Genetic Instability in the Mating Type System of *Tetrahymena pigmentosa*

Ellen M. Simon* and Eduardo Orias†

*Department of Ecology, Ethology and Evolution, University of Illinois, Urbana, Illinois 61801, and †Department of Biological Sciences, University of California, Santa Barbara, California 93106

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**ABSTRACT**

Selfing clones of *Tetrahymena pigmentosa* show several interesting genetic features, and provide some insight into the mechanisms of mating type (mt) determination. They differ significantly from those of *Tetrahymena thermophila*. They are distributed nonrandomly in crosses. Their rates of stabilization are highly variable, but most are much lower than those reported for *T. thermophila*. A number of subclones derived from nearly all the selfers have maintained stable mts in culture for several years. However, some subclones manifest persistent selfing, long after the calculated completion of allelic assortment for heterozygous loci. This phenomenon along with the perpetual maintenance of dominant mts in heterozygotes shows that phenotypic assortment is not involved in mt expression.—In crosses, many selfers exhibit quantitative and qualitative aberrations in the transmission of alleles to the gametes; some of the micronuclear changes underlying these aberrations occur during vegetative growth. There are rare illegitimate appearances of dominant alleles in sexual progeny, and more common illegitimate appearances of the most recessive phenotype.—Various models to explain mt determination in this species are considered. One which might account for the troubling phenomena of the system consists of an active mat expression site, with "cassettes" at other sites specific for the different dominant alleles and capable of transposition to the expression site.

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THE ciliated protozoa offer unique opportunities for genetic and developmental studies because most cells contain two kinds of nuclei, a diploid germline micronucleus and a large somatic macronucleus. During conjugation in the sexual species of *Tetrahymena*, the micronuclei undergo meiosis, haploid products of which participate in reciprocal fertilization. Following two postzygotic nuclear divisions two of the products (micronuclei) remain diploid. New macronuclei develop from the other products in which the genetically identical but each contains products of which participate in reciprocal fertilization. Conjugating pair, during conjugation in the sexual species of *Tetrahymena*, they form their vegetative progeny, known as clones, are maintained independently. Collectively they form a syncrone—the progeny of one conjugating pair.

The studies on mating type (mt) determination in the *Tetrahymena pyriformis* complex reveal two major classes of species, those like *Tetrahymena thermophila* that manifest karyonidal inheritance—the mt is determined independently in each developing macronucleus (NANNEY 1958; BYRD 1959; PHILLIPS 1969; SIMON and NANNEY 1984) and those like *Tetrahymena pigmentosa* that show direct genetic control of mts (HURST 1958; ORIAS 1959a, 1968; SONNEBORN 1974; SIMON 1980; D. NYBERG, personal communication; E. M. SIMON, unpublished data) with serial dominance. The mt specificities in *T. pigmentosa* are controlled by three alleles at the mat locus which Mendelize and show synclonal uniformity—all karyonides in a syncrone express the same mt—in more than 92% of the progeny. Two to 3% of the karyonides exhibit intraclonal mating (selfing) and approximately 5% express illegitimately the phenotype of the most recessive allele (ORIAS 1959a, 1963; SIMON 1980).

The evolutionary history of the complex is as yet incompletely rationalized (MEYER and NANNEY 1987). Some molecular differences suggest (NANNEY 1982) that some of the species diverged a very long time ago. Other studies, e.g., on the small ribosomal RNAs (VAN BELL 1985) indicate more recent speciation. The data derived from cytoskeletal proteins (WILLIAMS, BÜHSE and SMITH 1984) isozymes (MEYER and NANNEY 1987) rRNA restriction enzyme maps (NIelsen and ENGBERG 1985) and rRNA sequences (M. L. SOGIN, A. INGOLD, M. KARLOCK, H. NIelsen and J. ENGBERG, unpublished data; R. M. PREPARATA, E. B. MEYER, F. F. PREPARATA, C. R. VOSSBRINCK, D. L. NANNEY, E. M. SIMON and C. R. WOENSE, unpublished data) show clustering within each of the two groups of mating species but deep branching between the groups.

A long-standing problem in genetic studies of ciliates has been our ignorance of the macronuclear developmental process. The selfers of *T. thermophila* were of critical importance in the early genetic stud-
ies of Tetrahymena (Allen and Nanney 1958; Schensted 1958). The rate of stabilization to pure mt provided the basis for studying genetic assortment in the macronucleus, and for estimating its compound-ness. More recently the events in the developing macronucleus have been interpreted in terms of immunoglobulin-like DNA alterations at a complex mt locus and the independent and selective amplification of, usually, one or two determined copies in the macronucleus in each of the karyonides (Orias 1981; Orias and Baum 1985; Orias, Baum and Haller 1984).

An analysis of the origins and the assortment properties of selfing macronuclei in T. pigmentosa should be similarly illuminating with regard to synclonal, genic mt determination. A particular advantage of this species is that it includes at least two subspecies with substantial molecular differences (Borden et al. 1977), which nevertheless produce fertile hybrids (Orias 1959b; Simon 1980).

In this paper, the unpublished Orias (1959a) experiments on selfers, which exhibited abnormal gametic ratios, have been extended by E.M.S. By examining several additional selfers in more detail we have tried to answer the following questions: (1) are all "stabilized" segregants from selfers really stable? (2) are the kinetics of assortment of pure mt cells from selfers similar to those of T. thermophila? (3) are the testcross ratios of stable lines from different selfers always abnormal? and (4) have unexpected mt IIs from stabilized mat1/mat2 selfers lost mat1 from the micronucleus as did those from nonselfers (Orias 1963)?

Our results lead us to conclude that the answers to all four questions are negative and confirm that there are important differences in the mechanisms of mt determination and macronuclear development between T. pigmentosa and T. thermophila.

**MATERIALS AND METHODS**

The strains used and the methods for maintaining cultures and making crosses have been described earlier (Orias 1959a, 1963; Simon 1980). The methods for analyzing selfers of Allen and Nanney (1958) were followed by Orias (1959a). Single cell isolations (usually 30) from a selfing clone were serially transferred daily and stabilized or lost lines were replaced from parallel subclones. Lines not selfing for three successive transfers, 24 fissions at 25°C, were defined as stable. E.M.S. made serial transfers every other day until 450 fissions after selfing was first observed. Stable or lost subclones were not replaced.

Cytological preparations were made of samples from axenic or bacterized Cerophyl cultures. Staining methods utilized various combinations of published techniques (Sonneborn 1950 [Dippell and Chao's p. 141]; Ray 1956; Nissenbaum 1953; Bruns, Brussard and Merriam 1983).

**RESULTS**

Selfing clones that appeared in experiments on mt inheritance within syngen 8 and in intersyngenic hybrids (Orias 1959a; Simon 1980) provide most of the data in this report. Rare selfing and mixed mt synclones also occur in syngen 6.

The derivation of the "hybrid" and F2 progeny is outlined in Figure 1. Among the progeny of cross Z 2/21 (10%) were selfers as were 2/26 (8%) in cross Y; no selfers were found in cross X. In these experiments by E.M.S. exconjugants and karyonides were not separated. However, multiple subclones of 15 F1 selfers have been followed. In none of these did all subclones self. In most subsequent experiments only one subclone from each synclone was studied, which means that some selfers escaped detection.

**Occurrence of selfing:** Selfers comprised 1–2% of the progeny of nearly all crosses in Nanney and Caughey's (1955) study of T. thermophila families A and B if subclones were moderately starved between transfers. Approximately 19% appeared if no starvation was permitted. S. L. Allen and E. M. Simon (unpublished data) observed 1% or fewer selfers among several thousand progeny of 12 inbred families during routine inbreeding. In the crosses with T. pigmentosa considered in this report, the yield of selfers varied from 0/367 to 21% (11/53) and 23% (6/26) although starvation occurred routinely. Similar clustering of selfers was seen in Tetrahymena americana (Hurst 1958). Even more extreme variation occurred in other crosses in which several mt I clones which can transmit only mat3 alleles were testcrossed. Selfers and mixed synclones comprised 4% of 309 progeny; the extremes in individual crosses were 3/3 and 0/120. Selfing progeny can therefore be derived from two parents whose effective micronuclear genotype is mat3/mat3. Such progeny should be homozygous.

The manifestation of selfing in most progeny clones occurred approximately 10–20 fissions after the onset of maturity (usually 50–90 fissions after conjugation) which was monitored by the addition of mature tester strains to "left-over" cultures during serial transfers. There were, however, a few exceptions, e.g., one clone which matured at about fission 50 did not self until after fission 100. In addition, the cultures derived from three other pairs retained maturity and a parental mt. Although they apparently failed to complete

Eighteen primary subclones were initiated from each clone. When selfing occurred in several of these cultures, a second expansion provided >200 secondary subclones of each selfer. These lines were transferred every other day until 450 fissions after selfing was first observed. Stable or lost subclones were not replaced.

The rate of stabilization to pure mt provided the basis for studying genetic assortment in the macronucleus, and for estimating its compound-ness. More recently the events in the developing macronucleus have been interpreted in terms of immunoglobulin-like DNA alterations at a complex mt locus and the independent and selective amplification of, usually, one or two determined copies in the macronucleus in each of the karyonides (Orias 1981; Orias and Baum 1985; Orias, Baum and Haller 1984).
The data from these 14 selfers are pooled in Figure 2A. The average and weighted mean values increase steadily to about 200 fissions. A homogeneity \( \chi^2 \) test, even omitting fissions 1–62.5 by which time most total Rfs approach equilibrium in \( T. \) thermophila, yields a \( P \) value of 0.007. Similar calculations performed individually on two of the 8 \( \times \) 8, one 6 \( \times \) 8 F1 and two 6 \( \times \) 8 F2 selfers at 37.5 fission intervals yield \( P \) values of 0.02–0.05, <0.01, 0.2, <0.01 and 0.02–0.05. Therefore, not only do selfers differ from one another, but the Rfs do not stabilize within clones.

Figure 2B depicts the pooled stabilizations of the majority and minority mts in five 6 \( \times \) 8 F2 selfers yielding two mts. Similar data from \( T. \) thermophila are plotted in Figure 2C (redrawn from ALLEN and NANNY 1958). The rates at which minority types stabilize in \( T. \) pigmentosa do not approach a common equilibrium. In fact they may diverge in later fissions. These phenomena argue against a stabilization mechanism based exclusively on the random distribution of macronuclear elements with equal replication rates.

One other feature of the selfers in \( T. \) pigmentosa should also be mentioned. Although "stable" sublines apparently pure for mt are produced regularly under the conditions of the experiments, their stability is not always permanent. Seventy subclones of 8 \( \times \) 8 and F1 conjugation, subclones selfed—two of them beginning in the first transfer. These two abnormal selfers are not considered further in this report. Three subclones of the third, designated "21" in Table 2, maintained a stable mt I for 75 fissions; then one subclone destabilized. Strong selfing continued for at least 250 fissions during which a few sublines which expressed mt II or III were isolated. These observations indicate that destabilization may occur in "old" macronuclei.

**Stabilization of selfers:** Nearly all selfers of \( T. \) thermophila are able to produce stable sublines in almost any combination of two or more mts. The rates of stabilization per fission (Rfs) from cohorts of subcultures approach a common equilibrium within some 50 cell divisions from the expansion of a selfing culture, regardless of initial rate variations associated with different macronuclear compositions. The Rf varies from about 113 \( \times \) 10\(^{-4}\) [range 92–150 \( \times \) 10\(^{-4}\) over fissions 13 \( \rightarrow \) 262 (ALLEN and NANNY 1958)] for cells maintained in constant growth (at 27\(^\circ\)) to 130 \( \times \) 10\(^{-4}\) for cells allowed to starve regularly (transferred on alternate days). A major objective of the present study was to determine the Rfs for selfers of \( T. \) pigmentosa. Data accumulated by E.M.S. on 14 different selfing clones reveal Rfs from 8 to 169 \( \times \) 10\(^{-4}\) before 100 fissions and from 25 to 195 later. These 20- and 8-fold differences are incompatible with the \( T. \) thermophila model. All of these selfers produced lines with stable mts, but some produced only one stable type, even though they were maintained in multiple serial cultures long past the period of equilibration in \( T. \) thermophila. All the possible pairwise combinations of the three mts were recovered, but no more than two from a single selfer. The relative proportions of mts were systematically biased. The only "monotypic" selfers were mt I, the type associated with the most dominant mt allele. Moreover, in eight of nine ditypic selfers the more dominant mt (I or II) was the majority type. The overall ratio of dominant-recessive pure sublines in ditypic selfers was 4.6:1. If the monotypic selfers are indeed cryptic ditypes, the pooled ratio is close to 10:1. This biased ratio is not characteristic of only young selfing clones, but it continues at least as long as 350 fissions.

Figure 1.—Derivation of E.M.S. intersyngenic selfers. HG8 and 7152 are wild strains of subspecies (syngen) 6, IL3 is a wild subspecies 8 and AB6-7 an F1 from ORIAS (1959a). The three crosses among these parental strains and 36 crosses among their F1 progeny were reported earlier (see Tables 3 and 6, SIMON 1980). The parental crosses are now designated X, Y, and Z; new and old (1980) designations for the F1 clones selected for further study are ZI, 18, Z2, 27; Z3, 30; Z4, 32; Y1, 21, Y2, 22, Y3, 34; Y4, 36; X1, 48; X2, 49; X3, 50; X4, 60. The F1 \( \times \) F1 crosses whose progeny are considered here are listed under F2. Designations of F1 and F2 progeny which manifested selfing and are considered individually in this analysis are given in ( ). For the F2 selfers the numerals represent: cross no.-synclone no. If the progeny of a cross that yielded no selfers are considered, only the cross number is given (in [ ]). The fractions given for the F1 crosses are selfers/viable progeny.
hybrid selfers, tubed after apparent stability through two or three transfers, were reexamined 1 or 2 yr later. Nineteen of these clones were found to be selfing, to have changed mt, or to have become immature (indicative of prior mating within the culture). In the F2 selfer experiments we attempted to overcome this reselfing problem by carrying nonseleng sublines through 50 fissions before tubing. Even then a few selfed again and were discarded.

Two additional selfers were studied more extensively in experiments designed to provide data directly comparable to Doerder's (1979) computer simulation for macronuclear assortment in T. thermophila (see Materials and Methods). The results shown in Table 1 involve eight subclones of selfer S2 and six subclones of selfer S15. The difference in experimental design may have lowered the Rfs in comparison to those in earlier experiments. However, there was a fivefold difference in overall Rfs with >4500 opportunities to stabilize for each selfer and there are significant differences among subclones of a single karyonide. All these Rfs are below any reported for T. thermophila, by an average factor of more than 10. When analyzed for temporal trends (Table 1B) some inhomogeneity is apparent, but no systematic direction of change can be claimed. The Rfs remain low, and the direction of change stays strongly biased toward the mt of the dominant allele. Since sublines of the same clone can behave so differently, we need not be surprised that different selfers can give different rates of stabilization.

Here we see also, we believe for the first time, that perpetual selfers (S2-12 and S15-7) occur in Tetrahymena in laboratory crosses. At least some of those collected from natural habitats could be exceptional clones of “normal” species. Isozyme analysis (Meyer and Nanney 1987) has shown this to be so.

Figure 2.—Rates of stabilization (Rfs) of selfers. (A) Data from 14 E.M.S. selfers pooled according to source; the Rfs were calculated for five transfer (62.5 fission) intervals. The “odd” F2 is the only example in which the majority of stabilized lines expressed the more recessive mt. (B) Pooled data for the five ditopic 6 x 8 F2 selfers; they show the failure of the Rfs of the two mts to equilibrate. The lines were smoothed by combining data from successive pairs of transfers. (C) Stabilization in T. thermophila, Figure 2 (Allen and Nanney 1958, total data) redrawn with expanded vertical scale and the addition of estimated data points for the sum of the two mts. This selfer’s majority mt was VI, but in two of six 1–VI selfers the majority mt was I.

Table 1

Kinetics of stabilization of selfers in experiments done without replacement

<table>
<thead>
<tr>
<th>S2</th>
<th>S15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>Stabilized/</td>
</tr>
<tr>
<td>Subclone</td>
<td>opportunities</td>
</tr>
<tr>
<td>12</td>
<td>0/526 0</td>
</tr>
<tr>
<td>10</td>
<td>6/818 6</td>
</tr>
<tr>
<td>11</td>
<td>4/468 7</td>
</tr>
<tr>
<td>5</td>
<td>7/777 7</td>
</tr>
<tr>
<td>9</td>
<td>9/795 9</td>
</tr>
<tr>
<td>15</td>
<td>9/437 16</td>
</tr>
<tr>
<td>17</td>
<td>8/582 17</td>
</tr>
<tr>
<td>8</td>
<td>14/584 19</td>
</tr>
</tbody>
</table>

Homogeneity χ² = 21.47, 0.01 > P > 0.001

<table>
<thead>
<tr>
<th>B: Same data arranged in 100 fission intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–100</td>
</tr>
<tr>
<td>100–200</td>
</tr>
<tr>
<td>200–300</td>
</tr>
<tr>
<td>300–450</td>
</tr>
</tbody>
</table>

Homogeneity χ² = 11.56, P = 0.01

* Both selfers from a cross of an unexpected III to HG2, a mat2/ mat3 wild strain. One stabilized line was mt III; all others were mt II.
* Data from secondary subclones from 1° selfing subclone 12 isolated in the first expansion.
* Some lines discontinued at fission 240.
* For the interval 100–450 fissions: 47/3942, RF × 10⁴ = 12; χ² = 6.15, 2 df, 0.05 > P > 0.02.

In this experiment (S2 and S15) we also examined the question of stability more carefully. No lines were tubed unless stable for at least 50 fissions and transfers were continued on some "stabilized" lines for more than 200 fissions. The number of lines which selfed again or apparently remained stable are depicted in
lines of one of these continued as pure for mt. Twenty-four sublines immediately selfed strongly and continued selfing in all subcultures throughout the experiment. The remaining five sublines selfed weakly and erratically. In the second set of sublines all 38 selfed throughout the 112 fissions.

Micronuclear analysis of selfers and their stabilized sublines: Orias (1958a) reported that conjugants and crosses between stable subclones from selfing lines in T. pigmentosa gave aberrant mt ratios in their progeny, and he suggested that somatic instability of mt is associated with unconventional micronuclear behavior. To explore further the micronuclear composition of selfers, E.M.S. has obtained breeding data for 19 additional selfing clones, by isolating pairs from within the original selfing clones, by arranging crosses between stabilized sublines and homozygous mat3/mat3 tester stocks or by crossing sister stabilized sublines. Further inbreeding crosses were made with the progeny of two of these selfers in an effort to evaluate the micronuclear genotype more completely.

The data from 11 E.M.S. selfers are summarized in Table 2. They demonstrate that selfers which represent the three possible heterozygous mat allele combinations produce progeny which are generally compatible with the expected 1:1 ratio for testcrosses (X III) and 3:1 ratio for crosses between heterozygotes (S X Self). Moreover, the micronuclear instability which is responsible for selfing and is usually resolved in the stable expression in subclones of either of the two expected mts is not correlated with the loss of an allele from the micronucleus. Two examples are shown in Table 2C—the results of crosses involving the mt II subclones of 17-31 and 12-8 manifest mat1/mat2 genotypes. Thus the dominant allele is functional in the germinal but not in the somatic nucleus of lines which have stabilized to the more recessive mt. The exceptions to these generalizations are the following: (1) all three mat alleles appeared unexpectedly: an mt I from a mat2/mat3 testcross (16-9), two IIs from mat1/mat3 testcrosses (22 and 3-16) and 20 IIIs from mat1/mat2 inbreeding and testcrosses (17-31, 12-3, 5-4); (2) these illegitimate mt IIIs did not occur equally in all relevant crosses, e.g., 1.8% in 17-31 and 12-8 vs. 9% in 5-4; (3) selfers appeared—more commonly among intraclonal progeny of selfers (14%, 55/255) than in testcrosses of stabilized lines (1%, 7/751), and some stabilized lines differed from their sisters—the four selfers derived from 22 were among 30 progeny of one cross (13%) while the three sister lines yielded 0/62 progeny; (4) the fit to Mendelian ratios is often improved if the selfers and/or forbidden mt IIIs are combined with the mt I progeny instead of omitting them from the analysis (Table 2, B and C); and (5) the testcross of one subline of 5-16 yielded 57 Is and 6 IIIs which cannot be taken for a 1:1 ratio. Such a result could occur if the mating pairs failed to com-
plete conjugation. Under such circumstances the parental phenotype and full maturity are maintained. All synclones in our experiments were tested for immaturity with testers of the three mts (from both syngens for "hybrid" progeny). Those which mated well were discarded. In a few synclones from many crosses, weak mating reactions are seen. Unless the mixtures are examined very carefully, these reactions often escape detection. That most of this mating occurs because of the presence of a few "early mature" cells is shown by their 1:1 ratios of mt I:mt II in our crosses between matI/mat2 (I) and mat3/mat3 (III) parents which were performed simultaneously with the cross in question. This aberrant cross included more weak reactors than did most other crosses, but if they are eliminated, the 1:1 ratio becomes even more skewed (46:1). The chief significance of this observation is that it demonstrates a change from mat1/mat3 to mat1/mat1 in the germinal micronucleus that occurred during vegetative growth. To the times previously considered for mt genetic changes (meiosis and macronuclear DNA processing) must now be added some stage(s) of the mitotic cycle.

Three additional matI/mat2 selfers have been subjected to more intensive analysis. The data (see Tables 3 and 4) confirm and extend the observations made in the preceding paragraph. The original selfer pairs and testcrosses of stabilized sublines of selfer 13-3 (Table 3A) again give 3:1 and 1:1 ratios of expected mts, plus some forbidden mt IIIIs and selfers. Furthermore, the six stabilized sublines of this selfer that were analyzed by testcrosses provide evidence for at least two classes of matI/mat2 sublines; two mt I and two mt II lines give high frequencies (12%) of mt III, and two mt IIs produced no mt III (homogeneity \( \chi^2 \) calculated on individual crosses, \( P = 0.015 \)). These differences also arose vegetatively within a single clone, and are not correlated with the macronuclear stabilization events. Homogeneity \( \chi^2 \) tests on these six testcrosses with the mt II and selfer progeny pooled with mitochondrial value >0.99 and an excellent 1:1 ratio (128:124); with the aberrant progeny combined with mt II the homogeneity is drastically reduced (although still acceptable, \( P = 0.1 \)) and the ratio of 101:151 is not an acceptable 1:1 ratio.

Further evidence of vegetative micronuclear changes is seen in the testcrosses of selfer 20-25 (Table 3D). Unlike the 12 selfers considered so far, the selfer pairs and testcrosses of 20-25 did not yield the expected ratios of mts I and II. Instead one mt I stabilized subline produced no type I progeny; two produced no type II and three produced all three mts, although the proportions of mt III may differ. The type II clone lacks a functional matI allele in the macronucleus but produces mt I progeny.

The analysis of selfers 13-3 and 20-25 was continued with some of the progeny derived from selfer pairs (matI/mat2 × matI/mat2). The testcrosses of 13-

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**Table 2**

**Progeny tests of selfing clones of various genotypes**

<table>
<thead>
<tr>
<th>Selfing clone</th>
<th>mt of subline</th>
<th>Kind of cross</th>
<th>No. of crosses</th>
<th>Viability</th>
<th>Mating types of progeny*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. mat2/mat3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y15</td>
<td>II</td>
<td>× III</td>
<td>4</td>
<td>0.11–0.73</td>
<td>0           12  15         0  27</td>
</tr>
<tr>
<td>21</td>
<td>II</td>
<td>× III</td>
<td>3</td>
<td>0.22–0.50</td>
<td>0           24  14         0  38</td>
</tr>
<tr>
<td>16-9</td>
<td>II</td>
<td>× III</td>
<td>2</td>
<td>0.65, 0.55</td>
<td>(1)         32  42         0  75</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>× Self</td>
<td>1</td>
<td>0.30</td>
<td>0           8  1          0  9</td>
</tr>
<tr>
<td>P3</td>
<td>S</td>
<td>× Self</td>
<td>1</td>
<td>0.40</td>
<td>0           19  15         7  39</td>
</tr>
<tr>
<td>S2</td>
<td>S</td>
<td>× Self</td>
<td>1</td>
<td>0.29</td>
<td>0           41  10         11  62</td>
</tr>
<tr>
<td>S15</td>
<td>S</td>
<td>× Self</td>
<td>1</td>
<td>0.13</td>
<td>0           40  9          9  58</td>
</tr>
<tr>
<td>B. matI/mat2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z2</td>
<td>I</td>
<td>× III</td>
<td>4</td>
<td>0.25–0.71</td>
<td>36          (1)         51  4          92</td>
</tr>
<tr>
<td>3-16</td>
<td>I</td>
<td>× III</td>
<td>2</td>
<td>0.41, 0.85</td>
<td>30          (1)         26  0          57</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>× III</td>
<td>1</td>
<td>0.87</td>
<td>57          0           6  0          63</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>× Self</td>
<td>1</td>
<td>0.37</td>
<td>5           0           6  0          11</td>
</tr>
<tr>
<td>C. matI/mat2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-31***</td>
<td>I</td>
<td>× II</td>
<td>3</td>
<td>0.50–0.54</td>
<td>41          17         (2)  7          67</td>
</tr>
<tr>
<td>12-3**</td>
<td>I</td>
<td>× III</td>
<td>3</td>
<td>0.61–0.78</td>
<td>57          68         (3)  1          129</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>× III</td>
<td>2</td>
<td>0.65, 0.87</td>
<td>44          41         0   1          86</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>× Self</td>
<td>1</td>
<td>0.23</td>
<td>3           3           0  1          7</td>
</tr>
</tbody>
</table>

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* \( \chi^2 \) calculated using data from individual crosses are compatible with the expected 1:1 or 3:1 ratios (except P3, 0.05 > \( P > 0.02 \), and line 2 of 3-16) whether selfers are omitted or included with the more dominant mt.

* Pairs isolated from selfing clones (30 of 17-31, 60 of 12-3) all died; they may have been isolated too late—after those that survived conjugation had separated (F. P. DOERDE personal communication). Progeny of 17-31 were obtained by crossing matI/mat2 stabilized sublines of this clone, one mt I × three different IIs.
3 progeny (Table 3B) revealed one mat2/mat2 (clone 22), one mat1/mat1 (clone 7) with evidence of micro-nuclear instability—2 HIs, a III and a selfer, and a mat2/mat3 (clone 15). The apparently normal mat3 allele probably arose in the selfing parent strain.

In the testcrosses of 20-25 progeny (Table 3E), we have more examples (clones 26, 1, 24) of "normal" mat3 alleles which have replaced more dominant alleles. Unequivocal persistence of instability through meiosis and fertilization is manifested in clones 17 and 26. The anlagen giving rise to their macronuclei developed a stable type I expression, but the micronuclei retained (or regenerated?) an instability that is expressed in the tritypic progeny of testcrosses.

In crosses among these progeny the results are consistent with the genotypes suggested by the testcrosses (Table 3, C and F), and provide one additional item of evidence for the persistence of instability through meiosis—the mt I progeny of 20-25 in the 4 \times 23 (II \times III) cross.

Here we have also, this time among the progeny of single clones, direct evidence that more selfers appear in crosses between selfer derivatives than in crosses with one such parent—for clone 13-3, 8/169 (4.7%) vs. 1/216 (0.5%). The selfers summarized in Table 4, however, do not appear to be "unstable," as they were in the case of the first crosses. Perhaps the progeny of single clones of "normal" mat1 and mat2 exhibit an instability which is observed in crosses of different genotypes but not in crosses of the same genotype.
E. M. Simon and E. Orias

**TABLE 4**

Breeding analysis of 8 × 8 selfing clones EO-1 and EO-2

<table>
<thead>
<tr>
<th>Cross or number of crosses</th>
<th>Viability</th>
<th>Mating types of progeny</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EO1</strong></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>S</td>
</tr>
<tr>
<td>Stabilized lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I × III tester</td>
<td>0.97</td>
<td>77</td>
<td>47</td>
<td>39</td>
<td>12</td>
<td>175</td>
</tr>
<tr>
<td>II × III tester</td>
<td>0.96</td>
<td>84</td>
<td>77</td>
<td>51</td>
<td>10</td>
<td>251</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>161</td>
<td>124</td>
<td>90</td>
<td>286</td>
</tr>
<tr>
<td>I × II</td>
<td>0.71</td>
<td>45</td>
<td>17</td>
<td>2</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>II × mat1/mat2</td>
<td>1.00</td>
<td>40</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>II × mat1/mat3</td>
<td>0.75</td>
<td>54</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>Testcross progeny I × III tester</td>
<td></td>
<td>127</td>
<td>0</td>
<td>109</td>
<td>0</td>
<td>286</td>
</tr>
<tr>
<td>10</td>
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<tr>
<td>2</td>
<td>0.98</td>
<td>85</td>
<td>0</td>
<td>12</td>
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<td>1</td>
<td>0.98</td>
<td>28</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>3*</td>
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<td>50</td>
<td>26</td>
<td>25</td>
<td>0</td>
<td>81</td>
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<tr>
<td>2*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EO2</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selfer pairs</td>
<td>0.85</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Selfer progeny I × III tester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
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<td></td>
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</tbody>
</table>

* mt ratio is compatible with the 4:3:2 ratio of earlier testcrosses (lines 1 and 2, P = 0.4).
* † mt ratio different from 4:3:2 (P = 0.004); it fits 1:2:1 (P = 0.5).

0.74) ratios—4:3:2 for 1:1:III. Other crosses involving the two stable lines produced ratios consistent with these gametic ratios. (The only selfer studied by E.M.S., some of whose stabilized sublines demonstrated similarly aberrant gametic ratios, was 13-3 (see lines 2 and 5, Table 3A). The totals for these four lines 601:8311:20111 or 3:4:1 suggest that the mt IIIIs derived from mt I. Attempts to associate type III progeny with either mat1 or mat2 for a 1:1 ratio fail with EO-1.) A sample of 18 mt I testcross progeny of the stabilized EO-1 mt I subclone were again testcrossed. Ten of these behaved as normal mat1/mat3 heterozygotes. Three others gave progeny of types 1 and III, but with a strong bias for mt I and shown by a binominal exact analysis to differ from a 1:1 ratio (compare with Table 2B, line 3). Five yielded three mts in different relative frequencies; the ratio produced by three of them was similar (P = 0.4) to that of the original testcrosses; that produced by the last two lines was different (P = 0.004). Whatever the anomaly of the parental selfer might have been, it was transmissible through meiosis and fertilization; nearly half (8/18) of the progeny are still manifesting transmission irregularities following two successive conjugations.

A second selfer studied by Orias (EO-2) was analyzed in a different way. Testcrosses of seven mt I progeny (Table 4) like those of E.M.S. 13-3 and 20-25 (Table 3, B and E) gave varied results. However, three behaved like mat1/mat3 heterozygotes with an excess of type III progeny in contrast to those of EO-1. Clearly all three mat alleles were also present in selfer EO-2 and mat1 and/or mat2 were "lost" in some sexual progeny.

In summary, these micronuclear analyses (Tables 2, 3 and 4) have led to the following conclusions. (1) A great majority of selfers in syngen 8 and in 6 × 8 "hybrids," and subclones from them which have stabilized for the expression of one mt transmit mat alleles with results similar to those obtained with non-selfing strains; i.e., 1:1 or 2:1 ratios of expected mts, sometimes accompanied by a few illegitimate mts and/or selfers. (2) Selfing progeny are more frequent if both parents have been derived from selfers. (3) The proportion of unexpected mt III progeny produced by subclones of a clone can vary significantly. (4) Regardless of the genotype, the determinants for all three mts are retained at least in some strains and are "activated" in rare progeny. (5) While selfing is a manifestation of macronuclear instability which usually has no effect on micronuclear behavior, these experiments with T. pigmentosa have exhibited many examples of micronuclear instability during vegetative growth which are revealed only after conjugation and are not correlated with macronuclear stabilizations. Alleles are lost or gained, ratios of mts in testcrosses can be very skewed either to dominant or recessive alleles and some subclones transmit three mat alleles in ratios as nearly equal as 4:3:2. (6) These mitotic events are certainly much more frequent than conventional mutations.

**Cytological observations:** The ability of a clone to
transmit three alleles could be explained by any of several cytologically detectable conditions. A cell might have multiple micronuclei with different genotypes. It might be triploid or trisomic with different alleles on its three homologous chromosomes. We have made a considerable effort to detect chromosomal differences between stable and selfing lineages of *T. pigmentosa*. Bimicronucleate cells appear occasionally in both kinds of clones, but are not preferentially associated with selfing. They comprised less than 4% of the cells in eight nonselfing wild strains of syngens 6 and 8 (i.e., 0/118, 6/765, 4/115). Most counts made on selfers were similar (3 subclones of S2—1/33, 0/89 and 0/32 and 2 subclones of S15—1/140 and 0/157). However, in subclones 2 and 8 of S15 (see Table 1) which contained some abnormally shaped cells, about 20% of the cells had two micronuclei, or more than four meioic products. The micronuclear size and meiotic behavior are not systematically different in selfers, though aberrations are sometimes encountered in both kinds of clones. Selfers are certainly not triploid, and trisomics have not been observed. Selfing lines sometimes produce slow vegetative growth variants and morphologically deviant lines such as have been described in *T. thermophila* (NANNNEY 1957). The analysis of selfers in *T. thermophila* was made difficult by poor growth in many lines (ALLEN and NANNNEY 1958). Data from *T. pigmentosa* selfers with significant growth abnormalities were not included in this paper. Calculations of Rfs adjusted for the slow growth of a few lines did not change the values. Defective lines were not specifically associated with the selfing condition; approximately 50% of them returned to normal after a few transfers. So far as we can determine, selfers and irregular allele transmitters are cytologically different than stable lines.

**DISCUSSION**

The present study confirms previous analyses of mt inheritance in *T. pigmentosa* (ORIAS 1963; SIMON 1980), and clarifies somewhat the "exceptional" phenomena of selfing and "illegitimate" progeny. We also suggest a possible mechanism to explain the maintenance of dominant alleles during long periods of vegetative growth with amitotic macronuclear divisions. Since vegetative instability of mt has been intensively studied in *T. thermophila*, the selfing of *T. pigmentosa* provided the opportunity to build a bridge between the two major classes of mt systems in the species complex. Our analysis of the *T. pigmentosa* selfers shows, however, that they have different properties and must be explained in a different way.

**Selfing in ciliated protozoa other than**

**T. pigmentosa**

Intraclonal mating occurs in many ciliates under a variety of conditions. A few examples are: wild selfers in *Tetrahymena* (GRUCHY 1955; CHEN 1982; CORLISS 1960; BATSON 1983, 1985) and in *Euploites* (KATASHIMA 1959; NOBILI 1966); selfers showing transient changes of mt in *Paramecium* (BLEYMAN 1967; TAUB 1963; SONNEBORN, 1966); selfing triggered by environmental changes in *Paramecium* (BARNET 1966; HIWATASHI 1960); selfing in senescent clones of *Paramecium* (JENNINGS 1941) *Euploites* (HECKMANN 1967) and Tetrahymena (BYRD 1959; WELLS 1958).

Among the sexual species of the *T. pyriformis* complex the relatively ancient dichotomy of mt systems holds for the characteristics of selfing in the species for which data are available. *T. malacens* whose mt determination is karyonidal, as is that of *T. thermophila*, also produces selfers with characteristics of phenotypic assortment (SIMON and NANNNEY 1984). In the genically determined, synclonally uniform cluster, on the contrary, selfing in *T. pigmentosa* is not correlated with macronuclear assortment (this report). Nor is assortment involved in the expression of mts in *T. hyperangularis*, for immobilization antigens in this species or in *T. americanus* (SONNEBORN 1974) or for immobilization antigens in *T. pigmentosa* although rare unstable clones, with properties like those of selfers in this species do appear (E. M. SIMON unpublished data).

It is, therefore, unlikely that a single mechanism produces, or permits, instability even among the morphologically indistinguishable Tetrahymenases.

**Mating type determination in** *T. pigmentosa* **Somatic genetics (selfing)**

Most Tetrahymena selfers assort lines of pure alternative mts; thus the alternate specificities are present within the clones and are responsible for the mating. Whether or not these specificities are expressed in individual cells sequentially with the formation of heterotypic pairs [as in *Paramecium pentauralia* (BLEYMAN 1967), *P. septaurelia* (TAUB 1966) or *P. multimicronucleatum* (BARNET 1966)] or simultaneously with the formation of "homotypic" pairs has not been studied. Each cell in such pairs might have a mixture of recognition factors on their surfaces because their macronuclei may be actively transcribing two alleles for mt. A few experiments show that single cells do retain alternate potentialities. S. L. ALLEN (personal communication) found that cells from strong selfers of *T. thermophila* isolated into exhausted medium in capillary tubes undergo one (or two) cell divisions, starve and mate. A few *T. pigmentosa* selfing pairs were separated before they were committed to complete conjugation. Each small culture so derived selfed.

The *T. thermophila* selfers arise independently among the karyonides and are a manifestation of incompletely assorted mosaic macronuclei at maturity. (As the mt locus in *T. thermophila* is complex and controls an array of potentialities, mosaic macronuclei can occur in homozygotes. For other characters, e.g., serotypes or isozymes where, as in *T. pigmentosa* mts,
an allele is associated with a specific phenotype, phenotypic assortment is seen only in heterozygotes.) The expression of selfing in *T. pigmentosa* also varies among subclones (probably derived from different karyonides) of the same synclone. However, nonselfing progeny of selfers can transmit to their sexual progeny an increased frequency of somatic mt instability. We suggest that the basis for this selfing might be an inherent instability of DNA rather than assortment of stable subunits. Selfing can occur even when both parents are homozygous for *mat* alleles, and even when new macronuclei are developing from homozygous genotypes. No simple genetic mechanism for selfing is indicated by this analysis.

The *T. thermophila* macronucleus replicates and passively assorts variants with rates determined by the multiplicity of allelic copies. Because the production of pure lines differentially reduces a majority class of alleles in still mixed micronuclei, a cohort of mixed lines gradually approaches an equality of alleles and equal rates of production of the alternative pure derivatives. The “allelic” forms underlying mating type expression are apparently differentially modified forms of the compound mating expression locus in *T. thermophila*—only one of which, according to the hypothesis, is usually retained in the macronucleus (Orias 1981). The *T. pigmentosa* heterozygotes for alleles of two enzyme systems appeared in a limited experiment to assort as do the *T. thermophila* heterozygotes (Preparata, Nanney and Simon 1983), but the *T. pigmentosa* selfers do not behave in this way. Pure sublines of alternative types are produced by most selfers, with much more frequent stabilizations to the dominant mt than to the recessive, and with total Rfs nearly always much lower than the Rfs of *T. thermophila*. Moreover, different *T. pigmentosa* selfers produce “stable” sublines at greatly different rates; the vast majority of these have maintained stability for years, but a significant number of those sublines which were tubed after only two or three transfers selfed again. In addition, many other subclones from the same selfers did not stabilize although they were followed for several hundred generations. To explain such behavior by macronuclear assortment would require very large and variable numbers of elements (200 for an Rf of 25 × 10^{-4}, 27 for 190 × 10^{-4}). Such a hypothesis is strained by the different behavior of selfers from the same cross, the variable Rfs within many selfing clones and by the appearance of perpetual selfers. The biased outputs of dominant alleles could be explained by differential intranuclear selection for the alternative genetic elements, which should, however, accelerate stabilization.

An alternative to variable numbers of assorting units or the differential replication of the kinds of units lies in the concept of conversion. If one class of genetic determinant is occasionally altered, the nucleus will behave superficially as an assorter, but will in fact be “ever-sporting.” This interpretation seems the most reasonable one for the selfers of *T. pigmentosa*. The occasional activation of these latent mt specificities could prevent the perfect stabilization of assorting lines and yield low and variable Rfs depending upon the probabilities of conversion and the accidents of timing.

**Dominance and synclonal uniformity:** Unlike *T. thermophila*, in which codominance of alternative alleles and karyonidal determination are characteristic of many loci, the mt systems of *T. pigmentosa* and some closely related species manifest serial dominance and, in more than 92% of the progeny, synclonal uniformity of expression—all karyonides derived from a pair express the mt of the more dominant allele present. Both of these phenomena are incompatible with randomly assorting macronuclear elements. They could, however, be explained by one of three other mechanisms: (1) the influence of cytoplasmic determinants if massive exchange occurs during mating as proposed by Sonneborn (1957) to explain similar phenomena in *P. bursaria* but refuted by Siegel (1963), (2) selective replication of the more dominant allele in the very early development of anlagen in a heterozygote or (3) unilateral gene conversion of recessive alleles by more dominant alleles. All would produce homogenous macronuclei, alike in all karyonides. The frequently encountered illegitimate alleles are difficult to explain in alternatives (1) and (2) but can be readily accommodated in (3).

**Illegitimate alleles:** The analysis of selfers indicates that the macronuclei in rare clones retain the ability to regenerate alternative mt specificities throughout vegetative life. The breeding results lead to the belief that the cryptic maintenance of all unexpressed mt specificities is also possible in the micronucleus. All three *mat* alleles may appear in crosses between parents whose inferred genotypes would not permit them (see Table 2) and mt III appears in such progeny at a rate approximately 40 times that for mt I or II. A majority of selfers segregate germ line alleles in Mendelian ratios analogous to segregation from nonselfing strains; other selfers transmit all three mt alleles which are inherited vegetatively and sexually in different combinations and often with aberrant ratios. Although some of these irregularities might be explained on the basis of conjugal variations such as autogamy, similar variations were not found for isozyme markers for this species (Preparata, Nanney and Simon 1983), nor can they be attributed to the intersyngeneic source of many of these selfers. The aberrations were first described in studies limited to syngen 8 (Orias 1959a) and selling also occurs in syngen 6 (E. M. Simon, unpublished data).

The breeding performance of subclones shows that changes in the ability to produce gametes with illegit-
imate alleles occur during vegetative growth. Structural changes in 5S rRNA gene clusters during vegetative growth of *T. thermophila* have been described by ALLEN et al. (1985). In clone 13-3 (Table 3A) four of six stabilized lines, which should be *mat1/mat2*, produced 12% mt III progeny. In contrast, two other lines from the same clone produced no mt III progeny. The simplest interpretation is that the micronucleus of the original clone contained a *mat1* that was destabilized in some vegetative subclones. Perhaps subclones transmitting type III alleles are mixtures of two kinds of cells: some that produce only *mat1* or *mat2* gametes, and others that have changed so that their micronuclei are effectively *mat2/mat3* (SIMON 1980). The testcrosses of subclones of selfer 20-25 (Table 3D) suggest that either *mat1* or *mat2* can be lost vegetatively but the samples are too small to warrant analysis.

The argument for convertible alleles is strengthened by the occurrence of similar pairs of metastable triallelic subclones. Two subclones of E0-1 (Table 4, lines 1 and 2) yielded indistinguishable mt I:II:III ratios which are approximately 4:3:2. The testcross progeny of four subclones of 13-3 (Table 3A line 2 and footnote a) approximate a 3:4:1 ratio; although a homogeneity *χ*² (0.2 > *P* > 0.1) did not distinguish between the mt I and mt II subclones, larger samples might have demonstrated a significant difference. The subclones from the same source are not demonstrably different, but those from different sources have quantitative distinctions (homogeneity *χ*² test calculated on mt I:II:III progeny—*P* < 0.0005). E0-1 and 13-3 may represent two stability configurations that are alike in their qualitative features, but which are resolved with different proportions of the *mat* alleles. Although the triallelic nuclear states are capable of being maintained within a defined population, the data documenting their variability are also strong.

**Macronuclear and micronuclear misbehavior:** ORIAS (1959a) first showed that some selfers of *T. pigmen*osa have aberrant allele transmission. The present study on a larger sample of selfers supports the connection between misbehavior in the micronuclei and the macronuclei, but it also demonstrates that the two phenomena can be dissociated. Selfing macronuclei produce sublines that no longer self but that continue to be associated with peculiar allele transmission patterns. On the other hand, clones that are initially selfers and transmitters of three alleles, may undergo a macronuclear stabilization to a normal triallelic form. Both the micronuclear and macronuclear conditions are metastable—somewhat hereditary—but they may stabilize independently to provide more conventional genetic behavior. The association between the two kinds of irregular nuclei lies in their common origin in the synkaryon, not in their genetic or physiological interactions.

All of these considerations argue against a model of mt differentiation based on irreversible *mat* allele losses (*e.g.*, deletions) and support instead a model of *mat* allele interconversions (*e.g.*, duplication-transpositions). In the next section we engage in some pure speculation.

**Possible models:** I. Some selfers are trisomic or triploid. That triploidy is not characteristic of all selfers has been shown by cytological observation. ORIAS (1959a) considered simple trisomy to explain the transmission of all three *mat* alleles in testcrosses of E0-1 and E0-2 but rejected the hypothesis because mt ratios obtained were incompatible with those expected. In addition, a different mechanism is required to account for “biallelic” selfers and dominance.

II. The three-gene cluster model. All three *mat* genes can be expressed and are so closely linked that none is lost by assortment. However, evidence that genes shown to be closely linked by nullisomic analysis (BRUNS 1982) fail to assort is still lacking. Mobile genetic elements, not sequences coding for specific products, would activate or deactivate gene expression by insertion or excision at specific sites in the gene cluster during macronuclear development.

III. The duplication/transposition (cassette) model. There could be a transcribed locus (*mat*), which (a) might lack a coding sequence or (b) might control the most recessive mt, and silent cassettes with information specific for two or three different alleles. Some aspects would be similar to one yeast mt system (HABER 1983). In the more than 95% progeny of an average cross with stable mts a unilateral gene conversion of recessive alleles by more dominant alleles during macronuclear development might occur. The rare selfers could result from (1) possibly delayed cassette to *mat* conversions or (2) a duplication of *mat* in the germ line of some clones. The proposal of a detailed mechanism to explain several secondary complexities which we have described would require us to make too many assumptions at present.

**Other possibly related phenomena:** Hypotheses unifying mating phenomena in ciliates were proposed by NANNEY and ALLEN (1959) and ORIAS (1959a). In view of our present results we suggest that the major possibility for similarity is in changeable DNA albeit by differing mechanisms.

Extensive recombination among macronuclear "chromosomes" has been invoked to explain the somatic breakdown of macronuclear linkage in *Tetrahymena* by heteroduplex formation leading to gene conversion (DOERDER, LIEF, and DOERDER 1975). Physical evidence of DNA structural alterations during macronuclear development is accumulating rapidly for *T. thermophila*: fragmentation (PREER and PREER 1979), C4A2 repeats at termini (YAO, BLACKBURN and GALL 1981), regions flanking the tubulin genes (CALLAHAN, SHALKE and GOROVSKY 1984),
breakage and rejoining (Yao et al. 1984) internal rearrangements in fragments (Howard and Blackburn 1985), etc. Such alterations provide concrete evidence for structural changes which would explain the normal irreversible processes of macronuclear differentiation more readily than physiological processes as predicted by Nanney (1980). The selfers are rare exceptional clones.

Unidirectional gene conversion has been implicated in alterations in systems with mobile DNA: mt switching in Saccharomyces cerevisiae (Haber 1983), Schizosaccharomyces pombe where two silent loci are adjacent and separated from the expression site by a hot spot (Sonneborn and Schneller 1979) since about 1959 have noted probable analogies to the unstable phenomena in other systems, e.g., maize (Federoff 1983), which were known at the time, and which are now known to involve mobile genetic elements. However, we believe this to be the first attempt to develop a model involving duplication/transposition mobility of DNA to explain specific unstable genetic phenotypes in the ciliated protozoa. Cherry and Blackburn (1985) and Herrick et al. (1985) have described probable families of transposable elements in ciliates but no responsibility for coding or regulation of known cellular phenotypes was inferred. Borst (1983) while reviewing the trypanosome antigen system suggested that ciliate selfers could be analogous to mt switching in yeast and mentioned that, except for the trypanosomes, no other phenomena with mobile genetic elements were then known in protozoa—"the most diverse and versatile eukaryotic phylum."

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LITERATURE CITED


Bruns, P. J., T. B. Briggsard and E. V. Merriram, 1985 Nullisomic Tetrahymena. II. A set of nullisomics define the germinal chromosomes. Genetics 104: 257-270.


Howard, E. A., and E. H. Blackburn, 1985 Reproducible and variable genomic rearrangements occur in the developing so-
Tetrahymena Mating Type Instability

449


ORIAI, E., 1959b Mating interaction between varieties 6 and 8, *Tetrahymena pyriformis*. J. Protozool. 6 (Suppl.): 19.


YAO, M.-C., I. J. BLACKBURN and J. G. GALL, 1981 Tandemly repeated CCCCAA hexanucleotide of *Tetrahymena* is present elsewhere in the genome and may be related to alteration of the somatic genome. J. Cell Biol. 90: 515–520.


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