The genetics of mating type in the suctorian
*Tokophrya lemnarum*

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**SUMMARY**

The two clones produced by the two members of a conjugating pair are normally of the same mating type, about 50% of such synclones being mating type I, 50% type II. This ratio suggests (1) that mating type is determined by a pair of alleles at one locus or by a pair of mating type determining chromosomes, and (2) that one mating type is homozygous, the other heterozygous or hemizygous. Mating type ratios are unaffected by temperature or parental age. Exceptions are of three types: (1) mates that produce one clone of type I, the other of type II, (2) mates which fuse permanently and yield a clone pure for either mating I or mating type II, and (3) conjugation of three mates one of which is regularly non-viable, the two survivors producing either two clones of type I, two clones of type II or one of each type. The bases of these exceptional results remain obscure.

1. **INTRODUCTION**

*Tokophrya lemnarum* is a suctorian which exhibits a complex developmental life cycle. The adult form is sessile, stalked and attached to the substratum with a basal disk. It feeds on various ciliates by means of tentacles through which it ingests the cytoplasm and nuclear material of its prey. Asexual reproduction is by endogenous budding. A single, ciliated free swimming bud develops within the parental brood pouch and is released via the birth pore. The bud swims for several hours and then rapidly undergoes metamorphosis. Swimming stops, the cilia disappear, a stalk, basal disk and tentacles appear. The newly metamorphosed tokophrya becomes capable of asexual reproduction in about 12 h. Sexual reproduction takes the form of conjugation and follows a typical ciliate pattern: three pregonic divisions of the micronucleus, reciprocal fertilization and development of new micro- and macronuclei from division products of the synkaryon. The present paper deals with the mode of inheritance and determination of mating type in this species.

2. **MATERIALS AND METHODS**

A collection of *T. lemnarum* obtained from Yellowood Lake (near Bloomington, Indiana) contained two mating types designated I and II. The original clones of

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these two mating types, parents of later generations, were labelled P₁ and P₂, respectively. Individual F₁, F₂ and backcross synclones were assigned odd (MTI) or even (MTII) numbers on the basis of their mating with P₂ or P₁, respectively, or with other previously identified clones.

(i) Cultural conditions

The culture methods were the same as described by Colgin-Bukovsan (submitted to ACHIV für protistenkunde).

(ii) Crosses

Unless otherwise noted, crosses were made by mixing together cultures of the two mating types approximating 24 h after feeding. Cells were removed from mass Petri dish cultures by scarping them loose with a large bore micropipet. 700–1000 cells of each mating type were mixed together in the centre depression of a three-depression slide. The two mating types in comparable numbers were added separately to the end depressions as controls to verify that neither culture mated in the absence of the other. Most of the cells settled to the bottom of the depressions.

Mixtures of the two mating types were routinely left at room temperature (21.5 ± 1.5 °C) and observed 6–7 h after mixing. At this time pairs were isolated into lake water, one pair to a depression, and left at room temperature overnight. The next morning exconjugants were isolated and fed. Two to three days later when asexual reproduction began, daily reisolation lines were established (Sonneborn, 1950) and the exconjugant clones were placed at 27 or 31 °C to increase the fission rate and thus shorten the immature period. Clones were tested for maturity 10 days after conjugation and every 2 days thereafter until mating reactions were observed.

When it was desirable to know the mating type of the cytoplasmic parent of each exconjugant clone, cultures of mating type I were marked by feeding them 3–4 paramecia per cell 2 h before mixture to make them large and dark. Immediately before mixing, the culture fluid was poured off and replaced with lake water to eliminate any living paramecia and fragments of paramecia on which unmarked cells might feed. Because cells of mating type II had not been fed for 24 h, they were small, transparent and easily distinguished from the cells of mating type I. All pairs which formed consisted of one large, dark mating type I cell and one small, translucent mating type II cell. The exconjugants were still distinguishable when members of such a pair separated, so the mating type of the cytoplasmic parent of each exconjugant was easily recognized.

(iii) Temperature studies

The effects of temperature on the determination of mating type were studied by placing fed cultures at 13, 19, room temperature and 31 °C. When all of the paramecia had been eaten and all buds had metamorphosed, clones of complementary mating types were mixed at room temperature and quickly returned to their preincubation temperature. Isolation of mating pairs was carried out at the latter temperature. Exconjugants were isolated, fed and usually left at the tem-
perature at which they had conjugated until asexual reproduction resumed. The clones were then placed at 27 °C and daily isolation lines were established.

(iv) Aging studies

Aging studies compared the survival percentages and the mating type ratios of exconjugants obtained soon after the stocks were isolated from nature (approximately 25 fissions in the laboratory) and at 150, 300, 500, 650, 800 and 870 fissions after isolation. Each metamorphosed bud produced by a given parental cell was counted as one fission (Colgin-Bukovsan, 1969). Crosses between old (800 and 870 fissions in the laboratory) parental and relatively young (110 fissions) F₂ clones were also made and the results compared with those of the other crosses.

3. RESULTS

(i) Mating type inheritance: normal pairs

The results of three crosses between different sublines of the two original parental clones P₁ (mating type I) and P₂ (mating type II) are presented in Table 1. This table shows that: (1) synclones, i.e. the clones derived from the two exconjugants of a pair, are almost always uniform in mating type (two exceptions are dealt with later); (2) only the two parental types are found among the progeny; and (3) the two types occur in a ratio not significantly different from 1 : 1. This ratio is consistent with determination by a pair of allelic genes, homozygous in one mating type and heterozygous in the other, or by a pair of sex-determining chromosomes disomic in one mating type, monosomic in the other.

Backcrosses of the F₁ clones to the parental stocks and crosses between F₁'s of different mating types were made. Consistent with the hypothesis of genic or chromosomal determination, the predicted ratio of one mating type I synclone to one mating type II synclone was observed in all crosses (Table 1).

Unlike the marked differences in mating type ratios obtained in the Paramecium aurelia species-complex (Sonneborn, 1975) when conjugation and post-zygotic nuclear developments take place at different temperatures (Sonneborn, 1939, 1947; Nanney, 1957) in Tokophrya crosses made between F₂ clones at 13 °C, room temperature and 31 °C, yielded no significant deviation from equality of the two mating types (Table 2). Exceptional pairs, e.g. three which did not show synclonal uniformity will be dealt with elsewhere, as will the high mortality correlated with conjugation at 13 °C.

If mating type determination and inheritance has a purely genic or chromosomal basis, no appreciable difference would be expected between the ratios obtained when young clones are crossed and those obtained when older clones are crossed, provided that the latter did not involve more abnormalities during meiosis or more selective death of one mating type. The results in Table 3 are in agreement with this expectation. When one parent was clone P₁ or P₂ and the cross was made when these had been in the laboratory 800 fissions or more, although viability was reduced, the ratio of mating types is not significantly different from 1 : 1 (P = 0.25).
Table 1. Distribution of mating types among progeny of various crosses of *Toxophrya lemnarum*.

| Stocks crossed | Approximate age in fissions | Total no. of pairs | Viability (%) | No. of pairs |
|----------------|----------------------------|-------------------|---------------|--------------|
|                | MT I | MT II | MT I | MT II | MT I | MT II | P† |
| P₁ x P₂        | > 25 | > 25 | 10   | 90.0  | 5     | 4     | 1-0 |
| P₁ x P₂        | > 150 | > 150 | 31 | 100.0 | 13    | 18    | 0-5 |
| P₁ x P₂        | > 300 | > 300 | 7   | 100.0 | 5     | 2     | 0-5 |
| Total P₁ x P₂  | —    | —    | 48  | 97.9  | 23    | 24    | 1-0 |
| F₁-3 x P₂      | 60   | > 500 | 21  | 100.0 | 7     | 14    | 0-2 |
| F₁-3 x P₂      | 260  | > 650 | 31 | 100.0 | 16    | 15    | 1-0 |
| Total F₁ x P₂  | —    | —    | 52  | 100.0 | 23    | 29    | 0-5-0-3 |
| P₁ x F₁-4      | > 500 | 50   | 10+ | 90.0  | 7     | 1     | 0-1 |
| P₁ x F₁-4      | > 650 | 250  | 22  | 100.0 | 9     | 13    | 0-5 |
| Total P₁ x F₁  | —    | —    | 32  | 98-8  | 16    | 14    | 0-8 |
| Total all backcrosses | — | — | 84 | 97-9 | 39 | 43 | 0-7-0-5 |

* The two clones derived from a pair of exconjugants nearly always agree in mating type.

† The *P* value in all cases where the total number of pairs is less than 50 is derived from a binominal expansion as presented in Warwick's Tables (1932) and is the probability of getting a deviation this great or greater from a 1:1 segregation. Chi square was used to derive the *P* value in cases where the total is greater than 50.

+ Includes one pair which did not show synclonal uniformity and which is not included in the mating type results.

Table 2. The effect of temperature during conjugation upon mating type inheritance.

| Temperature (°C) | Age in fissions | Total no. of pairs | Viability (%) | No. of pairs |
|------------------|-----------------|--------------------|---------------|--------------|
|                  | MT I | MT II | MT I | MT II | MT I | MT II | P† |
| 13               | 50   | 60    | 20   | 45-0  | 2     | 7     | 0-2 |
| 13               | 125  | 135   | 21†  | 81-0  | 5     | 8     | 0-6 |
| 13               | 270  | 280   | 41§  | 75-6  | 16    | 13    | 0-7 |
| Total for 13     | —    | —     | 82   | 62-2  | 23    | 28    | 0-5 |
| Room temperature || 50 | 60 | 21 | 95-3 | 10 | 10 | 1-0 |

* All crosses were between F₄ clones 1 and 2. Three exceptional pairs which did not show synclonal uniformity are excluded from the mating type results.

† See text for temperature after conjugation.

‡ Includes three fused pairs which are not included in the mating type results.

§ Includes two fused pairs which are not included in the mating type results.

|| The room temperature pairs are also included in Table 1 as the F₄₋₁ x F₄₋₂ cross.
As appears in Table 1, these clones yielded the same ratio, with little or no mortality, when they were 150 fissions or more younger. The increased mortality thus appears to be a true aging effect, not a characteristic of the clones used. The same is true for fused pairs: about 10% of the pairs in Table 3 were fused, but no fused pairs occurred among the 200 listed in Table 1.

Table 3. Effects of age on mating type inheritance*

| Stocks crossed | Age in fissions | Total no. of pairs | Viability (%) | No. of pairs | No. of fused pairs | P |
|----------------|-----------------|--------------------|---------------|--------------|-------------------|---|
| MT I MT II     | MT I MT II      |                   |               | MT I MT II   |                   |   |
| P₁ P₂          | > 870 > 870     | 60                 | 63·3          | 21           | 14                | 3  |
| P₁ P₂          | > 800 > 800     | 50                 | 64·0          | 13           | 15                | 4  |
| P₁ F₂-2        | > 800 100       | 30                 | 66·6          | 12           | 7                 | 1  |
| F₂-1 P₂        | > 100 > 100     | 30                 | 60·0          | 9            | 7                 | 2  |
| Total          | —               | —                  | —             | 55           | 43                | 0·3-0·2 |

* The age in fissions of each clone is approximate. Ages of P₁ and P₂ are the approximate number of fissions in culture since collection from wild.

Table 4. Results of backcrosses of exceptional clones (derived from pairs which did not show synclonal uniformity of mating type) to stock cultures of mating type I and II

| Cross from which exceptional pair was obtained | Backcross | Total no. of pairs | Viability (%) | No. of pairs | P |
|----------------------------------------------|-----------|--------------------|---------------|--------------|---|
| MT I MT II                                   | Except. clone Normal clone |                   |               | MT I MT II   |   |
| P₁ F₁-1                                      | E₁ P₂     | 14                 | 100·0         | 8            | 6  |
| F₁-2                                        | E₁ P₁     | 14                 | 100·0         | 5            | 9  |
| F₂-2                                        | E₂ P₁     | 21*                | 76·2          | 6            | 9  |
| (Conj. at 31 °C)                             |           |                    |               |             |   |
| F₂-1                                        | E₄ P₂     | 20                 | 90·0          | 10           | 8  |
| F₂-2                                        | E₅ P₁     | 12                 | 100·0         | 8            | 4  |
| (Conj. at 13 °C)                             |           |                    |               |             |   |
| E₅ F₁                                       | E₆ P₂     | 12                 | 100·0         | 3            | 9  |
| Total                                       | —         | —                  | —             | 40           | 45 |

* Includes one pair which did not show synclonal uniformity.

(ii) Pairs not showing synclonal uniformity

As noted above, five pairs which did not show synclonal uniformity were found in these experiments. Another exceptional pair was obtained from a backcross of one of these first five exceptional pairs.

The first exceptional pair was found in an $F₁ \times F₁$ cross. In this cross the cells of the two mating types could be distinguished from each other because one mating type was well fed and dark and the other was unfed and light. The mating type of each exconjugant clone from the exceptional pair was the same as that of its cytoplasmic parent. Neither clone gave results differing significantly from a 1:1 ratio of mating types when crossed to a parental clone (Table 4).

The other exceptional pairs were not marked to permit identification of the stock
from which each exconjugant was derived. Genetic tests again gave 1:1 ratios of mating types suggesting the presence of the two parental genotypes in all six pairs (Table 4). The two exconjugant clones derived from one exceptional synclone were crossed to each other as a further test of the hypothesis that parental genotypes are present in these exceptional pairs. All 30 pairs originally isolated were represented by both exconjugant clones at maturity: 14 were mating type I and 16 were mating type II. This of course does not differ significantly from 1:1 ($P = 0.9$). Possible interpretations of these results will be presented later.

### Table 5. The derivation and mating types of clones produced by fused pairs

| Cross from which fused pair was obtained | Total no. viable pairs | Total no. fused pair clones | Mating types of fused pair clones | Average no. fissions from conj. to maturity | Cross no. |
|-----------------------------------------|------------------------|-----------------------------|-----------------------------------|-------------------------------------------|-----------|
| MT I                                    | MT II                  |                             | I       | II      | Fused pairs | Normal pairs |
| F$_{2-1}$ F$_{2-2}$ (Conj. at 13 °C)    | 17                     | 3                           | 1       | 2       | 52-66       | 55-33       | 1          |
| P$_1$ P$_2$ (Old clones – 800 fissions) | 9                      | 4                           | 2       | 2       | 54-75       | 50-6        | 2          |
| P$_1$ P$_2$ (Old clones – 870 fissions) | 38                     | 3                           | 0       | 3       | 52-33       | 53-77       | 3          |
| P$_1$ F$_{2-2}$ (P$_1$ old – 800 fissions) | 20                     | 1                           | 1       | 0       | 48-0        | 54-10       | 4          |
| F$_{2-1}$ P$_2$ (P$_2$ old – 800 fissions) | 18                     | 2                           | 1       | 1       | 57-6        | 54-06       | 5          |
| Total                                   | 102                    | 13                          | 5       | 8       |              |              |            |

(iii) **Fused pairs**

Paired *T. lemnarum* normally separate at the end of conjugation; but at 13 °C and in old clones some conjugating pairs fail to separate, fusing completely and permanently instead. A total of 13 fused pairs was found (Table 5). The first six buds produced by each were isolated and allowed to produce clones which were tested for mating type when they became mature. Both mating types were found among the progeny of different fused pairs, but each fused pair gave rise to only one type. To investigate the genotypes of clones derived from fused pairs, three clones of each mating type (each clone derived from a different fused pair) were backcrossed to cells of complementary mating type. In none of these crosses was the ratio significantly different from 1:1 (Table 6), indicating that clones derived from fused pairs have the same genotype for mating type as normal clones of the same mating type.

(iv) **Triplet conjugants**

When highly reactive cultures of complementary mating types are mixed, occasionally more than two cells fuse together in conjugation. Nineteen triplets,
i.e. groups of three conjugating cells, were studied genetically, but only two cells of each group survived. The third cell, although normal in appearance at the time of separation, lost its tentacles and died within 48 h. The two survivors of the

Table 6. The distribution of mating types among the progeny of clones derived from fused pairs backcrossed to stock cultures of mating types I and II

| Cross from which fused pair was obtained | Backcross | Viability (%) | No. of pairs |
|-----------------------------------------|-----------|---------------|--------------|
|                                         | Fused     | Normal        | MT I         | MT II       | P  |
| 1                                       | 1         | $P_2$         | 100·0        | 5           | 7  | 0·8 |
| 1                                       | 2         | $P_1$         | 100·0        | 5           | 8  | 0·6 |
| 3                                       | 4         | $P_1$         | 94·4         | 6           | 11 | 0·3 |
| 5                                       | 6         | $P_1$         | 100·0        | 3           | 7  | 0·3 |
| Total                                   |           |               |              | 37          | 44 | 0·7–0·5 |

Table 7. The results of backcrossing clones derived from triple conjugating groups by stock cultures of mating type I and II

| Cross from which triple pair was obtained | Stocks crossed | Total no. of pairs | No. of pairs |
|------------------------------------------|----------------|-------------------|--------------|
|                                          | Fused          | Normal            | MT I         | MT II       | P  |
| $P_1 P_2$                               | 1A*            | $P_2$             | 10           | 6           | 4  | 0·8 |
|                                          | 1B             | $P_2$             | 10           | 5           | 5  | 1·0 |
| $P_1 P_2$                               | 3A             | $P_2$             | 10           | 3           | 7  | 0·3 |
|                                          | 3B             | $P_2$             | 10           | 4           | 6  | 0·8 |
| $P_1 P_2$                               | 4A             | $P_1$             | 10           | 5           | 4  | 1·0 |
|                                          | 4B             | $P_2$             | 10           | 5           | 5  | 1·0 |
| $F_{1-3} P_2$                           | 5A             | $P_2$             | 10           | 3           | 5  | 0·7 |
|                                          | 5B             | $P_2$             | 10           | 7           | 3  | 0·3 |
| $F_{1-3} P_2$                           | 7A             | $P_2$             | 10           | 8           | 2  | 0·1 |
|                                          | 7B             | $P_1$             | 10           | 6           | 4  | 0·8 |
| $P_1 F_{1-4}$                           | 8A             | $P_1$             | 10           | 3           | 6  | 0·5 |
|                                          | 8B             | $P_2$             | 10           | 5           | 5  | 1·0 |
| $P_1 F_{1-4}$                           | 9A             | $P_1$             | 10           | 3           | 7  | 0·3 |
|                                          | 9B             | $P_1$             | 10           | 5           | 3  | 0·7 |
| $F_{1-3} F_{1-4}$                       | 11A            | $P_2$             | 10           | 7           | 3  | 0·3 |
|                                          | 11B            | $P_2$             | 10           | 4           | 5  | 1·0 |
| $F_{1-3} F_{1-4}$                       | 13A            | $P_2$             | 10           | 3           | 7  | 0·3 |
|                                          | 13B            | $P_1$             | 10           | 6           | 4  | 0·8 |
| $F_{2-1} F_{2-2}$                       | 15A            | $F_{1-3}$         | 10           | 2           | 8  | 0·1 |
|                                          | 15B            | $F_{1-3}$         | 10           | 5           | 5  | 1·0 |
| $F_{2-1} F_{2-2}$                       | 17A            | $F_{1-3}$         | 10           | 4           | 6  | 0·8 |
|                                          | 17B            | $F_{1-3}$         | 10           | 2           | 7  | 0·2 |
| Total                                   |               |                   | 101          | 111         | 0·5–0·3 |

* Each triplet was given a number so that stocks 1A and 1B are two of the three exconjugants from triplet 1.
triplets were grown to maturity and tested for mating type. Some pairs of clones were of the same type and others of complementary type. Six were mating type I, five were mating type II, while eight consisted of one mating type I member and one mating type II member. Members of each exconjugant clone were backcrossed to a standard stock of complementary mating type. The results (Table 7) indicate that these clones are capable of undergoing normal conjugation to produce synclones of either mating type. Interpretations will be considered in the Discussion section.

(v) Attempts to obtain a third genotype

Several methods were unsuccessfully employed to try to identify (by production of one, two or three genotypes and phenotypes, at self-fertilization) which mating type is homomorphic or homozygous (designated AA for convenience) and which heteromorphic or heterozygous (designated AB). The missing BB class was not unambiguously found in the exceptional pairs which did not exhibit synclonal uniformity or in the multiple conjugants mentioned above. The possibility that BB arises and is non-viable cannot be excluded.

Unsuccessful attempts were made to induce homozygotes by killing or cutting away one member of a conjugating pair after the first meiotic division but before fertilization.

4. DISCUSSION

(i) Interpretation of the major genetic results

No significant exceptions were found to the 1:1 segregation of the two mating types in any of the crosses undertaken. With few exceptions, to be dealt with later, inheritance was synclonal, i.e. both members of a pair of conjugants produce clones of the same mating type. Mating can be obtained only between a cell of mating type I and a cell of mating type II. In such a case, synclonal inheritance with a 1:1 ratio of mating types among progeny suggests either that one mating type is homozygous at a mating type locus while the other mating type is heterozygous or hemizygous at this locus, or that one is homomorphic for a pair of mating type chromosomes while the other is heteromorphic or monosomic. The data do not indicate which mating type is homozygous or homomorphic and which is not. Such information might be obtained if self-fertilization of the cells were possible. However, all attempts to induce self-fertilization met with failure.

An examination of other Ciliates that exhibit synclonal mating type determination and inheritance reveals a genic, not chromosomal, mechanism. The organisms studied include *Euplotes patella* (Kimball, 1942), *E. vannus* (Heckmann, 1963), *E. minuta* (Nobili, 1966), *Paramecium bursaria*, syngen 1 (Siegel & Larison, 1960), *P. caudatum*, syngens 3 and 12 (Hiwatashi, 1958, 1964), *P. tredecaurelia* (Sonneborn, 1966, 1975) and *Tetrahymena pyriformis*, syngen 8 (Orias, 1963). The details differ from species to species, but the genic basis is clear in all of them. Not a single example in Ciliates of mating type determination due to heteromorphic or monosomic chromosomes has been documented. On the other hand, chromosomal
mechanisms of several sorts do determine sex in various higher organisms and a comparable mechanism of mating type determination cannot be ruled out in the case of Tokophrya.

(ii) Exceptions to synclonal uniformity

All but six of the nearly 750 viable exconjug pairs examined produced two clones of the same mating type (exceptions found in triplet conjugations will be dealt with later). The exceptions yielded one clone of type I and one of type II. All six were tested by further crossing and yielded results identical with those of other clones of the same mating type. In the one exceptional pair in which the two members were morphologically identifiable before and after conjugation there was clearly no change of mating type following conjugation. The mating type of each conjugant persisted in the exconjugant clone it produced. Such information was not available for the other pairs.

Possible interpretations of the results are restricted by finding that all 12 clones examined went through a normal immature period. Immature periods in other Ciliates occur only when a new macronucleus develops from a micronucleus and this is known to occur only after meiosis, gamete nucleus formation and (usually) fertilization. These facts weigh heavily against concluding that the exceptional pairs failed to undergo meiosis, fertilization, and development of new macronuclei or that the prezygotic macronuclei persisted instead of being replaced by a new one developed from a product of the synkaryon. The usual Ciliate form of cytogamy, i.e. self-fertilization by union of two identical gamete nuclei derived from the mitotic division of a reduced nucleus, is also excluded because one member of each pair must have been heterozygous to give, on further breeding, the observed mating type ratios. However, results obtained could be interpreted as due to synkaryon formation by union of gamete nuclei derived from different, instead of the same, products of meiosis. In this way, either cross-fertilization or cytogamy could yield unlike members of a pair. Such an aberration of the cytogenetic processes, which is known to occur in other Ciliates, seems to be the most reasonable explanation of these exceptional pairs. The question could be resolved by following heterozygotes for other marker genes, if and when they are obtained in these stocks.

(iii) Fused pairs

Each of the 13 pairs in which the two conjugants fused permanently yielded a clone pure for mating type, some type I, others type II, and all breeding exactly like normal clones of these types. The possibility that in these pairs no fertilization occurred (as claimed by Davis (1942) for another Suctorian), can almost certainly be excluded because the clones from the fused pairs exhibited a typical post-fertilization immature period. The possibility that cytogamy occurred seems unlikely because no case was found in which a fused pair gave rise to both mating types, as would be expected in half the cytosomatic pairs. Other more plausible interpretations of the fused pair data cannot now be excluded.
(iv) **Triplet conjugants**

The main facts about triplets (Table 7) are: (1) one of the three exconjugants was always non-viable; (2) the two survivors produced two clones of type I (6 pairs) or two of type II (5 pairs) or one of each type (8 pairs); (3) both members of

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**Fig. 1.** Four conjugating tokophrya showing the micronuclei and the relative positions of the cells. A, nuclei which appear to be derived from second meiotic divisions; B, degenerating nucleus; origin unclear; C, spindles of the third pregamie division in which a single haploid nucleus divides to give 2 identical haploid nuclei.
all three classes of pairs when test-crossed, yielded the typical 1:1 ratio of mating types (total for all test-crossed, 101 type I to 111 type II synclones).

The occurrence among 19 pairs of 8 cases in which the two members of a pair yielded clones differing in mating type is in marked excess of the frequency (6 among 750 pairs) with which this result was obtained from ordinary paired conjugants. If the mate heterozygous for the mating type factor formed its two functional gamete nuclei from different products of the second meiotic division, instead of from a single product of this division, two-thirds of the resulting pairs of clones would differ in mating type. While this interpretation is possible, it fails to account for the regular death of one cell of each triplet and the rarity of unlike pair members in normal crosses.

An explanation for the death of one conjugant is suggested by cytological observations on one set of four conjugating cells (Fig. 1). The figure suggests that cell 3 will end up with two synkarya, cells 2 and 4 with one and cell 1 with none. The latter would probably develop its post-conjugation nuclei from a haploid nucleus. Since haploids would be expected to be usually non-viable in a presumably outbreeding diploid species like *T. lemnarum*, this could be the basis of death of one cell in triplet conjugants.

(v) The missing BB type

If one mating type in these organisms is AA and one AB, a third genotype, BB (phenotypes unpredictable), might be obtained under special circumstances. This problem will be dealt with elsewhere.

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