FACTORS DETERMINING CONJUGATION IN PARAMECIUM AURELIA III. A GENETIC FACTOR: THE ORIGIN AT ENDOMIXIS OF GENETIC DIVERSITIES

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INTRODUCTION

In a preceding paper of this series (SONNEBORN and COHEN 1936) it was shown that different stocks of Paramecium aurelia differ greatly in their innate tendency to conjugate. In the present paper it is shown that genetic differences in this respect sometimes (though rarely) arise within a single clone at endomixis.

Erdmann (1920), Caldwell (1933) and others have reported the occasional origin of genetic diversities at endomixis; the literature of the subject was reviewed by Caldwell. One of the cases described by Caldwell showed a change in relation to conjugation; that is, a biotype arising at endomixis showed early a reduced tendency to conjugate, and later a complete loss of the power to conjugate.

The cases here reported belong to the same stock R of Paramecium aurelia that was studied by Caldwell. Sonneborn (1936) has shown that in this stock conjugation can ordinarily be readily induced during several days immediately following endomixis; but that after this period has passed, another endomixis must occur before conjugation can be induced. In two cases, however, individuals emerging from endomixis gave rise to biotypes that were strikingly atypical in their behavior with respect to conjugation as well as in some other respects. These form the subject of the present paper. The methods and precautions employed have been earlier described (SONNEBORN 1936). As in previous work, conjugation tests were made by setting up, from the individuals produced in isolation cultures, small mass cultures which are kept at 31°C.

BIOTYPE T

Biotype T took origin at an endomixis occurring in an isolation culture on March 6, 1935; it was under observation in isolation lines or small mass cultures till August 27, 1935. Its characteristics were found to differ from those of other members of the stock, so that a detailed examination was made of it, in comparison with a number of lines closely related to it. The relationships of the various lines compared are shown in figure 1.

Between March 19 and May 27, thirteen standard conjugation tests were made with members of biotype T under conditions that induce conjugation in other biotypes of this stock (SONNEBORN 1936). The results
of these tests agreed with many additional tests made later under less rigorous conditions.

In none of these numerous tests did conjugation occur: not a single united pair was ever observed. Yet in most of the mass cultures, invariably in those observed for six days or longer, behavior occurred which is usually preliminary to conjugation. Groups of two to six individuals swam about in contact, as ordinarily occurs before completed conjugation. This behavior sometimes continued for as long as 12 hours in a given pair of animals, but conjugation was in no case completed. This type of behavior was characteristic of biotype T throughout the 5½ months that it was under observation.

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**Figure 1.**—Origin and relationship of biotypes T and O and collateral groups of biotype R₁. Horizontal lines represent daily isolation cultures of groups of one or more parallel lines of descent. These groups are labelled A, B, C, D, E, F, and G. Vertical dotted lines indicate the origin of new groups subcultured from older ones. The first to fifth endomixes since January 28 in any one direct line of descent are represented by the symbols e₁, e₂, e₃, e₄, e₅.

The ancestors of biotype T were typical in respect to conjugation. They were in isolation cultures from January 28, with endomixis on January 29, but not again till March 6. Between February 15 and March 5, conjugation tests were set up daily, by the same method employed for biotype T. In all 22 of these cultures endomixis occurred first and was followed by abundant conjugation. This is the behavior typical of stock R.

Three groups of lines descended from the ancestors of biotype T (groups A, B, and C of fig. 1) were similarly tested. In group A, which did not go through endomixis after the initial one of January 29, conjugation tests were made daily from March 6 to March 25. In all 21 of the tests endomixis occurred first and was followed by abundant typical conjugation. In
groups B and C, which had gone through endomixis on March 6 at the same time as had biotype T, 23 conjugation tests were made. The 11 tests made March 7 to 17 gave abundant conjugation within 24 hours; the remaining 12 tests (March 18 to 24) underwent first endomixis, then conjugation.

Thus it is clear that the peculiar behavior of biotype T under the standard conditions favoring conjugation did not occur in its ancestors nor in collateral lines descended from the same ancestors, regardless of whether they had or had not been through endomixis. The endomixis of March 6 altered a single individual in such a way that its descendants, when subjected to the conditions that ordinarily induce conjugation, make the initial steps toward but do not complete conjugation.

**BIOTYPE 0**

Biotype 0 arose from a single individual after the fifth endomixis (April 18) in the direct line of descent from individual A (fig. 1), isolated January 28, 1935; it was under observation till May 29. It showed certain unique features in its behavior under conditions favorable to conjugation, as appears from detailed comparisons with various groups of its relatives. Its relationship to biotype T and to other groups is shown in figure 1. Between April 25 and May 29, thirty-two standard conjugation tests were made with members of biotype 0. During this time the isolation lines from which the test cultures were made did not go through endomixis. The period most favorable for conjugation is known to be the first seven days after endomixis (Sonneborn 1936). Thus none of the tests made between the dates mentioned occurred in this favorable period, so that an endomixis in the mass cultures would be expected before conjugation could be induced.

The behavior of biotype 0 in the tests did not conform to that expected. Of seven tests made between the 7th and 16th days after endomixis, three yielded conjugants, though endomixis was infrequent. The 25 later tests all yielded endomixis in increasing proportions, but in only six did conjugation occur.

Furthermore, most of the conjugants did not go through the preliminary endomixis that is usual under these conditions though some of the cultures were set up as late as 34 days after endomixis. This was shown by the fact that conjugation as a rule occurred in the first two days of the culture, when other individuals were undergoing endomixis; and the conjugants when stained had the macronuclei whole or in early ribbon stages, while the non-conjugants were mostly in the ascending phase or climax of endomixis. Some of the conjugants included individuals that were undergoing endomixis, having anlagen of the new macronuclei well developed (a condition reported for other biotypes by Sonneborn 1936).
In none of the nine conjugating cultures were more than 10 pairs of conjugants found, though there were hundreds of non-conjugants; the usual occurrence of conjugation on a large scale after endomixis did not occur in biotype O. In one case four cultures having a majority of the animals in endomixis were combined to produce a culture of 750 animals: there resulted only two pairs of conjugants.

Thus in biotype O conjugation occurred only rarely, as compared with the usual large proportions of conjugants in similar cultures of other biotypes of stock R. Furthermore, in biotype O conjugation did not show the relation to endomixis usual for stock R, but could occur at practically any interval after endomixis.

For comparison with biotype O, its ancestors and collateral relatives (groups E and F, fig. 1) were tested daily between the second and third endomixes, for a period of 18 days (February 27 to March 16). The eight cultures set up between the third and tenth days after the endomixis of February 23 all showed the response typical for stock R: they yielded great epidemics of conjugation within 8 to 24 hours. The five cultures set up between days 11 and 14 gave the typical response for this period: high proportions of endomixis within 24 hours and at the same time a few pairs of conjugants. Similarly, the six cultures set up between days 15 and 20 all went into endomixis within 24 hours and yielded conjugants 12 to 48 hours later.

The lines of group B, which were derived from the ancestors of biotype O and had been through four endomixes, were tested daily after the endomixis of April 14, beginning April 18 and continuing until May 29. Altogether, 39 tests were made. The seven cultures set up between the 4th and 10th days after the climax of endomixis all yielded great epidemics of conjugation in 12 to 48 hours. The 32 cultures set up between the 11th and 45th days all went into endomixis within 24 hours. In the 30 of these cultures that were followed further, conjugants also appeared.

In the lines of group G, which were descended from the ancestors of biotype O and had been through five endomixes, conjugation tests were made daily after the endomixis of May 4, from May 5 to May 29. The ten cultures set up on the first ten days of this period all gave great epidemics of conjugation within 24 to 36 hours. Except for the appearance of a few conjugants in the culture set up on the 12th day, all 12 cultures set up from the 11th to the 24th day went first into endomixis. All of these that were followed two days longer soon conjugated.

Thus the peculiar responses of biotype O to the standard conditions favoring conjugation were confined to the descendants of a single individual in endomixis on April 18, 1935. They did not exist prior to that date in the ancestors of biotype O, nor did they exist either before or after a corresponding number of endomixes in collateral lines of descent. Endo-
# Table 1

Comparison of the mean daily fission rates of biotypes T and O with the mean daily fission rates of their ancestors and collaterals. $E = \text{endomixis.}$

<table>
<thead>
<tr>
<th>Period</th>
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<th>April</th>
<th>May</th>
</tr>
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<td>3.2</td>
<td>3.1</td>
<td>3.9</td>
</tr>
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<td>4.0</td>
<td>1.8</td>
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<td>Collateral groups B and C</td>
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<td>3.7</td>
<td>3.5</td>
<td>3.8</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Period</th>
<th>March</th>
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<tbody>
<tr>
<td></td>
<td>0-13</td>
<td>14-18</td>
<td>19-23</td>
</tr>
<tr>
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<td>Biotype O</td>
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<tr>
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<td>4.0</td>
</tr>
<tr>
<td>Collateral group G</td>
<td>3.9</td>
<td>3.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>
mixis altered one individual (and only one) in such a way that its descendants conjugated rarely and sporadically even at extraordinarily long intervals after endomixis.

**CHANGES IN OTHER CHARACTERISTICS, AND THEIR RELATION TO THE CHANGES IN TENDENCY TO CONJUGATE**

The changes in the tendency of biotypes T and O to conjugate were accompanied by changes in a number of other characteristics. These other changes in hereditary characters are of interest in themselves as illustrations of the types of genetic alterations producible by endomixis and they are also of interest as affording clues to the basis of the changes in tendency to conjugate. The main changes were in the following five characteristics.

1. **Fission rate**

Table 1 shows that the mean fission rates of the ancestors of biotypes T and O and of collateral groups from the same ancestors were maintained at between three and four fissions per line per day; but that the rate of biotype T fell to about 0.8 fission per line per day, and of biotype O to about 1.8 fissions per line per day. The fission rate in biotype O was thus about one-half as high as the controls, and in biotype T, about one-fourth as high as the controls.

2. **Mortality rate during the interendomictic period**

In biotype T there were under observation daily an average of 22.7 interendomictic lines for 42 days (April 9 to May 21). During these 952 line-days, 228 lines died; the mortality rate was thus 24.0 deaths per 100 line-days. In the controls of groups B and C during the same period there were but two deaths per 100 line-days. In biotype O there were under observation an average of 18.5 interendomictic lines for 42 days (April 18 to May 30). During these 776 line-days there were 44 deaths; the mortality rate was thus 5.7 deaths per 100 line-days. In the control groups B and G during the same period there were 1.9 deaths per 100 line-days. Thus the mortality rate in biotype T was 12 times as high as in the controls and in biotype O three times as high as in the controls.

3. **Mortality resulting from endomixis**

In biotype T, ten isolation lines went into endomixis between May 3 and May 19. In each of these lines, all individuals produced after the climax of endomixis were retained in isolation culture. Nine of the ten lines died within five days without going through a single fission. The remaining one was kept alive for 29 days, multiplying at a rate of less than 0.3 fission per line per day. The mortality rate in this line, however, was so high that only three animals were alive at the end of 29 days, when observations
were discontinued. During the same period, seven control lines of groups B and C went into endomixis and one line of descent from each of these was followed. Of the seven, three soon died and four lived and multiplied rapidly. These small samples thus suffered a rate of mortality due to endomixis of 90 percent in biotype T and 42.9 percent in the controls. In biotype 0, between May 12 and May 26, thirty-three isolation lines went into endomixis. As with biotype T, an attempt was made to culture all the progeny produced after the climax of endomixis; however, 21 failed to divide, nine divided once, two divided twice, and one divided three times; all died within three days. During the same period 12 isolation lines of the control groups B and G went into endomixis, and attempts were made to cultivate one line of descent from each. Of these, five soon died and seven lived and multiplied rapidly. These samples yield a rate of mortality due to endomixis of 100 percent in biotype O and 41.7 percent in the controls. That the mortality rate in biotype O was not always 100 percent was shown by a study of 20 endomictic animals removed from mass cultures. Of these, 18 died within three days (17 failed to divide and one divided three times); but two lived and multiplied well. Further studies on the descendants of these two viable endomictic individuals are given below.

4. Chain formation

In the isolation lines of biotype T there appeared 78 chains (that is, products of incomplete fission) in 952 line-days. In the controls of groups B and C, three chains were produced in 421 line-days. Chains thus appeared 8.2 times per 100 line-days in biotype T, 0.7 time per 100 line-days in the controls; hence, the frequency of chain formation was 11.7 times as great in biotype T as in the controls. Similar relations were shown by the mass cultures. Every mass culture of biotype T produced many chains, while these appeared rarely in the mass cultures of the controls.

In the isolation lines of biotype 0, chains appeared 23 times in 776 line-days. Among the controls of groups B and G these appeared only twice in 526 line-days. The frequency of chain production was thus 3.0 times per 100 line-days in biotype O, and 0.4 time per 100 line-days in the controls; hence chains appeared 7.5 times as frequently in biotype O as in the controls. Similar differences appeared in the mass cultures.

5. Variation in number of macronuclei

In daily stained samples from the isolation lines of biotype T, there appeared 61 individuals in which the macronuclear chromatin was organized into from two to five (usually two) large masses. Whether these are to be considered macronuclei or pieces of one macronucleus is not clear; but it is certain that they were not stages in endomixis. (According to the terminol-
ogy of Diller, 1936, they would be considered as stages in “hemixis”). In addition to these animals, seven individuals appeared which lacked all macronuclear chromatin. Altogether there were thus 68 individuals with aberrant numbers of macronuclei in 952 line-days, or 7.1 per 100 line-days. These variations were never observed in the control lines of groups B and C. In biotype O, there appeared 46 animals with extra macronuclei and six with no macronuclear chromatin, a total of 52 variants from the normal macronuclear condition in 776 line-days, or 6.7 per 100 line-days. Among the contemporary control lines of groups B and G, there were found five with extra macronuclei and none without macronuclei in 526 line-days, or 1.0 per 100 line-days. Variation from the normal number of macronuclei thus occurred 6.7 times as frequently in biotype O as in the controls. Similar relations appeared from comparisons of the mass cultures of both biotypes with their controls. Every mass culture of biotypes T and O contained individuals with aberrant numbers of macronuclei; the proportion of these ran as high as 34 percent in some cultures of T and 48 percent in some cultures of O. Among the control mass cultures running parallel to those of biotype T, not one contained a single individual of this type. Among 36 mass cultures run parallel to those of biotype O, 33 lacked individuals with an aberrant number of macronuclei, and of the remaining three, two contained a single aberrant individual, while the third contained twelve.

It appears possible to throw some light on the association of these various genetic characters and on the relation between them and the altered tendency to conjugate. The appearance of the same set of five characteristics, developed to different extents, in both biotypes T and O suggests at once that this is not an accidental similarity but is probably due to one and the same basic change. There is much to indicate that this change has been a slowing down of some of the physiological processes involved in fission. This takes two forms: a reduction in the frequency of fission, as shown in the mean fission rates, and a slowing down of the act of fission itself, as shown in the extreme case by chain production, the permanent failure of plasmotomy at fission. In the less extreme cases, plasmotomy is possibly not entirely prevented, but merely delayed for some time after nuclear division, so that there is considerable opportunity for unequal distribution of the products of nuclear division. The large number of animals showing extra macronuclei or no macronuclei are evidences that such unequal distribution did occur. Further, direct evidence was provided by an examination of the macronuclear condition in the chains. While some had one macronucleus in each unseparated daughter cell, many had unequal numbers in the two cells. Thus there were chains in which one cell had no macronucleus, the other from zero to three macronuclei; other combina-
tions observed were one in one cell and two to five in the other, two in one cell and two or three in the other, and three in one cell and five in the other. The two cells of a chain were frequently of unequal size, and in addition, unequal products of completed fission were found twenty-five times in the isolation lines of biotype T. The high mortality observed during the interendomictic period was certainly due, at least in part, to the production of non-viable chains and amacronucleate individuals. The apparently close connection between all these events leads us to interpret the individuals possessing macronuclear chromatin in two or more large masses as multimacronucleate rather than as possessing products of macronuclear fragmentation. On this view, we have here not a "hemictic" type of macronuclear reorganization (Diller 1936), but a multimacronucleate condition resulting from irregular distribution of the products of macronuclear division following delayed plasmotomy. In view of the irregularities observed in the distribution of cytoplasm and macronuclei, it would be surprising if the micronuclei were not also distributed irregularly. Our temporary aceto-carmine preparations viewed daily in large numbers did not permit detailed observation of micronuclei, but the high mortality following endomixis and especially the stage at which death occurred are consistent with this interpretation. Most of the endomictic individuals never proceeded beyond the climax stage of endomixis; in only a small proportion was there any evidence of the formation of a new macronucleus. Possibly many of those which failed to form a new macronucleus were unable to do so because they lacked a micronucleus. The point of view expressed above brings together into a unified interpretation all of the peculiar characteristics of the two atypical biotypes.

The absence of conjugation in biotype T and its rare occurrence in biotype O are in a large measure explicable in view of the low fission rate and high mortality of these biotypes. As shown by Sonneborn (1936), conjugation normally occurs in this stock of P. aurelia only during the week or so following endomixis (or conjugation). The earliest mass cultures for the induction of conjugation were set up 13 days after endomixis in biotype T and seven days after endomixis in biotype O. It was necessary, therefore, to bring about another endomixis before conjugation would occur in abundance. Endomixis was in fact induced in these cultures, but, as has already been stated, 90 percent to 100 percent of the endomictic animals died within three days. Most of them died without dividing, a few divided as much as three times, and only very exceptional ones divided more than this. Hence, a very small proportion of any culture was in condition to conjugate, and this proportion was subject to little or no increase because of the slow rate of fission and the high rate of mortality. It is of interest that the biotype with the lower fission rate and the higher
mortality produced no conjugants, while the one with the higher fission rate and lower mortality did produce conjugants in small numbers. It seems probable therefore that the absence of conjugants in biotype T and their rare occurrence in biotype O was a direct consequence of the absence or great rarity of viable recent exendomictic individuals.

The validity of the preceding explanation is further attested by two other types of observations. One type was made on certain clones that lost the tendency to conjugate after a period in which conjugation occurred but resulted only in non-viable exconjugants. Clones W64 and W103 were derived from two conjugants obtained July 16, 1931, during the second successive inbreeding of the slowly multiplying clone N21 (Sonneborn and Lynch 1934), also of stock R of *P. aurelia*. These two clones multiplied slowly, as did their grandparent clone N21, and they could readily be induced to conjugate during the first five months of their existence. All exconjugants derived from them proved non-viable. During the remaining 13 months that they were under observation, these clones could not be induced to yield a single pair of conjugants. The similarity of these clones to Caldwell’s (1933) biotype o9a is obvious. Accordingly, when Caldwell’s biotype o9a lost the power to conjugate, an attempt was made to investigate the cause of the loss. It was found that all endomictic individuals became abnormal by the time they reached the climax stage and never divided again. There was thus a complete extermination of the only animals that could possibly conjugate.

The other type of observation was made upon individuals that had not undergone endomixis for very long periods (more than 100 days). The technique of obtaining such individuals will be given in a later paper by Sonneborn, who found that these individuals never survive endomixis. When conjugation tests are made with mass cultures of such individuals from perfectly normal biotypes, no conjugation can ever be obtained. These results, combined with the observations on Caldwell’s biotype o9a and the known mortality rate following endomixis in biotypes T and O, strongly support the explanation proposed to account for the failure of biotype T to conjugate and the rarity of conjugation in biotype O. Why preconjugation behavior occurred in biotype T is a problem on which we can at present throw no light.

**Reversion to Ancestral Characters Following Endomixis, in Biotype O**

It will be recalled that although all exendomictics of biotype T either died or showed no change of characters, two of the 53 endomictic individuals of biotype O lived and multiplied. These gave rise to two new biotypes of extraordinary interest. Both attained a fission rate of three to four
fissions per day and were therefore indistinguishable in this respect from the ancestors and collaterals of biotype O. These clones were unfortunately followed for only nine days after the climax of endomixis. Breaking this into three periods of three days each, the mean fission rates were 2.1, 2.8, and 3.1 fissions per line per day. The gradual transition from the rate of biotype T (1.8 fissions per day) to that of the standard biotypes (more than three fissions per day) is comparable to the gradual transition in the reverse direction that occurred in biotypes T and O (table 1). Both of these transition periods following endomixis are comparable to the transition in change of characters following conjugation, as reported by Sonneborn and Lynch (1934) and Degaris (1935).

The reversion to the ancestral fission rate was accompanied by a similar reversion in the tendency to conjugate in at least one of the new biotypes. In the first test culture set up on the second day after the climax of endomixis, no conjugants appeared. In the second test, made on the third day, four pairs of conjugants appeared; the third, made on the fifth day, yielded seven pairs; the fourth, made on the sixth day, yielded more than 40 pairs; the final test, made on the eighth day, yielded a great epidemic of conjugation. It is noteworthy that the restoration of the tendency to conjugate, like the restoration of fission rate, came about gradually. The other biotype yielded only small numbers of conjugants during this period. Whether it could have produced more later was not determined.

The remarkable change of characters at endomixis and their occasional reversion at a subsequent endomixis are of much interest in relation to the similar appearance, disappearance, and reappearance of experimentally produced "Dauermodifikationen" after conjugation and endomixis, in the work of Jollos. This work has been reviewed by Jennings (1929) and by Jollos (1934). Jollos holds that special environmental factors can induce long-enduring modifications which eventually disappear under normal conditions. It is particularly striking that the reverse of this occurred in the present experiments: long enduring modifications arose in daily isolation cultures under what we found to be optimum cultural conditions and disappeared (but in only a few cases) after subjection to different environmental conditions (mass cultures at 31°C.). The question of whether these changes, occurring at endomixis, are dependent upon or independent of environmental conditions obviously calls for further investigation.

What is the physical basis of these changes of biotype within a clone? It might be interpreted, following Diller (1936) as recombination brought about by autogamy; following Jollos (1934), as "Dauermodifikationen"; or, following Raffel (1932), as gene mutations. Although Jollos implies that Jennings' school holds to the hypothesis of frequent gene mutations, the present authors have always shared Jollos' opinion that it is
no more than a formal explanation of the facts. DILLER's view is in even worse case. Not only is his evidence for autogamy far from convincing, but most extraordinary assumptions would be required to account both for the appearance and subsequent disappearance of the same characters by means of successive autogamous recombinations. Nor are we yet prepared to interpret the changes of biotype described in this paper as "Dauermodifikationen." At this time, we merely present the facts. In the future, we hope to subject the whole matter to sufficient experimental analysis to permit a decision as to the nature of such hereditary changes and as to whether their basis is in the micronucleus, the macronucleus, or the cytoplasm.

**SUMMARY**

In a large clone of stock R of *Paramecium aurelia* nearly all lines of descent can readily be induced to conjugate soon after endomixis, but not after longer periods have elapsed. Two exceptional lines of descent were found in this clone, each one arising from a single endomictic individual. In one (biotype T), the individuals could regularly be induced to swim together in pairs, but they never conjugated. In the other (biotype O) conjugation occurred, but only rarely. Under the same conditions, the ancestors and collaterals of these biotypes showed the behavior typical for stock R. Diversities in tendency to conjugate thus arose at endomixis only twice among the many exendomictics observed in this clone. Both of the new biotypes showed, in addition to the altered tendency to conjugate, reduction in fission rate, increase in mortality rate (both at endomixis and during the interendomictic period), increase in the frequency of chain formation, and variation in the number of macronuclei. These altered characteristics are all held to follow from a decrease in the rate of some of the physiological processes involved in fission.

The non-occurrence of conjugation in biotype T and its rare occurrence in biotype O are shown to follow naturally from the almost complete absence in biotype T, and the great rarity in biotype O, of the only kind of individuals that could be expected to conjugate, namely, viable animals that had recently been through endomixis. This explanation is supported by similar observations on other non-conjugating clones of the same stock.

Finally, no reversion occurred after subsequent endomixes in biotype T, while two exendomictic animals from biotype O showed complete reversion to the ancestral fission rate, and at least one of them also reverted in tendency to conjugate. Changes in both these characteristics, as well as the original changes at the origin of biotypes T and O, occurred gradually during the course of many fissions, just as did similar changes following conjugation.
LITERATURE CITED


