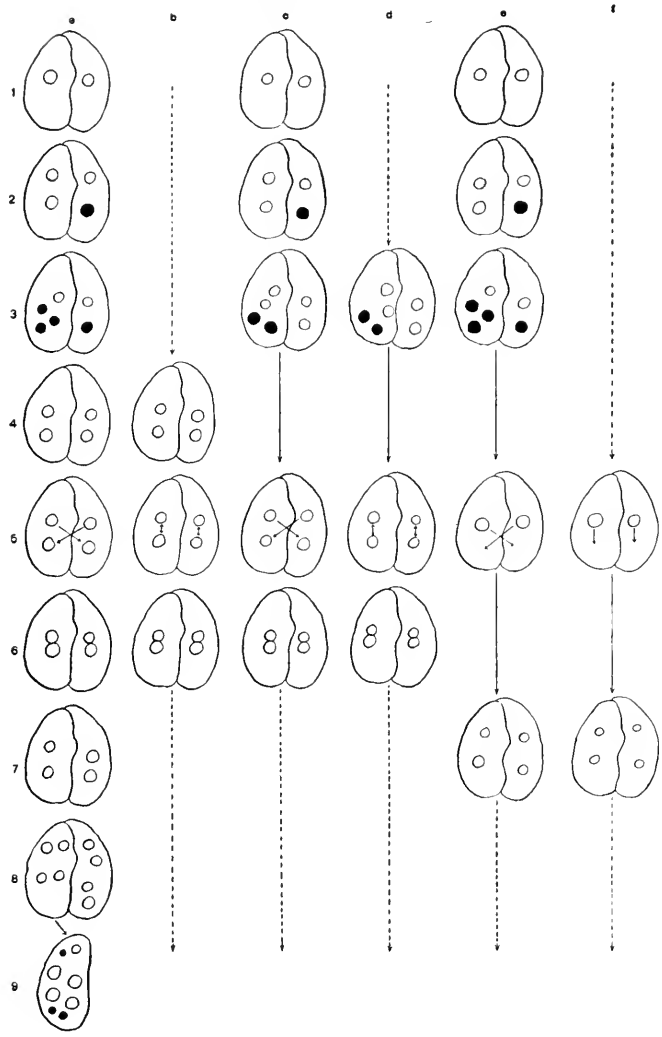


FIG. AC.

Variations in conjugation of *Paramecium* trichium, from Diller (1948), redrawn; macronuclei omitted; degenerating micronuclei black: column a, the more common scheme which is much like that of *P. caudatum*, with occasional degeneration of one nucleus after pregametic division, shown in 2 and 3, right; column b, similar to a, but with autogamy instead of cross fertilization; column c, third pregametic division omitted; column d, like c with autogamy; column e, like d for 1 and 2, but with single nucleus in each conjugant after second division—these pass to mates to develop parthenogenetically; column f, like e but with parthenogenetic development without passage to mate.

autogamy (colmn d) may occur. The haploid nuclei produced by the two pregametic divisions may fail to form pronuclei, but pass to their mates (column e) or develop parthenogenetically (column f.) Some of these variations are illustrated in Fig. AD. Transfer of a single nucleus into a mate with degenerating nuclei is shown at (1), leaving one conjugant without a nucleus. At (2) a binucleate specimen is conjugating with a uninucleate one. In (3) and (4) exchange of macronuclear material is taking place. In (3) one pronucleus has transferred without reciprocal exchange, producing a "haploid" individual at the left and a "triploid" at the right, as previously mentioned for *P. bursaria*. Diller also saw conjugation between a normal animal and the anterior member of a tandem pair, as described above for *P. bursaria*. Later Diller (1949) described an abbreviated process of conjugation in *P. trichium*, where exchange of nuclei took place after the first pregametic division. The products of the first division proceed directly to reconstitute the new nuclear apparatus by synkaryon formation or parthenogenesis, or a combination of the two. There is no degeneration of nuclei between divisions.

The account of conjugation in *P. putrinum* by Doflein and Reichenow (1949) follows closely the "standard" series of events in *P. trichium*, as shown in Fig. AC, column a. As stated by Diller, it is possible that Doflein's account refers to *P. trichium*. The identity of *P. putrinum* is still in doubt (Wenrich, 1926).

For *Paramecium aurelia*, with two small micronuclei, accounts of conjugation have been supplied by a number of authors, especially by Hertwig (1889), Maupas (1889), Diller (1936), and Sonneborn (1947). There is general agreement that the two micronuclei go through a crescent stage previous to the first pregametic division. The four nuclei produced by this division quickly go through the second division to produce eight haploid nuclei. According to Hertwig and Sonneborn seven of these nuclei degenerate while the remaining one divides to produce the pronuclei. However, Diller stated that a variable number, from two to five, of these eight nuclei at least begin the third division, but only two of the resulting nuclei, apparently those nearest the paroral cone, become pronuclei. As suggested by Diller (1936) and Sonneborn (1951), the position in the cell apparently determines which haploid nuclei become functional. Sonneborn (1951) cites this as an example of cytoplasmic control over nuclear activities. After exchange of pronuclei and syncaryon formation, the two conjugants separate and two mitotic divisions produce

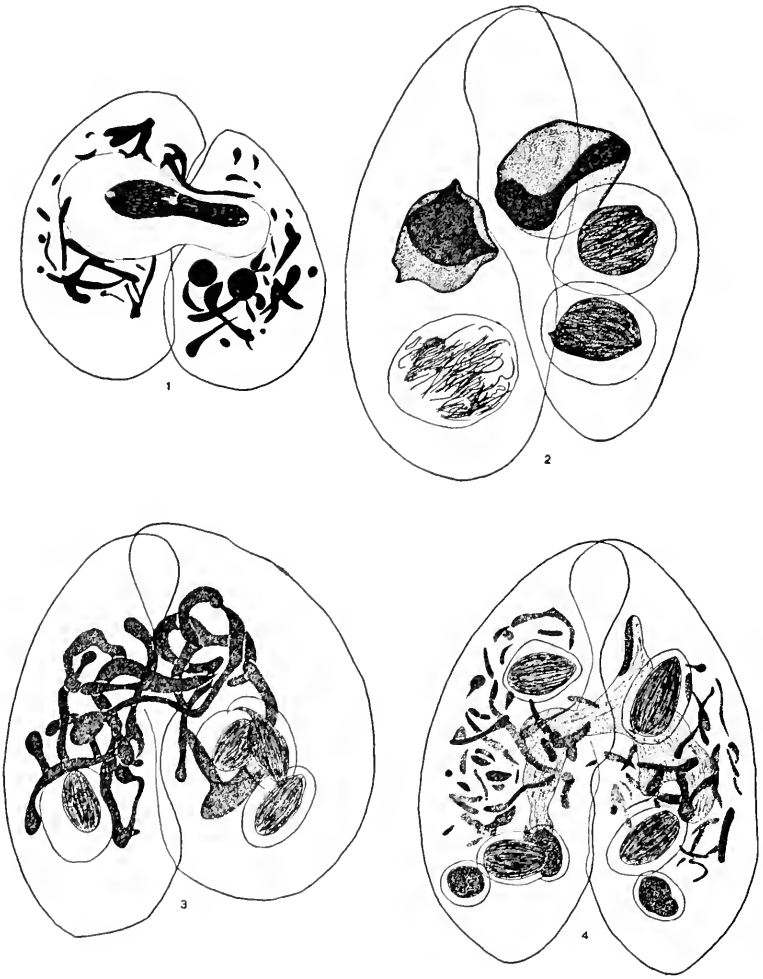


FIG. AD. Variations in conjugation of *Paramecium trichium*.

1, 2, 4, from Diller (1948); 3 from Diller (1949), redrawn. 1, transfer of a single gamete nucleus into a mate with degenerating nuclei, leaving one conjugant without a micronucleus; 2, a binucleate animal conjugating with uninucleate one; 3, one pronucleus has passed over without reciprocal exchange, producing a "haploid" animal at left and a "triploid" one at right, intermingling of macronuclear material; 4, two functional nuclei in each conjugant, large lobe of macronuclear material transferring from one to the other.

four nuclei from the syncaryon in each exconjugant. Two of these become macronuclear anlagen while the other two become functional micronuclei. Only one cytoplasmic division, accompanied by micronuclear division, is required to segregate the two new macronuclei into two daughter cells. Resolution of the old macronuclei into a skein begins about the time of pronuclear formation. The skein later breaks up into fragments which may persist to some extent until after establishment of the new nuclear condition in the exconjugants.

Paramecium multimicronucleatum usually has four micronuclei of the same type as those of *P. aurelia*. At the beginning of conjugation, according to Landis (1925), all four micronuclei go through the first division following crescent formation. All eight of the daughter nuclei undergo the second pregametic division. Of the sixteen nuclei so produced, usually four embark on the third division while the other twelve degenerate. Only one nucleus completes the third division to form the two pronuclei. Again, the location in the cell seems to determine which nuclei will become functional. After cross fertilization, the conjugants separate and three divisions provide eight nuclei from the syncaryon. Of these, most, probably seven, degenerate. Further divisions provide two macronuclear anlagen and two micronuclei. Four macronuclei are eventually produced, apparently by division of the two first formed, while four micronuclei are evident at the same time. By two successive cell divisions, accompanied by micronuclear divisions, the four new macronuclei are segregated into four cells, each with one macronucleus and four micronuclei. Müller (1932) found that, although the conjugations as described by Landis occurred in over 60 per cent of the animals, there was a very wide range of variation, especially in the exconjugants, in which macronuclear anlagen varied from two to fourteen, and micronuclei ranged up to twenty-eight. According to Köster (1933) the fragments of the old macronucleus are distributed among daughter cells during the six to eight divisions following conjugation and are eventually taken bodily into the new macronuclei.

Presumably all species of *Paramecium* will undergo conjugation under appropriate conditions, but no extended accounts have been published for *P. calkinsi*, *P. woodruffi*, or *P. polycaryum*. When *P. calkinsi* was first described and named by Woodruff (1921), he had not been able to induce conjugation or any other type of nuclear reorganization. Wenrich and Wang (1928) first reported conjugation for this species but gave no details. Recently Wichterman (1948b)

reported two mating types and conjugation among four races of *P. calkinsi*, but cytological details were not given. No accounts of conjugation in *P. polycaryum* or of *P. woodruffi* have been found. Gelei (1938) stated that conjugation occurs in *Paramecium nephridiatum* but gave no details.

Paramecium "Hybrids"

Various attempts have been made to hybridize different species of ciliates. In view of the existence of mating types in *Paramecium*, one would hardly expect successful hybridization to take place. Müller (1932) described conjugation between *P. caudatum* and *P. multimicronucleatum*, but none of the exconjugants survived. This was also true of the crosses between *P. caudatum* and *P. aurelia* obtained by DeGaris (1935). Considering that *P. caudatum* has a different type of micronucleus from those of *P. aurelia* and *P. multimicronucleatum*, these results are not surprising, although autogamy (cytogamy) or parthenogenesis might take place in such cross-species attachments. It will be recalled that Chen (1946b) found that crosses between two varieties of *P. bursaria* were also lethal.

Nuclear Reorganization Other than Conjugation in *Paramecium*

"*Endomixis*"

Woodruff was able to maintain a strain of *P. aurelia* for many years and for thousands of generations by the daily isolation method without conjugation occurring unless induced in side lines. However, cell division rate underwent a decrease or "depression" about every 30 days. Erdmann studied the nuclear phenomena during these depression periods and found that the old macronucleus broke up into fragments and a new nuclear apparatus developed from products of micronuclear divisions. There are some variations in the process but, as described by Woodruff and Erdmann (1914), the main events are as follows. While the macronucleus is breaking up, two micronuclear divisions produce eight small nuclei. Of these, all degenerate except one or two. A cell division produces two animals, each with one micronucleus. Two micronuclear divisions give rise to four small nuclei, two of which become macronuclear anlagen and the other two remain as micronuclei. A cell division, accompanied by micronuclear division, segregates the two anlagen into different cells, thus restoring the normal complement of one macro- and two micronuclei. These

authors emphasized the absence of any process of syngamy in "endomixis." In the same year Hertwig (1914) described a process of nuclear reorganization in this species which he called parthenogenesis.

"Endomixis" has also been described for *Paramecium caudatum*, first very incompletely by Erdmann and Woodruff (1916) and more fully by Chejfec (1930) (Fig. AE, 13 to 22). According to Chejfec the macronucleus begins to fragment while two micronuclear divisions produce four small nuclei (13 to 16). Three of these degenerate while the fourth undergoes division (17, 18). After a second micronuclear division (19) two nuclei become anlagen and the other two remain as micronuclei (20). One cell division distributes one of each type of nucleus to each of the two daughters (21, 22). The similarity of the micronuclear behavior in the earlier stages to that in conjugation is striking, and if a process of nuclear fusion should have been overlooked this might prove to be autogamy.

Erdmann's (1925) account of "endomixis" in *P. bursaria* is very incomplete, and apparently such a process is very rare in that species. Stranghöner (1932) described "endomixis" in *P. multimicronucleatum* involving eight to ten generations. Again the nuclear history is similar to that of conjugation, especially in the earlier stages. "Endomixis" has been reported to occur in *Paramecium polycaryum* by Woodruff and Spencer (1923) and in *P. nephridiatum* by Gelei (1938), but without details.

Autogamy

Diller announced the discovery of autogamy in *Paramecium aurelia* in 1934 and gave a full account of it in 1936 (Fig. AE, 1 to 12). This process occurs in single individuals and parallels the events described as "endomixis" by Woodruff and Erdmann (1914), except that a crescent stage develops in preparation for the first pregametic micronuclear division (2), and fusion of gamete nuclei takes place. After the second micronuclear division (3), most of the micronuclei degenerate, while a third division (5) produces two pronuclei (6) which unite (7) in self-fertilization or autogamy. Meanwhile the macronucleus passes through a series of skein stages (4 to 8) before fragmenting into small pieces (8 to 10). Pronuclear fusion takes place in the paroral cone which forms at the place where exchange of pronuclei occurs in conjugation. From the syncaryon (8), four nuclei are produced by two divisions (9, 10). Two of the four grow into anlagen (11), and a single cell division (12), accompanied by

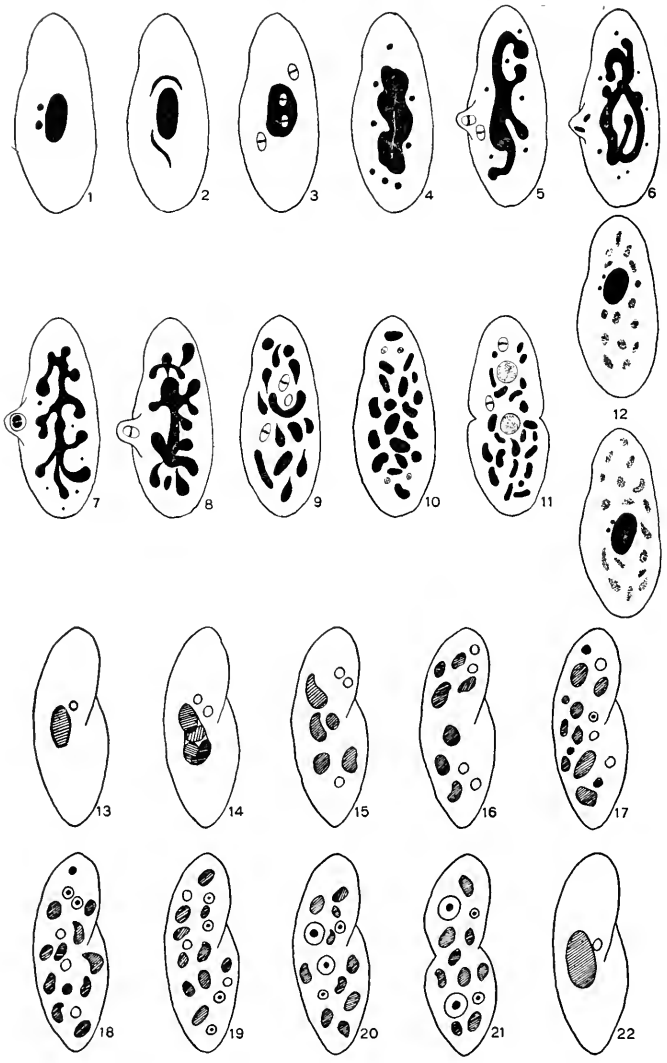


FIG. AE.

1-12, autogamy in *Paramecium aurelia*, from Diller (1936); 13-22 "endomixis" in *P. caudatum*, from Chejfec (1930), all redrawn. 1, normal animal with two micronuclei; 2, micronuclei in crescent stage; 3, second pregametic division, four nuclei in metaphase; 4, eight products of second pregametic division, beginning resolution of macronucleus; 5, six micronuclear products degenerate, two others divide; 6, pronuclei in paroral cone, early skein of macronucleus; 7, fusion of pronuclei in paroral cone, further resolution of macronucleus; 8, syncaryon in paroral cone; 9, two products of division of syncaryon; 10, four products of second postzygotic division; 11, two micronuclei and two anlagen; 12, products of first postzygotic cell

micronuclear division, segregates these into two daughter cells, each with the normal equipment of one macronucleus and two micronuclei (12). The similarity between this process and conjugation is very close.

Sonneborn and his associates made extensive studies of the conditions under which "endomixis" took place in *Paramecium aurelia*. After his discovery of mating types in this species (1937), Sonneborn made genetic studies of the consequences of this type of nuclear reorganization and found that in each case autogamy took place instead of "endomixis" (Sonneborn, 1947). It remains to be seen how many other cases of so-called "endomixis" may turn out to be autogamy or parthenogenesis.

Other Forms of Nuclear Reorganization

In addition to autogamy, Diller (1936) also described a series of nuclear reorganizations of the macronucleus which did not involve replacements from micronuclear sources. These reorganizations were called hemixis and could take place according to several patterns. Usually a partial or complete breakdown of the macronucleus occurs. Larger fragments, when segregated into daughter cells by cell divisions accompanied by micronuclear divisions, could develop into functional macronuclei. When the entire macronucleus fragments into balls, degeneration followed.

A slightly different form of nuclear reorganization was described by Sonneborn (1947) for *P. aurelia*. When animals undergoing conjugation or autogamy were kept at a temperature of 38°C after fertilization and during subsequent development, the growth of the macronuclear anlagen was arrested, not to be resumed until several cell divisions had ensued (Fig. AF). Because the micronuclei were less affected, cell division could continue. During these divisions some animals were separated which lacked any macronuclear anlagen (3). In these, one or more fragments of the old macronucleus began to grow, and, as they became segregated by subsequent divisions, they eventually developed into full-grown macronuclei (4 to 7). Sonneborn called this process macronuclear regeneration.

division, each with one macronucleus and two micronuclei. 13, normal specimen of *P. caudatum;* 14, two micronuclei formed, macronucleus breaking up; 15, 16, three and four micronuclei, fragments of macronucleus; 17, three micronuclear products degenerating; 18, 19, two micronuclear divisions; 20, growth of two anlagen; 21, cell division segregating one macronucleus and one micronucleus into each daughter; 22, normal animal.

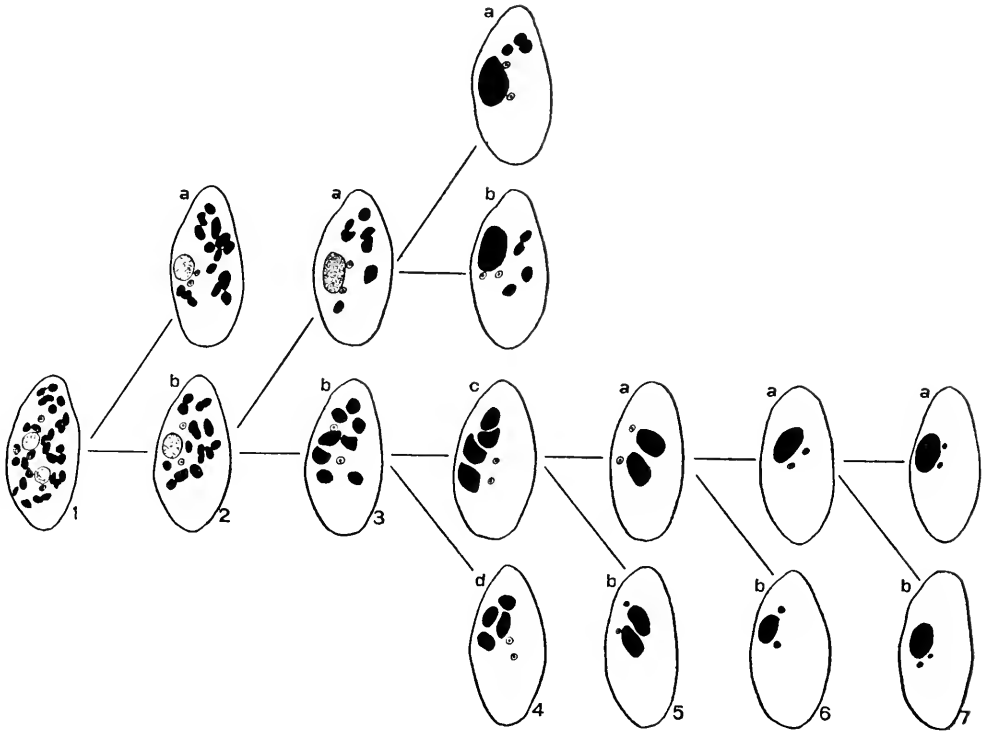


FIG. AF. Macronuclear regeneration in *Paramecium aurelia*, from Sonneborn (1947), redrawn.

1, reorganizing exconjugant or stage in autogamy with two macronuclear anlagen and two micronuclei; 2, cell division, segregating anlagen; 3, cell division with passage of anlagen to only one daughter (a); 4, (a, b) normal cell divisions, (c, d) products of division without anlagen, macronuclear fragments growing; 5, 6, further segregation of macronuclear fragments, each of which becomes a normal macronucleus; 6a, division to produce two normal animals, 7a, b.

Nuclear Reorganization in Other Ciliates

It would be reasonable to suppose that nuclear reorganization comparable to that in species of *Paramecium* would also be found in other ciliates, and it is. Woodruff (1941) gave a review of these processes under the head "Endomixis."

One approach to this question has been to separate animals after they had become associated for conjugation. Calkins (1921) accidentally separated a pair of conjugants of *Uroleptus mobilis* soon after they had come together. The individuals were isolated and found to undergo nuclear reorganization. Calkins cut off the anterior ends of fused pairs of this species, yet the separated animals regenerated and underwent nuclear reorganization. When the cutting was done in an earlier stage, Calkins assumed that autogamy took place, but when he cut off the anterior ends while the migratory nuclei were passing through that region, some other explanation was presumed to apply. Cutting off the anterior ends of exconjugants just after separation did not interfere with completion of nuclear reorganization. Ilowaisky (1926) also stated that individuals of *Stylonychia mytilis* underwent complete nuclear reorganization when forcefully separated after becoming associated for conjugation. The initial contacts between conjugants were presumed to stimulate the nuclear changes. On the other hand, Moore (1924) found no nuclear reorganization in individuals of *Spathidium spathula* when separated soon after becoming associated for conjugation.

Nuclear reorganization in single animals in the active state has been reported for a number of different ciliates. Heidenreich (1935) described various patterns of reorganization in several kinds of endozoic astomatous holotrichs during which the old macronucleus was replaced by a new one from micronuclear sources. He referred to the process as parthenogenesis. DaCunha and Muniz (1930) described endomixis in *Balantidium simile*, and I have seen a reorganization of that type in a similar form of *Balantidium*.

For the Hypotricha, Klee (1926) reported nuclear reorganization in *Euplotes longipes;* Ivanic (1929) described nuclear changes in *Euplotes charon* and *E. patella*, and Kay (1946) for *Oxytricha bifaria*. Horvath (1947, 1948a,b) produced amicronucleate races of *Kahlia simplex* either by treatment with ultraviolet light or by excess products of bacterial metabolism. By the first method, micronuclei were

destroyed or changed to macronuclei; by the second method, micronuclei grew into macronuclei. Amicronucleate animals were said to be capable of "endomixis" (presumably hemixis or macronuclear regeneration) with a repetition every 8 to 10 days. The macronuclei (normally two) broke up and new macronuclei developed from some of the fragments. Even amicronucleate animals were said to "conjugate," the "conjugants" being of different sizes. The joined animals fused into one, the macronuclei became resolved into ribbons, then fragmented, and new macronuclei regenerated from fragments the size of micronuclei. This process cannot properly be called conjugation in the absence of micronuclei. When amicronucleate animals conjugated with normals, there was also complete fusion of the associates, and presumably autogamy or parthenogenesis took place.

Among the Peritricha, "endomixis" was described for a species of *Trichodina* from tadpoles by Diller (1928). Later (1936) he thought that exconjugant and hemictic stages might have been lumped together as "endomixis," while Padnos and Nigrelli (1942), after a study of conjugation in *Trichodina spheroidesi,* thought that Diller's "endomixis" could be reorganization in exconjugants. Fauré-Fremiet (1930) recorded nuclear changes under the term "endomixis" in *Zoothamnium alternans,* and Seshachar (1946) reported hemixis in *Epistylis plicatilis* and *E. anastatica.* Willis (1948) recorded an apparent endomixis in *Lagenophrys tattersalli.*

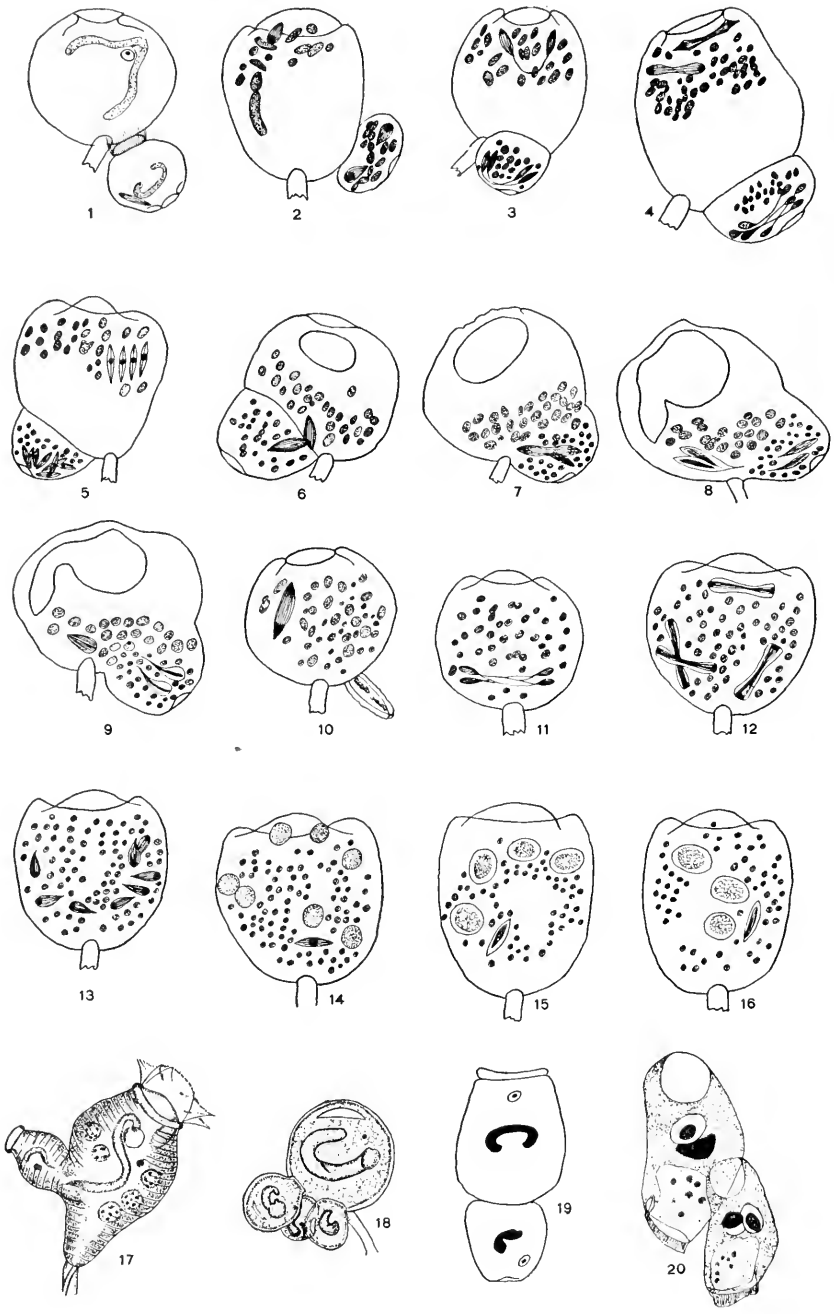
Nuclear reorganization seems to occur more commonly in cysts than during the active state. In my experience, it is common for the macronucleus to undergo fragmentation in ciliates after encystment. Some of the ciliates which have been reported as undergoing nuclear reorganization in the cyst stage are *Uroleptus mobilis* (Calkins, 1919); *Spathidium spathula* (Moore, 1924); *Euplotes longipes* (Klee, 1926); *Chilodonella uncinatus* (Ivanic, 1928, 1933a, 1935b); *Vorticella nebulifera* (Ivanic, 1929); *Urostyla grandis* (Tittler, 1935); and *Paraclevelandia simplex* (Kidder, 1938). In the last-named species, the reorganization was of an unusual type. The micronucleus divided into two. The macronucleus underwent a partial breakdown with about half of its chromatin being eliminated through the reservoir of the contractile vacuole. The remaining half rounded up and joined with one micronucleus to form a new macronucleus. This passed through a "ball-of-yarn" stage like that in the exconjugants of *Nyctotherus cordiformis* and some other ciliates. The other product of

micronuclear division became the new micronucleus. Most of these accounts are not sufficiently detailed to determine just what kind of reorganization takes place. They could be "endomixis," hemixis, autogamy, or parthenogenesis. However, Corliss (1952a, b) has recently described in detail an autogamous nuclear reorganization in the cysts of *Tetrahymena rostrata*. Apparently this is the first clear account of autogamy in a ciliate cyst. Doubtless other cases of autogamy will be found.

Order Peritricha

Since the time of Ehrenberg (1838) it has been observed that the members of the Peritricha (especially the Vorticellidae) conjugate in a manner different from the typical pattern. One or more preliminary cell divisions produce larger and smaller conjugants. Figure AG (17) shows a microconjugant and a macroconjugant being formed by a single division of a "neutral" individual of *Vorticella microstoma*, as described by Finley (1943). The microconjugant becomes detached, becomes motile, and swims about until it finds a macroconjugant with which it unites. Variations in the formation of microconjugants consist of one, two, or three divisions producing two, four, or eight microconjugants from the first smaller animal derived from a "neutral" individual. Finley and Nicholas (1950) found that four microconjugants were produced from the smaller product of the first "sex-segregating" division of *Rhabdostyla vernalis*. According to Finley (1943) the macroconjugant is attractive to the microconjugant for about 2 hours. Presumably a micro- and macroconjugant from the same parent could conjugate together. Several microconjugants may attach to one macroconjugant (Fig. AG, 18).

The nuclear details are shown in Fig. AG (1 to 16) as illustrated by Maupas (1889). Here it will be seen that there are three preliminary micronuclear divisions in the microconjugant but only two in the macroconjugant (1 to 5). After these preliminary divisions one nucleus in each conjugant survives and divides to produce pronuclei (6 to 8), but only one of those in the macroconjugant fuses with one from the microconjugant to form a syncaryon (9). By three divisions, the fusion nucleus gives rise to eight nuclei (10 to 13) of which seven enlarge as anlagen while one becomes the micronucleus (14). Accompanied by micronuclear divisions, the anlagen are segregated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into single individuals. Most of the material in the microconjugant enters the macroconjugant, but a remnant (10) is cast off. In the case of *Opisthonecta henneguyi*, a free-swimming vorticellid (Fig. AG 19), the microconjugant fused completely with the macroconjugant (Rosenberg, 1940).

In the Urceolariidae, which are free-swimming commensals on or in various hosts, the difference in size between conjugants is not so marked as for the sedentary vorticellids. The most recent accounts are those for *Trichodina spheroidesi* (Padnos and Nigrelli, 1942) and for *Urceolaria synaptae* (?) (Colwin, 1944). Padnos and Nigrelli reported only two preliminary micronuclear divisions in the microconjugant, whereas Colwin recorded three. Colwin found that exchange of pronuclei sometimes took place before nuclear material of the microconjugant passed over into the macroconjugant, leaving a remnant which was later discarded. In *Trichodina spheroidesi* the usual postconjugant development took place, eight nuclei being derived from the synearyon, seven of which became macronuclei and one the micronucleus. In *Urceolaria synaptae*, however, only four of the eight products of the syncaryon became macronuclei, three degenerated, and one became the micronucleus, following the pattern for *Paramecium caudatum* instead of that characteristic for vorticellids.

According to Awerinzew (1936), in a species of *Lagenophrys*, which lives in a lorica on the gill plates of a freshwater crab, *Tel-*

FIG. AG.

1-16, conjugation in *Vorticella monilata*, from Maupas (1889). 1, microconjugant attaching to macroconjugant; 2, first pregametic micronuclear division in microconjugant, break-up of macronucleus; 3, first pregametic division in macroconjugant, second in microconjugant; 4, second pregametic division in macroconjugant, third in microconjugant; 5, products of these divisions; 6, single nucleus left in each conjugant after others have degenerated; 7, 8, final pregametic division to produce pronuclei; 9, passage of one pair of pronuclei into macroconjugant where syncaryon is formed; 10, division of syncaryon, small remnant of microconjugant; 11, second postzygotic division; 12, third postzygotic division; 13, eight products from syncaryon; 14, seven products become macronuclear anlagen, one becomes the micronucleus; 15, 16, after first postconjugant cell division. Further divisions segregate anlagen into single cells. 17, 18, *Vorticella microstoma*, from Finley (1943). 17, division of a neutral individual into one micro- and one macroconjugant; 18, three microconjugants attached to one macroconjugant. 19, conjugation of macro- and microconjugants of *Opisthonecta henneguyi*, from Rosenberg (1940). 20, conjugants of unequal size in *Lada tanishi*, from Miyashita (1928), with unusual points of contact. (All illustrations redrawn.)

phusa, conjugation was preceded by an unequal division of an ordinary individual into a larger macroconjugant and a smaller "gametocyte," which then divided into two microconjugants. The gametocyte was not ciliated, but the micronconjugants were. There were two types of these with different ciliation and size. Only the larger type was seen to function as a micronconjugant. The nuclear phenomena were typical of the vorticellids.

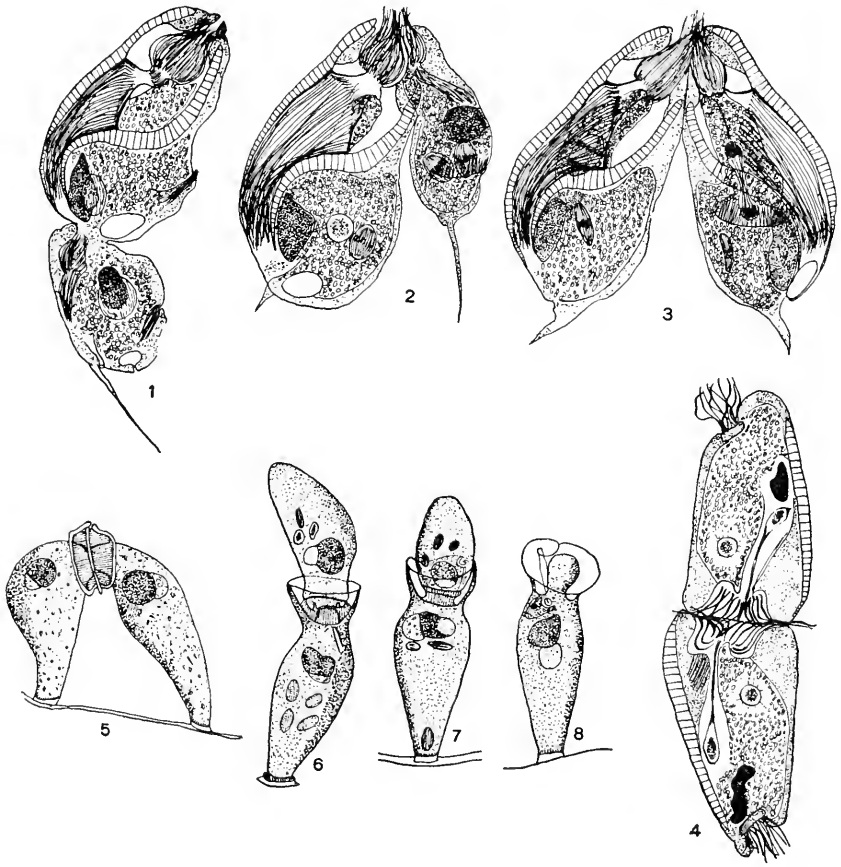

FIG. AH.

1-4 from Dogiel (1925). 1-3, stages in *Opisthotrichium janus:* 1, progamic division producing unequal conjugants; 2, conjugation of macro- and microconjugants; 3, conjugation of two macroconjugants; 4, conjugation of *Cyclopostbium bipalmatum* showing differentiation of migratory pronucleus into a sperm with tail; 5-8, stages in total fusion of conjugants of *Spirochona gemmipara*, from Plate (1886). (All illustrations redrawn.)

Order Chonotrica

According to Plate (1886) and Swarczewsky (1928), *Spirochona* of this epizoic group undergoes conjugation by complete fusion between the conjugants, as shown in Fig. AH (5 to 8). Nuclear details were not fully worked out.

CLASS SUCTORIA

Conjugation among Suctoria, which are usually sedentary and without cilia in the adult state, follows the common pattern in regard to nuclear behavior. There are a number of accounts, but the monograph by Collin in 1912 provides a review of the literature published up to that time.

When epidemics of conjugation occur, individuals of many species form cytoplasmic "arms," sometimes as long as the animal itself, which extend out from the body in various directions. If one of these contacts a similar extension from another individual, they become attached and pronuclei are exchanged across the "bridge" thus formed.

Collin recognized two general types of conjugation in this group; one is the common mode of exchange of pronuclei, separation, then reconstitution of the nuclear apparatus from the syncaryon in each exconjugant. In the other type, there is complete fusion with only one "synconjugant" surviving. In this type the two individuals may be equal in size ("isogamy") or unequal ("anisogamy").

In *Tokophrya cyclopum* (Fig. AI), complete fusion occurred. A first micronuclear division (1 to 3) was followed by a second (4). After fusion of the conjugants a division of the syncaryon produced a single micronucleus and a new macronucleus (5, center) while the macronuclei began to degenerate (5, at ends). Reconjugation could occur. In the case illustrated (6) a synconjugant (left) is shown conjugating with another individual which is the product of a previous reconjugation (right). The latter has two smaller and two larger old macronuclei besides a new macronuclear anlage and three micronuclei. In another illustration (not shown here) a larger synconjugant was in conjugation with two normal conjugants. The Suctoria thus show transitions between the type of conjugation common to the Peritricha and the more typical condition of the other Euciliata.

Note: The literature dealing with conjugation is so extensive that a comprehensive discussion is out of the question. Instead, a few topics have been selected for longer or shorter comments.

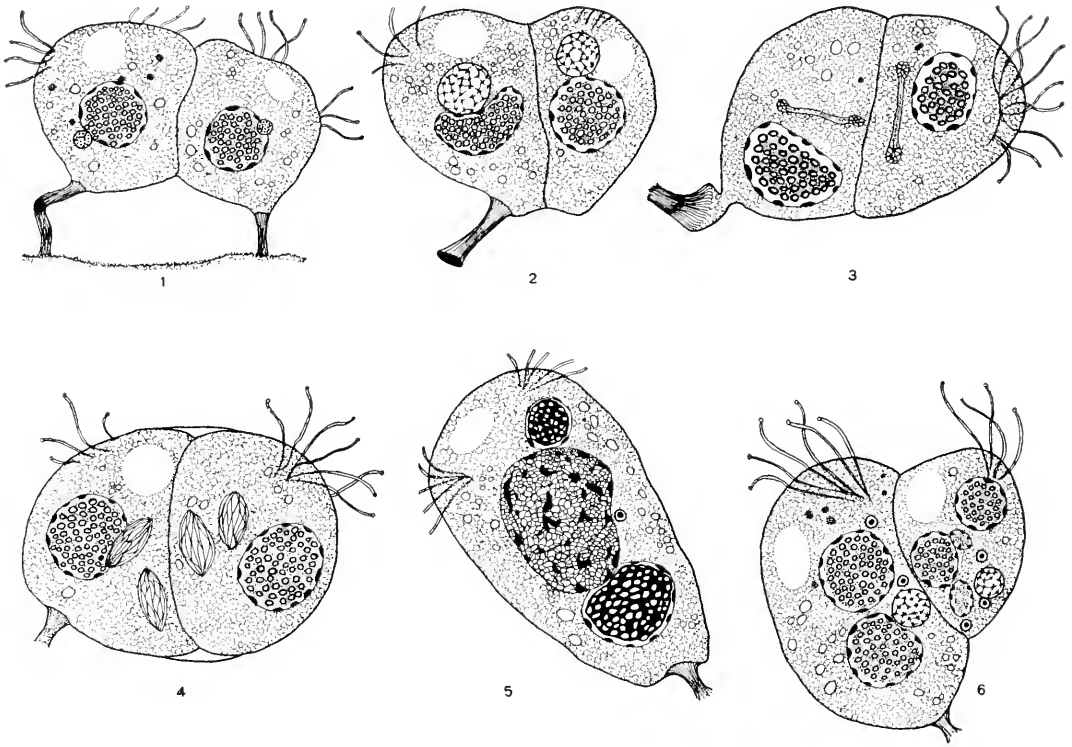

FIG. AI. Conjugation in *Tokophrya cyclopum*, from Collin (1912), redrawn.

1, early association; 2, more complete coupling, micronuclei in prophase of first pregametic division; 3, telophase of first pregametic division; 4, "metaphase" of second pregametic division, complete fusion takes place somewhat later; 5, synconjugant with new macro- and micronuclei in mid-region, degenerating old macronuclei at ends; 6, reconjugation: animal on left has two old macronuclei from an earlier conjugation, one anlage and two micronuclei; animal on right has two small degenerating macronuclei from a first conjugation, two larger macronuclei from a second conjugation, one anlage and three micronuclei.

Differentiation of Conjugants

Attention has already been called to the regularity with which conjugants of different sizes occur among members of the Peritricha and sometimes also in the Suctoria. Dogiel (1925) believed that conjugants were generally different, and he offered many tables of measurements, mostly in the Ophryoscolecidae, to support the concept. Some later writers have reported size differences between conjugants, as, for example, in various species of *Chilodonella* (MacDougall, 1925, 1936), *Cryptochilum echini* (Russo, 1926, etc.), *Lada tanishi* (Fig. AG, 20; Miyashita, 1928), *Ptychostomum* (=*Lada*) *chattoni* (Studitsky, 1932), *Conchophthirius mytili* (Kidder, 1933), and *Entorrhipidium echini* (Yagiu, 1940). According to Enriques (1908) conjugants of *Chilodonella uncinatus* did not differ in size until after attachment, and he thought that the contact stimulated size differentiation, which he called "hemisex." On the other hand, Ivanic (1933b) denied any sex differences in *C. cucullus*. In Dogiel's (1925) review some cases were cited where differences between conjugants were still more pronounced. In *Opisthotrichium janus*, an endozoic ciliate, a single preconjugant division produced a macroconjugant and a microconjugant of different morphology (Fig. AH, 1). However, Dogiel noted that not only would a micro- and a macroconjugant unite (2), but that two macroconjugants might associate together (3).

Maupas (1889) believed that in most ciliates the conjugants were smaller than non-conjugants and that this reduction in size resulted from one or more special preconjugant divisions. Although this idea has too many exceptions to be a valid generalization, a considerable number of examples can be found to support it. Special preconjugant divisions have been described or postulated for a number of ciliates including *Dileptus gigas* (Visscher, 1927), *Balantidium* sp. from the chimpanzee (Nelson, 1934), *Nyctotherus cordiformis* (Wichterman, 1937), and *Fabrea salina* (Ellis, 1937). According to Scott (1927), conjugants of *Balantidium caviae* were always smaller than non-conjugants, and Bogdanowicz (1930) reported the same condition for *Loxodes striata*. One wonders if these rapid fissions could be stimulated by abundance of food, then inhibited by subsequent lack of food, since decline of food after abundance has often been suggested as a stimulus to conjugation. However, Giese (1938) found no regular decline in size before conjugation in *Blepharisma undulans*, al-

though size of conjugants did depend on previous history, mostly relating to nutritional conditions. Thus, in many ciliates conjugants are smaller than non-conjugants but may be essentially of the same size, and Pearl (1907), Jennings (1911), and others have claimed that selective mating takes place, since, on the average, members of pairs of conjugants are more alike in size than non-conjugants chosen at random.

Pregametic Nuclear Phenomena

The number of pregametic mitoses in the Euciliata is relatively constant. There are certain other features that are generally found. The preparation for the first division (second in *Euplotes*) is a period of long duration, usually with special configurations of the intranuclear material. Species of *Paramecium* commonly show a "crescent" or "sickle" stage (Fig. AA, 3, at right) while many other ciliates show a "parachute" or "candelabra" stage (Fig. AJ, 13; AL, 1). Wichterman (1940) estimated that the time from first association of conjugants to the first metaphase lasted 7½ hours in *Paramecium caudatum*, the total time of attachment being about 13 hours. Since this first division is the first of the two meiotic or maturation divisions, and the micronucleus increases greatly in size, it is generally assumed that this size increase corresponds to the growth stage in metazoan gametogenesis. Most authors report "reduction" in the second of these divisions. In harmony with metazoan conditions, one would expect this first division to involve synapsis of homologous chromosomes and the formation and division of a haploid number of tetrads which would separate into dyads on the mitotic spindle. However, ciliate chromosomes frequently do not show the pattern of behavior that one finds in the typical metazoan first gametocyte nucleus. In *Euplotes patella* (Turner, 1930) there are four pregametic nuclear divisions after the conjugants have become joined. The first division follows a regular cell division pattern and shows eight chromosomes on the mitotic spindle (Fig. AJ, 1 to 8). The second division, then, parallels the first pregametic division found in most other ciliates, with a long-continued prophase (9 to 14) which involves a "parachute" stage (13). Instead of four tetrads appearing in the metaphase of this division, about thirty-two granules, most of them in pairs, make their appearance (15), and sixteen of them go to each pole (16). During the following division (17 to 20), only eight chromatin bodies show in the prophase (18), but in the metaphase four elon-

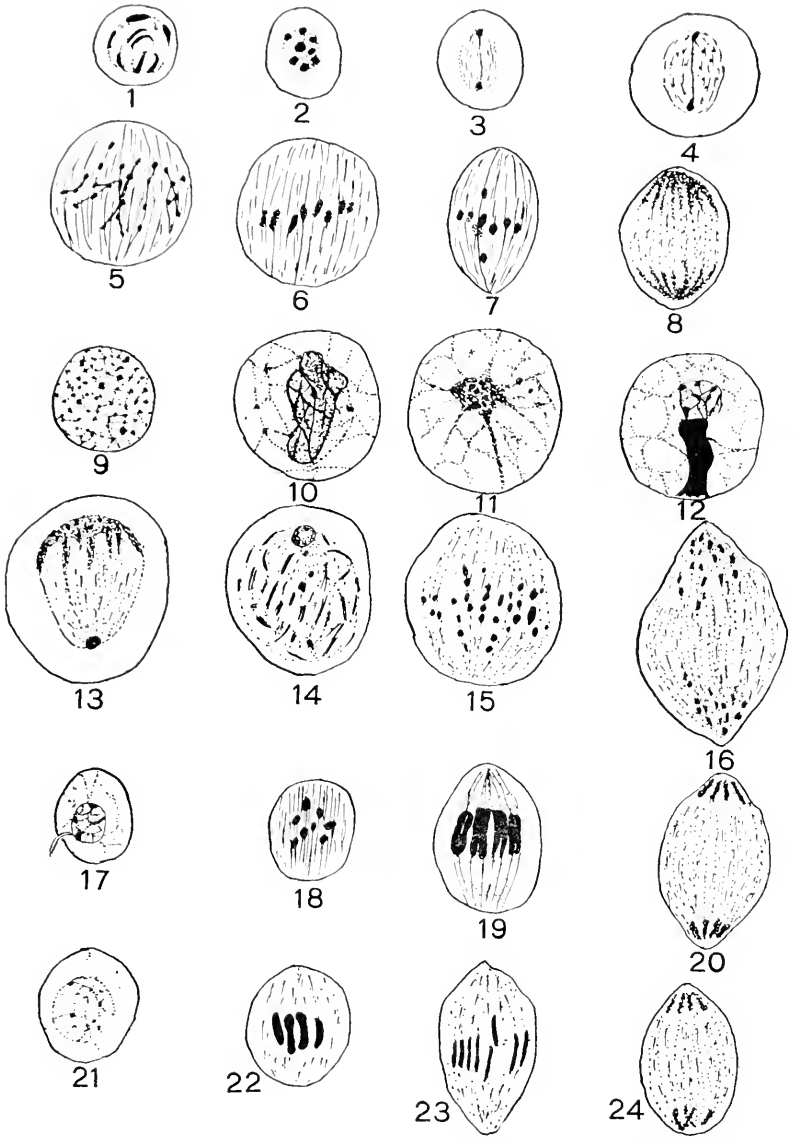

FIG. AJ. Nuclear divisions in conjugation of *Euplotes patella*, from Turner (1930), redrawn.

1-8, first (extra) pregametic micronuclear division showing eight chromosomes; 9-16, second pregametic (first meiotic) division, "parachute" stage at 13, thirty-two granules in metaphase (15), sixteen going to each pole (16); 17-20, third pregametic (second meiotic) division, "reduction" takes place; 21-24, fourth pregametic division with four chromosomes dividing.

gated pairs of chromatids appear (19) and four chromatids go to each pole (20). This is the third nuclear division, which corresponds to the second in other ciliates; it seems to be the "reduction" division. In the fourth and last division (21 to 24) only four chromosomes appear, and these divide to send four to each pole of the spindle (24).

The peculiar behavior of the chromatids in the first meiotic division has caused much confusion about chromosome numbers in meiosis. Turner considered the granules in the prophase of the first meiotic division (15) to be chromomeres, and stated that they usually are arranged in about eight groups. In the anaphase (16), the chromatin is still in the form of chromomeres. In the next division four pairs of chromatids appear (19). Presumably each pair is derived from four chromomeres. If the chromatin bodies which appear during the first maturation division are chromomeres, it is possible to fit the whole series of divisions into a scheme comparable to that in the Metazoa.

Gregory (1923) had still more difficulty with *Oxytricha fallax*, since she counted twenty-four dumbbells in the first maturation metaphase, twelve going to each pole; in the second division twenty-four dumbbells again appeared and twelve went to each pole; in the third division twelve dumbbells appeared and twelve granules separated to the poles. This case seems to involve double reduction or an extra division of chromatids between the first and second divisions. However, Kay (1946), in *Oxytricha bifaria*, reported forty-eight granules in the first maturation division, twenty-four going to each pole, and twenty-four chromosomes in the second, twelve going to each pole. She considered that reduction took place in the second division.

The tendency for protozoan chromosomes to appear as granules in one stage of mitosis and as more solid strands at another is illustrated by conjugation stages in *Nyctotherus cordiformis* (Wichterman, 1937). Two of these stages are shown in Fig. AK (1, 2). In (1) the anaphase in the conjugant at the left shows a group of granules passing to each pole in the third pregametic division, which produces the pronuclei. Metaphases of this division also show a similar number of granules. In the right-hand conjugant of this figure, the telophase of this division shows a "spireme." In the other figure (2) are seen the fusing pronuclei where there seems to be a single thread-like chromosome in each pronucleus. Noland (1927) found a parallel situation in *Metopus sigmoides*. These figures illustrate some of the difficulties involved in interpreting chromosome numbers in ciliates.

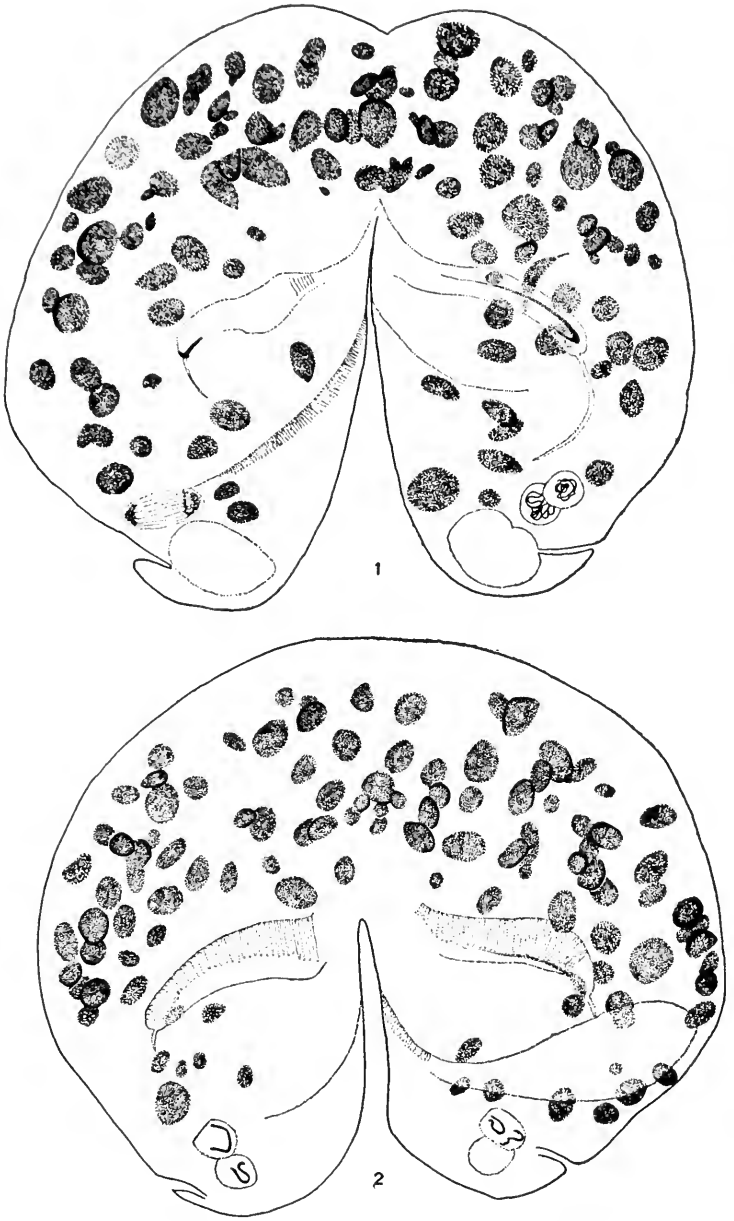

FIG. AK. Conjugating stages of *Nyctotherus cordiformis* from Wichterman (1937), redrawn.

1, anaphase stage of third pregametic micronuclear division at left, with many small chromosomes, telophase of this division at right; 2, conjugating pair showing fusion of pronuclei, one chromosome in each pronucleus; note mingling of macronuclear fragments at anterior ends of the conjugants in both figures.

Recently these difficulties have been the subject of a special study by Devidé and Geitler (1947) and Devidé (1951). In the account of Devidé (1951) it is stated that the chromosomes of euciliates in the earlier literature are not and cannot be chromosomes, but chromosome aggregates, the transverse division of which, so far as it occurs, and the inconstancy of shape and number, do not present any problem for modern karyology. The "true" chromosomes are said to be visible chiefly during meioses, when they pair and form tetrads seen in metaphases where dyads separate. The masking observed in somatic, metagamic, and postmeiotic mitoses can also apply sometimes to meioses.

Devidé used aceto-carmine mostly and described micronuclear divisions and meioses for *Colpidium compylum*, *Euplotes charon*, *Oxytricha* sp., *Chilodonella dentata* (= *C. uncinata*), and mitoses for *Opalina ranarum* and *Cepedea dimidiata*. His demonstration of typical tetrads in the first meiotic division of *Colpidium compylum* and *Euplotes charon* are impressive, but not so satisfying for *Oxytrica*, *Stylonychia*, or *Chilodonella*, since clearly defined tetrads are not indicated for these other species. Hence, even with Devidé's technique, satisfactory details of meiosis were not always forthcoming. The idea of chromosome aggregates is a useful one and would help to explain such a situation as that seen in *Nyctotherus*, referred to above (Fig. AK). In the prophase of the first meiotic division of *Stylonychia*, Devidé states that chromatic bodies number well over 100 and possibly 300 to 400. With such large and indefinite numbers, it would be very difficult to work out meiotic counts, and one wonders if, after all, in such stages, the chromatic bodies are not chromomeres instead of chromosomes.

Another feature of the meiotic period of conjugation deserves to be mentioned. As already noted, Diller showed transfer of macronuclear material from one conjugant to another in *Paramecium trichium* (Fig. AD, 3 to 4). In *Nyctotherus cordiformis* (Wichterman, 1937) conjugants eventually became completely fused in the anterior region so that, presumably, cytoplasm and macronuclear fragments are free to transfer from one conjugant to the other (Fig. AK). In those cases where conjugants fuse partially or completely, as in *Metopus sigmoides*, the Peritricha (Fig. AG, 7 to 9), the Chonotricha (Fig. AH, 5 to 8), and the Suctoria (Fig. AI), cytoplasmic and macronuclear material of the two conjugants may become mixed

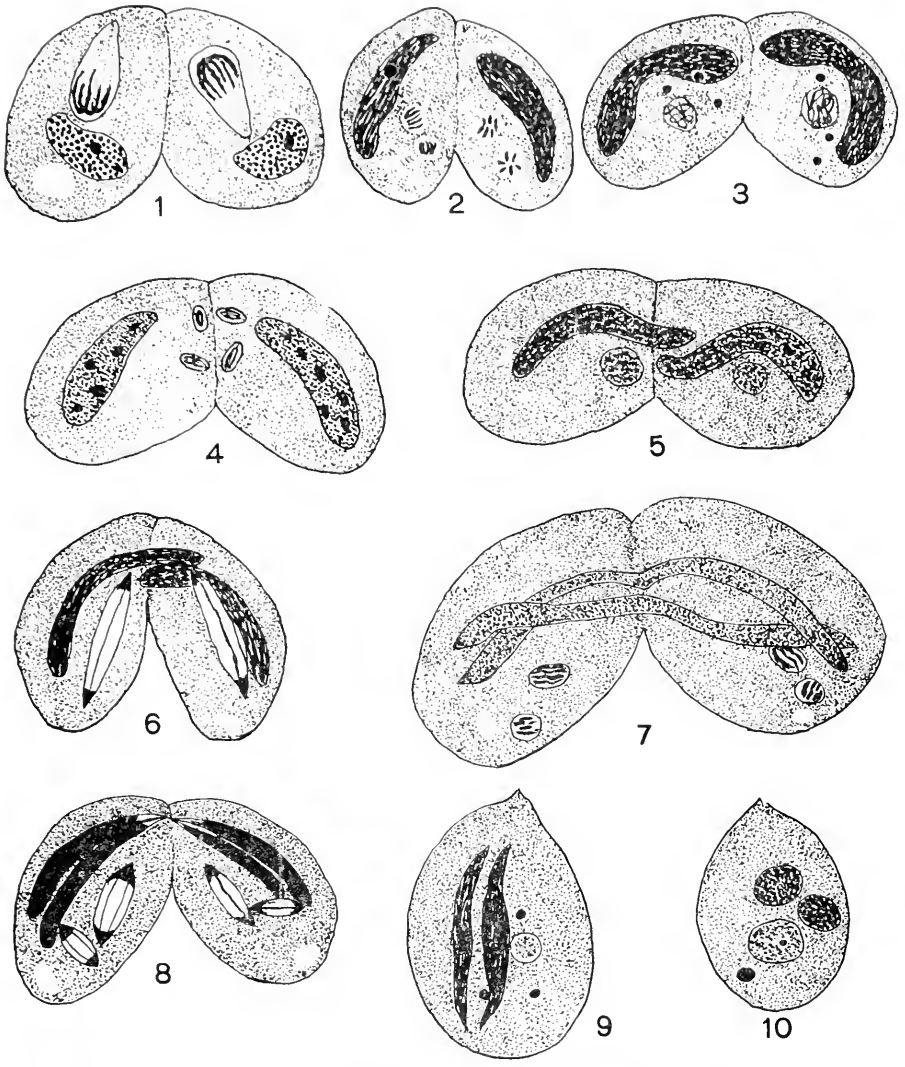

Fig. AL. Conjugation in *Anoplophrya branchiarum* from Collin (1909), redrawn.

1, "candelabra" stage of micronuclei; 2, metaphase of second pregametic micronuclear division, macronuclei elongating; 3, prophase of third pregametic nuclear division, three nuclei degenerating in each conjugant; 4, pronuclei before exchange; 5, prophase of division of syncaryon, one macronucleus projecting into other conjugant; 6, telophase of division of syncaryon; 7, second postzygotic division, elongated macronuclei stretching through both conjugants; 8, constriction of macronuclei, telophase of second postzygotic division; 9, exconjugant with old half-macronuclei, three small nuclei, one enlarging to become an anlage; 10, exconjugant with rounded-up macronuclear remnants, one macronucleus and one micronucleus.

together. In certain ciliates, however, conjugation is accompanied by a regular exchange of portions of the macronucleus between the conjugants, as described, for example, for *Anoplophrya branchiarum* (Collin, 1909). As shown in Fig. AL, each macronucleus becomes much elongated and then projects itself into the cytoplasm of its mate (5 to 8). The result is that each exconjugant contains half of its own macronucleus and half of that of its partner (9). Summers and Kidder (1936) found a similar condition in *Anoplophrya orchestiae*, and Macdougall (1936) in *Chilodonella labiata*. Just why certain ciliates should regularly exchange portions of their macronuclei during conjugation would be difficult to explain.

Differentiation of Gametes

Often there is no obvious difference in the appearance of the two pronuclei which form in a conjugant, but in many cases there are more or less pronounced differences. Sometimes there is merely a slight difference in size, as in *Paramecium caudatum* (Maupas, 1889; Calkins and Cull, 1907, and others), or there may be a special cytoplasmic area in connection with the migratory pronuclei as described for *Uropleptus mobilis* (Calkins, 1919). But perhaps the most extreme case of gamete differentiation is that in *Cyclopostbium bipalmatum* (Dogiel, 1925) where the migratory pronucleus becomes sperm-like with a long tail, breaking out of the endosarc into the joined peristomal cavities and passing down the cytopharynx to the endosarc of its partner (Fig. AH, 4). The differentiation of the male gamete in this case is comparable to that in many Metazoa.

Different Patterns of Behavior in Conjugation

Some of the variations in the behavior of the nuclei during conjugation have been referred to in the previous pages. No attempt has been made in this review to classify them. Dogiel (1925), in his extensive review of conjugation, recognized five different types of nuclear behavior leading to synkaryon formation and ten types of nuclear reconstruction in exconjugants. Since there is often a great deal of variation within any one species, as shown, for example, by Diller (1948) for *Paramecium trichium*, any attempt to make use of or expand Dogiel's classifications does not seem profitable.

Evolution of Conjugation

When one tries to compare conjugation with other forms of syngamy, one is struck by the fact that in typical examples most of the potential gametes fail to develop. Since three pregametic divisions in each conjugant seem to be the rule, potentially eight gametes could be produced from each original micronucleus, whereas normally only two become functional. This suggests that bringing the gamonts (conjugants) together reduces the hazards to potential gametes, since an exchange of gametes can be effected with dangers of loss greatly reduced. Conjugation might therefore be thought of as an evolutionary product derived from more primitive ancestral conditions where a brood of gametes was produced, somewhat like that in

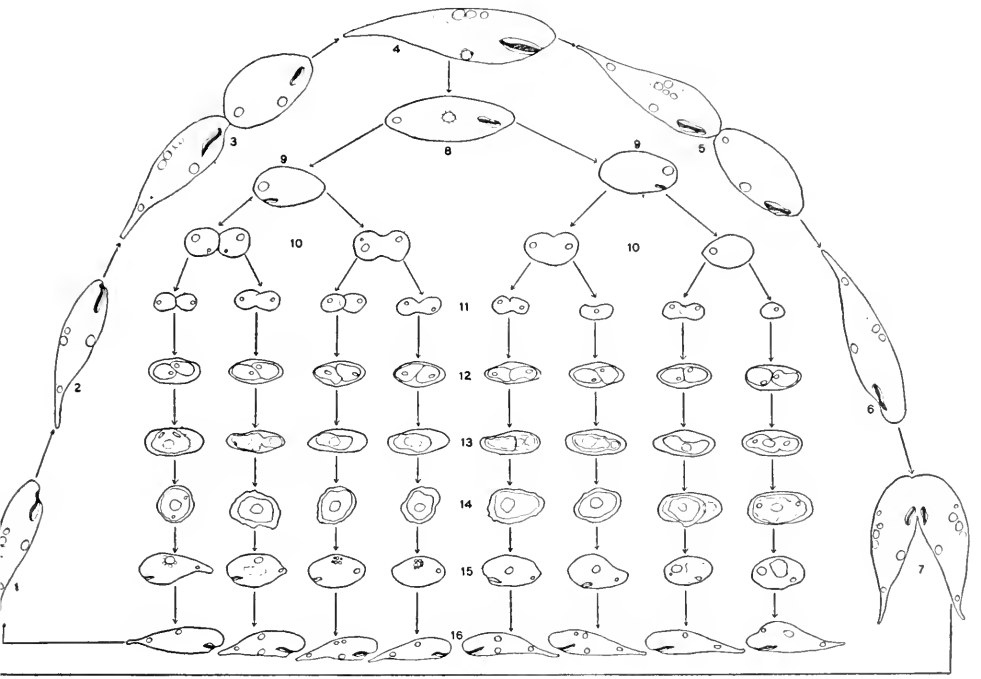

Fig. AM. Scheme of life cycle of *Dallasia frontata* including gamete brood formation, from Calkins and Bowling (1928), redrawn.

1, 2, young "tailed" forms; 3-5, division of "tailed" forms; 6, preconjugant; 7, conjugation of "tailed" forms; 8, "boat" form, 9-11, divisions without growth, to produce gametes; 12, encystment of sister gametes; 13, 14, zygote formation; 15, 16, excystment and development of new "tailed" forms.

chlamydomonad flagellates (see paper by Lewin), or the formation of uninucleate gametes in the Opalinidae.

Fortunately, we have in the unusual behavior of *Dallasia frontata* (Calkins and Bowling, 1928), the development of just such a brood of gametes (Fig. AM). In this case the cell body divides with each nuclear division, leading to the formation of gametes. Isogametes are formed which, however, unite with sister gametes in a process of autogamy (called paedogamy by Calkins and Bowling) to produce each zygote. Normal conjugation also occurs in this species (Calkins and Bowling, 1929). In typical conjugation, nuclear divisions take place without cell divisions; hence gamete development is telescoped into the cell body of each conjugant.

Calkins and Bowling (1928, 1929) suggested that the peculiar brood formation in *Dallasia* is related to "endomixis" and that both might relate to ancestral gamete brood formation. Attention has already been called to the situation in the vorticellids, where an unequal division of a "neutral" individual produces macro- and microconjugants. In many cases, four or eight microconjugants are produced. These divisions might also reflect the supposed ancestral gamete brood formation pattern. Furthermore, it will be recalled that in the microconjugant there is an extra micronuclear division, which may indicate a definite trend toward multiple gamete formation. Just why there should be manifested such an ancestral trend in the microconjugant and not in the macroconjugant is an interesting question. A survival value might be postulated since the microconjugant dies if it does not find a mate, whereas the macroconjugant, after a few hours of receptiveness toward microconjugants, reverts to a "neutral" status, capable of continuing the line by normal binary fission (cf. discussion by Finley, 1952). The extra micronuclear pregametic division in each conjugant of *Euplotes* can be interpreted as a part of this ancestral trend, as can also the one or more preconjugant cell divisions that have been reported for a good many ciliates (see discussion of differentiation of conjugants).

The relationship between the peculiar gametogeny of *Dallasia* and "endomixis," suggested by Calkins and Bowling, is not so readily discerned. However, since Diller (1936) and Sonneborn (review, 1947) have shown that "endomixis" in *Paramecium aurelia* is autogamy, and the so-called paedogamy of *Dallasia* is also seen to be autogamy, then that aspect, at least, is common to both processes. But

autogamy also occurs as a variation of conjugation, as previously shown for various species of *Paramecium*. Furthermore, autogamy is widely distributed among the Protozoa, seemingly being characteristic for the Cnidosporidia and Haplosporidia, and occurring in Heliozoa, and possibly in the Radiolaria, and various animal flagellates (Cleveland's studies). If ciliates have evolved from flagellates where autogamy as well as union of gametes of diverse parentage occurs, the appearance of autogamy in ciliates should not be surprising. Unfortunately, Calkins and Bowling found that the zygotes resulting from autogamy were mostly non-viable. This might suggest that conjugation, providing for cross fertilization, would have a greater survival value than autogamy. One could not say the same for the Cnidosporidia and Haplosporidia, in which autogamy is the rule.

In the preceding consideration of sexual phenomena in ciliated Protozoa, no attempt has been made to describe the occurrence and behavior of mating types, since discussions and interpretations of mating-type phenomena are provided by Dr. Nanney and Dr. Metz in the next two papers.

SUMMARY

Among the Phytomastigina, syngamy is common in the Phytomonadina (see paper by Lewin). It is uncommon in the Chrysomonadina, Euglenoidina and Dinoflagellata, and in these groups no haploid-diploid cycles have been fully demonstrated except for the chrysomonad, *Ochrosphaera neopolitana*.

In the Zoomastigina, cell fusions have been described for scattered representatives of the Rhizomastigina, Protomonadina and Polymastigina, but without adequate cytological details. According to Cleveland, however, the flagellates that inhabit the gut of the wood-feeding roach, *Cryptocercus punctulatus*, exhibit sexuality whenever the host molts, and detailed cytological descriptions have been provided for certain species. Haploid flagellates undergo postzygotic meiosis, some kinds with one, some with two divisions. Diploid flagellates show pregametic meiosis which requires two divisions in some species, and only one in others. Autogamy occurs in some species. In *Trichonympha* and some other haploid flagellates, each gametocyte produces a male and a female gamete by a sex-differentiating mitosis during which each parental chromosome gives rise to dif-

ferently staining chromatids; the more darkly staining male chromatids are segregated to one pole and the more lightly staining female chromatids to the other pole by a special mitotic mechanism.

In the Sarcodina, cell fusions without adequate chromosome data have been described for various members of the Proteomyxa, Amoebina and Testacea. In the Foraminifera, syngamy is common, usually alternating with asexual multiple fission. Diploid-haploid sequences have been worked out for a few species. Gametes are amoeboid in some species, flagellated in others. In the Heliozoa, meiosis and approximate isogamy have been reported for *Actinosphaerium eichhorni* and for *Actinophrys sol* with full chromosome details described for the latter species. Formation of flagellispores has been reported for various kinds of Radiolaria by several authors, but satisfactory evidence for syngamy has not been found.

In the Sporozoa, sexual reproduction appears to be general. In the Gregarinida both isogamous and anisogamous unions and both pregametic and postzygotic meioses have been described. In the Coccidia and Haemosporidia, anisogamy prevails with postzygotic meiosis the rule in the Coccidia. Meiosis has not been satisfactorily worked out for the Haemosporidia. Attention is called to evidence indicating that sex determination takes place early in the development of gregarines and coccidia, possibly at the first postzygotic nuclear division. In certain cases, development of a parasite is seen to be correlated with developmental stages of the host. In the Myxosporidia, Actinomyxidia, Microsporidia and Haplosporidia, autogamy seems to be the rule, with isogamy or slight anisogamy occurring. No sexual reproduction has been reported for the Helicosporidia or Sarcosporidia.

In the Ciliophora, certain members of the Protociliata, all of which are endozoic, are said to show isogamy or anisogamy of gametes which are produced by fission of gamonts that excyst in new hosts. In the Euciliata and Suctoria, sexual reproduction commonly takes the form of conjugation during which mutual fertilization takes place between the conjugants. Conjugants are often similar but may be dissimilar in size and morphology. They frequently are smaller than nonconjugants, the result, in some cases at least, of special preconjugant divisions. In the Peritricha, conjugants are regularly dissimilar in size, the microconjugant fusing partially or wholly with the macroconjugant, which alone survives. In certain members of the

Chonotricha and of the Suctoria, complete fusion of conjugants takes place to produce a single synconjugant. Other variations in conjugation include cytogamy (autogamy in each conjugant) and parthenogenesis in each member of a pair. Single individuals may undergo autogamy. Nuclear reorganization without sexuality may also take place as in endomixis, hemixis and macronuclear reorganization. Formation of a brood of small gametes by a single parent, as in *Dallasia frontata* (and other evidence), suggests that conjugation has evolved from a brood-forming ancestral condition.

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Mating Type Determination in Paramecium aurelia A Study in Cellular Heredity *

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THE GENETICS of *Paramecium*, in its modern sense, had its beginning in the discovery of mating types in *P. aurelia* (Sonneborn, 1937), and the determination and inheritance of mating type was the first problem attacked with the newly acquired possibility of cross-breeding analysis. Although many fundamental discoveries essential for the understanding of this problem were made by Sonneborn (1947), the determination and inheritance of mating types in this species have remained less well understood than the genetics of other characteristics, such as the killer and the antigenic traits (Sonneborn, 1950b).

Two major difficulties have been encountered in trying to understand the mating type system. One of these has been to account for differences among nuclei arising by mitotic divisions from a common source. Another has been to account for what seemed to be two very different systems of determination and inheritance of mating type in different races of this species. Recently (Nanney, 1953)

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an attempt was made to give a complete, if formal, explanation for these and other problems. Although this explanation appeared promising and early attempts at verification were encouraging (Sonneborn, 1951), more recent studies have shown that the interpretation is certainly not correct in detail. On the other hand, strong support has been received for the more general features of the model proposed.

Before considering any explanation in detail, it is first of all necessary to review the available information concerning mating type determination in this species. Many of the observations are not available in the literature, and the author is deeply indebted to Prof. T. M. Sonneborn for the use of his unpublished data. It is to be understood that the present treatment of Sonneborn's unpublished observations is subject to his later modification or extension. After reviewing the observations we will return to a consideration of detailed explanations.

PATTERNS OF MATING TYPE DETERMINATION AND INHERITANCE

General Background, the Varietal System and Cytogenetic Considerations

Paramecium aurelia is a "taxonomic" species composed of at least eight so-called varieties which are themselves effective genetic species (Sonneborn and Dippell, 1946; Sonneborn, 1947, 1950a). (See also Metz, 1954.) Each variety contains no more than two mating types and in relatively few combinations can these conjugate with the mating types of other varieties. Such intervarietal conjugations as are completed usually result in the death of the F1 or F2 generations. However, enough intervarietal combinations give some degree of reaction to provide evidence of mating type homologies for most of the varieties. The two mating types within a single variety are designated by Roman numerals, an odd and an even number, and it is clear that the odd mating types in most varieties are similar though not identical. The even mating types in most varieties are also similar but not identical.

The eight varieties fall into two readily distinguished classes, those which have been characterized as showing little cytoplasmic inheritance: Group A, composed of varieties 1, 3, 5, and 7, and those

which show considerable cytoplasmic inheritance; group B, composed of varieties 2, 4, 6, and 8. Most of the study of mating types has been concentrated on variety 1 in group A and variety 4 in group B. The mating types in variety 1 are designated as I and II; those in variety 4 as VII and VIII.

The chief reason for Sonneborn's (1945) conclusion that group A and group B varieties have fundamentally similar systems of determination and inheritance of traits, including mating types, is their possession of identical major cytogenetic processes. These consist chiefly of conjugation, cytogamy, autogamy, and macronuclear regeneration (Sonneborn, 1947). The details of these processes are discussed elsewhere in this symposium (Wenrich, 1954) and will be presented here only briefly.

Conjugation occurs when cells of complementary mating types are mixed under appropriate conditions. Initially clumps and eventually pairs are formed. Within each member of a conjugating pair the micronuclei undergo meiosis, and all except one of the haploid nuclei disintegrate. This remaining nucleus divides mitotically to produce the migratory and stationary nuclei of identical genetic constitution. The migratory nuclei of the pair members are exchanged and fuse with the stationary nuclei to form the syncarya. From the fact that the nuclei fusing in the two pair-members are alike, it will be clear that the syncarya must be of the same genotype.

Cytogamy is essentially similar to conjugation except that an exchange of gamete nuclei fails to take place and mutual self-fertilization results. In this case, therefore, the syncaryon is formed by the fusion of two sister haploid nuclei of identical genic constitution. The syncarya must, therefore, be homozygous for all their genes.

Autogamy occurs periodically in all the stocks studied. This process is identical with cytogamy except that it occurs in single unpaired cells.

In all three of these reorganization processes the syncaryon normally gives rise to four mitotic division products, two of which become micronuclei and two of which become macronuclei. The anlagen of the two macronuclei are regularly separated into different cells at the first postzygotic cell division and develop into mature macronuclei which divide, apparently amitotically, at subsequent cell divisions. The animals whose macronuclei are derived from a single macronuclear anlage are called a caryonide.

The fate of the fragments of the old macronucleus is the same in all three fertilization processes. They are passively distributed to the products of successive fissions. Since the old macronucleus breaks down ordinarily into about 30 to 40 fragments, the cells contain on the average 15 to 20 after one fission, 7 to 10 after two fissions, and so on. Meanwhile the fragments slowly distintegrate, but their disintegration usually does not begin until after the first or second cell division.

In 1942 Sonneborn reported a variation in the behavior of these fragments and of the new macronuclear anlagen which occurs in both groups of varieties and which provides a useful tool in genetic analysis. The new macronuclear anlagen may either fail to arise from products of the syncaryon or their development may be temporarily inhibited so that, although segregated at the first cell division, they may fail to divide at the second or third cell division. In either case, cells arise that lack macronuclear anlagen but contain fragments of the old macronucleus. In these the fragments fail to disintegrate. Instead they grow and continue to be segregated until only one is present in each cell. By this time each fragment has reached the size of the normal macronucleus and thereafter divides at each cell division. The whole process of development of a new macronucleus from a single fragment of the old macronucleus is known as macronuclear regeneration.

In so far as present information goes, regenerated fragments control the same hereditary traits as the macronucleus from which they were derived. Presumably, therefore, each fragment contains at least one complete set of nuclear genes. These results would be expected on either of two very different hypotheses. Sonneborn (1942) originally interpreted macronuclear regeneration as indicating a compound macronuclear structure, i.e., a macronucleus composed of discrete genetically balanced subnuclei. Kimball (1943) proposed as an alternative explanation that the macronucleus contains a great many sets of chromosomes randomly arranged and assorting more or less at random when the macronucleus divides or breaks down into fragments. It is apparent that either of the postulated nuclear structures could result in macronuclear regeneration as found, but that the necessary number of chromosome sets would be much greater in a macronucleus constructed according to Kimball's hypothesis.

Macronuclear regeneration occurs under ordinary conditions

with a very low frequency. However, Sonneborn (1942) increased its frequency greatly by exposing cells to high temperatures during the period when the syncaryon undergoes its divisions and the new macronuclear anlagen are developing. If the exposure is begun earlier in the fertilization processes, the micronuclei may be lost. Macronuclear regeneration may also be induced by exposure to very low temperatures at the same period (Sonneborn, unpublished).

Group A Pattern

In some varieties of group A a few stocks have been found which are pure for one mating type (Sonneborn, 1938; Sonneborn and Dippell, 1946). These few stocks are of the odd mating type. In all other stocks any isolated animal can, according to a definite pattern, produce both mating types. To these "two-type" stocks we will turn first. The main features of inheritance of mating types in such stocks were first reported by Sonneborn (1937) and Kimball (1937).

Animals of these two type stocks usually undergo no change of mating type during vegetative reproduction. However, changes of mating type may occur at nuclear reorganization. The kind of nuclear reorganization (conjugation or autogamy) has little or no influence on the frequencies of the mating types produced. A single reorganized animal may give rise to either or both mating types. If both are produced, segregation of the mating type determiner nearly always takes place at the first postzygotic cell division, and the two products of this division are, during subsequent vegetative reproduction, pure for mating type. It is at this same division that the independently developing macronuclear anlagen separate. Hence, the unit of mating type inheritance is the caryonide, and this sort of inheritance is said to be caryonidal. This fact at once suggests that the macronuclei may be differentiated in such a way as to determine different mating types. In further support of this view, Sonneborn (1937, 1938, 1939, etc.) marshalled a number of lines of evidence which will be briefly reviewed.

When macronuclear regeneration occurs the mating type of the parent cell is perpetuated and no change is observed (Sonneborn, 1942). This same result is obtained whether macronuclear regeneration occurs as the result of treatment with high temperatures or as

the result of reorganization in amicronucleate lines. These observations show that new mating types arise whenever new macronuclei are formed from micronuclei and that new mating types do not arise at nuclear reorganization when new macronuclei fail to form. Moreover, it is seen that mating type is perpetuated through any piece of the old macronucleus.

In some stocks mating types fail to segregate at the first postzygotic division, but segregate at the second or third division. In these stocks multiple macronuclear anlagen have been found and mating type distribution in these, as well as in "normal" stocks, is correlated with the distribution of independently developing macronuclear anlagen (Sonneborn, 1938, 1939). In rare cases in some stocks mating type segregation continues and is clearly not due to the segregation of diverse whole macronuclei (Kimball, 1939). Caryonides showing this kind of mating type segregation contain both mating types and conjugation occurs regularly within the caryonides. These are called, therefore, "selfing caryonides." A more detailed consideration of these selfers will be presented later. Except for the selfing caryonides, the facts of mating type inheritance in group A clearly require the conclusion that macronuclear differences determine the two mating types.

The relative frequencies with which the different mating types, and hence the diverse macronuclei, arise after fertilization varies in dependence upon the temperature prevailing at this time (Sonneborn, 1939, 1942). The relative frequency of the even mating type increases with the temperature over the range of 12° to 32° C. Although this implies some effect of temperature on the developing macronuclei, the effect could be either direct or indirect, through the mediation of some cytoplasmic constituent.

Regardless of the temperature within this range, and consequently regardless of the relative frequencies of the two mating types, the two independently developing macronuclei in a single reorganizing cell are independently determined as to which mating type they will control. This is shown by the fact that the relative frequencies of the various possible combinations for the two macronuclei agree with the calculations based on simple probability considerations.

An anomalous result was obtained when animals of variety 3 underwent both conjugation and subsequent reorganization below

10° C. (Sonneborn, 1939). Under these conditions very little change of mating type occurred at conjugation, each mate producing a clone predominantly of its own mating type. These observations were made before the discovery of macronuclear regeneration, and since mating types do not change at macronuclear regeneration, it seems possible that some, perhaps all, of this failure to change mating types at low temperature is due to the occurrence of macronuclear regeneration.

In sum, the evidence available on mating type determination in group A agrees in indicating macronuclear control of the differences in mating types. The problem is then raised as to how different macronuclei arise from genetically identical micronuclei. Pair members are identical in their nuclear genes after fertilization, as required by the cytogenetic details and as shown by genetic analysis (Sonneborn, 1939, 1947), and yet pair members may differ in their mating types. Even more remarkable, sister macronuclei developed from presumably identical mitotic products of a single fertilization nucleus may determine different mating types. The fact that mating types may change at any autogamy, despite the fact that complete homozygosis is established by a single autogamy, argues that no micronuclear differences or genic recombination can account for the differences in mating types. This conclusion is reinforced by an additional fact set forth in the next paragraph. Several tentative hypotheses have been advanced to account for macronuclear differences, but none is satisfactory. This then remains the chief unexplained phenomenon of mating type determination in group A, the manner in which unlike macronuclei arise from identical micronuclei.

The additional fact referred to in the previous paragraph is of special importance, although the reason for its importance will not become evident until later. The mating type of an animal before reorganization is not correlated in any way with the mating type of its progeny. An animal of mating type I gives rise to the same kinds of progeny and in the same proportions as animals of mating type II (Sonneborn, 1937; Kimball, 1937). In other words, animals in the two-type stocks of group A which differ in mating type do not differ from each other with regard to either the types of progeny which they produce or the relative frequencies of these progeny.

The preceding account shows that the micronuclei in animals of different mating types in group A are alike, while the macronuclei

are in some unspecified way different. No mention has yet been made of particular gene differences affecting mating type inheritance. One effective gene difference has, however, been reported (Sonneborn, 1939). The difference in the mating type phenomena in the two-type stocks fully discussed above and in the one-type stocks briefly mentioned at the beginning of this section is due to a difference in a single pair of allelic genes. The recessive allele, for which the one-type stocks are homozygous, restricts mating type to type I: the dominant allele (homozygous in two-type stocks) permits development of either mating type according to the pattern set forth above. The mode of action of these alleles remains unknown.

The results of previous investigations on mating type determination in stocks of group A may be summarized as follows:

1. The differences between the complementary mating types are due to some as yet unknown differences in the macronuclei.

2. These macronuclear differences arise at the time the macronuclei develop from products of the syncaryon.

3. Temperature increases at this time increase the probability of origin of a macronucleus that will control the even mating type.

4. The two macronuclei which develop synchronously in the same fertilized cell are independently determined as to which mating type they will control.

5. A single gene difference determines whether both mating types can be produced or only one, the odd mating type.

6. In stocks in which both mating types can be produced, there is no effect of the mating type of an individual on the mating type of its sexually produced offspring.

Group B Pattern

Many of the features of mating type determination and inheritance in group A are also found in group B (Sonneborn, unpublished). As a rule mating types are strictly inherited during vegetative reproduction. Stocks of varieties 4 and 8 show a small percentage of selfing caryonides, just as some stocks of variety 1. Varieties 2 and 6, like variety 5 in group A, show a much higher frequency of these selfers. With the exception of the selfers, mating types change only at the time of nuclear reorganization. After nuclear reorganization the sister caryonides from a single reorganized cell may show differ-

ent mating types. Marked temperature effects on mating type determination have also been shown for the B varieties under certain conditions and again, as in the A varieties, the higher temperatures favor the even mating types (Nanney, unpublished).

In only one essential feature have differences between the A and B groups been noted (Sonneborn, 1947). In group A there is no correlation between the mating types of parents and their progeny, nor between the mating types of sister caryonides. In group B, changes occur rarely at reorganization, and when they do occur they usually involve both sister caryonides. A strong correlation is thus found between the mating type of the cytoplasmic parent and the mating type of the progeny. Similarly, the mating types of sister caryonides are strongly correlated. At conjugation this parent-progeny correlation usually results in one of the cytoplasmic parents giving rise to two caryonides of one mating type and the other giving rise to two caryonides of the other mating type. Since exconjugants of a single pair are known to be alike in regard to the kinds of genes which they possess, Sonneborn concluded that the differences which characterize the mating types, though clearly due to differences in the macronuclei, are inherited through some cytoplasmic mechanism. This interpretation was supported by the observation that pair members which are allowed to exchange massive amounts of cytoplasm usually give rise to the same kinds of progeny.

The presence of a cytoplasmic component in the group B system of mating type determination introduces special problems, and diversities of interpretation have existed in regard to its significance. Because of the special features of the cytoplasmic involvment, we will return later to a more extended consideration of nucleocytoplasmic interactions in the B system.

The general features of the B system may be summarized as follows:

1. As in group A, mating types are determined by alternative macronuclear conditions.

2. Higher temperatures, as in group A, favor the formation of macronuclei controlling the even mating types.

3. In contrast to the group A pattern, mating types in group B show a strong parent-progeny correlation. Mating types are not redetermined at random at nuclear reorganization, but tend strongly to be maintained through reorganization.

+. This parent-progeny correlation is due to cytoplasmic conditions characteristic of the two mating types which determine the manner in which the new nuclei develop.

NATURE OF MACRONUCLEAR DIFFERENCES

The facts presented in the previous section show a consistent pattern of mating type determination and inheritance in both groups of varieties, but leave unanswered a central question: In what ways do the macronuclei in cells of different mating types differ? A possible clue to these differences comes from a study by Chao (1953) on the killer character in variety +. Sonneborn (1947) showed that the killer character is determined by the presence of self-duplicating particles, called kappa, in the cytoplasm. He further showed that the presence of kappa is conditioned by the presence of a gene, K, in the macronucleus. Preer (1950) developed technics for visualizing the particles cytologically, and these technics have been used by Chao to study directly the factors influencing kappa concentration. Two of his observations are pertinent here. (1) Cells of a given mating type will support twice as many kappa particles when they have the genotype KK as when they have the genotype Kk. This observation shows a direct numerical relationship between the number of K genes in the nucleus and the number of kappa particles in the cytoplasm. (2) Cells of a given genotype will support twice as much kappa when they show the odd mating type as when they show the even mating type. These observations suggested (Nanney, 1953) that macronuclei in cells of the odd mating type contain twice as many K genes as those of the even mating type. Alternatively, it is possible that certain genes in one mating type are precisely twice as active as those in the other mating type, but this explanation seems less reasonable.

Chao's observations form the basis for a gene-dosage hypothesis of mating type determination. This hypothesis can take a number of different forms. Differences in the number of K genes could be achieved though a doubling or halving of the chromosomes bearing the K gene, thus yielding an aneuploid constitution for one of the mating types. Similarly, differences in the number of K genes could be brought about by a doubling or halving of the entire chromosome complement. This interpretation would ascribe significance to dif-

ferent ploidy levels, either in subnuclei or in the macronucleus as a whole.

An evaluation of these alternative formulations is complicated by the fact that the macronucleus undoubtedly contains many sets of nuclear genes (Sonneborn, 1947), and the manner in which these are organized in the macronucleus is not well understood. Nevertheless, certain conclusions seem warranted.

1. The polyploid interpretation in any simple form is untenable. This conclusion is derived from several different lines of evidence. (*a*) The experimental production of cells with different ploidy levels did not give the results expected on this interpretation (Sonneborn, 1953). (*b*) The mating type system in *Tetrahymena pyriformis*, which shows a remarkable series of parallels to that in *P. aurelia*, involves at least seven different mating types (Nanney and Caughey, 1953). Although two ploidy levels, characteristic for different mating types, are conceivable, the necessity for postulating seven or more different ploidy levels becomes too great a burden for the hypothesis to sustain. Certainly ploidy alone is not sufficient to account for caryonidal inheritance of mating types in *Tetrahymena*. (*c*) It has not been possible to demonstrate any significant differences in the deoxyribose nucleic acid content of cells of different mating types in variety 4 of *P. aurelia* (Guthe, Tefankjian, and Nanney, unpublished).

2. An aneuploid interpretation that postulates the loss of a chromosome while the macronuclear anlagen are still diploid cannot be supported. This is shown by the fact that all heterozygotes studied, including specifically the Kk heterozygotes (Sonneborn, 1947), have shown the dominant phenotype. If the eliminated chromosome contained the dominant allele (as it should at least in some cases), the recessive allele would have been manifested.

3. An aneuploid interpretation that holds that chromosome doubling occurs either in the diploid stage or later could account for the observations. Similarly, the loss of certain chromosomes after the macronucleus has developed could account for the observations. On the other hand, neither of these interpretations can be tested with present technics and the mechanism whereby some one type of chromosome could be regularly, quantitatively and specifically increased or eliminated is obscure. Hence, the gene-dosage hypothesis must be considered to have lost its chief utility.

4. Chao's remarkable observations concerning mating types and kappa concentration indicate some precise quantitative distinctions between the mating types, but the nature of the distinctions remains a puzzle.

NUCLEOCYTOPLASMIC INTERACTIONS IN THE B SYSTEM

There can be no doubt that a cytoplasmic component occurs in the group B system of mating type determination, nor that this cytoplasmic component acts on the developing macronuclei so as to determine their manner of development. Differences of interpretation are possible only in regard to the manner in which the cytoplasmic specificity is maintained. Either the cytoplasmic conditions are self-perpetuating, or they are to some extent under the control of the nuclei.

Interpretations regarding the means of perpetuation of the cytoplasmic conditions center around the subsequent behavior of the caryonides which have changed mating type at nuclear reorganization. Sonneborn (unpublished) observed two kinds of changed caryonides in regard to their stability at subsequent reorganizations. The first group were those that maintained the changed type with as great stability as normal clones and included nearly all caryonides that had changed from mating type VIII to mating type VII and the majority of those which had changed from type VII to type VIII. Many caryonides that had changed from type VII to type VIII showed a considerable amount of reversion from VIII to VII at the next autogamy. More rarely, a caryonide of type VII showed a considerable change to type VIII at autogamy. Since in these clones the cytoplasmic conditions usually associated with one mating type were at least partially maintained in the presence of a macronucleus controlling the other mating type, Sonneborn concluded that the cytoplasmic conditions necessary for the inheritance of mating type are self-maintaining and at least to some extent independent of the nuclear condition. Although failing to manifest its activity at one nuclear reorganization, the cytoplasmic determiner maintains itself and is manifested at a subsequent reorganization.

This interpretation is called into question first of all by recent investigations regarding the nature of the caryonides which show considerable reversion at reorganization. In a study of the inheritance of

mating types at conjugation in selfing caryonides (Nanney, unpublished), it was observed that the mating type VIII individuals in such caryonides change to type VII with high frequency. All selfing caryonides studied, regardless of the amount of selfing observed, showed this instability. In some caryonides one of the mating types was so infrequent that routine observation for selfing was not sufficient to demonstrate that these were in fact selfers and observations on several subcultures for a period of days were required to detect selfing. Since most such selfing caryonides appear superficially to be pure type VIII cultures, but revert largely to type VII at reorganization, it appears possible that many of the unstable VIII's studied by Sonneborn were of this kind. If so, the reversion is in these cases intimately connected with the question of the nature of selfing caryonides and is not due simply to the fact that these clones have recently changed mating type.

Sonneborn's original interpretation is also called into question in so far as it focuses attention upon the occasional aberrant clones and ignores the behavior of the majority of clones which have changed mating types. Usually when changes occur from one pure mating type to another pure mating type, not only are the mating types changed but the changed types are perpetuated normally through nuclear reorganization. Even when sister caryonides, sharing a common source of cytoplasm, show different mating types, the type VII caryonide produces almost exclusively type VII progeny and the type VIII caryonide produces almost exclusively type VIII progeny (Nanney, unpublished). One finds the usual high correlation between the mating type of the parent and that of its sexual progeny.

Sonneborn (1953) has recently provided a similar but more elegant test for the hypothesis of nuclear control, and the results seem conclusive. This test involves inducing at conjugation (a) cytoplasmic exchange between the mates and (b) macronuclear regeneration. The cytoplasmic exchange results in the change of mating type in one member of the pair, so that new macronuclei controlling one mating type share the same cytoplasm with fragments of a macronucleus controlling a different mating type. The conditions under which macronuclear regeneration was induced were such as to suppress division of the new macronuclei, but not to abort them. This suppression of division of the new macronuclei results in the formation of diverse lines of descent from a single cell, some containing a new

macronucleus controlling one mating type and some containing regenerated macronuclei controlling the other mating type. Since it seems reasonable to assume that the cytoplasmic elements, if these are indeed independent of nuclear control, will be equally assorted in the various lines of descent, all lines of descent should at a subsequent reorganization give rise to the same kinds of progeny. This result was not obtained. Instead, each line at a subsequent reorganization regularly produced progeny of the same mating type as the parental line. Therefore, the cytoplasmic conditions must have been modified to bring them into agreement with the nuclear constitution.

Since the mating type of the parent is clearly controlled by the macronucleus, and since the cytoplasmic condition necessary for the inheritance of mating type through nuclear reorganization is strongly correlated with the parental mating type, it follows that the cytoplasmic condition is ultimately controlled by the old macronucleus. Thus, though the cytoplasm determines the nature of the new macronucleus, the cytoplasm is in turn controlled by the old macronucleus. This results in a cyclical interdetermination of the nucleus by the cytoplasm and of the cytoplasm by the nucleus. This system transcends "maternal inheritance" in that the cytoplasmic influence alters the new nucleus in such a way as to perpetuate a trait indefinitely through both vegetative and sexual reproduction. Regardless of the explanation eventually given for those rare clones which revert to an original mating type, the situation observed in the majority of changed clones argues strongly for the macronuclear control of the cytoplasm.

The precise nature of this macronuclear control is problematical. The nucleus could produce directly some substance or substances necessary for mating type inheritance, but it is also possible that the macronucleus merely controls the rate of production of a self-reproducing factor such as has been demonstrated for the killer character in this same organism (Sonneborn, 1947; Chao, 1953). In the absence of evidence for a self-reproducing factor, it appears advisable to adopt the simpler interpretation.

NATURE OF SELFING CARYONIDES

One of the perplexing problems concerning mating types, both in *P. aurelia* and in other ciliates, involves the nature of the selfing

caryonides. These caryonides are clones deriving their macronuclei from single macaronuclear anlage; yet within such clones are found cells of diverse mating types (Kimball, 1939; Jennings, 1941; Nanney and Caughey, 1953). Since the macronuclei control the mating types, it is presumably some macronuclear instability that permits cells within a single clone to manifest different mating types. The fact that diverse pure types may be derived from the selfers indicates that the unstable macronuclei may "stabilize," though it is not clear what the stabilization involves.

A recent formal explanation for the selfers in *P. aurelia* (Nanney, 1953) was based on the idea of structural inhomogeneity. Since the most widely held interpretation of macronuclear structure considers that the macronucleus is compound, i.e., contains many sets of nuclear genes associated into subnuclei with a certain degree of integrity, it appears plausible to postulate differences between mating types as due to some characteristic of the subnuclei. If this were correct, some macronuclei might contain diverse types of subnuclei, and such "mixed" macronuclei could provide a basis for the observed vegetative segregation of mating types.

In an attempt to test this interpretation (Nanney, unpublished), macronuclei which would ordinarily control different mating types were allowed to fuse. This fusion was induced through starving exconjugants (Sonneborn, 1947). Stock 90 in variety 1 was used in this study because in this variety mating types are determined at random at reorganization, giving many cells with diverse sister nuclei, and because selfing is rare in this stock. After macronuclear fusion many clones were found which behaved like the spontaneous selfers in other stocks of the same variety. This production of selfers by the fusion of diverse macronuclei seemed strongly to support the hypothesis of structural inhomogeneity for the spontaneous selfers, but did not exclude other interpretations.

Further information from another source has cast considerable doubt upon this interpretation. The hypothesis offered hope that an analysis of the pattern of segregation of mating types in an unstable clone would be profitable. It seemed reasonable to assume that the probability of origin of a "stable" macronucleus would depend upon the original number of subnuclei, the relative numbers of the different kinds and the number of divisions the macronuclei had undergone. The analysis in *P. aurelia* was, however, complicated by a relatively

short period of vegetative growth before autogamy intervened and replaced the macronuclei being studied. When it was discovered that selfing caryonides occurred in *Tetrahymena pyriformis* and that autogamy did not (Nanney and Caughey, 1953), it seemed that an analysis would be profitable here. Hence, studies were undertaken with this end in view. In the course of these studies (Nanney and Caughey, unpublished) it was found that the mating types in selfing clones can be stabilized readily simply by starving the cells. This was not anticipated on the structural hypothesis, since starvation of macronuclei with *stable* but diverse subnuclei could not result in the establishment of macronuclei with only one type of subnucleus. Hence, the hypothesis is of no value for the selfers in *T. pyriformis*, and since the systems of determination in *P. aurelia* and *T. pyriformis* are so much alike, the hypothesis for *P. aurelia* is severely discredited.

These observations indicate that, while the systems leading to the manifestation of the different mating types are mutually antagonistic, they may under certain circumstances be maintained simultaneously for long periods of growth. The failure of the structural hypothesis suggests that the differences characterizing macronuclei controlling different mating types are to be sought in physiological rather than structural features.

SUMMARY AND CONCLUSIONS

A survey of the patterns of mating type determination and inheritance in *Paramecium aurelia* is presented with an examination of the significance of these patterns in an understanding of cellular heredity. The major conclusions may be summarized as follows:

1. Nuclei containing the same genetic materials may be differentiated in regard to the phenotypes (mating types) that they control.

2. The differentiated nuclei normally breed true in vegetative growth, i.e., the nuclear characteristics are hereditary.

3. An important factor in determining the manner in which the nuclei develop (in the group B varieties) is the kind of cytoplasm in which they develop.

4. The significant cytoplasmic conditions are in their turn determined by the kind of nucleus that previously occupied the cell.

Thus, mating type perpetuation through both vegetative and sexual reproduction is shown to be due to a series of nucleocytoplas-

mic interactions, in which the cytoplasm determines the nature of the macronucleus and is in turn redetermined by that macronucleus. This pattern of nucleocytoplasmic cooperation emphasizes the conclusion that the cytoplasm is not a passive, but an active partner in cellular heredity.

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Mating Substances and the Physiology of Fertilization in Ciliates *

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IN METAZOAN FERTILIZATION the egg and sperm meet, these two cells adhere, the egg is activated, and the gametes fuse. Similarly, protozoan fertilization involves an initial adhesion, a series of physiological and morphological changes, and finally partial or complete cellular fusion. It appears then that no profound physiological difference should exist between the process of fertilization in metazoa and ciliate protozoa and that information regarding fertilization in these two groups of organisms might profitably be considered together. Such an approach seems timely since much of the available information on fertilization in ciliates (Kimball, 1943; Sonneborn, 1947, 1949; Metz, 1948) and in metazoa (Tyler, 1941, 1948, 1949; Runnstrom, 1949; Rostand, 1950; Rothschild, 1951a,b; Chang and Pincus, 1951; Bielig and von Medem, 1949) has been reviewed extensively, but independently, in recent years. In keeping with this view certain problems in the physiology of fertilization are outlined here, the methods employed to solve these are presented, and the degree to which these efforts have succeeded is considered and compared. Since the reviewer's main thesis has developed from studies on *Paramecium*, the physiology of fertilization in this form is discussed in some detail, pertinent studies on other ciliates are then considered, and finally an attempt is made to relate these investigations to metazoan fertilization.

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PROBLEMS AND METHODS

The primary problem in the physiology of fertilization is the problem of the activation initiating mechanism. Associated with this primary event are certain other phenomena which constitute special problems in themselves. Thus: (1) the metazoan gamete or the ciliate must be sexually ripe or reactive before fertilization can occur; (2) some degree of attachment or union of the reacting cells would appear to be essential for their interaction; (3) fertilization is universally characterized by a high order of specificity; (4) an astonishing series of biochemical and morphological changes follows immediately upon activation in most forms; and finally (5) passage of nuclear material from one cell to another presupposes some degree of functional fusion of cells. In passing it may be noted that some of these problems also apply to several other major biological phenomena, notably embryonic induction, host-virus relationships, and specific tissue affinities. Although major interest has centered about the problem of the activation-initiating mechanism, a comprehensive account of fertilization must provide not only for the initiating reaction itself but for these associated phenomena as well. The second, third, and fourth of these phenomena, as well as the activation initiating mechanism, will be considered in this review.

The various morphological, physiological, and biochemical changes that occur at fertilization all follow in a very precise and orderly sequence under favorable circumstances. This has led to the view that most, if not all, of these many changes are interrelated, that they all proceed from a few or even a single event—a chemical reaction between the interacting cells. Considered in this fashion, it would not seem excessively difficult to identify the activation-initiating reaction or reactions. However, this and the associated problems have been studied for over fifty years and many competent investigators have directed their attention toward them, but no comprehensive theory based on substantial experimental data is yet available to describe them. Four general methods have been employed by various investigators in their attempts to reveal the mechanism of fertilization. These are outlined below.

The first of these may be called the biochemical approach. By studying the end effects of activation, particularly the physical and metabolic effects, and by tracing these back to their initial causes,

one might eventually achieve a biochemical description of the activation-initiating mechanism. This biochemical approach began with Warburg's (1908) demonstration of the increase in respiration that follows activation of the sea urchin egg. Although a great deal has been learned about the biochemistry of the unfertilized as compared with the activated egg, and although the approach has even been extended to fertilization in ciliates (Boell and Woodruff, 1941), it is still largely in the descriptive stage. Even in the most thoroughly studied form, the sea urchin, general agreement on metabolic pathways has yet to be reached (Rothschild, 1951a; Cleland and Rothschild, 1952). Evidently, then, it will be some time before this approach to the problem may be expected to yield the desired answers to the fertilization problem.

The second approach to the problem of the activation-initiating mechanism is through the medium of artificial parthenogenesis. Artificial parthenogenesis was first achieved with the silkworm egg (Tichomiroff) in 1886. However, it was not until the turn of the century, with Loeb's (1899) experiments on the sea urchin egg, that this field of investigation showed real promise. During the early part of the century it was anticipated that parthenogenesis would provide the key to the mechanism of action of the sperm in fertilization. However, a very large number and variety of effective physical and chemical agents were soon discovered, and as yet no common factor has been found among them. Parthenogenesis has made the important contribution of demonstrating that the egg contains within itself all essentials for development and has given substance to the stimulus or trigger concept of sperm action, but as yet no comprehensive scheme to explain parthenogenesis or to relate it to sperm activation has been forthcoming.

A third approach to the activation problem involves the investigation of cell extractives and substances liberated spontaneously by gametes or protozoa. This field is an attractive one because the effects obtained are frequently striking and, like the biochemical studies, the results are interesting in their own right whether or not they contribute to an understanding of fertilization. The usual rationale here is to attempt to ascribe a role in fertilization to such substances once they are obtained. The most thoroughgoing application of this approach is to be found in the studies on the sperm and egg isoagglutinins, fertilizin and antifertilizin. But, even after forty years of pain-

staking research, no investigator has presented convincing evidence that these substances are essential for fertilization.

The fourth avenue of attack follows logically from the preceding one and, in fact, represents a more systematic development of it. This approach involves the partial or preferably complete isolation of the activation-initiating mechanism and a study and characterization of its parts. So far this method has not been employed with success on metazoa, in spite of many attempts to activate eggs with sperm extracts or dead sperm. However, this method has met with rather striking success in the ciliate *Paramecium*. Since this approach and the associated preceding one are concerned with various sex substances, a further discussion of these agents will be presented before a comprehensive account of fertilization in *Paramecium* and other ciliates is given.

SEX SUBSTANCES AND MATING SUBSTANCES

Beginning with Frank Lillie's (1913, 1914, 1919) now classic studies on fertilizin in the sea urchin and the annelid worm, *Nereis*, the most fruitful approach to many of the problems of fertilization has been through an analysis of specific interacting substances of sex cells. Indeed, these have produced the only comprehensive, though now outmoded, theory of fertilization, namely, Lillie's fertilizin theory. Such sex substances have been demonstrated or their presence inferred in many organisms, both plant and animal, unicellular and multicellular. In ciliates these substances are produced only when the organisms are in the sexual or mating condition and are capable of fertilization. These agents, the mating substances, have specific action upon ciliates of complementary sex or mating type and presumably perform some function in fertilization. Correspondingly in metazoa, agents may be obtained from eggs and sperm which specifically affect gametes of the species. Again these agents are produced (presumably) by the gametes themselves and are believed to function in fertilization.

In both groups of organisms the more spectacular and more readily studied sex agents are those which appear in the fluid containing the gametes or sexually reactive protozoa. Such water-soluble substances are actively produced and secreted or dissolve passively from the cell or its surface into the fluid. Among ciliates the only well-established example of such diffusible mating substances is in

the hypotrich *Euplotes patella.* In this form Kimball (1942) has shown that the fluid from certain clones will induce animals of certain other clones to conjugate. The most intensively studied agent of this sort from metazoan gametes is the fertilizin obtained from eggs. In its most spectacular form such fertilizin specifically agglutinates the sperm of the species.

Although freely diffusible, water-soluble sex substances of this sort occur in the most diverse animal groups (Protozoa and Vertabrata), they have not been demonstrated universally in either protozoa or metazoa (see Tyler, 1948, concerning metazoa). In the demonstrable absence of such agents, specific sexual reactions, either between protozoa or metazoan gametes, may be attributed again to specific sex substances. However, in such cases the agents must be insoluble in the fluid medium, firmly bound to the cell, or both. It will be seen in the account to follow that sex substances in *Paramecium* are exclusively of this type, and on proper analysis this condition may be expected to be found widely among metazoa and protozoa alike.

FERTILIZATION IN *PARAMECIUM*

Normal Sexual Phenomena

Two sexual processes occur normally in *Paramecium:* conjugation and autogamy. The essentials of these are presented here as background for the analysis to follow. For a more detailed account of normal conjugation and autogamy and a thorough treatment of the literature, the reader should consult Sonneborn's (1947) excellent review.

Conjugation. Conjugation in *Paramecium* involves several types of union between mates and a variety of internal changes in these animals. The first step in conjugation is the initial adhesion. Under suitable conditions this takes the form of the striking mating reaction described first by Sonneborn (1937). Dozens, even hundreds, of animals stick together to form large masses or agglutinates. After some time these mating reaction clumps break down, releasing mating pairs and single animals. Animals in mating pairs are united at first only at the holdfast region (Jennings, 1911; Wichterman, 1940; Hertwig, 1889; Metz, 1947) near their anterior ends (Fig. 1a). This holdfast union is firmer and more intimate than the mating reaction union,

but the mates can still be separated without injury. Subsequently each mate produces a paroral cone (Diller, 1936) more posteriorly (Fig. 1b). The cones of the mates overlap and finally fuse. After paroral cone fusion the mates cannot be separated.

After breakdown of the agglutinates the released animals will not give mating reactions. Clearly they have undergone a physiological change, a loss of mating reactivity. At this time (*P. aurelia*) the first signs of nuclear activity appear. These involve a migration of the micronuclei from their usual position in the vicinity of the macronucleus and an enlargement of the micronuclei in preparation for the first meiotic prophase. The meiotic divisions, the post-meiotic division

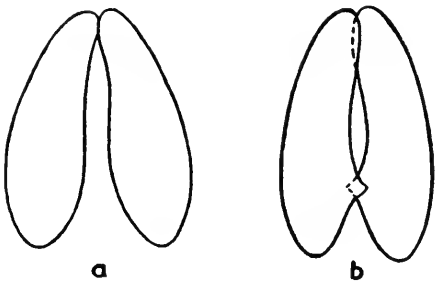

FIG. 1. Types of union in conjugating and pseudo-selfing paramecia.

(a) Holdfast union in early conjugants and pseudo-selfing animals. (b) Holdfast and paroral cone union in more advanced conjugants.

forming the two genetically identical (Sonneborn, 1939a) pronuclei, macronuclear breakdown, and the passage of the migratory pronucleus to the paroral region then follow.

The holdfast and paroral unions, loss of mating reactivity, and meiotic and macronuclear events all follow in a very precise and orderly sequence in conjugation. The obvious facts that these changes are induced in the conjugation process and that they occur in a predictable sequence suggests that these various changes are interrelated and that they may be initiated by a few or even a single reaction in conjugation. In other words, conjugating paramecia are activated in the same sense that the metazoan egg is activated by the fertilizing sperm. Indeed, some manifestations of activation in the metazoan egg and in *Paramecium* are strikingly analogous.

Autogamy. Essentially the same series of physiological and

morphological changes occurs in conjugation and autogamy. The autogamous animal undergoes loss of mating activity, meiosis, macronuclear breakdown, pronuclei formation, and paroral cone formation (Diller, 1936). It appears, then, that the autogamous animal, like the conjugant, is activated and that both undergo the same physiological cycle. However, autogamy differs from conjugation in three important essentials. (1) Autogamy occurs spontaneously, but only under certain physiological conditions, in single isolated animals. No contact or union with a mate is involved. (2) Autogamy results in self-fertilization. The two pronuclei fuse to produce an homozygous syncaryon. (3) Autogamy and conjugation are initiated through different mechanisms (Metz, 1948; Metz and Foley, 1949).

It should be noted that exchange of pronuclei fails to occur in a number of abnormal conjugation processes. The usual genetic result in such instances is identical with natural autogamy, that is, self-fertilization. All such phenomena are sexually induced and are considered to be natural or experimental variants of normal conjugation. They are conveniently termed cytogamy (Wichterman, 1940). They are to be distinguished from natural autogamy, which occurs spontaneously and without sexual contact between animals of opposite mating type.

Cellular Adhesion and Mating-Type Substances in *Paramecium*

The initial phase of the sexual reaction in *Paramecium* is adhesion of potential conjugants through the mating reaction union. There is no evidence for action at a distance, no chemotaxis leading to this reaction. Adhesion occurs only upon random contact. Furthermore, it is not mediated by any agent from the medium. Thus culture filtrates or supernates have no effect upon the mating behavior of *Paramecium* (Sonneborn, 1937, 1939b; Metz, 1947; Kimball, 1943), and repeated washing does not alter their mating reactivity (Metz, 1947 and unpublished). Therefore the initial adhesion, the mating reaction, must result from direct interaction of surfaces. Indeed it may be attributed to the interaction of surface substances. Evidence for such surface substances is derived from several sources. Sonneborn (1937, 1942b, c) has observed that an animal of one mating type can adhere briefly to an animal of the same mating type if it has first clumped

with an animal of opposite type. This suggests the transfer of surface substances from one animal to another at the contact. Studies of the effect of various agents on the mating behavior of *Paramecium* lend further support to this view. Paramecia that have been killed by appropriate treatment with a wide variety of agents will give specific mating reactions with living animals of opposite mating type. Furthermore this reactivity can be blocked by treatment with certain mild agents such as antiserum and protein group reagents. Although no extract with mating substance activity (inhibition of the mating reaction, action on animals of opposite type) has yet been prepared, the evidence presented above and other characteristics of the mating reaction to be outlined below leave no reason to question the view that the initial adhesion depends upon reactions at the molecular level. Therefore the initial adhesion, the mating reaction, can be attributed to an interaction of substances or at least molecular configurations attached to or built into the surface structure of the paramecium. For convenience these are called the *mating-type substances*. The following sections will deal mainly with the role of these mating-type substances in other aspects of fertilization.

Specificity of the Mating-Type Substances
Breeding Systems in *Paramecium*

The initial adhesion, the mating reaction, is the first step in conjugation. If the mating reaction does not occur, the succeeding events are not observed. Conversely, completion of conjugation rarely fails to follow the mating reaction under optimal conditions. In view of this relationship the specificity of the mating reaction is a limiting factor in conjugantion, and, since the mating reaction results from interaction of the mating-type substances, the primary specificity in conjugation will be determined by these mating-type substances. It will be evident from the account to follow that this is a very high order of specificity.

Most species of *Paramecium* have been examined for mating reactions, and breeding systems have been worked out in nearly all of these. Almost from the time of the discovery of mating types in *Paramecium*, two distinct breeding systems were recognized. In one of these the morphological species consists of a number of sexually isolated varieties each of which contains two interbreeding mating types.

Ordinarily the mating reaction and conjugation occur only between the two mating types in a variety. *P. aurelia* (Sonneborn, 1937, 1938a, 1939a, 1942a, 1947), *P. caudatum* (Gilman, 1939, 1941, 1950; Y. T. Chen, 1944; Hiwatashi, 1949a), *P. woodruffi* (Woodruff, cited by Sonneborn, 1939b), and *P. calkinsi* (Sonneborn, 1939b; Wichterman, 1951) follow this type of behavior.

In the second group of animals, sexually isolated varieties are again found, but in this group several mating types are present in certain varieties and any one of these will mate with all others in the variety. *P. bursaria* (Jennings, 1938, 1939a; Jennings and Opitz, 1944; Chen, 1946a), *P. trichium* (Sonneborn, 1938a, 1939b), and *P. multimicronucleatum* (Giese, 1941) show this type of behavior.

Aurelia-Type Systems. Among the aurelia-type species (two mating types per variety) *Paramecium aurelia* has been examined most thoroughly. The fifteen known mating types of this species fall into eight sexually isolated varieties. With the exception of variety 7, which contains one (type XIII), all varieties contain two mating types (Table I). On the basis of experiments on the breeding systems and certain genetic studies, Sonneborn and Dippell (1946a) have separated the several varieties into two distinct groups, the group A and the group B varieties (Table I). In some respects the group A varieties (Sonneborn's odd-numbered varieties 1, 3, 5, 7) are the more interesting, for in this group intervarietal mating can occur in certain combinations of types. Indeed every one of the group A mating types gives at least one such reaction. These intervarietal mating reactions are exceptional. They are never as intense as their intravarietal counterparts, they occur only under unusually favorable conditions, and only four of them (types I x X; II x V; II x IX; II x XIII) ever lead to complete conjugation (Sonneborn and Dippell, 1946a). Sonneborn and Dippell have noted the following interesting relationships in these group A reactions: (1) all seven group A mating types give a unique set of reactions and are therefore different (types I and IX react with different intensities with types II and X); (2) types I, V, IX, and XIII are similar since they all react with type II; likewise types II, VI, and X all react with type XIII; (3) mating reactions and conjugation only occur between odd- and even-numbered types, never between two odd- or two even-numbered types. From these observations Sonneborn and Dippell (1946a) conclude that the several group A mating types consist of two general types, or two series of homologous

TABLE I

BREEDING SYSTEMS IN *Paramecium aurelia*

Group	Variety	Mating type	I	II	V	VI	IX	X	XIII	XV	XVI	IV	V	VII	VIII	XI	XII	General Mating Type
A	1	I	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−
		II		−	+	−	+	−	+	−	−	−	−	−	−	−	−	+
	3	V			−	+	−	−	−	−	+	−	−	−	−	−	−	−
		VI				−	−	−	+	−	−	−	−	−	−	−	−	+
	5	IX					−	+	−	−	−	−	−	−	−	−	−	−
		X						−	+	−	−	−	−	−	−	−	−	+
	7	XIII							−	−	−	−	−	−	−	−	−	−
Intermediate	8	XV								−	+	−	−	−	+	−	−	−
		XVI									−	−	−	+	−	−	−	+
B	2	IV										−	+	−	−	−	−	
		V											−	−	−	−	−	
	4	VII												−	+	−	−	−
		VIII													−	−	−	+
	6	XI														−	+	
		XII															−	

types, which they designate plus (even-numbered) and minus (odd-numbered).

In contrast to the group A varieties the three group B varieties (Sonneborn's even-numbered varieties 2, 4, and 6) show no cross reactions among themselves. However, Sonneborn and Dippell (1946b) have described one race (race 31), which conjugates readily with variety 4 animals of group B and surprisingly also gives a weak mating reaction, but never complete conjugation, with variety 3 of group A. On the basis of this cross reaction and certain genetic properties, Sonneborn and Dippell (1946b) regard race 31 as constituting a distinct variety, variety 8, which links the group A and B varieties. Finally, this series of cross reactions admits the mating types of the intermediate variety 8 and the group B variety 4 to positions in the two series of general mating types of group A (Sonneborn, 1950). Therefore, eleven of the fifteen mating types in *P. aurelia* may be assigned general mating-type designations. Types II, VI, X, XVI, and VIII are plus types; types I, V, IX, XIII, XV, and VII are minus types (Table I).

From the foregoing account it is evident that a very high order of specificity obtains in the mating reaction in *P. aurelia;* an order of specificity that compares very favorably with that of antigen-antibody reactions, fertilizin-antifertilizin reactions, and fertilization in other organisms. Since this specificity is determined by surface substances, the mating-type substances, these must be highly complex substances or molecular configurations.

According to the simplest scheme (Metz, 1948) the mating reaction within a variety in *P. aurelia* should result from interaction of a pair of such substances, *A* and *a*, which are complementary in structure and combine in antigen-antibody-like fashion (Fig. 3a). To include the two general mating-type systems (plus and minus) in this concept it is only necessary to assume that the mating substances in a homologous series are structurally similar but not identical. Thus the plus series should consist of five structurally similar but not identical substances A^1, A^3, A^4, A^5, A^8 (superscripts designate the variety). Correspondingly the minus series of general types should contain six similar but distinct substances a^1, a^3, a^4, a^5, a^7, a^8, which are all complementary in structure to the members of the plus series. In the "lock and key" terminology of immunology, near perfect complementariness obtains only between the two mating types (A^1 and a^1) in a

variety. Less perfect "fits" resulting in weaker linkages and less strong mating reactions should, and in most cases do, occur in intervarietal combinations of plus and minus mating types.

In *P. caudatum* an even larger number of sexually isolated varieties has been reported. Gilman (1950) has described eleven varieties and has tested these against the four Japanese varieties found by Hiwatashi (1949a). Two of the latter failed to conjugate with any of Gilman's cultures (Gilman, 1950) and are therefore distinct varieties. This gives a total of thirteen varieties with twenty-five mating types (only one mating type has so far been found in variety 10). Aside from these Y. T. Chen (1944) has described four Chinese varieties. These have not been tested against the American or Japanese varieties and may include additional varieties.

Although mating generally occurs only between the two types in a variety, Gilman (1949) does report seven intervarietal reactions. One of these links varieties 3 and 6; the remaining six reactions link varieties 2, 8, 9, and 10 in such fashion that two general types appear (the reaction between types XVIII and XX is so irregular that the writer suspects an error). Gilman's (1949) data are summarized in Table II, and the similarity to the situation in *P. aurelia* is apparent. As in the latter species the mating reactions between types within a

TABLE II

INTERVARIETAL MATING REACTIONS IN *Paramecium caudatum*

Varieties which give no intervarietal reactions are not included in this table. Type XIX, variety 10, has not yet been discovered.

Variety		3		6		2		8		9		10		
	Mating Type	V	VI	XI	XII	III	IV	XV	XVI	XVII	XVIII	XIX	XX	General Mating Types
3	V	−	+	−	−	−	−	−	−	−	−		−	
	VI		−	+	−	−	−	−	−	−	−		−	
6	XI			−	+	−	−	−	−	−	−		−	
	XII				−	−	−	−	−	−	−		−	
2	III					−	+	−	+	−	−		−	−
	IV						−	+	−	+	−		−	+
8	XV							−	+	−	−		+	−
	XVI								−	+	−		−	+
9	XVII									−	+		+	−
	XVIII										−		+	+
10	XIX													−
	XX												−	+

variety are most readily explained by assuming interaction of pairs of complementary surface substances and at least in the varieties that are linked by intervarietal reactions, two homologous series of substances may be assumed. Finally, as Sonneborn (1947) points out, De Garis' (1935) report that *P. aurelia* will mate with *P. caudatum* suggests the interesting possibility that the two homologous series of mating types may extend across species lines.

The breeding pattern in *P. calkinsi* does not differ from the systems in *P. aurelia* and *P. caudatum*. Wichterman (1951) finds four isolated varieties in this species. Mating reactions and conjugation occur only between the two complementary types within the varieties. Breeding systems in *P. woodruffi* have not been described in detail.

Bursaria-Type Systems. Immediately after Sonneborn's (1937) discovery of mating types in *P. aurelia*, Jennings (1938) examined *P. bursaria* for similar breeding behavior. This study and those that extended it (Jennings and Opitz, 1944; Chen, 1946a) revealed a very interesting and different system of mating specificity in this form. As mentioned above, sexually isolated varieties occur in *P. bursaria* as they do in other species. However, the number of mating types within a variety is not limited to two. When more than two mating types occur in a variety they interbreed freely. Thus four mating types occur in varieties I, III, and VI. Any one of the mating types within one of these varieties will mate with the other three members of the variety. Similarly, each of the eight mating types in variety II will conjugate with the remaining seven types.

The specificity relations in *P. bursaria* are therefore of an unusual sort. There is a high order of specificity between the varieties, but an apparent lower order of specificity within four of the six varieties. However, if the multiple types are examined in terms of specific interacting substances, a formal explanation for this apparent low-order specificity is evident. The key to the problem lies in the observed number of interacting mating types in a variety. This number conforms to the geometric progression 2^n, which in turn suggests that n pairs of substances are involved in mating-type specificity. According to the proposed scheme two independent pairs of specific, complementary, interacting substances would be required for a four-type variety. Each mating type would possess two mating-type substances, one substance of each independent pair. Assuming a random relation

between the two independent systems, four interacting mating types may be obtained. This hypothesis is presented for variety 1, *P. bursaria* (a four-type variety) in Table III. α designates the reaction between the two complementary substances, *A* and *a*, of one pair (the α pair); similarly β represents reaction between the members, *B* and *b*, of the second pair (β pair) of substances.

TABLE III

PROPOSED EXPLANATION OF MULTIPLE MATING TYPES IN *P. bursaria*, Var. I

Two independent pairs of specifically interacting substances, α and β, are so distributed that each mating type possesses one α substance (either *A* or *a*) and one β substance (either *B* or *b*). Mating occurs when *A* reacts with *a* (α reaction) and/or *B* reacts with *b* (β reaction). α substances (*A*, *a*) cannot react with β substances (*B*, *b*).

Mating[a] Type		A	B	C	D
	Substances Present	*AB*	*Ab*	*aB*	*ab*
A	*AB*	—	β	α	α, β
B	*Ab*		—	α, β	α
C	*aB*			—	β
D	*ab*				—

Note. α, substance *A* reacts with substance *a*; β, substance *B* reacts with substance *b*.
[a] Jennings' alphabetical system for designating mating types in *P. bursaria* is used here.

This concept may be extended to include an eight-type variety (variety II) by postulating three independent pairs of specifically interacting substances. Each mating type should possess three mating-type substances, one from each independent pair. By proper random arrangement eight mating types should result. Any one of these types should react with all the others, but not with itself, through specific interaction between one or more pairs of substances.

Support for this hypothesis is found in the mating behavior of "adolescent" clones (Jennings, 1939a) and the intervarietal reactions reported in *P. bursaria* (Jennings and Opitz, 1944).

Exconjugant clones in *P. bursaria* pass through an "immature" period of weeks or months during which they will not conjugate or give mating reactions. This may be followed by a period of "adolescence" during which the clones mate poorly and in some cases fail

to give the complete or "adult" spectrum of mating reactions. Such clones are reported in variety I, a four-type variety. Here the adolescent clones in question mate at first only with two of the four mating types. Upon reaching maturity they mate with a third type, as well, giving the full spectrum of reactions. As Jennings points out, it is of considerable interest that such clones mate either with types A and B or types C and D. The adolescent clones are therefore type CD or AB, respectively. CD clones mature to C or D, and AB clones mature to A or B. No BC or AD adolescent clones are reported. These observations fit the α-β concept on the assumption that the two systems of substances do not appear simultaneously as the clones reach adolescence. Thus the synthetic mechanism which produces the α system of mating substances may appear at adolescence, whereas the mechanism for β substance formation does not function until later (maturity). During adolescence, then, the clones have one or the other of the α substances (*A* or *a*) and will mate with the two mature types possessing the complementary substance (*a* or *A*) indicated in Table III. Upon reaching maturity, one or the other of the β substances (*B* or *b*) is also produced and the final mating type is established.

Only one series of intervarietal reactions has been reported in *P. bursaria* (Jennings and Opitz, 1944). One of the two mating types in variety IV (type R) cross reacts with four of the eight types (types E, K, L, M) in variety II. Therefore four of the eight mating types in variety II are similar. They may have one mating substance in common which in each cross reaction combines with the same complementary substance of variety IV, type R, animals.

Thus in the irregular "adolescent" and intervarietal reactions the results support the hypothesis to the extent that half of the mating types in a multiple-type variety should have one mating substance in common. Unfortunately, no other data pertaining to this problem are available.

Activation-Initiating Mechanism in *Paramecium*.

Until recently little was known regarding the activation-initiating mechanism of fertilization in *Paramecium*. However, since the systematic development of methods for partially isolating this system (Metz, 1946, 1947) our understanding has increased to the point where more is now known regarding the activation-initiating mecha-

nism in *Paramecium* than in any other organism. Partial isolation of the activating system is achieved by killing and fixing paramecia without destroying their ability to activate living animals. Such dead paramecia may be regarded as a collection of highly specific substances adsorbed to or built into an inert carrier, the bulk of the dead animal. By utilizing this partially isolated, static system, the activation-initiating mechanism has been examined most thoroughly in *P. aurelia* (Metz, 1946, 1947, 1948; Metz and Foley, 1949) and somewhat less extensively in *P. calkinsi* (Metz, 1948; Metz and Butterfield, 1951) and *P. caudatum* (Hiwatashi, 1949b, 1950).

When properly prepared dead paramecia are mixed with reactive living animals of complementary mating type, the living and dead animals promptly adhere and under favorable conditions form large mating reaction agglutinates as in normal conjugation. These mating reaction agglutinates break down after 1 to 2 hours (*P. aurelia*), releasing the living animals. These may be freed as single individuals or as "pseudo selfing" pairs (Fig. 1a) joined only at the holdfast region* (*P. aurelia* and *P. calkinsi;* Metz, 1947, 1948). The released animals (singles as well as pairs) then proceed to undergo meiosis and macronuclear breakdown in normal fashion and according to the time schedule of normal conjugants (Metz, 1947). None of these events is observed in mixtures of living and dead paramecia of the same mating type. This activation of living by dead animals of opposite type has been reported in types 7 and 8, variety IV of *P. aurelia* (Metz, 1947); type II of *P. calkinsi* (Metz, 1948); and types 2, 4, 5, and 8 of *P. caudatum* (Hiwatashi, 1949b). With this brief outline of the newer methods we may now attempt to analyze the activation-initiating mechanism in *Paramecium.*

Activation through sexual processes in *Paramecium* requires contact of potential mates. This follows from the facts that culture fluids, filtrates, and the like have no specific effect upon paramecia of opposite mating type (Sonneborn, 1937, 1939b; Kimball 1943; Metz, 1947); that animals which have been killed, fixed, and repeatedly washed retain their ability to activate living animals; and finally that the ability of dead (or living animals) to activate is directly related to their ability to give mating reactions. Since the process of activation in conjugation requires contact or union of potential conjugants, the

* Hiwatashi (1951a) reports "pseudo selfing" pairs in *P. caudatum* which are united at both the holdfast and the paroral regions.

various manifestations of activation must be initiated by one of the following mechanisms: (a) direct interaction of fixed surface substances, (b) transfer or diffusion of substance(s), (c) a combination of surface interactions and transfer of substances.

As Sonneborn (1949) points out, none of these possibilities has been excluded by direct experiment. Nevertheless, the constitution of the dead animals which are capable of activating living animals and the nature of the reaction between them render the first of these possibilities highly probable. The reaction between living and dead animals is a superficial one to the extent that mates can be separated mechanically at any time during their union. As seen in Table V certain of the effective killing agents are strong fixatives. After treatment with these agents the dead animals must be washed repeatedly to remove the killing agent. In this process of killing and fixation, any substance that is appreciably soluble in water would be removed from the dead animals. Thus, any essential, diffusible agent would necessarily be a rather special, relatively water-insoluble substance. Unfortunately, dead animals that have been thoroughly extracted with lipid solvents have never been tested for their ability to activate living animals. However, it is not unlikely that the activating properties will withstand such treatment, since lyophilized animals (*P. aurelia*) give good mating reactions after extraction with absolute acetone, ether, chloroform, or benzene (Metz and Fusco, 1949). In view of these considerations it appears highly probable that the activation-initiating substances are surface substances and that the essential activating reaction(s) is a reaction between these surface substances. The question then arises as to what surface substances interact to initiate activation. Experimentally, the problem is most readily approached from a consideration of the three types of union that occur in conjugation. These are: (1) the mating reaction union, (2) the holdfast union (Fig. 1a), and (3) the paroral union (Fig. 1b).

Of these several types of union the last is clearly unnecessary for activation, as shown by the following three observations: (1) Paroral union occurs after the holdfast union is formed, mating reactivity is lost, and meiosis has begun. (2) Dead animals which are capable of activating living animals do not possess paroral cones. (3) The third animal in conjugating "threes" (Chen, 1940a, 1946b; Metz, 1947) is usually joined to the primary pair only by its holdfast region.

Nevertheless, this third animal undergoes a complete nuclear cycle (cytogamy).

Holdfast union, the second union in conjugation, is also not an essential preliminary to activation in *Paramecium*. This is evident from the fact that the reaction between dead and living animals, which leads to activation of the latter, does not involve a holdfast attachment. Although this evidence clearly eliminates typical holdfast union as a possibility, it cannot be considered a critical elimination of holdfast substances from participation in the activating reaction. Since the dead animals are derived from normal cultures whose members can form holdfast unions, it is possible that they possess special preformed holdfast substances. These might interact with similar substances on the living animals even though this interaction does not lead to lasting union. The same argument may be applied to paroral substances.

Both holdfast and paroral substances were clearly eliminated as essential factors in the initiation of activation by a study of a mutant stock of *P. aurelia* (Metz and Foley, 1949). Animals of this CM ("can't mate") stock give good mating reaction under appropriate physiological conditions. However, these animals are incapable of proceeding further in the conjugation process. Thus CM animals never form holdfast or paroral unions with CM or normal animals. Furthermore the CM animals do not undergo loss of mating activity, meiosis, or macronuclear breakdown through sexual association with CM or normal animals. In short, CM animals cannot be activated by CM or normal animals of opposite mating type. Nevertheless, CM animals can induce loss of mating activity, holdfast union ("pseudo

TABLE IV

ACTIVATING PROPERTIES OF CM AND NORMAL ANIMALS OF OPPOSITE TYPE (*P. aurelia*, Var. 4)

Living Formalin-killed	Normal Normal	Normal CM	CM Normal	CM CM
Mating reaction	+	+	+	+
Loss of mating reactivity	+	+	−	−
Holdfast union	+	+	−	−
Meiosis	+	+	−	−
Macronuclear breakdown	+	+	−	−

selfing" pair formation), meiosis, and macronuclear breakdown in normal animals. These relations were discovered in mixtures of living and dead animals and are so summarized in Table IV, but they apply equally well to living-living mixtures of animals of opposite type.

Since the CM animals cannot form holdfast or paroral unions, it is reasonable to conclude that the CM animals lack any special holdfast or paroral cone substances. Nevertheless, the CM animals can activate normal animals, and therefore they possess the activation-initiating mechanism. Consequently, interaction of holdfast or paroral substances is not essential for activation. The only other known substances which could interact to initiate the various changes in conjugation, then, are the mating-type substances. Therefore, activation in *Paramecium* must result from interaction of some as yet unknown substances, or from interaction of the mating-type substances. The latter alternative is accepted as the simpler hypothesis.

Interrelation of the Activation Phenomena

The interrelationship of the events which follow the activating reaction have not been investigated as thoroughly as their importance warrants. However, some information is available and more may be expected in the future.

Since the subsequent events in conjugation follow in an orderly sequence from the initial reaction, it is reasonable to suppose that

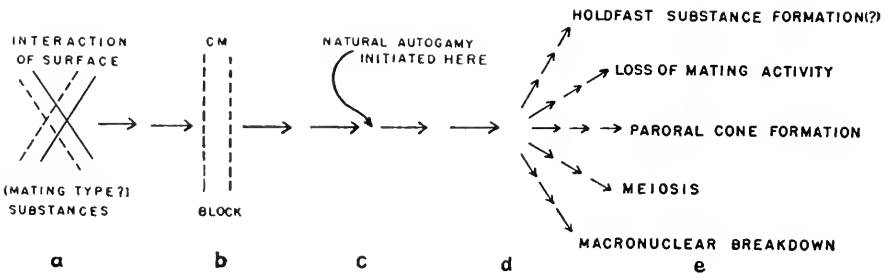

Fig. 2. Scheme for activation in *Paramecium*.

(a) Initiating reaction (mating-type-substance interaction?) in sexually induced activation. (b) CM block, here assumed to lie "internal" to the initiating reaction, (a). (c) Position where chain is activated in natural autogamy. (d) Breakup of main activation chain into side reactions leading to (e) the various end effects of activation.

they are related to this initial event through a predetermined chain of reactions. One form of such a scheme has been presented graphically by Metz (1948), and his figure is reproduced here (Fig. 2). Support for this type of scheme is derived from the study of abnormal or mutant stocks. Of these the CM stock in *P. aurelia* has been examined most thoroughly (Metz, 1948; Metz and Foley, 1949). It will be recalled (Table IV) that the CM animals can activate normal animals but that they cannot themselves be activated by sexual means. Apparently some block, the CM block, prevents activation from proceeding much beyond the initial stages in the CM animals.

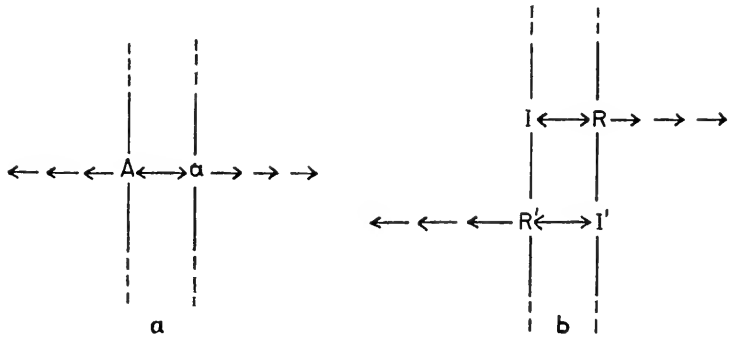

Fig. 3. Two possible activation-initiating mechanisms.

Each series of arrows represents the main activation chain in one conjugant. (a) Simultaneous activation of conjugants by interaction of a single pair of surface substances. (b) Simultaneous activation of conjugants by interaction of two pairs of surface substances.

The CM block is of particular interest because the CM animals regularly undergo natural autogamy. Two important facts may be deduced from this: (1) Activation in conjugation (and its experimental variants) and natural autogamy are initiated through different mechanisms. This will be discussed under parthenogenesis. (2) The activating system "internal" to the CM block is intact. This suggests that the block may be a relatively simple deficiency such as the lack of an enzyme or essential substrate.

At least two possibilities exist for the position of the block. These depend upon the nature of the activation-initiating mechanism. If activation is initiated simultaneously in both mates by interaction of a single pair of substances (Fig. 3a), the CM block must lie internal

to the initiating mechanism. This follows from the fact that CM animals can activate normal animals. A second possibility requires that each conjugant activate its mate by an independent system. Thus, an inducing substance *I* in one animal combining with a reacting substance *R* in its mate might activate the latter. A reciprocal arrangement of similar substances (*R'*, *I'*) would be required to activate the second animal. These relations are represented in Fig. 3b, and it is apparent that the CM block could reside in the activating mechanism if the CM animals lacked *R*. Since there is no evidence that two such independent systems operate in activation, the first alternative view is accepted. Thus the CM block is placed internal to the initiating mechanism in Fig. 2.

Sonneborn (1942b,c) has described another "can't mate" stock in *P. aurelia*. Animals of this type I (variety 1) stock gave good mating reactions with normal type II animals, but they failed to form lasting pairs. Sonneborn describes pairs of type II animals from the mixtures, but his account is not sufficiently detailed to determine if these were pseudo selfing pairs. Sonneborn's (1942b) statement that the clumps "continually break up and reform" suggests that loss of mating reactivity was not induced in the type II animals, but this is difficult to establish with certainty except by a controlled experiment (Metz and Foley, 1949). Unfortunately neither the mutant type I or the normal type II animals from such mixtures were examined cytologically for nuclear evidence of activation.

As Sonneborn (1949) points out, little information is available regarding the relationship between holdfast substance formation, loss of mating reactivity, paroral cone formation, meiosis, and macronuclear breakdown. These may arise independently from a main chain as indicated in Fig. 2, or one or more of them may be sequential to another. The available information regarding each of these will be given in the order listed.

Holdfast Substances. Holdfast union may result from interaction of special holdfast substances which are produced as an early manifestation of activation and which are not mating-type specific (Metz and Foley, 1949). Such holdfast substance formation may branch from the main activation chain between *a* and *c* (Fig. 2) since autogamous animals never form holdfast or other unions. However, the intimate association of animals obtained only in mating reaction agglutinates may be necessary for holdfast union. The action of cer-

tain "killer" fluids may bear upon this question (Chen, 1945; Jacobson, 1948; Preer, 1948). The killer fluids in question induce activation with and without pairing in certain "sensitive" stocks (see further under parthenogenesis). The description (Chen, 1945) of the resulting pairs suggests that pairing involves a holdfast union. Therefore it is possible that, in these exceptional pairs, holdfast substances are produced in response to the activating action of the killer fluids and that the animals unite directly through holdfast substance interaction. The fact that pairs form only after fluid and animals have been mixed for some time favors this view. If this explanation should prove correct, the mating reaction could not be a prerequisite for holdfast unions, and failure to find holdfast unions in autogamy could best be explained by assuming that holdfact substances are not formed in this process. Since CM animals do not form holdfast unions, holdfast substance formation must arise beyond *b* (Fig. 2). Thus the argument presented here suggests that holdfast substance formation arises between *b* and *c*.

Loss of Mating Reactivity. This reactivity occurs in conjugation, its natural and experimental variants, and in natural autogamy. No statement is available regarding loss of mating reactivity in response to "killer fluids" and no case of activation without loss of mating reactivity has been reported. However, mating reactivity can be regained in a remarkably short time after conjugation, as Diller (1942) and others have shown. Loss of mating reactivity does occur in certain abnormal material (*P. bursaria*) which fails to undergo the normal nuclear cycle and in which conjugants separate prematurely (Chen, 1946d). Furthermore, Tartar and Chen (1941) found that living enucleate fragments (*P. bursaria*) lost mating reactivity after clumping for a short time with animals of opposite type. From these observations it appears that loss of mating reactivity can follow directly from the mating reaction and is not dependent upon other activation phenomena. It may be sequential to but not dependent upon holdfast substance formation, since it occurs in natural autogamy. Thus it might arise between *c* and *d* in Fig. 2. Paroral cone formation, macronuclear breakdown, and meiosis could be sequential to loss of mating activity.

Paroral Cone Formation. Observations on paroral cone formation are limited. Hertwig (1889) clearly described these structures in conjugants. They are formed also in pseudo selfing pairs (Metz,

1947), animals activated by "killer" fluids (Chen, 1945), and naturally autogamous animals (Diller, 1936). Paroral cone formation should be distinguished from the process of cone fusion, since the presence of cones does not automatically insure fusion. Actual fusion occurs only in conjugation and possibly certain cases of "pseudo selfing" (Hiwatashi, 1951a). The presence or absence of cones has not been reported specifically in abnormally conjugating material. However, cones presumably form in conjugating amicronucleate animals since pronuclei pass to these from normal mates (Chen, 1940c). A similar situation obtains in "abbreviated" conjugation (Diller, 1949), where micronuclei are exchanged at precocious stages of meiosis. It is clear from these cases that paroral cone formation does not depend upon the presence of a micronucleus or particular meiotic stage.

Macronuclear Breakdown. The macronucleus breaks down in a characteristic way (see Hertwig, 1889; Maupas, 1889; Metz, 1947; Sonneborn, 1947, for figures) in conjugation, "pseudo selfing" (Metz, 1947), natural autogamy (Hertwig, 1914; Diller, 1936), and in "killer" fluid activation (Preer, 1948). Presumably macronuclear breakdown follows the normal pattern in conjugating amicronucleate animals (Sonneborn, 1938b; Chen, 1940c) and therefore is not dependent upon a micronucleus. Macronuclear breakdown followed by some degree of macronuclear regeneration apparently can occur without other obvious manifestations of activation (Diller, 1936). However, such hemixis does not follow the normal breakdown pattern, according to Diller's account, and therefore its relation, if any, to activation is uncertain.

Meiosis. None of the normal non-meiotic events of activation depends upon the micronucleus, since all these apparently occur in conjugating amicronucleate animals. The initiation of meiosis may be sequential to holdfast substance formation and loss of mating reactivity for the reasons given above, but its serial relation to other events has not been determined. In any event, the meiotic process itself is subject to many independent variations. It may consist of nothing more than a swelling of the micronucleus in abnormal stocks (Chen, 1946d), or it may progress in normal or abnormal fashion through one or both divisions in intervarietal crosses between normal stocks of *P. bursaria.* Chen (1946c) suggests that this results from mixture of incompatible cytoplasms. In "abbreviated" conjugation (*P. caudatum*) the meiotic process may be out of phase with other events

(Diller, 1949), and, in *P. calkinsi*, type II, activated by formalin-killed animals, meiosis is frequently arrested in the prophase of the first division in spite of the fact that the macronucleus subsequently breaks down in normal fashion (Metz and Foley, unpublished). Far more spectacular deviations from the normal process have been described by Nanney (1952) in centrifuged *Tetrahymena* conjugants.

In summary form, the evidence presented in this section shows that holdfast substance formation could arise independently from a main activation chain at a point between *b* and *c* in Fig. 2. Loss of mating activity certainly arises beyond *c*. It may arise before *d*. Therefore paroral cone formation, macronuclear breakdown, and meiosis could be sequential to holdfast substance formation and loss of mating reactivity, but there is no evidence for sequential order or dependence among these three phenomena. Clearly none of the other activation phenomena is dependent upon meiosis or a micronucleus since all occur in amicronucleate animals.

Physical Basis for the Mating Reaction

According to the views presented here the mating-type substances perform three major functions in fertilization: (1) They effect the initial adhesion of potential conjugants. (2) They supply the primary specificity in conjugation. (3) Their interaction triggers the entire series of activation changes.

In view of their primary role in fertilization it is now essential to characterize the mating-type substances in more clear-cut physicochemical terms. Specifically, three items of information are needed, namely, the location of the mating-type substances, their chemical nature, and their manner of interaction. These are considered below.

Location of the Mating-Type Substances. All workers agree that the initial adhesion involves the cilia of animals of complementary mating type. Jennings (1939a) and Tartar and Chen (1941) noted that the surfaces of *P. bursaria*, when united in the mating reaction, were separated by a space that approximated the length of one cilium. These observations are readily confirmed by casual observation, but unfortunately there are no detailed descriptions of the mating reaction union in the literature. Therefore, Metz and Pitelka (unpublished) undertook to examine this reaction in *P. calkinsi* with phase contrast optics. In this form the cilia appeared to agglutinate tip to

tip. Not infrequently the tips of several cilia of two mating animals all adhered together to form a tight knot at the point of union. Even single isolated cilia prepared by sonic treatment of reactive formalin-killed animals adhered to living animals of opposite type. Again the point of attachment involved one end of the isolated organelle. When tension was placed upon two joined cilia, they drew apart but remained attached by a fine, apparently elastic, thread which finally broke. This is believed to represent stretching and final breaking of a cilium sheath. If such a break did not occur at the original point of union of the cilia, a piece of the cilium surface membrane would necessarily be transferred from one animal to another. This would readily account for Sonneborn's (1937, 1942b,c) observation that a paramecium of one mating type can clump with another of the same mating type after it has first clumped with one of the opposite type.* Unfortunately, these observations, though quite suggestive, do not exclude other regions of the cilium or even the pellicle from participation in the mating reaction. The animals were necessarily under considerable compression when observed. Furthermore, the behavior of individual mating cilia could be studied only on very loosely united animals or at regions where the pellicles of the mates were relatively far apart. These observations (Metz and Pitelka, unpublished) leave no doubt that the cilium surface possesses mating-type substances, and that further study should be directed toward the structure of these organelles, particularly their tips.

Several workers have examined paramecium cilia with the electron microscope, but none of these studies has revealed any mating-type-specific organization. Jakus and Hall (1946) found that cilia contain a number of fine fibrils, but these workers were unable to detect a membrane or limiting sheath about the cilium. Recent studies, however, leave no doubt that such a structure forms the limiting boundary of the cilium. Such a membrane has been observed in sectioned cilia by Lansing, Hillier, and Sonneborn (unpublished), and its presence has been confirmed (Heilbrunn, 1952; Wichterman, 1953) in material prepared by the CO_2-critical-point method (Ander-

*Sonneborn's observation has been confirmed repeatedly (Metz, unpublished) in mixtures of living and lyophilized (Metz and Fusco, 1949) *P. calkinsi* of opposite type. The living and lyophilized animals clump on mixing, and shortly thereafter numbers of living animals may be seen adhering to one another.

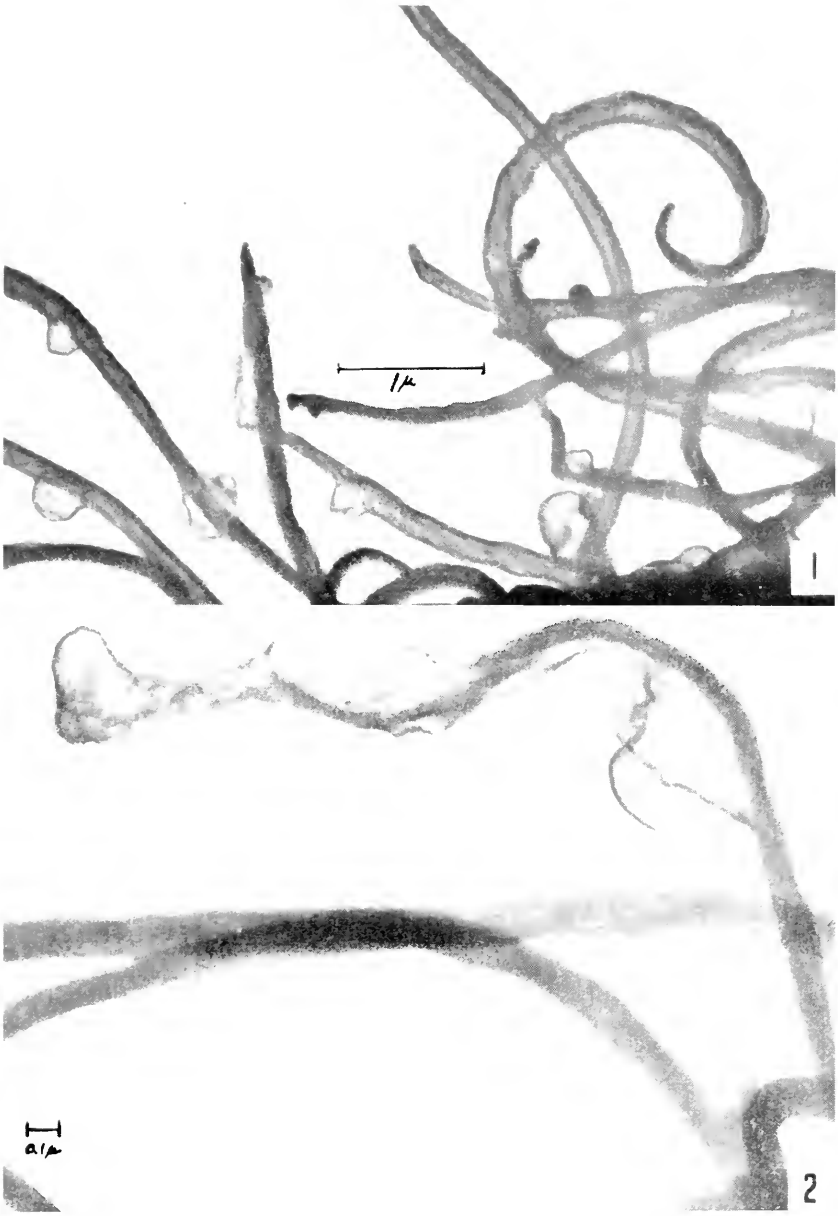

PLATE I. Electron micrographs of *P. calkinsi*, type II, prepared to show the cilium membrane.

Reactive *P. calkinsi*, type II, were killed in 5% formalin, frozen in liquid air, and lyophilized to the collodion membrane. The "blisters" in (1) and the expanded membrane in (2) probably resulted from partial melting of the preparation during lyophilization.

son, 1950) and by Metz and Pitelka (unpublished) in lyophilized preparations of *P. calkinsi* (Plate I). The last study is the only serious visual attempt that has so far been made to detect mating-type differences in paramecium cilia. The material was prepared for electron microscopy in several ways including drying living and formalin-killed paramecia in air and lyophilizing living and formalin-killed animals directly to the collodion membrane. Comparisons were then made among (1) cilia of animals from reactive and unreactive cultures, (2) cilia from reactive animals of the two complementary mating types, and (3) cilia of control animals with others that had been removed from mating reaction agglutinates. These comparisons revealed no consistent differences within the three categories. These observations thus confirm the original supposition, namely, that the mating-type substances constitute part of the molecular pattern of the cilium surface membrane. No evidence was obtained for an extracellular coat or capsule about the cilium. The tips of the cilia likewise showed no specialized organization. However, a sharp reduction in diameter was observed approximately 1 micron from the cilium tip. This narrow terminal part of the cilium is evidently more delicate than the remainder of the organelle, since it was distorted in many of the preparations.

Chemistry of the Mating-Type Substances. A proper chemical study of the mating-type substances should reveal the general chemical nature of these substances. Furthermore it might be expected to demonstrate specific differences between complementary substances. Such differences might account for the specificity of the mating reaction and provide some information about the manner of interaction of these agents.

Ordinarily, characterization of substances of biological origin is achieved by appropriate extraction and purification followed by an identification and description based on physical and chemical properties. A number of different physical and chemical agents have been employed in attempts to extract mating substances from paramecia. They include breaking up the reactive animals by mechanical means (Metz, 1946; Metz and Butterfield, 1950; Hiwatashi, 1950), by freeze-thawing (Metz, 1946), irradiation with x-rays (Wichterman, 1948), heating (Metz, 1946), acid and alkaline extraction (Metz, 1946), digestion with enzymes (Metz and Butterfield, 1950), extraction with salt solutions (Metz, 1946), detergents (Metz, unpub-

lished), and a variety of organic solvents (Metz, 1946). In spite of these efforts no extract has yet been obtained which inhibits the mating reactivity or otherwise specifically affects animals of complementary mating type.

Since all attempts at extraction have uniformly met with failure, other methods for characterizing the mating-type substances are required. The most fruitful of these has been a study of the effect of various agents on the mating reactivity of intact paramecia. Of the agents employed (see Table V), enzymes and "specific group reagents" have provided the most positive information regarding the nature of the mating substances.

Evidently the mating-type substances (*P. calkinsi*) are either proteins or substances closely associated with protein, since mating substance activity is destroyed by proteolytic enzymes (Metz and Butterfield, 1951).

If mating substances are proteins, amino acid residues might well be essential for their activity. To test this possibility certain "protein group reagents" were examined for their effect on mating substance activity. These agents, the conditions under which they were employed, and their effects are given in Table V and discussed below. The various limitations in this type of analysis may be found in several recent works (Glick, 1949; Danielli, 1949, 1953; Barron, 1951; Herriott, 1947; Olcott and Fraenkel-Conrat, 1947) and numerous short papers.

Oxidizing and reducing agents do not affect mating reactivity. Likewise, rather drastic treatment with mercuric ion and iodoacetate treatment sufficient to render paramecia nitroprusside negative failed to destroy mating reactivity. Therefore sulfhydryl and disulfide groups are not essential constituents of the mating-type substances (*P. calkinsi*). All the amino and phenolic group reagents tested inactivated *Paramecium* when used under the mild conditions generally employed in protein group studies. Therefore it is probable that both of these groups are "essential groups" in the mating-type substances (*P. calkinsi*). Thus nitrous acid, dinitrofluorobenzene, and diazonium compounds react with both amino and phenolic groups. Iodine reacts by substitution in phenolic groups (the inactivating action of iodine cannot result from oxidation of sulfhydryl groups since these are not essential), and benzoyl chloride and formalin combine with amino groups. It should be noted that formalin-killed, reactive animals (*P.*

TABLE V

EFFECT OF VARIOUS AGENTS ON THE MATING REACTIVITY OF *Paramecium*

Agent	Organism	Preliminary* Treatment	Effect and Conditions of Experiment	Reference
Temperature	*P. aurelia*, Var. 4	L	*Not reactive*, 52°C and above, 5 min	Metz, 1946
			Reactive, from freezing (solid CO_2-acetone) to 52°C	Metz and Fusco, 1949
	P. calkinsi	L, F	Similar to *P. aurelia*	Metz, unpublished
				Metz and Fusco, 1949
	P. caudatum	F	*Not reactive*, 50°C, 5 min	Hiwatashi, 1950
pH	*P. aurelia*, Var. 4	L	*Reactive*, only from pH 3.6 to pH 9.8	Metz, 1946
	P. calkinsi	L, F	Similar to *P. aurelia*	Metz, unpublished
	P. caudatum	F	*Reactive*, only from pH 4.8 to pH 9.5	Hiwatashi, 1950
X-rays	*P. aurelia*, Var. 4	F	*Reactive* up to 500,000 to 1,000,000 r	Metz, unpublished
	P. bursaria	L	*Reactive* up to 500,000 r	Wichterman, 1948
UVL	*P. aurelia*, Var. 4	F	*Not reactive*, after irradiation with Sterilamp, no detailed study	Metz, unpublished
Enzymes				
Trypsin	*P. calkinsi*	F, P	*Not reactive*	Metz and Butterfield, 1951
Chymotrypsin	" "	" "	" "	
Hyaluronidase	" "	F	*Reactive*	
Lysozyme	" "	F, P	"	
Ribonuclease	" "	" "	"	
Ptyalin	" "	" "	"	
Lecithinase	" "	F	"	

TABLE V (Cont.)

Agent	Organism	Preliminary Treatment	Effect and Conditions of Experiment	Reference
Sulfhydryl agents				
Iodoacetate	*P. calkinsi*	F, P	*Reactive*, 0.17 *M*, pH 7.5, animals nitroprusside negative	Metz, unpublished
H_2O_2	" "	F	*Reactive*, 3%; at higher conc. animals become hydrophobic and cannot be tested	" "
$HgCl_2$	" "	L	*Reactive*, 0.02 *M* (1/12 saturated), higher conc. not tested	" "
Disulfide agents				
Cysteine	*P. calkinsi* I	F	*Reactive*, 0.25 *M*, pH 6.3, higher conc. not tested	" "
Thioglycolate	" " "	"	*Reactive*, 0.26 *M*, pH 8, higher conc. not tested	" "
Na_2SO_3	" " "	"	*Reactive*, 0.5 *M*, pH 8, 1 hr, 25°C	" "
Amino and phenolic agents				
Formalin	*P. calkinsi* I	L	*Strongly reactive*, 5% formalin, amino groups free *Not reactive*, 12% and above, 30 min to 2 hr, 25°C	Metz and Butterfield, 1950 and unpublished
	P. calkinsi II	L	*Weakly reactive* at best, 5%, 30 min to 2 hr, 25°C	Metz and Butterfield, 1950 and unpublished
	P. aurelia Var. 4	L	*Strongly reactive*, 2% *Not reactive*, 12% and above, 30 min to 2 hr, 25°C	Metz, 1947

TABLE V (Cont.)

Agent	Organism	Preliminary Treatment	Effect and Conditions of Experiment	Reference
	P. caudatum	L	*Reactive*, 1.5% to 6%	Hiwatashi, 1949b
	Types 1, 3, 6, 7		*Not reactive*, below 1.5% or above 6%	" "
	Types 2, 4, 5, 8		*Not reactive*, all formalin concentrations	" "
Benzoyl Cl	*P. calkinsi* I	F	*Not reactive*, excess of reagents, pH 4.5, 10 min, 25°C	Metz, unpublished
Dinitrofluorobenzene	" " "	F	*Not reactive*, excess of reagent, pH 4.2, 3 hr, 40°C	" "
Diazosulfanilic acid	" " "	"	*Not reactive*, excess of reagent, pH 8.0, 5 to 30 min, 25°C	" "
	P. calkinsi II	F, P $HgCl_2$	*Not reactive*, excess of reagent, pH 8.0, 5 to 30 min, 25°C	
Iodine	*P. calkinsi* I	F	*Not reactive*, above 0.006 *M*, pH 8, 1 hr, 25°C	" "
	P. calkinsi II	F	*Not reactive*, above 0.0004 M, pH 8, 1 hr, 25°C	" "
Nitrous acid	*P. calkinsi* I	F, P	*Reactive*, 0°C, 1 *M*, pH 4, 30 min to 2 hr *Not reactive*, 25°C	" "
	P. calkinsi II	F, P $HgCl_2$	*Not reactive*, 0°C *Not reactive*, 25°C } 1 *M*, pH 4, 30 min to 2 hr	" "

TABLE V (Cont.)

Agent	Organism	Preliminary Treatment	Effect and Conditions of Experiment	Reference
Periodate	*P. calkinsi* I	F, $HgCl_2$	*Reactive*, 3% periodic acid, pH 5, 5 min, 25°C; animals positive to Schiff's reagent	Metz, unpublished
Organic solvents				
Absolute ether acetone, benzene, chloroform	*P. calkinsi*, *P. aurelia*	D	*Reactive*, 30 min, 25°C	Metz and Fusco, 1949
Ethyl alcohol	*P. calkinsi*, *P. aurelia*	D	*Not reactive*, 30 min, 25°C	" " " "
Glycerine	*P. caudatum* III	L	*Reactive*, 50% glycerin	Hiwatashi, 1950

Note: For methods used in unpublished experiments, see text and Metz and Butterfield, 1950, 1951.

* F = reactive formalin-killed animals used in the experiment.
P = reactive picric-acid-killed animals used in the experiment.
$HgCl_2$ = reactive $HgCl_2$-killed animals used in the experiment.
D = reactive lyophilized animals used in the experiment.
L = reactive living animals used in the experiment.

calkinsi I) stain deeply (Metz, unpublished) with azo-carmine G (Monné and Slautterback, 1951), and up to 10 per cent of the Kjeldahl nitrogen may appear as free amino nitrogen (standard formol titration) in such animals, a value which compares favorably with that for alcohol-killed control animals (Metz, unpublished). Evidently, then, the amino groups of formalin-killed, reactive animals are not bound to any appreciable extent.

Possible action of periodate on β-hydroxy amino acid residues (Edsall, 1942) has not been investigated, but apparently hydroxyl groups of carbohydrates are not essential (*P. calkinsi* I), for animals retain reactivity at periodate concentrations which render them strongly positive to Schiff's reagent.

The failure of several organic solvents to remove or destroy mating substance activity (Table V; Metz and Fusco, 1949) would seem to eliminate loosely bound fat-soluble substances as essential constituents. However, Chargaff's (1944) report that alcohol splits lipoprotein, whereas various other solvents do not, is of some interest, for this agent destroys mating reactivity even at low concentrations (Metz, 1946; Metz and Fusco, 1949; Hiwatashi, 1950).

Although a large number of agents have been examined for inactivating action on the mating-type substances of *Paramecium*, only two of these have shown clear-cut differential action on complementary mating types. These agents are formalin and nitrous acid. Formalin-killed type II *P. calkinsi* never give strong mating reactions regardless of the formalin concentration employed for killing. However, type I formalin-killed animals give intense reactions. On the other hand, type II animals killed with $HgCl_2$, picric acid or by lyophilization mate as intensively as similarly treated type I animals (Table V; Metz and Butterfield, 1950). The differential action of formalin is even more striking in *P. caudatum*, for Hiwatashi (1949b) reports that one of the two mating types in each of four different varieties is completely unreactive after formalin killing, whereas the complementary type in each variety gives strong reactions after formalin treatment. This action of formalin indicates two series of homologous mating types in *P. caudatum* and suggests the interesting possibility that these two series correspond to the two general mating types established by intervarietal matings (Table II). Unfortunately the data of Gilman and Hiwatashi are insufficient to establish this relationship. Finally, it should be recalled that formalin has no dif-

ferential action on the two mating types of variety 4, *P. aurelia* (Metz, 1947).

A differential action of nitrous acid was found in *P. calkinsi* (Table V). Type I animals mate after treatment with nitrous acid at 0°C, whereas type II animals fail to do so. The mating reactivity of both is destroyed by nitrous acid at 25°C. It is tempting to ascribe the differential action of formalin and nitrous acid to inactivation of amino groups. However, the action of both these agents is complex (see French and Edsall, 1945; Olcott and Fraenkel-Conrat, 1947), and no such conclusion is warranted until this very interesting relationship has been subjected to a more thorough biochemical analysis.

From the chemical studies reviewed in this section it is evident that mating-type-substance activity is dependent upon protein integrity. Among amino acid residues sulfhydryl and disulfide groups are not essential, but free alpha amino groups or terminal groups of basic amino acids, phenolic groups, and perhaps other amino acid residues appear to be essential.

Immunological Studies on the Mating Reaction. Since the mating-type substances of *Paramecium* cannot be extracted, an immunological analysis of these substances *in situ* might be expected to yield valuable information regarding their nature. In some experiments antisera affect complementary mating types differentially (Bernheimer and Harrison, 1941; Hiwatashi, 1951b); in others the two types behave alike (Bernheimer and Harrison, 1941; Sonneborn, personal communication). Such variable results are not surprising since the experiments were based on the immobilization of living animals, and the presence of a given immobilizing antigen is known to depend upon both the genotype and the environment (Sonneborn, 1950; Beale, 1951), at least in *P. aurelia*. In view of the absence of mating-type specificity in these studies and the ambiguity of the immobilization test in this regard, Metz and Fusco (1948) designed a direct test for anti-mating substance antibodies in immune sera. *P. aurelia* was used in the original study, and the results have been confirmed on a less extensive scale with *P. calkinsi*. Reactive formalin-killed animals were treated with antiserum for a substantial period (30 minutes to 2 hours) and then washed free of the excess antiserum and tested for mating reactivity with living reactive animals of the same and complementary types. In some tests the antiserum-treated animals failed to mate. This clearly indicates that the mating-type

substances can be blocked by antiserum. However, this action of antiserum was not mating-type specific. In fact, several lines of evidence suggest that the reactive groups of the mating-type substances do not combine directly with the inhibiting antibody. Thus, inhibiting antisera can be prepared from both reactive and unreactive paramecia; both can absorb out the inhibiting antibody; antisera which inhibit the reactivity of one race and mating type fail to inhibit animals of this same mating type but of another race; finally, animals of a single race and mating type may gain or lose the ability to give the blocking effect. Granting a chemical constancy of mating substances within a type, these results must mean that the inhibiting antibodies react with antigens other than the mating-type substances. Therefore it is concluded (Metz and Fusco, 1948) that the inhibiting antibody combines with some "neighboring" antigen and prevents intimate contact in the mating reaction by a mechanical masking or steric hindrance of the mating-type substances.

No direct attempt has yet been made to relate the mating-reaction-inhibiting antigens to the immobilizing antigens of Sonneborn, but in some cases, at least, the two appear to differ. Thus cultures were obtained by selection (*P. aurelia*) in which the mating reaction was inhibited by a given antiserum but was not immobilized by the same serum.

It is evident from this study that the mating-type substances are associated with antigenic material, but until specific anti-mating substance antibodies are obtained, this approach is not likely to be rewarding. Perhaps such antibodies can be prepared by immunizing animals other than the rabbit.

Parthenogenesis

The eggs of many metazoa can develop without the mediation of a spermatozoon. The developmental machinery may be set in motion spontaneously (natural parthenogenesis) or through the experimental application of various physical or chemical agents (artificial parthenogenesis). In view of the many other parallels that exist between fertilization in *Paramecium* and metazoa, it is not surprising that phenomena comparable to natural and artificial parthenogenesis also occur in *Paramecium*.

The protozoan counterpart of natural parthenogenesis is natural

autogamy.* This process occurs frequently and regularly only in *P. polycaryum* and *P. aurelia*, and it has been studied intensively only in the latter. The conditions which induce autogamy in *P. aurelia* are identical with those which bring animals into mating condition, namely, mild starvation. However, autogamy can be induced only after a certain minimum number of fissions following the previous autogamy or conjugation. This suggests that autogamy functions in a "life cycle" in *P. aurelia*, and this is borne out by Sonneborn's (1938c) demonstration that, when autogamy is prevented, the clone eventually dies.

Conjugating and autogamous animals undergo nearly identical physiological and morphological changes. In both processes the animals lose mating reactivity, form paroral cones, and undergo macronuclear breakdown and meiosis in identical order. This indicates that the same chain of reactions is operative in conjugation and autogamy (Fig. 2). However, the chain of reactions is set in motion by different mechanisms in the two types of behavior. In conjugation, initiation of activation requires interaction of surface substances on mating animals; in autogamy, no mate is present. Of greater significance is the fact that different routes or receptors are clearly operative in conjugation and autogamy. This is evident from the behavior of the mutant CM stock of *P. aurelia* (Metz, 1948; Metz and Foley, 1949). These CM animals possess the activation-initiating mechanism, at least so far as their ability to activate normal mates is concerned, yet they cannot themselves be activated by sexual means (Table IV). Evidently some block, the CM block (Fig. 2b), prevents sexually induced activation from proceeding beyond initial stages in CM animals. Nevertheless, these CM animals regularly undergo natural

* Some may object to this use of the term parthenogenesis since self-fertilization occurs in autogamy. This distinction between parthenogenesis and autogamy has considerable genetic significance but no physiological importance. The behavior of pronuclei at the critical fertilization stages is probably controlled by the cytoplasm (see Nanney, 1952) and follows a definite pattern. Whether or not this pattern includes pronuclear fusion depends upon the number of pronuclei present. Thus, when amicronucleate and normal animals conjugate, two haploid exconjugants are produced. These develop by parthenogenesis in the genetic sense, for no zygote nucleus has been formed. Yet the animals have been activated and have passed through all the other fertilization changes (Chen, 1940b,c). For present purposes this is considered a variant of the normal sexual process. As used here, parthogenesis means non-sexual activation followed by a normal nuclear cycle with or without pronuclear fusion.

autogamy. Therefore, the main reaction chain is activated beyond the CM block in natural autogamy through a mechanism and receptor other than the normal sexual one. These relations are presented graphically in Fig. 2.

No systematic effort has been made to induce activation in paramecia by physical or chemical agents under conditions that would exclude natural autogamy. However, one group of agents is known to activate under such conditions. These agents may therefore be regarded as artificial parthenogenetic agents for *Paramecium.* These are culture fluids from certain "killer" stocks of *Paramecium.* The effect has been reported in *P. bursaria* (Chen, 1945) and in *P. aurelia* (Preer, 1948; Jacobson, 1948). Induction of paroral cone formation (Chen, 1945), normal type macronuclear breakdown (Preer, 1948; Jacobson, 1948), and probably meiosis (Chen, 1945) with or without pairing are reported. When pairs are formed, they appear several hours after addition of the "killer" fluid. These are not normally conjugating animals (see under subsequent activation phenomena). They may be united by the holdfast region. An interesting relationship exists between the animals producing the fluids and those which give the parthenogenetic response. In the effective combinations the fluid and animals are of different varieties. In *P. bursaria,* fluid from certain variety 5 animals activated animals of two mating types in varieties 2 and 3 and of one mating type each in varieties 4 and 6. Chen (1945) states that certain stocks in these varieties failed to respond, but he gives no details. In *P. aurelia* the relationship is highly stock specific. Killer stock G, variety 2 animals activate stock P variety 1, type I sensitive animals. Fluids from stock G animals of both mating types (III, IV) are effective. It is clear from these observations that the parthenogenetic effect is unrelated to mating type. The parthenogenetic agent in such fluids is probably paramecin, since the effect is produced by killer lines but not sensitive lines of stock G. Why this agent activates certain sensitive stocks and not others is unknown, and its mode of action has been investigated to a limited extent only. Paramecin-treated animals undergo macronuclear breakdown several hours later than conjugating controls mated simultaneously. Evidently the action of paramecin is delayed in some way. Jacobson (1948) suggests that the site of paramecin action is internal and that this delay represents time required for the paramecin to penetrate to this site. If this interpretation is correct, it means that

paramecin initiates activation through some other receptor than the one operative in sexually induced activation. A thorough study of the parthenogenetic action of paramecins is greatly to be desired, and a systematic search for other agents with similar action should be made. Since paramecin is a desoxyribonucleoprotein (van Wagtendonk, 1948), other substances of this nature might well be investigated. A study of the effect of parthenogenetic agents on blocked stocks such as the CM stock should be particularly rewarding.

As Metz and Foley (1949) suggest, these studies on parthenogenesis in *Paramecium* have certain interesting implications for parthenogenesis in metazoa. The CM study shows that natural parthenogenesis (natural autogamy) in *Paramecium* and sexually induced activation are initiated through different routes. This situation may obtain in other forms, such as hymenopterous insects where the egg can develop either parthenogenetically or by sperm activation. Likewise artificial parthenogenetic agents need not necessarily act through the same receptor as the sperm (Metz and Foley, 1949; Runnstrom, 1949). In fact, different parthenogenetic agents may act specifically at different points in an activation chain.

FERTILIZATION IN OTHER CILIATES

Breeding systems containing clear-cut mating types have been described in detail in only one form other than *Paramecium*, namely, *Euplotes patella*. Mating types also occur in *Leucophrys patula*, *Onychodromus grandis*, *Stylonichia pustulata*, and *Loxophyllum fasciola* according to Jennings' (1939b) interpretation of Maupas' (1889) observations. Recently mating types have been reported briefly in *Tetrahymena* (Elliott and Nanney, 1952; Elliott and Gruchy, 1952), *Stylonichia putrina* (Downs, 1952) and *Euplotes harpa* (Katashima, 1952).

Mating types were first reported in *Euplotes patella* by Kimball (1939). Subsequently Kimball (1941, 1942) and Powers (1943) described the breeding system and inheritance of mating type in greater detail. Kimball (1943) and Sonneborn (1947) have both reviewed these studies extensively. Therefore, this discussion will be confined primarily to a comparison of the physiology of fertilization in *Paramecium* and *Euplotes*.

Kimball (1943) reports two (possibly three) non-interbreeding

varieties in *Euplotes patella* and two more sexually isolated varieties in a morphologically distinct but similar form. Mating types occur within all these varieties, but only one of the *E. patella* varieties has been studied in detail. In this variety six "mating types" are reported. When animals (with some culture fluid) of one type are mixed with animals (and fluid) of any one of the other five types, conjugation occurs in the mixture. The course of events in such mixtures differs in three ways from what has been described for *Paramecium:* (1) no sex reaction of any kind occurs for at least 90 minutes (Kimball, 1939); (2) mass mating reactions never occur; and (3) animal-free fluids induce conjugation (selfing) in at least some of the other types. The later events in conjugating *Euplotes* approximate the usual ciliate pattern (Turner, 1930; Kimball, 1941; Katashima, 1952) and need not be considered further, but the three points mentioned above depart radically from the scheme devised for *Paramecium* and warrant serious treatment.

Since the action of culture fluids is the striking feature in *Euplotes*, this subject will receive primary consideration. Three conjugation-inducing agents are found among the six mating types. Animals may produce one or two of these, depending upon their genetic constitution (Kimball, 1942). The production of the substances depends upon three allelic genes, one allele for each substance. There are, then, six kinds of animals (the six mating types), three which produce one kind of substance (homozygous animals) and three

TABLE VI

CONJUGATION-INDUCING ACTION OF ANIMAL-FREE CULTURE FLUIDS IN *Euplotes patella*

Conjugation ("selfing") is indicated by +; failure to observe conjugation by −.

Animal-Free Fluids		Animals					
Fluid Agent	Mating Type	IV	VI	III	I	II	V
1	IV	−	+	+	−	−	+
2	VI	+	−	+	−	+	−
3	III	+	+	−	+	−	−
1, 2	I	+	+	+	−	+	+
1, 3	II	+	+	+	+	−	+
2, 3	V	+	+	+	+	+	−

which produce two of the three substances (heterozygous animals). A particular fluid agent induces conjugation only in those animals which do not produce the same agent. These relations are summarized in Table VI. In view of this action of fluids the constitution of conjugating pairs in mixtures of mating types is subject to uncertainty. Pairs may be composed of animals of the same mating type (induced by fluid of the other type), or of different type. As Sonneborn (1947) points out, this is an important distinction which Kimball (1942) and Powers (1943) have not made in all combinations of mating types.

In a searching analysis of Kimball's and Powers' data Sonneborn (1947) concludes that true interbreeding between animals of different mating type occurs only in combinations of mating types in which "each one induces the other to self, that is, when each produces a conjugation-inducing substance that the other one does not produce" (Sonneborn, 1947).* Sonneborn marshals considerable support for this view and finds only one exception which may not be serious in view of other exceptional conjugations reported by Kimball and Powers.

Kimball (1943) discusses the mechanism of action of the conjugation-inducing fluids in *Euplotes* and concludes that these may function as agglutinins or that they may induce the formation of other adhesive agents. The former possibility seems unlikely since the action of fluids is not immediate and since it does not readily account for conjugation between animals of different mating types. Therefore Kimball's second proposal is developed here in accord with the newer knowledge of fertilization in *Paramecium*.

If one overlooks exceptional cases of "unexplained" conjugation and intraclonal selfing in the data of Kimball and Powers, it develops that conjugation occurs only in situations were fluids could induce the conjugation. This is implicit in Sonneborn's (1947) scheme. It seems likely, therefore, that *all* conjugation in *Euplotes* is mediated by a fluid agent whether the mates are of the same or different types. In other words, two animals cannot conjugate unless each has been

* According to Sonneborn's scheme animals of any one "mating type" will actually conjugate (hybridize) with animals of only three of the other five "types." All conjugation in mixtures with the remaining two types is selfing. Thus the term mating type has a rather special meaning in *Euplotes*. Two clones are of different type if conjugation occurs in the mixture, even if the conjugation is only selfing among animals of the same clone.

exposed independently to the action of a fluid agent which it does not itself produce. Possibly a particular conjugation-inducing agent activates animals which do not produce this agent. The substances involved in the initial adhesion might well be formed as a result of such activation. If these adhesive substances acted in non-specific fashion, any two activated animals, regardless of mating type, should adhere on contact and might then proceed to conjugate. The effect of the fluid agents on *Euplotes* would then be roughly analogous to the activating action of mating substance interaction in *Paramecium.* Initial union (by ventral cirri, Turner, 1941) of *Euplotes* would then correspond to holdfast union in *Paramecium.* Viewed in another way, the conjugation-indulging agents in *Euplotes* are analogous in action to the killer fluids which induce pairing and nuclear reorganizations in *Paramecium* (see under parthenogenesis in *Paramecium*).

This view accounts for the failure to observe an immediate reaction when fluids and animals or complementary types of *Euplotes* are mixed. No effect is observed for at least 90 minutes. According to the scheme this is the time required for synthesis of the "non-specific adhesive substances." No mass mating reactions occur in mixtures of *Euplotes* mating types, and this also might be expected since large numbers of animals would not necessarily be in the adhesive stage simultaneously. In any event, mass agglutination of *Euplotes* presents mechanical difficulties for the adhesive organelles (ventral cirri) are present only on one side of the animal. The importance of this factor may be seen in Kimball's (1941) observation that double animals do form small groups or chains.

It might be expected from this view that fluids would induce other activation phenomena in responsive *Euplotes* in the absence of conjugation. Such action has not been reported. In fact Kimball (1941) describes exconjugants (probably animals from pairs split in early conjugation stages) which failed to show any nuclear changes. Similarly Katashima (1952) separated conjugating *E. harpa* by killing one pair member and then studied the nuclear behavior of the surviving member. When the operation was performed after the "preliminary micronuclear division" (2 to 5 hours after beginning of conjugation) but before the first meiotic division, the nuclear composition of the surviving isolate promptly returned to the vegetative condition. When the operation was performed at later stages, the micronuclei sometimes completed the nuclear cycle (cytogamy). Clearly, then,

intimate union of conjugants is required for micronuclear changes in *E. harpa*. Nevertheless, some physiological change does occur in *E. harpa* even before permanent union is established, for pairs separated at this early stage did not reunite or conjugate with other animals. Whether this is a delayed effect of a fluid agent or a result of initial union remains to be determined. Indeed, fluid agents have yet to be demonstrated in *E. harpa*.

The possible relationship between conjugation-inducing agents from fluids and the responsive animals presented above can be formalized in terms of specific interacting substances. As Kimball (1943) is aware, this relationship forms a system resembling the blood groups in man. However, further speculation on *Euplotes* is pointless because Kimball's stocks appear to have been lost and there is little prospect of further investigation in the immediate future.

Downs (1952) has described five mating types in another hypotrich, *Stylonichia putrina*. These five types constitute a variety since conjugation occurs when any two of them are mixed. Apparently Downs has not yet tested animal-free fluids for conjugation-inducing action. Therefore any comparison with the related *Euplotes* would be premature.

CONCLUSIONS REGARDING METAZOAN FERTILIZATION

Thirty years ago the major problems in metazoan fertilization seemed on the threshold of solution, for Glaser (1921) confidently wrote "whatever transformations our views on the initiation of development may undergo within the next few years, the zone within which we seek for understanding is now marked off by the reaction capacities of perfectly definite physiological compounds." Unfortunately the present outlook is not so optimistic, for the factors responsible for initial adhesion, specificity, activation, and most other aspects of sperm-egg interaction remain obscure.

Of the various "sex substances" that have been obtained from metazoan gametes only the egg membrane lysins from sperm have well-understood action in fertilization (for recent discussions see Krauss, 1950; Berg, 1950; Swyer, 1951.) These egg membrane lysins remove mechanical barriers that would otherwise block the approach of the fertilizing sperm. In certain cases at least, these agents supply a specificity factor in fertilization.

Among other agents the sperm and egg isoagglutinins, fertilizin and antifertilizin (obtained from eggs and sperm respectively), have been studied most extensively. The highly specific nature of their interaction and the dramatic character of the agglutinating action suggest that these substances are essential factors in metazoan fertilization. This may prove to be the case, but as yet substantial experimental evidence to support this view is wanting. Initial adhesion of gametes may involve a fertilizin-antifertilizin reaction (Tyler, 1948), and a modest case may be made for a role in specificity determination for these agents, but again experimental support for these possibilities is lacking. A clear demonstration of the function or functions of these extraordinary substances is much needed and may well prove to be the turning point in the analysis of fertilization in metazoa. Unfortunately, we have no more positive information concerning the role of specific substances in the attachment of sperm to egg, specificity determination, and activation initiation in metazoan fertilization. For detailed accounts of the present status of these problems, the reader should consult the reviews listed on page 284.

For *Paramecium* our understanding of the mechanism of fertilization has progressed to a somewhat more satisfactory position. The analysis of fertilization in *Paramecium* clearly demonstrates the primary role of the cell surface in fertilization. It appears that initial adhesion, fertilization specificity, and initiation of activation depend upon the interaction of complementary surface substances, the mating-type substances. No diffusible agents of any kind appear to operate. *Paramecium* then exemplifies the classical concept of a "trigger reaction" in fertilization. As many workers have pointed out, a similar interaction of surface substances may function in metazoan fertilization. It is certainly clear from the many observations on normal fertilization in the sea urchin (see Dan, 1950, for a recent account), *Nereis* (Lillie, 1911, 1912) and the starfish (Fol, 1879; Chambers, 1930; Hörstadius, 1939) that activation of the egg results from a rather superficial sperm-egg contact. Apparently this reaction does not even require a firm union between sperm and egg, for Goodrich (1920) was able to remove the activating sperm intact (?) from the surface of the *Nereis* egg by microdissection. Similarly, Reverberi (cited by Runnstrom, 1949) found, in certain interspecific tunicate crosses, that the activating sperm not only failed to penetrate but actually fell from the egg surface in some cases. Perhaps clarifica-

tion of some of the problems of fertilization may be obtained by analyzing the "partially isolated" system as was done for *Paramecium*. Many scattered attempts to activate eggs with dead sperm and sperm extracts are recorded in the literature. Although these have been unsuccessful, a more systematic effort would now seem in order. Indeed, a beginning in this direction has been made by Hutner and Provasoli (1951) in the alga *Clamydomonas*. These investigators found that gametes killed by formalin, heat, ultraviolet light, or Streptomycin clumped strongly with gametes of complementary mating type. Likewise Metz (1945) and Metz and Donovan (1951) showed that sea urchin sperm killed with heat (*Strongylocentrotus purpuratus*) or with Bouin's fluid (*Arbacia*) agglutinated specifically with fertilizin. Evidently the sperm retained its specific organization when killed by these agents, at least so far as one surface group is concerned. As yet attempts to activate eggs with such dead sperm have failed, but further efforts may yet demonstrate the utility of the method in such material.

Regardless of the outcome of such attempts with eggs and sperm, the ciliates offer a wealth of material for the investigation of sexual phenomena that should not be neglected. In this material a number of different sexual mechanisms appear to exist, any one of which may serve as a useful model for studies on other forms. In *Paramecium* distinct mating types occur, and sexual reactions depend upon interaction of surface substances. In *Euplotes* "mating types" of a different sort are found, and diffusible substances appear to mediate conjugation. *Vorticella* produces macro- and microconjugants by a single division, and these may then copulate (Finley, 1939, 1952). The possible relation of sexual cycles to hormonal activity of the host in parasitic forms such as *Nyctotherus* (Wichterman, 1937) presents new opportunities for the investigation of the role of defined substances in sexual control. As a final attribute mutant stocks deficient in their sexual mechanisms occasionally become available in this material. As Metz and Foley (1949) have shown, analysis of such defective stocks can provide valuable information concerning fertilization. Apart from such examples as those cited here, great numbers of ciliates remain to be studied by modern experimental methods. A thorough examination of this reservoir of material certainly should extend our general understanding of the physiology of fertilization.

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Addendum

Material that appeared too late for the manuscript includes new information on sexuality in *Tetrahymena* from the Nanney-Elliott group, a detailed study of the role of irradiations in the mating rhythmicity of *Paramecium bursaria* (Ehret, 1953), and a comprehensive review of the cell surface in relation to metazoan fertilization (Runnstrom, 1952).

Elliott and Hayes (1953) found three interbreeding mating types in *Tetrahymena pyriformis* material collected at Woods Hole. From the F_1, F_2, F_3, and back cross progeny obtained by mating two of these (Type I, stock WH-6 x type II, stock WH-14) Nanney and Caughey (1953) isolated a total of seven pure mating types. These seven types form an interbreeding system; any one of the types will conjugate with the remaining six types. These workers emphasize that the first sign of a sexual reaction (pairing) does not occur for at least 90 minutes after mixing complementary types. Immediate agglutinative mating

reactions are never observed when the types are mixed. These investigators suggest that conjugation may be mediated by substances released into the medium by the organisms. These papers give an account of the cytology of conjugation in the Woods Hole stocks and in a third paper Nanney (1953) describes the conjugation cytology, including the conjugation of three animals, and the effects of centrifugation on nuclear development of conjugants in a selfing stock (AA 1-8).

Unlike certain *P. aurelia* stocks which ordinarily mate only at night and are inhibited by light (Sonneborn, 1938a), *P. bursaria* generally mates only during the day. However, Ehret (1953) finds that animals maintained in continuous darkness exhibit the normal rhythm but with reduced intensity. The reactive period may be shifted and its intensity enhanced or diminished by appropriate exposure to light. The action spectrum for these effects suggests a flavin type photosensitive substance. Ehret presents quantitative ultraviolet and x-ray inactivation data for mating reactivity and describes a phenomenon, "autoagglutination" (sticking together of animals of the same mating type), which results from ultraviolet, x-irradiation, or high-intensity visible irradiation. Since this reaction may be induced in unreactive and immature clones and is associated with various pathological changes (vesiculation, bleb formation, immobilization), its relation, if any, to the specific mating reaction is problematical. It would not appear to be related to the x-ray induced change of mating type reported by Lee (1949). The observation that formalin killed *P. bursaria* give mating reactions (Metz and Foley, 1949) takes some force from Ehret's hypothesis that mating substance is continuously lost and synthesized anew at the cilium surface during the reactive phase.

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Comments on the Origin and Evolution of "Sex"

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In the preceding discussions in this volume the reader found that many variations occur in the way that "sex" expresses itself; as a result, many different interpretations of its meaning and significance can be offered. Here some ideas are presented which indicate that there are many as yet unsolved problems in connection with "sex."

It is difficult to give a definition of sex or of sexuality because of the variations in the process and the differences of opinion about the subject. In the foreword of his book on sexuality, Hartmann (*Die Sexualität*, 1943) states that a controversy between certain botanists and himself involved the definition of sexuality. He indicates that one botanist defined fertilization and sex as the change from a haploid to a diploid phase. Hartmann's definition was the union of two sexually different haploid cells to form a diploid one. It would be difficult to decide just where sexuality begins in the series of conditions found by Cleveland for the flagellates in *Cryptocercus* because the change from haploidy to diploidy by one endomitotic division (*Barbulanympha*) would fit into the definition of sex by the botanist cited by Hartmann. Consequently, no definition of "sex" will be attempted here.

Naturally there has been much speculation about the origin and evolution of "sex," most of which has been based upon conditions in higher organisms. Geddes and Thomson (1889) in their book on the evolution of sex made the following statement (p. 117): "The number of speculations as to the nature of sex has well-nigh doubled since Drelincourt, in the last century, brought together two hundred and sixty-two 'groundless hypotheses,' and since Blumenbach quaintly remarked that nothing was more certain than that Drelincourt's own theory formed the two hundred and sixty-third. Subsequent investigators have, of course, long ago added Blumenbach's '*Bildungstrieb*'

to the list; nor is it claimed that the generalization we have in our turn offered has yet received 'final form,' if that phrase indeed be ever permissible in an evolving science, except when applied to what is altogether extinct." Like many other authors, Geddes and Thomson held that sex differences were primarily metabolic in character.

Just how many additional "theories" about sex have been offered since 1889 is not known, but only three will be outlined here and they are based largely on conditions in the Protozoa, the group with which I am most familiar. The first may be designated the "hunger" theory; the second may be referred to as the "chance fusion" theory; and the third consists of the ideas suggested by Cleveland and based on his studies of sex phenomena in the animal flagellates living in the gut of the wood-feeding roach, *Cryptocercus punctulatus*.

THE "HUNGER" THEORY

Dangeard employed a metabolic concept in discussing the possible evolution of "sex." He repeatedly expressed himself on this subject but made a somewhat more extended statement in 1913-14. He based his theory to a considerable extent on the life history of the chlamydomonad flagellates; in this group rapidly ensuing fissions produce "spores" (zoospores) which, as gametes, fuse together to produce zygotes.

Dangeard stated that, with simple bipartition, the intermediate period of nutrition suffices to maintain nutritive equilibrium. However, the nutritive condition finds itself unbalanced at the end of a series of sporulating divisions. The "spores" are enfeebled, famished, incapable of development. This diminution of vital energy of ordinary "spores," under the influence of insufficient nutrition, is, according to him, the cause which has provoked, in the course of evolution, the appearance of sexuality.

In his general conclusions, Dangeard expressed himself essentially as follows: (1) Sexual reproduction has had for its cause a nutritive origin, the results of successive bipartitions without intermediate periods of nutrition. (2) The gametes are like ordinary "spores" (zoospores) but are weak and incapable of continuing their own development. (3) Sexual reproduction has been derived from asexual reproduction. (4) The attraction which unites the gametes is of the same nature as that which carries an organism toward its prey or

leads to the presence of food. (5) Fertilization in its primitive significance is then a phenomenon of "autophagy." (6) This autophagy has introduced into the cycle of development a new stage which constitutes sexual reproduction. (7) Development of a gamete without union with another gamete constitutes parthenogenesis. Dangeard considered the haploid state the basic one.

THE "ACCIDENTAL FUSION" THEORY

This theory is fairly well expressed by Rhumbler (1898), who made a study of shelled rhizopods (Testacea). Referring to the close similarity of the process of syngamy as described by Schaudinn (1896) for *Actinophrys sol* with the sexual reproduction characteristic of the Metazoa, he stated his belief that the ancestral type of sexuality was to be found in the testate rhizopods.

Rhumbler takes as a starting point the tendency of certain rhizopods to cluster together in "nests" of individuals. He says that he has seen as many as fifty individuals of *Amoeba verrucosa* clustered together but without fusions. This tendency to form clusters he calls "cytotropism."

The next step is fusion of cytoplasm, or plasmogamy, which is very common among the Testacea and has been reported by a number of observers; and it has also been reported for other members of the Sarcodina, as, for example, some heliozoans. Rhumbler states that he has seen hundreds of cases of plasmogamy in *Difflugia lobostoma;* also that during several years many pairs of different species of *Difflugia* had been fixed and stained, but no changes in their nuclei or other structures could be recognized; that is, there were no more variations than occurred among single individuals. All possible combinations were seen—large nuclei, small nuclei, much reserve food, little reserve food, and so on.

Rhumbler recognized that a complete series of stages of cell fusion and reduction divisions had not been established, but believed that they did take place. The evolutionary steps might be: (1) cytotropism, or the clustering of cells without fusion, caused by the production of chemotactic substances; (2) "accidental" fusion of cytoplasms, or plasmogamy (eventually such fusions became necessary); (3) mixing of cytoplasms might induce nuclear divisions with one daughter nucleus degenerating, thus providing for "reduction";

(+) finally, nuclear fusion or karyogamy (such nuclear fusion would eventually become a necessity).

Apparently Rhumbler believed that ordinary individuals are diploid as they are in *Actinophrys sol.* Possibly they are haploid. It might be pointed out that cytoplasmic fusion could be followed by nuclear fusion producing diploidy. Haploidy could be restored by a single segregating nuclear division of the type described by Cleveland.

Since Rhumbler's paper was published, several observers have reported complete fusion in various shelled rhizopods. In 1910 Dangeard denied the occurrence of karyogamy in *Arcella*, as claimed by Hertwig and his followers (see Swarczewsky, 1908), but later (1937) he stated that "conjugation" including karyogamy took place in *Difflugia globulosa.* Ivanic (1935) described complete fusion including karyogamy in *Cochliopodium digitatum*, and Penard (1938) reported complete fusion for *Euglypha scutigera.* Other reports are mentioned in the section on Testacea in the paper "Sex in Protozoa."

CLEVELAND'S IDEAS ON THE ORIGIN OF SEX

In his discussion of the sexual cycle of the hypermastigote flagellate, *Urinympha*, Cleveland (1951) indicates some of his ideas about the evolution of sex. In *Urinympha*, which is diploid, the sexual cycle consists of meiosis in one division producing haploid nuclei and the fusion of these nuclei in a process of autogamy. No cytoplasmic divisions or fusions occur, although the "gametocyte" complement of extranuclear organelles is lost as is also one centriole. New extranuclear organelles are produced by the centriole which remains.

Referring to the situation in *Barbulanympha*, which is haploid, variations in its sexual cycle include an endomitotic duplication of chromosomes producing diploidy. This condition persists for about 10 days, then meiosis restores haploidy. *Barbulanympha* also has two other cycles. In one, two gametic nuclei are produced by the mitotic division of the haploid nucleus. These nuclei fuse autogamously without cytoplasmic division, then meiosis follows. The other cycle is similar, but cytoplasmic division accompanies the gametogenic mitosis, producing separate gametes which can fuse in a random manner. Nuclear fusion and meiosis follow.

As indicated by Cleveland, this group of sexual cycles suggests an evolutionary series with the following steps: (1) meiosis to relieve

endomitotic diploidy; (2) meiosis followed by autogamy, but without cell division, thus without gamete formation; (3) gametogenesis in haploid cells followed by fertilization, then meiosis, restoring haploidy; (4) establishment of diploidy, followed by simultaneous gametogenesis and meiosis, then fertilization, restoring diploidy; (5) differentiation of the "sexes" as to gonads and secondary sexual characters.

Cleveland states that, before the appearance of meiosis, unicellular organisms were perhaps haploid. Diploidy could arise by endomitosis in a haploid nucleus without compensating meiosis. When asexual cells are haploid, meiosis and gametogenesis cannot occur simultaneously; meiosis is zygotic. When asexual cells are diploid, gametogenesis and meiosis must occur concomitantly.

In sexual cycles, as pointed out by Cleveland, even when no gametes are produced, a centriole is lost, as occurs in higher types of cycle, since one gamete loses its centriole somewhere during the process of gamete formation and fusion. Hence the loss of a centriole, like meiosis, antedated gametogenesis in sexual evolution. Therefore a study of the origin of sex should begin with a consideration of environmental agents responsible for meiosis, that is, those that induce suppression of duplication of centromeres and chromosomes. Of secondary importance are the agencies responsible for differentiation into male and female gametes, their union, and the formation of male and female characters.

EVOLUTION OF "SEX"

With these theories of the origin of syngamy before us, we may pursue the subject of the evolution of this process further.

An examination of the status of sexuality in the Protozoa reveals that the groups in which syngamy is most commonly found are those which are more highly evolved. Among the Sarcodina these are the Foraminifera, Heliozoa, and possibly the Radiolaria, with only scattered examples, often unconfirmed, in the Proteomyxa, Amoebaea, and Testacea. Among the Sporozoa syngamy seems to be general in the Gregarinida, Coccidia, and Haemosporidia; also common or general in the Myxosporidia, Actinomyxidia, some Microsporidia and Haplosporidia. In the Ciliophora, syngamy of a relatively primitive type is described for the Protociliata, whereas in the Euciliata and

the Suctoria it usually takes the modified form of conjugation. Among the Mastigophora, in the Phytomonadina, which includes the rather highly evolved Volvocidae, syngamy is extensively represented, whereas examples are very few and often questionable in the more primitive Chrysomonadina; syngamy is apparently absent in the Cryptomonadina, is seldom found or not well authenticated in the Euglenoidina, and not reported for the Chloromonadina. In the animal flagellates syngamy was not well-established until Cleveland described the sexual cycles of the flagellates living in the woodroach, *Cryptocercus*.

Since the higher groups of Protozoa are supposed to have evolved from animal flagellates, and the latter from plant-like flagellates, we may well ask whether or not sexuality (syngamy) has been developed independently in the highly evolved groups mentioned above, or was passed on from the plant-like flagellates to animal flagellates, and from the latter to the Sarcodina, Sporozoa, and Ciliophora. Are the different orders of plant-like flagellates sufficiently closely related to have been derived from a common ancestor, or did they evolve somewhat independently? In either case, what were their ancestors? Some of the primitive fungi, at least, with primitive sexuality (see discussion by Raper) are thought to have evolved from a "flagellate complex" (Cantino, 1950), and now we find that there is evidence for sexuality in the bacteria and possibly in the viruses. What were the ancestors of the bacteria; of the viruses?

Is it profitable to suppose that "sex" arose only once in some ancestral group and has been handed down to all descendants, or is it more probable that it has arisen sporadically in many different groups? In either case, can we consider any of the three previously mentioned theories of the origin of "sex" as having exclusive validity, or partial validity, or any validity at all?

According to Cleveland, the first step in the evolution of "sex" could have been the production of diploidy by endomitosis, in which chromosomes are duplicated but not the centrioles. This could be followed by meiosis, which would restore haploidy. As this could happen in a single individual, no gametes would be involved. In case diploidy had become established by endomitosis, meiosis could be followed by autogamy, without the production of separate gametes. The other two theories mentioned would have diploidy result from cell fusions. If nuclear fusion followed, meiosis could restore hap-

loidy. Apparently Cleveland is the only author to provide a detailed description of one-division meiosis in the Protozoa. This may well be the primitive type, whether diploidy arose by endomitosis or by fusion of the nuclei of previously independent cells.

All three of the theories mentioned are based upon the study of organisms with definite nuclei which divide by mitosis. Is this the most primitive condition? There is genetic evidence for recombination of genes when different races of bacteria are mixed, suggesting syngamy and meiosis in that group (see discussion by Lederberg and Tatum). Genetic evidence suggests syngamy in viruses (see discussion by Visconti). Are we to suppose that bacteria and viruses have nuclei with chromosomes and that not only mitosis but also syngamy and meiosis take place in these organisms? Hutchinson and Stempen, in this volume, have shown that cell fusion may take place in bacteria; and there is some evidence for mitosis in bacteria (see discussion by Lederberg and Tatum). Can viruses have nuclei, chromosomes, mitosis, and syngamy? Recently Fraser and Williams (1953) claimed to have identified strands of nucleic acid, or chromosomal material from viruses when host bacteria were broken up on an electron microscope screen and photographed.

If "sex" exists in bacteria and viruses, is the phenomenon a sporadic and isolated one, or is it more general? If these organisms represent the most primitive types of living things, and "sex" is found to be general among them, did "sex" appear with the origin of life? Is "sex" therefore the universal characteristic of living things that has often been postulated? If this is so, can there be "sex" without nuclei and chromosomes, or did the first living beings have nuclei and chromosomes? If the first forms of life did not have nuclei and chromosomes, could they have had "sex"? In the blue-green algae (Cyanophyta), in which organized chromosomes apparently are absent, no sexual phenomena have been found. Are the blue-greens to be regarded as degenerate, having lost chromosomes and "sex," or are they primitive, having been derived from still simpler ancestors in which chromosomes and "sex" did not exist? Dodson (1952) has suggested that the blue-greens represent the ancestral type from which all the other algae have been derived. If this is so, when and where did well-defined nuclei, chromosomes, and "sex" come into existence? Why is syngamy so uncommon in the Chrysomonadina, Cryptomonadina, Euglenoidina, Amoebina, and so on? Have these groups evolved in

such a way that "sex" has been largely lost? If so, why was it lost? How can these organisms flourish without "sex," which Nature seems to have found so advantageous in the evolution of higher forms of life? Possibly "sex" is more prevalent than present evidence indicates and more research will bring more examples to light. However, there is ample evidence that many unicellular organisms can multiply asexually for indefinite periods of time without syngamy. Frankly, we do not have satisfactory answers to many of the questions asked above. Future research may possibly provide the answers.

SEX DETERMINATION AND SEX DIFFERENTIATION

Since the formation of gametes and their fusion to produce new individuals is widespread among microorganisms, what light can be shed on how gamete determination and differentiation have arisen? The very expression "sex determination" implies a difference between the sexes, and much of modern study of lower and higher organisms indicates that sex determination is genetic and therefore presumably due to different genic composition of the chromosomes; and indeed, in many animals, there are visible differences in the chromosomes of the two sexes, as pointed out by McClung in 1902.

The terms male and female indicate a morphological difference between the gametes which unite to produce a zygote and, in higher organisms, differences in the individuals which produce the gametes and in the organs in which they are developed. In cases of hermaphroditism the sex organs of both sexes occur in the same individual. Apparently most of the numerous theories of the nature of sex suggested by the quotation from Geddes and Thomson (1889) dealt with the development of maleness and femaleness.

In cases of "isogamy" morphological differences between uniting gametes are not apparent, although physiological differences are presumed to exist. For example, the attraction that brings gametes together may be centered primarily in one gamete or may involve both. According to Hartmann's theory of relative sexuality, both male and female potencies are present in each gamete, and the way in which they unite depends upon the relative amounts of these potencies possessed by each gamete. However, with most organisms, male and female characteristics are fairly stable and definite (see review by Smith, 1951).

One of the most interesting results of Cleveland's studies was the finding of a differentiating nuclear division in the gametocytes of haploid flagellates such as *Trichonympha* and *Leptospironympha* during which male and female sets of chromosomes are segregated from each other. Each parental chromosome gives rise to a chromatid of each sex.

The existence of sex-differentiating mitoses was postulated by Prokofieva-Belgovskaya (1946). Starting with binucleate cells in the bark parenchyma of potatoes, in which, after aging, two sister nuclei come to have different appearances and behavior, then citing examples from the literature, this author presents some interesting interpretations. The two sister nuclei in the potato cells, by the end of starch formation, become considerably shrunken. The basophily of the chromonemata and chromomeres is greatly diminished. In many cases these sister nuclei differ from each other considerably, for example in number of nucleoli and in stainability. The heterocyclicity of these nuclei is also shown when they divide. The differences between the two nuclei in their resting and divisional conditions are interpreted to indicate that the mitosis which produced them was a differentiating process. In anaphase, one set of chromosomes is supposed to be "younger" and is segregated from the other, which is "older"; that is, chromatids derived from the same parental chromosomes are not identical. In line with some of the more recent ideas about gene and chromosome duplications (for example, Delbrück, 1941), it is postulated that a new unit forms as a "copy" of the original instead of the two units being formed by "splitting." Thus we have the concept of "mother" chromosomes producing "daughter" chromosomes by a sort of budding, and the latter may differ somewhat from the "mother." These supposed differences in molecular makeup are thought to account for the differences in appearance and behavior of the sister nuclei described above.

As a matter of fact, Cleveland's description of the separation of male from female chromatids in the gametocyte division of *Trichonympha* and other species indicates a mechanism that results in the separation of one group of sister chromatids from the other. A system of joining into two groups operates to prevent random orientation of pairs of chromatids and to insure the separation of all the "male" from the "female" chromatids. Apparently this process is not

always perfect however, since in *Trichonympha* Cleveland sometimes found intersexes or "gynandromorphs" among the "gametes."

Just how far the application of this process can be extended is problematical. Prokofieva-Belgovskaya applied the principle to the segregation of "male" from "female" sets of chromosomes in various Protozoa such as *Actinophrys sol* (Bělař, 1923, 1926), *Amoeba diploidea* (Hartmann-Schilling, 1917), *Adelea ovata* (Jollos, 1929), *Stylorhynchus longicollis* (Léger, 1904), and *Bursaria truncatella* (third division of the micronucleus, Poljansky, 1934), as well as to various other animals and plants. Prokofieva-Belgovskaya also suggested that the segregation of "mother" from "daughter" chromosomes was the initial step in the evolution of "sex." Is the "mother-daughter" system in the potato cells a sex-segregating system? If not, why does it occur? Is that system of duplication a general one? In Cleveland's flagellates, sex-segregating mitoses take place only under the influence of the molting hormone of the host; at other times the ordinary type of mitosis takes place. Cleveland's cases apparently show that there are, then, two types of mitosis, a sex-segregating type and a non-segregating type. What the stimuli to segregation would be in a potato cell, or in the other cases cited by Prokofieva-Belgovskaya, is not apparent.

Although heterocyclicity may indicate sex differences, and many other instances could be cited, it does not necessarily indicate that "mother-daughter" origin and separation of complete sets of chromosomes are always required to differentiate the sexes. A difference of one chromosome, or a part of a chromosome, or even one or a few genes may be enough to differentiate male and female nuclei from each other. The heterocyclicity, then, could well be the result of sex-determining influences other than the "mother-daughter" system.

In a note appended to the paper by Prokofieva-Belgovskaya, H. J. Muller remarks that, if such mother and daughter chromosomes are distinguishable, it would be most surprising if all the mother chromosomes should go to one pole and all the daughter chromosomes to the opposite pole of the spindle. Although such a segregation is possible, it would involve a new principle which might be as important as the apparent differences between the two sets of sister chromosomes. It remained for Cleveland to describe a mechanism whereby such a segregation actually takes place.

Division of a haploid nucleus giving rise to one migratory "male" and one stationary "female" pronucleus has long been known for the third division in the gametogenesis of ciliates, but apparently the mechanism described by Cleveland has not been recognized in such cases, although it might apply. However, Sonneborn (1951) has suggested that, since these pronuclei are presumably genetically equivalent, local cytoplasmic differences would constitute the sex-determining factor. Maupas (1889) and Diller (1936) had earlier suggested cytoplasmic determination of the differential behavior among the nuclei derived from the original micronuclei of conjugants and animals undergoing autogamy.

In many higher animals the sexes differ in the chromosomal content of their cells, the so-called X and Y chromosomes apparently being the differentiating feature. These differentiating chromosomes and the evidence for random segregation of chromosomes on the mitotic spindle, especially in meiotic divisions, would militate against the general occurrence of the "mother-daughter" type of segregation. Thus the evidence indicates that in most cases of chromosome duplication the products are identical, while under certain conditions the two groups of daughter chromatids may be different. Here, surely, is an intriguing field for further research.

As presented in this volume, "sex" is seen to have a very wide distribution among microorganisms. On the other hand, in certain groups it is very uncommon or apparently absent altogether. Its evolution is as unclear as that of many of the groups of organisms themselves. Continued research, both extensive and intensive, may be expected, in time, to find answers to many of the problems that remain to be solved.

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