SCALAR CONSTRAINTS IN *TETRAHYMENA* EVOLUTION

Quantitative Basal Body Variations Within and Between Species

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ABSTRACT

Tetrahymenas of 17 species of the *T. pyriformis* complex have been stained with protargol and analyzed for numbers of basal bodies in half cells just before cell division. At this stage, cells of all strains manifest considerable variation in numbers of basal bodies; the coefficient of variation ($\sigma/\bar{m}$) is usually between 0.05 and 0.10. Much of this variability is observed in cells in the same nutritional state, at the same stage of the growth cycle, and in the same part of the life cycle. The basal body variability may be related to the variation in macronuclear DNA content that results from the imprecise amitotic macronuclear division.

With a few exceptions, strains of different species are difficult to distinguish on the basis of basal body numbers. The species means in the samples examined show a range only from 234 (*T. furgasoni*) to 481 (*T. capricornis*), about a twofold difference. This limited variation in the means suggests that these organisms are constrained within narrow limited by some scalar function of their organismic design, which prevents an evolutionary size dispersion—even though molecular scrambling has occurred in the complex at an appreciable rate for a very long evolutionary interval.

KEY WORDS *Tetrahymena* · evolution · basal bodies · size regulation · nucleocytoplasmic interaction

The basal bodies (kinetosomes), located at the proximal ends of the cilia, are readily visualized cortical elements that provide a useful means of assessing the numbers of cell structures and patterns of organellar association in the ciliated protozoa. In *Tetrahymena*, the basal bodies are associated with the specialized cilia composing the oral membranelles, with the longitudinal rows of cilia (kineties) making up the somatic ciliature. We are here concerned with the number of basal bodies in the somatic ciliature.

Previous studies have provided information concerning the regulation of basal body number. New somatic basal bodies arise anterior to preexisting basal bodies (1, 14). In *Tetrahymena* they arise throughout the cell cycle (24, 32). Although in some organisms the new basal bodies are produced only in specialized regions of the cell, in *Tetrahymena* they arise in all parts of the cortex. With respect to the distribution of new basal bodies in time and space, *Tetrahymena* is relatively relaxed, but some regions are more active in basal body production than others, and the rates of production may not be completely constant throughout the life cycle and the cell cycle (16, 23, 28, 30).

The number of ciliary rows varies over a considerable range within a single strain of *Tetrahymena*, and sometimes even within a recently cloned culture. The number of basal bodies per ciliary row varies inversely with the number of rows (25, 27). Particularly, the total number of basal bodies is independent of the number of rows but is maintained at approximately the same level in cells with, e.g., 16, 18, or 20 ciliary rows. Because the number of basal bodies appears to be reasonably constant within a strain (so long as the growth conditions are maintained constant), the question arises as to whether basal body number might be a useful quantitative trait in genetic studies and in systematic comparisons.

We are here concerned particularly with two questions – the differences in basal body numbers within a single clonal culture, and the differences between strains belonging to the same and different species. As will be shown, substantial variation occurs within a culture, and it is not related to previously identified variables. Nevertheless, relatively little difference in the mean numbers is observed even between species with remote common ancestors. We suggest that the intraclonal variation in basal body number may be tied to intraclonal variation in DNA content (8), and that the similarity in the means for different species is due to a common constraint of scale operating on this particular organic design (26).

MATERIALS AND METHODS

The number of basal bodies in a *Tetrahymena* is markedly dependent upon culture conditions. The number may fall from several hundred to a few dozen under sustained starvation conditions (23), but the immediate response to a non-nutrient medium may be an increase in basal bodies associated with an elongated and streamlined dispersive form (30). To avoid nutritional effects, the following studies were carried out on populations of cells in the fast exponential growth phase. The cells were loop-inoculated into 10-ml tubes of axenic 1% proteose peptone at room temperature (~23°C) and harvested 2 or 3 days later by centrifugation in a clinical centrifuge (half speed).

The number of basal bodies doubles during the cell cycle, so that randomly chosen cells are expected to vary over at least a 100% range. This variability may be greatly reduced by using staged cells. Staging is most easily accomplished by using Frankel's stomatogenic stages, which divide the cell cycle into seven unequal periods, 0–6. Stage 6 is the last and most easily recognized stage, identified by the familiar peanut profile (Fig. 1) and makes up about 12% of the cell cycle (18). During the survey phase of this study, we inadvertently interpreted stage 6 somewhat more broadly than did Frankel and Williams, and probably included some late stage 5 cells in the analysis.

The only reported variation in basal body numbers associated with the life cycle occurs in young hybrids between inbred strains in *T. thermophila* (28). These show a 10% increase in basal body number which is lost by 100 fissions. All the strains included in the present report are long-maintained laboratory cultures that should not be manifesting such a "heterotic" augmentation.

The staining precedure employed was similar to that described by Jerka-Dziadosz and Frankel (21) as a modification of the Dragesco protargol procedure.

The strains examined included representatives of the ten genetic species or "syngens" (13, 17) recently assigned Latin binomials by Nanney and McCoy (29). Some information has been published on these strains (27), but the procedures of ascertainment did not permit an asesssment of the variation among individuals within a culture, and hence of the reliability of the population mean.

Several asexual species, previously referred to as phenosets A, B, C, D, and E (6) were also studied. Phenosets A, B, C, and E are amicronucleate and have also been assigned Latin binomials (29), but no information has been published concerning basal body numbers in amicronucleate strains.

We also include data from a new "syngen" 14 recently isolated by Dennis Nyberg (personal communication), which represents a new genetic species that has not yet been assigned a Latin name. Finally, we report data from syngen 5, which was considered of doubtful status (29) before Nyberg's recent collection of additional strains. Altogether, 17 "genetic species" are thus included in this survey.

RESULTS

The Numbers of Basal Bodies in Proters and Opisthes at Division

Previous estimates of basal body populations used pooled data from several different individuals at the same stage of the cell cycle having the same number of ciliary rows. All ciliary rows were not counted on any cell, because all are not stained adequately and because some were always obscured by the underlying macronucleus which also stained darkly with the protargol procedure. In fact, only ventral ciliary rows were counted, and the total basal body count was estimated on the assumption that the ventral ciliary rows were equal (excepting the short postoral rows) to the dorsal rows.

To examine variability of basal body number among individuals, one must be able to estimate

the total number from those on a part of the individual, and preferably from a small sample of easily accessible rows. To approach this objective, a more complete examination of basal body distribution in stage 6 cells was necessary. The availability of some particularly well-stained cells made possible an almost complete count of basal bodies on a limited number of cells.

The first question raised was whether the anterior and posterior division products (proters and opisthes) in this material showed any systematic differences in the number of basal bodies. The results of near-complete inventories of 5 cells (Table I) show that neither the proter nor the opisthe is equipped with a significantly larger number of basal bodies, but in four of the five cells the proter had a slight advantage. The correlation between the daughter cells ($r = 0.988$) was remarkably high.

The results do demonstrate a substantial difference between cells of the same strain, in the same culture, at the same stage of the cell cycle.

One might expect, solely because of the duration of stage 6, a range of 12% difference between cells due to differences in the time of fixation, and that the range might be somewhat greater because of the inclusion of some late stage 5 cells. The observed difference between cells 1 and 2 is nearly twice that value, however, though the average difference is much smaller. A greater spread of values within stage 6 might be expected if the formation of basal bodies during this stage is more rapid than in an average time segment of the same length elsewhere in the cell cycle.

Because proters and opisthes are not systematically different in basal body numbers, basal body counts of the opisthe alone provide a satisfactory basis for estimating the total population. The opisthes are much easier to count because the ciliary rows do not yet converge into a closely packed anterior junction as in the proter. An additional 10 opisthes were counted as completely as possible (Table II) to give a total sample of 15 nearly completely ascertained opisthes. The range of variation of the augmented sample is nearly the same as that of the original sample. As has been previously shown (27), the total number of basal bodies in a cell is not affected significantly by the number of ciliary rows. (The linear regression of total basal bodies on numbers of ciliary rows in

TABLE I

Complete Basal Body Counts on Proters and Opisthes in Stage 6 Dividers of Strain C3-368

	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5	
Row number	proter	opisthe	P	O	P	O	P	O	P	O
1	13	14	17	16	10	12	8	13	15	13
2	22	24	25	27	22	22	19	19	22	22
3	26	23	27	25	22	22	22	21	21	23
4	20	24	28	31	22	23	22	18	24	24
5	19	19	26	20	20	18	20	18	19	18
6	21	20	24	24	20	23	(20)	17	20	20
7	(21)	20	26	24	18	22	19	17	19	22
8	(21)	22	25	23	20	17	19	21	18	19
9	22	22	24	21	17	18	18	17	18	18
10	22	20	24	27	18	19	(19)	18	18	18
11	21	22	23	25	19	16	21	18	18	20
12	23	24	26	26	21	20	20	21	20	20
13	23	22	(24)	25	22	21	20	(17)	20	21
14	22	(21)	(24)	(24)	21	17	10	(19)	19	21
15	21	20	22	(24)	21	20	19	19	20	21
16	20	21	24	23	19	20	19	20	19	(21)
17	19	20	25	25	18	(19)	(19)	19	(19)	20
18	14	15	(22)	21	20	(19)	(19)	(19)	19	22
19			17	17	20	18	(19)	(19)	21	20
20					20	16	13	18	20	21
21					12	11	11	12	13	13
Total	370	373	453	448	402	393	386	379	420	417

Values in parenthesis represent interpolations made where direct counts were not possible.

TABLE II

Complete Basal Body Counts in Opisthes in Stage 6 Dividers of Strain C3-368

Row number	Cell number									
	6	7	8	9	10	11	12	13	14	15
1	15	12	11	14	15	15	15	14	13	12
2	25	22	23	(21)	25	21	(22)	26	24	22
3	26	22	21	21	24	22	(22)	23	20	19
4	(25)	20	21	19	22	24	(22)	23	21	21
5	(24)	21	21	21	21	20	23	20	23	16
6	23	21	17	(19)	20	21	23	24	20	19
7	21	19	18	(19)	21	21	23	21	19	19
8	19	20	20	19	20	19	20	18	20	19
9	21	20	18	17	20	19	23	20	19	19
10	19	20	19	18	(21)	19	21	25	19	17
11	20	20	17	18	(21)	20	20	23	19	18
12	(21)	21	(19)	21	22	21	19	23	19	17
13	23	23	21	18	22	23	21	24	23	17
14	21	21	19	23	23	20	22	21	21	15
15	22	(21)	22	21	21	21	22	24	21	19
16	21	(21)	20	21	21	24	21	24	21	(19)
17	17	21	21	20	21	(23)	22	(23)	23	(19)
18	15	19	22	19	21	(23)	19	(23)	(22)	20
19		13	20	20	14	20	21	(23)	(22)	16
20			12	14	13	10	14	23	(22)	16
21							14	13	20	15
									10	11
Total	378	377	378	383	408	408	429	459	441	388

this sample of 15 opisthes is positive and has a numerical value of ~8, but the scatter is so wide that the slope is not significantly different from zero.)

The Correlation of Basal Body Numbers in Rows within a Cell

The availability of full body counts allows us also to examine the question of correlation among the basal body numbers of rows in the same cell. If the correlation is high, all rows need not be counted to provide a reasonable estimate of the total basal body number. A simple way to explore this matter is to estimate the total basal body number using a few rows, and to compare the estimate with the full count. For this exercise we arbitrarily selected rows number 3, 7, 10, and 13. These were added and divided by 4 to give a mean row count, and the mean was then multiplied by the row number, less 1. The subtraction of one row is required by the observation that the two postoral ciliary rows are substantially shorter than normal somatic rows, and together contain about the number of basal bodies found in an average row.

This analysis (Table III) shows that the mean basal body count estimated from these four rows (403) is scarcely different from the direct count of all the rows (404). As might be expected, the standard deviation of the estimates (σ = 34) is greater than that of the direct counts (σ = 29), but only slightly. These results are consistent with a regular distribution of basal bodies within the cell, and a high correlation among the rows. They justify the use of a few rows to estimate the total basal body population.

The Numbers of Basal Bodies in Different Somatic Rows

The question must still be asked as to whether the particular rows examined affect the estimates. A preliminary study done earlier indicated that all the somatic rows have about the same number of basal bodies, but the observations were not made on stage 6 cells. Recently, Kaczanowski (23) has shown a gradient of basal body proliferation during stomatogenesis, with excessive numbers of

TABLE III

Total Basal Bodies in Opisthes at Stage 6 Enumerated by Direct Counts and by Estimates Using Only Dorsal Rows, Only Ventral Rows, and Combinations of Dorsal and Ventral Rows

Cell	Rows	Direct count	Estimate based on rows 3, 7, 10, 13	Estimate based on dorsal rows 8, 9, 10, 11	Estimate based on ventral rows 2, 3, n-3, n-4
1	18	373	361	366	374
2	19	448	455	428	446
3	21	393	420	380	410
4	21	379	365	365	390
5	22	417	420	385	435
6	18	378	378	344	404
7	19	377	378	360	387
8	20	378	375	352	404
9	20	383	361	342	394
10	20	408	418	390	432
11	20	408	404	366	428
12	21	429	435	425	425
13	21	459	465	430	475
14	22	441	425	404	462
15	22	388	378	383	420
Mean		404	403	381	419
SD		29	34	29	38

Regression analysis of Direct Count vs. Row numbers: linear regression = 0.388, slope = 8.4, $P = 0.623$.

new basal bodies on the ventral surface. He in fact invokes this ventral-dorsal gradient, in combination with a central-terminal gradient to explain the localization of the new oral anlagen. Kaczanowski's observations were made on severely starved cells, but a more subtle manifestation of such a gradient might be expected even in fast exponential cells. A test for a gradient is provided by an estimate of total basal bodies employing ventral rows, and another estimate using only dorsal rows. The dorsal row estimate is systematically low—with a mean of 381; the ventral row estimate is systematically high—with a mean of 419. The variability of the estimates is not very different. Because all previous published estimates of basal body counts are based primarily on 7 ventral "index rows", they must slightly inflate the population estimates, at least when they employed stage 6 cells. The amount of such inflation is of the order of 15–20 out of 400, or perhaps as much as 5%. The data provided here on strain comparisons are more accurate than those previously published, and give slightly lower estimates.

The Variability of Basal Body Numbers in More Nearly Synchronized Cells

The counts in the previous observations were made on stage 6 cells, estimated to comprise at least 12% of the cell cycle. The variability among the cells was somewhat greater than 12%, but a combination of timing difference plus observational and staging error might account for a 20% differential, particularly if basal body proliferation during stage 6 is responsible for more than 12% of the total proliferation. We wished to be assured that the variability in basal body numbers reflected real differences between cells at the same time in the cell cycle, and particularly at the time of division.

A more precise staging of the cells may be achieved by utilizing the nuclear changes associated with the terminal phases of fission. In protargol-stained preparations the micronucleus is usually not visible in vegetative cells (stage 0), as it is closely associated with the macronucleus and is obscured by the surrounding granular dark-staining material, perhaps ribonucleoprotein. At the start of stomatogenesis, the micronucleus moves away from the macronucleus and develops a characteristically staining spindle apparatus (Fig. 1 *a*). Micronuclear division occurs before stage 5. In early stage 5 (Fig. 1 *b* and *c*) the macronucleus retains its spherical shape and the first discontinuities appear in the ciliary rows. In late stage 5 the macronucleus begins to undergo its amitotic program, elongating into a cylindrical structure (Fig. 1 *d* and *e*). A nuclear constriction appears, converting the cylinder into an hourglass figure characteristic of early Stage 6 (Fig. 1 *f*). Finally, as the cortical constriction develops, the connection between the daughter nuclei is reduced to a thin filament, and the stage is called late Stage 6 (Fig. 1 *g* and *h*).

Using the techniques of estimating basal body populations from counts of characteristic rows, we have examined the basal body numbers in more fully staged cells of two strains (Table IV). The *T. canadensis* strain has a higher mean population at each stage than does the *T. pigmentosa* strain. It is also somewhat more variable (the standard deviation is higher, even with larger sample sizes). Nevertheless, the pattern of increase is similar in the two strains. The number of basal bodies probably increases from stage 5 to stage 6, but the variability within the substages almost obscures the increase.

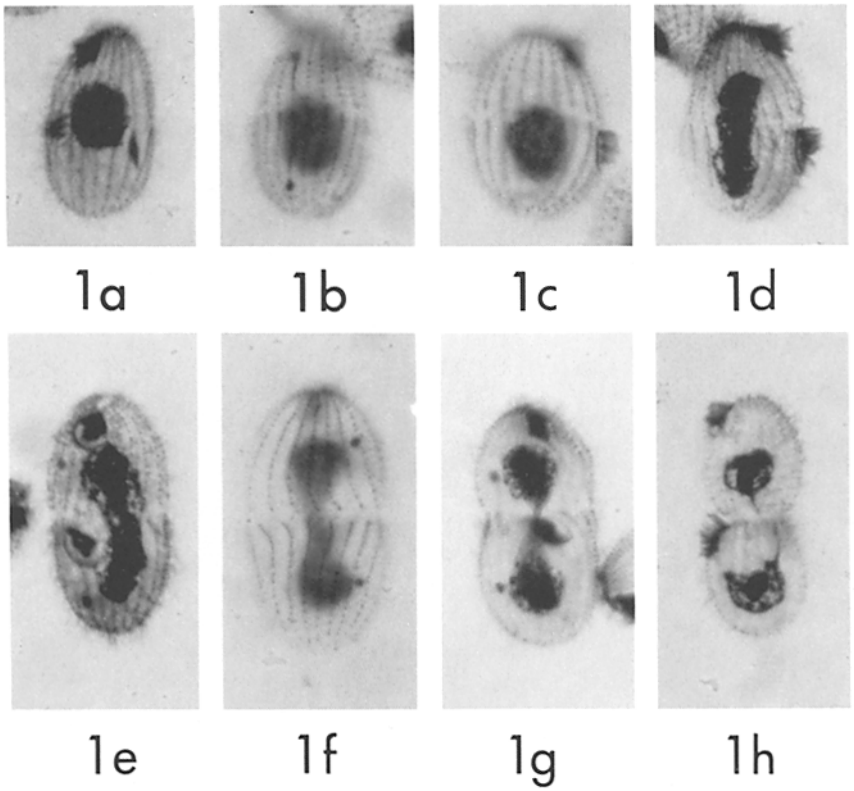

FIGURE 1 Photographs of cells of *T. thermophila*, strain C, approaching cell division. Stage 4: the micronucleus has moved away from the macronucleus and has formed a spindle; the macronucleus is spherical; the new oral apparatus possesses most of its major components (Fig. 1*a*). Stage 5: the macronucleus is still spherical (Fig. 1*b* and *c*) but elongates later into a cylindrical structure (Fig. 1*d* and *e*); the fission zone becomes more prominent. Stage 6: the macronucleus constricts to a thin strand (Fig. 1*b*); cortical and macronuclear constriction continues; the new oral structures become functional (Fig. 1*g* and *h*).

Some of the variability among the stage 6 cells must be accounted for by the time within the stage at which they were examined, but this temporal component accounts for only a fraction of the variability within stage 6 cells. In each strain the individual cells in the substages overlap broadly. Cells at the time of division do not have precisely the same number of basal bodies; in the *T. canadensis* strain, cells destined to divide within a few minutes have population estimates ranging between 385 and 576, i.e., differences as high as 20% on either side of the mean are apparent. Such large differences were not observed in the strain of *T. pigmentosa*.

Comparisons of Basal Body Numbers of Strains within Species

The largest sample of strains from a single species (Table V) is that for *T. thermophila* (syngen 1). The 11 strains examined give estimates ranging from 332 to 404 for opisthes at stage 6. Measurements of individual cells overlap extensively, even when the most dissimilar strains are compared. Nearly all the strain means lie within about one standard deviation (σ = 24) of the species mean (369). The variations between strains may be reasonably interpreted as the consequence of sampling errors combined with the substantial intrastrain variation. One should not be surprised at the similarities among these strains, however, for they all share some common ancestors in their laboratory derivation.

The next largest array of strains (syngen 5) does not include strains derived in the laboratory, however, but is composed of a sample of seven strains independently isolated from the wild. In this array, the strain means range somewhat

TABLE IV

Estimated Basal Body Populations in Opisthes at Finely Staged Intervals in Two Strains: Tetrahymena canadensis, Mating Type II and T. pigmentosa, Subspecies 6, Strain UM 1091

	T. canadensis			*T. pigmentosa*		
Stage	Basal body estimate	Substage mean	σ	Basal body estimate	Substage mean	σ
Early 5	436			370		
	391			320		
	413	406	29	330	358	34
	418			365		
	355			405		
	425					
Late 5	400			337		
	460			385		
	405	430	79	378	368	19
	594			385		
	402			375		
	355			370		
	391			347		
Early 6	415			333		
	387			390		
	480			390		
	465			430		
	537	467	47	350	379	38
	480					
	480					
	470					
	424					
	527					
Late 6	470			399		
	522			415		
	446	481	62	405	401	15
	576			400		
	456			415		
	515			375		
	385					

lower, from 315–387. This range is similar in extent and overlaps that observed in *T. thermophila*, but the species mean (352) is somewhat lower. One would have difficulty assigning a strain to one of these two species on the basis of basal body numbers.

T. pigmentosa is represented by five strains derived from two different subspecies (syngens 6 and 8). Although the means of the subspecies differ (400 vs. 437), the difference is not significant ($P = 0.11$), and a composite mean for the species (424) may be calculated. This mean is significantly higher ($P < 0.01$) than that for the first two species discussed, but estimates for some strains overlap.

T. capricornis is represented by three strains with a relatively high basal body number (481), significantly different from that of *T. thermophila* and *T. pigmentosa* ($P < 0.01$), though again, individual strain estimates show overlap. *T. capricornis* provides the highest estimate of mean basal body number among the 17 species surveyed.

Two other species are represented by as many as three strains: *T. pyriformis* (*sensu stricto*), phenoset A, and *T. lwoffi*, phenoset E. Both of these species are amicronucleate and asexual. Each is reasonably homogeneous with respect to strain differences but the two species are clearly distinct. The intraspecific homogeneity, like that for *T. thermophila*, may not be meaningful. In each case the three strains may have been derived in laboratories from a single wild strain (6). *T. furgasoni* has the lowest species mean for basal body number (234), and the lowest strain mean (201). Generally, amicronucleate strains have fewer basal bodies than do micronucleate strains, though one of the four amicronucleate species (*T. pyriformis*) has a slightly larger mean number (362) than two of the 13 micronucleate species (*T. cosmopolitanis* with 355 and syngen 5 with 352).

These studies demonstrate significant differences among species of this complex (Fig. 2), but the range of the species means is remarkably narrow from 234 to 474. Moreover, because of the large coefficient of variation for individuals within a clone, the confidence limits for the means of strains are large. Counting basal bodies is not an efficient means of classifying strains to species, and strain differences in numbers of basal bodies are not good genetic markers.

DISCUSSION

Intrastrain Variability in Basal Body Number

The observations summarized here disclose a considerable variation in the numbers of basal bodies present in cells of the same strain, at the same phase of the cell cycle, in fast exponential growth. The variation is not due exclusively to a difference in time within the cell cycle, for even finely staged cells show large discrepancies. Nor is the variability due to variations in the numbers of ciliary rows, for row number and basal body counts are not significantly correlated (25, 27).

The mechanism whereby the number of basal bodies is controlled is not known. Probably some

TABLE V

Estimates of Basal Body Numbers for Strains of 17 Species of the T. pyriformis Complex

Species	Strain	N	Strain mean	Strain σ	Species mean	Species σ
T. thermophilia	A1-17685K	5	362	42		
(syngen 1)	A1-178681b	5	332	18		
	B2-8685k	5	347	30		
	B2-8687a	5	353	23		
	B3-3683	5	404	21		
	C2-4682	5	378	20	369	24
	C3-368	15	404	29		
	D-1968	5	389	24		
	D1-17683	5	371	27		
	D1-17685	5	346	28		
	F1-16685	5	378	28		
T. americanis	III	5	468	28	434	48
(syngen 2)	VIII	5	400	40		
T. borealis	VI	5	400	24		
(syngen 3)						
T. cosmopolitanis	UM981	5	320	36	355	49
(syngen 4)	UM914	5	389	35		
Syngen 5	UM30	5	376	35		
	WF7	6	367	33		
	WV0	7	315	19		
	KP9	6	337	18	352	27
	WF2	7	360	32		
	WF1	9	387	30		
	WI1	6	325	28		
P. pigmentosa	UM1060	5	488	64		
(syngen 6)	UM1091	11	391	29		
	UM1147 or UI7152	5	442	19		
(syngen 8)	I	5	441	34	418	32
	II	5	359	49		
T. canadensis	UM1215	5	421	30	447	37
(syngen 7)	II	17	473	52		
T. tropicalis	TC-160	5	377	23	381	5
(syngen 9)	TC-89	5	384	32		
T. hyperangularis	EN-101	5	362	40	365	4
(syngen 10)	EN-10II	5	368	52		
T. australis (syngen 11)	AU-1-24	5	452	31	447	8
	AU-50-1	5	441	33		
T. capricornis	AU-$F_1$2	5	410	29		
(syngen 12)	AU-3-4	5	506	27	481	42
	AU-115-3	10	505	78		
Syngen 14	x3-AL	5	393	63		
T. pyriformis	GL-Iowa	5	345	19		
(phenoset A)	GL-Zeuthen	6	360	59	362	18
	H-CCAP	12	381	42		
T. elliotti	E-CCAp	9	326	20	317	13
(phenoset B)	GL-Eichel	5	308	18		
T. furgasoni	W-ATCC	4	201	5	234	47
(phenoset C)	GL-ATCC	4	268	17		
(phenoset D)	HSM-Cameron	5	408	33	371	52
	HS-Eichel	6	334	28		
T. lwoffi	Chs	12	262	38		
(phenoset E)	H-ATTC	4	281	15	282	20
	Gfj-CCC	5	302	19		

In a few cases, slide preparations that lacked complete strain designations were available. These were included in the analysis and are listed by mating type only.

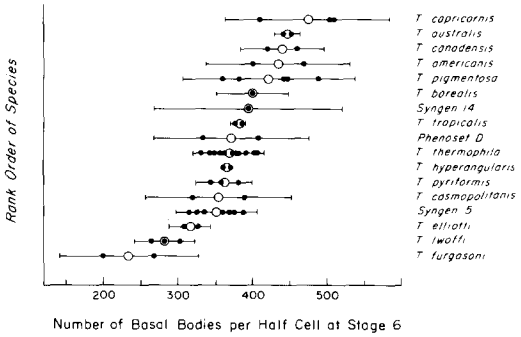

FIGURE 2 Comparison of basal body numbers in strains of 17 species of the *T. pyriformis* complex. The large white circles represent the species means; the small round circles represent strain means. The bars signify two standard deviations from the mean.

limiting cellular constituent, that is itself coupled to cell division regulators, also controls the number of basal bodies. The intercellular variation suggests that the critical cellular constituent is some component that is coupled to cell division only loosely.

Although any number of candidates for basal body regulators might be considered, the known variability of macronuclear DNA content (4, 9, 15) makes macronuclear DNA, or some component directly proportional to macronuclear DNA, a plausible candidate. Although macronuclear DNA replication is all-or-none in *Tetrahymena* (10) unlike in *Paramecium* (5), macronuclear division is only approximately equal, with an average difference in the DNA quantities of daughter cells of ~8% (8). Thus, although in normal growth the macronuclear DNA replicates once in each cell cycle, the amounts of DNA per cell do not remain equal, but gradually diversify. Variability in DNA quantity is constrained within certain limits, however; when these limits are reached, mechanisms are activated to restore DNA quantities to normal levels. When the DNA content falls below some threshold, the coupling between DNA synthesis and cell division is relaxed; two replication cycles occur before cell division occurs. In like manner, when cells obtain too large an amount of DNA (by segregational error or by fission blockage), two cell divisions may occur with a single DNA replication. The chromatin extrusion bodies often formed at macronuclear division probably provide an additional and more subtle way of reducing an excess DNA charge (11).

From the present perspective, macronuclear DNA content is a well-documented variable within a *Tetrahymena* culture, independent to a large degree of nutritional, cortical, or cell cycle variables. Any cellular organelle or component could plausibly be regulated in proportion to the amount of macronuclear DNA present and could for that reason show a cell-to-cell variation similar to that of DNA already documented. To postulate that basal body number is proportional to macronuclear DNA does not of course require that macronuclear DNA directly regulates basal body synthesis, only that some component proportional to macronuclear DNA is involved. Moreover, DNA replication and basal body production may be uncoupled under some special circumstances (30). Unfortunately, we are not now able to assess DNA content and basal body populations on the same cells.

The Narrow Range of Basal Body Numbers in Tetrahymena Species

The twofold range of basal body numbers here documented may seem to be a fairly broad range, unless one examines other kinds of differences among the species (26). The DNA base ratios of these species of the *T. pyriformis* complex span a range of ~8% for G + C/total bases (12). This range is about twice that found among all the vertebrates. Allen and Li (2) have reported that DNA-DNA reannealing studies show as little as 10% cross-annealling between some of these species, even when unqiue sequences are studied. Mitochondrial DNA's have completely different patterns following treatment with restriction enzymes, and may cross-anneal at less than 10% of the homologous level (20). The rDNA molecules of the different species can often be readily distinguished (19), as may be some of the histones (22). The structural proteins making up the epiplasmic skeleton of the cell show wide variations (31). Isozyme analyses (3, 6, 7) may show no common mobilities between strains of two species when as many as 10 enzyme activities are assayed.

All these evidences of molecular diversification argue for either an extremely remote common ancestor or very rapid molecular diversification. Yet certain features of all these sibling species have remained invariant or extremely conservative: the metabolic patterns, the nutritional requirements, the chromosome number, the cytoarchitecture (26). Also among these conservative features is the scale of the organism which, to-

gether with the others, contributes to the primordial "design" of a tetrahymena.

The scale of a cilium—once perfected—has persisted through numerous evolutionary transitions to give a structure possessing not only the same relative proportions of parts in a wide assortment of organisms, but also nearly the same absolute sizes of parts. In a similar manner, the larger organismic design of a ciliate has persisted through numerous molecular substitutions. We suggest that part of this *Tetrahymena* design is a scalar function, perhaps based substantially on the scalar design of its motile organelles. We cannot define precisely the selective pressures required to maintain the organismic scale—whether they involve velocities necessary to capture prey or to elude predators, to search for security, or to escape hostile environments. But the evolutionary persistence of scale strongly argues for its selective significance in this particular organic design.

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