MATING TYPE DETERMINATION IN VARIETY 8, *TETRAHYMENA PYRIFORMIS*¹

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TWO major kinds of mechanisms controlling the expression of hereditary mating type specificities have been described among the ciliates. In *Euplotes patella* (KIMBALL 1942; POWERS 1943; SONNEBORN 1947), *Euplotes vannus* (HECKMANN 1961) and *Paramecium bursaria* (JENNINGS 1942; SIEGEL and LARISON 1960; SIEGEL 1963), mating types are inherited as if uniquely controlled by simple Mendelian differences. In *Paramecium aurelia* (SONNEBORN 1947) and variety 1 of *Tetrahymena pyriformis* (NANNEY and CAUGHEY 1953; NANNEY, CAUGHEY and TEFANKJIAN 1955), by contrast, only an array of mating type potentialities is under genetic control. Which mating type is actually expressed by any given offspring depends not only on the set of potentialities it inherits, but also on the function of a mechanism which ultimately prevents the expression of all but one of the potentialities. The selection of different phenotypes in different lines with the same array of potentialities represents a case of cellular differentiation.

The ability to cross differentiated cells, so far restricted to the single-celled organisms, has made possible a further clarification of the process involved. In *P. aurelia*, SONNEBORN (see 1947 review) has demonstrated that the cellular organelle which controls the expression of mating types is the macronucleus (somatic or vegetative nucleus). The micronucleus (germinal or sexual nucleus) serves as the reservoir of the array of genetic potentialities for the macronucleus of the next sexual generation. Thus, the process which, at every generation, limits the number of expressed potentialities is accompanied by a (macro-) nuclear differentiation. Furthermore, a given differentiated state is inherited throughout the life cycle of the macronucleus, i.e. until the next sexual reorganization, when a new macronucleus is differentiated *de novo* from the germ line. Mechanisms which perpetuate phenotypic differences in the absence of genotypic differences, have been designated as "epigenetic" (NANNEY 1958a, b).

The object of the present study was to analyze genetically the mechanism of mating type determination in variety 8, *T. pyriformis*, and its relationship to the

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basic types previously described. It was found that the mechanism is very similar to that of *P. bursaria* and *Euplotes*, i.e. a genetic kind of mating type determination. This came rather as a surprise, considering that variety 1 of *T. pyriformis*, undoubtedly a close relative of variety 8, shows an epigenetic kind of mating type determination. Rare anomalies have been detected, however which suggest the operation of an epigenetic component.

MATERIALS AND METHODS

Variety 8 of *T. pyriformis* was described by GRUCHY (1955). Strains belonging to this variety have been collected in Minnesota, Michigan and Wyoming. Three clones from the Alpena, Michigan collection (Alp. 1, 2 and 17) were selected for this study. They represented the three mating types described for this species, designated by the roman numerals I, II and III.

The experimental techniques employed in culturing, crossing and typing clones in this study were identical to those described in variety 1 of *T. pyriformis* (NANNEY, CAUGHEY and TEFANKJIAN 1955). Conjugation is followed by a period of sexual immaturity which, in variety 8, lasts for about 150 cell divisions (about one month under the nearly maximal growth conditions used). The occurrence of such a period of sexual immaturity following the process of sexual reorganization provides an opportunity for the screening of nonconjugants, i.e. clones derived from conjugants which separated prior to nuclear exchange. The frequent occurrence of such clones, if not detected, can lead to serious errors of interpretation of the breeding results (see NANNEY 1963). Tests for sexual immaturity were routinely performed on exconjugant clones approximately between 12 and 25 cell divisions after conjugation. Nonconjugants, which appeared with an average frequency of less than 1 percent, were excluded from further consideration.

The events of conjugation in *T. pyriformis* are similar to those described for other ciliates (MAUPAS 1889; ELLIOTT and HAYES 1953; NANNEY 1953; RAY 1956). The macronuclei of the paired cells do not participate in conjugation and are eventually resorbed. The micronucleus in each conjugant undergoes two mitotic divisions to give rise to four nuclei. Of these, three degenerate and are resorbed; one survives and divides mitotically, giving rise to a migratory and stationary pronucleus in each member. The conjugants exchange migratory pronuclei and each migratory nucleus eventually fuses with the stationary pronucleus of the other conjugant. The fusion nucleus then divides twice. Since the second division spindles are longitudinally oriented, two nuclei become located at the anterior end and two at the posterior end. The former then differentiate morphologically into new macronuclei; of the latter, one disintegrates and the other remains as the new micronucleus. Morphological differentiation of the macronucleus is accompanied by a marked increase of its DNA content. At the first cell division after separation of the exconjugants, the two new macronuclei do not divide but are distributed to the daughter cells, while the new macronucleus divides mitotically. At succeeding cell divisions the micronucleus continues to divide mitotically and the macronucleus "pinches" amitotically into approximately equal halves.

The salient feature of the events of conjugation is the production of two exconjugants of identical genotype. This follows from the fact that, in each conjugant, the stationary and migratory pronuclei are mitotic sisters and, therefore, identical; hence the reciprocal exchange and the ensuing nuclear fusion result in two exconjugants with identical fusion nuclei, from which both the macro- and micro-nucleus are derived, as explained above.

RESULTS AND CONCLUSIONS

Caryonidal analysis: Three Alpena strains collected in the same sample of water and representing the three mating types described for variety 8 of *T. pyriformis* were selected for genetic analysis mainly on the basis of the excellent viability of their offspring. These were crossed in all possible combinations, and 30 pairs from each cross were isolated. Following conjugation, the two exconjugants from each pair were isolated; subsequently, when these divided, the two daughter cells from each exconjugant were likewise isolated. The four clones thus obtained from each pair are known as "caryonides". Fifty-three out of the original 90 pairs isolated were represented by the complete set of caryonides, and these were selected for further growth. These caryonides were serially transferred through 150 cell divisions (to get past the period of sexual immaturity) and then tested for mating type. With only one exception, the four caryonides derived from any given pair were of the same mating type. (The only exception was a set in which three of the caryonides were of the same mating type, while the fourth was a selfer, i.e. a clone whose members have the ability to mate with one another.)

Since conjugation yields exconjugants of identical genotype, this pattern of synclonal uniformity (i.e. the two exconjugants of a pair yielding identical progenies [SONNEBORN 1957]) is indeed what would be expected if the mating type were uniquely determined by the genotype. But the genotypic identity of the exconjugants is not the only mechanism which could account for this synclonal uniformity. If the expression of mating type were under cytoplasmic control and if cytoplasm was regularly exchanged between the two members of the pair during conjugation, then synclonal uniformity would also be the expected outcome. A more complete breeding analysis had to be carried out in order to distinguish between these two possibilities.

Breeding analysis: The phenotypic ratios obtained in the original crosses are given in Table 1, according to the mating type of the caryonidal set. One F_1 clone of each mating type derived from each of the three original crosses between the

TABLE 1
The mating types of the progeny of the original crosses

Parental clones	Offspring			Total typed
	I	II	III	
Alp. 17 (I) $\times$ Alp. 2 (II)	8	7	0	15
Alp. 17 (I) $\times$ Alp. 1 (III)	9	3	5	17
Alp. 2 (II) $\times$ Alp. 1 (III)	0	13	8	21

Alpena strains was selected for further study. The clones were designated by their mating types (in roman numerals) and a superscript (in arabic numerals) which indicates the mating type of their parent. Thus, for example, I¹² is the F₁ clone of mating type I selected from the original I × II cross. The seven clones thus obtained were crossed to one another in all 16 possible combinations, and one caryonide from each pair was isolated for 60 or 90 pairs for each cross. The results of these crosses (Table 2) can be arranged in blocks reflecting similar breeding performances. The results of chi-square tests for homogeneity of the crosses within each block are given; in all cases the departure from homogeneity was statistically insignificant.

The results of these crosses (Table 2) can be summarized as follows: the three clones of mating type II behave alike in all crosses; similarly the two type III clones show no significant differences in breeding performances. In contrast, the two mating type I clones differ, but their differences are detected with greater

TABLE 2
Mating types of the F₂ clones produced in crosses of selected F₁ clones

Parental clones†	Offspring				Total typed	Total isolated	Probability of chi-square	
	I	II	III	S*			Homogeneity	Genetic ratio
I ¹² × II ¹²	26	33	0	0	59	60	.3 - .2	
× II ¹³	28	30	0	0	58	60		
× II ²³	18	25	0	1	44	60		
	72	88	0	1	161	180		.3 - .2
I ¹³ × II ¹²	25	0	33	2	60	60	.98-.95	
× II ¹³	32	1	39	0	72	75		
× II ²³	25	0	31	3	59	60		
	82	1	103	5	191	195		.2 - .1
I ¹² × III ¹³	40	22	21	3	86	90	.9 - .8	
× III ²³	43	23	18	4	88	90		
	83	45	39	7	174	180		.6 - .5
I ¹³ × III ¹³	36	4	45	1	86	90	.4 - .3	
× III ²³	42	3	40	1	86	90		
	78	7	85	2	172	180		.6 - .5
III ¹³ × II ¹²	0	29	25	3	57	60	.8 - .7	
× II ¹³	0	25	32	0	57	60		
× II ²³	0	25	29	1	56	60		
III ²³ × II ¹²	0	32	27	1	60	60		
× II ¹³	0	32	28	0	60	60		
× II ²³	0	28	32	0	60	60		
	0	172	173	5	350	360		.98-.95

* Selfers.

† See text for nomenclature of parental clones.

efficiency in crosses with the type II clones than in those with the type III clones. The differences are quantitative in the latter case, and qualitative in the former. Excluding the selfers and the rare mating type II clones from I^{13} , the numbers found are in good agreement with simple 1:1 or 2:1:1 ratios, as shown by the last column of chi-square probabilities in Table 2.

The simplest interpretation of the results is that mating type differences in this species are controlled by multiple allelic differences at a single locus, designated as the *mt* (mating type) locus. A clone carrying at least one *mt^A* allele expresses mating type I; a clone carrying at least one *mt^B*, but no *mt^A*, allele expresses mating type III, while the homozygote for the *mt^C* allele expresses mating type II. Figure 1, showing the pedigree of the F_1 clones, provides no contradiction of this hypothesis.

This interpretation yields a number of predictions, the most crucial of which have been successfully tested.

(1) The type I progeny of the original $I \times III$ cross ($mt^A/mt^C \times mt^B/mt^C$), should fall into two different genotypic classes, mt^A/mt^B and mt^A/mt^C , in equal frequencies. These types should be distinguishable by testcrossing to mating type II clones (mt^C/mt^C). Specifically, the testcross of the former should yield type I and type III offspring (in equal frequencies), while that of the latter should yield type I and type II offspring (in equal frequencies). Eight type I clones were subjected to the progeny tests described, and 15 offspring were scored in each testcross. Three of the testcrosses fell into the first category, and the remaining five into the second.

(2) All the type I progeny from $mt^A/mt^B \times mt^C/mt^C$ crosses should be identical: mt^A/mt^C . Five clones from each of the three crosses of this type shown in Table 2 ($I^{13} \times II^{12}$, II^{13} and II^{23} , respectively) were tested, and all 15 behaved as predicted.

FIGURE 1.—Pedigree of selected F_1 clones.

(3) The type III progeny of the $mt^A/mt^B \times mt^B/mt^C$ crosses should fall into two equally frequent genotypic classes, mt^B/mt^C and mt^B/mt^B , the latter representing a genotype not encountered before. Fourteen such clones of mating type III were testcrossed; nine were shown to belong to the former class, and five behaved as predicted for the second class, i.e. when testcrossed with type II clones (mt^C/mt^C) they produced only type III offspring.

(4) A clone of genotype mt^A/mt^A , if one could be obtained, should yield progeny of only mating type I when testcrossed to a type II clone. Such a clone would not be expected to occur normally, since such a genotype can only arise from the mating of two parents carrying at least one mt^A allele, which would make both parents express the same mating type (I). But a clone behaving in the predicted manner was obtained by crossing a clone of genotype mt^A/mt^B with a mating type III clone derived from a selfer. (This unusual type III clone had been shown by an independent analysis, to be published elsewhere, to carry an mt^A allele in its germ line.)

Table 3 summarizes the data from all crosses, including those from the testcrosses described above, classified according to the inferred parental genotypes. The comparison of "total typed" with the "total isolated" columns shows that the viability of the offspring was good in all cases. The probability values are for chi-square tests performed on the appropriate expected ratios, excluding selfers and the rare, unexpected type II offspring noted before.

One of the crosses resulting in the greatest heterogeneity of offspring types ($I^{12} \times III^{23}$), originally carried out at 25°C, was repeated at 14° and 22°. No significant differences among the three crosses were obtained. Offspring ratios are thus insensitive to temperature over most of the range throughout which conjugation is possible in variety 8. This observation is in perfect agreement with the hypothesis of direct genetic control of mating type determination, and contrasts strikingly with the temperature sensitivity of mating type ratios in *P. aurelia* and in variety 1 of *T. pyriformis*, where the determination is "epigenetic".

Thus the Mendelian hypothesis presented above provides an adequate frame of reference for the major features of the mechanism of inheritance and determi-

TABLE 3
Summary of crosses, classified according to parental genotypes

Parental genotype	Offspring				Total typed	Total isolated	Expected ratio	Probability† of chi-square
	I	II	III	S*				
$mt^A/mt^A \times mt^C/mt^C$	83	1	0	1	85	90	1:0	
$mt^A/mt^B \times mt^B/mt^C$	102	7	114	4	227	240	1:1	.6 - .5
$mt^A/mt^B \times mt^C/mt^C$	98	1	113	5	217	225	1:1	.3 - .2
$mt^A/mt^C \times mt^B/mt^C$	160	99	86	12	357	360	2:1:1	.3 - .2
$mt^A/mt^C \times mt^C/mt^C$	439	507	0	6	952	1175	1:1	.05-.02
$mt^B/mt^B \times mt^C/mt^C$	0	0	151	2	153	165	1:0	
$mt^B/mt^C \times mt^C/mt^C$	0	349	370	13	732	850	1:1	.3 - .2

* Selfers.

† Selfers and rare type II offspring excluded from calculations.

nation of mating types in variety 8. Further explanation must be sought, however, for those rare discrepancies which have been noted.

DISCUSSION

Excluding the rare appearance of unexpected offspring of mating type II and selfers, the breeding results presented support strongly the hypothesis that mating type is ordinarily determined by alleles of a single mating type locus, transmitted in normal Mendelian fashion. This mode of mating type determination is very similar in its essential features to those analyzed in *Paramecium bursaria* (JENNINGS 1942; SIEGEL 1963) and in *Euplotes vannus* (HECKMANN 1961). In the former species, there are four mating types, normally determined by two alleles each at two unlinked loci. In the latter, five alleles of a single mating type locus have been shown to determine the five mating types described for that species. As in the present case, the alleles of *E. vannus* can be arranged in a linear series of increasing dominance.

Such a pattern of genetic control of mating type expression is in marked contrast to that found in *P. aurelia* (SONNEBORN 1947) and variety 1 of *Tetrahymena pyriformis* (NANNEY and CAUGHEY 1953). In these species the mating type locus determines only the mating type potentialities. Which of these potentialities is expressed by a given offspring is the result of a process of macronuclear differentiation (with or without cytoplasmic mediation depending on the species studied; see review by NANNEY 1957). The difference in pattern of mating type determination between varieties 1 and 8 of *T. pyriformis* is puzzling, considering the fact that, morphologically, they are similar enough to be included within the same taxonomic species. Since both varieties must be closely related, one would expect a homology between the *mt* loci, and hence an underlying similarity between such apparently diverse mechanisms of mating type determination. This is the context in which the findings in variety 8 will be discussed below.

The progressive series of dominance in which the three alleles of the mating type locus can be arranged poses an interesting problem concerning the mechanism that controls their expression. The following arguments suggest that the *mt* locus controls, at least, the *mechanism of expression* of the genes responsible, directly or indirectly, for the specificity of the mating reaction: (1) Almost certainly the differences among the three mating type "alleles" fall within the same cistron (as defined by BENZER 1955). Otherwise, one would expect unusual phenotypes for at least some of the heterozygous combinations, such as the expression of a novel mating type (different from that controlled by either of the two "alleles" of the heterozygote), or the simultaneous expression of two specificities. (2) If these are three alleles of the same cistron, then *at most one* of them could be a "null" allele (if one of them is, *mt^c* is the most likely candidate, since it is the most recessive). Hence at least two of the alleles must be "active". (3) Yet, the heterozygote for the other two "active" alleles expresses only one of them. Hence the action of the more dominant allele must *actively* inhibit the action of the more recessive one. This indicates that one function of the *mt* locus is to regu-

late the differential rate of synthesis of substances responsible for the mating type specificity, and hence the suggestion that it is part of the mechanism that selects that specificity to be expressed. In this context, a selfer must represent an occasional failure in the operation of this control system. These arguments suggest a basic similarity in the function of the *mt* locus in variety 8 to that in variety 1, whose regulatory function has been amply documented (NANNEY 1957; NANNEY and ALLEN 1959).

In the absence of any evidence to the contrary, the simplest hypothesis is that the alleles of the *mt* locus, in addition to their regulatory function, also code the specificity of the substances involved in the mating reaction; whether or not this is the case remains to be shown. On the other hand, a functional and structural differentiation between genes which code for specific sequences of amino acids of specific enzymes, and the genes which control the differential rate of synthesis of those enzymes, appears to be of frequent occurrence among the bacteria (JACOB and MONOD 1961; see also Volume 26 (1961) of the Cold Spring Harbor Symposia on Quantitative Biology). In view of these findings, the hypothesis that the primary information for the mating type specificities may be contained in loci *other* than the *mt* locus seems well worth entertaining. The fact that the difference between two alleles of the *mt* locus of variety 1, *T. pyriformis*, *simultaneously* result in a difference in the ability to express *three* different mating types (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1959) lends some support to this hypothesis.

The complete description of the mechanism of mating type determination will eventually have to include an explanation for the occasional and unexpected appearance of mating type II offspring. Progeny tests of six of these type II clones indicate that their genotype is mt^c/mt^c , i.e. no different from "ordinary" mating type II clones; the remaining three exceptions were not studied further. Certain abnormalities of conjugation already studied in other ciliates, such as selfing, autogamy or cytogamy, could account for them. The inability to follow the segregation of additional markers makes it impossible to rigorously exclude such cytogenetic abnormalities as the cause of the appearance of these specific exceptional clones. However, had such cytogenetic abnormalities occurred with comparable frequency in all crosses, their occurrence would have been detectable in other ways. For example, the frequency of occurrence of cytogamy which would be necessary to account for the exceptional type II clones would have led to a certain (predictable) frequency with which exconjugants from the same pair would differ in mating type. The absolute absence of such differences among a total of 166 pairs (in various experiments) for which both exconjugants were typed is highly significant (ORIAS 1959). An analogous type of analysis of the data shows that autogamy and selfing do not occur with the frequency necessary to account statistically for the high frequency of exceptional type II clones. Nor can their pattern of occurrence be explained by a simple assumption of recombination between two different and closely linked sites (ORIAS 1959: Appendix).

One hypothesis which cannot be easily excluded is that unusual type II clones arise as a result of "mutation" from mt^A (or mt^B) toward mt^c . If so, this heritable

change must occur at the unusually high rate of at least 10^{-3} per allele per cell division to account for a mutant frequency of the order of 5 percent. Heritable changes, resembling these in their high frequency, have also been detected in *P. bursaria* (SIEGEL 1963). The mechanism through which these arise is not yet understood, but their high frequency again leads one to consider the hypothesis that they are heritable changes in the functional state of genes regulating the expression of mating type specificities which are *not* due to random accidents of DNA replication. The existence of such mechanisms appear to require no substantially new assumptions beyond those properties already established for nucleic acids and proteins. DELBRÜCK (1949) and, more recently, MONOD and JACOB (1961) have pointed out that systems of mutual inhibition are capable of existing in either of two (or more) mutually exclusive, self-perpetuating states; whichever pathway gets established first will suppress the potential inhibitions that would result from the operation of the other (or others). In theory, any degree of reversibility is possible between these states, depending on the detailed properties of the components of the system. A system which appears to possess many of these characteristics is that which controls the alternative expression of two loci, which in turn determine the specificity of two flagellar antigens in *Salmonella* (phase variation, LEDERBERG and IINO 1956). Whether or not the similarities between these frequent heritable changes in *Tetrahymena* and the phenomenon of phase variation in *Salmonella* will turn out to be more than superficial is, at this point, a matter of speculation.

SUMMARY

The results of a crossbreeding analysis of wild strains of variety 8, *Tetrahymena pyriformis*, indicate that the three mating types described for this species are determined by three different alleles of a single mating type locus. The three alleles can be placed in a linear series of increasing dominance. The latter observation, in conjunction with that of the rare appearance of selfers and unexpected phenotypes among the offspring, has been taken as evidence that, as in variety 1 of *T. pyriformis*, the function of the *mt* locus is regulatory in nature, i.e. it controls the *selection* of which mating type specificity is to be expressed. It is not known whether the alleles of the *mt* locus also contain the information for the specificities themselves.

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