THE GENETICS OF THE H SEROTYPE SYSTEM IN VARIETY 1 OF *TETRAHYMENA PYRIFORMIS*

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PREVIOUS studies on serotypes of variety 1, *Tetrahymena pyriformis*, demonstrated that a single strain grown under different conditions could manifest any of several different serotypes even though any one cell normally expressed only one serotype at any one time (LOEFER, OWEN and CHRISTENSEN 1958; MARGOLIN, LOEFER and OWEN 1959; INOKI and MATSUSHIRO 1958). Strains of independent origin, moreover, manifest different arrays of serotypes and can often be distinguished by their responses to antisera. In particular, the available strains of variety 1 can now be classified into five distinct categories when grown at 25°C in an Aerobacter-Cerophyl medium (LOEFER and OWEN, in press). These serotypes are designated as the H serotypes, specifically, Ha, Hb, Hc, Hd and He. This report is concerned in part with the genetic basis for the strain differences in H antigens.

The H serotype system is also characterized by "vegetative instability" of the phenotypes in the progeny of certain crosses. Preliminary analysis of the intra-clonal variation within the serotype system suggests that a novel form of nuclear differentiation is involved.

MATERIALS AND METHODS

The Ha serotype was first identified in the wild strain WH-6 and is now established in an inbred strain designated as Family A. Hb was detected in strain WH-52; it has not been established in an inbred series, and the prospects for doing so are meager since WH-52 is now virtually sterile. Hc first appeared in an early generation of an inbred series called Family B; it is absent from the terminal members of the same series, but has been established in another inbred line called Family D1. Serotype Hd was originally detected in a wild strain, WH-14, and is now available in the inbred series designated as A1, B and D. Finally, He was discovered in strain UM-226 and is established in inbred series C. These serotype designations differ from those in the original publication (MARGOLIN, LOEFER

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and OWEN 1959) in several respects and reflect the newly acquired information concerning the genetic relationships and distribution of the serotypes.

The inbred "families" of variety 1 have been established on the basis of the *mt* allele which they carry (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1959). Those with *mt*^A are called "Family A"; those with *mt*^B are called Family B, etc. With the identification of H serotypes, it became apparent that some of the sublines of these families, derived after intercrossing, were distinguishable from the primary stem lines. Moreover, additional sublines have been, and are being, developed deliberately to combine serotype and mating type characteristics. Therefore, when two sublines bearing the same *mt* alleles are serologically distinguishable, they are given different designations. For example, inbred series A and A1 have the same *mt* alleles but differ in serotypes. Families D and D1 are similarly distinguished.

The breeding procedures and culture methods are identical with those routinely used in *Tetrahymena* genetic studies (NANNEY and CAUGHEY 1955; NANNEY, CAUGHEY and TEFANKJIAN 1955). The procedures for preparing antisera and for identifying the serotypes are presented elsewhere (LOEFER and OWEN, in press).

RESULTS

The analysis of the Hc-Hd strain differences: Serotype Hc was established in an inbred line by crossing the original strain manifesting the trait to an inbred Family D strain. This was followed by selective inbreeding from the progeny manifesting the Hc serotype. In the course of inbreeding, the mating type locus, *mt*^B, originally associated with the Hc serotype was replaced by the *mt* locus of Family D, *mt*^D. The new inbred series is called, therefore, Family D1. At various times in the breeding studies crosses were made back to the Family D strains and also to other strains showing the Hd serotype. Since the results of these various crosses are essentially identical, no effort is made to distinguish them in the following account.

Initially the exconjugants and caryonides of each pair were separated, allowed to give rise to small clones in depression cultures, and were then tested with both anti-Hc and anti-Hd sera. Since no marked differences could be detected among the progeny of single pairs, this practice was discontinued, and all the progeny of a pair were permitted to grow as a single culture. Within Family D1 all the progeny are Hc (72 pairs); within Family B all the progeny are Hd (98 pairs). All the offspring (76 pairs) of crosses between these two families are, when tested in the first depression cultures, immobilized or greatly retarded by both anti-Hc and anti-Hd serum. They are designated, therefore, as Hcd in phenotype.

The next step in the analysis was to cross the F₁ progeny and to ascertain the serotypes in the F₂. This could be accomplished only after the F₁ cultures were allowed to mature. The maturation was brought about by making single cell transfers from serial cultures on alternate days for 7-8 transfers. In most cases several different lines from each original pair were maintained to maturity. All the cultures were then tested for both mating type and serotype. These tests

yielded a surprising result. All the clones originally tested Hcd, but a large majority of the lines at maturity were either Hc or Hd. The remaining Hcd lines, showing responses to both antisera, were highly heterogeneous; in some, most of the cells gave slight reactions to anti-Hc and strong reactions to anti-Hd; others gave the reciprocal response or some response intermediate between the extremes. Even a single culture, tested with a single antiserum, showed some heterogeneity, in that the responses of individual cells were variable. The variability within a culture was, however, relatively much less obvious than that between cultures.

In spite of this complication, to which we will return later, the F_2 generation was produced and the progeny examined as before. Crosses were made between F_1 clones of various phenotypes to determine whether the phenotypic diversity was in any way significant in determining the outcome of the crosses. The results (Table 1) show that the inheritance of the H serotypes follows a simple Mendelian pattern; although the Hd phenotype is in slight excess, the P value for a 1:2:1 ratio is 0.1–0.2. The inbred Hc strains can be designated as H^c/H^c , the inbred Hd strains as H^d/H^d , and the F_1 clones, regardless of phenotype, as heterozygotes, H^c/H^d . It should be apparent, of course, that the genotype cannot be fully determined from the phenotype in mature cultures.

These results show that the phenotypes of the heterozygotes have no detectable influence on the phenotypes in the next generation. The genetic capacities, at least of the micronuclei, are not modified when a heterozygote undergoes the "differentiation" involved in changing its serotype expression. Two further kinds of crosses were undertaken to establish this point more firmly and to complete the evidence for a simple single gene control of antigenic potentialities. Crosses were made between heterozygotes in various phenotypic phases to homozygotes of various origin. The results (Table 2) are again consistent with the simple genetic model, and no evidence of phenotypic carry over is apparent.

Evidence for additional alleles at the H locus: Preliminary studies of crosses between strains manifesting other combinations of serotypes have been carried out. The most extensive of these studies involves the serotypes Ha and Hc. A representative of Family A showing serotype Ha was crossed with one from Family D1 showing serotype Hc. From a first cross, 41 pairs were isolated, and the first depression cultures were found to be affected by both anti-Ha and anti-Hc. Two lines were subsequently derived from each of the 41 pairs. In another

TABLE 1

The results of crosses between H^c/H^d heterozygotes manifesting various phenotypes

Parental serotypes	Serotypes of progeny			Total
	Hc	Hcd	Hd	
Hc $\times$ Hc	35	70	51	156
Hc $\times$ Hd	29	65	34	126
Hcd $\times$ Hd	9	27	17	53
Hd $\times$ Hd	24	53	24	100
Totals	95	215	125	435

TABLE 2

Results of crosses between homozygotes and H^C/H^D heterozygotes showing various phenotypes

Parental genotypes	Parental phenotypes	Serotypes of progeny			Total
		Hc	Hcd	Hd	
$H^C/H^C \times H^C/H^D$	Hc $\times$ Hc	32	33	0	65
	Hc $\times$ Hd	12	12	0	24
Totals		44	45	0	89
$H^C/H^D \times H^D/H^D$	Hc $\times$ Hd	0	16	15	31
	Hcd $\times$ Hd	0	16	13	29
	Hd $\times$ Hd	0	17	19	36
Totals		0	49	47	96

experiment, both exconjugant clones from 26 pairs were examined and found to react to both antisera. As in previous crosses, the initially double response disappeared in many of the sublines during the growth to maturity. After nine transfers, and about 120 cell divisions, only 21 of the 133 lines followed were still Hac. The ratios of the two pure types were eccentric; 103 of the pure lines were Ha and only nine were Hc.

Various crosses were made among the F₁ clones, and the serotypes of the F₂ clones were determined as before. The F₂ pairs presented certain complications in classification not encountered in the Hc-Hd study. Three major classes of pairs were again discerned, those in which both initial exconjugant clones were Ha, those in which both clones were Hc, and those in which the progeny manifested some degree of reaction to both anti-Ha and to anti-Hc. However, these latter pairs were heterogeneous; in some the two responses were clear-cut for both exconjugant clones; in some the reactions to anti-Hc were weak in one or both clones, and, in a few, one clone tested Hac and the other Ha. Nevertheless, the ratios of the three classes of F₂ pairs (Table 3) correspond to a 1:2:1 phenotypic ratio, again indicating a single gene difference between strains pure for the Ha and strains pure for the Hc serotypes. As in the previous study, no influence of the phenotype of the parent on the phenotypes of the progeny is apparent. One small-scale backcross of a heterozygote to the Hc parent yielded 14 type Hc progeny

TABLE 3

The results of crosses between H^A/H^C heterozygotes manifesting various phenotypes

Parental serotypes	Ha	Serotypes of progeny		Total
		Hac	Hd	
Ha $\times$ Ha	18	31	17	66
Ha $\times$ Hc	17	30	12	59
Hc $\times$ Hc	5	16	7	28
Totals	40	77	36	153

and 13 type Hac. It appears reasonable to conclude, therefore, that the Ha serotype is determined by an allele at the *H* locus, to be designated as H^A .

All the F_2 and backcross cultures in this study were maintained by single-cell isolations through about 120 cell divisions and again tested for serotypes. While the initially pure Ha and Hc cultures remained unchanged, most of the cultures having the Hac phenotype became pure Ha or pure Hc. The ratio of the two types was eccentric; as in the F_1 , the Ha type was 5–6 times as frequent as the Hc type.

Mention must also be made of certain aberrant F_2 clones not included in the above tabulations. One clone failed to show reactions to either anti-Ha or anti-Hc in repeated tests. Three showed typical Ha reactions except for a slight but significant retardation in anti-Hd. Four showed good reactions to anti-Hc, but also showed reactions to anti-Hb and anti-Hd. Several explanations for these aberrant clones are possible, but further tests are required to establish or to disprove them.

Finally, a few additional crosses have been made to further associate the various H serotypes. Twenty pairs from a cross between pure Ha and pure Hd clones were examined and found in the first depression to be Had. A total of 120 sublines were then followed to maturity and retested. All except eight had become Ha; the remaining sublines consisted of five type Hd and three type Had. In the same manner eight F_1 pairs from Hc $\times$ He were studied. In the first depressions, all showed strong reactions to anti-He but weak and variable reactions to anti-Hc. From the expanded lines (120), all eventually became pure He except four which became Hc.

Although incomplete, the available information is consistent with the hypothesis that the H serotypes are associated with multiple alleles at a single locus, the *H* locus. The H^A , H^C and H^D alleles are the most firmly established. An H^E allele is suggested, and a distinctive allele, H^B , may exist in the sterile wild strain, WH-52. All heterozygous combinations appear to be associated with a peculiar variability of phenotypic expression; some sublines "differentiate" to express the activity of one allele, and other sublines differentiate to express the other. Differences in the relative probabilities of expressing (or excluding) particular specificities are apparent. These may depend upon the combination of alleles, background genetic effects, or perhaps environmental variables. In the situations thus far tested, however, the phenotypic differentiation in one generation appears not to be significant in determining the differentiation in the next.

The mechanism of vegetative assortment of H serotypes: The crucial problem exposed in the previous sections is the vegetative change of phenotypes associated with heterozygosis at the *H* locus. An evaluation of alternative interpretations of the phenomenon requires a fuller description than has thus far been provided. All the studies to be reported refer specifically to the Hc and Hd serotypes, but preliminary observations on the other dual serotypes provide qualitative confirmation.

To provide quantitative data on the serotypic changes, 16 different caryonidal cultures manifesting the dual serotype Hcd were expanded to provide a total of 240 sublines. After two-days growth each of these sublines was reinitiated from a single cell, and the process was continued serially. After each reisolation the

"leftover" culture was tested by mixing one drop of the culture with anti-Hc serum and one drop with anti-Hd serum in concentrations sufficient to immobilize pure cultures in 30–60 minutes. The tests were read at about 45 minutes.

Considerable diversity of response was observed, and an arbitrary classification was devised. The range of responses constituted a continuous series from complete immobilization in anti-Hc and no reaction to anti-Hd (c responses) to the opposite extreme (d responses). The intermediate responses, immobilization or retardation of most of the tested population in both antisera, could be classified into three major categories. A cd response indicates a stronger response to anti-Hc than to anti-Hd; a cd response indicates essentially equal effects of the two sera; *cd* responses are predominant responses to anti-Hd. Certain difficulties are inherent in this classification. The *cd* responses are difficult to distinguish from pure c responses at one extreme and from *cd* responses at the other. Similarly, *cd* responses grade into both d and *cd* responses. The breadth or narrowness of the *cd* category depends on how fine a distinction one wishes to make. Nevertheless, the classification scheme serves a useful descriptive purpose. The classified data from the entire series are summarized in Table 4. The departures from a total of 240 clones classified reflect the clones which died or were so degenerate that classification was difficult. When clones died, they were replaced by isolations from sister cultures which were vigorous.

The general features of the time course of serotype change are clear. Cells isolated from cultures 18-fissions-old usually produce cultures giving intermediate responses. Upon further isolation, however, smaller and smaller fractions give intermediate responses. At first the pure cultures are mainly type Hd, but eventually pure cultures of type Hc are produced.

The individual subseries, derived from different caryonides, do not behave uniformly. A few produced type Hd exclusively and quickly; most produced a larger fraction of Hd than of Hc, but a few gave more Hc than Hd.

The array of serotypes at any one time does not provide all the information needed for an analysis. An examination of individual pedigrees is required to determine the stability of the phenotypes. All subcultures of a particular phenotype, regardless of their positions in the pedigrees, were grouped into a single

TABLE 4

The distribution of serotype phenotypes in subclones derived from single cells at various times since conjugation

Phenotypes	18	31	44	Fissions since conjugation			96	109	122
				57	70	83			
c	0	15	17	20	29	42	41	40	41
cd	1	9	25	42	35	18	14	18	18
cd	6	58	35	20	17	13	13	12	11
<i>cd</i>	4	33	40	33	19	17	24	20	9
d	5	123	120	126	139	144	147	149	157
Totals	16	238	227	231	239	234	239	239	236

class, and the phenotypes of their next serial derivatives were examined. The intermediate categories (*cd*; *cd*; and *cd*) each produced progeny with scattered phenotypes, centering about the phenotype of the original culture, but including less than 50 percent in that class. The intermediate phenotypes show, therefore, only moderate heritability. In contrast, the cultures at the extremes of the range, the pure *c* and pure *d* cultures, produce largely cultures of the same type, and only occasionally produce derived cultures of intermediate rank. Two *a priori* interpretations of these "reversions" are possible; they could represent imperfect classification of the cultures so that occasional *cd* cultures are classified as *c*, and some *cd* cultures are classified as *d*. In this case their production of intermediate phenotypes in derived cultures would not represent true instability but only the imperfections of the techniques. The occasional misclassification of clones can scarcely be doubted. The more crucial question is whether some "reversions" are real, i.e., whether clones which have for a period of time produced no antigen of a particular kind can reinitiate its production. In this latter case one might expect that any pure culture regardless of the time since it became pure would have a constant probability of producing intermediate derivatives. If, the reversions are artifacts, the more times a culture had been tested, the smaller should be the possibility that it is erroneously classified and the smaller should be the probability that it will yield intermediate derivatives. To test this point, all pure cultures were classified according to the number of times in sequence they had tested pure. This gives an array of "pure" cultures ranging from those which had been so classified for the first time through those which had been classified pure for as many as ten successive transfers. The behavior of the next serial isolation in each case was noted (Table 5). Clones classified as pure for the first time revert in over 20 percent of the cases; those classified pure for the second time yield intermediate progeny in less than ten percent. Less than one percent revert after five pure classifications, and no reversions were found after six or more pure classifications. These results are consistent with the hypothesis that the reversions are artifacts of the techniques, reflecting the limitations of the immobilization tests. The possibility of true reversions still exists, but thus far no evidence for them has been obtained. If they occur at all, they must occur at a very low rate, at least after a period of "pure" growth. These observations also provide an opera-

TABLE 5

The phenotypes of the next isolation cultures in lineages which had tested "pure" for different numbers of transfers

No. of previous pure cultures	Pure	Phenotypes of derived cultures	
		Intermediate	Percent intermediate
1	205	56	21.5
2	225	23	8.9
3	226	9	3.8
4	183	6	3.2
5	187	1	0.5
6 or more	505	0	0.0

tional definition for fixation: clones which have tested pure for six successive serial cultures are certainly pure; those which have tested pure for even three or four transfers have a very high probability of being pure.

This analysis permits a study of the kinetics of the fixation process, even though certain problems remain. A clone which has been classified as pure in six or more serial cultures is certainly pure in the terminal culture, but the precise time in the sequence that it became pure cannot be determined with precision. In some cases it must have been pure the first time it so tested; in other cases it must not have become pure until after one or more further transfers. If all fixations are assumed to have occurred at the initiation of the first pure culture, fixations are actually scored in some instances prior to their occurrence, and the absolute time axis in a fixation curve is slightly distorted. If, however, one is primarily interested in absolute rates of fixation, this distortion is of little consequence so long as the fraction stabilizing is small. In any case, adjustments can be made when they seem to be indicated.

For the following tabulations R_t (Rate of fixation per transfer) is defined as the fraction of cells isolated at any time from cultures of intermediate phenotype which yield persistently pure cultures in further transfers (in most cases six or more transfers, but occasionally only three-five when a culture was lost before completing the sequence).

R_t has two components, R_{tc} and R_{td} , representing the rates of fixation to Hc and Hd, respectively. R_t is found (Table 6) to remain approximately constant throughout the interval investigated at a value of 0.17 ± 0.02 . The components of R_t vary, however, with R_{td} starting high and gradually becoming smaller and R_{tc} beginning at a very low figure and rising gradually until it is equal to R_{td} . No tests of partial rates beyond the convergence point have been made; conceivably, the rates cross, but this is considered unlikely. The rate per transfer, R_t , can be converted into a rate per fission, R_f , by the simple expedient of dividing the rate per transfer by the number of cell divisions occurring before transfer. Under the conditions employed in these experiments (transfers on alternate days), the number of cell divisions between transfers is about 13, and R_f has a value of approximately 0.013. This is a significant figure to which we will return subsequently.

A very similar pattern of vegetative assortment of phenotypes has already been studied in these strains (ALLEN and NANNEY 1958) during examination of the mating type system. Since the two systems appear so similar, it is important to

TABLE 6

The relationships between rates of fixation of types and fissions since conjugation

Rates	18	Fissions since conjugation			Total
		30-56	69-94	107-133	
R_{tc}	.00	.032	.040	.105	44/935
R_{td}	.19	.137	.107	.088	115/935
R_t	.19	.169	.147	.193	159/935

determine whether they are indeed quantitatively alike. The rate of mating type fixation per fission (R_f) had been found to be about 0.0113, slightly lower than the R_f determined for the serotype system. These figures are not, however, directly comparable since the conditions of the experiments were slightly different. In the mating type studies the transfers were made daily to prevent the selfing which occurs in lineages unstable for mating type and to avoid the slight starvation encountered with alternate day transfers. Severe starvation had been found to increase the rate of fixation in some clones. To provide more nearly comparable data an additional experiment was designed, identical to the previous one in general procedures, but involving daily transfers of single cells. In this experiment 39 fixations were scored for 433 opportunities, yielding an R_f of 0.090 ± 0.014 . Since the cells undergo about eight fissions per day under these conditions the R_f is about 0.0112 ± 0.0018 , indistinguishable from the R_f of 0.0113 ± 0.0004 (calculated from Table 1, ALLEN and NANNY 1958) in the mating type system.

This experiment was also designed to provide information on the behavior of sister caryonides from a single pair. Each caryonide in the previous experiment was taken from a different pair, and one could not determine whether the differences in frequencies of the pure types at the end of experiment were due to differences between pairs or could arise within a single pair. The latter experiment included all four caryonides from three different pairs. The distributions of serotypes in the terminal cultures from different caryonides are given in Table 7. Although all caryonides showed evidence for some Hc and some Hd antigen, the relative amounts in the different caryonides were strikingly different. Caryonide 1a, for example, was predominantly type Hc in the terminal cultures while caryonide 1b, a sister caryonide from the same pair, produced more pure Hd lines than pure Hc lines. Similar observations hold for caryonides 3a and 3b. These differences cannot reflect differences in initial genetic constitutions and must

TABLE 7

The distribution of phenotypes among lineages derived from related caryonides

Caryonide	c	Phenotypes of sublines				Total
		cd	cd	cd	d	
1a	20	7	0	0	1	28
1b	4	10	2	1	13	30
1c	9	5	1	1	7	23
1d	7	18	4	1	0	30
2a	18	6	2	0	1	27
2b	12	5	2	0	2	21
2c	18	4	1	2	2	27
2d	7	12	7	0	3	29
3a	3	9	0	1	15	28
3b	22	6	1	1	0	30
3c	24	1	2	1	2	30
3d	12	6	0	1	7	26

reflect differences which arise subsequent to the establishment of the fertilization nucleus. Such a conclusion does not eliminate the possibility that initial genetic differences may influence the probabilities for different kinds of "differentiation." Indeed, it should be noted that most of the first caryonides studied produced predominately Hd lines in the terminal cultures, while the second cross produced predominantly Hc pure lines. The factors, genetic and environmental, significant for these differences will have to be explored systematically.

DISCUSSION

The mating type and serotype systems in variety 1: The interpretation of the results relies heavily upon earlier studies of intraclonal variation in ciliates, particularly on serotype determination in *Paramecium* (BEALE 1957; SONNEBORN 1957) and mating type determination in *Tetrahymena* (NANNEY 1958). It is necessary, therefore, to point out the essential features of these earlier analyses. The most direct parallel is between the mating type and the H serotype systems in variety 1 of *T. pyriformis*.

All the available inbred strains of variety 1, although essentially homozygous, continue in each generation to produce an array of mating types. The arrays in the different "families" are, however, distinctive, either in regard to the types which are expressed or the frequencies in which the types are produced (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1959). Genetic analysis of strain differences locates the major determinants of mating type specificities at a single chromosomal locus, designated as the *mt* locus.

The manner in which diverse mating types are distributed among the progeny of a single pair are instructive in defining the physical basis of the mating type differences. As SONNEBORN demonstrated long ago in *Paramecium* (SONNEBORN 1937, 1947, 1957), mating type diversities assort in *Tetrahymena* primarily at the time new macronuclei are assorted following conjugation. The distribution of mating types among the progeny of a pair demonstrates that the mating type differences among cells of identical genotype are localized within the macronucleus, and mating type determination must be viewed as a process of nuclear differentiation (NANNEY 1956, 1958).

Further insight into the nature and localization of the macronuclear differentiation came from an analysis of clones (caryonides) which were unstable in their mating types and produced pure derivatives during cellular proliferation (NANNEY and CAUGHEY 1955; ALLEN and NANNEY 1958). Most unstable lineages are capable of producing two pure mating types, but any combination of two mating types may be produced in different unstable clones. Regardless of the types produced, the kinetics of the fixation process appear identical. Specifically, after an initial period of variability, all clones begin to produce pure types at a rate calculated to be 0.0113/cell division. Furthermore, the components of this rate (the rates to the two different mating types being produced) are often initially very unequal but gradually approach equality in a time course experiment.

To explain these observations, a particulate segregation model was developed (SCHENSTED 1958), and new experiments were designed to test its applicability. Thus far all the data are consistent with the following hypotheses: (1) the macronucleus consists of a population of diploid subnuclei; (2) the subnuclei are capable of differentiating to yield a heterogeneous macronucleus; (3) once differentiated the subnuclei breed true and are assorted approximately at random to the daughter nuclei produced at fission. One significant property of the model is the relationship between the number of assorting subnuclei (N) and the equilibrated fixation rate (R_t), shown to be $R_t = \frac{1}{2N-1}$. The number of subnuclei in a newly divided cell is calculated to be about 45.

The studies on the H serotype system show remarkable similarities to those on the mating type system. Again the specificities for a particular kind of trait are associated with a particular genetic locus, in this case the *H* locus. Again some mechanism of mutual exclusion prevents the persistent expression of all specificities simultaneously. The differences in the behavior of sister caryonides suggests, as for the mating type system, that the critical differentiations are macronuclear, but further studies are required to establish this point with certainty. In both cases, pure lines of cells are produced during cellular proliferation, and these maintain their phenotypes during further cell division. Most significantly, the rates of production of pure lineages in the two systems are apparently the same, about 0.0113/cell division, and the same kind of covariation of partial rates of fixation is observed. Finally, in neither case do the differentiations occurring in one sexual generation influence the differentiations in the next generation.

In the face of these parallels, one can scarcely doubt that a similar mechanism underlies the two kinds of differentiation. If the vegetative assortment of mating types is to be explained by the assortment of differentiated diploid subnuclei, then the vegetative assortment of serotypes must also be explained in this way. The simplest interpretation of the serotype system involves the following postulates: (1) that the macronucleus consists of a population of diploid subnuclei, (2) that the subnuclei become differentiated by the suppression of one of the pair of alleles in each subnucleus; (3) that once established, the differentiations are perpetuated indefinitely in further subnuclear replications, and (4) that pure lines are produced by the random assortment of differentiated subnuclei. The interallelic suppression may be viewed as kind of "persistent indeterminate dominance."

This interpretation is predicated on a particular view concerning the organization of the ciliate macronucleus, specifically the idea that the macronucleus consists of many discrete diploid subnuclei. Alternative views are that the subnuclei are, or can become, haploid, or even that subnuclei are nonexistent and the macronucleus is simply a disorganized bag of individual chromosomes. If diploid genomes are disrupted and an assortment of chromosomes or genomes occurs during cellular division, one would expect that any heterozygote could be resolved into its components by continued cellular division. The behavior of the serotypes in *Tetrahymena* would then simply reflect a process of chromosomal segregation common to all macronuclear genes. This explanation cannot be ruled out directly

for the serotypes, but it is inapplicable to the mating type system. Somatic assortment occurs for mating types in nearly all newly constituted clones, even after ten generations of inbreeding by crosses of sister caryonides—at a time when heterozygosis is extremely improbable. Moreover, the production of three or four different mating types from some caryonides could not be explained on the basis of a single pair of segregating alleles.

Unfortunately, a direct test for segregation of other genes in the macronucleus of *Tetrahymena* is not yet possible, and most of the arguments for intact diploid genomes are indirect and fail to be compelling. It is necessary, therefore, to rely on the studies on the one ciliate which has been examined intensively on this point. Exhaustive attempts to demonstrate macronuclear segregation in *Paramecium aurelia* (SONNEBORN, SCHNELLER and CRAIG 1956), utilizing a variety of different genes, have failed to reveal a single case of genetic assortment. SONNEBORN did observe certain serotypic variants comparable in some ways to those reported here, but in no case was the ability to produce a particular antigen lost completely or irrevocably. Unless the macronucleus in *Paramecium* is organized on a different plan from that in *Tetrahymena*, we must conclude that diploid genomes are usually maintained intact, and that the phenotypic variants observed in *Tetrahymena* reflect nuclear differentiation rather than genetic recombination.

One further word should be said concerning the relationship between the serotype and mating type systems in variety 1. The many similarities might suggest that they are indeed the same systems assayed in different ways. This contention can be disproved by a detailed comparison, but more compelling information is being developed. Preliminary studies (NANNEY, unpublished) indicate that the *H* and the *mt* loci are unlinked and that differentiations in one system are independent of those in the other system. The parallels are not, therefore, based on a single common mechanism but are based on the operations of similar but separate mechanisms.

The serotype systems in Tetrahymena and Paramecium: The serotype system in *Tetrahymena* shows many similarities to the serotype system in *Paramecium* (BEALE 1954, 1957; SONNEBORN 1957) but also some significant differences. In *Paramecium* a single clone has a wide variety of antigenic potentialities but normally expresses only one at a time. The antigens which are expressed can often be called forth by particular modifications in the environment, but intra-clonal variants may persist for long periods of time in the same environment. Genetic analyses of antigenic potentialities show that a large number of independent genes are involved. Serotype differentiation may be viewed, therefore, as a special kind of "conditional epistasis"; the expression of a gene at one locus ordinarily prevents the expression of genes at other loci. On the other hand, in *Paramecium* the expression of a gene does not usually prevent the expression of an allelic gene; indeed, with a few conspicuous exceptions (SONNEBORN, SCHNELLER and CRAIG 1956; FINGER 1957), the two alleles at a given locus are expressed simultaneously, no matter how diverse their products may appear serologically. Finally, crosses between strains of identical genotype manifesting

different serotypes show that the cytoplasm plays an important role in maintaining serotypic "states."

In *Tetrahymena* (LOEFER, OWEN and CHRISTENSEN 1958; MARGOLIN, LOEFER and OWEN 1959; INOKI and MATSUSHIRO 1958), many of the same observations are available. Again a single clone can differentiate into a variety of serotypes, but a particular cell normally expresses only one antigen at a time. Differentiated subclones can be maintained for a period of time in the same environment. Thus far the genetic basis for only the "H" serotypes has been examined; whether the genes for the other serotypes are linked or are widely distributed is not yet known. Within the H system at least, the interallelic relations appear distinct from those usually encountered in *Paramecium*; in *Tetrahymena* the exclusion of diverse serotypes seems to hold not only for nonallelic genes but even for allelic genes. Whether allelic exclusion operates at other loci has not yet been determined, but it appears to operate in all allelic combinations for the *H* locus.

The available information, though meager, suggests that the mechanisms of interlocus exclusion and interallelic exclusion are distinct. The interallelic exclusion appears to be a strictly nuclear phenomenon; no evidence for a cytoplasmic carry-over from one generation to the next has been found. In contrast, the one breeding study involving presumably interlocus exclusion (INOKI and MATSUSHIRO 1958) suggests that, as in *Paramecium*, the cytoplasm can determine the phenotype of a new cell following conjugation. This study was, however, a small scale study and certain necessary precautions against "nonconjugation" were not reported. Further work will be necessary before this point can be considered established. Another significant difference between the interallelic and interlocus differentiations is their relative reversibility. No cases of reversion of interallelic differentiations have been encountered, if one accepts the interpretation that early "reversions" actually represent cases of misclassification. Interlocus differentiations are, on the other hand, freely reversible. Preliminary observations (NANNEY, unpublished) suggest that the interallelic differentiations persist even during a period when the locus is not being expressed, i.e., when a cell in the "H" state is transformed to the "L" state and back again. Quite possibly the two systems of serotype differentiation find parallels in the two major classes of mating type differentiation in the ciliates—the group A system being localized strictly within the nucleus, and the group B system having a major cytoplasmic component in its mechanisms of perpetuation (SONNEBORN 1954, 1957; NANNEY 1954, 1958).

SUMMARY

Strains of variety 1 of *T. pyriformis* manifest different immobilizing antigens when grown at 25°C in an Aerobacter-Cerophyl medium. Breeding analyses show that the ability to produce these particular antigens, the H antigens, is associated with a single genetic locus, the *H* locus. Four distinctive alleles, H^A , H^C , H^D , and H^E , appear to be established in different inbred strains.

Although the transmission of antigenic potentialities in sexual processes is

simple and uncomplicated, the expression of the *H* alleles poses problems. Heterozygous clones initially express both antigenic specificities, but in the course of cellular proliferation they produce some sublines expressing only one of the antigens and others expressing only the other. The phenotypically "pure" lineages maintain their characteristics in further growth. The kinetics of antigenic fixation are identical with the kinetics of mating type fixation in clones of "unstable" mating types, and a similar explanation must be applied to both. The preferred explanation for serotype "differentiation" is that one allele in each subnucleus of the compound macronucleus is suppressed, that the suppression becomes an hereditary character of the subnucleus and that the fixation process is the result of an assortment of differentiated subnuclei at macronuclear division. The phenomenon is considered as a kind of "persistent indeterminate dominance." The differentiations in one sexual generation appear not to influence the differentiations in a subsequent generation.

The system of mutual exclusion among the H serotypes is not to be confused with the system of mutual exclusion of presumably nonallelic antigen determining genes, which has very different properties. Specifically, the interlocus differentiations appear much more readily reversed and probably involve a cytoplasmic component in a feedback system.

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