GENETIC FACTORS AFFECTING MATING TYPE FREQUENCIES IN VARIETY 1 OF *TETRAHYMENA PYRIFORMIS*¹

D. L. NANNEY

*Zoology Departments, University of Michigan, Ann Arbor, and University of Illinois, Urbana*²

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PREVIOUS studies on mating type determination in variety 1 of *Tetrahymena pyriformis* have shown that cells with identical genotypes may manifest different mating types, and that the "hereditary" expression of mating type depends upon a persistent nuclear alteration of an unknown kind (NANNEY and CAUGHEY 1953; NANNEY 1956, 1958). Other studies have shown that the differences in the array of mating types that can be expressed by two homozygous inbred strains derived from a common source are determined by differences at a single chromosomal locus, designated as the *mt* locus (NANNEY, CAUGHEY and TEFANKJIAN 1955). Strains of "Family A" are homozygous for an allele which permits the development of clones (caryonides) of mating types I, II, III, V and VI in characteristic frequencies; strains of "Family B" are homozygous for another allele and produce caryonides of types II, III, IV, V, VI or VII.

Since these two alleles were obtained from the progeny of only two cells, isolated from the same pond, the existence of many alleles in natural populations seemed a likely possibility. Since such alleles might prove of considerable value in the analysis of the "epigenetic" system regulating mating type expression, a search was begun for new mating type alleles in other strains. Some evidence for relevant genic diversity has been obtained, but these genes do not determine new mating types or new combinations of the previously known mating types; they merely affect the frequencies with which the same combinations of mating types arise among the caryonides of a strain.

MATERIALS AND METHODS

The number of strains of variety 1 from diverse natural sources is not large. WH-6 (I) and WH-14 (II) were collected from the same pond near Woods Hole, Massachusetts (ELLIOTT and GRUCHY 1952). WH-52 (III) was collected from a nearby pond at the same time (ELLIOTT and HAYES 1953). From extensive collections over much of the United States GRUCHY (1955) identified an additional variety 1 strain only from Vermont; UM-226 (VI) was chosen to represent this source. In the spring of 1955 the author made collections in Michigan and

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² Present address.

obtained variety 1 from Lake Huron near Alpena, Michigan, and from Indian Lake in Michigan's Upper Peninsula; strains Alp-4 (VI) and I.L.-12 (III) were chosen to represent these sources.

The procedures used in maintaining, crossing and testing cultures for mating types have been described previously (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1956) and will not be presented here.

RESULTS

The mating type potentialities of strains of diverse origin

The first objective of this study was to define as precisely as possible the capabilities of the various strains, in terms of the mating types which they could produce. One indication of capacity is the mating type expressed by the strain; thus WH-6 expresses type I; WH-14 expresses type II; WH-52 and I.L.-12 express type III; Alp-4 and UM-226 express type VI. Since earlier work had shown that a strain can express only one of its several potentialities, the phenotype of a clone provides only limited information concerning its capacities. Only breeding performance can provide information on the full range of potentialities.

The most direct breeding analysis would consist of crossing a single wild strain with itself, but this procedure is not possible in the absence of autogamy or selfing. Secondly, one might initiate a breeding analysis by crossing two clones of different mating type derived from the same source; but only in the case of WH-6 and WH-14 have diverse mating types been isolated from the same container. Hence, the only procedure available was to cross the wild strains to inbred strains of known capabilities. This procedure permits some additional information to be obtained, but does not allow a definition of the full range of potentialities. Thus, Family A cannot produce types IV or VII; the appearance of either of these types among the progeny of crosses between a wild strain and Family A would indicate that the wild strain carried the potentiality for the type. Similarly, if a new type appeared under these conditions its determinant must have come from the wild strain. Identical considerations apply to crosses with Family B. If type I or a new type appears in a cross to Family B, the determinant must have come from the wild strain. A combination of crosses with Families A and B yields information about the ability to produce types I, IV, VII and new types, but provides no evidence concerning capacities to produce types II, III, V or VI.

The analysis involved examination of both F_1 and F_2 generations. Earlier studies on variety 1 had shown that the ability to produce a mating type behaved as a dominant (or semidominant) trait, i.e., the F_1 produced in crosses of homozygous strains included some caryonides of each mating type possible in either parental line when sufficient clones were examined. If this observation could be generalized, the definition of mating type arrays in wild strains could be accomplished by ascertaining the mating types of the F_1 progeny. If, however, the ability to express some mating type should depend on a recessive gene, the F_1 strains would have to be crossed to yield an F_2 . New genotypes might be found among the F_2 which might potentiate new mating types. In no case, however,

was a different array of types found in the F_1 and F_2 when adequate samples of caryonides were examined; for this reason the two sets of data are not separated in Table 1.

Among the F_1 and F_2 caryonides examined no new mating types appeared. Either the wild strains possess no new potentialities or they express them too infrequently to be detected under the conditions employed. Some of the samples are small, primarily because of difficulties with viability, but further studies with the derived strains have also failed to yield new types. Potentialities for new types in these strains must, therefore, have a very low probability of expression if they occur at all.

Table 1 also suggests that of the six strains examined only WH-14 has the ability to produce types IV or VII, and that all strains can produce mating type I. Since WH-6 and WH-14 were the progenitors of Families A and B, and since only WH-14 can produce types IV and VII, WH-14 must have been the source of the Family B *mt* allele. WH-14 also produces mating type I, however, a capacity not associated with the Family B allele. WH-14 is, therefore, probably heterozygous, carrying one Family B *mt* allele and one similar to or identical with the Family A allele. WH-6 may be homozygous for the Family A allele.

The other four strains lack the Family B allele, i.e., they cannot produce types IV and VII. They may have alleles similar to the Family A allele, but this possibility requires further exploration.

TABLE 1

Screening tests for mating type potentialities by crosses of wild strains to inbred strains of known capabilities

A. Crosses to Family A							
Strain	Mating type	Generation	Mating types of progeny				Total
			Family A types	IV	VII	New	
WH-6	I	F_1	26	0	0	0	26
WH-14	II	F_1, F_2	28	9	1	0	38
WH-52	III	F_1	45	0	0	0	45
UM-226	VI	F_1, F_2	13	0	0	0	13
Alp-4	VI	F_1, F_2	73	0	0	0	73
I.L.-12	III	F_1	85	0	0	0	85
B. Crosses to Family B							
Strain	Mating type	Generation	Mating types of progeny				Total
			Family B types	I	New		
WH-6	I	(P_1)	..	(1)	.		(1)
WH-14	II	F_1	14	3	0		17
WH-52	III	F_1, F_2	77	6	0		80
UM-226	VI	F_1, F_2	88	17	0		105
Alp-4	VI	F_1, F_2	108	19	0		127
I.L.-12	III	F_1, F_2	90	19	0		109

The extraction of mating type determinants from wild strains

In the absence of better "indicator strains" the full array of mating type potentialities of a strain cannot be directly assessed. Procedures are available, however, for extracting genes from such strains and establishing them in homozygous condition. A wild strain capable of producing type I can be crossed to Family B. The F_1 progeny should contain a *mt* allele from Family B and also a *mt* allele from the wild strain. One quarter of the F_2 should lack the Family B allele entirely and should be homozygous for the corresponding gene in the wild strain. These homozygous segregants can be identified in progeny tests by their failure to produce types IV and VII. Their identification is facilitated by selecting F_2 segregants which lack types IV and VII among the four caryonides of a pair. This extraction procedure has been carried out for *mt* alleles from Alp-4 and UM-226. The performance of the derived strains is summarized in Tables 2 and 3.

The UM-226 derivative, identified by its failure to produce types IV and VII in the F_2 , failed to produce these types in further inbreeding. One may be confident, therefore, that the Family B allele had been replaced. These strains continued to produce types I, II, III, V and VI, the same array of types found in Family A. The different progenies show significant variations in mating type frequencies, suggestive of further segregation of factors related to mating type

TABLE 2

Mating type frequencies among caryonides produced in crosses of clones homozygous for the UM-226 mt allele

Parental mating type	Generation	Mating types of progeny							Total
		I	II	III	IV	V	VI	VII	
II $\times$ VI	F_3	15	9	1	0	5	9	0	39
I $\times$ III	F_4	4	9	3	0	0	19	0	34
I $\times$ III	F_5	8	12	4	0	2	23	0	49
V $\times$ VI	F_6	12	19	2	0	3	21	0	58
I $\times$ II	F_7	7	30	56	0	8	31	0	132

TABLE 3

Mating type frequencies among caryonides produced in crosses of clones homozygous for the Alp-4 mt allele

Parental mating type	Generation	Mating types of progeny							Total
		I	II	III	IV	V	VI	VII	
I $\times$ VI	F_3	37	11	11	0	0	13	0	72
I $\times$ II	F_3	35	7	5	0	0	17	0	64
I $\times$ III	F_4	29	4	4	0	1	16	0	54
I $\times$ II	F_4	12	1	3	0	0	3	0	19
I $\times$ VI	F_4	41	6	2	0	0	5	0	54
II $\times$ VI	F_4	36	7	2	0	0	6	0	51
I $\times$ II	F_5	22	6	4	0	0	10	0	42

expression, but the array of types appears to be fixed at the time homozygosis is established at the *mt* locus. Whether the *mt* allele from UM-226 is identical with that in Family A cannot be determined from the available information.

The derivatives from Alp-4 behaved much like those from UM-226. Types IV and VII did not reappear, and types I, II, III and VI were produced regularly. However, mating type V appeared in neither of the progeny tests (F_3) on segregants and appeared only once in a subsequent generation. The mating type frequencies in the Alp-4 lineages were less erratic than those observed in the UM-226 derivatives. Again the data are suggestive of an allele similar to the Family A allele in the array of mating types potentiated, but perhaps differing from it in the probabilities of expressing the different mating types. An evaluation of this possibility required further study.

The genetic analysis of the Alp-4 derivatives

Family A produces five mating types, I, II, III, V and VI; under "standard" conditions the frequencies of caryonides manifesting these types are, respectively, 25%, 17%, 17%, 9% and 30%. The derived Alp-4 strains produce the same types but in very different frequencies, i.e., 60%, 12%, 9%, 0.3% and 20%. Such differences could be due to differences at the *mt* locus, or they could reflect differences at one or more other genetic loci. This question is susceptible to direct attack.

One could cross the Alp-4 derivatives with Family A and examine the F_2 segregants to determine whether the differences in frequency segregate as if controlled by a single pair of genes. This approach offers difficulties, since the arrays of types produced by the two series are identical, and an unambiguous classification of *mt* genotypes in the F_2 would require very large numbers, even assuming that they are distinctive. Fortunately, a simpler procedure is possible. Previous studies (NANNEY, CAUGHEY and TEFANKJIAN 1955) have shown that Families A and B are not distinguished by factors other than the *mt* locus which sensibly affect mating type frequencies; the homozygous F_2 segregants derived from an intercross of Families A and B are indistinguishable from the original Family A or Family B homozygotes. Hence, a demonstration of differences in supplementary genetic factors between the derived Alp-4 strains and Family B is equivalent to a demonstration of differences between the Alp-4 strains and Family A; and most importantly, the F_2 *mt* classes are much easier to identify.

Hence, a strain of the derived Alp-4 series (F_4) was crossed to an inbred (F_7) representative of Family B. Three of the first generation pairs from this cross were chosen to produce the F_2 by crosses of sister caryonides. Sixty-four of the viable true-conjugant pairs were then subjected to study for mating type potentialities. The first task was to verify the control of the mating type array by a single locus.

Certain simplifying assumptions were introduced to minimize the work involved in assigning potentialities. In previous studies the ability to produce type I and types IV and VII behaved as alternatives. Some F_2 pairs failed to produce type

I, some failed to produce types IV and VII, and some produced all three types. Since IV and VII potentialities had not been found to dissociate in previous crosses, their inseparability in these crosses was assumed. Thus, if type IV appeared, the potentiality for type VII was considered to be present, and *vice versa*. The adoption of this assumption prevents the use of these data in estimating the frequency of separation of the components of a possibly compound locus. It makes possible on the other hand, the assignments of Family B alleles to the F_2 without the exhaustive tests sometimes required before the relatively rare type VII appears.

The second assumption was that types I and IV would appear in reasonably high frequencies or not at all. This assumption was also based on the previous studies which indicated that these types were either common (10 percent or more) or absent. The importance of the assumption lies in its use in classifying certain F_2 pairs without progeny tests, using only the mating types expressed in the four caryonides of the F_2 . Thus, if among the caryonides of a single pair both types I and IV, or types I and VII were observed, the pair was classified as a heterozygote. Fifteen of the 64 pairs were of this type.

If type I did not appear in the F_2 , or if neither IV nor VII appeared in the F_2 , further tests were required. Whenever possible these consisted of crosses of sister caryonides to yield an F_3 . In some cases, however, all the F_2 caryonides were alike in mating type and could not be crossed; in other pairs only one of the four caryonides survived; even in some cases where crosses between sister caryonides were possible, all or most of the progeny died. In most such instances backcrosses could be carried out. If neither type I nor types IV or VII appeared in the F_2 , backcrosses to both parents were required, but if type I was present only the backcross to the Alp-4 derivative was necessary; if either type IV or VII was present, only the cross to Family B was made.

Of the 64 pairs studied, 16 produced neither IV nor VII in progeny tests which usually involved from 20 to 60 caryonides; these all produced type I. Twelve failed to produce type I in samples of similar size, but did produce IV and, in most cases, VII. Thirty-four produced both I and IV or VII; these include the fifteen pairs classified without progeny tests. Two pairs were sterile both on inbreeding and in backcrosses; both of these pairs, however, included a type I caryonide. None of the 64 segregants was negative for I, IV and VII. The appearance of the "double negative" would be expected on a two-factor basis, and its absence—as in the crosses between Families A and B—supports the interpretation that the I and IV–VII determinants are allelic factors. Moreover, the 16:34:12 ratio in the F_2 is consistent with the 1:2:1 ratio expected on a single factor hypothesis. Hence, we may assume that one of the *mt* alleles had been extracted from Alp-4 and that it carried with it the potentiality for mating type I.

The question remains, however, whether the frequencies of the mating types, or even the potentialities for the other mating types, are determined in whole or in part by the *mt* locus. Conceivably the *mt* allele in Alp-4 is actually the same allele found in Family A, and the striking differences in mating type frequencies

are determined by genes at other loci. An analysis of the frequencies of the mating types produced in the progeny tests of the F_2 segregants permits a partial answer to these questions.

If Alp-4 and Family B differ in supplementary factors, they should segregate in the F_2 , in part at least independently of the *mt* locus. The recovered Alp-4 homozygotes should differ from the original inbred parents, and the recovered Family B homozygotes should differ from their inbred parents. Such differences should be manifested in mating type frequencies in progeny tests, both in backcrosses and to a greater extent in F_3 progeny tests. The pooled data are presented in Table 4. They demonstrate significant differences between the parental homozygotes and the corresponding F_2 homozygotes. Specifically, when the Family B mating type allele is combined with factors from Alp-4, the frequency of type IV increases and that of type VI declines. When the Alp-4 allele is combined with factors from Family B, the frequency of type I declines and the frequency of type VI rises. Less striking differences may occur with other mating types. Since these modifications in mating type frequencies were detected with a limited number of F_2 pairs, and since these pairs are probably not a random sample of F_2 segregants, little can be said about the magnitude of the differences between Alp-4 and Family B. The two series do, however, differ in factors other than the *mt* genes which affect mating type frequencies.

Some additional information regarding the supplementary factors might be obtained by analyzing the variations among the progeny tests from different F_2 pairs. Although the sample sizes are too small for detailed quantitative analysis, borderline values in significance tests for homogeneity have been found. The available data are not adequate for an estimation of the number of additional genes segregating.

The evidence for supplementary factors does not apply apparently to mating type V. The vanishingly small fraction of this type in both the original Alp-4 line and in the recovered *mt* homozygotes strongly suggests that the factor re-

TABLE 4

Comparison of mating type frequencies in crosses in which the parents have the same genes at the mt locus, but in which the parents may differ in other genes segregating in the cross between Family B and Alp-4 derivatives

Parents	Mating types of progeny							Total
	I	II	III	IV	V	VI	VII	
Inbred Family B	.000	.149	.093	.473	.045	.139	.100	1090
F_2 (B) Backcross	.000	.123	.123	.556	.035	.082	.071	171
F_2 (B) Intercross	.000	.179	.052	.553	.043	.069	.107	307
	Chi-square: 28.5		D.F.: 10		P: <.01			
Inbred Alp-4	.596	.119	.087	.000	.003	.198	.000	356
F_2 (Alp-4) Backcross	.454	.113	.088	.000	.005	.321	.000	218
F_2 (Alp-4) Intercross	.498	.145	.045	.000	.002	.312	.000	401
	Chi-square: 20.1		D.F.: 6		P: <.01			

sponsible is either closely linked to or equivalent to the Alp-4 *mt* allele. Hence, all the differences between Alp-4 and Family A may not be due to supplementary genetic factors; some could be due to differences at the *mt* locus itself. At least one must conclude that the Alp-4 chromosome is different from the Family A chromosome. This chromosome could also have significantly different effects on other mating types, but this has not been established.

DISCUSSION

The designation of genotypes

The observations just reported raise certain terminological problems. The two alleles at the *mt* locus previously recognized were given descriptive designations; the Family A allele was called $mt^{(IV,VII)-}$ and the Family B allele was called $mt^{(I)-}$, to indicate the types which were not produced when the alleles were homozygous. The allele derived from Alp-4 is identical with the Family A allele in the array of types which it permits, but it may differ in the probability of expression of those types. Fully descriptive allelic designations would require not only the mating type array, but also the frequencies of expression of the various mating types under standard conditions of residual genotype and environment. The information necessary for such designations is not yet available and the symbols would, in any case, be too cumbersome for manipulation. Since all the information about the effect of an allele cannot be put into its designation, a simpler but less informative designation will be employed in future work. The allele in Family A will be designated as mt^A ; that in Family B will be called mt^B . The derived Alp-4 strains will be designated as Family C and the allele which they carry will be called mt^C . Similarly, the derived UM-226 strains will be called Family D and their allele, mt^D .

This study suggests that a number of genes influence the frequencies with which mating types appear. Thus far, however, no evidence indicates that loci other than the *mt* locus determine the array of types which can be expressed. The significance of the genetic background must be recognized, even though the mode of action of the additional genes is not yet understood. For the present, the genetic background may be described as a series of "polygenic complexes", apparently fixed in Families A, B and C, but still labile in Family D. Since Families A and B seem to have equivalent backgrounds and arose from a common source, their complex may be called the Massachusetts-1 complex. Family C, although derived from an intercross between a Michigan and a Massachusetts strain, appears to have fixed a distinctive set of supplementary factors, which will be referred to as the Michigan-1 complex. If a true-breeding series can be established in Family D, the background will be called the Vermont-1 complex.

The data now available relating genotypes to the frequencies of mating types produced at conjugation at 26°C in variety 1 are summarized in Table 5. All the data for Family A are not included; in particular, the Family A clones produced after a long series of inbreeding generations (NANNEX 1957) have been excluded since they show slightly different mating type frequencies than the original

TABLE 5

Summary of data relating genotype and mating type frequencies within inbred strains of variety 1, Tetrahymena pyriformis

Genotypes of progeny	Mating type frequencies							Total
	I	II	III	IV	V	VI	VII	
<i>mt^A/mt^A</i> (Mass.1/Mass.1)	.253	.173	.174	.000	.095	.304	.000	809
<i>mt^A/mt^B</i> (Mass.1/Mass.1)	.116	.136	.146	.236	.070	.179	.121	399
<i>mt^B/mt^B</i> (Mass.1/Mass.1)	.000	.149	.093	.473	.045	.139	.100	1090
<i>mt^B/mt^C</i> (Mass.1/Mich.1)	.215	.116	.071	.260	.007	.296	.050	284
<i>mt^C/mt^C</i> (Mich.1/Mich.1)	.596	.119	.087	.000	.003	.198	.000	356
<i>mt^C/mt^A</i> (Mich.1/Mass.1)	.245	.208	.107	.000	.044	.396	.000	159

Family A clones. These differences may account for the slight heterogeneity noted when all Family A progenies are compared (NANNEY 1956) and probably reflect some residual genetic heterogeneity within the clones from which the highly inbred strains were derived.

(Since this manuscript was submitted for publication, information from two independent studies has suggested that a clerical error, possibly at the F_1 level, was involved in the derivation of Families C and D. Specifically, the esterases (ALLEN unpublished) and the serotypes (LOEFER unpublished) of Family C resemble those of UM-226, not those of Alp-4. Analyses of differences among the inbred strains would not be affected by such considerations, but in any future attempt to derive similar strains, the possible misidentification of origin of Families C and D should be noted.)

The number of mating types within a variety

Several apparently different patterns of mating type determination occur in the various species of Paramecium and Tetrahymena, but they all show peculiarities which are difficult to explain in conventional genetic terms, and they all agree in certain significant features. Before work was initiated on Tetrahymena, regularities were observed in the numbers of mating types found within varieties of Paramecium (See METZ 1954; SONNEBORN 1957). Only two mating type specificities are known to occur within a variety of *P. aurelia*, *P. multimicronucleatum* or *P. caudatum*, but multiple mating types are common in *P. bursaria*. One variety of *P. bursaria* contains only two types, but three varieties contain four types and one contains eight types. This regularity in numbers of mating types, corresponding to a geometric progression, 2^n , suggested an explanation for the basis of mating type specificity which involved complementary pairs of substances, one pair in a two-type system, two pairs in a four-type system and three pairs in an eight-type system. This explanation received support from other observations and is an important suggestion concerning the chemical basis for mating behavior in these ciliates. If the hypothesis of paired complementary substances has general applicability, then all systems of multiple mating types in these ciliates should contain numbers of mating types fitting the 2^n progression.

Such regularities in numbers of mating types have not been demonstrated, however, in *Tetrahymena*. Of the nine varieties now known, two have two mating types, three have three mating types, one each has five, seven, eight and nine mating types (GRUCHY 1955; ELLIOTT and HAYES 1955; HURST 1958; ORIAS 1958). The failure to demonstrate a geometrical progression is not yet critical. Collections of some varieties are very few in number and almost certainly do not contain representatives of all the possible mating types. Moreover, only three varieties have been subjected to intensive breeding analysis; such breeding tests may increase the known number of mating types in some varieties. Nevertheless, none of the three most intensively studied varieties has produced a number of types in agreement with the hypothesis.

The study just reported yields a pessimistic prognosis for obtaining critical evidence on this problem in *Tetrahymena*. Certainly all the types possible within a variety cannot be obtained from crossing any two strains within the variety. Even when a type can be produced by a particular cross, its frequency may be very low (e.g., type V in Family C), and that type may not be found with limited sample sizes. The failure to find new types in additional wild strains of variety 1 does not rule out the existence of undiscovered types and does not constitute evidence sufficient to reject a geometrical progression. However, the data available from *Tetrahymena* provide no support for the universality of such progressions.

The observations on *Tetrahymena* cannot be disregarded because of the taxonomic distance which separates *Tetrahymena* from *Paramecium*. The pattern of mating type determination in variety 1 of *T. pyriformis* (NANNEY 1956) is essentially identical to that in variety 1 of *P. aurelia* (SONNEBORN 1947, 1957), and the pattern in variety 2 of *T. pyriformis* (HURST 1958) corresponds in major respects to that in variety 1 of *P. bursaria* (JENNINGS 1942). The problem of mating type determination in this group of ciliates must be approached as a single problem.

The mechanism of mating type determination

This study extends the factual basis upon which an understanding of mating type determination must be built. The evidence for the primary significance of the *mt* locus in determining mating type arrays supplements that previously available in this variety (NANNEY, CAUGHEY and TEFANKJIAN 1955) and also that in variety 1 of *P. aurelia* (BUTZEL 1955). Unlike the possibly related systems controlling antigenic specificities in these ciliates (See BEALE 1954; SONNEBORN 1957) the factors potentiating alternative specificities are not distributed throughout the genome, but are localized on one particular chromosome and have not yet been separated, although in admittedly inadequate tests. This localization, which can scarcely be fortuitous, suggests that the persistent nuclear alterations involved in mating type determination may be alterations at, or near, these chromosomal sites. Certain other facts support the opinion that the nuclear alterations are, in fact, chromosomal alterations of some kind (NANNEY 1958). In any case, one need not postulate long routes of communication between genes controlling

mutually exclusive potentialities. As yet no judgment can be rendered concerning the complexity of the *mt* locus; each mating type might be represented by a genetically separable element or the mating type locus might be, in some functional sense, unitary. In the latter case, mating type determination might involve the establishment of some stable, but poorly understood "allelic configuration", the array of allelic states being dependent in some way on the allelic structure. For example, the DNA at the *mt* locus might determine the sequence of amino acids in a single protein molecule which is capable of several different folding configurations; materials or conditions in the vicinity of the locus might determine the patterns of folding and might be perpetuated through feed-back mechanisms.

The supplementary genetic factors may influence the establishment of different nuclear states by altering the intracellular environment. Effects similar to these genetic effects have been achieved by modifying the extracellular environment (SONNEBORN 1947; NANNEY unpublished) and the cytoplasmic environment in which the nuclei differentiate (SONNEBORN 1957; NANNEY 1954). The supplementary factors are of limited analytical use, however, until they can be defined in more specific terms and manipulated to show relationships between mating types.

SUMMARY

New mating types have been sought in breeding experiments with wild strains of variety 1 of *T. pyriformis* from four natural sources in Michigan, Vermont and Massachusetts. No mating types beyond the seven derived from the original strains of variety 1 (WH-6 and WH-14) have been found.

Alleles at the *mt* locus have been extracted from two of the wild strains and established in homozygous condition in inbred series. The new inbred series are designated as Families C and D. They are similar to Family A, previously described, in producing mating types I, II, III, V and VI. Families A, C and D differ, however, in the frequencies of caryonides of the various types produced at conjugation. The differences between Families A and C are shown to depend in part on the chromosome bearing the *mt* locus and in part on other genetic factors.

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