mutagenic and carcinogenic (*11*) activity. These data provide additional support for the predictive value of mutagenic assays.

Additional toxicological tests conducted with NHDC and xylitol would provide evidence whether or not safer alternatives to saccharin as sweeteners are already available.

ROBERT P. BATZINGER
SUH-YUN L. OU
ERNEST BUEDING
Departments of Pathobiology and Pharmacology and Experimental Therapeutics, Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

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1 July 1977

Macronuclear Subunits of *Tetrahymena thermophila* Are Functionally Haploid

Abstract. *In* Tetrahymena thermophila *the major argument for the existence of diploid subunits has been that some loci show a delay in the accumulation of stable subclones during macronuclear assortment. This delay is based on the assumption that throughout the life cycle there are 45 subunits. We find that for at least 50 fissions after conjugation there is sufficient DNA for 66 haploid subunits. These additional subunits early in the life cycle are sufficient to explain the observed accumulation of stable subclones in all instances. This removes the need to invoke diploidy to explain assortment, thus resolving the question of subunit ploidy in favor of haploidy.*

Although there is consensus that the macronucleus of *Tetrahymena thermophila* (*1*) consists of independent subunits, the genetic organization of these subunits has been unclear. Equally persuasive arguments for their organization as either haploid or diploid sets of micronuclear genes, as well as arguments that they are individual gene loci, have been presented (*2*). Resolution of this problem is of special importance not only because of the current interest in the evolution of the ciliate macronucleus (*2*), but also because *Tetrahymena* is increasingly used in genetic studies (*3*). In this report we present evidence that the argument for diploidy is based on an illusion created by the presence of 66 rather than 45 subunits early in the life cycle. We conclude that macronuclear subunits behave as haploid sets of genes throughout the life cycle.

The existence of subunits is inferred largely from the phenomenon of macronuclear assortment (*2-6*). With the exception of one locus (*7*), all heterozygotes, regardless of dominance relationships, are initially unstable for phenotype (which in ciliates is controlled by the macronucleus) and give rise to stable subclones which irreversibly express only one allele. The available evidence is best explained by a stochastic model (*4, 8*) in which, following their replication, subunits are randomly distributed at each macronuclear division. Thus, in an unstable heterozygote there are two kinds of subunits, those expressing one gene and those expressing its allele. With repeated random subunit distribution stable subclones whose macronuclei consist of only one type of subunit are eventually produced (*9*). This model allows calculation of the number of assorting units from the equilibrium rate of assortment (*10*). For all assorting loci, the equilibrium rate of assortment is experimentally the same (*3*) and implies the presence of 45 subunits in a G_1 macronucleus. The observation that assortment for all loci yields 45 subunits suggests a single common mechanism.

Since macronuclear DNA behaves with haploid reassociation kinetics (*11*), and since G_1 macronuclei have an average DNA content of 45C (*12, 13*), the conclusion that there are indeed 45 haploid subunits would appear straightforward. However, macronuclear assortment also provides a powerful argument in favor of diploidy (*2, 3, 6, 13, 14*). Specifically, for some loci the accumulation of stable subclones is significantly slower than expected if 45 subunits were to begin the assortment process immediately after conjugation. It has been argued (*5, 15*) that, since for these late-assorting loci the products of both alleles can be detected in all clones for 30 to 50 fissions after conjugation, the delay must mean that subunits are heterozygous and, therefore, diploid. It was proposed that either a genetic mechanism to delete an allele or an epigenetic mechanism to repress permanently one allele had to exist (*5*).

Several attempts to reconcile the diploidy implied by late assortment with the concept of haploid subunits have been made, but none has been successful (*13, 16-18*). The hypothesis that 23 diploid subunits might in some way mimic the assortment of 45 haploid subunits is inconsistent with the random nature of assortment (*8, 19*).

The resolution proposed here is based on the results of cytofluorimetric measurement of the DNA content of G_2 macronuclei of heterozygous cells (*20, 21*). For the first 50 fissions after conjugation the mean DNA content of G_2 macronuclei is 132C (Fig. 1). This is considerably more than the 90C found in older cells and is sufficient DNA for 66 haploid subunits in a G_1 macronucleus. The importance of these subunits is made clear in Figs. 2 and 3. Figure 2 shows that when the ratio of types of subunits is close to 1 : 1 at the initiation of assortment, the accumulation of stable subclones (also in a 1 : 1 ratio) is initially slower than for other ratios. For ratios that are close to 1 : 1, as are the ratios observed for stable types for all late-assorting loci (*3*), the accumulation of stable types is a direct function of the number of subunits (Fig. 3). For 66 subunits there is an additional delay of 10 to 15 fissions in the accumulation of stable subclones as compared to the assortment of 45 subunits.

It is our contention that these additional subunits early in the life cycle are sufficient to account for the reported delay in assortment. Figure 3 shows the reported proportions of stable subclones for all late-assorting loci for which naturally occurring codominant alleles are available (*22*). Despite the fact these proportions represent small numbers of stable subclones, the agreement with the assort-

ment of 66 subunits is generally excellent. The agreement is more impressive when these proportions are compared with the results of computer simulation which can show the variability inherent in the assortment process. These simulations show (Fig. 3), first, that there can be considerable variation with respect to the appearance of a small number (arbitrarily chosen to conform to most of the reported assortment experiments) of stable subclones and, second, that the proportion of stable subclones at 50 fissions can also vary considerably. With the exception of the P-1 locus, all of the reported proportions are consistent with the assortment of 66 subunits. The exceptional P-1 assortment is not observed in all cases. In a study previous to the one shown in Fig. 3 (*14*), stable subclones were found to appear much earlier, but the data were too incomplete to include in Fig. 3. The difference between these two reports is difficult to rationalize (*23*), but it may be the result of either experimental design (*24*) or other peculiar properties of the P-1 alleles (*25*). Regardless of the explanation, we do not consider this one apparent exception to be sufficient grounds for rejecting the conclusion that for late-assorting loci the assortment process begins early after conjugation (*26*).

Although the presence of 66 subunits can account for the late assortment of some loci, it is necessary to explain why for other loci stable subclones appear early in the life cycle. It is significant that for all early-assorting loci the ratio of stable types of subclones deviates significantly from 1 : 1 (*3*). Such asymmetry means that the starting ratio of types of subunits must also be asymmetric (*10*),

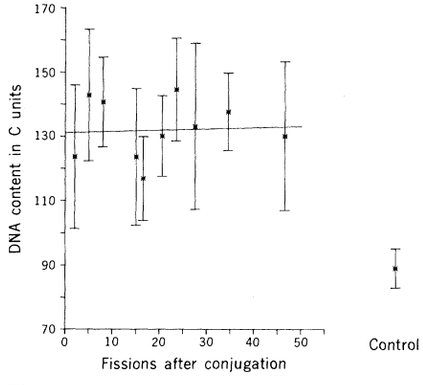

Fig. 1. The DNA content of G_2 macronuclei for the first 50 fissions after conjugation. Ordinate values are calculated with the G_2 micronucleus being used as a 4C standard (*13*). Bars are 95 percent confidence limits, and the solid line is least-squares regression of the means ($Y = 0.046X + 131.1$). The control culture was approximately 150 fissions after conjugation.

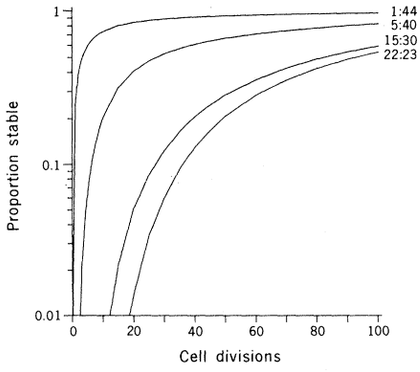

Fig. 2. The theoretical accumulation of stable subclones from unstable heterozygotes with increasing clonal age. Each curve represents a different starting ratio of types of subunits. Values were obtained by raising the appropriate matrix [equation 3 in (*10*)] to the appropriate number of powers.

and, as shown in Fig. 2, the greater the asymmetry, the earlier the appearance of stable subclones. However, increasing asymmetry also means that the kinetics of early assortment become largely independent of the total number of subunits (*27*). Therefore, so long as the ratio of alleles is highly asymmetric, early appearance of stable subclones is expected whether the number of subunits is 45 or 66 early in the life cycle.

Therefore, we contend that for all loci the assortment process begins shortly after conjugation. The need to invoke diploidy to explain late assortment thus no longer exists.

The most reasonable alternative to the diploid subunit is an arrangement of genetic material that behaves as a haploid set of genes. The argument that subunits cannot be smaller than haploid sets of genes (*16, 17*) is simple (*2*). If subunits were, for example, individual genes or chromosomes, independent, random distribution at macronuclear division would result in aneuploidy and therefore greatly increase cell mortality. Since it is well established that clones of *T. thermophila* can be kept in culture for decades without loss of vigor that is attributable to the macronucleus (*28*), there must be a more ordered arrangement of genetic material. The most likely arrangement is as functionally haploid subunits; this arrangement would be consistent with biochemical data (*11*), quantitative measurements of macronuclear DNA content (*12, 13*), and the identity of equilibrium rates of assortment (*3*).

The mechanism which forms haploid subunits must account for the variation in ratios of alleles. Three observations are pertinent.

1) The asymmetry of allele ratios is normally directional. In the best-studied case, the locus for the H immobilization-antigen, the four alleles can be ordered with respect to the proportion of stable subclones produced (*29*). When all possible heterozygotes are compared, subclones expressing H^E are much more frequent than those expressing any of the other alleles; similarly, H^A subclones are more frequent than H^C or H^D.

2) Meiotic coupling relationships appear not to be maintained in the phenotype of fully assorted macronuclei (*6, 14*). In dihybrids for two linked loci, four phenotypic classes are produced in equal numbers.

3) A small portion (no more than 10 to 15 percent) of micronuclear DNA sequences are not included in a fully developed macronucleus (*30*). As in other ciliates (*2*), these sequences are apparently not needed by the amitotic macronucleus and are discarded during macronuclear development.

We suggest that during macronuclear development the chromosomes contributed by each gametic nucleus undergo extensive (somatic) recombination or fragmentation, or both, after which new linkage groups are assembled. These

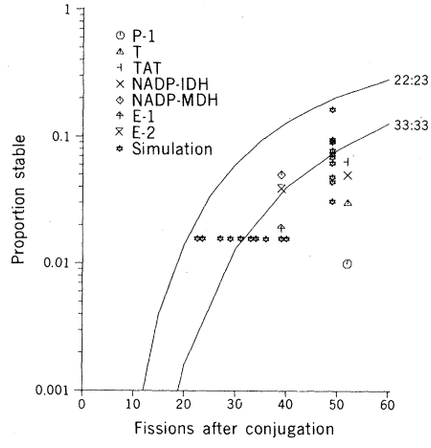

Fig. 3. Expected and observed accumulation of stable subclones. The two theoretical curves for 1:1 starting ratios for 45 and 66 subunits were calculated as in Fig. 1. Observed proportions are those reported for loci for which codominant alleles are available: E-1 and E-2 esterase (*14*); P-1 phosphatase (*16*); T ciliary antigen (*34*); *TAT* (E.C. 2.6.1.5; L-tyrosine:2-oxoglutarate aminotransferase); *NADP-MDH* (E.C. 1.1.1.40; L-malate: NADP-oxidoreductase); and *NADP-IDH* (E.C. 1.1.1.42; L-isocitrate:NADP oxidoreductase) (*35*). For each computer simulation a model in which each subunit is independently replicated and randomly distributed to new macronuclei was used (*8*). In each of the ten simulations shown, the starting ratio was 33:33, and a total of 128 computer-generated clones was monitored. For each simulation two parameters were recorded: the fission at which a total of two stable subclones was reached and the proportion of stable subclones at 50 fissions (plotted at 49 fissions for clarity).

may be composite chromosomes (*31*) each consisting of a haploid set of genes or, alternatively, they may be smaller units that behave as a larger linkage unit at macronuclear division, perhaps through attachment to a common site on the nuclear membrane (*32*). During the formation of new linkage units some DNA sequences may be eliminated, and others may form heteroduplexes which lead to gene conversion. Since the behavior of H alleles implies that not all aspects of the process are random, we suggest that early-assorting loci are located near fragmentation or recombination sites which are particularly prone to gene conversion or gene conversion-like events (*33*). Tests of these ideas require further biochemical characterization of macronuclear development, particularly with respect to restriction-like endonucleases, further characterization of the mechanism of macronuclear division, and construction of detailed maps of both micronuclear and macronuclear genomes.

F. PAUL DOERDER
JONATHAN H. LIEF
Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260
L. E. DEBAULT
Department of Pathology, University of Iowa, Iowa City 52242

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22. The DNA content decreases from 132C to 90C between 50 and 100 fissions after conjugation (*21*). This would contribute to the delay in accumulation of stable subclones. However, since the matrix used to calculate the theoretical curves (*10*) cannot accommodate variable numbers of total subunits, points beyond 50 fissions are not shown in Fig. 3. Although they would be useful, they are not essential for the argument presented here.
23. J. W. McCoy (personal communication) has found evidence suggesting that the initial accumulation of subclones can vary with genetic background. Although the literature is unclear, apparently the same strains were used for the experiments in both (*14*) and (*16*). However, very few assortment experiments have been repeated, and the most consistent interpretation is that all assortment begins early after conjugation.
24. In assortment experiments clones are usually serially recloned only every 8 to 13 fissions, and often fission rates of individual clones are not monitored. Thus, it is possible that a stable, recloned cell could be the linear descendant of a stable cell that arose shortly after the previous transfer but, because of the length between transfers, such stability at an earlier fission would not be recorded. In computer simulations (Fig. 3) each fission is monitored.
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26. A careful reading of the literature shows that the delay in the onset of assortment is in fact overstated on the basis of 45 subunits. Specifically, the slower accumulation of stable subclones for 1 : 1 ratios (Fig. 2) has not been adequately considered. For example, Allen (*14*) found that the average delay between the expected and observed proportions of stable subclones for E-1 and E-2 heterozygotes was 40 fissions, and therefore concluded that for these loci each diploid subunit began to express only one allele at 40 fissions after conjugation. Yet in the same paper it is reported that at 40 fissions 1.9 and 3.9 percent of subclones heterozygous for E-1 and E-2, respectively, were stable. Since the probability of observing stable subclones soon after the onset of assortment is vanishingly small when the starting ratio of subunits is 1 : 1, the assortment process must have begun earlier. Similarly, for the P-1 locus assortment is said to begin at 50 fissions, although at 50 fissions 1 percent stable subclones were present (*16*). Overstatement also applies to loci identified by recessive mutations (not shown in Fig. 3). For example, Doerder (*6*) reported that assortment at two regulatory loci, r1 and r3, began between 20 to 30 and 30 to 40 fissions, respectively. However, close examination of the original data reveals patterns of gene expression that can only be accounted for by an ongoing process of assortment which began at least 15 to 25 fissions earlier.
27. Random distribution means that the replicated products have equal probability of moving to opposite poles (segregation) or the same pole (nondisjunction). Thus, in the case where a G_1 macronucleus posesses only one of a kind of subunit, there is a probability of 0.25 that one of the progeny cells will have no copies of it; that is, the probability of a stable subclone is 0.25. This behavior is independent of the total number of subunits.
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32. Independent behavior of subunits smaller than haploid genomes would, through random assortment and unequal macronuclear division, lead to aneuploidy unless there were compensatory differential subunit replication at each division. This appears unlikely since in *T. pyriformis* it appears that DNA molecules replicate only once each cell cycle [(*18*); H. A. Andersen and E. Zeuthen, *Exp. Cell Res.* **68**, 309 (1971)].
33. Since both alleles are almost always recovered from each new macronucleus, we suggest that the process leading to the formation of haploid subunits occurs after the 4C stage of macronuclear development (*13*). If gene conversion were to occur when two copies of each allele are present, a ratio of 3:1 could result; if conversion were also to occur at later stages, even further distortion could result. Alternatively, it is possible that following the assembly of new linkage groups, certain of these are preferentially over- or underreplicated. If such differential replication were a function of DNA sequences surrounding particular genes, deviation from 1 : 1 would result. This would be a gene-conversion-like event.
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22 March 1977; revised 18 July 1977

Hormonal Inhibition of Feeding and Death in *Octopus*: Control by Optic Gland Secretion

Abstract. *Female* Octopus hummelincki *lays eggs, broods them, reduces its food intake, and dies after the young hatch. Removal of both optic glands after spawning results in cessation of broodiness, resumption of feeding, increased growth, and greatly extended life-span. Optic gland secretions may cause death of most cephalopods and may function to control population size.*

It is well known that the female octopus spawns once in its life, eats less while caring for the eggs, and invariably dies shortly after the eggs hatch. This report indicates that death is due to a secretion or secretions from the optic glands. When these glands are removed after eggs have been laid, the female ceases to brood the eggs, begins to eat again, gains weight, and lives for a prolonged period.

The optic glands are the only definitely