INHERITANCE OF T SEROTYPES IN TETRAHYMENA

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PREVIOUS work on serotypes of *Tetrahymena pyriformis*, syngen 1, has shown that a single strain manifests different serotypes at different temperatures. Specifically, all strains have a different serotype at 15°C (the L type) from that at 25°C (the H type) (LOEFER, OWEN and CHRISTENSEN 1958). This paper is concerned with a third serotype, expressed at 40°C, which will be called the T serotype.

Studies of the H serotype system have revealed four different H types distributed among the 11 different inbred families of *T. pyriformis*, syngen 1. These represent different alleles at a single genetic locus. Heterozygotes in this system are unstable in expression and during vegetative multiplication sublines differentiate so that some express one *H* allele, and others express the other *H* allele (NANNEY and DUBERT 1960).

Three different T phenotypes among the 11 inbred families can be distinguished with T specific antisera. This paper will examine the genetic basis of these traits and possible linkage relationships with the other known loci in Tetrahymena. Differentiation in T serotype heterozygotes during vegetative multiplication will be considered in the following paper.

MATERIALS AND METHODS

The strains used in this study were representatives of 11 inbred families of *T. pyrformis*, syngen 1. The allelic constitution of these families is given in Table 1.

TABLE 1

*Allelic constitution of the inbred families**

Inbred family	*mt*	*H*	*T*	*E-1*	*E-2*	*P-1*
A	mt^A	H^A	T^A	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^A$
A1	mt^A	H^D	T^A	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^A$
A3	mt^A	H^E	T^B	$E\text{-}1^B$	$E\text{-}2^C$	$P\text{-}1^B$
B	mt^B	H^D	T^A	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^B$
B2	mt^C	H^C	T^C	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^A$
C	mt^C	H^E	T^B	$E\text{-}1^C$	$E\text{-}2^C$	$P\text{-}1^B$
C1	mt^C	H^A	T^A	$E\text{-}1^C$	$E\text{-}2^B$	$P\text{-}1^B$
D	mt^D	H^D	T^C	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^A$
D1	mt^D	H^C	T^C	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^A$
E	mt^E	H^D	T^A	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^B$
F	mt^F	H^D	T^A	$E\text{-}1^B$	$E\text{-}2^B$	$P\text{-}1^B$

* From ALLEN (1964 and unpublished), BLEYMAN, SIMON and BROSI (1966) and NANNEY (1963a and unpublished).

In order to test for the T serotypes, cells were grown in axenic liver peptone media (0.4% liver L extract plus 1% peptone in deionized water) in a 40°C incubator. Tubes containing 10 ml of liver peptone were inoculated using a sterile platinum loop from room temperature axenic peptone tube cultures and allowed to grow at 40° for 24 hours. The serological testing methods were the same as those described previously for the H serotypes (LOEFER, OWEN and CHRISTENSEN 1958). The cells were allowed to stand at room temperature one hour before testing to recover from the temperature shock.

The T specific antisera were obtained by injecting cells grown at 40°C into rabbits. The cells were grown in two liter volumes of axenic liver peptone in 3-liter flasks on an incubator shaker at 40°C. After two days the flasks were checked for bacterial contamination and the contents collected by refrigerated centrifugation at 5,000 rpm. The packed cells were diluted 1:5 in distilled water and frozen in 0.5 ml aliquots for injection. Undiluted cells were frozen for absorptions. The two different types of immunization schedules followed yielded similar results. In both cases the rabbits were first given 0.2 ml of packed cells intramuscularly with Freunds adjuvant. In one group the succeeding injections were all intravenous; in the other group they were all subcutaneous. These later injections consisted of 0.1 ml of packed cells and were administered every week. Bleedings were collected every one to two weeks after the third injection and serum was obtained as described previously (LOEFER, OWEN and CHRISTENSEN 1958). Using this method, antisera with titers varying from 1/40 to 1/2,500 were obtained. The procedure for absorption of antisera was as follows: 0.25 ml of frozen concentrated cells were added to 0.5 ml of undiluted antiserum in a centrifuge tube and incubated overnight at 4°C. The cells were spun down in a clinical centrifuge the next day and the supernatant decanted. This procedure was repeated until an antiserum negative to cells of the type used in the absorption was obtained.

The exact temperature at which cells switch from the H serotype to the T serotype was ascertained by growing cells in axenic liver peptone tubes in a water bath. Studies on transformation from H to T and *vice versa* employed the test system described above, with the exception that tubes of cells in log culture (as determined by cell counts) at one temperature were transferred to the other temperature and tested for serotype after regular intervals of time.

The methods for making crosses in bacterized media, and testing for mating types in the progeny have been described elsewhere (NANNEY and CAUGHEY 1955). The bacterized medium used in this study was bacterized peptone, a one-day culture of *Aerobacter aerogenes* in axenic peptone diluted 1:80 with deionized water. In order to test bacterized synclones (pair cultures) for the T serotype, the following procedure was used. The bacterized tube cultures were centrifuged at half speed on the clinical centrifuge for 5 minutes and most of the liquid was poured off immediately. The resulting concentrated cell suspension was transferred by loop inoculation to a tube of axenic liver peptone containing antibiotic (1×10^6 units penicillin and 1 g streptomyocin per liter). The tubes were then put in the 40°C incubator and tested as described above. (If the cultures are starved, centrifugation is unnecessary. Three drops of bacterized culture fluid is added to a 10 ml tube of liver peptone plus antibiotic with a Pasteur pipette.) Although this method was satisfactory for short term purposes, tube cultures prepared in this way often showed visible contamination in 4 to 7 days. Therefore, in order to transfer stocks from bacterized peptone to axenic peptone permanently, a single-cell washing method was employed. This procedure consisted of transferring about six cells into a depression containing two drops of axenic peptone plus antibiotic at the same concentration as above. The cells were transferred to another such depression after 2 to 4 hours. Finally after another 2 to 6 hr, a single cell was transferred into a full depression of axenic peptone plus antibiotic. Usually the depression contained about 2^{10} animals in 2 days and a loop inoculation to an axenic peptone tube was made at this time.

In order to eliminate the above procedure and obtain synclones which could be maintained indefinitely in room temperature peptone tubes, crosses were performed in axenic peptone at room temperature. The methods for these crosses were the following: antibiotic solution was added to peptone tubes of the parent cultures to give the final concentration described above. The cultures were then centrifuged for 5 minutes at low speed, the supernatant discarded, and the cells resuspended in distilled water at one fifth to one third of the former volume. Four drops of each parental culture were mixed in depression slides and pairs were isolated the next day.

The pairs were isolated into depressions containing peptone medium with antibiotic. The depresresion slides were irradiated with ultraviolet light before the medium was added to inhibit growth of molds. After 3 days, the synclones were loop inoculated to axenic peptone and liver peptone tubes and a few drops of the culture were added to tubes containing one ml of bacterized peptone. The bacterized tubes were used for testing H serotypes and for nonconjugation tests as described in earlier papers (NANNEY and CAUGHEY 1955; NANNEY and DUBERT 1960; NANNEY 1963b). The "nonconjugation" test is simply a test for sexual maturity; cells which have undergone a complete reorganization usually enter a period of sexual immaturity. Nonconjugation tests do not exclude certain cytogenetic irregulariites (genomic exclusion, ALLEN 1963) when they result in a complete nuclear reorganization. However, completed reorganization with genomic exclusion requires two "rounds of mating" and is usually found only when conjugating pairs are isolated very late in a mating culture (ALLEN 1967). Efforts were made to isolate pairs soon after the initiation of conjugation in order to avoid this complication. The axenic cultures were tested for the T serotype as described above. Each synclone was tested for H and T serotypes three times before being designated as a given type.

The axenic cultures were also used in the preparation of enzyme extracts for starch gel electrophoresis. This was done in order to test for possible linkage of the T serotype locus with the esterase-1, esterase-2 and phosphatase-1 loci (ALLEN 1960, 1961; ALLEN, MISCH and MORRISON 1963a). The preparation of the extracts, starch gel electrophoresis and identification of the isoenzymes were performed as described by ALLEN (1964) with one minor exception. The cells used in the extracts of the esterase-1 and phosphatase-1 enzymes were grown on a shaker at 30°C for 4 days, rather than in unshaken cultures at 30° for 5 to 7 days. Controls consisting of extracts of parental cultures were run simultaneously with extracts from F_2 synclones. In all cases the expected parental bands were obtained.

RESULTS

Temperatures at which T is expressed: As described in METHODS, the T serotype is manifested at 40°C in axenic peptone, and this was the test system used in the work described in this paper. Studies on axenic cultures grown at various temperatures in a water bath revealed a sharp discontinuity between the expression of the H and the T serotypes. No cells grown at temperatures between 30° and 35° reacted with T antisera and no cells grown between 38° and 40° reacted with H antisera. Reactions to both antisera were obtained at 36° and 37°. The 36° cells gave a stronger H reaction and the 37° cells gave a stronger T reaction. The 11 inbred families behaved alike in this respect.

Transformation between the H and the T serotypes: Transformations were studied by taking a culture in log growth at one temperature, transferring it to the other temperature, and testing the culture for H and T serotypes at regular intervals thereafter. When cells in log culture were used, reproducible results were obtained. For this study incubators set at 26° and 40°C, were used. The tests were performed at room temperature and the number of hours refers to the time the cultures remained at a particular temperature before being tested. Cells grown at 40°, and then transferred to 26° showed the following pattern. After 1 to 2 hours at 26°, all the cells reacted with T antisera and none with the H antisera. After 3 to 6 hours, most cells reacted with both antisera. At 3 hours more

cells reacted with T antisera than with H, but this was reversed at 6 hours. After 7 hours all the cells reacted with H antisera and none with T antisera.

The reverse experiment, in which cells were grown at 26°C, and then transferred to 40°, gave the following results. After 1 hour all the cells reacted with H antisera and none with T antisera. After 2 to 4 hours most cells reacted with both antisera. After 5 to 6 hours all cells reacted with T antisera, and none with H antisera. The longer time needed to transform from T to H appears to be a function of the longer generation time of the cells at 26°. The importance of generation time was first suggested by the observation that when cells grown at 40° for 24 hours (in late log or plateau stage) were transferred to 26°C, 10 to 12 hours were required for them to transform completely to H. The first reaction to H antisera appeared at 4 to 5 hours. To estimate the generation time at the respective temperatures in relation to transformation, cell counts were performed as follows: Pasteur pipettes were calibrated and the number of animals in a single droplet from one of these pipettes was counted. Serial 1:10 dilutions were made until the number of animals per droplet was less than ten, and ten such droplets were counted for each culture. Using this method to ascertain population density, the maximum generation time at 40° was found to be about 1½ hours, while at 26°, it was about 3 hours. The observations on cell counts during transformation are summarized in Table 2. At least one doubling occurred before any transformation was detectable and two to three doublings occurred before the transformation was complete. These observations resemble those on transformation in *Paramecium aurelia* (BEALE 1957). No striking differences were detected among the inbred families in the time of transformation.

T Specific antisera: The possibility of a T serotype was suggested when it was discovered that cells grown at 40°C were not immobilized by the appropriate H antisera. Only one H antiserum affected these cells, and this cross reaction could be removed by absorption with 40° cells. The presence of a T serotype at 40° was confirmed by the properties of antisera prepared against the inbred families grown at 40°. Thirteen T antisera were obtained with homologous titers ranging from a pooled value of 1/40 to 1/640. Only one of these antisera showed any cross reaction with H cells and this small cross reactivity could be removed by

TABLE 2

The number of fissions required for transformation of cells between H and T serotypes

Type of transformation	Number of hours	Serotype expressed	Number of fissions
T to H at 26°C	1, 2	T	0 to 1
	3,4,5,6	T and H	1 to 2
	7+	H	2 to 3
H to T at 40°C	1	H	0 to 1
	2,3,4	H and T	1 to 2
	5, 6	T	3+

absorption, leaving the T specific reaction. Thus with minor exceptions, antisera prepared against H and T cells did not crossreact with each other.

Antisera prepared against the 11 inbred families grown at 40°C revealed three different T phenotypes. The types were closely related, for they all cross reacted with each other. Cross reacting antisera often produce positive responses for one serotype at dilutions which produce intermediate responses for another type. Absorptions were usually required to render unambiguous the typing of cultures: a systematic two dilution difference was considered satisfactory. With absorbed antisera the inbred families were divided into three groups as follows: Ta (families A, A1, B, C1, E, and F), Tb (families A3 and C), and Tc (families D, D1 and B2). The absorption data are consistent with a scheme in which determinant (α) is assigned to the Ta families, ($\alpha\,\beta$) to the Tb families and (γ) to the Tc families. The results expected from this scheme are summarized in Table 3. When antisera against group Ta (anti-α) were absorbed with cells of type Ta (α) or Tb ($\alpha\,\beta$) all reactivity was removed; while absorption with cells of type Tc (γ) left the reaction to types Ta and Tb. Antisera against type Tc cells (anti-γ) lost all reactivity when absorbed with Tc (γ) cells, but absorptions with Ta (α) or Tb ($\alpha\,\beta$) cells resulted in an antiserum specific for Tc cells only. Antisera against type Tb cells (anti-$\alpha\,\beta$) lost reactivity if absorbed by Tb ($\alpha\,\beta$) cells. Absorption with Ta (α) cells produced an antiserum specific for type Tb ($\alpha\,\beta$) cells only; while absorption with Tc (γ) cells left an antiserum specific for Ta (α) and Tb ($\alpha\,\beta$) cells. Samples of antisera against ten of the inbred families grown at 40° were absorbed with one of each of the following types of cells: A3 and C (Tb), D1 (Tc) and A1, C1 and E (Ta). Results obtained with these antisera were in agreement with the scheme presented above. That these reactions are characteristic of the inbred families and not of particular strains within the families was shown by the fact that different strains of the same family reacted identically to the T antisera.

Demonstration of allelism of the T phenotypes: The results of crosses between inbred families of different T phenotypes showed that these serotype differences were controlled by different alleles at a single locus. The F_1 crosses were carried out in bacterized peptone medium. F_1 synclones were tested for failure to complete conjugation and for H serotypes. Samples of the synclones were centrifuged and inoculated into axenic liver peptone tubes with antibiotic (1 g streptomycin

TABLE 3

Prediction of antibodies remaining in antisera to cells grown at 40°C after absorption with different inbred families

Absorbant cells	Determinant	Phenotype	Antiserum against cells of phenotype		
			Ta (anti-α)	Tb (anti-$\alpha\beta$)	Tc (anti-γ)
A, A1, B, C1, E, F	α	Ta	0	anti-β	anti-γ
A3 and C	$\alpha\beta$	Tb	0	0	anti-γ
D, D1, B2	γ	Tc	anti-α	anti-$\alpha\beta$	0

and 1×10^6 units penicillin/liter) for testing the T serotypes. All true F_1 synclones reacted with both types of H and T antisera. F_1 synclones heterozygous for the T serotypes were obtained in the following types of crosses: $T^B/T^B \times T^C/T^C$ (B2 × C, A3 × D1) $T^A/T^A \times T^C/T^C$ (B2 × A, B2 × C1); $T^A/T^A \times T^B/T^B$ (A3 × F).

In order to obtain an F_2, the F_1 cultures were allowed to mature by isolating single cells and carrying these secondary lines through five serial transfers until they had undergone approximately 65 fissions. At this time most of the F_1 lines manifested only one *H* allele, as was expected from earlier work, but most of the lines still reacted with both T antisera. However, a few F_1 lines manifested only one T serotype, indicating that the T serotypes also differentiate in vegetative multiplication. This differentiation will be discussed in the following paper. In order to make a F_2 cross in axenic peptone, single F_1 cells were washed and new sublines inoculated into axenic peptone tubes. The axenic crosses were made as described in METHODS.

The expected Mendelian ratios for allelic genes of 1:2:1 for F_2 crosses and 1:1 for testcrosses were obtained, regardless of the phenotype of the F_1 cells at the time the cross was made. Crosses between heterozygotes with different *T* alleles also yielded the expected 1:1:1:1 ratio for the different genotypes. The families used in these crosses were B2 × C ($T^B/T^C \times T^B/T^C$); B2 × A, B2 × C1 ($T^A/T^C \times T^A/T^C$) and A3 × F ($T^A/T^B \times T^A/T^B$). The results are summarized in Table 4. The T^A/T^B and T^B/T^B phenotypes were difficult to distinguish with the available antisera. A culture was scored Tb if it gave a positive reaction to an anti-Tb antiserum absorbed with Ta cells, and scored Ta on the basis of a negative reaction to such an antiserum. T^A/T^B heterozygotes gave an intermediate response; testing the cells with two consecutive antiserum dilutions was necessary to distinguish them from the two homozygotes. For the T^A/T^B F_2 cross, numerous progeny tests were made to resolve the ambiguous cases. For the $T^A/T^B \times T^B/T^C$ double heterozygote cross, no effort was made to distinguish between the T^A/T^B and T^B/T^B synclones.

Several of the crosses involving families B2 × C (including two in the table) gave aberrant results for the H serotype locus. In all cases the number of Hc progeny was less than expected. For example, in the F_2 cross between parental serotypes Tc and Tc, there were 34 He:52 Hce:8 Hc, and in the F_2 cross between parental serotypes Tb and Tb, there were 16 He:25 Hce:9 Hc. In both of these crosses there was considerable death; 12% in the first case and 16% in the second. However, the crosses differed most markedly from the others in the exceptionally high numbers of nonconjugants (50% in the first case and 40% in the second). This was in contrast to the Tbc × Tbc cross in which there was 15% death, but only 6% nonconjugation and to the Tac × Tc (B2 × A) cross in which there was 6% death and 6% nonconjugation. The reduced number of Hc progeny and high rate of nonconjugation suggested some kind of abnormality associated with the *H* locus. The fact that the T ratios were unaffected in these crosses is evidence against close linkage of the *T* locus with the *H* locus. That some kind of degeneration was taking place was evident because five to six months after the B2 × C

TABLE 4

Distribution of T phenotypes in F_2 and testcrosses

Parental genotypes	Parental phenotypes	Progeny serotypes						Total	χ^2	P
		Ta	Tb	Tab	Tac	Tbc	Tc			
$T^B/T^C \times T^B/T^C$ (B2 × C F_2)	Tbc × Tb	..	3	..	..	16	6	25	2.2	.05<P<.2
	Tbc × Tbc	..	21	..	..	54	20	95	1.8	.2<P<.5
	Tc × Tc	..	23	..	..	44	27	94	.72	.5<P<.8
	Tb × Tb	..	12	..	..	23	15	50	.68	.5<P<.8
	Totals	..	59	..	..	137	68	264	.99	.5<P<.8
$T^A/T^C \times T^A/T^C$ (B2 × A F_2)	Tac × Tc	28	..	..	53	..	24	105	.32	.8<P<.95
(B2 × C1 F_2)	Tac × Tac	13	..	..	29	..	11	53	.66	.5<P<.8
	Totals	41	..	..	82	..	35	158	.68	.5<P<.8
$T^A/T^B \times T^A/T^B$	Tab × Tab	18	20	33	..	..	..	71	.50	.5<P<.8
(A3 × F F_2)										
$T^A/T^A \times T^A/T^C$ (B2 × A)	Ta × Tac	47	..	..	43	..	..	90	.18	.5<P<.8
$T^C/T^C \times T^A/T^C$ (B2 × A)	Tc × Tac	..	..	..	15	..	19	34		
	Tc × Ta	..	..	..	10	..	8	18		
	Totals	..	..	..	25	..	27	52	.08	.5<P<.8
$T^C/T^C \times T^C/T^B$ (B2 × C)	Tc × Tc	..	..	..	..	9	6	15		
	Tc × Tbc	..	..	..	..	12	9	21		
	Totals	..	..	..	..	21	15	36	1.0	.2<P<.5
$T^A/T^A \times T^A/T^B$ (A3 × F)	Ta × Ta	13	..	13	..	..	..	26		
	Ta × Tab	23	..	27	..	..	..	50		
	Totals	36	..	40	..	..	..	76	.21	.5<P<.8
$T^A/T^B \times T^B/T^C$	Tab × Tbc	..	22*	..	17	14	..	53	1.86	.2<P<.5
$T^A/T^C \times T^B/T^C$	Ta × Tbc	..	10	..	10	11	14	45	1.01	.5<P<.8
$T^A/T^B \times T^A/T^C$	Tab × Tac	6	10	..	8	11	..	35	1.68	.5<P<.8

* Tab or Tb.

heterozygotes had been obtained, almost all of them gave aberrant results in F_2 crosses. Even a repetition of the Tbc × Tbc cross using cells descendant from those used in the first cross resulted in 30% death, 50% nonconjugation and no Hc progeny. At this time the original B2 parent was used in the cross of B2 × A which gave excellent results, while a repetition of the B2 × C cross gave very few normal conjugants. Thus, family C appeared to be the defective parent.

Inbreeding degeneration has been observed in *T. pyriformis* in earlier work, and certain strains of family C have been giving anomalous results in crosses for some time (ALLEN 1963; NANNEY 1963a; NANNEY and NAGEL 1964). The phenomenon of genomic exclusion in which F_1 progeny are homozygous for the genes

of one parent (unilateral genomic exclusion) or in which some are homozygous for one parent's genome and others for the genome of the other parent (bilateral genomic exclusion) was first observed in crosses involving family C (ALLEN 1963). In cases of unilateral genomic exclusion the abnormal strain does not contribute to the zygotic fusion nucleus. In the case of a heterozygote mated with an abnormal strain of C called C*, 1:2:1 ratios for the alleles of the normal mate are sometimes observed. In crosses between two heterozygotes, and with the possibility of bilateral genomic exclusion, various ratios might be produced. In the crosses described above there seems to be a selection against the haploid nuclei containing the Hc marker. The mechanism for this is not understood.

Relationship of the T serotype locus to the H serotype locus: Tabulation of the H and T serotypes of the F_2 synclones obtained from several crosses gave no evidence for linkage between the *H* and the *T* loci. The observed values given in Table 5 corresponded to those expected on the basis of independent assortment of the two serotypes.

Relationship of the T serotype locus to the mating type locus: F_2 progeny from several crosses were tested for mating type in order to investigate possible linkage between the *T* locus and the mating type (*mt*) locus. Unfortunately, testing for the *mt* genotype is more difficult than testing for serotypes. Each mating type genotype determines an array of five to seven possible mating types occurring in definite frequencies (NANNEY 1959). Among the progeny of a single pair there may be one to four different mating types, which are manifested only after

TABLE 5

Relationship between H serotypes and T serotypes in F_2 crosses

Parental genotypes	F_2 progeny H serotypes		F_2 progeny T serotypes Tb	Tbc	Tc	Totals	χ^2	P
$H^C/H^C, T^C/T^C \times H^E/H^E, T^B/T^B$								
(B2 × C F_2)	He	Obs	7	13	5	25		
		Exp	6	12	6			
	Hce	Obs	5	28	10	43		
		Exp	12	24	12			
	Hc	Obs	8	14	5	27		
		Exp	6	12	6			
	Total		20	55	20	95	6.6*	.5<P<.8
			Ta	Tac	Tc			
$H^C/H^C, T^C/T^C \times H^A/H^A, T^A/T^A$								
(B2 × A F_2)	Ha	Obs	5	13	3	21		
		Exp	6.6	13.2	6.6			
	Hac	Obs	17	25	14	56		
		Exp	13.2	26.5	13.2			
	Hc	Obs	6	17	5	28		
		Exp	6.6	13.2	6.6			
	Total		28	55	22	105	4.24	.8<P<.95

* χ^2 is calculated on the basis of independent assortment.

a maturation period of about 65 fissions. The mating types found in the different *mt* genotypes are given in Table 6. Qualitatively, the *mt* loci differ in that cells of genotype mt^B/mt^B may be any of the mating types II, III, IV, V, VI or VII, but never mating type I; while cells of genotype mt^A/mt^A or mt^C/mt^C may be any of the mating types I, II, III, V or VI, but never IV or VII. Although these potentialities are inherited on a simple one gene basis, control of the actual mating types is epigenetic and caryonidal.

A brief review of the conjugation process in Tetrahymena is in order at this point (ELLIOTT and HAYES 1953; NANNEY 1953; RAY 1955). Each normal vegetative cell has a compound macronucleus and a diploid micronucleus. The macronucleus controls the vegetative phenotype; the micronucleus serves as a germinal nucleus. During conjugation meiosis takes place in the micronucleus and four haploid micronuclei are produced by each member of the conjugating pair. In each cell all but one of these meiotic products disintegrate and the remaining one divides once mitotically. In each member of the pair, one of the nuclei crosses over the conjugation bridge to the other cell, and the other nucleus remains behind. In each cell, a diploid zygotic nucleus is then formed by nuclear fusion. Thus, the members of a pair are genetically identical after conjugation with respect to the micronucleus. When the pairs separate, this zygotic nucleus divides twice to form four nuclei: two become new micronuclei and two undergo rapid growth and become new macronuclei. At this time the old macronuclei disintegrate. At the first postzygotic division the two new macronuclei in each exconjugant are assorted to their two daughter cells, called caryonides.

There is no correlation among the mating types of caryonides from a single pair, but most of the descendants of a given caryonide have the same mating type (NANNEY and CAUGHEY 1953). Thus, mating type differences assort at the same division at which the newly developed macronuclei are segregated into daughter cells. Therefore, in order to determine the mating types produced by a pair, caryonides should be isolated and transferred to maturity. However, the cells do not divide synchronously, and caryonidal isolations are laborious. Therefore a simpler procedure has been adopted in which three or six cells are isolated from each pair culture (synclone) and transferred to maturity. However, to determine the *mt* genotype of a given pair, one must ascertain whether it has the potentiality to produce cells of the critical mating types I, IV and VII. If IV or VII appears among the cells derived from one pair, the presence of a mt^B allele is shown, if I

TABLE 6

Mating type frequencies in different mt *genotypes**

Genotype	Mating type frequencies							Total
	I	II	III	IV	V	VI	VII	
mt^A/mt^A	.253	.173	.174	.000	.095	.304	.000	809
mt^B/mt^B	.000	.149	.093	.473	.045	.139	.100	1090
mt^C/mt^C	.596	.119	.087	.000	.003	.198	.000	356

* From NANNEY (1959).

appears, mt^C or mt^A is present. If both I and IV or VII appear, then the pair can be classified as a heterozygote. As can be seen from the mating type frequencies, progeny of some pairs would be expected to yield none of the distinguishing mating types. To ascertain the genotypes unambiguously, several cells from each pair must be typed and then progeny tested to determine their mating type potentialities.

However, linkage can be determined in an easier way. If three cells are isolated from each F_2 synclone, transferred to maturity and scored for mating type, a comparison of the frequencies of the critical types with their T genotype can be made. The expectations on the basis of no linkage, complete linkage, and 25% recombination are given in Table 7. For example, consider mating type I, found only in cells with an mt^A allele, for the case of complete linkage with the T locus. The original parents were mt^A/mt^A, T^A/T^A and mt^B/mt^B, T^C/T^C. If *mt* and T are completely linked the F_2 should consist of only three genotypes: mt^AT^A/mt^AT^A; mt^AT^A/mt^BT^B and mt^BT^B/mt^BT^B in a ratio of 1:2:1. A clone of mating type I cannot be in the third class. If mating type I were equally frequent in clones homozygous and heterozygous for mt^A, the type I clones would be distributed in a 1:2 ratio with respect to the linked marker, T^A, in the homozygous and heterozygous condition. However, mating-type frequencies are dosage dependent: types such as I, IV or VII are usually about twice as frequent in homozygotes as heterozygotes (NANNEY 1959). Because homozygotes are half as frequent as heterozygotes, but twice as likely to produce type I, clones of type I will be distributed about equally in the homozygote and heterozygote classes. In contrast, if no linkage occurs between *mt* and T, the mating types should be distributed among the T genotypes strictly in proportion to their frequencies; that is, type I clones would be distributed in a 1:2:1 ratio instead of a 1:1:0 ratio. Moderate linkage would yield intermediate values. The method is not sensitive enough to identify distant linkage, but is adequate to disprove close linkage; e.g. 25% or less. The

TABLE 7

Expected mating type frequencies for different T *genotypes in* F_2 *progeny for different cases of linkage of the* T *locus with the* mt *locus*

Case	T serotypes	Mating types		
		I	IV	VII
I. Complete linkage	Ta	.5 (32.5)*	.0 (0.0)	.0 (0.0)
	Tac	.5 (32.5)	.5 (49.5)	.5 (7.5)
	Tc	.0 (0.0)	.5 (49.5)	.5 (7.5)
II. No linkage	Ta	.25 (16.25)	.25 (24.75)	.25 (3.75)
	Tac	.50 (32.50)	.50 (49.50)	.50 (7.50)
	Tc	.25 (16.25)	.25 (24.75)	.25 (3.75)
III. 25% recombination	Ta	.375 (24.4)	.125 (12.4)	.125 (1.9)
	Tac	.500 (32.5)	.500 (49.5)	.500 (7.5)
	Tc	.125 (8.1)	.375 (37.1)	.375 (5.6)

* Numbers in parentheses indicate expected numbers on the basis of total cultures examined (see Table 8).

TABLE 8

Relationship between mating type and T *serotypes in* F_2 *crosses*

Parental genotypes	F_2 progeny T serotypes	Mating types I	II	III	IV	V	VI	VII	Total pairs
$T^C/T^C, mt^B/mt^B \times T^B/T^B, mt^C/mt^C$ (B2 × C F_2)	Tb	6	12	4	13	0	5	2	
	Tbc	13	13	1	21	1	19	4	
	Tc	8	9	2	12	0	8	0	90
$T^C/T^C, mt^B/mt^B \times T^A/T^A, mt^A/mt^A$ (B2 × A F_2)	Ta	7	6	9	12	3	8	2	
	Tac	7	18	4	19	3	17	0	
	Tc	6	6	2	9	3	7	1	104
$T^C/T^C, mt^B/mt^B \times T^A/T^A, mt^A/mt^A$ (B2 × C1 F_2)	Ta	3	3	1	1	0	6	1	
	Tac	11	3	2	12	2	10	3	
	Tc	4	3	1	0	0	4	2	54
Totals for B2 × A and B2 × C1 F_2	Ta	10	9	10	13	3	14	3	
	Tac	18	21	6	31	5	27	3	
	Tc	10	9	3	9	3	11	3	158
Totals for all F_2	Ta,b	16	21	14	26	3	19	5	
	Ta,b/c	31	34	7	52	6	46	7	
	Tc	18	18	5	21	3	19	3	248

Mating type	χ^2 (Case II)	P	χ^2 (Case III)	P
I	.261	.8<P<.95	15.06	P<.01
IV	.739	.5<P<.8	23.14	P<.01
VII	.495	.5<P<.8	10.72	P<.01

expectations for the three cases are given in Table 7. Figures in brackets are numbers expected on the basis of the observed totals. Table 8 shows the results from three different crosses scored for mating type and T serotype. The observed values correspond to those expected on the basis of independent assortment of the *mt* and the *T* loci.

One additional point concerning the method must be made. As described above, three cells from each synclone were isolated and transferred to maturity and scored for mating type. If two or three of these sublines are of the same mating type, they could be from the same caryonide; therefore, only the first subline of a particular mating type was tabulated. Since the purpose of the experiment was to compare the mating types found among F_2 synclones of different *T* genotypes and not to determine absolute mating type frequencies, this does not bias the results.

Relationship of the T *serotype locus to the enzyme loci:* In addition to the mating type and *H* serotype loci, three unlinked genetic loci controlling enzyme specificities have been analyzed in *T. pyriformis*, syngen 1. These are the

esterase-1 locus, controlling two groups of isoenzymes which split alpha naphthyl acetate and alpha naphthyl propionate and are inhibited by eserine sulfate; the esterase-2 locus, controlling two alternative esterases which split alpha naphthyl butyrate and are unaffected by eserine sulfate; and the phosphatase-1 locus, controlling alternative forms of an acid phosphatase (ALLEN 1965).

The possibility of linkage of the *T* serotype locus with one of the enzyme loci was investigated by making the appropriate crosses in peptone and then growing samples from the F_2 synclones in axenic peptone flasks at 30°C for the enzyme extractions. The enzyme extractions and starch gel electrophoresis were performed as described in METHODS, and the F_2 synclones were scored for their enzyme type. Tabulation of the T serotypes and enzyme types of F_2 synclones from the two crosses gave no evidence for linkage between the *T* locus and any of enzyme loci, although loose linkage was not excluded (Table 9.) The observed values corresponded to those expected on the basis of independent assortment of

TABLE 9

Relationship between the T *locus and the enzyme loci in* F_2 *crosses*

Parental genotypes	F_2 progeny T serotypes		F_2 progeny enzyme types E–1b	E–1bc	E–1c	Totals	χ^2	P
$T^C/T^C, E\text{–}1^B/E\text{–}1^B, P\text{–}1^A/P\text{–}1/ \times$ $T^A/T^A, E\text{–}1^C/E\text{–}1^C, P\text{–}1^B/P\text{–}1^B$ (B2 × C1 F_2)	Ta	Obs	3	9	1	13		
		Exp	3.31	6.63	3.31			
	Tac	Obs	5	15	9	29		
		Exp	6.63	13.25	6.63			
	Tc	Obs	3	8	0	11		
		Exp	3.31	6.63	3.31			
	Total		11	32	10	53	5.63*	.5>P>.2
			P–1a	P–1ab	P–1b			
	Ta	Obs	1	8	4	13		
		Exp	3.31	6.63	3.31			
	Tac	Obs	9	15	5	29		
		Exp	6.63	13.25	6.63			
	Tc	Obs	1	6	4	11		
		Exp	3.31	6.63	3.31			
	Total		11	29	13	53	3.06	P= .8
			E–2b	E–2bc	E–2c			
$T^B/T^B, E\text{–}2^C/E\text{–}2^C \times$ $T^A/T^A, E\text{–}2^B/E\text{–}2^B$ (A3 × F F_2)	Ta	Obs	3	10	4	17		
		Exp	4.31	8.62	4.31			
	Tab	Obs	13	14	6	34		
		Exp	8.62	17.24	8.62			
	Tb	Obs	5	10	4	18		
		Exp	4.31	8.62	4.31			
	Total		21	34	14	69	4.42	.8>P>.5

* χ^2 is calculated on the basis of independent assortment.

the T serotypes and the enzyme types. In the calculation of the chi-squares for the experiments involving the enzyme loci, cells were lumped according to the rationale that classes expected to be large with linkage were put together and *vice versa*. The corresponding degrees of freedom were subtracted.

DISCUSSION

Previous work on high temperature serotypes in Tetrahymena: Two previous studies suggested the presence of a high temperature serotype. MARGOLIN, LOEFER and OWEN (1959) reported that an antiserum against a 20°C bacterized peptone culture of WH-6 (Ha) had no effect on an axenic 35° culture of the same strain. The results of WATSON, ALEXANDER and SILVESTER (1964) are discussed in the next section.

Nature of the T *antigens:* Little information concerning the nature of the gene product of the *T* locus is available. Preliminary immunodiffusion and immunoelectrophoresis experiments (PHILLIPS, unpublished) showed that each T antiserum gives what appears to be a unique array of bands with 40° cells. Unpublished results of immunodiffusion experiments using H antisera showed that bands corresponding to the different *H* allelic types can be identified among the array of bands obtained with room temperature cells (NANNEY, unpublished).

Much more extensive work has been done with the immobilization antigens of *Paramecium aurelia*. They have been partially purified and subjected to gel diffusion and fingerprinting analysis. They were found to be proteins of about 250,000 molecular weight localized in the cilia and cell walls (PREER 1959; BISHOP 1961). Reduction and alkylation of antigen A and antigen D of syngen 1 yielded molecules of 35,000 and 80,000 molecular weight respectively, suggesting that these antigens are composed of smaller subunits held together by disulfide bonds (STEERS 1965; JONES 1965).

Recently an elegant fractionation of *Tetrahymena pyriformis cilia* has been accomplished (WATSON, ALEXANDER and SILVESTER 1964). Antiserum to whole cilia yielded six precipitin bands with crude ciliary extracts in gel diffusion plates, but only three bands with an acetic acid extract. Antisera to the acetic acid fraction immobilized WH-14, one of the parents of the inbred families which is known to have the H^D allele. Cells of WH-14 grown at 37°C were not affected by the antiserum. Therefore this fraction appears to contain the H immobilization antigen, but not the T antigen. The acetic acid fraction gave three peaks on the ultracentrifuge and the ultraviolet absorption curves at pH 2 were of a general protein type. Further fractionation by precipitation with TCA and extraction with ethanol yielded two additional fractions. An ethanol insoluble fraction gave two lines on gel diffusion plates and a broad line on the ultracentrifuge. It had an amino acid composition similar to that of whole cilia. The ethanol soluble fraction appeared to be a protein of unique amino acid composition which gave only one precipitin band and one peak in the ultracentrifuge. Unfortunately, there is no report of antisera made to the ethanol fractions. However, the work strongly suggests that the H immobilization antigens of *Tetrahymena pyriformis* are

proteins found on the cilia. Preliminary work on the characterization of the H and T antigens is underway (BRUNS, unpublished).

Relationship between the H and T antigens: The chemical relationship between the T antigens produced at high temperatures and the H antigens produced at room temperature is also unknown. It is possible that the H and T antigens are in reality the same polypeptide chain which either assumes a different conformation or is chemically modified at the different temperatures, and thus elicits different antibodies. On the other hand they may be completely unrelated molecules or they may have subunits or portions of the molecule in common. The idea of a simple conformational change in a single polypeptide chain is implausible, since the H and T antigens are apparently controlled by unlinked loci. However, further work will be necessary to distinguish between these possibilities.

Striking parallels to the serotype systems in *T. pyriformis* are found in the extensive literature on serotypes in *P. aurelia* (see PREER 1967). In this ciliate as many as 12 alternative and mutually exclusive antigens controlled by unlinked genes are found in a single strain under different environmental conditions. In syngen 1, there are three mutually exclusive antigens expressed at different temperatures: S at 10° to 19°C, G at 20° to 25° and D at 26° to 35°. Different stocks have different alleles at each of these loci such as 30D, 60D and 50D. BISHOP and BEALE (1960) showed electrophoretic differences between the partially purified antigens 60D and 60G, while the four allelic types 60D, 90D, 103D and 33D were not electrophoretically distinguishable. JONES and BEALE (1963) found gross differences between peptide maps of D and G antigens and small differences among nine D antigens, including five which were immunologically indistinguishable. Thus in *P. aurelia* both genetic and chemical evidence demonstrates differences between the antigens expressed at different temperatures. The strong correlation between serological relationship and similarity of peptides suggests that antigenic sites are found throughout the molecule and makes the idea that mutually exclusive antigens such as G and D in *P. aurelia* or H and T in Tetrahymena have large portions of the molecule in common less probable. In view of the heterogeneity of both the antibodies and cell extracts used to obtain the antisera, few conclusions can be drawn about the nature of the immobilization antigens at this time.

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SUMMARY

Cells of *Tetrahymena pyriformis*, syngen 1, grown axenically at 40°C express a new serotype, (T), different from the H serotype expressed at room temperature. Among the 11 inbred families three T phenotypes are controlled by three alleles at a single locus, not closely linked to other known loci.

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