

Virginia were used. All these animals have similar micronuclei. During conjugation the pronuclei in the two conjugants are alike. Under such circumstances evidence of exchange of pronuclei was less convincing although there were many pairs in which an exchange evidently occurred. The migratory pronucleus was usually slender, the stationary pronucleus relatively short and broad. In many cases, the migratory pronuclei were observed crossing from one conjugant to the other.

Exceptional cases found in the conjugation between animals having similar micronuclei further strengthen the evidence of exchange of pronuclei in *P. bursaria*. There is usually a simultaneous movement toward each other of the two migratory pronuclei in the two conjugants, but there are a few exceptional cases in which the movement is not simultaneous. One pronucleus completes its migration by the time the other begins. Such a behavior gives rise to pairs in which one conjugant appears to have three pronuclei whereas the other has one, clearly indicating that an exchange of pronuclei takes place during conjugation in *P. bursaria*.

¹ Diller, W. F., *Anat. Record* (supp.) **60**, 92-93 (1934).

² Wichterman, R., *Biol. Bull.*, **73**, 396-397 (1937); *Id.*, *Ibid.*, **75**, 376-377 (1938).

³ I am greatly indebted to Prof. H. S. Jennings for these races of *P. bursaria*.

⁴ See Woodruff, L. L., *Quart. Jour. Micros. Sci.*, **74**, 537-545 (1931).

⁵ In some cases the two pronuclei in *Gr14* remain in the same conjugant and fuse to form a syncaryon.

*CONJUGATION IN PARAMECIUM BURSARIA BETWEEN
ANIMALS WITH VERY DIFFERENT CHROMOSOME
NUMBERS AND BETWEEN ANIMALS WITH AND
WITHOUT MICRONUCLEI*

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(1) *Conjugation between Animals with Very Different Chromosome Numbers.*—In *Paramecium bursaria* different races may have very different chromosome numbers. A study of ten races of *P. bursaria* shows that in one race (*Fd*) the chromosome number is apparently about eighty while in each of the other nine races the chromosome number is very much greater, running up to several hundred. A probable explanation of this increased number of chromosomes is the occurrence of polyploidy.¹

Races of *P. bursaria* with very different chromosome numbers can conjugate with each other, for example, the two races—*McD*₃ and *Fd*.² These

two races belong to two different mating types and differ in the size of the micronucleus and in the quantity of chromatin contained in it. These differences are correlated with the difference in chromosome number. *McD₃* is a polyploid race having several hundred chromosomes. It has a large, deeply staining micronucleus which contains a large quantity of chromatin. *Fd* has apparently about eighty chromosomes. This race has a small, lightly staining micronucleus which contains a small quantity of chromatin.

Under appropriate conditions, individuals belonging to these two races will immediately agglutinate when mixed and soon form pairs. In such a mixture the progress of nuclear changes is quite uniform in all the pairs; this is especially true in the earlier stages of conjugation. By isolating and fixing every hour a number of pairs from such a mixture (with subsequent staining) it is possible to trace with considerable accuracy the successive nuclear changes during conjugation and the time intervals between these different stages. In the present investigation this has been done. Many other preparations were also made of pairs which were isolated from other mixtures made at different dates which may be far apart. One or more sets of slides were made of the conjugants from each mixture, each set containing animals which were fixed at one time. From the same mixture a number of conjugants may be fixed about six hours after onset of conjugation, another group of conjugants fixed about eleven hours after onset of conjugation, etc. The time intervals between the onset of conjugation and the time of fixation are known for all the material used in the present study.

During conjugation between animals with very different chromosome numbers the nuclear changes in both conjugants are normal. The micronucleus in each conjugant undergoes three pregamic divisions. The first pregamic division is a very long process while the second and the third divisions require a very much shorter length of time for their completion. After the first pregamic division, one of the two micronuclei degenerates while the remaining one undergoes the second pregamic division, giving rise to two micronuclei. One of the two micronuclei from the second division degenerates, leaving only one micronucleus which undergoes the third pregamic division. This last pregamic division gives rise to two pronuclei. Throughout these stages it is clearly observed that the micronucleus in one conjugant (*McD₃*) is much larger and contains a much greater quantity of chromatin than the micronucleus in the other conjugant (*Fd*). The two pronuclei in the *McD₃* conjugant are also much larger and stain much more deeply than those in the *Fd* conjugant. At room temperature exchange of pronuclei usually occurs 28-31 hours after the onset of conjugation. After the exchange of pronuclei each conjugant contains one large, darkly staining pronucleus and one small, lightly staining pronucleus. These two pronu-

clei (different in size, staining capacity and in quantity of chromatin) in each conjugant fuse and form a syncaryon. The syncaryon in one conjugant appears to be the same as that of the other conjugant and they both divide three times before the conjugants separate. After the first division of the syncaryon, one of the nuclei degenerates, and the other divides twice, giving rise to four nuclei. The nuclei resulting from the divisions of the syncaryon are alike in both conjugants. Thus before conjugation the two conjugants are different in the size of the micronuclei and in the number of chromosomes but after conjugation they are alike.

(2) *Conjugation between Animals with and without Micronuclei.*—In *P. bursaria* animals with and without micronuclei can conjugate with each other, for example, the two races—*Gr14* and *S*. They belong to two different mating types. Race *Gr14* has a characteristically large, darkly staining micronucleus. In race *S* most of the animals do not have a micronucleus, while some have a “ghost” micronucleus which does not stain with haematoxylin. Conjugation is readily obtained between these two races.³ A *Gr14* individual can conjugate either with an *S* individual without a micronucleus or an *S* individual with a “ghost” micronucleus.

During conjugation between *Gr14* and the amiconucleate *S*, the micronucleus in *Gr14* shows a normal behavior. It undergoes three pregamic divisions, resulting in the formation of two pronuclei. These pregamic divisions are similar to those found in conjugation between races *McD₃* and *Fd*. The amiconucleate conjugant produces no pronuclei. In most of the pairs one of the two pronuclei in the *Gr14* migrates to the amiconucleate conjugant. After this migration each conjugant possesses a single pronucleus (“hemicaryon”).⁴ Since *Gr14* is a polyploid race, each “hemicaryon” probably still contains several sets of chromosomes. The “hemicaryon” in each conjugant behaves like a syncaryon. It undergoes three divisions before the conjugants separate. After the first division, one of the nuclei degenerates, leaving one nucleus which undergoes two divisions, giving rise to four nuclei. The nuclei resulting from these divisions of the “hemicaryon” in one conjugant appear to be the same as those of the other conjugant and they all seem to contain the same number of chromosomes. Thus before conjugation the two conjugants are different in the nuclear apparatus but after conjugation they are alike.⁵

The account given above of the transfer of one pronucleus from the *Gr14* conjugant to the amiconucleate conjugant is true for most of the conjugating pairs. In a small number of exceptional cases, no such transfer takes place. The two pronuclei in *Gr14* remain in the same conjugant and fuse to form a syncaryon. A syncaryon resulting from such autogamy behaves like those formed after an exchange of pronuclei. After the first division of the syncaryon, one of the two products degenerates and the remaining nucleus divides twice, giving rise to four nuclei. The syncaryon

and its products of divisions are larger and contain many more chromosomes than the "hemicaryon" and its products of divisions. This is to be expected since there is no reduction of chromosome number in autogamy, whereas in the case in which the transfer of pronucleus takes place the chromosome number is probably halved.

The significance of conjugation between animals with very different chromosome numbers and between animals with and without micronuclei seems to be twofold:

(1) There is considerable flexibility in conjugation of *Paramecium*. Animals with a great number of chromosomes can conjugate with animals with relatively few chromosomes. Animals with the usual micronucleus can conjugate with animals without a micronucleus.

(2) One of the results of conjugation in *Paramecium* is the elimination of the great diversities in the nuclear apparatus and great difference in chromosome number that may exist between the two conjugants.

¹ See Chen, T. T., *Proc. Nat. Acad. Sci.*, **26**, 239-240 (1940).

² I am greatly indebted to Prof. H. S. Jennings for the races of *P. bursaria* used in the present investigation.

³ The technique used in the present study of conjugation between *Gr14* and *S* is essentially the same as that used for the study of conjugation between *McD₃* and *Fd*.

⁴ As soon as this pronucleus in each conjugant starts to divide it should no longer be called a "pronucleus." A new term "hemicaryon" is hereby used in contradistinction to syncaryon. A syncaryon is made up of two (or more) pronuclei, whereas a "hemicaryon" is composed of only one pronucleus.

⁵ Diller reported in *P. aurelia* cases of conjugation in which one member of the pair was normal while the other member lacked micronuclei entirely. He did not describe the nuclear changes in the normal conjugant nor the result of such conjugation. See Diller, W. F., *Jour. Morph.*, **59**, 11-51 (1936).

NOVEL TYPES OF NERVE REFLEXES

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The idea of the nerve reflex has since the time of Descartes played a rôle of first importance in neurophysiology (Fearing, 1930). Like the much later concept of the neurone, as formulated by Waldeyer in 1891, it has afforded a basis of first significance for the analysis of nervous activities. Neurones have proved to be of many kinds, but the nerve reflex has remained almost true to type. Sherrington has called it the unit of functional nervous integration. Pavlov divided reflexes into the well-known