Genomic Exclusion and Other Micronuclear Anomalies Are Common in Genetically Defective Clones of *Tetrahymena thermophila*

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ABSTRACT

Genomic exclusion (GE) is an abnormal form of conjugation which has previously been described in detail for three hypodiploid strains of *Tetrahymena thermophila*. These strains cannot form gametic nuclei and by failing to participate in normal reciprocal fertilization their genes are excluded from exconjugants. To determine whether GE is a general property of infertile strains, we surveyed genetically and cytogenetically 19 additional strains of *T. thermophila* to determine why they failed to contribute genes to sexual progeny. Crosses to genetically marked tester strains showed that seventeen of these strains undergo GE. In each case GE appears to be due to the failure of the defective partner to form functional gametic nuclei. The normal conjugant, however, contributes to its defective partner a haploid nucleus identical to its own, and following diploidization of the unfertilized nuclei, the conjugants separate retaining the old macronuclei. Cytofluorimetric measurement of micronuclear DNA content in 18 strains suggests that aneuploidy is the proximate cause of GE; eleven strains were hypodiploid, five were diploid and three were hyperdiploid. Many irregular cytogenetic events were observed in conjugants presumably not undergoing GE, including, in some instances, abnormal meiosis in the normal partner. Since genomic exclusion was found in both wildtype and mutant clones, the results suggest that it should be possible by appropriate crosses to identify genomic exclusion strains of any genotype.

CLONES of *Tetrahymena thermophila* inevitably lose the ability to transmit genes to sexual progeny (NANNEY 1963, 1974; SIMON and NANNEY 1979). Because such infertile strains are useless in ordinary genetic analysis, they are usually discarded without determining the reason for their infertility. However, for some clones infertility is due to the loss at conjugation of the germinal micronucleus in a process called genomic exclusion (GE). The genetic details of GE were first described by ALLEN and coworkers in C*III (pronounced as "C star") (ALLEN 1963, 1967; ALLEN, FILE and KOCH 1967; ALLEN and WEREMIUK 1971; ALLEN, WEREMIUK and PATRICK 1971). Subsequently, Doerder's group described two additional GE strains, A*III and A*V (WEINDRUCH and DOERDER 1975; DOERDER and SHABATURA 1980), and SCHOLNICK and BRUNS (1982) showed that GE is the most frequent outcome of matings in which macronuclear development is aborted. GE is of interest not only for the insights it provides into germinal aging and the cytogenetics of conjugation, but also, paradoxically, for its considerable practical utility in the formation of heterokaryons and whole-genome homozygotes as described below. In this paper 19 wild-type and mutant strains of *T. thermophila* chosen because they failed to undergo normal conjugation are shown by segregation analysis to be GE strains. These results support the hypothesis of NANNEY (NANNEY 1963, 1974; SIMON and NANNEY 1979) that GE is a common manifestation of germinal (micronuclear) aging in *T. thermophila*.

In order to understand the genetic and cytogenetic events of GE, it must be recalled that exconjugants formed as a result of normal conjugation are (1) genotypically identical, (2) sexually immature and (3) depending upon the genotypes involved, phenotypically different from parental conjugants. Genetic identity results from the reciprocal exchange of haploid gametic nuclei between two cells temporarily fused at the oral apparatus. The gametic nuclei are formed in each conjugant by mitosis of a single meiotic product and therefore are identical in each conjugant. Hence, upon reciprocal exchange of single gametic nuclei, conjugants become genetically the same. Sexual immaturity and phenotypic change in each exconjugant clone result from discarding the old macronucleus and the development of new ones from products of the fertilization nucleus. Although phenotypic change is manifest within a few fissions, sexual immaturity lasts for 40–60 fissions.

Genomic exclusion can be thought of as micronuclear transplantation that requires two rounds of mating to become phenotypically manifest. The micro-

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nuclei of C*III, A*III and A*V are severely hypodiploid, containing less than half the diploid number (*2N* = 10) (RAY 1956) of chromosomes (ALLEN 1967; ALLEN and WEREMIUK 1971; WEINDRUCH and DOERDER 1975; DOERDER and SHABATURA 1980). Conjugation with a normal partner results in the meiotic destruction of the aneuploid micronucleus and its replacement with a nucleus derived from the normal conjugant. Because it cannot form functional gametic nuclei, the "star" cell does not participate in reciprocal fertilization. Instead, it simply receives a gametic nucleus from its partner, and the partner, meanwhile, is left with an unfertilized nucleus identical to the one given to the "star" cell. The failure of fertilization does not, however, result in death as might be expected. Rather, the gametic nuclei in both conjugants are diploidized by endoreplication, and the conjugants separate retaining the old macronuclei. Because the old macronuclei are retained, no new life cycle is initiated; the exconjugants are sexually mature and display parental phenotype. This process is referred to as genomic exclusion because destruction of the "star" micronucleus effectively "excludes" its micronuclear genome from future sexual progeny.

In round 2, as in normal conjugation, the new micronucleus of the "star" exconjugant forms gametic nuclei and reciprocal fertilization occurs; in addition, round 2 exconjugants discard the old macronucleus and develop new ones. Thus, in contrast to normal conjugation which results in sexually immature progeny in a single round of mating, GE requires two rounds to initiate a new life cycle. The only genetic differences from normal conjugation are (1) that the genes from the original "star" micronucleus are excluded, and (2) that when exconjugants from the same round 1 pair undergo round 2, the progeny are completely homozygous, since both round 2 conjugants possess identical micronuclei derived from a single round 1 haploid meiotic nucleus.

Genomic exclusion is of both practical and theoretical interest. On the practical side GE can be used to form heterokaryons. After round 1, the "star" cell is a heterokaryon which, by virtue of macronuclear retention, has the phenotype of the original cell and a micronucleus that may differ genetically from the macronucleus at many loci depending on the choice of the normal partner. Heterokaryons permit, for example, the construction both of nullisomic strains missing one or more micronuclear chromosomes (BRUNS, BRUSSARD and MERRIAM 1983) and of strains in which the micronucleus carries mutations (including lethals) not present in the macronucleus. Another practical use of GE is the construction, in round 1, of whole genome homozygotes, and in round 2, of exconjugants expressing such a genome. On the theoretical side, GE is of interest as an alternative genetic pathway to normal reciprocal fertilization.

Genetically defective strains of *T. thermophila* appear rather frequently, either early in the life cycle, or inevitably after hundreds to thousands of asexual fissions (SIMON and NANNEY 1979) and are routinely identified by the effects of macronuclear retention, *i.e.*, parental phenotype and lack of sexual immaturity. In this paper, a survey of 19 such "star" strains shows that GE as previously described for C*III, A*III and A*V is the major outcome of conjugation. The results also suggest that further study of the cytogenetics of "star" clones may yield a greater understanding of normal conjugation.

MATERIALS AND METHODS

Strains: The strains of *T. thermophila* used in this study are listed in Table 1. Fertile strains were of two types, wildtype and mutant. Wildtype strains A and B were originally obtained from DAVID NANNEY (University of Illinois). Mutant strains B280-1a, B280-1b and B367 were derived in our laboratory and have a strain B genetic background. They are fertile strains heterozygous (B280) and homozygous (B367) for serotype (*rseC1*), cell shape (*con*), and temperature-sensitive cell division arrest (*cdaA1*). With the exception of the WH strains, "star" strains were initially identified as unable to transmit genes to progeny in either our laboratory or that of DAVID NANNEY by ELLEN SIMON.

Wild-type and defective strain designations are coded as follows. Roman numerals designate the mating type, the last 2 Arabic digits indicate the year the strain was last inbred, and the preceding 1–2 digits represent the inbreeding level of the strain at that time. A "star" in the name means that the clone is infertile, and the beginning combination of letters and numbers designates the strain. For example, B*1463-VII is an infertile member of strain B derived from the 14th generation of inbreeding in 1963 and is mating type VII. The lower case letter in B280 strains designates sibling clones of different mating type. WH-6*I and WH-14*II are wild isolates that have not been inbred since shortly after isolation from nature. They are, in fact, the original parents of strains A and B, which are in turn one of the parents from which other inbred strains are derived. For the sake of brevity, stocks will be referred to by their strain and mating type, with a "star" if necessary.

Media: PPY = 1% w/v proteose peptone (Difco Laboratories) and 0.15% w/v yeast extract powder in distilled water; autoclaved. PPYPS = PPY containing 1 mg/ml each of penicillin-G and streptomycin sulfate added after autoclaving. BP (bacterized peptone) = a 1:70 dilution in sterile distilled water of a PPY culture of *Klebsiella pneumoniae*. The bacterial cultures were inoculated, grown until cloudy and then refrigerated at 4° until needed.

Maintenance of stocks: Because micronuclear deterioration is more likely to be observed with increasing fission age (and in the absence of liquid nitrogen storage when this study was performed), it was important to minimize the fission age of stocks, particularly fertile strains B280-1a and B280-1b, during the experimental interval. Therefore, for all stocks a "conservation ritual" (HUTNER *et al.* 1973) was used to minimize the number of fissions occurring from beginning to end. The longest period of such growth in BP without establishment of new cultures by this routine was one month. The B280 tester stocks aged less than 400 fissions during the course of this study.

Protocol for genetic analysis: The triple heterozygotes B280-1a and 1b were used as tester stocks to measure genetic viability and genetic segregation ratios. Using BP media, crosses were performed at room temperature essentially as previously described (WEINDRUCH and DOERDER 1975). Sexual maturity of Rounds 1 and 2 progeny was tested by adding cells of nonparental mating type to day-old BP replicates (and controls) and scoring the next day for the presence of pairs. In some cases immature progeny were grown to maturity in BP.

Genetic markers were scored as follows. The *con* (conical) cell-shape marker was scored visually on the original plate on which pairs or single cells were isolated (DOERDER *et al.* 1975). The temperature-sensitive markers *cdaA1* and ts-1 were scored on replica microtiter plates containing drops of PPYPS after 18 hr of incubation at 39–40°. Homozygotes for *cdaA1* become large, deformed cells and remain alive at the restrictive temperature (FRANKEL *et al.* 1976), whereas ts-1 homozygotes become small and die rapidly (MCCOY 1973). To examine expression of the *rseC* locus (DOERDER 1979), the original plates were replicated to microtiter plates containing BP at room temperature. Two days later the cells were examined for expression of the L immobilization antigen by adding anti-L serum. The L antigen expressed by *rseC* homozygotes was considered present if the cells were affected (immobilized).

Measurement of micronuclear DNA content: The micronuclear DNA content was measured by cytofluorimetry of Feulgen-stained log-phase cells as previously described (DOERDER and DEBAULT 1978). Measurements of slides of each staining batch were standardized to the G2 mean DNA content of 4C as based on diploid strain B control cells. G2 micronuclei were recognized by their proximity to the notch of the macronucleus. Particularly small hypodiploid micronuclei were often impossible to measure.

Cytogenetics of conjugation: PPY cultures in early stationary phase of strains selected for cytological examination were washed into DRYL's (1959) solution, resuspended to 25–33% or their original volume and starved for 12 hr. DRYL's solution was then added to bring cultures to equivalent densities, and the cells were mixed for conjugation. Samples from each of four mating mixes set up at different times were taken at two-hour intervals. Samples were dried on microscope slides, fixed as for cytofluorimetry and stained following Dippell and Chao's modification of Delameter's basic fuchsin stain (SONNEBORN 1950).

RESULTS

Genetic analysis: When the normal partner is heterozygous, GE as previously described for C*, A*III and A*V results, in two rounds of mating, in the visible manifestation of a 1:1 segregation of genetic markers (ALLEN 1967; WEINDRUCH and DOERDER 1975). To test the 19 additional strains listed in Table 1 for GE, two triply heterozygous tester strains, B280-1a and B280-1b, differing only in mating type were used. Both strains were heterozygous for recessive cell shape (*con*), cell division (*cdaA1*) and serotype (*rseC1*) markers and expressed the dominant (wild type) allele in the macronucleus. Expression of one or more recessive markers after conjugation, therefore, could only be the result of genetic events leading to homozygosity.

Control and "star" strains were mated to one of two fertile, heterozygous tester strains B280-1a and B280-1b and single conjugating pairs were isolated into separate drops of culture medium. From growing cultures of such round 1 exconjugants, two single cell isolates were removed into separate drops and, if necessary, later scored for phenotype, sexual maturity and mating type. If remating occurred in the leftover drop, 2–3 round 2 pairs were isolated into separate drops. After growth, cells in the drops were scored for maturity and phenotype. The results of control, round 1 and round 2 crosses are shown in Table 2. Genetic segregation ratios observed in round 2 exconjugants are shown in Table 3.

As expected (and desired) control crosses of the B280 testers to wildtype and mutant strains yielded 98–100% immature progeny (Table 2), and the genetic markers were transmitted in expected Mendelian ratios (Table 3). These results verify that the B280 strains are highly fertile, and that they correctly transmit the marker genes. By contrast, round 1 exconjugants in "star" × B280 matings either died, or if they survived, were of parental phenotype and mating type. In most instances, the surviving exconjugants underwent a second round (round 2) of mating, and

TABLE 1

Fertile and defective stocks

Strain	Genotype
Fertile strains	
A-1777-I	Wild type
A-1977-VI	Wild type
B-2077-II	Wild type
B-2077-V	Wild type
B-2178-VII	Wild type
B280-1a-II	*+/rseC1, +/con, +/cdaA1*
B280-1b-III	*+/rseC1, +/con, +/cdaA1*
B367-7a-VII	*rseC2/rseC2, con/con, cdaA1/cdaA1*
Defective strains	
A*1868-III	Wild type
A*1868-V	Wild type
A3*865-II	Wild type
B*1767-VI	Wild type
B*1463-VII	Wild type
B2*666-V	Wild type
C*557-III	Wild type
C2*368-I	Wild type
F*1365-I	Wild type
F*1567-II	Wild type
con*273-II	*con/con*
rseA1*371-VI	*rseA1/rseA1*
rseA1*271-II	*rseA1/rseA1*
rseB*271-III	*rseB/rseB*
rseB*476-V	*rseB/rseB*
rseC*172-V	*rseC/rseC*
rseC1*173-II	*rseC1/rseC1*
rseC1,con,cdaA1*IV	*rseC1/rseC1, con/con, cdaA1/cdaA1*
rseC2*III	*rseC2/rseC2*
ts-1*II	*ts-1/ts-1*
WH-6*I	Wild type
WH-14*II	Wild type

TABLE 2

Breeding performance of "star" strains at round 1 and round 2 GE

Strains crossed	Round 1[a] Immature (%)	Round 1[a] Mature (%)	Round 1[a] Dead (%)	Round 2 Immature (%)	Round 2 Mature (%)	Round 2 Dead (%)	N
Control crosses							
A-I × B-V	100	0	0				
B367-7a × B280-1a	98	0	2				
B367-7a × B280-1b	98	2	0				
"star" × B280-1a or B280-1b							
A*III	0	89	11	30	9	62	71
A*V	0	41	59	40	0	60	35
A3*II	0	90	10	10	27	63	30
B*VI	0	86	14	49	4	47	81
B*VII	0	97	3	49	1	50	82
B2*V	0	47	53	36	5	59	42
C*III	2	91	7	25	5	70	83
C2*I	0	74	26	48	5	47	66
F*I	0	99	1	0	88	12	34
F*II	2	67	31	54	12	34	59
con*II	0	12	88	17	8	75	12
rseA1*II	0	91	9	40	18	42	55
rseA1*VI	0	79	21	49	10	41	49
rseB*III	0	88	12	42	0	58	66
rseB*V	0	5	95	67	0	33	3
rseC*V	0	4	96	50	0	50	4
rseC1*III	0	62	38	51	4	46	57
rseC1,con,cdaA1*IV	1	17	82	39	15	46	13
rseC2*III	16	59	25	63	8	29	52
ts-1*II	0	91	9	47	13	40	87
WH-6*I	0	8	92				0
WH-14*II	0	85	15	37	1	62	79

[a] 94–96 round 1 progeny were isolated in each cross.

except for F*I which appeared never to participate in reciprocal fertilization and macronuclear development, the round 2 exconjugants usually either were immature or died. The 1:1 ratio of genetic markers among the round 2 immature progeny is consistent with development of new macronuclei from micronuclei derived solely from the B280 cell in round 1.

Since two rounds of mating (Table 2) and the pattern of genetic segregation (Table 3) are consistent with GE as previously described for C*III, A*III and A*V, 17 of the 19 new strains are shown to be capable of genomic exclusion.

Among the 19 strains, four yielded immature round 1 progeny. There are two explanations for such progeny: (1) normal cross fertilization with macronuclear development and (2) a variant form of GE called short-circuit genomic exclusion. The latter, which has previously been found in C*III (BRUNS, BRUSSARD and KAVKA 1976), is the result of (rare) successful macronuclear development following micronuclear replacement and is detectable by complete homozygosity after just one round of mating. C*III and F*II immature progeny were shown by the phenotype and mating type of the single cell isolates to be the result of this rare process (data not shown). RseC2*III was shown by these same tests to have yielded immature progeny through normal conjugation. Progeny of the fourth strain, rseC1,con,cdaA1*IV, were lost before they could be tested.

As shown in Table 2, most strains yielded round 1 progeny that underwent round 2 as soon as the food supply was exhausted. This simple result indicates that both exconjugant cells survived and divided after mating. This was confirmed by appropriate tests in crosses in which the defective cell had an easily scored phenotype (*e.g.*, con*II and ts-1*II) as well as by mating type tests of the single cell isolates. Mating types in these instances were always those of one or the other parent; this rules out the remote possibility of macronuclear development followed by early maturity.

By contrast, with A3*II and WH-6*I most of the Round 1 progeny did not remate even though they were clearly mature as judged by the mating of the single cell isolates to mating type tester. Further mating type tests (data not shown) showed that A3*II and WH-6*I exconjugant cells preferentially died after conjugation, leaving the B280 mate as survivor.

Micronuclear DNA content: The best characterized GE clones, C*III, A*III and A*V, are severely

TABLE 3

Genetic segregation in control and round 2 crosses of "star" strains to B280-1a or B280-1b

Cross	Number observed +:rseC1	+:con	+:cdaA1	*N* scored
Control crosses				
B-V	75:3[a]	78:0	78:0	78
B367-7a × B280-1a	35:51	37:49	40:46	86
B367-7a × B280-1b	42:41	49:34	40:43	83
Round 2 crosses				
A*III	10:11	13:8	13:8	21
A*V	6:8	12:2[b]	12:2	14
A3*II	1:0	1:0	1:0	1
B*VI	22:18	20:20	21:19	40
B*VII	21:19	19:21	23:17	40
B2*V	4:9	6:7	6:7	13
C*III	10:9	10:9	9:10	19
C2*I	17:15	17:15	15:17	32
F*I				0
F*II	19:13	13:19	13:19	32
con*II	1:1	0:2	1:1	2
rseA1*VI	7:14	11:10	14:7	21
rseA1*II	9:9	7:11	8:10	18
rseB*III	10:16	12:14	21:5	26
rseB*V	0:2	0:2	2:0	2
rseC*V	1:1	1:1	2:0	2
rseC1*III	13:16	13:16	17:12	29
rseC1,con,cdaA1*IV	2:3	3:2	2:3	5
rseC2*III	15:16	18:13	13:18	31
ts-1*II	23:18	20:21	22:19	41
WH-6*I				0
WH-14*II	13:16	12:17	16:13	29

[a] The 3 rseC1 clones are the result of macronuclear assortment.
[b] Underlined ratios indicate significant deviation from 1:1 segregation.

TABLE 4

Micronuclear DNA content of "star" strains

Strain	Haploid equivalents of DNA Mean	95% C.I.	Range	Sample
B control	4.0	0.10	2.8–5.4	89
A*III	2.1↓	0.71	0.6–4.1	13
A*V	1.9↓	0.61	0.8–4.6	15
A3*II	2.0↓	0.18	1.0–3.7	33
B*VI	1.9↓	0.22	1.2–2.9	22
	3.1↓	0.17	1.7–4.2	45
B*VII	3.6↓	0.23	2.6–4.3	22
B2*V	4.1	0.23	3.0–5.2	31
C*III	1.0↓	0.56	0.4–1.8	6
C2*I	4.1	0.21	2.9–5.5	43
F*I	2.7↓	0.23	1.9–4.8	32
F*II	3.7↓	0.18	2.8–5.0	41
con*II	3.9	0.14	3.2–4.7	25
rseA1*VI	2.5↓	0.31	1.3–4.0	20
rseA1*II				
rseB*III	4.5↑	0.50	2.1–5.5	16
rseB*V	3.8	0.17	1.9–4.5	32
rseC*V	2.7↓	0.27	1.7–4.3	33
rseC1*II	3.7	0.36	1.7–6.1	30
rseC1,con,cdaA1*IV	3.3↓	0.25	2.9–3.7	7
rseC2*III	4.8↑	0.27	3.7–6.1	24
ts-1*II	1.9↓	0.19	1.2–3.3	32
WH-6*I	5.7↑	0.47	4.9–6.7	10
WH-14*II	1.3↓	0.11	0.9–1.9	26

↓ indicates hypodiploid value; ↑ indicates hyperdiploid value. C.I. = confidence interval.

hypodiploid as measured by chromosome counts, cytophotometry and physical appearance of the micronuclei (ALLEN and WEREMIUK, 1971; WEINDRUCH and DOERDER, 1975; DOERDER and SHABATURA 1980). Cytological examination showed that many of the present clones were also hypodiploid, but some strains had normal looking micronuclei. Since ciliates are difficult to karyotype, microspectrophotometry was used to measure micronuclear DNA content. The results shown in Table 4 are for G_2 micronuclei, since micronuclei are in this phase for most of the cell cycle. As expected, hypodiploidy was observed in most strains, although the degree of hypodiploidy as measured by average DNA content is probably underestimated since the very small micronuclei close to the macronucleus could not be measured and therefore are not included in the mean. Some strains, however, had diploid amounts of DNA (B2*V, C2*1, con*II, rseB*V and rseC1*II), and a few were significantly hyperdiploid (rseB*III, rseC2*III and WH-6*I). In addition to differences in mean micronuclear DNA content, DNA content of the "star" strains was also more variable than the control. This, too, is consistent with aneuploidy.

Contrary to what might have been expected, there is no clear correlation between micronuclear DNA content and breeding behavior at either round of mating.

Cytogenetics of round 1 mating: Several strains were chosen for cytogenetic examination. Defective strains were mated to a normal control and samples taken at timed intervals were prepared for cytological examination as described in MATERIALS AND METHODS. In the majority of pairs, the defective mate and its fertile partner were readily distinguishable by nuclear size and/or cytogenetic behavior (*i.e.*, events were normal in one cell, abnormal in the other). To permit comparison with GE, the following brief description of normal conjugation is provided (RAY 1956; DOERDER and DEBAULT 1975). After pairing, the micronucleus of each conjugant moves away from its position close to the macronucleus and elongates into a crescent (parachute) 1–2 times as long as the cell. Following condensation of the crescent, meiosis I and II proceed with spindles oriented along the anterior-posterior axis. At the end of meiosis II, one of the haploid nuclei lies close to the oral membranes at the anterior attachment point uniting the conjugants. This nucleus undergoes a mitotic division to form the migratory and stationary gametic nuclei; the three

remaining meiotic products move posteriorly and are destroyed. Following reciprocal exchange of migratory gametic nuclei, fertilization occurs anteriorly to the macronucleus and the fertilization nucleus immediately undergoes two mitotic divisions along the anterior-posterior axis. The two division products in the anterior of the cell immediately initiate macronuclear development, while the two nuclei in the posterior remain as micronuclei. At the end of conjugation each cell (and each exconjugant) contains two macronuclear anlagen, two micronuclei and an old macronucleus undergoing autolysis. By the end of the first cell cycle, the old macronucleus and one micronucleus are completely destroyed. At the ensuing cell division, the remaining micronucleus divides and the macronuclei are distributed to each cell.

A*III, A*V, B*VII and C*III: The cytogenetics of mating in these strains was essentially similar to previously published descriptions for strains C*III and A*III (ALLEN 1967; DOERDER and SHABATURA 1980). Essentially, the prezygotic events were normal in the diploid mate and the hypodiploid micronucleus of the "star" cell was destroyed during meiosis. Following transfer of the normal migratory gametic nucleus into the "star" cell, one of two pathways was followed. In the first, the gametic nuclei behaved as fertilization nuclei and underwent two mitotic divisions giving rise to macronuclear anlagen. However, except for rare instances (short-circuit genomic exclusion), these conjugants died. In the second pathway, the gametic nuclei did not divide ("arrested" division) and following endoreplication to restore diploidy, cells separated without further cytogenetic maneuvers.

C2*I: Unlike the previous strains, the GE strain C2*I had a diploid amount of micronuclear DNA. It also showed variability in cytogenetic behavior. For example, in one experiment, this strain appeared to follow the pattern of A*III, etc., described above. Out of 30 pairs, 20 had developed macronuclear anlagen and 10 (33%) had what appeared to be gametic nuclei that failed to divide. Among 45 pairs isolated at the same time, 33 died and 12 (27%) survived. Such close agreement in survivorship is consistent with the idea that in GE strains, round 1 conjugants which develop macronuclei die. This survivorship, however, contrasts to the 74% reported in Table 2. In another experiment, cytogenetic events were normal in both conjugants until meiosis II, after which in about 25% of pairs, one or, more frequently, both conjugants lacked micronuclei at the attachment membrane and failed to undergo the mitotic division to form gametic nuclei. The subsequent cytogenetic events were impossible to sort out and rationalize with survivorship.

F*I: F*I never produced immature progeny at either round of conjugation (Table 2). By the end of meiosis II, the mating mixture consisted predominantly of two types of pairs, neither of which is likely to be the result of transfer of gametic nuclei, either reciprocal or unilateral. In the first type, as in C2*I described above, one or both conjugants lacked a micronucleus at the attachment membrane. Subsequently, in the same proportion of pairs, no normal micronuclei were present; micronuclei that were present were clearly undergoing autolysis in the posterior region of the cell (the normal position for micronuclear autolysis). These cells presumably died. In the second type, one or both of the conjugants possessed 1–4 large, darkly staining micronuclei, generally in the anterior of the cell. Because of their close resemblance in size and staining intensity to gametic nuclei, presumably autolysis of one or more meiotic products failed to occur. These micronuclei, and the old macronucleus, were retained by exconjugants. Because remating pairs were observed in which conjugants possessed two or more crescents, these retained meiotic products apparently assumed normal micronuclear function.

rseC2*III: This strain possessed a diploid amount of micronuclear DNA and yielded the highest frequency of immature pairs at round 1. Cytogenetic events were normal until meiosis II, after which in 10% of pairs neither conjugant had a micronucleus at the attachment membrane. In late conjugation, virtually all pairs were of two kinds: (1) those in which both partners resembled C*III and A*III by having one arrested micronucleus just anterior to the macronucleus and (2) those undergoing macronuclear development. In this case, however, whereas the former presumably are the products of GE, the latter account for the immature, recombinant progeny described above.

Loss of micronuclear function: As mentioned in the Introduction, all clones of *T. thermophila* eventually lose the ability to transmit genes to progeny if they are not permitted to mate and initiate a new life cycle through macronuclear development. Three months after the crosses in Tables 2 and 3 were completed, the two B280 strains were crossed to B367 to determine whether they remained fertile. B280-1b was completely fertile, but the other strain, B280-1a, yielded, only 58% ($N = 96$ pairs) immature exconjugants (*cf.* 98% in Table 2). Thirty-eight percent of the pairs died, and 4% yielded mature exconjugants that remated. Among the 4 pairs that underwent round 2, 3 died, and the other yielded immature cells that were conical in cell shape and wild type for cell division arrest and serotype. Since B367 was homozygous for the mutant markers, this exceptional recombinant represents neither GE nor normal fertilization and will be discussed in the DISCUSSION.

Among the 58% immature progeny, 51 pairs were

TABLE 5

Abnormal segregation in immature progeny of a later cross of B280-1a

Phenotype:	Number observed		
	+:rseC1	+:con	+:cdaA1
	14:37	9:42	9:42

scorable for the three genetic markers. For each locus segregation was distorted significantly ($P < 0.001$) (Table 5). The identical cross made months before showed no significant distortion, although there was a similar bias toward the mutant allele (Table 3). No systematic bias was observed in the ratios shown in Table 3 in which B280-1a was the donor strain. These marker loci are all located on chromosome *4* (BRUNS 1984), and, curiously, the distortion was in favor of the recombinant mutant chromosome. A possible explanation for this distortion is presented in the DISCUSSION.

Genetic linkage of *con*, *rseC* and *cdaA*: Because homozygous GE progeny possess genes derived from a single meiotic product, round 2GE progeny are the equivalent of unordered tetrads and therefore are suitable for linkage analysis. Because the method of nullisomics (BRUNS, BRUSSARD and MERRIAM 1983) shows that the marker loci are all on chromosome *4* (BRUNS 1984), the data used to derive Table 3 were analyzed for evidence of genetic linkage among the marker loci. They show that *rseC* and *con* could be linked by a minimum of 40 map units; all other combinations show independent segregation. This result is similar to that reported previously (DOERDER 1979) and is consistent with the location of *con* and *rseC* on the left arm of chromosome *4* and *cdaA* on the right.

DISCUSSION

The major conclusion of this study is that most genetically defective strains of *T. thermophila*, whether wild type or mutant, undergo GE at conjugation. GE was demonstrated in 20 out of 22 strains by the presence of two rounds of conjugation and, in the second round, by sexual immaturity and the appearance in 1:1 ratios of genetic markers present in the normal round 1 partner. The exceptional strains, WH-6*I and F*I produced no immature round 2 progeny.

The second important conclusion is that the cytogenetic reason for GE appears to be the same in all instances: the failure of the defective strain to produce functional gametic nuclei and the subsequent unilateral transfer of a haploid gametic nucleus to the "star" cell from its normal partner. In GE, gametic nuclear transfer is followed by diploidization of the unfertilized gametic nuclei and retention of the old macronucleus in both exconjugants. Macronuclear development, if it occurs, is, for unknown reasons, usually lethal.

GE has both practical and theoretical consequences. On the practical side, GE strains are exceedingly useful both as a way of producing "instant" homozygotes and in the production of heterokaryons in which micronuclei and macronuclei are of different genotypes. The recent Tetrahymena literature contains numerous examples of heterokaryon usage. For example, the recent description of mutants in which the cytoplasm affects macronuclear development required the use of heterokaryons derived from A*III and B*VII (DOERDER and BERKOWITZ 1987). The present results show that it should be possible to obtain a GE strain of any genotype, wild type or mutant. All that is necessary is to test the genetically defective clones for two rounds of mating and for segregation of markers solely from the control conjugant. The only caveats are that some strains exhibit higher round 1 and round 2 mortality than others and that heterokaryons can themselves become genetically defective (WEINDRUCH and DOERDER 1975).

On the theoretical side, GE has implications for the mechanisms of cell-cell communication and nucleocytoplasmic interactions during conjugation. The cytogenetic events of normal conjugation are highly synchronized between conjugants (RAY 1956) and are presumably checkpointed through mutual feedback. The relative lack of irregularities at round 2, when new micronuclei are present, suggests that these checkpoints are affected by the defective micronucleus of the "star" cell. A possible checkpoint might be at the attachment membrane. This is suggested by the failure of meiosis and the subsequent mitosis to place a gametic nucleus at the attachment membrane of the "star" cell at the same time as one is placed there in the normal cell. The cytogenetic importance of the oral region, aside from its obvious role as the point of cell-cell contact, has been previously emphasized (NANNEY and NAGEL 1964; DOERDER and SHABATURA 1980). An example illustrating yet another aspect of cell-cell communication is the behavior of strains such as C2*I, F*I and rseC2*III in which micronuclear products in *both* conjugants failed to reach the attachment membrane. In these instances, anomalous behavior in one conjugant appeared to induce similar behavior in the normal cell. NANNEY and NAGEL (1964) observed similar bilateral effects in other defective strains. The reason for such coordinate behavior in these strains and not others is unknown.

The proximate cause of micronuclear dysfunction appears to be aneuploidy, since most GE strains have hypo- or hyperdiploid DNA amounts and also show

considerable variation in micronuclear DNA content. Aneuploidy, particularly if accompanied by chromosomal rearrangements, can explain the failure of both meiosis (through asynapsis) and the subsequent mitosis. However, aneuploidy, *per se*, does not readily explain the failure to place a micronuclear product at the attachment membrane, nor does it mechanistically account for the cytogenetic events subsequent to micronuclear destruction. Moreover, there is no adequate explanation for the origin of aneuploidy except to say that the micronucleus is dependent upon the macronucleus for its replication (WEINDRUCH and DOERDER 1975).

Micronuclear aneuploidy is the likely cause of the loss of fertility and abnormal segregation of syntenic markers in strain B280-1a. Since the segregation distortion involved three linked loci, single-gene effects must be discounted and a chromosomal mechanism must be considered. For example, a monosomic missing the wild-type homolog would, when mated to a homozygous mutant, yield monosomic and diploid progeny all of the mutant phenotype. Wildtype phenotypes would appear in reduced frequency according to the proportion of heterozygotes (disomics) in the cell population. Unfortunately, karyotypic data to test this hypothesis are not available, but since aneuploidy is a common feature of GE strains, chromosome loss seems to be the most reasonable explanation. With the availability of easily scorable markers on all 5 micronuclear chromosomes, it should be possible to design experiments to test whether chromosome loss occurs in a programmed way.

Four exceptional "star" strains yielded immature progeny after a single round of mating. For strains C*III and F*II (and presumably rseC1,con,cda A1*IV), immaturity was probably due to the short-circuit variant of genomic exclusion (BRUNS, BRUSSARD and KAVKA 1976) in which rare cells apparently complete macronuclear development. The present results confirm short-circuit GE in C*III and show that it is rare among GE strains. By contrast, normal conjugation, rather than short-circuit GE, accounts for the immature progeny in the rseC2*III cross. This strain apparently consists of two types of cells, those capable of normal conjugation and those resulting in GE. Such heterogeneity is expected during micronuclear deterioration (WEINDRUCH and DOERDER 1975). SCHOLNICK and BRUNS (1982) also described strains which produce both normal and GE progeny. Moreover, these authors found, as did we, that GE is the most frequent outcome in infertile crosses.

Although GE and death are most commonly found in aging, infertile strains, other nuclear anomalies occur in some strains. For example, in F*I micronuclear divisions terminated with meiosis II and without the destruction of micronuclear products. At round 2, which in GE resembles normal conjugation, these cytogenetic events were repeated, again resulting in mature exconjugants. Another example is the irregular genetic transmission observed in tester strain B280-1a (Table 5) as discussed above. In addition to the segregation distortion, unexpected segregation was also observed in the single pair of immature round 2 exconjugants. Although the precise cytogenetic origin of the observed recombination cannot be determined, the outcome is similar to instances of abnormal cross-fertilization and macronuclear retention described by SCHOLNICK and BRUNS (1982).

The ability of a strain to undergo GE appears to be stable. C*III and A*III have been in existence for over 25 and 13 yr, respectively, and show no significant change in the GE behavior. Although most of the strains described here are no longer extant (having been lost in laboratory moves), B*VI, B*VII and F*I are still available, and both B* strains continue to yield GE progeny (DOERDER and BERKOWITZ 1987; F. P. DOERDER, unpublished results). However, like ALLEN (ALLEN and WEREMIUK 1971), we find that there is variability in the frequency of GE progeny; since subclones can be selected that yield high frequencies of GE progeny, the variability is attributed to clonal heterogeneity. The important point is that the ability to induce GE appears not to be lost. A recent test of F*I suggests that although it may yield some GE progeny, it behaves unpredictably (F. P. DOERDER, unpublished results).

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