

mouse ovaries. This introduces a method that could be used for the genetic identification of some lethals and certain types of embryos that have to be killed for examination. It should prove of value in studies on differences in maternal environments, in experiments on the relation of hormonal and other factors to the development and functioning of the ovary and possibly for investigations on the origin of the germ cells. An extension of the method over successive generations, to produce animals descended from any number of unborn direct female ancestors, would provide still further possibilities for research.

\* Aided by a grant from the Rockefeller Foundation and by grants to the Roscoe B. Jackson Memorial Laboratory from the National Cancer Institute, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the International Cancer Research Foundation.

<sup>1</sup> Russell, W. L., and Hurst, J. G., these PROCEEDINGS, 31, 267-273 (1945).

<sup>2</sup> Grüneberg, H., *The Genetics of the Mouse*, Cambridge Univ. Press, 1943, 412 pp.

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*INDUCTION OF CONJUGATION IN PARAMECIUM BURSARIA  
AMONG ANIMALS OF ONE MATING TYPE BY FLUID FROM  
ANOTHER MATING TYPE\**

BY TZE-TUAN CHEN

DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF CALIFORNIA, LOS ANGELES

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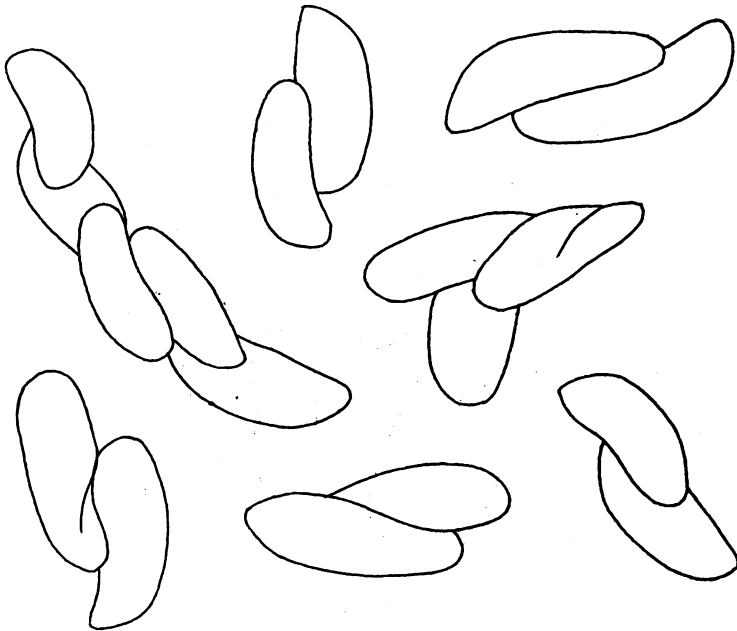
In these experiments, it was found that animal-free fluid from a Russian clone (Ru22) induces clotting and conjugation among animals of another mating type, even though the latter belongs to a different variety.<sup>1</sup>

Most of the present work was done on the effects of fluid from cultures of a Russian clone (Ru22) on animals of mating types belonging to four other varieties. This Russian clone (together with a number of other Russian clones) was sent to the present writer from Moscow by Professor G. F. Gause. Jennings and Opitz<sup>2</sup> found that this clone did not mate with any of the four varieties known at that time and they were of the opinion that this clone possibly belongs to a fifth variety. In the present paper it is called the fifth variety. (Apparently only one mating type of this variety has been found.)

The animals of this Russian clone (as well as those of other clones referred to in this paper) were cultured in essentially the manner described by Jennings.<sup>3</sup>

In testing the effect of fluid from the Russian clone on animals of another mating type, the fluid is first taken out of the culture (Ru22) with a micro-

pipette; it is then placed in depression slides and carefully examined under a dissecting microscope. All the animals, if any are accidentally included in the fluid, are removed. Then a number of animals of the clone to be tested, together with a very small quantity of fluid from this clone, are added to the Ru22 fluid. The depression slides are then left in a moist chamber and examined from time to time. As a control, fluid from the clone to be tested is first taken out of the culture, placed in a depression slide, and then animals from the same culture (or another culture of the same clone) are added.



|a

FIGURE 1a

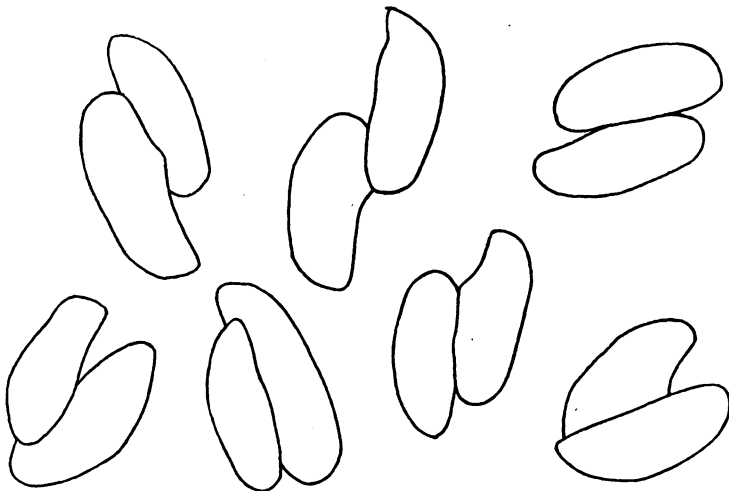
Clotting and pair-formation among animals of an English clone (En1) induced by fluid from a Russian clone (Ru22).

The fluid from cultures of the Russian clone (Ru22) was tested on animals of a number of mating types belonging to four other varieties (II, III, IV, VI) and especially on animals of an English clone (En1) belonging to the sixth variety.

*Effects of the Ru22 Fluid on Animals of the English Clone (En1).*—This English clone (En1) was collected by Professor E. G. Pringsheim in Cambridge, England, and was sent by him to the present writer, who subsequently made numerous tests on it. The results so far obtained show that it will not mate with animals of any of the known varieties but will con-

jugate readily with animals of a clone collected from Prague, Czechoslovakia (also sent by Professor Pringsheim). It seems clear that this English clone together with the Czechoslovakian clone constitute a new variety, which is designated as Variety VI.

**Clotting:** The animal-free fluid from Ru22 cultures apparently renders the surface of the animals of the English clone sticky so that the animals adhere to one another, forming clots and pairs (Fig. 1*a*). No such clotting or pair-formation was ever observed in the controls. The clotting or group-formation of animals of the English clone (one mating type)<sup>4</sup> induced by the fluid of the Russian clone (another mating type) differs somewhat from the clotting which occurs when animals of diverse mating types are mixed.



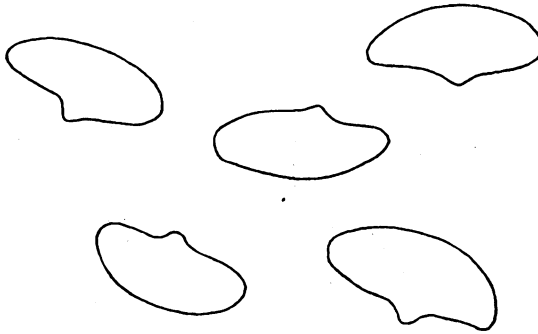
1b

FIGURE 1b

Pairs formed among animals of an English clone (En1) induced by the fluid from a Russian clone (Ru22).

The difference is twofold: (1) The clotting among animals of the same mating type occurs much later than that which usually occurs when animals of diverse mating types are mixed. In the former case, agglutination occurs 4-6 (usually more) hours after the animals of one mating type are introduced into the fluid of another mating type; in the latter case, agglutination occurs immediately or almost immediately. (2) The clots formed by animals of the same mating type are much smaller than those formed as a result of mixing animals of diverse mating types. In induced group-formation, each clot usually consists of 2-7 individuals. Clots formed as a result of mixing animals of diverse mating types are usually larger; each of the larger clots often consists of many individuals.

Conjugation: Pair-formation among animals of the same mating type<sup>5</sup> differs somewhat from pair-formation when those of diverse mating types are mixed. In the first place, in induced conjugation, the pairs are formed much later (4-6 or more hours after the animals are introduced into the fluid of another mating type). When animals of diverse mating types are mixed, usually many pairs are formed in one or two hours. Secondly, in induced conjugation many of the pairs are atypical. Although some of the pairs are hardly distinguishable from those formed as a result of mixing animals of diverse mating types, other pairs are atypical in that the two conjugants do not have the relative positions typical of normal conjugating pairs (Fig. 1*b*). Thirdly, in many pairs, the conjugants are not held as firmly together as those formed in ordinary conjugation. As a result, in a number of cases the conjugants become separated when fixed. This is true even after the animals have been conjugating for more than 20 hours.

1*c*FIGURE 1*c*

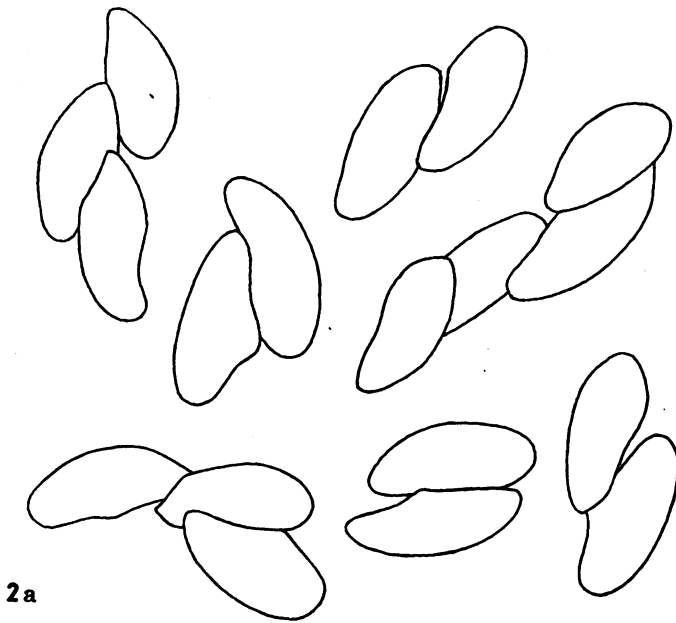
Single animals of an English clone (En1) undergoing perhaps autogamy or endomixis or some other type of nuclear processes induced by the fluid from a Russian clone (Ru22).

Fixed and stained preparations of induced pairs show that nuclear changes occur in the conjugants and anlagen are formed in the ex-conjugants. The nuclear changes in induced conjugation are being studied and will be reported on separately.

Induction of Nuclear Changes in Single Animals: The Ru22 fluid induces not only clotting and conjugation, as already described, but also nuclear changes in some individuals not associated with any others. These single animals develop a paroral cone (Fig. 1*c*). This structure has been reported in *Paramecium aurelia* undergoing autogamy (endomixis).<sup>6</sup> Preliminary cytological studies show that nuclear changes take place in these single animals. It remains to be determined whether they undergo autog-

amy, or endomixis, or some other type of nuclear processes. These single animals may be found together with the conjugating pairs; at other times, however, they may be found alone.

In addition to the induction of clotting and conjugation among animals of the same mating type and of nuclear changes in some single animals, there are other effects of the Ru22 fluid on the members of the English clone. These additional effects are recognizable even before clotting and conjugation occur. The animals become (1) sluggish in movement, (2) darker in color, and (3) distorted in form to a greater or less extent. No such changes were found among the animals in the controls.



2a

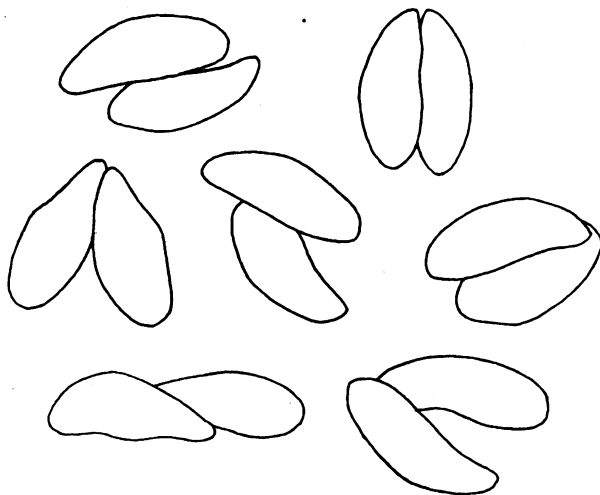
FIGURE 2a

Clotting and pair-formation among animals of an American clone, MM17 (Variety II), induced by fluid from a Russian clone (Ru22).

It should be noted that only a percentage (usually small) of the animals are visibly affected by the Ru22 fluid, and hence only a small number of them undergo clotting and conjugation; while a few solitary animals undergo nuclear changes. It is difficult to understand why only a percentage of animals are obviously affected. It seems probable that there are differences in physiological condition even among the animals of the same culture. It thus seems that those individuals which are affected differ from others added to the fluid at the same time.

*Effect of Washing Animals.*—In order to determine whether or not induction of clotting and pair-formation among members of the English clone En1 by the Ru22 fluid might be due to some impurities in the cultures of the Russian clone, on November 23, 1944, a number of the animals of this Russian clone were carefully washed and then recultured. Some weeks later the fluid was tested again on the animals belonging to the English clone En1. Clotting and pair-formation were again observed, as described above.

*Effects of the Ru22 Fluid on Animals Belonging to Some Additional Varieties.*—The fluid of the Russian clone Ru22 is apparently effective in inducing conjugation throughout the species since it induces conjugation in all the four varieties so far tested. The one other variety (I) is being tested.



2b

FIGURE 2b

Pairs formed among animals of an American clone, MM17 (Variety II), induced by the fluid from a Russian clone (Ru22).

In addition to inducing conjugation among the animals of the English clone (En1) belonging to the sixth variety, this Ru22 fluid also induces clotting and conjugation in each of the following clones: MM17 (mating type F) and Gr13 (type K) of the second variety, clones Gr1 (type N) and Pi3 (type O) of the third variety, and clone Ru3 (type R) of the fourth variety.<sup>7</sup> Clotting and pair-formation in these varieties are essentially the same as those which occur among animals of the English clone as described above (cf. Figs. 1a, 1b and 2a, 2b). No clotting or conjugation was ever observed in the controls. Some other clones of these three varieties were

also tested but as yet no clotting or conjugation has been observed. These negative results might be due to differential effects of the Ru22 fluid on different clones of the same variety.

*Discussion.*—The phenomena in *P. bursaria* described in this paper are possibly related to those of "sex stuffs" found in certain algae (Geitler,<sup>8</sup> Moewus<sup>9</sup>) and Protozoa (Kimball<sup>10</sup>). In *Tetraspora lubrica* Geitler found that centrifugates of culture fluid of one sex cause atypical group-formation among cells of the other sex. Copulation, however, does not follow this type of group-formation. In *Chlamydomonas eugametos* Moewus discovered the same effect of filtrates or centrifugates of culture fluid of one sex on individuals of the other sex. In *Euplotes patella* Kimball found that conjugation can be induced between animals of the same mating type by placing them in fluid in which another mating type has been living.

Another possibility is that the phenomena in *P. bursaria* described in this paper are similar to those of the "killers" as reported by Sonneborn<sup>11</sup> for *P. aurelia* in view of the following facts: (1) The Ru22 fluid makes the animals of the English clone En1 sluggish, darker and distorted in shape and these phenomena are similar to those in lethal interactions between diverse stocks in *P. aurelia*. (2) In *P. aurelia* Sonneborn (unpublished) found that one of the "killers," among other effects, induced pairing as described in this paper for *P. bursaria*.

\* Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the University of California.

<sup>1</sup> The fluid of two other Russian clones (Ru21, Ru30) appears to have the same effects as those of Ru22 fluid. These three clones belong to the same mating type and are characterized by a large body and an unusually large micronucleus. These clones are vigorous; flourishing cultures can be easily obtained.

<sup>2</sup> Jennings, H. S., and Opitz, P., *Genetics*, **29**, 576-583 (1944).

<sup>3</sup> Jennings, H. S., *Ibid.*, **24**, 202-233 (1939).

<sup>4</sup> Of course, it is also possible, though not probable, that the Ru22 fluid causes a change in mating type in some of the animals of the English clone, and hence in this case it is merely a conjugation between animals of diverse mating types.

<sup>5</sup> In addition to the conjugating pairs, some "threes" were also observed. (A group of three animals in conjugation is to be called a "three.") In each "three," nuclear changes occur in all three conjugants and anlagen are formed in the ex-conjugants.

<sup>6</sup> Diller, W. F., *Jour. Morph.*, **59**, 11-67 (1936); Caldwell, L., *Jour. Exper. Zool.*, **66**, 371-407 (1933).

<sup>7</sup> As yet only a few clones of these three varieties have been tested. It is likely that induced conjugation will be found in more clones when a greater number of clones are tested.

<sup>8</sup> Geitler, L., *Biol. Zentralbl.*, **51**, 173-187 (1931).

<sup>9</sup> Moewus, F., *Arch. Protistenk.*, **80**, 469-526 (1933).

<sup>10</sup> Kimball, R. F., *Genetics*, **27**, 269-285 (1942).

<sup>11</sup> Sonneborn, T. M., *Amer. Nat.*, **73**, 390-413 (1939).