THE RELATIONSHIP BETWEEN THE MATING TYPE AND THE H SEROTYPE SYSTEMS IN TETRAHYMENA

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TWO systems of cellular differentiation in variety 1 of *Tetrahymena pyriformis* are remarkably similar (ALLEN and NANNEY 1958; NANNEY and DUBERT 1960). In both cases cells of the same clone, and presumably of the same genotype, may come to manifest highly distinctive cellular phenotypes, even when grown in identical environments. In both cases sublines of a clone gradually diverge and eventually become clearly and persistently distinguishable. The detailed kinetics of the "fixation" of pure types in the two systems, so far as they have been studied, are alike; after an initial period of equilibration the rate of fixation is about 0.011 per cell division. In one case (mating types) compelling evidence is available for a macronuclear basis for the variation; suggestive evidence for a macronuclear basis of serotype differences has been reported. Finally, single chromosomal loci appear to set the limits of variation in the differentiations, specifically, the *mt* locus for the mating types and the *H* locus for the serotypes.

The similar behavior of the two systems requires a similar explanation. The interpretation proposed and supported by previous studies is that diploid subnuclei become differentiated during the development of the macronucleus, that these differentiations become hereditary characteristics of the subnuclei, and that the fixation of pure sublines results from the random assortment of differentiated subnuclei at cell division, a process of "intranuclear genetic drift." The segregation model (SCHENSTED 1958) permits an estimate of the number of assorting units, about 45 in newly divided cells.

In spite of the similarities in the two systems, a detailed comparison demonstrates some significant differences between them. For example, the somatic variation in H serotypes appears to be restricted to heterozygotes, but in the mating types occurs in both homozygotes and heterozygotes. Moreover, only two stable types, corresponding to the specificities of the two alleles present, have been extracted from a single clone showing serotype variation; but in rare cases as many as four distinctive mating types have been extracted from a single clone (caryonide). These observations suggest that the details of subnuclear differentiation are different in the two cases, and that the similarities noted merely reflect a common mode of assortment of differentiated subnuclei. To further elucidate the relationship between these two systems, experiments were designed to answer two questions: (1) What is the genetic relationship between the *H* and the *mt* loci? and (2) Are serotype and mating type differentiations correlated in any way?

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MATERIALS AND METHODS

Two sets of strains were employed in this study, representatives of an inbred series designated as Family D1 (mt^D/mt^D ; H^c/H^c) and representatives of an inbred series designated as Family B (mt^B/mt^B ; H^D/H^D) (NANNEY and DUBERT 1960). Strains homozygous for the H^c allele, when grown at 25°C in an Aerobacter-Cerophyl medium, are uniformly immobilized by homologous antiserum; they are said to manifest the Hc phenotype. Similarly, strains homozygous for the H^D allele are immobilized under the same conditions by their homologous antiserum and are designated as Hd in phenotype (LOEFER and OWEN, in press). The two antisera show essentially no cross-reactions at the concentrations employed. The F_1 hybrids between the strains are initially immobilized by either antiserum, and their phenotype is indicated by the symbol Hcd. Following successive single cell transfers, however, the heterozygotes eventually produce many phenotypically pure sublines of type Hc and Hd. The initial phenotypes of the F_2 are Hc, Hcd and Hd, in approximately a 1:2:1 ratio.

The mating type genotypes are determined with much greater difficulty than the serotype genotypes. A particular allele potentiates the production of five or six different mating types in various frequencies, and only a fraction of the possible mating types usually appear among the progeny of a single pair. Moreover, the mating types of the progeny cannot be ascertained until after a period of sexual immaturity lasting 60 or more cell divisions. A total of four different alleles of the mt gene have been established in inbred lines (NANNEY 1959), but only three have thus far been involved in breeding analyses: mt^A , mt^B and mt^C . A secondary purpose of these experiments was to obtain segregation data for the mt^D allele; unfortunately, this allele is the most difficult of the four to identify.

The gene mt^B potentiates the production of the six mating types—II, III, IV, V, VI and VII. The mt^D allele, on the other hand, makes possible the production of types I, II, III, V and VI. The heterozygote may produce any of the seven types. The genotype of an F_2 segregant is ascertained by its manifestation—either directly in the F_2 or in progeny tests—of the critical types I, IV and VII. If type IV or VII is produced, at least one mt^B allele is present. Similarly, if type I appears, mt^D must have been present. If both I and IV or VII appear, the pair is classified as a heterozygote, mt^B/mt^D .

Establishing the *absence* of an allele is a more complicated procedure and requires progeny tests. Progeny tests of two kinds have been used. The most efficient tests are those by inbreeding from a single F_2 pair to produce an F_3 . This is possible only when two or more mating types are present in the F_2 and when the cross is fertile. If inbreeding cannot be carried out, backcrosses to the parental strains can often be employed, but this requires at least twice as many progeny to yield the same result. Backcrosses to the mt^B/mt^B parent yield information only regarding the ability of the tested clone to produce type I, i.e., they determine the presence or absence of the mt^D allele. Backcrosses to the mt^D/mt^D parent yield information only about the presence of the mt^B allele.

The reliability of progeny tests depends on two factors—the size of the sample

and the probability of expression of the critical mating types. The absence of mt^B can be established with relative ease because either type IV or VII indicates its presence; and type IV is a common type, being produced in nearly 50 percent of caryonides of homozygotes and about half this frequently in most heterozygotes. Hence, if neither IV nor VII appears in a progeny test of 20–30 caryonides, one may be reasonably certain that mt^B is absent from the tested zygote.

In contrast, the inbred mt^D/mt^D strains used in these crosses produce only about 14 percent of type I, and in heterozygotes with mt^B even fewer might be produced. Establishing the absence of the mt^D allele would require large progeny tests and was not considered necessary in these studies, particularly since the alternative allele could be scored readily. Although no systematic effort was made to determine the distribution of the mt^D allele among the F_2 pairs, direct evidence for its presence was sometimes obtained incidentally while ascertaining the mt^B distribution. A more efficient study would have employed mt^C (producing 60 percent type I in some homozygotes) or mt^A (producing about 25 percent type I in some homozygotes).

RESULTS AND ANALYSIS

Genotype distributions: A cross was made between strains of genotype H^C/H^C ; mt^D/mt^D and H^D/H^D ; mt^B/mt^B . Three F_1 pairs of the genotype H^C/H^D ; mt^B/mt^D were selected to produce the F_2 . No attempt was made to isolate the exconjugants and caryonides of the F_2 pairs, and all the progeny of a single pair were allowed to grow in a single culture. After routine tests for “nonconjugation,” the initial cultures were tested with both anti-Hc and anti-Hd serum and classified on the basis of their responses. Of the 122 pairs examined, 29 were Hc, 65 were Hcd and 28 were Hd. Since the phenotype of *initial* cultures is directly related to their genotype, the “H” genotypes of the F_2 pairs were immediately assigned, i.e., H^C/H^C , H^C/H^D , and H^D/H^D . The 1:2:1 ratio ($P = 0.7\text{--}0.8$) confirms previous studies on the segregation of these alleles.

The remaining, and more difficult, problem was to determine the *mt* genotypes. The first approach to this objective consisted of isolating nine individual cells from the first cultures produced by each of the 122 pairs, to continue these secondary lines through eight serial transfers, and to test the terminal mature cultures for mating type. If all the four independently differentiated caryonides are represented in the sample from a pair, most pairs should be assignable to a “partial genotype.” Unfortunately, considerable caryonidal death or differential growth must have occurred; many of the pairs were represented by nine sublines of a single mating type. Nevertheless, partial genotypes could be assigned to many of the F_2 pairs on the basis of the F_2 phenotypes. Those pairs which produced IV or VII, but no I, were of genotype $mt^B/?$; those which produced I, but no IV or VII, were of genotype $mt^D/?$; those producing both I and IV or VII were completely defined as mt^B/mt^D . Unfortunately, 38 of the 122 pairs produced neither I nor IV or VII. A preliminary test of correlation showed no relationship between the *H* genotypes and these partial *mt* genotypes.

To refine further the genotypic determinations, progeny tests (either F_3 or backcrosses to Family D1) were carried out on 16 of the 17 $mt^D/?$ pairs and on the 38 pairs for which no information was provided in the F_2 tests. One $mt^D/?$ pair could not be tested. The progeny tests consisted of the progeny of 30 pairs, usually with only one subline representing a pair. If neither type IV nor VII appeared in the F_2 or the progeny tests, the pair was classified as mt^D/m^D . If both I and IV or VII appeared under proper circumstances, the pair was classified as mt^B/m^D . No effort was made to resolve the $mt^B/?$ pairs. This procedure established three categories of F_2 pairs: mt^D/m^D , mt^B/m^D , and $mt^B/?$. The frequencies found were 22:28:72, respectively. Since the mt^D segregation was not completely analyzed, the only test of significance for this distribution is that on the partition of F_2 pairs with at least one mt^B allele against those with no mt^B allele. The observed distribution of 100:22 is only a fair fit to a 3:1 ratio ($P = 0.05-0.10$).

Even after this refinement of the mt genotypes, no preferential association of mt and H genotypes could be detected (Table 1). The three H genotypes were distributed in an approximate 1:2:1 ratio in each of the mt genotypes ($P = 0.9-0.95$). Even if the distributions are lumped by pooling the classes expected to be large on a linkage hypothesis (mt^D/m^D ; H^C/H^C : mt^B/m^D ; H^C/H^D ; and $mt^B/?$; H^D/H^D) against those with expectedly low values (the remaining genotypes), the P value is still 0.5-0.7. Thus, no evidence for linkage is provided, and any reasonably close linkage is rendered improbable. The mt and H loci are transmitted essentially independently.

Phenotype distributions: Even though the H and mt genotypes show no preferential association in the F_2 of a dihybrid cross, the possibility remains that a phenotypic association exists between the H serotype and the mating type systems. For example, one might find in lineages giving "vegetative assortment" of both mating types and serotypes that certain mating types would be associated with a particular serotype more often than expected by chance. This in turn would suggest that the conditions responsible for particular serotype and particular mating type determinations were related. The experiment reported in the previous section presented an opportunity to test this possibility.

TABLE 1

The distribution of H genotypes among partial mt genotypes

Partial <i>mt</i> genotypes	Serotype genotypes	Found	Expected
mt^D/m^D	H^C/H^C	7	5.5
	H^C/H^D	11	11
	H^D/H^D	4	5.5
mt^B/m^D	H^C/H^C	7	7
	H^C/H^D	15	14
	H^D/H^D	6	7
$mt^B/?$	H^C/H^C	15	18
	H^C/H^D	39	36
	H^D/H^D	18	18

The 65 F_2 pairs of genotype H^c/H^D assorted serotypes during the growth to maturity; they had also undergone mating type differentiation, and the survivors of the original nine isolations from each pair could be scored for both mating type and serotype. The first step in the analysis was to determine whether the serotype frequencies varied in the different mt genotypes. A chi-square test failed to reveal significant differences between serotype frequencies in the mt genotypes ($P = 0.05-0.10$), even though the mt^D/mt^D genotype had a somewhat higher frequency of Hd serotypes than the other two classes. Since no striking differences were found in serotype frequencies among the mt genotypes and since an analysis of distributions among individual mt genotypes was complicated by the large numbers of classes and the relatively small sizes of the samples, the total distributions of mating types and serotypes (Table 2) were tabulated and analyzed. A chi-square test for homogeneity on this distribution revealed no obvious preferential association of the H and mt phenotypes ($P = 0.5-0.7$).

Secondary interrelations: The two analyses just reported appear to eliminate any direct relationship between the H serotype and the mating type systems; i.e., the H and mt loci are not the same locus, indeed they are not even closely linked, and subnuclear differentiations in the two systems appear not to be correlated. One final question remains: do factors on the H chromosome appreciably influence the course of mating type differentiation? The converse of this question, concerning the influence of the mt chromosomes on serotype differentiation, has already been asked in connection with the analysis of phenotype distributions. In that case no clear decision was possible with the evidence available.

In previous studies of mating type determinants (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1959), strains differing in mating type *arrays* were found to differ at the mt locus; strains differing in mating type *frequencies* were found to differ, in part, in genetic factors elsewhere in the genome. Similar information concerning the differences between Families D1 and B was provided in

TABLE 2

The distribution of mating types and serotypes among the vegetative progeny of H^c/H^D heterozygotes

Mating types	Hc		Serotypes Hcd		Hd		Total
	Found	Exp.	Found	Exp.	Found	Exp.	
I	23	24.2	8	11.1	31	26.7	62
II	66	56.2	21	25.6	56	61.7	143
III	19	23.5	13	10.8	28	25.8	60
IV	53	60.0	27	27.4	73	67.0	153
V	7	6.3	4	2.9	5	6.9	16
VI	43	42.8	18	19.5	48	47.0	109
VII	9	8.3	8	3.8	4	9.1	21
Selfer	4	3.1	3	1.4	1	3.4	8
Total	224		102		246		572

TABLE 3

Mating type frequencies within Family D1, within derived clones of the same mt genotype, and in backcrosses to Family D1

Crosses	I	II	Mating types III	V	VI	Total
Family D1	F. 36	24	92	12	90	254
	E. 53	41	60.2	12.2	88	
Backcross	F. 24	17	24	9	44	118
(4 progeny tests)	E. 24.7	19	28	5.7	41	
F ₂	F. 38	44	36	13	55	186
(22 expanded pairs)	E. 38.9	30	44.2	9	64.5	
F ₃	F. 106	72	79	13	148	418
(17 progeny tests)	E. 87.4	67.4	99.4	20.1	145	

this study, and a test of association between the supplementary factors and the *H* locus is possible.

It is first of all possible to demonstrate that the derived *mt^D/mt^D* homozygotes differ ($P \leq 0.01$) from the original homozygotes (Family D1) in the frequencies of the mating types (Table 3). Generally speaking, the F₂ segregants produce more I's and II's and fewer III's than the original Family D1's. These differences are to be ascribed to the segregation of supplementary factors different for the two parental inbreds. Since the *mt^B/mt^B* homozygotes were not identified, a direct demonstration of the effect of the supplementary factors on this genotype is not possible.

Next one may ask whether mating type frequencies within *mt* genotypes are correlated with *H* genotypes. An affirmative answer would suggest a significant contribution of factors on the *H* chromosome toward the determination of mating type frequencies. For this analysis the F₂ data will be ignored; the nine derivatives of a single pair were often alike, suggesting derivation from a single caryonide, and produced "jackpots" of particular mating types which invalidate the usual tests for significance. The F₃ and backcross tests can, however, be used in several situations. Comparisons can be made, for example (Table 4), between the mating type frequencies in the F₃ progenies of *mt^D/mt^D* segregants with different *H* genotypes; in the F₃ progenies of *mt^B/mt^D* segregants; and in the backcross tests of the *mt^B/?* pairs. Other smaller scale tests were available and yielded similar results. In no case was evidence obtained for significant differences in mating type frequencies among the *H* genotypes. This analysis does not exclude the possibility that supplementary factors with slight effects occur on the *H* chromosome, but the determinants of mating type frequencies are not preferentially clustered on this chromosome.

TABLE 4

The relationship between H genotypes and mating type frequencies in progeny tests

Series	H genotype	I	II	III	Mating type IV	V	VI	VII	Total
<i>mt^D/mt^D</i>	<i>H^C/H^C</i>	32	22	26	0	4	38	0	122
<i>F₃</i>	<i>H^C/H^D</i>	62	34	38	0	7	83	0	224
	<i>H^D/H^D</i>	12	16	15	0	2	27	0	72
		$\chi^2 = 6.1$		d.f. = 7	P = 0.5 — 0.7				
<i>mt^B/mt^D</i>	<i>H^C/H^C</i>	11	17	18	34	3	23	2	108
<i>F₃</i>	<i>H^C/H^D</i>	22	33	28	46	4	47	12	192
	<i>H^D/H^D</i>	8	7	5	1	2	7	0	30
		$\chi^2 = 13.0$		d.f. = 8	P = 0.1 — 0.2				
<i>mt^B/?</i>	<i>H^C/H^C</i>	13	7	12	19	3	30	3	87
Backcross	<i>H^C/H^D</i>	25	34	21	27	5	60	0	176
	<i>H^D/H^D</i>	23	39	21	31	8	43	6	171
		$\chi^2 = 15.0$		d.f. = 10	P = 0.1 — 0.2				

SUMMARY AND CONCLUSIONS

This analysis demonstrates that the H serotype and mating type systems in variety 1 of *T. pyriformis*, in spite of their many similarities, are in fact distinct and independent systems of cellular differentiation. The *H* and *mt* loci are not the same loci; they show no evidence of linkage and are probably located on different chromosomes. Moreover, in genotypes permitting both kinds of differentiation, no correlation is found between the serotypes and mating types of terminal cultures. This observation would indicate that the events responsible for subnuclear differentiations of one kind are independent of the events responsible for the subnuclear differentiations of the other kind. Finally, no marked effects of *H* chromosomal elements on mating type frequencies or of *mt* chromosomal elements on H serotype frequencies have been demonstrated.

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