AN ABBREVIATED CONJUGATION PROCESS IN PARAMECIUM TRICHIUM

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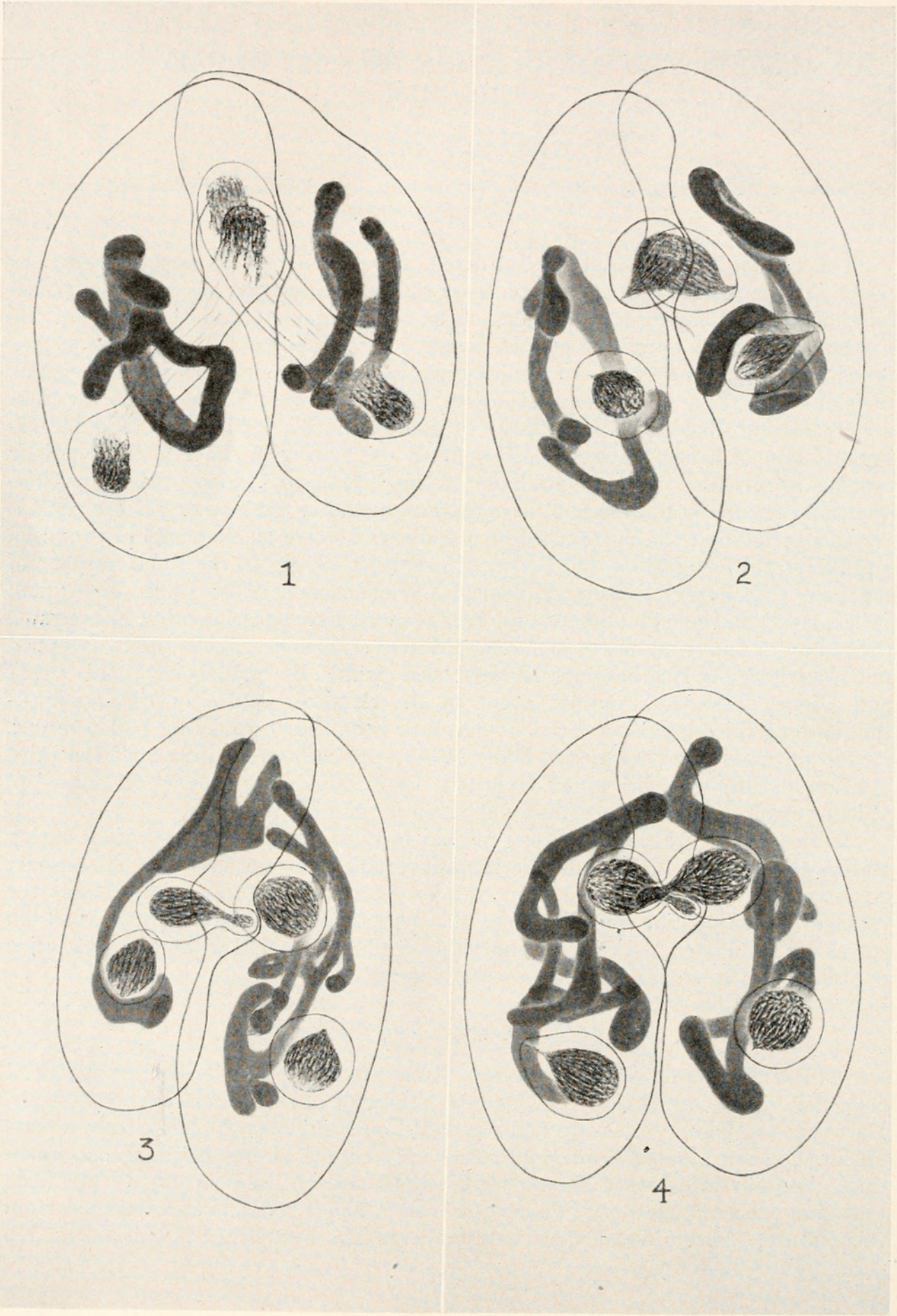
The remarkably constant, well-ordered, complex series of nuclear processes which are characteristic of ciliated protozoa during conjugation has been established by a host of cytological investigators. The almost monotonous regularity of the maneuvers in many species of ciliates during conjugation has led to a fairly stereotyped concept of the events of conjugation in ciliates generally; three pregamic divisions producing the pronuclei (with degeneration of nuclei after the first and/or second divisions), interchange of gametic nuclei, fertilization, and the reorganization of a new nuclear complex from the synkaryon after a characteristic number of divisions. The invariability of this "standard" process was called into question recently by a number of investigators including the author (Diller, 1936), who suggested that conjugation might not always involve an exchange of pronuclei and reciprocal fertilization, but fusion of pronuclei arising in the same member of the pair (autogamy). Both cytological (Wichterman, 1940; Chen, 1946; and Diller, 1948) and genetic (Sonneborn, 1947) studies have subsequently demonstrated the reality of autogamy in conjugation. Moreover, genetic effects due to cytoplasmic interchange during conjugation have been claimed by Sonneborn (1943, 1945) and Dippell (1948). Another event in the classical picture of conjugation—the puzzling third pregamic division—has now been shown to be not indispensable. In certain races of *P. trichium* (Diller, 1948) the conjugants may omit the third division and proceed with either reciprocal fertilization, autogamy ("cytogamy" of Wichterman) or parthenogenetic development of gametic nuclei.

In view of the great versatility of nuclear behavior shown by *P. trichium* during conjugation (Diller, 1948) and the large favorable micronuclei which this species possesses, it would seem to be of interest to describe a further variation from the "standard" conjugation behavior. In this heretofore undescribed process certain stages are eliminated and the micronuclei proceed directly and without degeneration of their products to establish a new nuclear apparatus.

Source of Material and Techniques

All the material on which the present study was made, was derived from a pond collection kindly furnished by Dr. Hannah Croasdale of the Department of Zoology, Dartmouth College. The collection was taken on September 20, 1946, from a pond on Dr. Carleton's grounds in Hanover, N. H. Some of this material was introduced into hay infusion on September 23, 1946, and on the next day about fifteen pure-line mass cultures, each descended from a single animal, were isolated from this culture. Several small mass cultures were also established at this time. No

PLATE I



significant differences in cytological behavior between the various lines were noticed, although most of them were examined from time to time and the selection of material to be studied was more or less random. Very shortly after their establishment, conjugation occurred in many of the cultures; for instance, in isolation culture No. 10, conjugation was in progress by October 3.

In most of the lines conjugation occurred in at least small numbers, at all times. The cultures were maintained on hay and malted milk, boiled in pond water. Several of them (isolation cultures Nos. 5 and 6) were mixed. Although there were small numbers of conjugants in each culture at the time of admixture, the combination resulted in a rather heavy incidence of conjugation so that it would seem as if these two cultures may have been opposite mating types. The cultures were maintained until June, 1947, when they were abandoned. Toward the end of their life span the cultures showed a more conventional behavior and finally did not conjugate at all. Temperatures in the laboratory became rather high and this may have been responsible for the decline of the cultures.

All the observations reported in this paper were made on killed and stained material. The animals were pipetted from the cultures into a centrifuge tube, concentrated, allowed to stand for a few minutes, and then fixed in Perenyi's fluid. They were sometimes subsequently treated with Schaudinn's fluid, and stained in acetic orcein or in Grenacher's alcoholic borax carmine. Both stains gave very good results. Usually fast green or indulin were used as counterstains. All the technique was carried out in the centrifuge tube and the animals mounted on slides in diaphane or clarite.

EXPLANATION OF PLATES

These are camera lucida drawings of stained whole animals from six isolation cultures started Sept. 24, 1946, and a small mass culture started Sept. 28, 1946, all derived from Carleton Pond, Hanover, New Hampshire. Magnification about 1200 times. All the figures illustrate *Paramecium trichium* during "abbreviated" conjugation. The animals were fixed in Perenyi's fluid and stained with Grenacher's alcoholic borax carmine or acetic orcein and counterstained with indulin or fast green. The specimens shown in Figures 1, 2 and 9 are representatives of small mass culture A; Figure 3, isolation No. 15; Figures 4 and 5, isolation No. 10; Figures 6, 7, 10, 11 and 16, isolation No. 14; Figure 8, isolation No. 6; Figures 12 and 15, isolation No. 9; Figures 13 and 14, isolation No. 17. Only the nuclear structures have been drawn. In some of the figures, particularly the later stages, old macronuclear fragments lying on top of the structures which were intended to be illustrated were omitted for the sake of clarity.

PLATE I

EXPLANATION OF FIGURES

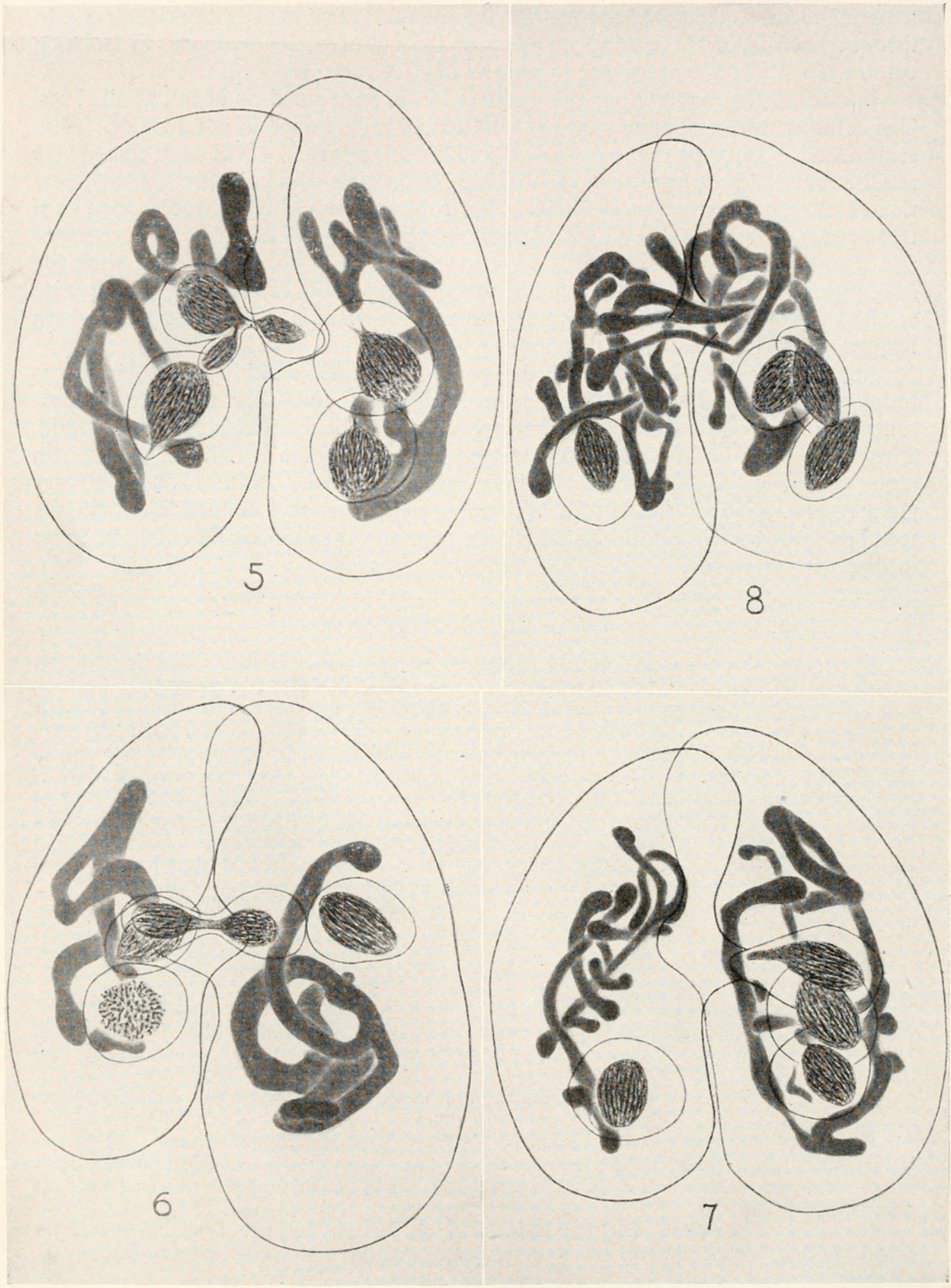
FIGURE 1. Telophase of first maturation division in a culture engaging in "abbreviated" conjugation. The macronuclear skeins (simple) seem to develop earlier than they do during "standard" conjugation. A daughter nucleus is found in the paroral cone region of each conjugant.

FIGURE 2. Two nuclei resulting from the first division. Each has a tail indicative of recent separation.

FIGURE 3. Two nuclei in each conjugant. Twisted chromosomes and a knob on the nucleus in the paroral cone region of the left conjugant are suggestive of its impending passage. Since the corresponding nucleus in the right conjugant is not in a similar condition, this pair suggests a one-way passage.

FIGURE. 4. "Migratory" nuclei produced after the first division. A nipple-like process on each extending toward external boundary of cone. Suggestive of reciprocal transfer.

PLATE II



Observations

Although no observations were made on the length of time that the members of the pair remain attached in abbreviated conjugation, it is my distinct impression that the time is much less than for the conventional method. It is probable that the first division of the micronucleus consumes less time than ordinarily is the case. The macronucleus in abbreviated conjugation seems to be somewhat precocious in its skein formation. By the time of the telophase of the first division, a simple macronuclear skein (Fig. 1) has formed. One sister chromosome group of each spindle is likely to be found in, or near, the paroral cone. The daughter nuclei, immediately after separation, frequently have "tails" on them (Fig. 2). This is true also for the corresponding post-telophase stage of the other divisions. Normally, the first division is not followed by degeneration. I have seen only one or two pairs, in abbreviated conjugation, in which degeneration of nuclei was evident. This is rather unusual because in conventional conjugation, as well as in the type in which only the third pregamic division is omitted, degenerating nuclei after the first and second divisions are the rule.

A reorganization of the two nuclei in each conjugant leads to a premetaphase condition (Fig. 3 and others). A knob-like, nipple-like, or handle-like process (Figs. 3, 4, 5, 6, 7, 8 and 10) is formed on one (Fig. 3) or two (Fig. 4) of the nuclei. This modification marks the nucleus as a potential migratory gametic nucleus. Undoubtedly, it is reflective of cytoplasmic stresses, pressures and/or currents in the cone regions. The narrow pointed process may be directed toward the cell boundary or toward the interior of the cell. The chromosome threads are frequently arranged in a spiral fashion, suggesting a twisting influence on the nucleus. In Figure 3, the presence of a single nucleus in the paroral cone of the left conjugant, with a terminal knob, and the absence of a similar structure in the right conjugant suggest an imminent one-way passage. Frequently, the macronucleus of one conjugant is a little more advanced in skein formation than is the other (Fig. 3). In contrast, two such migratory nuclei (Fig. 4) may be present, indicating an approaching reciprocal transfer.

Occasionally the pinching effect appears to be so severe as to cause a disruption of the migratory nucleus into several parts. Such an instance is represented in Figure 5. It is conceivable that this process may be the means whereby small accessory nuclei arise, by a purely amitotic mechanism. This possibility will be considered later in connection with subsequent stages. It is probable that most of these constricted nuclei would recondense and adjust to the normal condition after

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Plate II

Explanation of Figures

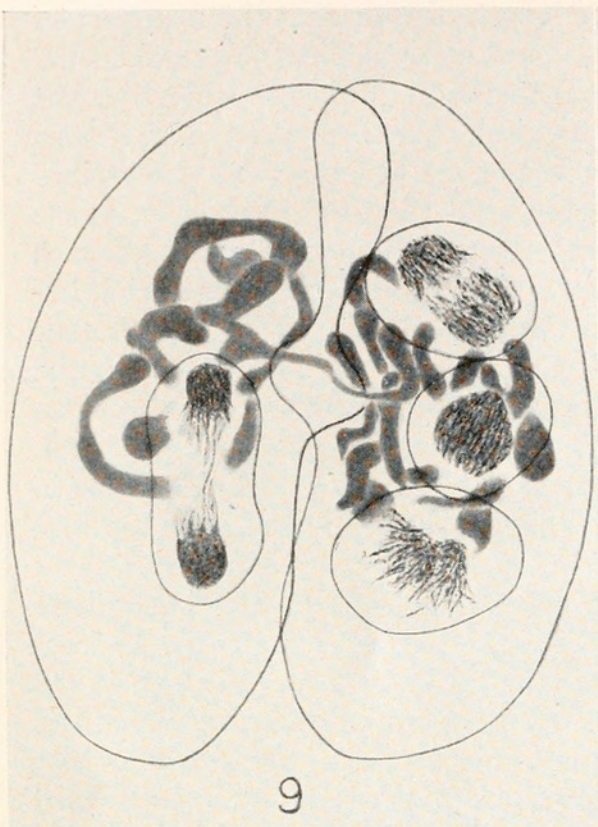
Figure 5. The "migratory" nucleus of the left conjugant pinching off two small portions, each connected to the larger section. One of the small accessory nuclei is lying in the cone.

Figure 6. Macronuclei in simple skeins. No degenerating micronuclei. Migratory nucleus, constricted in the middle, passing through the cone from the right conjugant to the left. One-way passage.

Figure 7. One-way passage of migratory nucleus, after first division, from the left conjugant to the right one. Tail of migratory nucleus still in tip of cone.

Figure 8. One-way passage of a migratory nucleus. Interchange of rather coarse macronuclear strands in both directions.

PLATE III



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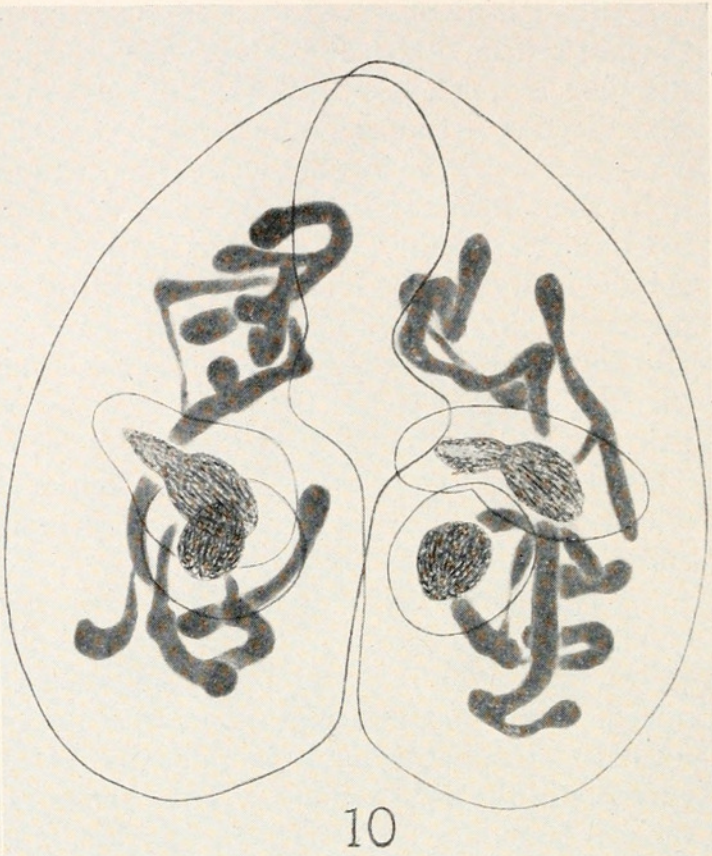

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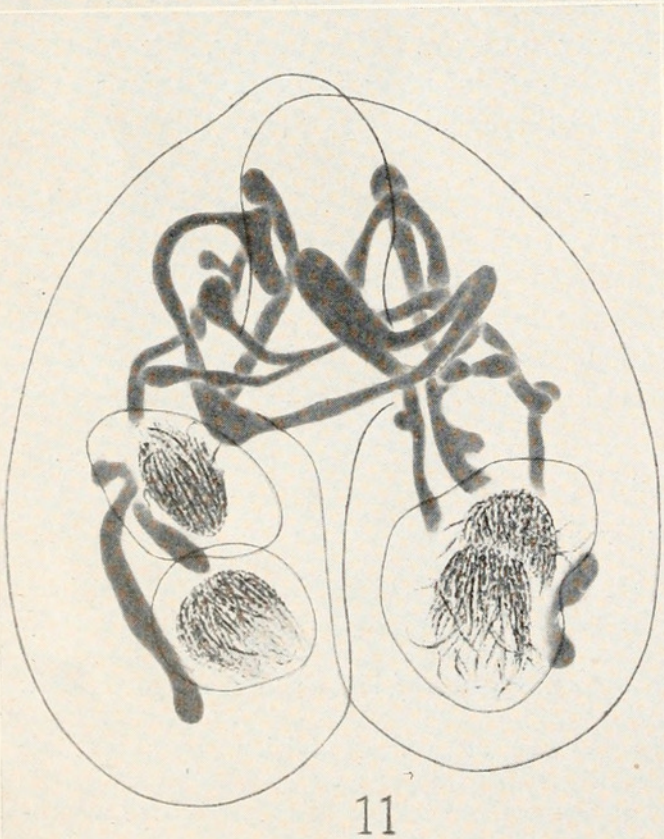

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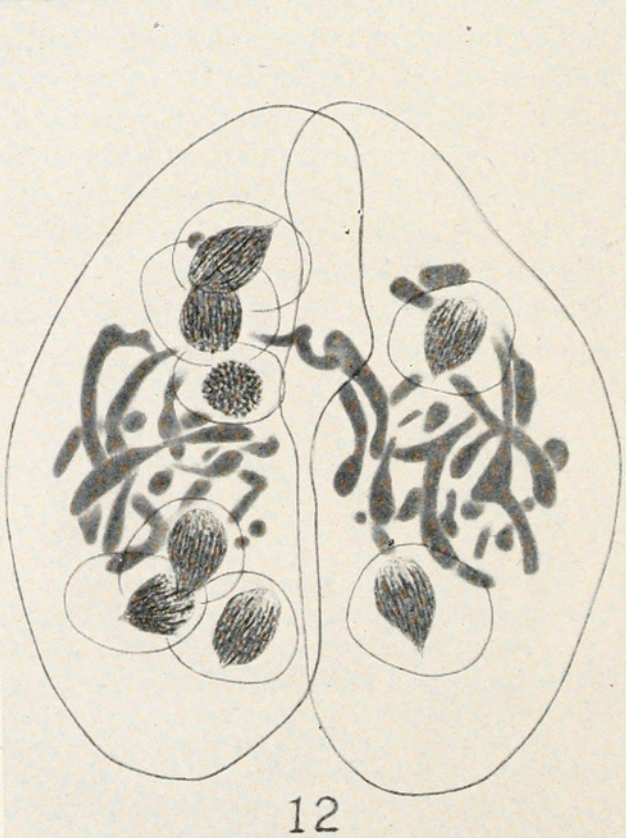

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the temporary stresses had been relieved. I have seen one other instance, not figured in this paper, which could produce a similar result. One member of the pair had two normal nuclei from the first division. The other had a tripolar telophase of the first division. One of the three sister chromosome groups was smaller than the other two.

At the time when the migratory nuclei are actually passing through the paroral cones they often show an equatorial attenuation. Such a dumbbell effect is shown in Figure 6. The migratory nucleus is passing into the small left conjugant. There is no indication of nuclear passage in the reverse direction. The macronuclear skeins are still relatively simple and coarse. In this case there is cytoplasmic continuity at one level only. Figure 7 illustrates a slightly later stage of one-way passage of a migratory nucleus after the first division. Its tail is still in the cone region. Probably in this pair there is cytoplasmic continuity between the conjugants at two levels.

It is difficult to ascertain the frequency of the occurrence of the different modes of behavior of the nuclei, after the first division, in the Carleton Pond stock of *P. trichium*. One-way passage of a nucleus, leading to the spectacular unbalanced condition of three nuclei in one member and one nucleus in the other conjugant—a situation which first attracted the author's attention to this process—is by no means a rarity in these stocks. Two-way passage (interchange), as suggested by Figures 4, 10 and others, is also quite common. In the event of an original heteroploidy of the micronuclei of the two conjugants, it is possible to determine at a later stage whether interchange had occurred. A third alternative is evident: the failure of nuclei in both conjugants to migrate and their development in the same conjugant in which they arose. This possibility is, in the author's opinion, a valid one, but seems to be more rare than the other two. Apparently, breakdown of the tips of the paroral cones, cytoplasmic currents, and/or internal pressures at the proper stage are the factors which determine the movements of nuclei at this time. The nature of these forces is entirely conjectural but it is of interest to note that they may be unequal in the two members of the pair.

Shortly after the passage of the micronuclei, or their non-passage, strands of the macronuclear skein may, or may not, become stretched across the cone regions from one conjugant to the other in much the same fashion as in unabbreviated conjugation (Diller, 1948). Passage of the macronuclear skein may be unidirectional

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PLATE III

EXPLANATION OF FIGURES

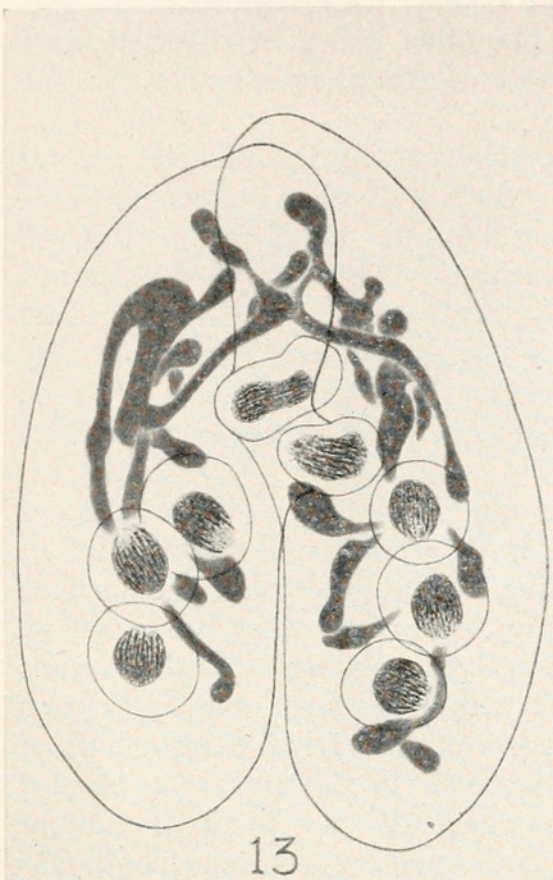
FIGURE 9. One micronuclear figure in left conjugant; three dividing nuclei in right conjugant. Second division. Macronuclear strand passing from left conjugant into right. All the nuclei are in slightly different mitotic stages.

FIGURE 10. Two gametic nuclei in each conjugant. Either cross-fertilization, autogamous fertilization, or parthenogenesis, is imminent. Uncertain whether there is cytoplasmic continuity in oral cone regions. The "migratory" nuclei are retaining the constrictions characteristic of the migratory condition.

FIGURE 11. Synkaryon formation in right conjugant. Two separate nuclei in left conjugant. In the latter, either nuclear fusion is delayed or the gametic nuclei are going to develop parthenogenetically. Macronuclear interchange in both directions.

FIGURE 12. Six nuclei in left conjugant; two in right conjugant. Macronuclei in short complicated strands.

PLATE IV



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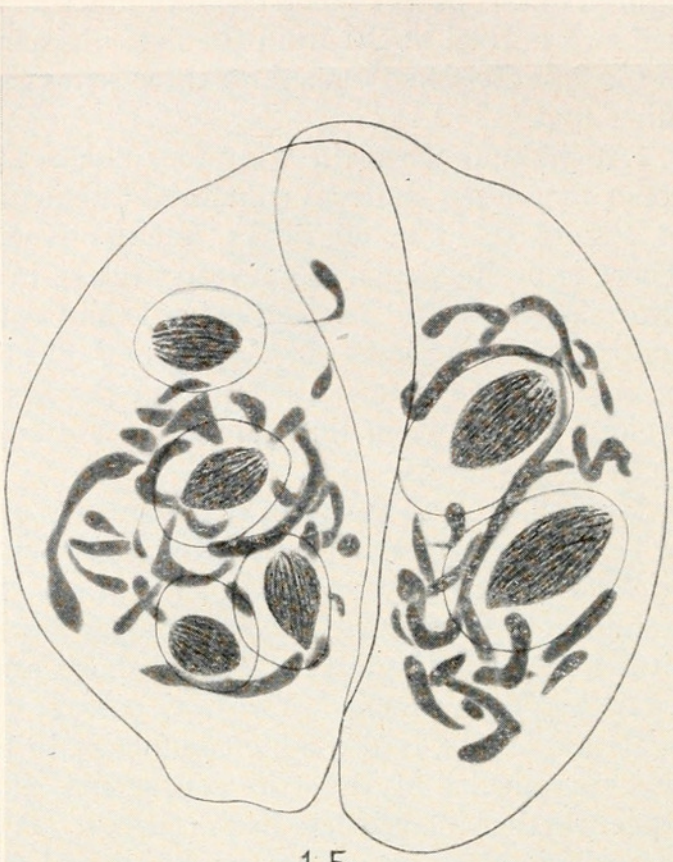

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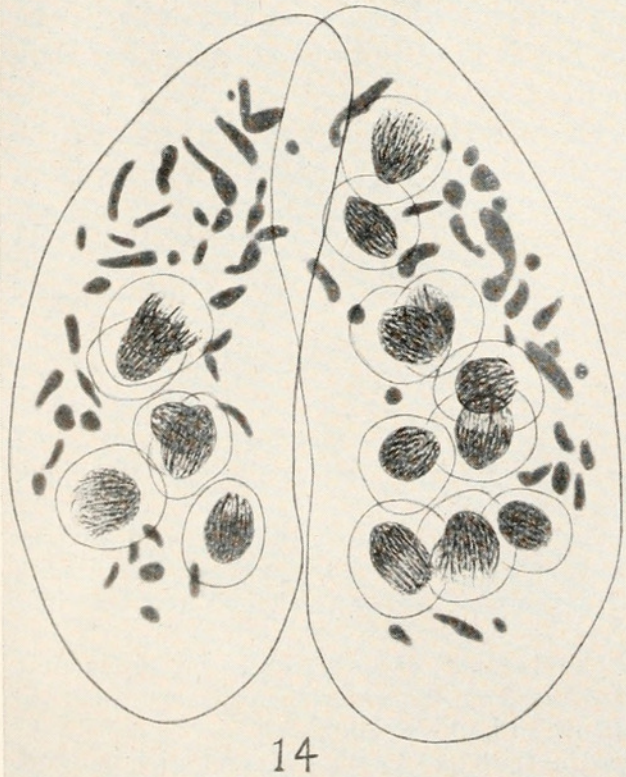

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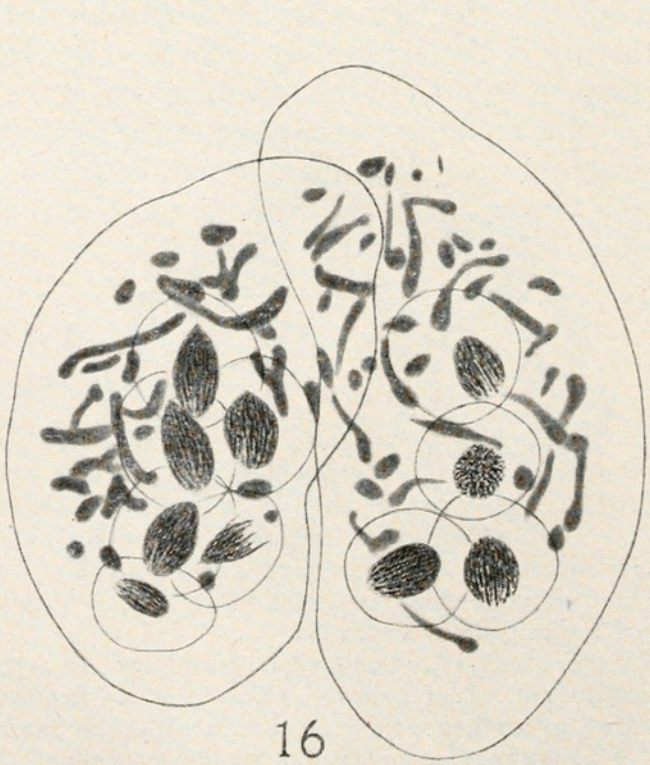

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(Fig. 9) or may extend in both directions (Figs. 8 and 11). Macronuclear exchange was very frequent in the Carleton stock. In case it is unidirectional, the strands can pass either from the uninucleate conjugant into the trinucleate member, as in Figure 9, along the path which the single migratory nucleus took, or in the reverse direction. The direction of macronuclear movement seems not to be directly correlated with the direction of micronuclear movement. Exchange of macronuclear material apparently marks the end of interconjugant micronuclear movement.

As remarked above, there is normally no degeneration of nuclei at this stage, or any other stage, in abbreviated conjugation. One can detect several alternative modes of behavior of the nuclei from this point, keeping in mind the possibilities that the nuclei of one member of the pair may be behaving differently from those of the other member, and even that the nuclei in the same conjugant may be diverse in their activities. First, fertilization (synkaryon formation) may occur. Such a condition is shown in the right conjugant of Figure 11 and such was probably the ancestry of the two nuclei in the right member of Figure 15. Depending on whether interchange had occurred, cross-fertilization or self-fertilization would be accomplished. Second, parthenogenetic development of the nuclei may take place. This seems to be the most frequent type of activity in abbreviated conjugation. Third, combinations of fertilization and parthogenetic development may be adopted. Although the critical stages are rare, it is possible by reason of size differences to reconstruct previous history.

Figure 9 illustrates the micronuclear activity of the second division. The conjugant on the left contains a late anaphase micronucleus, while that on the right has three dividing nuclei in slightly different mitotic stages. It is a little unusual for the micronuclei to show such asynchrony. Probably the two anaphase nuclei, one in each conjugant, are sisters. It seems likely that all of these nuclei are developing without fertilization (parthenogenetically).

It is difficult to be sure about the exact history and the immediate fate of the nuclei of Figure 10. The "tailed" nuclei may have been interchanged, or not, and may be on the point of fusion with the stationary nuclei. Otherwise, parthenogenetic development would be expected to follow.

Figure 11 shows synkaryon formation in the right conjugant and two separate

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Plate IV

Explanation of Figures

Figure 13. Unusual interchange at the end of the second division, with no degenerating nuclei apparent. The nucleus at the top is pressing against the cone moving toward the left, while the nucleus directly below it is part-way through the cone, passing into the right conjugant.

Figure 14. Six nuclei in left conjugant. Ten nuclei in right conjugant. Presumably, this condition arose from a one-way passage of a gametic nucleus at the stage represented in Figure 13. Old macronuclei represented by closely packed short rods and spheres (many omitted).

Figure 15. Four nuclei in left conjugant. Two very large nuclei in right conjugant. Probably parthenogenesis has occurred in the left conjugant while synkaryon formation has taken place in the right member.

Figure 16. Four nuclei in right conjugant. Four large nuclei and two small ones in the left conjugant. The latter may have arisen from pinched-off parts of nuclei after the first division, as suggested by Figures 3 and 5. (They may have originated in the right conjugant).

nuclei in the left conjugant. In the latter, either nuclear fusion is delayed, or the gametic nuclei are going to develop parthenogenetically. The latter alternative seems to me the more probable, since my observations suggest that very little time elapses before nuclear fusion is completed.

Occasionally I have found that one or both of the conjugants at later stages possess nuclei of different sizes. Aside from the explanation of original heteroploidy of the conjugants, this can best be interpreted by assuming that the larger nuclei have arisen from synkarya, while the smaller ones have developed parthenogenetically. Assuming that the number of nuclear generations is the same in both conjugants, synkaryon formation in one and not the other will result in different numbers of nuclear products in the two members at later stages (cf. Fig. 15). Although this is not the only explanation, I believe that asymmetric synkaryon formation is a valid one. One wonders whether an extra postzygotic division is required for final reorganization since there has been a reduction in nuclear number in the conjugant which produced a synkaryon. Another device for bringing about unequal nuclear numbers in the two conjugants is for the mitotic stages to become slightly out of step with each other. I believe this to be a real, but rather rare, happening. However, in the uninucleate-trinucleate pairs the single nucleus seems often to be ahead of the three others (Fig. 9).

Figure 12 shows completion of the second division in a pair in which there has been one-way transfer. By this time the macronucleus has usually fragmented into complicated short strands and rodlets and no longer can be traced to the opposite cell. Apparently the paroral cone intercommunications heal over at this time. The conjugants separate after this stage or during the next (third) division.

Migration of nuclei in abbreviated conjugation is not completely restricted to the time immediately after the first division. Very rarely, interchange can occur after the second division. Two such cases are shown in Figures 13 and 14. In the former, each conjugant has four nuclei, one of which is located in the paroral cone and is projecting into the other animal, apparently on the verge of effecting interchange. A one-way transfer of this type would result in five nuclei in one conjugant and three in the other. At the conclusion of the third division of such a hypothetical case, six and ten nuclei, respectively, would be found in the conjugants. That is apparently the explanation of the asymmetric condition of the pair illustrated in Figure 14.

A rather unusual and interesting asymmetrical case is illustrated in Figure 16. Four nuclei, following the second division, are present in each conjugant. In addition, there are two small nuclei in the left conjugant. These may have arisen as "buds" pinched off the nucleus at the time of the first division, as suggested earlier, which have persisted through division. (See Fig. 5.) They seem perfectly viable and normal.

The events subsequent to the third division, when the animals separate, have offered no special points of interest. Presumably reorganization, anlagen formation, and disappearance of the old macronucleus are similar to the standard processes characteristic of *P. trichium* (Diller, 1948), although I have made no particular effort in these studies to work out the post-conjugant stages. Regularly, the mature exconjugants would be expected to have four macronuclear and one micronuclear anlagen.

Discussion

The abbreviated conjugation process in the Carleton race of *P. trichium,* reported in this paper, accomplishes the ends of nuclear reorganization in a remarkably simple and direct manner without wastage of micronuclei and without unnecessary stages. However, it is so unorthodox and so divergent from the conjugation pattern of other ciliates, and even of other races of the same species, as to pose problems about its general significance, and, in fact, about the meaning of certain phases of the conjugation process as a whole. The standard or conventional scheme of micronuclear activity in the ciliates involves three pregamic divisions (two have been reported in a certain race of *P. trichium,* Diller, 1948), and a variable number of postzygotic divisions which reconstitute the definitive nuclear complex. The term "postzygotic divisions" is here extended to include parthenogenetic divisions or generations as well as those of fertilization nuclei. It is borne in mind, of course, that variation in numbers of macronuclear and micronuclear anlagen is common but is fairly constant for a given species. In *P. trichium,* in the standard process, there are two or three pregamic divisions and three postzygotic divisions. In abbreviated conjugation three divisions, simply, are required to complete the process. (Possibly an extra division is appended in case synkaryon formation is involved.) Similar numbers of final nuclear products arise in both processes. The failure of nuclei to degenerate in the abbreviated process, generally, accounts for the end products being the same in number as in standard conjugation. The question then arises as to the homology of the nuclear generations in abbreviated conjugation with those in the standard process. In both, the first division shows a characteristic polarized (not crescentic) prophase stage. This may well be indicative of a maturation or a reductional process and is followed, in the standard conjugation, by one or two other divisions before fertilization or parthenogenetic development. However, in abbreviated conjugation there is no further division before nuclear exchange and fertilization (or parthenogenesis) are accomplished. If one were to assign the exchange period as a central reference point in both processes, then one can consider the first division in abbreviated conjugation as a maturation division and the second and third divisions as being homologous with the postzygotic divisions of the standard process. If this interpretation is valid, what can be inferred about the chromosomal cycle in abbreviated conjugation? A comparable problem was raised before (Diller, 1948) in connection with the omission of the third division in certain races of *P. trichium* and the parthenogenetic development of reduced nuclei; it was concluded that under these circumstances each conjugation would be expected to result in a progressive diminution of chromosome number. Unfortunately, direct observation of chromosome numbers in the various generations is very difficult, if not impossible, to make, and even estimates of nuclear size are not very satisfactory in spite of the large and comparatively favorable micronuclei of *P. trichium.* It has been considered axiomatic that two maturation divisions are necessary to bring about chromosomal segregation and reduction in mature gametes. This is undeniably accomplished in the standard conjugation process, even when the third pregamic division is omitted, but is doubtful in abbreviated conjugation.

Two possibilities suggest themselves. First, that reduction is completed in the later divisions and that the final nuclei are haploid, unless fertilization occurs. In

the latter eventuality, the awkward situation of the occurrence of a maturation division before fertilization and another after fertilization would exist. A second, and more probable, speculation is that the gametic nuclei are not reduced but diploid, and the nuclei arising by parthenogenesis would remain diploid while those derived from synkaryon formation would be tetraploid (cf. Fig. 15). Although a good deal of heteroploidy was evident in these cultures, hypoploidy was not nearly as extreme nor as conspicuous as in certain other stocks which I have been studying.

In correlation with the shortened morphological manifestations, it would be interesting to know how the time relationships of abbreviated conjugation compare with those of the standard process. I have the impression that abbreviated conjugation takes a shorter time than the standard process, but no positive evidence on this point. Unfortunately, the cultures were discarded before this information was obtained, in fact, before it was realized that abbreviated conjugation was happening; and I have not been able to secure any more stocks from the Carleton Pond, although several collections were made. The causes of the induction of abbreviated vs. standard conjugation are also entirely unknown at present. It seems to be not entirely a racial or genetic effect, since there were some instances of standard conjugation in certain of the Carleton Pond stocks.

I know of no other conjugation study in ciliates in which nuclear transfer has been observed at the end of the first division. The mechanism of conjugation activity has apparently been accelerated to bring about nuclear passage two generations ahead of the time usually required: the tips of the paroral cones have broken down and the macronuclear skein is far advanced. The latter seems to be precocious and attuned to the prospective activity of the micronuclei. Transfer of the micronuclei may be unidirectional, resulting in the asymmetrical condition of one nucleus in one member and three in the recipient, or reciprocal (interchange), or, probably, there can be non-passage. Such a selection suggests a chance determination. A pinching or constriction of the "migratory" nucleus before and during passage may be extreme —so severe as to cause a complete separation of fragments from the nuclei. These may persist and continue an apparently independent existence. (One hesitates to apply the terms "migratory" and "stationary" nuclei to the products of the first division, with the implication that these are reduced nuclei and that they invariably are involved in interchange.)

As in other accounts of conjugation in *P. trichium* (Diller, 1948), macronuclear passage may occur in abbreviated conjugation, after micronuclear migration. The macronuclear exchange may be either reciprocal, unidirectional, or, probably, omitted. In case of unidirectional micronuclear passage, macronuclear exchange is not necessarily along the same path, i.e., from the conjugant with one nucleus into the one with three nuclei, but may be in the opposite direction from the trinucleate to the uninucleate conjugant.

Also, as in other processes of conjugation in *P. trichium*, the subsequent micronuclear activities may be variable: fertilization (cross-fertilization or autogamy), parthenogenetic development, or combinations of fertilization and parthenogenesis in the two members of a pair or even, probably, in the same member of the pair. The versatility, lability and variability of micronuclear activity in *P. trichium* should be susceptible to experimental attack and analysis.

SUMMARY

1. A process of "abbreviated" conjugation occurs in one race of *P. trichium* in which the number of micronuclear divisions is reduced to three (or possibly four) from the "standard" pattern of five or six.

2. There may be exchange of micronuclei at the conclusion of the first division. Frequently, unidirectional passage of a gametic nucleus occurs at this time so that an asymmetry results in the two conjugants, one of them having three micronuclei and the other conjugant one micronucleus.

3. The products of the first division proceed, directly, to reconstitute the new nuclear apparatus. This they do by synkaryon formation, parthenogenetic development or a combination of the two, usually dividing twice. There is no degeneration of nuclei between divisions.

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