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Direct Observation of the Transfer of Pronuclei in Living Conjugants of *Paramecium bursaria*[1]

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At the present time three sexual processes have been described for *Paramecium* in which three micronuclear (pregamic) divisions lead to synkaryon formation. In one, called conjugation (cross-fertilization), there is a temporary union of two individuals involving three micronuclear divisions, *nuclear exchange,* and the establishment of a new synkaryon in each conjugant. Thus, a sine qua non of conjugation is nuclear exchange. The second process, called autogamy (self-fertilization), was described by Diller (*2*) for *P. aurelia* as occurring in *single* animals only. Here, three micronuclear divisions lead to the formation of gametic nuclei which fuse and form a synkaryon in the single animal without the cooperation of another individual.

Earlier investigators used killed and stained paramecia for their cytological studies. Beginning in 1936 the author approached the problem of cytological study of nuclear behavior in joined paramecia with an entirely new method. Studies were made on *living* joined pairs of paramecia which were isolated and placed in a precision microcompression chamber so that direct observations could be made under the microscope. This method led to the discovery of a third and new sexual process called *cytogamy.* In this process, the three pregamic divisions occur as in conjugation, but, instead of a transfer of pronuclei as in true conjugation, the pronuclei remain within the confines of each joined individual and a synkaryon is formed in each (*8*). The classical accounts of conjugation wherein nuclear exchange is supposed to be a constant feature were questioned.

More recently, a rather lively interest has developed in the problems of exchange of pronuclei and cytoplasm during the conjugation process. On the general problem papers have appeared giving cytologic, genetic, and serologic evidence of the transfer of pronuclei or cytoplasm or both during conjugation. Chen (*1*), using fixed and stained preparations of *P. bursaria* presents cytological evidence of pronuclear transfer. In addition, he reports some cases in which the two pronuclei remain in the same conjugant and fuse to form a synkaryon (cytogamy). Sonneborn (*7*) presents genetic evidence to show that there is nuclear and cytoplasmic transfer in *P. aurelia* during conjugation, and he also shows genetic evidence for cytogamy. He reports that the transfer of cytoplasm is crudely measured by the extent of the time interval between separation of conjugants at their anterior ends and separation at their paroral cones. If the interval is less than 3½ minutes, exchange of cytoplasm is not detected, regardless of what races were crossed, but when the interval is 20 minutes or more, "cytoplasmic factor" is invariably transferred. Further, he reports that exchange of cytoplasm at conjugation never occurs in crosses in certain races (although nuclei are exchanged in these crosses) but does occur in others. Concerning nuclear exchange, Sonneborn (*6*) reported that there is a definite temperature factor involved in cross-fertilization and some indication that calcium increases the frequency of it and that sodium decreases it. Harrison and Fowler (*4*) present serologic evidence of cytoplasmic interchange during conjugation of *P. bursaria.* They believe that the antigen involved in the reaction is very largely, if not exclusively, cytoplasmic in character and that there is an extensive interchange of cytoplasm.

In view of what has been done thus far, it was thought desirable to study the conjugation process in the *living* condition in an effort to obtain information on nuclear and cytoplasmic behavior and determine the time relationships involved. From a large number of races of five different species of *Paramecium,* two races of *P. bursaria,* of opposite mating type, seemed pre-eminently suited for direct observation on living conjugants when placed in the microcompression chamber. One race, B9, is composed of large green specimens due to the presence of zoochlorellae, while the other race, 255 (kindly supplied by Dr. J. A. Harrison), consists of smaller paramecia which are colorless because of the complete absence of zoochlorellae. At any time or in any stage one is able to identify the members of each race in the conjugation process. The mating reaction is much like that reported for this species by Jennings (*5*). Here the organisms begin showing a feeble mating reaction at 4:45 A.M. at 25° C., concomitant with the appearance of daylight. The reaction gradually increases in intensity until at 8:00 A.M. it is strong

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and continues so through the noon hour. The intensity of the reaction gradually decreases after 1:30 P.M., becoming feeble again at approximately 4:30 P.M. For this study, single joined pairs were isolated as soon as formed and then placed in the microcompression chamber for observation.

In conjugation the three pregamic divisions occur as follows at 25° C.: completion of first division, approximately 14 hours after mating; completion of second division, an hour after the first; the third division, involving exchange of pronuclei and syngamy, 16–18 hours after mating. One can see that the micronuclear product, which remains near the paroral region after the first and second divisions, generally is the one destined to undergo a succeeding division. The one farthest from that region degenerates. The dividing spindle of the third pregamic division is seen to arrange itself at nearly right angles to the long axis of the conjugant and press against the membrane at the point of crossing. It takes about 20 minutes for the migratory pole of the spindle to pass from one conjugant to the other. Cyclosis in each conjugant is slower than usual, and crystals are seen to bunch themselves around the dividing spindles.

After syngamy, the three amphinuclear (metagamic) divisions are completed, respectively, as follows: the first, approximately 20 hours after mating; the second, 22 hours; and the third, 24 hours. Conjugants frequently are seen completing the third amphinuclear division at the time of separation 24 hours after mating. All of the time relationships given here were recorded at a temperature of 25° C. and differ widely from those of Hamburger (*3*). Temperature is an important factor regarding the length of time the animals remain joined together in the process.

It is noteworthy that in no case was a visible transfer of cytoplasm or of zoochlorellae observed during the conjugation of these mating types.

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News and Notes

The recently organized Emergency Committee of Atomic Scientists of which Albert Einstein is president and Harold C. Urey is vice president, met on 17 November in Princeton, New Jersey, to devise means of raising a $1,000,000 educational fund to aid in enlightening the public on the social implications of nuclear energy. The Committee has announced six objectives of the educational campaign and various members of the committee and others are available to speak on these points in various parts of the country.

The six statements of fact follow:

1) Atomic bombs can now be made cheaply and in large number. They will become more destructive.

2) There is no military defense against atomic bombs and none is to be expected.

3) Other nations can rediscover our secret processes by themselves.

4) Preparedness against atomic war is futile, and if attempted, will ruin the structure of our social order.

5) If war breaks out, atomic bombs will be used, and they will surely destroy our civilization.

6) There is no solution to this problem except international control of atomic energy and ultimately, the elimination of war.

Aside from Prof. Einstein and Dr. Urey, the other members of the Committee are: Selig Hecht, Columbia University; Victor F. Weisskopf, Massachusetts Institute of Technology; Leo S. Szilard, University of Chicago; Hans A. Bethe, Cornell University; Thorfin R. Hogness, University of Chicago; Philip M. Morse, on leave from Massachusetts Institute of Technology; and Linus Pauling, University of California.

A Committee on a Junior Scientists' Assembly, with Morris Meister, president of the National Association of Science Teachers, as chairman, has recently been appointed by the AAAS. This Committee is working to bring together young scientists who are still in the midst of their scientific training, so that they may share their experiences and opinions. The Assembly of Junior Scientists was planned in realization of the increasing importance of the role of young scientists. Many young persons during the war were taken directly from their studies and placed on important research teams where they assumed great responsibility. Most of these young men and women have now returned to school and are in a position to look back at their recent secondary studies and evaluate them for teachers and for science-minded high school pupils. They are also looking ahead toward