CYTOGAMY: AN INDUCIBLE, ALTERNATE PATHWAY OF CONJUGATION IN *TETRAHYMENA THERMOPHILA*

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ABSTRACT

We report the occurrence of cytogamy in *Tetrahymena thermophila*. By analogy to *Paramecium*, cytogamy generates exconjugant clones that derive their entire genetic information from a single meiotic product of their cytoplasmic parent. Thus, "instant" whole-genome homozygotes are created. Cytogamy has been induced in every strain of *T. thermophila* tested, and most of the excytogamous progeny have exhibited high fertility. The high frequency with which cytogamy can be induced by hyperosmotic shock, coupled with the foregoing genetic properties, make this process a practical (and already proven) method for the isolation of recessive mutants in *T. thermophila*. We also report that the cytogamy-inducing treatment induces other rare abnormalities of genetic transmission, which have not yet been characterized.

NORMAL conjugation in the ciliates involves meiosis and subsequent biparental fertilization, generating progeny in ratios that conform to the standard Mendelian rules of genetic transmission (reviewed by SONNEBORN 1975). A uniparental variation of normal conjugation called *cytogamy* has long been known in Paramecium (SONNEBORN 1941). In cytogamy, the entire nuclear genetic information of an exconjugant is derived from a single meiotic product of its cytoplasmic parent. Thus, an "instant" whole-genome homozygote results.

The isolation of recessive mutants has been a challenging problem in *Tetrahymena thermophila* because of its diploid germ line. Successive advances have exploited unique aspects of the mechanisms that handle the genetic information in *T. thermophila* (reviewed by ORIAS and BRUNS 1975). The most efficient method, "short-circuit genomic exclusion" (BRUNS, BRUSSARD and KAVKA 1976; BRUNS and SANFORD 1978), is a self-fertilization induced by mating to the so-called C* strain (ALLEN 1967a,b). While this method has, for many practical purposes, solved the problem of isolation of recessive mutants, it is less than ideal because only a small fraction (around 5%) of the conjugating pairs follow this pathway and, in our experience, most of the resulting clones have low fertility.

While performing crosses of *T. thermophila* for a routine genetic analysis, some rare progeny were found with phenotypes suggestive of cytogamy. Because of the practical importance of uniparental transmission for the generation of homozygotes expressing recessive mutations, we decided to study the process

in more detail in order to learn how to induce it in high frequency. We document here the existence of a process in *T. thermophila* with genetic consequences identical to those of cytogamy. The process generates homozygotes generally exhibiting high fertility. Separately (ORIAS, HAMILTON and FLACKS 1978), we report that cytogamy can be induced by exposing pairs to a hyperosmotic shock during a brief period of sensitivity approximately midway through the conjugation process. Recessive, temperature-sensitive phagocytosis mutants have been isolated using cytogamy (SUHR-JESSEN 1977; SUHN-JESSEN and ORIAS, in preparation).

MATERIALS AND METHODS

Strains: All the clones used are derived from inbred strain B of *Tetrahymena thermophila* (formerly syngen 1 of *T. pyriformis*; NANNEY and MCCOY 1976). A list of the clones and their genetic description is given in Table 1.

Media: *2% PP* medium (proteose peptone containing metal salts; ORIAS and BRUNS 1975) was the nutrient medium used. *2% PP + cycl* and *2% PP + 6mp* are 2% PP medium containing 15 μg of cycloheximide (Sigma) or 6-methylpurine (Sigma) per ml, respectively. Stock solutions of cycl and 6mp were prepared as 30 mg per ml in methanol and 7.5 mg per ml in water, respectively, and stored indefinitely at —20° without loss of activity. Aqueous solutions of cycloheximide were used only on the same day they were made.

Cultures to be crossed were starved in Dryl's medium (DRYL 1959), which contains 1 mM sodium citrate, 1 mM $MgCl_2$, 2 mM Na_3PO_4 buffer, pH, 7.1. Bacterized medium (2% BP; ROBERTS and ORIAS 1973a) was used for mating type tests.

Culture maintenance and other routine procedures: Most of these have been previously described in detail (ORIAS and BRUNS 1975).

Mating-type tests were done by a slightly improved method, as follows. Clones to be tested (available as stationary phase cultures in 100 μl of 2% PP medium in 96-well plates) were replicated to approximately 50 μl of 2% BP medium in flatbottom 96-well plates. At the same time, stationary phase cultures of each mating type tester were diluted 50-fold into 2% BP.

TABLE 1

List of clones used

Clone name	Genetic description*	Source
BIII	Inbred wild type strain	D. L. NANNEY
CU329	*ChxA2/ChxA2* (cycl-S, II)	P. J. BRUNS
CU324	*Mpr/Mpr* (6mp-S, IV)	P. J. BRUNS
CU330	*ChxA2/ChxA2* (cycl-S, IV)	P. J. BRUNS
CU325	*Mpr/Mpr* (6mp-S, V)	P. J. BRUNS
SB100	*ChxA2/ChxA2* (cycl-S, CAR, II)	CU329 Mutagenesis
SB101	*Mpr/Mpr* (6mp-S, CAR, IV)	CU324 Mutagenesis
SB103	*ChxA2/+*, *Mpr/+* (cycl-S, 6mp-S, II)	CU329 × CU324

Locus names: Chx: cycloheximide (cycl) resistance (ROBERTS and ORIAS 1973b; BYRNE, BRUNS and BRUSSARD 1978); *Mpr*: 6-methylpurine (6mp) resistance (BYRNE, BRUNS and BRUSSARD 1978). *ChxA* and *Mpr* are unlinked (MCCOY 1977).

Other abbreviations: CAR: chloramphenicol resistance (ORIAS and ROBERTS 1973a); R: resistant; S: sensitive.

* The first string of symbols refers to the micronuclear genotype. The parenthesized string refers to the phenotype. Roman numerals indicate the mating type.

Both types of cultures were incubated at 30°. Two days later, about 50 μl of each tester were separately mixed with replicate cultures of each unknown. Six hr later, most of the pairing reactions were fully developed and could be scored. By scoring again the next day, most of the remaining positive reactions could be identified.

Standard cross: Cultures of each of the strains to be crossed were prepared by inoculating 10 ml of 2% PP with 0.01 ml of a weekly transferred stock culture. The cultures were incubated at 30°. Two days later, the two strains were separately washed and resuspended in 10 ml of Dryl's medium and kept overnight at 30° in standard petri plates. Next morning, equal numbers of cells from the two cultures, each at concentrations of between 2 and 5 × 10^5 cells per ml, were mixed at 30°. Within 30 to 60 min, the first pairs were seen. At the appropriate time after mixing (285 min if cytogamy is to be induced at high frequency), the mating culture was diluted with 2% PP and pairs were immediately isolated into drops of 2% PP medium in petri plates (see ORIAS and BRUNS 1975, for details). Three days later the cultures were replicated for drug-resistance tests. All incubations were done without shaking.

When exconjugants were to be isolated, the previous procedure was modified as follows. The cultures were mixed at room temperature (about 22°). At the desired time (nine hr after mixing if cytogamy is to be induced with high frequency), pairs were isolated as above, but kept at room temperature. Exconjugants began to separate about 24 hr after the cultures were mixed. Before the first postconjugation fission, one exconjugant was removed to a separate drop. The plates were incubated at 30° for three days and then replicated.

Isolation and genetic characterization of chloramphenicol-resistant derivatives: In order to determine the cytoplasmic parents of cytogamous clones, chloramphenicol-resistant derivatives of clones CU329 and CU324 (SB100 and SB101, respectively) were obtained. They were induced by nitrosoguanidine mutagenesis (10 μg per ml for one hr at 30° according to the method previously described (ROBERTS and ORIAS 1973a).

To show that these two mutations do exhibit cytoplasmic inheritance (as do all other chloramphenicol-resistant mutations isolated in *T. thermophila*), the crosses SB100 × CU324 and SB101 × CU329 were performed. Exconjugants were separated and tested for resistance to chlor, cycl and 6mp. Sixty-four and 67 pairs of exconjugants that had undergone sexual reorganization were obtained from the two crosses, respectively. In all cases, one exconjugant was chlor-R and the other chlor-S, as expected.

RESULTS

Genetic system for detecting cytogamy: Two very useful, genetically marked clones (CU324 and CU329) were used to investigate the occurrence of cytogamy. Such clones have been termed functional heterokaryons (BRUNS and BRUSSARD 1974), since they express a phenotype (determined by the macronucleus) different from their micronuclear genotype (Table 1). These strains allow the phenotypic distinction of pathways that conjugated pairs can follow (normal cross-fertilization, cytogamy and nonconjugation), as explained in Figure 1. For the experimental distinction between the various outcomes, we used either of two different protocols: the exconjugant separation (XCS) or the mixture resolution (MXR) assays (Figure 2).

The XCS assay (Figure 2, upper panels) was performed by separating exconjugants as described in MATERIALS AND METHODS and separately replicating to 2% PP + cycl and 6mp media, respectively. The cultures were kept at 30° for three days and then scored. The MXR assay (Figure 2, lower panels) was performed on cultures derived from individual pairs without exconjugant separation. It is less rigorous, but also less laborious. Pairs were isolated into drops as described in MATERIALS AND METHODS. Three days later, the cultures were repli-

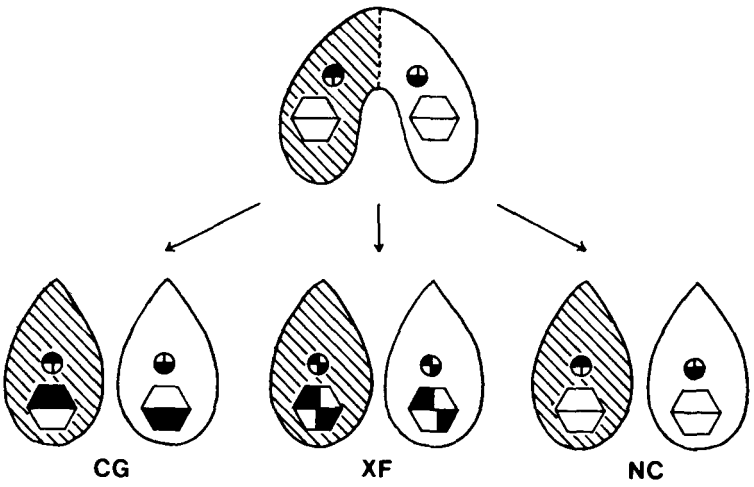

FIGURE 1.—Various genetic compositions expected for the pairs of exconjugants derived from the cross of CU324 and CU329, depending on the conjugation pathway followed. Key to the symbols used. Hexagon: macronucleus; circle: micronucleus; "northern hemisphere": *Chx* locus; "southern hemisphere": *Mpr* locus; solid color: resistance (dominant); white: sensitivity; the cytoplasm of one conjugant has been shaded to indicate the origin of each exconjugant.

Normal cross-fertilization (XF), involving meiosis, exchange of gametic nuclei and fertilization, yields two doubly heterozygous exconjugant clones, both expressing resistance to cycl and 6mp (since the resistance alleles are dominant). In cytogamy (CG), meiosis occurs, but the exchange of gamete nuclei fails; self-fertilization yields exconjugants expressing the genetic information originally contained in the micronucleus of their respective cytoplasmic parents; one becomes phenotypically cycl-R, 6mp-S, and the other cycl-S, 6mp-R. In "nonconjugation" (NC) the two exconjugants fail to develop new macronuclei and retain their old one; both exconjugants remain phenotypically sensitive to both drugs. (The NC micronuclei are shown as expected when no exchange occurs prior to macronuclear retention; alternatively, a doubly heterozygous micronucleus could be present, as in the XF pair.)

cated to 2% PP + cycl and 2% PP + 6mp, respectively, and incubated at 30°. Three days later, the survivors of each drug were recorded. The plates were then replicated to the other drug, incubated at 30° for three more days and scored again. Frequently, only one exconjugant of a cytogamous pair survives. This occurrence is directly detected with the XCS assay, and inferred with the MXR assay. Detailed genetic studies were done only with pairs from which both cytogamous exconjugant clones were recovered.

Clones apparently resulting from cytogamy were initially obtained at a low and unpredictable frequency (1–10%). The timing of the transfer of conjugating pairs from starvation buffer to 2% proteose peptone medium was subsequently found to be critical for the induction of cytogamy, and this explained most or all of the variability previously observed (ORIAS, HAMILTON and FLACKS 1979). Most of the cytogamies studied here represent events induced by a shift to 2% PP medium at or near the critical time (285 min at 30°, or nine hr at room temperature, after mixing the two initiated cultures).

Evidence for separate, uniparental transmission to each conjugant: The macronucleus (somatic nucleus) and the micronucleus (germinal nucleus) are norm-

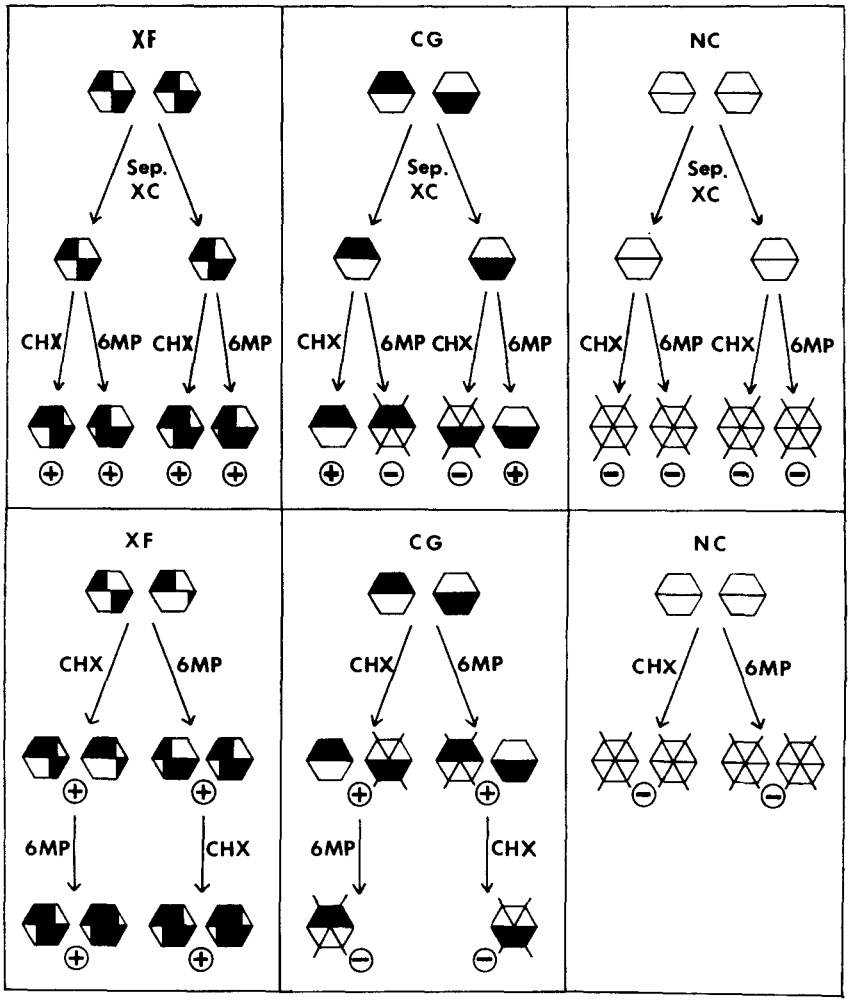

FIGURE 2.—Experimental distinction among possible conjugation pathways followed by a given conjugating pair in the cross CU324 × CU329. Upper panels: exconjugant separation assay. Lower panels: mixture resolution assay. Key to symbols used. XC: exconjugants; CHX and 6MP: culture replicated to 2% PP + cycl or 6mp, respectively; + and —: growth or no growth, respectively; other symbols as in Figure 1. Hexagon pairs represent the macronuclei (phenotype) of the exconjugant clones derived from one conjugating pair.

ally derived from the diploid zygote nucleus during conjugation. Both nuclei were independently examined to confirm the genetic consequences predicted for cytogamy, namely micronuclear homozygosity and macronuclear homogenetity for the appropriate markers (Figure 1).

Macronuclear genotype analysis: This analysis was based on phenotypic assortment (SONNEBORN 1975), a phenomenon whereby heterozygous clones, upon vegetative multiplication, segregate subclones expressing only one of the two alleles. This segregation is due to the random distribution of allele copies

during macronuclear division (ALLEN and NANNEY 1958; NANNEY and DUBERT 1960; ORIAS and FLACKS 1975). The equilibrium rate of production of assortants from a heterozygote having a mixed macronucleus is around 0.011 per fission (see SONNEBORN 1975; NANNEY and PREPARATA 1979).

The detection of macronuclear S (sensitive, recessive) and R (resistant, dominant) alleles in resistant and sensitive clones, respectively, poses different problems. A phenotypically sensitive clone (by the replication test) may include cells having some macronuclear R allele copies, but too few to confer resistance. This possibility can be efficiently detected by allowing additional fissions (so that random distribution generates descendants with enough macronuclear R copies to confer resistance), and by retesting a larger population sample. This test was performed by adding about 30 μl of culture in 2% PP medium to 1 ml of the same medium containing cycl or 6mp, as appropriate ("mass test"). The culture was incubated three days at 30° and scored for survivors. A culture derived from one cell whose macronucleus had any (even only one) R alleles would almost certainly be identified by this mass test with regard to cycl-R (ORIAS and NEWBY 1975) and likely also for 6mp-R.

Detecting macronuclear S allele copies in phenotypically resistant cells is a more tedious and uncertain proposition, based on the detection of sensitive phenotypic assortants. The most effective procedure, devised by ALLEN and NANNEY (1958) and adapted to drug-R by ORIAS and NEWBY (1975), involves serial daily subcloning of the culture, accompanied by regular tests for the drug-S phenotype. The large number of progeny obtained in this study dictated a limited test (six to eight subclones from each clone).

Clones whose phenotype suggested cytogamy were subjected to assortment analysis (Table 2). Out of 55 pairs of presumptively cytogamous progeny, 52 failed to produce assortants with the opposite phenotype. As a control, data were

TABLE 2

Frequency of phenotypic assortment among presumptive ex-cytogamous clones and unselected heterozygotes

Type of clone	Total clones studied		Clones that showed assortment		Total subclones tested		Drug-sensitive assorted subclones	
	cycl	6mp	cycl	6mp	cycl	6mp	cycl	6mp
Cytogamous* (exconjugant separation assay)	16	16	0	0	98	99	0	0
Cytogamous* (mixture resolution assay)	39	39	3†	0	236	239	3	0
Unselected normal conjugants	21	31	16	23	130	193	31	53

At about 40 fissions after conjugation, 6 to 8 single cells were isolated from each clone to be tested into drops of 2% PP. The plates were incubated three days at 30° and then replicated to the appropriate drug medium. The results were scored after three days at 30°.

* Both exconjugants of each pair were studied.

† Double heterozygotes that gave negative results in 6mp test. (One case was detected by mass tests; the other two would almost certainly have been detected had mass tests been run.) The other experiment in each case was cycl-S, 6mp-R and failed to assort.

obtained under identical circumstances, using unselected heterozygotes (Table 2). About 75% of heterozygous clones gave at least one drug-sensitive segregant subclone and about one-third of all the subclones tested for any one drug showed assortment (*i.e.*, became sensitive). Although the conclusion of R homogeneity of any clone is only tentative (based on the failure to find assortants in a limited sample), the presumptively cytogamous clones behaved, as a class, as if they had a homogenous macronucleus for the two markers used in the cross.

Micronuclear genotype analysis: Seven randomly selected exconjugant pairs that behaved as excytogamous and failed to show phenotypic assortment were testcrossed in order to determine their micronuclear genetic composition. In testcrosses to wild-type clone BIII, progeny that had undergone sexual reorganization were identified by a change in mating type (a positive test), rather than by the less reliable maturity test (a negative test). This procedure underestimates the number of normal conjugant progeny, particularly when one parent is mating type IV (the most frequent type). Some testcrosses were performed using homozygous heterokaryons (see Table 1). True progeny could be distinguished from either parent by the expression of the drug-R marker derived from the heterokaryon, but the cross informs only about the locus for which the heterokaryon is genotypically sensitive.

Five of the seven pairs were fertile and have the expected marker combination in doubly homozygous form (Table 3). One exconjugant in each of two pairs had limited or no fertility, and the genotype was not unequivocally determined, but the other exconjugant behaved as expected in each case.

In summary, then, the clones presumptively identified as cytogamous by the two assay methods behaved (with rare exceptions), as if (1) they were homozygous for the two markers used and (2) all their genetic information were of uniparental origin.

Parental source of the genetic endowment of excytogamous progeny: Having demonstrated uniparental transmission, it became necessary to demonstrate that the nuclear genetic information of a presumed excytogamous clone was derived from its cytoplasmic parent. To this end, one of the parents was labeled with chloramphenicol resistance, a trait that shows cytoplasmic inheritance (ROBERTS and ORIAS 1973a). Two types of crosses were done: SB100 (CAM-R derivative of CU329) × CU324, and SB101 (CAM-R derivative of CU324) × CU329. Among 368 pairs isolated in three experiments, nine pairs, for which both exconjugants were recovered, were identified as possible cytogamies: three by the exconjugant separation method and six by the mixture resolution method (see MATERIALS AND METHODS). All nine clone pairs were tested for phenotypic assortment of *ChxA* and *Mpr*, and the two members of each pair behaved as if they were homogeneous for the reciprocal phenotypes. Eight of the nine clone pairs were testcrossed and gave results consistent with micronuclear homozygosity for the corresponding markers (results not shown). All 18 clones had the chloramphenicol phenotype expected if the cytoplasmic parent were responsible for the uniparental contribution. The result also indicates that the osmotic shock

TABLE 3

Testcrosses of presumptive excytogamous clones

Clone and MT		Parental phenotype cycl	6mp	Tester parent	Testcross progeny phenotypes cycl R	S	6mp R	S	NC	X	V	T	Inferred genotypes *Chx*	*Mpr*
1A	VI	R	S	BIII	Sterile								—	—
1B	V	S	R	BIII	0	10	9	1*	1	18	11	46	S/S	R/R
				CU329	—	—	19	0	11	16	19	46		
2A	II	R	S	BIII	34	0	0	34	1	1	44	46	R/R	S/S
				CU324	38	0	—	—	5	3	38	46		
2B	VI	S	R	BII	0	33	33	0	2	6	38	46	S/S	R/R
				CU329	0	37	—	—	2	7	37	46		
3A	VI	S	R	BIII	0	25	24	1*	1	2	43	46	S/S	R/R
				CU329	—	—	35	0	5	6	35	46		
3B	IV	R	S	BIII	17	0	0	17	1	9	32	46	R/R	S/S
4A	IV	R	S	BIII	21	0	0	21	0	5	34	44	R/R	S/S
4B	IV	S	R	BIII	0	25	25	0	0	3	41	44	S/S	R/R
				CU329	—	—	35	1*	8	2	36	46		
5A	IV	S	R	BIII	16	0	0	16	1	1	27	44	S/S	R/R
5B	IV	R	S	BIII	0	16	16	0	0	2	26	44	R/R	S/S
				CU329	—	—	35	0	4	7	35	46		
6A	IV	S	R	BIII	0	16	16	0	1	3	26	44	S/S	R/R
				CU329	—	—	41	0	1	4	41	46		
6B	IV	R	S	BIII	7	0	0	7	2	24	11	44	R/R	S/S
7A	VII	S	R	BIII	0	10	9	1*	6	29	10	44	S/S	R/?
				CU329	—	—	4	4*	16	22	8	46		
7B	IV	R	S	BIII	15	0	0	15	2	7	24	46	R/R	S/S

Key to symbols. NC: nonconjugant (drug-S, III progeny from BIII crosses; cycl-S or 6mp-S progeny from CU329 or CU324 crosses, respectively); X: dead; V: viable progeny; T: total pairs isolated. A and B are the exconjugants of a presumptively cytogamous pair. R and S: resistance and sensitivity. MT: mating type.

All clones were testcrossed to BIII and some to homozygous heterokaryons CU324 or CU329 (Table 1). In BIII crosses, only clones that changed mating type and/or phenotype are tallied as S or R. The viability column is the sum of these and true progeny with parental mating type, estimated from the relative frequency of the six possible mating types under our experimental conditions (N=93; frequencies of mating type II–VII: 0.22, 0.05, 0.38, 0.08, 0.24 and 0.03, respectively). The difference between T and (NC + X + V) in BIII crosses is the estimated number of nonconjugants derived from the non-BIII parent.

* Excytogamous clone (derived from the tester parent), or immediate 6mp-S assortment, or clone falsely classified as 6mp-S. With the exception of clone 7A (which is otherwise quite unfertile), the frequency of these abnormalities is within the range observed for crosses for normal clones.

does not induce with high frequency an exchange of the cytoplasmic determinant for chloramphenicol resistance between the two conjugants.

Is the uniparental endowment derived from a single meiotic product? In conjugation, the micronucleus of each member of a pair undergoes two meiotic divisions, yielding four haploid meiotic products. Normally, three of the four are destroyed, and the entire nuclear genetic information of the progeny is derived from a single meiotic product from each parent. The same could be true

in the cytogamies considered here, the essential difference being a failure to exchange nuclei between the two cells. On the other hand, the nuclear information of an excytogamous clone might well be derived, for example, from the fusion of two haploid meiotic products, if some of the nuclei normally destroyed remained functional. The previous experiments do not test this question, because all the meiotic products in any one cell were identical.

How many meiotic products are involved was addressed using a double heterozygote for *ChxA* and *Mpr* (unlinked loci; McCoy 1977). If all pairwise fusions of four meiotic products were equally likely, only one-ninth to one-third of the cytogamous clones should be double homozygotes (depending on the extent of centromere linkage: none or complete, respectively). But if only a single meiotic product participates, then every cytogamy should yield whole-genome homozygotes.

To perform the test, the double heterozygote (clone SB103, Table 1) was constructed with a phenotypically sensitive phenotype. This clone was then crossed to CU324 at 23°. The phenotypes of 255 pairs of exconjugants were separately determined. Three-fourths of the presumptive cytogamies are identifiable because one exconjugant (derived from CU324) should be cycl-S, 6mp-R and the other should be something else. (Cytogamies where both exconjugants become cycl-S, 6mp-R cannot be phenotypically distinguished from normal conjugants.) Sixteen exconjugant clone pairs had phenotype combinations consistent with cytogamy. Their macronuclear composition was investigated by looking for phenotypic assortment, and the micronuclear genotype was examined by testcrosses, as previously described. Six of these presumptive cytogamous clones were disregarded because the cycl-S, 6mp-R exconjugant carried alleles of both parents either in its macro- or micronucleus (data not shown), and they were not examples of the uniparental transmission previously identified. These clones represent abnormalities of a different sort, which have not yet been fully characterized.

The complete genetic characterization of the remaining ten apparently excytogamous pairs is shown in Table 4. In seven of these pairs, the non-(cycl-S, 6mp-R) exconjugant was homozygous at both loci tested. Furthermore, both exconjugants had the macro- and micronuclear genotypes expected from cytogamy. Thus, the seven pairs had the homozygous genetic endowment that would be exclusively expected if one meiotic product were involved, and only rarely expected if fusion of two or more products were involved.

The other three pairs in Table 4 (Nos. 2, 4 and 9) showed some heterozygosity in both the macro- and micronucleus of the non-(cycl-S, 6mp-R) exconjugant, and one of them (No. 9) shows a genetic segregation at the *Chx* locus consistent with triploidy or trisomy (5:1 ratio). If such clones are the result of uniparental transmission, then more than one meiotic product was involved. However, all three clones could also have resulted from a biparental nuclear fusion, accompanied by some other cytogenetic abnormality (see DISCUSSION). Thus, we cannot conclude that two different meiotic products from one cell fused in these three pairs.

TABLE 4

Testcrosses of presumptive excytogamous progeny of a doubly-heterozygous clone

Exconjugant pair	Initial phenotype cycl	Initial phenotype 6mp	Phenotypic assortants* cycl	Phenotypic assortants* 6mp	Testcross progeny phenotypes† cycl R	cycl S	6mp R	6mp S	NC	X	Inferred genetic composition Micronucleus *Chx Mpr*	Macronucleus§ *Chx*	Macronucleus§ *Mpr*	Homo- or heterozygote‖
1A	S	R	—	0/6	0	20	19	1‡	1	11	S/S,R/R	S	R	
1B	S	S	—	—	0	16	0	16	4	12	S/S,S/S	S	S	D. Hom.
2A	S	R	—	0/5	0	5	5	0	14	52	S/S,R/R	S	R	
2B	R	R	0/5	2/5	14	5	12	7	6	35	R/S,R/S	R	M	D. Het.
3A	S	R	—	0/6	0	14	14	0	1	13	S/S,R/R	S	R	
3B	S	S	—	—	0	8	0	8	12	12	S/S,S/S	S	S	D. Hom.
4A	S	R	—	0/5	0	12	12	0	2	10	S/S,R/R	S	R	
4B	R	R	1/6	0/6	11	10	20	1‡	1	23	R/S,R/R	M	R	S. Hom.
5A	S	R	—	0/6	0	10	10	0	6	19	S/S,R/R	S	R	
5B	R	R	0/6	0/6	8	0	8	0	2	16	R/R,R/R	R	R	D. Hom.
6A	S	R	—	0/6	0	7	7	0	9	12	S/S,R/R	S	R	
6B	S	S	—	—	0	17	0	17	5	10	S/S,S/S	S	S	D. Hom.
7A	S	R	—	0/5	0	17	17	0	17	30	S/S,R/R	S	R	
7B	R	S	0/6	—	16	0	0	16	38	8	R/R,S/S	R	S	D. Hom.
8A	S	R	—	0/6	0	32	32	0	0	3	S/S,R/R	S	R	
8B	R	S	0/6	—	16	0	0	16	3	9	R/R,S/S	R	S	D. Hom.
9A	S	R	—	0/6	0	8	8	0	9	24	S/S,R/R	S	R	
9B	R	R	1/6	0/6	31	7	38	0	2	21	R/R/S,R/R	M	R	Tri.
10A	S	R	—	0/6	0	7	7	0	5	21	S/S,R/R	S	R	
10B	S	S	—	—	0	13	0	13	4	16	S/S,S/S	S	S	D. Hom.

Symbols as in Table 3.

* —: No growth in the mass test for R subclone assortment.

† Exconjugants from 46 pairs were isolated (exceptions: 92 pairs for clones 2A, 2B, 7A and 7B and 184 pairs for clone 9A). Pairs were tallied if both XC survived (cycl, 6mp, NC) or died (X). The balance are pairs whose XC divided before they could be separated or, more commonly, where only one XC survived; true progeny were not distinguished from NC in these cases. In testcrosses of cycl-S, 6mp-S clones (1B, 3B, 6B and 10B), true progeny were distinguished from NC by a changed mating type.

‡ See * footnote on Table 3.

§ S or R: homogeneous for the S or R allele, respectively. M: mixed (both R and S alleles present).

‖ D. Hom.: double homozygote; D. Het.: double heterozygote; S. Hom.: hom. for one locus, het. for the other; Tri: possible triploid or trisomic.

TABLE 5

Universality of cytogamy induction

Cross	XF	CG	NC	X	Total
CU329 × CU324	51	14	7	19	91
CU329 × CU325	44	9	12	25	90
CU330 × CU325	60	8	5	19	92

Crosses were made at 30°. Pairs were diluted ten-fold in 2% PP medium at five hr after mixing the initiated cultures.

Abbreviations: XF: cross-fertilization; CG: cytogamy; NC: nonconjugants; X: dead.

In summary, the results are consistent with the assumption that a single product of meiosis is involved in most (or all) of the cases where uniparental transmission is observed.

Universality of cytogamy induction: Can any clone undergo cytogamy? Crosses were carried out with other heterokaryons and the previously described cytogamy assays were used. Cytogamy was induced in every case (Table 5). It has also been observed with other clones involving other genetic markers (HAMILTON and SUHR-JESSEN, in preparation; ROBERTS and ORIAS, in preparation).

DISCUSSION

Characterization of cytogamy in Tetrahymena thermophila: Our genetic study has documented the existence of an alternate pathway of conjugation in *T. thermophila*, designated as *cytogamy*, in which the entire nuclear genetic information of each exconjugant is derived exclusively from the micronucleus of one parent, *i.e.*, its cytoplasmic parent. This conclusion has now been confirmed autoradiographically (HAMILTON and SUHR-JESSEN, in preparation). Our genetic study also indicates that in cytogamy the entire genetic information of each exconjugant originates from a single haploid nucleus, generated by meiosis. The micronucleus of each exconjugant behaves as homozygous diploid, and the macronucleus has the expected genetic homogeneity, as evidenced by the lack of phenotypic assortment. Each exconjugant becomes an "instant" whole-genome homozygote (*i.e.*, homozygous at every locus). The postulated sequence of events in cytogamy in Tetrahymena is illustrated in Figure 3.

We consider the term cytogamy useful because operationally it has the same genetic consequences as the cytogamy known in *Paramecium aurelia* (SONNEBORN 1941). In Paramecium, the basis for cytogamy is the failure of the conjugating cells to exchange migratory gametic nuclei. Fusion of the sister migratory and stationary nuclei generates an instant homozygote. Although we observe similar genetic consequences in Tetrahymena, it should be stressed that we do not know whether the cytogenetic basis for cytogamy is identical in both organisms, or whether a unique pathway is involved in Tetrahyman. Other departures from the normal events of conjugation could lead to the same genetic results. For example, two successive rounds of DNA synthesis in the surviving haploid meiotic product, unaccompanied by nuclear exchange between conjugants, could be followed by normal postzygotic mitoses to generate new homozygous micro- and macronuclei. The results of testcrosses do not rigorously determine whether the micronucleus of cytogamous clones is haploid or diploid. We favor the latter because the maximum fertility of haploid clones is around 20%, when crossed to normal diploids (NANNEY and PREPARATA 1979).

Two other ways of generating instant whole-genome homozygotes have been previously described in *T. thermophila*: genomic exclusion (ALLEN 1967a,b) and short circuit genomic exclusion (BRUNS, BRUSSARD and KAVKA 1976). Cytogamy is distinguishable from the other two in that both exconjugants need

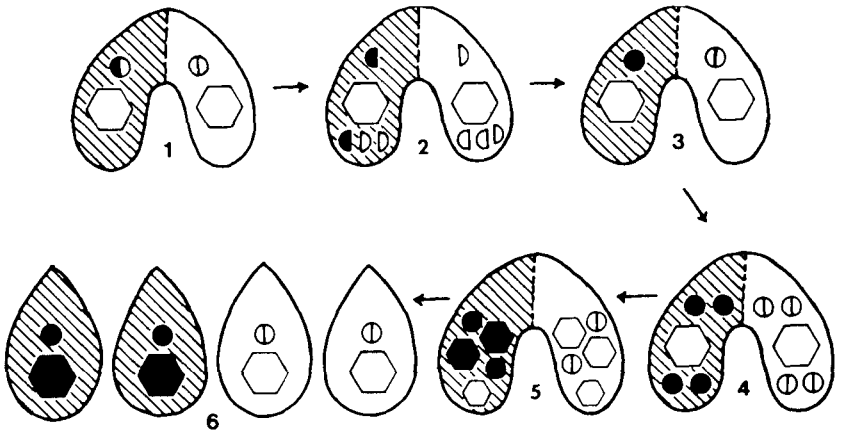

FIGURE 3.—Diagrammatic representation of the postulated sequence of events in cytogamy in *T. thermophila*. Symbols as in Figure 1, except that only one locus is considered. (1) Pairing between a micronuclear heterozygote (with wild-type macronucleus) and a wild-type strain. This heterozygote could have originated as a functional heterokaryon or could be the result of a micronuclear mutation. (2) The micronucleus of each conjugant has undergone meiosis. The three posterior nuclei will be destroyed and are not shown further. (3) A diploid nucleus is formed from the surviving meiotic product, either by diploidization or by mitotic division and subsequent fusion. (4) Two mitotic divisions of the diploid nucleus generate four nuclei, destined to become two macro- and two micronuclei. (5) Differentiation of new macronuclei. The old macronucleus migrates posteriorly and will be destroyed. (6) The two exconjugants have separated and undergone the first binary fission, giving rise to the four caryonides.

not be (and in general will not be) genetically identical. In this respect, cytogamy differs also from normal conjugation.

Practical implications of cytogamy in T. thermophila: Perhaps the most useful consequence of cytogamy is that it allows the isolation of recessive mutants. The formation of an instant whole-genome homozygote from a single meiotic product allows the expression of a previously induced micronuclear mutation, regardless of whether it is dominant or recessive (Figure 3). Cytogamy has become a practical tool because (1) it can be induced in a large fraction of the pairs (approximating 50%) under simple and repeatable experimental conditions (ORIAS, HAMILTON and FLACKS 1979), and (2) the excytogamous progeny are generally fertile. Cytogamy has already been used in our laboratory to isolate Tetrahymena mutants with a heat-sensitive capacity to phagocytize (SUHR-JESSEN, 1977; SUHR-JESSEN and ORIAS, 1979).

Short circuit genomic exclusion (BRUNS, BRUSSARD and KAVKA 1976) (SCIGEX for short) is another useful method for generating instant genome homozygotes. Its basis is a (cytogenetically uncharacterized) departure from normal events induced by conjugation with the C* strain. This strain has a defective micronucleus and is unable to contribute any of its nuclear genetic information to the progeny. In SCIGEX, all the nuclear genetic information of the progeny is derived from a single meiotic product from the normal mate. Drawbacks of SCIGEX for mutant isolation are its low frequency (about 5%

of the conjugating pairs) and, in our experience, the low fertility of mutants isolated by this method. SCIGEX has the useful feature that the products of the other common pathways of conjugational events can be efficiently selected against in mass cultures. This can also be accomplished for cytogamy by using recently isolated, recessive drug-resistance markers (*far*, conferring 2-fluoroadenosine resistance, BRUNS, BRUSSARD and KAVKA 1976; *gal*, conferring 2-deoxygalactose resistance, ROBERTS and ORIAS, unpublished; *gal* behaves as dominant or recessive depending on the conditions of exposure to 2-deoxygalactose). The isolation of a phenotypically wild-type strain homozygous for a dominant lethal mutation in the micronucleus would provide an alternative method of eliminating most (if not all) normal conjugants.

Cytogamy may also find use in speeding up genetic analysis of new mutants, testcrossing for mapping purposes and the construction of new strains. At present, however, the need to build markers into the strains so that cytogamies can be easily identified makes this method somewhat cumbersome. The development of a dominant lethal heterokaryon could be very useful here also.

The converse problem is avoiding cytogamy when normal conjugants are desired. In the cross used here, normal conjugants can be selected from a mixture that also contains excytogamous progeny by their resistance to both drugs. The absolute frequency of cytogamy can be minimized by isolating pairs into 1% (instead of 2%) proteose peptone, and/or delaying the isolation until well past the time of gametic nuclei exchange (eight or more hours after mixing initiated cultures at 30°; ORIAS, HAMILTON and FLACKS 1979).

Why has cytogamy in Tetrahymena not been identified earlier? Several reasons can be given. The first is that if the conjugating pairs are treated gently, the frequency of cytogamy is low, in the neighborhood of 1% of conjugating pairs (ORIAS, HAMILTON and FLACKS 1979). Rare genetic results (probably cytogamies) were described by NANNEY (1963). He considered cytogamy as a possible explanation, but favored an explanation based on genomic exclusion.

A second factor has been the recent technical advances in Tetrahymena genetics (ORIAS and BRUNS 1975). Foremost among these is the development of techniques for the efficient manipulation of large numbers of clones, such as routine growth in pure (axenic) culture, miniaturization of growth vessels, simultaneous replica-plating of many cultures, and the use of drug-resistance markers for mass selection of desired phenotypes. Another important advance has been the construction of heterokaryons (BRUNS and BRUSSARD 1974), that enable us to identify rare genetic events or to select against undesired ones. It was the availability of these methods and strains that allowed us to detect, with a reasonable degree of assurance, the occurrence of cytogamy and encouraged us to study it, even when its frequency was initially only about 1% of conjugating pairs.

Finally, the fortuitous discovery that cytogamy could be induced with high frequency by a carefully timed transfer to 2% proteose peptone (ORIAS, HAMILTON and FLACKS 1979) greatly simplified and speeded up the present analysis. By including morphological markers (*i.e.* ts "monster" mutations; FRANKEL *et al*

1976) into our homozygous heterokaryons, we now have clones that allow even faster identification of cytogamous clones.

Other cytogenetic abnormalities: Progeny with unexpected phenotypes and/or genotypes have been seen in the course of this study. Most of these appear to have been induced by the cytogamy-inducing treatment (unpublished observation) although at a lower frequency than cytogamy. Some of the conjugation abnormalities yield exconjugants that phenotypically appear to be cytogamous.

One likely occurrence is the one-way (*i.e.*, nonreciprocal) donation of a gametic nucleus, so that one exconjugant is in effect cytogamous, while the other has biparental endowment. This mechanism can explain the three exceptional pairs in Table 4 and many other clone pairs showing analogous phenotype combinations but not further characterized (*e.g.*, three exceptions in Table 2). A triple fusion in the exconjugant that retained its Chx^+ Mpr^+ migratory nucleus could explain the testcross results obtained with pair No. 9 in Table 4. Triploid strains occur in *T. thermophila* and generally have high fertility (PREPARATA and NANNEY 1977; NANNEY and PREPARATA 1979). Of course, the three pairs in Table 4 could be due to the uniparental fusion of two different meiotic products, as mentioned before, or other abnormalities, such as meiotic chromosomal nondisjunction.

Conjugation among ciliates is a remarkable developmental program, involving the differential manipulation of three types of nuclei with different functions and fates. Cytogamy and the other abnormalities mentioned here represent different failures of this program that, if brought under experimental control, could provide unique insights into the cellular mechanisms involved, as well as their regulation.

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