THE GENETIC CONTROL OF MATING TYPE POTENTIALITIES IN TETRAHYMENA PYRIFORMIS¹

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GENES controlling mating type potentialities in *Paramecium aurelia* (SONNEBORN 1939) were the first mendelizing factors demonstrated in the Ciliates. Although other genes have since been discovered in *P. aurelia*, the mating type genes were of particular significance in that, for the first time, they provided unequivocal evidence for classical genetic mechanisms in the Ciliates and a firm basis for subsequent studies in cellular heredity.

Genes have also been demonstrated in another Ciliate, *Euplotes patella* (KIMBALL 1942), and these include factors concerned with mating type inheritance. The modes of action of the mating type genes in these forms appear to be different. The *Paramecium* genes confer on the cell containing them a potentiality, the realization of which may depend upon other circumstances; the mating type genes in *Euplotes* appear directly to determine the mating type of a cell.

MATERIALS AND METHODS

Materials

This report is concerned with evidence for genes controlling mating type potentialities in variety 1 of *Tetrahymena pyriformis*. This species, like several species of *Paramecium* (SONNEBORN 1947), is composed of a number of non-interbreeding groups designated as "varieties" (GRUCHY 1954). Within each of the varieties from 2 to 8 different mating types have been identified; conjugation occurs, within a variety, when any two clones of different types are combined. At the present time 7 mating types are known for variety 1 (NANNEY and CAUGHEY 1953). The strains used in this study were derived from two clones isolated from a pond near Woods Hole, Massachusetts, by ELLIOTT and GRUCHY (1952) and are designated as the WH strains. All the 7 known mating types have appeared among the progeny of these clones.

Methods

Since the culture methods and techniques used to determine the mating types of unknown cultures are presented elsewhere (NANNEY and CAUGHEY 1955), the general procedures will be indicated only briefly here. Immediately following conjugation the exconjugants and the first fission products are separated into individual containers and allowed to give rise to cultures. These exconjugant cultures are immature and must undergo about 80 fissions before their mating types can be determined.

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The procedure for obtaining mature cultures is to make serial isolations from the exconjugant cultures, single animals being isolated—usually at two day intervals—for a total of 8 to 10 transfers. At the end of this time the cultures are placed in test tubes. Finally these mature cultures are mixed with samples of each of the standard mating types and are examined for conjugants. Every culture examined has mated with 6 of the 7 standard types and has been classified as belonging to the type with which it did not mate.

Cylogenetics

The cytological events occurring at conjugation in *Tetrahymena* have been discussed in detail by NANNEY (1953), by ELLIOTT and HAYES (1953) and by RAY (1954). The cytological events of genetic significance are as follows. Meiosis occurs in each member of a conjugating pair and results in the formation from the single original diploid micronucleus of four haploid nuclei, each with 5 chromosomes. Three of the four haploid nuclei disintegrate and the fourth undergoes an equational division—giving rise to a migratory and a stationary nucleus. An exchange of the migratory nuclei occurs between the two members of a pair and is followed by a fusion of the migratory and stationary nuclei of diverse origin. The cytological events lead to the expectation that pair members, regardless of their original genetic constitutions, will become alike following conjugation. If the original parents are homozygous for different alleles at a particular locus, the exconjugant F_1 will be heterozygous. Segregation will occur when F_1 clones are crossed; one fourth of the F_2 pairs will be homozygous for one allele, one half will be heterozygous and one fourth will be homozygous for the other allele.

The fertilization nucleus produced at conjugation undergoes two divisions before the exconjugant divides. Two of the four nuclei so produced become macronuclei and two become micronuclei; one of the micronuclei disintegrates. At the first cell division following conjugation the single remaining micronucleus divides, but the two new macronuclei are separated into different cells to divide at all subsequent divisions. This first cell division is, therefore, unique in that diverse lines of cells (caryonides), deriving their macronuclei from two different primordial macronuclei, are separated at this division. In *Tetrahymena* (NANNEY and CAUGHEY 1953), as in *P. aurelia* (SONNEBORN 1937), sister caryonides derived from the same exconjugant may show different mating types, but—in general—the cells within a caryonide are of the same mating type. Occasionally clones are found which contain two or more mating types and which conjugate (self) in clonal cultures. These selfing clones are discussed at length elsewhere (NANNEY and CAUGHEY 1955) and only those selfers from which pure types have been derived are utilized in the present analysis. Caryonidal inheritance (SONNEBORN 1947; NANNEY 1954) indicates that the mating types in these forms are controlled by the macronuclei and that diverse mating types may be developed on a common genetic basis. On the other hand, it has been shown for *P. aurelia* that the mating type *potentialities* (SONNEBORN 1947) and the mating type *frequencies* (BUTZEL 1953) are determined in some cases at least by nuclear genes. Evidence presented below demonstrates that the spectrum of possible mating types in *Tetrahymena* is also controlled by genes.

Tests for conjugation

The two strains on which the present study is based are designated as WH-6 (mating type I) and WH-14 (mating type II). Crosses involving these two strains yield many pairs with cytological irregularities and few viable progeny are produced. It has been possible, however, to derive some apparently normal exconjugant clones from such crosses. Further crosses with these derived strains show relatively little inviability and few cytological anomalies. Nevertheless, occasional pairs are found which separate without forming new macronuclei. Such pairs must be detected and eliminated if a genetic analysis is to be significant. Several tests for nuclear reorganization have been utilized in these experiments. In the earliest crosses, after conjugation, one member of each pair was killed, stained and examined for new macronuclei. Examination of hundreds of pairs stained just prior to separation has revealed no cases in which one member had produced new macronuclei and the other member had not. Hence, it appears reasonable to conclude that if the killed exconjugant has new macronuclei, its living mate has also undergone a nuclear reorganization. This procedure is the most direct method for demonstrating that reorganization has occurred, but it requires the sacrifice of half the information a pair might provide.

The second test for reorganization was derived from an observation made during the experiments described above. Every cell stained and found to contain new macronuclei had been paired with a cell which gave rise to an immature clone, i.e., a clone which would not mate until many fissions later. Conversely, every cell stained and found to contain no new macronuclei had been paired with a cell which gave rise to a clone which was mature as soon as it could be tested. Therefore, an examination of "exconjugant" clones for early maturity constitutes a satisfactory screening procedure for non-conjugants. Approximately half the crosses to be reported were screened in this fashion.

A third test, also based on the initial observations, has been used extensively but has not proved very satisfactory. This test is based on the fact that pairs which do not undergo reorganization do not change mating type; when sub-lines are derived at the first cell division following separation, four clones are derived from a pair—two of one parental mating type from one cell and two of the other parental mating type from its mate. Any pair which produces clones which deviate from this pattern of mating type distribution may be considered to have undergone a nuclear reorganization. Since several different mating types are produced in all crosses and since these are distributed among the caryonides approximately at random, relatively few conjugants will be falsely excluded so long as all four caryonides are available for testing. This is particularly true if one or both of the parental types is rare. The chief difficulty arises from the fact that all four caryonides are not always available for testing; one or more caryonides may die, or may be accidentally lost in making isolations. Moreover, it is not always desirable to obtain all four caryonides when representatives from a maximum number of *pairs* are desired. Under these circumstances some pairs excluded as possible non-conjugants are certainly rejected falsely. These false exclusions result in a distortion of the relative frequencies of the mating types among the progeny—to different degrees and in different directions depending upon the mating types of the parents. In the experiments reported here, the significant data are largely

qualitative and the frequency distortions are not critical. The chief bearing of the excluded clones is upon the total number of progeny used to conclude that a particular mating type *cannot* appear. The exclusion of clones as possible non-conjugants is, therefore, a conservative factor.

None of the above tests for nuclear reorganization adequately distinguishes between true conjugation (involving an exchange of genetic material between pair members) and cytogamy (mutual self fertilization). In certain instances it is clear that an exchange of material has occurred, but no generally valid procedure exists for excluding cytogamous pairs. Evidence will be presented later, however, which indicates that most, perhaps all, of the reorganization is conjugation.

Progeny tests

The studies on caryonidal inheritance in *Paramecium aurelia* (SONNEBORN 1947) demonstrate that clones with the same genotype may possess different mating types. Moreover, clones with the same mating type may have different genotypes. As will be shown, a similar situation occurs with Tetrahymena. Under such circumstances the "phenotype" governed by a particular genotype is not adequately manifested by any one clone. The genotype determines a spectrum of possibilities which can be discerned only by examining large numbers of clones. Whenever possible these clones have been obtained through the use of progeny tests in which sister caryonides of different mating types are crossed.

Crosses between sister caryonides should yield progeny exclusively of the parental genotype only if the parents are homozygous. If the parents are heterozygous for one or more factors a variety of genotypes would be expected among the progeny, and the parental genotype might be rare. Hence, if certain mating type potentialities are determined by heterozygous genotypes, a parental potentiality might be lost through recombination, or a potentiality not possessed by the parental genotypes might appear among the progeny. Fortunately, this complication has not arisen under conditions suitable for its detection. Specifically, heterozygous determination of a potentiality would be detected in a cross of diverse inbred strains by the appearance of a mating type which neither inbred strain alone could produce. Such novel types have not been detected.

In certain instances, it is either impossible to cross sister caryonides or to examine their progeny. Under these circumstances, the clones must be outcrossed to other clones of known potentiality. For example, a clone of unknown potentiality may be crossed to a clone which *cannot* produce mating type I; if mating type I appears among the progeny, one may conclude that the tested clone possessed the potentiality for that type. This outcross procedure has been validated by outcrosses of clones which can also be subjected to analysis by crosses of sister caryonides. The outcross method is much less economical than the sister $\times$ sister progeny tests, but it is the only practicable method for classifying some clones.

THE GENETIC ANALYSIS

Two reasonably homogeneous strains have been obtained through inbreeding the F_1 cultures. The first inbred series, designated as "family A", has been carried through

six generations; crosses among the various related clones regularly produce 5 of the 7 mating types known for this variety; only types IV and VII are lacking. The second inbred series, designated as "family B", has been carried for five generations; crosses between clones in this group produce all the known types except type I. Only a fraction of the possible combinations of parental mating types in either family have been examined in the crosses made; only 4 of the 10 possible combinations in family A have been studied and only 4 of the 15 combinations in family B. Since cells of the same mating type could be of different genotype, an exhaustive study of the mating type potentialities within the families would require a very large number of crosses. It is possible, therefore, that some as yet unexamined crosses will yield types other than those thus far observed. On the other hand, the data available (table 1) suggest a reasonable uniformity among the progeny within a family, regardless of the parental mating types. Occasionally some type which should appear is missing from a particular cross. Thus in family A, the cross F_3 II $\times$ VI did not yield type V. Since the types missing are in each case rare types, their absence is probably due to sampling error. Moreover, in some cases the crosses were repeated at different temperatures and the missing types were found. At no temperature, however, were types IV or VII found in family A or type I in family B. That a real genetic difference characterizes these families is further borne out by the subsequent breeding analysis.

The plan for analyzing the genetic basis for the observed differences in mating type potentialities was as follows. A representative of family A was crossed with a representative of family B and the progeny of 20 pairs were tested for mating type. These progeny constituted the F_1 generation. Three of these F_1 pairs were chosen to produce the F_2 by crosses of sister caryonides. The F_2 pairs were then analyzed for mating type potentialities by conducting progeny tests.

The mating types of their progeny indicated that all the 20 F_1 pairs had undergone a nuclear reorganization. Eight of the 20 pairs showed evidence of genetic transfer; in these 8 pairs, either one conjugant produced mating types originally found only in different families (I and IV or I and VII) or both conjugants produced mating types found originally in only one parental family (I and I; IV and IV; VII and VII or IV and VII). More of the pairs would probably have been clearly judged to be true conjugants if all the caryonides had been available for analysis, but 19 of the 80 caryonides either selfed, were accidentally lost or died before reaching maturity. All 7 mating types appeared in the F_1 . This fact at once suggests that if the capacities to produce these mating types are under genetic control, and if the parents were homozygous for different mating type potentialities—the genes giving a cell a particular potentiality are expressed in the heterozygous condition.

The F_1 pairs chosen as parents for the F_2 were selected for good viability and a diversity of mating types. Two of the pairs were among the 8 giving evidence of genetic exchange; the third pair had undergone a reorganization, but cytogamy could not be excluded. The F_2 was derived from the following crosses: cross 1—between types II and V from the same member of an F_1 pair (the possibly cytogamous pair), cross 2—between types III and IV, also sister caryonides, and cross 3—between types I and VII from a single member of a third pair. Each of these crosses produced in either the F_2 or a subsequent generation some progeny of each of the 7 mating types. Since

TABLE 1

The distribution of mating types among the progeny of crosses within inbred strains of Tetrahymena pyriformis, variety 1

Parents	Mating types of progeny							
	I	II	III	IV	V	VI	VII	Total
<i>A. Family A</i>								
F ₁ II × VI	3	1	4	0	2	2	0	12
F ₂ I × III	4	6	1	0	10	7	0	28
F ₃ II × VI	9	6	9	0	0	3	0	27
F ₃ II × V	12	2	4	0	2	4	0	24
F ₄ III × VI	17	11	10	0	8	27	0	73
F ₄ I × III	9	9	15	0	2	17	0	52
Totals	54	35	43	0	24	60	0	216
<i>B. Family B</i>								
F ₁ IV × V	0	16	0	29	7	9	5	66
F ₁ IV × F ₂ VII	0	7	6	31	6	24	12	86
F ₂ II × V	0	10	0	9	1	1	5	26
F ₃ IV × VII	0	2	1	2	2	8	4	19
F ₃ IV × V	0	5	7	28	0	3	4	47
F ₄ IV × VI	0	50	21	154	7	19	32	283
Totals	0	90	35	253	23	64	62	527

the parents in each cross were derived from a *single* F₁ exconjugant, an exchange of mating type factors must have occurred in the initial cross, at least in these cases. For practical purposes we may assume that both parents (P₁) were homozygous and that the F₁ pairs used were heterozygous for factors differentiating the two families.

Two of the 45 F₂ pairs were excluded from further analysis as probable non-conjugants; all 4 caryonides were available, two of one parental mating type from one member and two of the other parental type from its mate. Six of the F₂ pairs failed to produce progeny viable at the time of maturity. Four of the F₂ pairs produced only clones of an abnormal kind, designated as "semi-amiconucleate". These clones contain large numbers of amiconucleate cells, which are visibly abnormal; the amiconucleates do not multiply indefinitely under our cultural conditions and the clones are perpetuated from the cells having micronuclei. The semi-amiconucleate condition is usually detected only after 40 to 50 fissions and is usually observed in all or none of the caryonides from a pair. Both the normal and the amiconucleate cells in such cultures can conjugate, but the viability of the progeny is extremely low. These clones were, therefore, eliminated from the analysis. Thus, of the 45 original pairs obtained, only 33 were analyzed fully, 11 from each of the 3 crosses.

Progeny tests on the F₂ pairs revealed only 3 obvious classes: *class 1*—lacking the ability to produce types IV and VII, *class 2*—producing all 7 types and *class 3*—lacking the ability to produce type I. The potentialities observed in the P₁, the F₁ and the F₂ are summarized in figure 1. In this figure the mating type frequencies are represented as sectors in a circle. The P₁ frequencies are derived from the accumu-

FIGURE 1.—Diagrammatic representation of crosses; mating type frequencies indicated as sectors in a circle. The P_1 frequencies are derived from the accumulated data for families A and B; the F_1 frequencies are derived directly from the F_1 ; the F_2 frequencies for the three classes are based on progeny tests in which sister caryonides from single F_2 pairs were crossed. The horizontal hatching designates the type found uniquely in family A; the vertical hatching designates the types found uniquely in family B.

lated data for families A and B. The F_1 frequencies are derived from the 20 F_1 pairs examined. The F_2 frequencies are the average frequencies observed for each class when crossed to sister caryonides; the outcross data are not considered. Class 1 in the F_2 resembles family A both in the kinds and the frequencies of mating types; class 3 closely resembles family B.

The results of progeny tests on individual F_2 pairs are summarized in table 2. The data are given only for the 3 critical mating types, but all the pertinent progeny for these types are included, whether produced in the F_2 , the F_3 , the F_4 or in outcrosses. The confidence limits shown are the 95% confidence limits of the observed frequencies, expressed in percentages. Considerable variation is found in the frequency of a type, even within a class. This is probably due to the fact that some F_2 pairs were analyzed almost exclusively by outcrosses, some almost exclusively by sister $\times$ sister crosses and some by both means. If an F_2 pair were heterozygous for a capacity, it should produce different frequencies of a type when crossed with itself and when crossed to a homozygous recessive clone. The data may also be slightly biased by the procedures involved in determining the sizes of the samples. Each F_2 was first studied by an examination of 20 to 30 progeny (caryonides). Later, another sample of similar size was obtained. If these small samples included relatively large numbers of the critical types no further progeny tests were made. If few or none of a particular type appeared, still further samples were added until a reasonably large total was available. Because of this selective increase in sample size, the frequencies of certain types in small samples may be unduly high. Finally, some bias may have resulted from the procedures discussed above in the section on Tests for Conjugation.

TABLE 2

The frequencies of the significant mating types produced in progeny tests of the F_2 . The first numbers in the pair designations refer to the three crosses (discussed in the text) from which the F_2 was derived

F_2 pairs	Mating type I			Mating type IV			Mating type VII		
	No.	Total	Conf. lim.**	No.	Total	Conf. lim.**	No.	Total	Conf. lim.**
<i>Class 1</i>									
1-1*	7	43	7-31	0	89	0-4	0	89	0-4
1-6	27	94	20-40	0	94	0-4	0	94	0-4
1-10*	9	77	6-21	0	110	0-3	0	110	0-3
2-9*	17	72	15-35	0	85	0-4	0	85	0-4
3-4*	7	28	11-45	0	63	0-6	0	63	0-6
3-5*	14	86	9-26	0	144	0-3	0	144	0-3
3-10*	8	61	6-14	0	88	0-4	0	88	0-4
3-12*	13	80	10-26	0	80	0-5	0	80	0-5
<i>Class 2</i>									
1-2*	6	98	2-13	16	94	10-26	6	94	2-13
1-3*	4	87	1-11	11	90	6-21	7	90	3-15
1-4	8	41	9-35	10	41	12-40	3	41	2-20
1-7	6	49	5-25	10	49	10-34	3	49	1-17
1-13*	5	81	2-14	7	78	4-18	3	78	1-11
1-14	11	65	9-28	19	65	19-42	7	65	4-21
1-15*	7	45	6-30	7	115	2-12	9	115	4-16
2-1*	8	54	7-27	12	83	8-24	4	83	1-12
2-3	11	74	8-26	11	74	8-25	7	74	4-19
2-6	8	67	5-22	10	67	7-25	2	67	<1-10
2-10	9	51	8-31	19	51	24-52	2	51	<1-13
2-11	13	80	9-26	18	80	14-33	4	80	1-12
2-13	14	95	8-23	8	95	4-16	3	95	1-9
2-14	3	73	1-12	27	73	25-49	9	73	6-22
2-15	7	71	4-19	13	71	10-30	9	71	6-23
3-1*	10	86	6-20	17	115	9-23	8	115	3-13
3-7	12	87	7-23	16	87	11-29	5	87	2-13
3-13	10	66	7-26	19	66	19-42	1	66	<1-8
3-15	10	49	10-34	19	49	26-54	2	49	<1-14
<i>Class 3</i>									
1-11*	0	74	0-5	11	36	16-47	5	36	5-30
2-4	0	80	0-5	38	80	35-58	8	80	4-19
2-7*	0	81	0-4	7	37	8-35	8	37	10-38
3-6	0	98	0-4	40	98	31-51	8	98	4-16
3-8	0	107	0-3	55	107	41-61	14	107	8-21
3-9	0	78	0-5	33	78	31-54	10	78	6-22

* F_2 pairs tested wholly or in part by outcrosses.

** 95% confidence limits expressed in percentages.

In spite of these difficulties in quantitative interpretation, certain general conclusions seem secure. (1.) Certain F_2 pairs (class 3) lack the ability to produce mating type I, while others (classes 1 and 2) produce this type in appreciable numbers. Little overlap is observed in the confidence limits for these two groups. (2.) Certain other F_2 pairs (class 1) lack the ability to produce type IV. Again, little overlap is observed

in the confidence limits for those pairs which did and those which did not produce this type. (3.) Certain F_2 pairs (class 1) produced no mating type VII, but—because type VII is a rare type—the confidence limits of the frequencies for pairs producing this type overlap considerably the limits for pairs failing to produce the type. Nevertheless, it is significant that every pair producing type VII also produced type IV and no pair failing to produce type VII produced any type IV. (4.) Every F_2 pair produced at least one of the three critical types. (5.) The frequencies of type I are generally highest among the progeny of pairs which produced none of types IV and VII. Conversely, the highest frequencies of types IV and VII are found among the progeny of pairs failing to produce type I.

Three interpretations for these data may be considered. The simplest interpretation would attribute the observations to the existence of a pair of alleles, $ml^{(I)-}$ and $ml^{(IV, VII)-}$, at the same locus. According to this scheme cells homozygous for the $ml^{(I)-}$ allele would be unable to produce mating type I, but could produce any of the other 6 known mating types; family B and class 3 of the F_2 would have this genotype. Cells homozygous for the other allele, $ml^{(IV, VII)-}$, would be unable to produce types IV and VII, but could produce any of the other 5 mating types; family A and class 1 of the F_2 would have this genotype. Finally, the heterozygote would be able to produce any of the 7 known types; the F_1 in the cross between families A and B and class 2 of the F_2 would have this genotype. The F_2 should consist of these three classes in a ratio of 1:1:2; since 33 F_2 pairs were examined, the expected ratios are 8.25:8.25:16.5. This is in satisfactory agreement with the observed ratio, 6:8:19.

According to a second interpretation genes would occur at two independent loci, one locus determining the ability to produce type I and the other determining the ability to produce types IV and VII. A dominant allele at a particular locus would permit the expression of a given type (or types) and the recessive allele would fail to permit that type. Family A would be homozygous for the allele permitting mating type I and also for the allele failing to permit types IV and VII; family B would have the reciprocal homozygous genotype. The F_1 would be heterozygous for both factors. The F_2 would consist of four phenotypic classes, the three observed plus a fourth—corresponding to the double recessive—which would lack the abilities to produce mating types I, IV and VII. The expected phenotypic ratio among the 33 F_2 pairs examined would be 6.2:6.2:18.6:2.1. The observed ratio, 6:8:19:0, does not differ significantly from this expectation.

The distinction between these two hypotheses involves the presence or absence of the F_2 class unable to produce types I, IV and VII. Since the F_2 data were not sufficiently numerous to exclude its existence, further crosses had to be undertaken. The rationale for these crosses was as follows. According to the two-factor hypothesis class 2 of the F_2 , which is capable of producing all 7 mating types, should consist of 4 genotypic classes. Only one of these, the double heterozygote, could produce the missing genotype through recombination. This double heterozygote should make up $\frac{4}{9}$ of the class 2 pairs and should have a probability of $\frac{1}{16}$ of producing the double recessive at sexual reorganization. Hence, the probability for a double recessive among the sexual progeny of this F_2 class is ($\frac{4}{9} \times \frac{1}{16}$) or about 0.028.

Similarly, class 3 of the F_2 , which is incapable of producing type I, should be com-

TABLE 3
Evidence against the existence of a double recessive genotype

Source	Probability for double recessive on two-factor hypothesis		Total examined	Number	
				Expected	Found
F ₂	0.062		33	2.1	0
	Unadjusted	Adjusted			
F ₃ —Class 1	0.17	0.43	7	2.9	0
Class 2	0.028	0.09	8	0.7	0
Class 3	0.17	0.85	3	2.5	0
Totals			51	8.2	0

* Adjusted for screening procedure described in text.

posed of two genotypic classes, one of which should comprise $\frac{2}{3}$ of the total and have the probability of $\frac{1}{4}$ of producing the double recessive. Therefore, the probability for a double recessive among the sexual progeny of class 3 is ($\frac{2}{3} \times \frac{1}{4}$) or about 0.17. Identical considerations hold for the progeny of class 1.

Although each of these probabilities is low, they may be greatly enhanced by a judicious selection of F₃ pairs for further analysis. For example, among the progeny of class 2, 70% of the pairs with two or more caryonides available showed one or more of the three critical types and could be excluded from consideration. The probability that one of the remaining 30% is a double recessive is thereby increased from 0.028 to about 0.09. Similar considerations hold for the progeny of the other two F₂ classes. 60% of the progeny of class 1 could be excluded, thus raising the probability for a double recessive among the remaining 40% from 0.17 to about 0.42. 80% of the progeny of class 3 could be excluded, thus raising the probability for a double recessive from 0.17 to about 0.85.

These considerations and the results of the analysis of selected F₃ pairs are summarized in table 3. The phenotypic class postulated on the two-factor hypothesis has not appeared under conditions under which it would be reasonably expected. It must be concluded, therefore, that the class does not exist, at least in the frequency demanded by the hypothesis in its simplest form. One reservation should be kept in mind, however. Present data are not sufficiently good to eliminate the possibility that the double recessive has not appeared because it has reduced viability. The hypothesis of two independent loci is, therefore, rejected primarily because it requires more assumptions than the hypothesis of alleles at a single locus.

A third general explanation for these results may also be considered, that two or even three loci on a single chromosome are responsible for the results observed. This hypothesis would be applicable, even if many more data were available, by assuming very close linkage of the various loci involved. In the absence of good screening techniques it will be difficult to render this explanation even improbable. If, on the other hand, progeny attributable to crossing-over eventually appear, this hypothesis will be strongly supported. At the present time, however, the simplest interpretation of the data is that involving alleles at a single locus.

TABLE 4
Evidence for the equivalence of class 1 of the F₂ and family A

Crosses	Mating types					
	I	II	III	V	VI	Total
Family A × Family A	54	35	43	24	60	216
Family A × Class 1	93	63	61	32	117	366
Class 1 × Class 1	58	42	37	21	69	227
Totals.....	205	140	141	77	246	809

$$\chi^2 = 2.4$$

$$\text{d.f.} = 8$$

$$P = 0.95-0.98$$

TABLE 5
Evidence for the equivalence of class 3 of the F₂ and family B

Crosses	Mating types						
	II	III	IV	V	VI	VII	Total
Family B × Family B	90	35	253	23	64	62	527
Family B × Class 3	18	14	73	4	26	9	144
Class 3 × Class 3	46	35	156	20	44	38	339
Totals.....	154	84	482	47	134	109	1010

$$\chi^2 = 12.7$$

$$\text{d.f.} = 10$$

$$P = 0.2-0.3$$

The single locus hypothesis receives some additional support from certain quantitative considerations. If class 1 is genetically equivalent to family A, crosses of class 1 × class 1, class 1 × family A and family A × family A should yield not only the same mating types among the progeny, but the same frequencies of these mating types. If other factors were involved, this agreement might not be expected. Although, as pointed out above, quantitative treatment of present data is not entirely justified because of possible frequency distortions, attempts to verify this expectation have been made (table 4). The results are in satisfactory agreement with the hypothesis. Similarly a genetic equivalence of class 3 and family B is suggested by the data in table 5.

DISCUSSION

The data presented in the previous section indicate that differences in mating type potentialities in two inbred strains of *Tetrahymena pyriformis* are dependent upon differences in a single chromosome. The strains designated as family A are homozygous for an allele $mt^{(IV, VII)-}$; this genotype permits the expression of mating types I, II, III, V and VI in characteristic frequencies. The strains designated as family B are homozygous for the allele $mt^{(I)-}$; this genotype permits the expression of mating types II, III, IV, V, VI and VII. Any of the 7 known types may appear in the hetero-

zygote. Although the available data are consistent with this interpretation, they do not rule out the possibility that the *ml* locus consists of two or more linked genes. Because screening techniques for recombinants have not been perfected, it is unlikely that this situation will be clarified soon. It should be possible, however, to find other genes and perhaps other mating types in collections from the wild. Particularly, it is to be anticipated that an 8th mating type will appear, since the similar but more extensively studied multiple mating systems in *Paramecium bursaria* contain 2, 4 or 8 mating types (see METZ 1954 for discussion and references). The information now available for the varieties of *T. pyriformis* (GRUCHY 1954) suggests a similar "numerological" system for this species. If several genes can be obtained for this system, they will be of great value in studying the mechanisms of mating type determination in the Ciliates.

Even the limited information now available on the mating type genes in Tetrahymena is worth considering in a discussion of Ciliate sexuality. According to METZ's (1954) interpretation of the peculiar geometric progression of numbers of mating types, systems of two mating types may be explained on the basis of the presence or absence of a single substance; systems of four mating types may be explained as due to the presence or absence of two substances independent of one another; and systems of eight mating types would require various combinations of three substances. This interpretation suggests that the elimination of one substance in an 8-type system will reduce it to a 4-type system, simultaneously removing four of the mating types. Since it seems reasonable to assume that genes would influence the production of these hypothetical substances, it also seems reasonable to believe that genes would preferentially affect four mating types at once. Yet the available data on the Tetrahymena genes lend no support to this notion. The gene *ml*^{(I)-} blocks the appearance of only mating type I (and perhaps VIII, if this type is subsequently found); the gene *ml*^{(IV, VII)-} blocks the appearance of only IV and VII (and perhaps VIII). It is of course possible that these genes are not concerned with the production of primary mating type substances but rather with other cellular conditions influencing which of the various substance-combinations will be expressed. Available information does not disprove METZ's hypothesis, but it offers it no support.

In the absence of information regarding the action of the mating type genes, their designation poses a problem. *A priori* the genes could be either "potentiating" or "inhibiting". Thus the *ml*^{(I)-} factor could either inhibit the development of type I or it could make possible the production of types IV and VII (and perhaps others). The negative designations were chosen largely because they were simpler; they should not be interpreted as indicating anything regarding the mode of action of the genes. In fact, it would seem odd that allelic genes controlling inhibitors are mutually recessive. Of only one thing can we be reasonably sure; since mating type differences within a genotype are determined by persistent macronuclear differences, these genes must influence in some way the course of macronuclear differentiation.

Perhaps the chief significance of this study at the present time is the demonstration for *T. pyriformis* of a classical genetic mechanism, operating as would be expected from the cytological observations. Therefore, an essential step has been accomplished in preparing this organism for fruitful studies in genetics and cellular differentiation.

SUMMARY

Evidence is presented for the first nuclear genes to be found in *Tetrahymena pyriformis*. These genes, designated as $mt^{(I)-}$ and $mt^{(IV, VII)-}$, behave as alleles and act in determining mating type potentialities. Cells homozygous for the allele $mt^{(I)-}$ lack the ability to produce mating type I but can produce the other six mating types now known for this variety. Cells homozygous for the other allele lack the ability to produce types IV and VII. Any of the seven known mating types may be produced by heterozygous cells.

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