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Quantitative predictions of random segregation models of the ciliate macronucleus

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SUMMARY

Models of the macronucleus in *Paramecium tetraurelia* which assume known levels of ploidy and random segregation of subunits smaller than a haploid set at both fission and macronuclear regeneration (MR) are not consistent with the hypothesis that senescence is due to aneuploid imbalance. Either senescence has some other basis or there is a mechanism for regular distribution of subunits at MR. Random segregation models for fission and MR are consistent with most data on survival and heterozygote stability, but if the ploidy level is 860 they fail to account for the data of Nyberg (this volume). Since the ploidy level may be higher than 860, models of random segregation cannot be ruled out for *Paramecium*. Models of the macronucleus in hypotrichs which assume randomly segregating chromosome fragments are consistent with data on survival and on stability of heterozygous genotypes at fission.

1. INTRODUCTION

Most ciliated protozoa have both micronuclei and macronuclei, but the main physiologically active nucleus is the macronucleus. The macronucleus is remarkable in that it usually exhibits no well-defined chromosomes or other clearly visible subunits at cell division. It is generally assumed that, in the absence of mitosis, its constituent units, whatever their nature, are distributed at random to the products of fission. Fauré-Fremiet (1953) pointed out that random assortment of subunits smaller than a whole genome should result in macronuclear imbalances. He suggested that a main function of conjugation and autogamy is to replace periodically the old imbalanced macronuclei with new nuclei derived from the mitotically dividing diploid micronuclei. He postulated that such imbalances might be the cause of the reduced fission rate and death seen in senescence which occurs in both hypotrichs and *Paramecium* (but not *Tetrahymena*) when they are prevented from undergoing conjugation or autogamy (Sonneborn, 1954). However, it is not known whether senescence in ciliates is due to such imbalances or some other cause (Sonneborn, 1975). Another consequence of the random assortment of subunits smaller than a diploid set should be the chance segregation after many vegetative fissions of heterozygous clones into lines pure for each of the alternative alleles.

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Heterozygote segregation is the rule in *Tetrahymena* (see discussion in Orias & Flacks, 1975), but its existence is doubtful in other ciliates.

Macronuclei generally contain much more DNA than do micronuclei. Amounts vary widely in different ciliates and have been tabulated by Raikov (1969). If there were no differential loss or gain in any base sequences during the formation of a macronucleus from a diploid micronucleus the amount of DNA in the macronucleus divided by half the amount in the micronucleus should be a measure of the level of ploidy in the macronucleus. Losses, however, have been clearly demonstrated in the hypotrich, *Stylonychia* (see below). Errors arising from differential increases or losses are avoided if the macronuclear genome size is determined by renaturation kinetics (kinetic complexity) and then divided into the amount of DNA per macronucleus to give an estimate of the level of macronuclear ploidy.

Orias & Flacks (1975) have recently reviewed the literature on the nature of the macronucleus in *Tetrahymena*. Estimates based on both renaturation kinetics and relative amounts of DNA in the macronuclei and micronuclei indicate that there are about 45 genomes in the macronucleus at G 1. Apparently no major loss of DNA occurs when the macronucleus is formed from the micronucleus. Tetrahymena can divide vegetatively indefinitely without accumulating deleterious aneuploid imbalances. Heterozygotes (with one exception) always segregate into pure lines after many vegetative fissions. It is concluded that the macronucleus consists of 45 haploid subunits which assort randomly at fission. Mathematical predictions of the rate at which heterozygous genes should segregate at fission agree with observed rates. The model fails, however, to account for the fact that different genes begin segregation at different times.

Prescott, Ammermann and others (see Prescott, Murti & Bostock, 1973) find that in the hypotrich *Stylonychia* the macronuclear subunits are small chromosome fragments. Ammermann *et al.* (1974) give estimates based on kinetic complexity and amount of DNA per macronucleus which indicate that there are about 4000 genomes in the macronucleus. Much of the DNA in the micronuclei is repetitive and is lost during formation of the macronucleus. Although hypotrichs show senescence, heterozygote segregation has not been demonstrated (Heckmann, 1967). Several years ago Ammermann (1971) considered the mathematical consequences of random segregation of chromosome fragments on senescence and heterozygote segregation in hypotrichs, but at that time reliable estimates of macronuclear ploidy were not available.

It is well established from the amounts of DNA in the micronucleus and macronucleus in a number of stocks of the *Paramecium aurelia* complex that a macronucleus contains about 860 times the haploid amount (Woodard, Gelber & Swift, 1961; Woodard, Woodard & Gelber, 1964; Behme & Berger, 1970; Allen & Gibson, 1972). If, however, unlike the case of *Tetrahymena* but like *Stylonychia*, many sequences are selectively reduced in the formation of the macronuclei, 860 may not represent the true level of ploidy in the macronucleus. In fact, it has been reported recently that losses in DNA occur during formation of the macronucleus in *P. bursaria* (Schwartz & Meister, 1975*a*). According to Allen & Gibson (1972)

860 does represent the true level, for the amount of DNA found in the macronucleus divided by the size of the macronuclear genome determined from kinetic complexity is also approximately 860. Soldo & Godoy (1972), however, find from determinations of kinetic complexity that the macronuclear genome is much smaller. Applying their determination to the amount of DNA they find in a macronucleus they conclude that the number of genomes in the macronucleus is 1640. If their value for the genome is applied to the determinations of the amount of DNA in the macronucleus determined by Allen & Gibson (1972) or Behme & Berger (1970), the estimate of the number of genomes goes up to approximately 4000. *Paramecium* exhibits senescence, but neither Sonneborn, Schneller & Craig (1956) nor Nobili (1960, 1961) could find convincing evidence for heterozygote segregation.

Kimball (1943) suggested that the macronucleus in *Paramecium* is polyploid and implied that its chromosomes are distributed at random at cell division. Kimura (1957) calculated the number of fissions required to make likely the complete elimination of given chromosomes and concluded that his results were compatible with the assumption that chromosomal imbalance produces senescence in *Paramecium*. Kimura, however, assumed that the number of genomes is about 100 – a value which we now know is much too small. It has also been proposed that the macronucleus in *Paramecium* consists of diploid subunits (Sonneborn, 1947). Sonneborn based his hypothesis in part on the fact that *Paramecium* shows high viability even when undergoing the process of macronuclear regeneration (MR). In MR new macronuclei may reform from each of the approximately 20–40 fragments of the old macronucleus produced at autogamy or conjugation. Failure to find heterozygote segregation, of course, also supports the diploid model.

Although adequate quantitative predictions of the models have been made for *Tetrahymena*, the higher levels of ploidy found in hypotrichs and *Paramecium* have not been satisfactorily treated. Kimura's treatment (1957) is presented in a general form and it allows an approximation of a value he calls omega, the probability that all kinds of subunits are retained after a given number of fissions. The estimates are said to be valid only for very low values of omega. Omega, then, can indicate only extreme imbalances. It is the purpose of this paper to present calculations for high levels of ploidy and different degrees of imbalance that make possible the evaluation of data which bear on the possible effects of aneuploidy and heterozygote segregation at both fission and macronuclear regeneration.

2. MODELS

(i) *Fission*

The mathematical model for random segregation of subunits is the same, irrespective of whether the subunits are fragments of chromosomes, chromosomes, or sets of chromosomes. We start with a cell in G 1 containing a total of N subunits. Some of the total, M in number, are of a particular type whose distribution we wish to follow. Later, before the first fission, the numbers have increased to $2N$

and $2M$. The cell now divides in half so that each of the daughter cells gets exactly N subunits. It is assumed that each of the $2M$ subunits is just as likely to go to one of the two daughters as the other. The process is repeated at each cell division. We wish to calculate the probabilities that a cell has 0, 1, 2, ..., N subunits of the particular type after g cell generations.

The solution to the problem is well known and has been given by Michaelis (1955), Kimura (1957) and Schensted (1958). It may be expressed as a tabular array of probabilities, $P(f, g)$, where the rows $f = 0, 1, 2, 3, \ldots, N$ give probabilities of all the different possible numbers of the type being followed and the columns, $g = 0, 1, 2, 3, \ldots$, give probabilities at successive fissions. The first column, $P(f, 0)$, may be written down at once, for the probabilities at 0 fissions are 0 for all subunit numbers except the chosen initial number $f = M$, for which $P(M, 0) = 1{\cdot}0$. To calculate further columns we make use of the following array,

$$p(j, k) = \frac{\binom{2j}{k}\binom{2N-2j}{N-k}}{\binom{2N}{N}},$$

where rows, $j = 0, 1, 2, 3, \ldots, N$, give probabilities for the number of subunits of a particular kind in the parent, and columns $k = 0, 1, 2, 3, \ldots, N$, give probabilities of k subunits in a daughter cell – all in G 1. Each of the probabilities in subsequent columns may be computed by iteration as follows:

$$P(f, g) = \sum_{j=0}^{N} P(j, g-1)\, p(j, f).$$

If N is large, the computational requirements are prohibitively large; the $p(j, k)$ array itself containing N^2 elements. Moreover, algebraic solutions relating N, M and $P(f, g)$ directly are not available. We have found, however, that if terms in the above equations which make insignificantly small contributions are omitted, the computations are well within the capacity of a modern high-speed digital computer. The procedure we have used is briefly as follows. Terms in which $P(f, g-1)$ is less than 10^{-10} are ignored. The $p(j, k)$ array is partly stored, partly recomputed as needed, and limited as follows. It is evident that the maximum probability in each row of $p(j, k)$ corresponds to $j = k$, or the diagonal of the table, i.e. $p(0, 0)$, $(p1,1)$, $p(2, 2)$, ..., $p(N, N)$. Only terms of the diagonal beginning with (0, 0) and going up to values found empirically to be of significance are used; they are computed and stored. Additional entries in each row decrease to the left and to the right of the stored diagonal values, and are computed by iteration from the diagonal values whenever needed. Since most have very low probabilities they are limited by taking $4j^{\frac{1}{2}} = 4k^{\frac{1}{2}}$ terms to the right and left of the diagonal term in each row, others having been found empirically to represent negligibly low probabilities. We have also taken advantage of the fact that successive rows from the top read to the right are the same as successive rows from the bottom read to the left, and the fact that the terms greater than zero in the rows are symmetrical about the diagonal. The final test of whether only unimportant terms have been eliminated

by these procedures is to see that the sum of the calculated probabilities in each column of $P(f, g)$ is close to 1·0. Sums are indeed close to 1·0 in all cases, justifying the method. Further checks on the computations were carried out using computer simulation for selected models, and direct calculations of $P(0, g)$ according to the model described in the next paragraph. All methods agreed.

If we assume that each of the $2M$ subunits has an independent and equal chance of going to either daughter,

$$p(j, k) = \binom{2j}{k}(\tfrac{1}{2})^{2j}.$$

Kimura (1957) also gives a relation which makes it possible to approximate $P(0, g)$. His approximation is not particularly useful, however, for $P(0, g)$ may be computed exactly and easily by iteration according to the method of Otter (Preer, 1948). Other values can be computed as above, letting N approach infinity. In this model we no longer require that each daughter receive exactly N subunits. The model is equivalent to the special case of the preceding in which N is made to become very large relative to M. We have computed the distributions according to the first model for $N = 36\,980$ and $M = 430$ and according to the second model for $M = 430$, both through 280 generations. The results proved to be exceedingly close. In general, the effect of holding M constant and reducing N is to reduce the variance.

(ii) *Macronuclear regeneration*

Macronuclear reorganization apparently begins while cells are in G 1 (Kimball & Vogt-Köhne, 1961; Berger, 1973). The nucleus is then partitioned into a total number of F fragments, each of the N subunits having an equal probability of being one of the N/F passed to a given fragment. Every subunit is then increased by a multiple of F to restore the G 1 number. The probability that a macronucleus derived from a fragment after macronuclear regeneration (MR) will have $k = 0, 1, 2, \ldots, N$ subunits is given by the array

$$p(j, k) = \frac{\binom{j}{k/F}\binom{N-j}{N/F-k/F}}{\binom{N}{N/F}}.$$

$p(j, k) = 0$, except for integral values of k/F. Computations for successive macronuclear regenerations are carried out like those for fission.

3. PREDICTIONS OF VIABILITY IN *PARAMECIUM*

If the level of ploidy in the macronucleus of stock 51 of *Paramecium tetraurelia* is 860, then each of the approximately 43 chromosomes constituting a haploid set (Dippell, 1954) in a macronucleus newly formed from a micronucleus should be represented 860 times. If the subunit is the chromosome and the subunits assort at random at fission, then chance deviations from 860 will occur. Deviations from 860 can thus be thought of as aneuploidy. If aneuploidy is severe, viability should be impaired. Stock 51 reproduces vegetatively after conjugation or autogamy for

about 100 fissions with gradual reduction in viability, but during the next 100 fissions senescence becomes extreme. Most clones do not live beyond 200 fissions (Smith-Sonneborn, 1971), but with selection clones can sometimes be maintained for as many as 300 fissions (T. M. Sonneborn, personal communication). Is the degree of aneuploidy predicted by a model of chromosomal subunits consistent with these facts? We are interested in the distribution of the chromosomes of a set, so $M = 860$. The total number of chromosomes is $N = 860 \times 43 = 36980$. Results of a calculation based on these numbers appear in Table 1. The table gives probabilities after different numbers of fissions in the form of frequency distributions.

Table 1. *Frequency distributions for an initial number of 860 subunits and a total number of 36980*

Upper class limit*	Number of fissions				
	50	100	150	200	250
0·00	0·000	0·000	0·000	0·000	0·000
0·17	0·000	0·000	0·000	0·000	0·001
0·33	0·000	0·000	0·003	0·008	0·016
0·50	0·000	0·009	0·025	0·043	0·058
0·67	0·017	0·062	0·092	0·109	0·117
0·83	0·146	0·182	0·182	0·175	0·166
1·00	0·355	0·271	0·227	0·199	0·179
1·17	0·322	0·243	0·203	0·177	0·159
1·33	0·131	0·146	0·139	0·130	0·122
1·50	0·026	0·061	0·075	0·080	0·081
1·67	0·003	0·020	0·034	0·043	0·049
1·83	0·000	0·005	0·013	0·021	0·027
2·00	0·000	0·001	0·004	0·009	0·013
2·17	0·000	0·000	0·001	0·004	0·006
2·33	0·000	0·000	0·000	0·001	0·003
2·50	0·000	0·000	0·000	0·000	0·001
S.D.	0·168	0·238	0·292	0·337	0·376

* Actual limits divided by the initial number of subunits.

Upper class limits appear in the first column, each having been divided by the starting number of 860. The standard deviation (S.D.) is given in the last row. It is noted that after 150 fissions the probability that a specific one of the 43 chromosomes will be reduced to between 0·17 and 0·33 of its starting number of 860 is 0·003. Since there are 43 chromosomes, the probability that one or more of the 43 sets will be reduced to this level in a single paramecium is on the order of $1-(1-0{\cdot}003)^{43} = 0{\cdot}22$. (This computation assumes that the probabilities are independent, which they are not. Nevertheless, computer simulation shows that the error is only slight.) Although it is possible that aneuploidy at this level might be lethal, the severity of imbalance is surely not sufficient to be certain that such cells would die. Consideration of this and other probabilities in the table lead to the conclusion that the model cannot be ruled out on the basis that it predicts excessive imbalance at fission.

Berger (1973) reports 19–48 macronuclear fragments, with a mean of 35, at conjugation in stock 51. The chromosome number is taken as 42 instead of 43 in order that $N/35$ will be an integer. Predictions for a model in which $M = 860$, $N = 36120$ and the number of fragments at macronuclear generation (MR) is 35 are given in Table 2. In order to conserve space, only probabilities for numbers of subunits up to the mean of 1·00 are given. Most of the distributions are skewed towards the highest subunit numbers. In the computations it was assumed that 15 fissions precede the first MR and then that the MRs occur one immediately after the other. No great error is introduced by this procedure for the effects of a few fissions are small relative to that of an MR. It is noted that the distribution after 15 fissions and one MR is much like that in which cells have undergone 100 fissions.

Table 2. *Frequency distributions for an initial number of 860 subunits, a total number of 36120 and 35 macronuclear fragments*

Upper class limit	Number of macronuclear regenerations				
	1	3	5	7	10
0·00	0·000	0·000	0·000	0·003	0·014
0·17	0·000	0·001	0·010	0·025	0·047
0·33	0·000	0·014	0·038	0·058	0·076
0·50	0·006	0·051	0·079	0·092	0·098
0·67	0·050	0·111	0·119	0·117	0·109
0·83	0·170	0·165	0·144	0·128	0·110
1·00	0·285	0·186	0·147	0·125	0·104
S.D.	0·216	0·351	0·445	0·514	0·584

Inspection of the computer printouts shows that generally each MR has roughly the same effect as approximately 70 fissions. Nobili (1960, 1961) has induced ten successive MRs, each separated by ten fissions, in derivatives of stock 51. The lines exhibited progressive decline in viability during the experiment. Although all paramecia finally died after the last MR in unselected lines they underwent 15–20 more fissions than the controls undergoing fission, which died after 132 divisions. Table 2 shows that cells which have undergone ten MRs according to the model are indeed markedly aneuploid. In fact, on the order of $1-(1-0{\cdot}014)^{42} = 0{\cdot}45$, or 45 % of all cells should have lost completely one or more of the 43 kinds of chromosomes. However, such cells do die, and it is not felt that the model is eliminated. Moreover, it is likely that diversity is over-estimated in the table, for Sonneborn (personal communication) has observed that the number of macronuclear fragments decreases in aged lines. Only if it could be shown that the model predicts severe imbalance before the actual onset of senescence could the model be rejected. Nevertheless, the data do clearly rule out the possibility that senescence at fission is due to aneuploid imbalances, for ten MRs should cause the same degree of imbalance as roughly $10 \times 70 = 700$ fissions, whereas, in fact, Nobili found that MR had virtually no effect on the rate of senescence. Stock 51, of course, rarely lives beyond 200–300 fissions.

If the ploidy level is more than 860, then predicted aneuploid imbalances will be less severe, and the same general conclusions are reached: during their life-cycle paramecia could survive the degree of aneuploidy predicted, but senescence at fission does not result from aneuploidy.

Table 3. *Frequency distributions for an initial number of 430 subunits and a total number of 36980*

Upper class limit	Number of fissions				
	50	100	150	200	250
0·00	0·000	0·000	0·000	0·000	0·001
0·17	0·000	0·000	0·004	0·012	0·023
0·33	0·000	0·008	0·025	0·043	0·058
0·50	0·009	0·044	0·071	0·087	0·095
0·67	0·064	0·110	0·123	0·124	0·121
0·83	0·180	0·172	0·155	0·141	0·129
1·00	0·271	0·199	0·164	0·142	0·127
S.D.	0·240	0·339	0·415	0·479	0·536

Table 4. *Frequency distributions for an initial number of 430 subunits, a total number of 36120 and 35 macronuclear fragments*

Upper class limit	Number of macronuclear regenerations				
	1	3	5	7	10
0·00	0·0000115	0·004	0·022	0·055	0·117
0·17	0·001	0·023	0·053	0·070	0·078
0·33	0·009	0·056	0·080	0·088	0·086
0·50	0·041	0·092	0·098	0·095	0·086
0·67	0·106	0·120	0·108	0·096	0·082
0·83	0·178	0·133	0·108	0·092	0·076
1·00	0·211	0·131	0·102	0·085	0·070
S.D.	0·307	0·500	0·637	0·748	0·883

4. PREDICTIONS OF HETEROZYGOTE SEGREGATION IN *PARAMECIUM*

If the level of ploidy is 860, then in a heterozygote 430 of the 860 chromosomes of one kind have one allele and the remaining 430 chromosomes have the other allele. Therefore in considering heterozygosity we wish to start with $M = 430$ out of a total of $N = 36980$ chromosomes and determine the expected frequency after subsequent fissions and MRs. The results of computations for fission are given in Table 3. If a dominant allele must be completely eliminated before a recessive can be expressed, cells expressing a recessive allele would appear after 250 fissions in a frequency of only 0·001. If the recessive genes can be expressed when the dominants fall to 1/6 (= 0·17) or less of their normal value, the frequency only rises to 0·023 + 0·001 = 0·024. So demonstration that the model fails at fission is difficult unless dominance is incomplete. Nyberg (this volume) presents results of a case showing incomplete dominance; furthermore, he is able to show that the model for a ploidy

level of 860 is incompatible with his data. Nevertheless he is unable to rule out models which assume higher levels of ploidy.

Computations for MR on the basis of $M = 430$, $N = 36120$, and a fragment number of 35 have been carried out. The results appear in Table 4. Nobili (1960, 1961) reports that no segregation was observed in close to 1000 MR lines marked with a total of four heterozygous genes. If all the MRs had been the first MR the expected number of pure segregants (taking the probability of 0·0000115 from Table 4) would be $0{\cdot}0000115 \times 1000 \times 4 = 0{\cdot}05$ among the 1000, clearly too few to rule out the model. On the other hand many non-segregants (but we do not know how many) were from later MRs where Table 4 shows the probability is much higher. Furthermore, great reductions, but not complete eliminations, might also be expected to be manifested phenotypically. Nevertheless, the model cannot be properly evaluated without more details concerning the relations between the numbers of fissions, numbers of MRs, numbers of macronuclear fragments, and numbers of non-segregating clones.

A model for randomly dividing haploid subunits ($M = 430$, $N = 860$) was computed and appears in Table 5. It reveals slightly less tendency for segregation than the model for $M = 430$ and $N = 36980$, and leads to the same conclusions. Models for higher levels of ploidy show still less tendency for segregation and also lead to similar conclusions.

Table 5. *Frequency distributions for an initial number of 430 subunits and a total number of 860*

Upper class limit	Number of fissions				
	50	100	150	200	250
0·00	0·000	0·000	0·000	0·000	0·000
0·17	0·000	0·000	0·001	0·003	0·007
0·33	0·000	0·001	0·007	0·017	0·026
0·50	0·001	0·015	0·034	0·050	0·061
0·67	0·023	0·067	0·090	0·100	0·103
0·83	0·141	0·164	0·158	0·149	0·140
1·00	0·337	0·254	0·211	0·184	0·165
S.D.	0·169	0·238	0·289	0·331	0·368

5. MODELS FOR HYPOTRICHS

Since Ammermann *et al.* (1974) have estimated that in *Stylonychia* the number of copies of each gene in the macronucleus is about 4000, a model in which $M = 4000$ can be computed to measure chromosome imbalance, and a model in which $M = 2000$ can be used to test heterozygote segregation. We have chosen $N = 43 \times 4000 = 172000$ (because we also wished to apply the model to stock 51 of *Paramecium tetraurelia*). Computations show that the actual value of N is relatively unimportant so long as it is much larger than M. Senescence in *Stylonychia* occurs at around 500 generations according to Ammermann (1971). According to the model the degree of imbalance expected after 500 fissions is not severe, the

probability being less than 0·000005 that subunits of a specific kind are reduced to less than 1/6 the euploid number after 500 fissions. If, say, there were even as many as 10000 different kinds of essential subunits, the chance of one or more having been reduced to this level is less than $1-(1-0{\cdot}000005)^{10000} = 0{\cdot}05$. The probability of eliminating completely a subunit originally present in 4000 copies is computed to be 0·00001 after 1375 generations according to the Otter model which assumes that N is very large. These computations all show that the level of ploidy found in *Stylonychia* would make it extremely resistant to aneuploid changes resulting from random distribution of chromosome fragments at cell division.

Heckmann (1967) reports that some lines of *Euplotes* can undergo as many as 800 fissions without showing segregation of the mating-type locus. Assuming that M is 2000 and N approaches infinity, the probability that a given allele has been completely eliminated is only 0·00005 after 800 fissions. If N is smaller the probability will be smaller. Even assuming that a recessive allele will be expressed before the dominant is completely reduced to 0, it is unlikely that we would choose to reject the model because of predicted instability without further data.

6. CONCLUSIONS

Attempts to solve the structure of the macronucleus cytologically have not been conclusive for most ciliates. See, for example, the studies by Wolfe (1967) on *Paramecium* or the review by Raikov (1969). Many investigators have therefore turned to the more indirect approach of examining models. Virtually all models have assumed randomly segregating subunits which either are diploid genomes, haploid genomes, chromosomes, or fragments of chromosomes. Any model of randomly segregating macronuclear subunits must be consistent with data on the size of macronuclear DNA and levels of ploidy. Furthermore, the models must not predict mathematically imbalances which would result in reduced viability or heterozygote segregation before they are known to occur. Models for *Paramecium* in which the individual subunits are chromosomes, each present in 860 or more copies, are consistent with most available data. However, Nyberg in this volume presents data on heterozygote stability at fission which makes models with up to 860 copies unlikely. But if the ploidy level is above 860, as indeed it may be, then the models cannot be eliminated. We must conclude that no available data clearly rule out the possibility that the macronucleus of *Paramecium* consists of randomly segregating chromosomes.

Certain of the assumptions of the models presented here cannot be exact. Known losses of DNA from the macronucleus of *Tetrahymena* (Cleffmann, 1968) during fission indicate non-randomness. Non-randomness, depending on its nature, could either speed up or slow down the calculated increases in variances in numbers of units. It has also been assumed that the products of fission have a constant and equal number of subunits. But both inequalities and fluctuations in the amount of DNA are known for *Paramecium* (Kimball & Barka, 1959; Schwartz & Meister, 1975*b*) and *Tetrahymena* (Cleffmann, 1968). Inequalities in division would increase the variance.

It should also be noted that although the models assume a constant rate of fission in all lines, such constancy is not found in real cultures. As long as determinants with alternative alleles are not correlated with differences in fission rate the assumption is reasonable. However, if aneuploid changes occur, one would certainly expect that reduced fission rate and death would lead to overestimation of the frequency of unbalanced genotypes. Since we have concluded in all cases that predicted imbalances are not sufficiently great to rule out random models, such overestimation would not affect our conclusions.

It is not known whether the basis for senescence in ciliates is nuclear or cytoplasmic, although Sonneborn has most recently been inclined to the view that the basis is cytoplasmic (Sonneborn, 1975). In any event the computations presented here make it virtually certain that if random assortment of parts of the genome occurs at both fission and MR, then random assortment cannot be the cause of senescence. If it were the cause, then MR should have a very large effect on accelerating senescence. Instead, it has no effect (Nobili, 1960, 1961). The hypothesis that senescence arises from random assortment of units at fission can only be salvaged by assuming that unknown mechanisms lead to regular assortment of units amongst fragments at MR.

Finally, computations have been made for the model in hypotrichs of randomly segregating chromosome fragments, each present at a ploidy level of 4000 (Ammermann *et al.* 1974). The model is consistent with the data on senescence in *Stylonychia* and *Euplotes*, and also the data on heterozygote stability in *Euplotes*.

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