TRANSITORY HETEROSIS IN NUMBERS OF BASAL BODIES IN *TETRAHYMENA PYRIFORMIS*[1]

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ABSTRACT

The numbers of kinetosomes in ciliary rows on the ventral surface of cells of *Tetrahymena pyriformis*, syngen 1, are greater by about 10% in young heterozygotes established by crossing two inbred strains than in either homozygote. The difference, apparent at 20 fissions post-fertilization, is essentially gone by 100 fissions. This observation is consistent with a generalized "allelic exclusion" at all loci completed by perhaps 60 fissions after fertilization.

SIEGEL (1958) first documented the occurrence of hybrid vigor in a ciliated protozoan by showing that F_1 clones produced by crossing certain homozygous strains of *Paramecium aurelia*, syngen 1, manifest a higher mean fission rate than either parental strain. This report is concerned with another quantitative characteristic in another ciliate. Heterosis in the present instance is primarily of interest in connection with the ill-understood phenomenon of "allelic exclusion". NANNEY and DUBERT (1960) first observed in *T. pyriformis* syngen 1 that heterozygotes for the *H* antigenic locus give rise during vegetative growth to sublines manifesting one or the other alternative allele. Since that time heterozygotes for nearly every locus examined have manifested "vegetative assortment" of phenotype, though remaining demonstrably heterozygous for the micronuclei. Although nearly all loci behave alike in manifesting allelic exclusion and in the kinetics of the assortment process once it is initiated, they are idiosyncratic with respect to the time of onset of assortment. The assortment of H allelic specificities begins immediately after fertilization, but assortment related to other loci occurs after a delay ranging up to about 50 cell divisions. One assortment is reported as occurring only after 200 fissions for a peculiar acid phosphatase isozyme (ALLEN 1971), but it probably represents a different kind of event than the usual clean separation of allelic specificities. Both the mechanism and the selective significance of somatic assortment are unknown, but the behavior of heterotic characters is relevant to both considerations. If allelic exclusion occurs for all loci (and not just selected polymorphic loci) heterosis based on heterozygosity should erode in time.

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The opportunity to probe this question arose during a quantitative analysis of the distribution and pattern of increase of basal bodies (kinetosomes) on the surface of *T. pyriformis* (NANNEY 1971a,b). Greater numbers of basal bodies were found on young heterozygotes (20 fission clones) than on mature (100+ fission) clones of the parental strains. This previously unreported observation indicated either that basal body number was a heterotic characteristic, or that the number of basal bodies declined with time in all genotypes. As will appear, young heterozygotes do indeed possess more basal bodies than do young homozygotes, but the heterozygotes unlike the homozygotes gradually lose basal bodies until they approximate the homozygous state.

MATERIALS AND METHODS

The cortical system subject to analysis here has been recently described in detail (NANNEY 1971a,b) but can be briefly reviewed. The ciliate cortex is composed of patterned arrays of "cortical units," each of which contains one or more basal bodies and associated microtubular assemblies. The cortical units may be visualized by various silver impregnation methods. The Chatton-Lwoff staining procedure (described by CORLISS 1953) apparently stains surface features only, while the protargol stain developed by DRAGESCO (1962) and used primarily in this study stains the basal bodies beneath the surface. The ventral surface of a Tetrahymena, which provides the

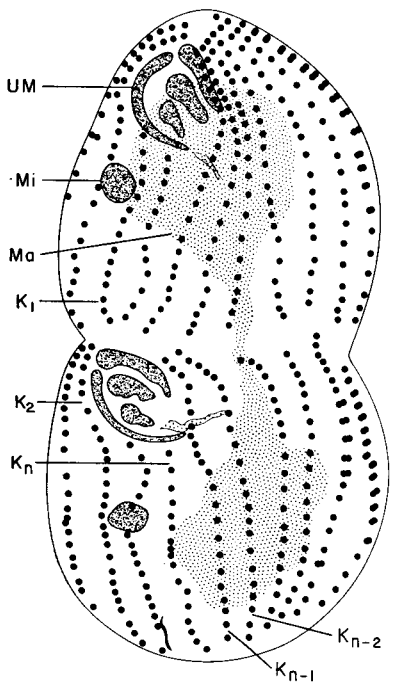

FIGURE 1.—Semidiagrammatic representation of a stage 6 (prefission) Tetrahymena stained with the protargol technique. UM—undulating membrane of the tetrapartite oral apparatus of the anterior daughter (proter); Mi—the micronucleus of the anterior daughter; Ma—the anterior portion of the almost divided macronucleus; K_1—the first kinety (ciliary row) of the proter; K_2, K_n, etc.—ciliary rows of the posterior daughter (opisthe). Drawn by ALICE BOATRIGHT from a photograph; details obscured by underlying nuclear material provided by interpolation.

ciliary rows (kineties or meridians) for the present analysis, is illustrated in Figure 1. All ciliary rows except (usually) two reach from the posterior end of the cell to the anterior apex; these two postoral rows terminate at the oral apparatus. The postoral row on the cell's right (your left) is designated as row #1 and the rows are enumerated in order to the cell's right, all the way around to row #*n*, which is the other postoral row. The number of ciliary rows is maintained with considerable stability within a cell lineage, but different numbers of ciliary rows may be maintained in cells with the same genotype (NANNEY 1966). The number of basal bodies in a ciliary row is not independent of the number of ciliary rows, but declines as the number of rows increases. This decrease in basal bodies per row approximately compensates for the increase in rows to maintain a constant number of basal bodies per cell in a particular genotype (NANNEY (1971b).

In Tetrahymena, unlike in some other ciliates, new basal bodies are added continuously during the cell cycle (NANNEY 1971a); comparisons must, therefore, be made between strains at comparable stages of the cell cycle. For present purposes one stage (FRANKEL's 1964 stage 6) only was studied. This stage is conspicuous and easily identified under low magnification by the "peanut" appearance characteristic of "dividing" cells. It represents a fairly small fraction of the cell cycle and hence reduces the variation considerably.

At this stage the fission furrow separates the anterior (proter) from the posterior (opisthe) daughters and permits the half-cells to be counted separately. Seven rows were counted wherever possible—rows #1 and 2, *n*, *n*—1, *n*—2, *n*—3 and *n*—4. The anterior and posterior daughters were recorded separately but no systematic differences could be demonstrated. All fourteen counts from a particular cell were seldom available because the underlying nuclear apparatus usually obscures some of the rows. Attempts were made to obtain at least ten half-cell counts for each row from each clone examined.

The strains used were representatives of Strains (Families) A and B of syngen 1 of *T. pyriformis*, and F_1 hybrids between the strains. Crosses were made within and between strains in bacterized peptone (1% proteose peptone inoculated with *Enterobacter aerogenes*, incubated overnight at room temperature and diluted 1/70 with distilled water prior to use). Approximately five fissions after conjugation, a single cell was transferred to axenic medium by washing the cell 3 times in 1% peptone containing 1,000,000 units penicillin/l and 1 g streptomycin/l. The growing cultures were transferred from the slides containing the peptone-antibiotic mixture to test tubes containing proteose peptone. They were harvested in log growth and stained by a modified Dragesco protargol technique developed by FRANKEL and DZIADOSZ (personal communication). To obtain aged clones new tubes were serially inoculated until the clones had undergone about 100 cell divisions (about 6 tube transfers) and were again harvested in log phase and stained. Numbers of ciliary rows were determined on the clones and sets of slides were selected to give as large an array of ciliary row classes (corticotypes) as possible.

RESULTS

Properties of stage-6, protargol-stained cells: A characteristic sample of the results obtained is displayed in Table 1. Data from four clones of predominantly 19-rowed stage-6 strain-A immature cells are shown here. The numbers of half-cells studied varied from about 10 to about 20. The means of the individual rows vary in a regular pattern. The postoral rows (1 and *n*) have fewer basal bodies per row than do the others. The number of basal bodies per row increases progressively to the left of the oral apparatus (from *n*—1 to *n*—4) and approaches the value in row 2, the ciliary row immediately to the right of the mouth. The standard errors of the half-cell means range from about 0.5 to 1.0, and thus provide a basis for rejecting a significant difference between clones for counts on a particular row. In comparing any two clones, one is usually consistently higher in mean counts for every row, but this consistency is misleading. Within a single

TABLE 1

Analysis of basal bodies in four immature clones of strain A with 19 ciliary rows

Clone		Row Number 2	1	*n*	*n*—1	*n*—2	*n*—3	*n*—4	Σ
2	Mean	25.1	14.3	12.4	20.8	21.9	21.8	20.8	137.1
	N	17	23	21	13	15	14	11	
	S.E.	.20	.59	.45	.99	.58	.82	.69	
	C*	.13	.53	.34	.61	.23	.18	.25	2.27
5	Mean	26.3	14.8	13.5	22.0	23.5	24.8	24.9	149.8
	N	10	17	18	8	11	12	12	
	S.E.	1.12	.58	.49	.85	.55	.75	.98	
	C	.48	.38	.32	.26	.14	.27	.46	2.31
8	Mean	24.9	14.0	12.8	21.0	22.1	23.8	24.1	142.7
	N	11	12	12	11	11	10	11	
	S.E.	.77	.64	.61	.51	.61	.71	.69	
	C	.26	.35	.34	.14	.19	.21	.22	1.71
11	Mean	26.5	13.8	13.6	21.6	23.1	23.8	24.6	147.0
	N	11	12	14	10	11	10	11	
	S.E.	.98	.64	.54	.48	.54	.33	.41	
	C	.40	.35	.30	.11	.14	.05	.08	1.43
Mean of Means		25.7	14.2	13.1	21.4	22.7	23.6	23.6	144.2
	Mean C	.32	.40	.33	.28	.18	.18	.25	1.94

* Coefficient of variation = Variance/Mean.

cell the counts for various rows are very highly correlated (NANNEY 1971a) and the counts were usually made on several rows of the same cell. Although all 14 counts possible on a single cell were seldom realized, 10 or more were often available. Hence the counts on the different rows do not represent independent ascertainments. The number of cells sampled is small and these must vary somewhat in their placement within stage 6, if for no other reason. The sums of the seven index rows in half-cells (last column) are judged not to reflect meaningful differences among the clones.

Because of a concern over the homeostatic capacities of heterozygotes relative to homozygotes, an index of variability in numbers of basal bodies was developed. This index is generated by summing the variance ratios (coefficients of variability) for the half-cell samples (last column). The statistical significance of this measure is problematical because of the correlation between half-cell counts, but the index provides an empirical measure which can be directly assessed. The index of clone variability for the four clones analyzed here ranges from 1.43 to 2.31.

Ten clonal samples of strain A were examined at 20 fissions (Table 2). The number of ciliary rows varied from 17 to 22 in different clones and most clones were essentially monotypic. In accordance with previous experience the number of basal bodies per row in a corticotypic series declines as the number of rows

TABLE 2

Summary of strain A analysis

Ciliary rows		Immature clones Mean	C*	Mature clones Mean	C*
17		168	1.87	142	1.91
		162	1.67	152	2.34
	Mean	165		147	
18		155	2.41	142	1.84
		144	1.16		
	Mean	150			
19		137	2.27	145	2.31
		150	2.31	144	1.59
		143	1.71	132	2.23
		147	1.42	132	1.48
	Mean	144		138	
20		145	2.21		
21				114	1.37
22		117	2.33	116	2.05
			Mean 1.94 ± 0.43		1.90 ± 0.37

* See footnote, Table 1.

increases. Nine clones were also examined at 100 fissions, wherever possible the same clones studied at 20 fissions. Again a decline in numbers of basal bodies is coincident with higher numbers of ciliary rows. For many clones fewer basal bodies were observed at 100 fissions than at 20, but the percentage difference was small and a *t*-test provides no support for a significant difference in the mean number of basal bodies at these different clonal ages. Moreover, the clonal variance index indicates no significant change in stability of basal body number as the clones mature.

Only three corticotypic classes were obtained among the strain B clones (Table 3) and the decline in numbers of basal bodies with higher numbers of ciliary rows is not so evident. Strain B provides even less evidence of change in mean numbers of basal bodies with age. Direct comparisons between the strain A and strain B clones is complicated by the different corticotypic modes of the distributions. These differences between the A and B samples are not due to intrinsic strain differences but are a consequence of historical accidents and corticotypic inertia (see NANNEY 1968). In spite of this difficulty the two strains appear by projection to be reasonably similar in numbers of basal bodies at equivalent corticotypes. The average clonal variance index is higher in strain B than in strain A and is higher in mature than in immature clones. *t*-tests, however, fail to establish these differences as significant. Generally speaking, strains A and B are very much alike, both in mean numbers of basal bodies and in the

TABLE 3

Summary of strain B analysis

Ciliary rows		Immature clones Mean	C*		Mature clones Mean	C*
19		133	1.64		140	1.86
		136	1.38		134	3.13
	Mean	135			137	
20		143	4.17		129	3.31
		124	2.39		133	2.91
		130	1.00		128	1.94
		135	2.62		128	2.28
					140	1.91
	Mean	132				
				Mean	132	
21		131	2.65		118	2.48
		132	1.31		122	1.72
		126	1.01			
	Mean	129		Mean	120	
		Mean	2.10 ± 1.35			2.39 ± 0.20

* See footnote, Table 1.

variability of basal body number, and neither strain manifests a significant effect of clonal age.

The heterozygote data (Table 4) provide a different result. Mature clones show lower basal body means than immature clones, and these differences are demonstrably significant; $P = <.01$ for corticotype 20 and about .05 for corticotype 21. The clonal variance moreover increases in the mature clones, but this difference just misses significance ($P \cong .10$), primarily because the variability of the mature clone is itself so variable.

If immature heterozygotes are compared with homozygotes of the same corticotype, the heterozygotes have more basal bodies. Although not many clones are available at the corticotypes appropriate for comparison, *t*-tests show a P value of .02 for corticotype 21 and .05 for corticotype 20. Mature heterozygotes do not differ from mature homozygotes in numbers of basal bodies or in variability.

Supplementary data: As mentioned in the introduction, earlier observations on cells from various stages of the cell cycle indicate that young heterozygotes have more basal bodies than do old homozygotes. The data just reported were collected to confirm this indication and do in fact support it. However, sampling vagaries reduce the direct comparisons available in some of the most significant categories, and provoke an effort to employ also the data previously assembled to test the same issues. The data available are not, however, directly comparable to those just reported.

Cells were previously stained, for example, with the Chatton-Lwoff silver pro-

TABLE 4

Summary of heterozygote analysis

Ciliary rows		Immature clones Mean	C*		Mature clones Mean	C*
20		157	1.50		134	2.61
		156	1.82		135	1.28
		144	1.84		130	2.90
	Mean	152			133	1.76
					142	1.92
				Mean	135	
21		160	1.50		127	2.41
		156	1.75		141	1.48
		138	2.62		137	3.08
		154	1.54			
	Mean	152		Mean	135	
22		135	1.16		127	1.80
		131	1.83			
		140	2.14			
	Mean	135				
		Mean	1.76 ± 0.13			2.14 ± 0.21

* See footnote, Table 1.

cedure and the equivalence of the counts made on the two kinds of preparation had to be checked. In fact, preliminary analyses indicate that the protargol preparations yield about 18% more basal bodies than those from equivalent cells stained with the other technique. This difference probably reflects the ability of the protargol stain to penetrate the surface and expose new basal bodies before they develop cilia. But in any case, Chatton-Lwoff data need to be transformed into protargol equivalents.

Secondly, data previously assembled were gathered from all stages of the cell cycle, and relatively few represented stage-6 cells. Hence, to compare stage-2 cells with stage-6 cells, for example, another data transformation is required. This is achieved by employing the observation (NANNEY 1971a) that the increase in total ciliary units is approximately linear through the cell cycle. Using FRANKEL and WILLIAMS (1972) estimate of the time spent in the various morphological stages, one may convert counts made on earlier stages into mid-stage-6 equivalents. The counts on stage-0 cells are multiplied by 1.41, stage-1 cells by 1.19, stage-2 cells by 1.12, stage-3 cells by 1.11, stage-4 cells by 1.08 and stage-5 cells by 1.04.

Thirdly the task of comparing strains whose samples happen to be available in different corticotypes may be accomplished by employing the regular relationship between numbers of basal bodies and numbers of ciliary rows (NANNEY 1971b).

A first approximation is provided by using a 12.7 unit transformation between adjacent corticotypes. This figure is obtained from the regression of basal body number in the six index rows against the total numbers of ciliary rows.

Finally an adjustment of the protargol data is necessary to render them comparable to the transformed Chatton-Lwoff data. The regression analysis was based on six rather than seven index rows; row one was excluded because of the difficulties in distinguishing between somatic and buccal basal bodies during stomatogenesis. The numbers used for the protargol data were those from the appropriate six rows. Moreover, the numbers were transformed from their half-cell basis to a whole-cell basis by doubling.

One final problem concerns the use of strains other than A, B and their hybrid. Considerable information had been acquired on a heterozygote between strain A3 and B, but no information was available on homozygous A3. The heterozygote was, however, indistinguishable from the AB hybrid where comparisons were available. Moreover, strain A3 has a background very similar to that of A and B, though differing from each in a few known alleles. No *a priori* grounds are available for believing it different from A or B with respect to numbers of basal bodies.

TABLE 5

Index row counts for various clones converted to stage 6, corticotype 19, Protargol-stain equivalents

Homozygous young	Homozygous old	Heterozygous young	Heterozygous old
274.8	229.6	290.5	230.4
262.0	247.0	302.1	253.2
266.3	243.7	288.0	250.0
247.7	271.0	281.0	248.4
245.6	259.6	278.0	256.9
270.0	259.2	284.9	251.5
257.4	239.0	294.5	246.9
266.4	240.0	294.1	252.5
241.0	252.4	270.7	268.7
246.0	241.0	271.5	256.4
274.3	285.2	253.4	
270.1	243.3	270.2	
233.9	251.3	262.6	
247.9	242.3	277.0	
257.3	242.5	273.8	
258.4	247.5	270.2	
264.2	232.6	272.3	
254.2	240.0	275.4	
245.9	243.2	279.9	
	245.9		
	245.0		
	261.4		
Mean 257.0	248.3	278.3	251.5
S.D. 11.9	12.6	11.9	9.6
95% 251–263	243–254	273–284	245–258

Granting the inevitable loss of accuracy and credibility occasioned by such finagling, the results are not entirely uninteresting (Table 5). As noted in the more limited data already considered, the young heterozygotes have more basal bodies than do any of the other arrays—which are very similar. The 95% confidence limits of the young heterozygotes do not overlap with any of the others. Compared with old heterozygotes, a t-test gives a value of 5.24 with 27 df, well beyond the 1% level of confidence. In contrast, the young and old homozygotes yield a t-value of 2.29 with 39 df. This difference is significant at the 5% level but not at the 1% level. Old homozygotes and old heterozygotes are indistinguishable ($t = 0.95$; $\mathrm{df} = 27$).

DISCUSSION

Allelic exclusion in Tetrahymena heterozygotes yields subclones in which the products of one or the other allele are not detectable (see NANNEY 1964, 1968b; BLEYMAN 1971). Whether the silent allele is present but repressed, or is physically absent, has not yet been established (see ALLEN and GIBSON 1972). In no case, however, has an excluded allele come back to expression after a period of non-expression; the possibility of physical removal must be seriously entertained. Mechanistically, loss and repression need not be drastically different; a regulatory molecule might, for example, interfere in one case with a replicase and in the other with a transcriptase.

This is not an appropriate occasion to discuss all the evidence bearing on the structure and function of the ciliate macronucleus. The present data were assembled, however, against the backdrop of these issues and make a modest contribution toward their resolution. Allelic exclusion has been interpreted as a means of achieving phenotypic plasticity in a genetically buffered outbreeding diploid (NANNEY 1963). The polymorphic loci which are phenotypically scrambled in sublines of a genetically (micronuclearly) homogeneous but heterozygous clone, provide preadaptational responses to a multiplicity of environmental vicissitudes. If this interpretation were correct, allelic exclusion might be observed only for those loci, and perhaps only for those alleles which had been selectively placed under such a discipline. So long as phenotypic assortment had been found only for certain natural genetic polymorphisms, this interpretation had some attraction. Now, however, that induced mutations (CARLSON 1971; DOERDER unpublished) have been shown to undergo allelic exclusion, the presumption is strong that allelic exclusion is not limited to a few selected loci. In fact, only one locus for which an adequate test has been made, appears not to undergo vegetative assortment (McCOY unpublished). The generality of the phenomenon decreases the likelihood of a locus-specific regulatory mechanism, and supports rather the probability of a generalized mechanism, of which haploidization (or nonreplication) might be an example.

The haploidization hypothesis finds some support from cytochemical observations (WOODARD, GOROVSKY and KANESHIRO 1972), but encounters a major difficulty in application. The time at which assortment begins within a clone is specific for the locus in question (ALLEN 1965, BLEYMAN, SIMON and BROSI

1966). This observation might be interpreted as due to a chromosome-specific haploidization, in which case no more than five occasions of assortment (representing the five chromosome pairs) would be expected. Moreover, loci on the same chromosome would be expected to begin assortment at the same time, and probably to maintain their coupling arrangements. ALLEN's (1965) test of this point for the one linkage then available, showed no correlation in the behavior of the *mt* (mating type) and *E-1* (esterase) alleles. That linkage, however, was loose and has not been unambiguously confirmed in this laboratory. Other and closer linkages (DOERDER unpublished) do, however, confirm the general result. Thus far no evidence has been found for a *chromosomal* exclusion mechanism. The resolution of this problem may eventually be found in the persistent suggestions that the macronuclear chromosomes are smaller parts of the meiotic (micronuclear) chromosomes which yield the linkage results (ALLEN and GIBSON 1972, BOSTOCK and PRESCOTT 1972).

The contribution of the results presented here is, as indicated, modest. The increase in numbers of basal bodies in the hybrids is probably, though not demonstrably, the effect of heterozygosity at several different loci. If all loci assort, and if the assortment is always initiated by 50–60 cell divisions after fertilization, the heterotic effect would be expected to decline thereafter until it was entirely lost. The results are consistent with such an interpretation, though they do not establish it.

The stability analysis is less decisive. Certain considerations (LERNER 1954) suggest that stability of developmental pattern may be associated with the heterozygous condition. Regardless of whether allelic assortment in Tetrahymena is genetic or epigenetic, its consequences should be similar in so far as developmental stability is concerned. The only suspicious change in variability of basal body number is in the heterozygotes as they become older. This change is in the direction predicted, but falls just short of statistical significance.

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