Comparison of *Phyllomedusa trinitatus* albumin with the albumins of representative hylid frogs

Species tested	Immunological distance
Phyllomedusinae	
Phyllomedusa tarsius [a]	6
P. guttata	49
P. palliata	56
P. bucklei	58
P. lemur	61
Pachymedusa dachnicolor	127
Agalychnis callidryas	153
A. annae	163
Amphignathontinae	
Gastrotheca riobambae	> 200
Anotheca spinosa [b]	> 200
Hylinae	
Hyla regilla	172
H. chrysoscelis	145
H. arborea (Japan)	152
H. arborea (France)	155
Pseudacris triseriata	170
P. nigrita	175
Litoria aurea	170
L. booroolongensis	180
L. caerulea	180
L. verreauxii	176
Trachycephalus jordani	178

[a] Formerly *P. edentula*. W. E. DUELLMAN, Herpetologica *30*, 105 (1974); [b] This species has been shown to be a Hyline frog (MAXSON, in press).

large distance to both species of *Agalychnis* is similar to that seen between hyline and amphignathodontine subfamilies. If *Phyllomedusa* were erected to familial status, then *Agalychnis* should be considered a separate subfamily of this new family. This would not be in conflict with the traditional anatomical and behavioral information available on both of these genera. Before more definite molecular conclusions can be made, however, additional antisera would be needed to both *Pachymedusa* and *Agalychnis*.

The average distance of *Phyllomedusa trinitatus* albumin to albumins of both amphignathodontine and hyline frogs is nearly as large or larger than the distance seen between *Phyllomedusa* and *Agalychnis* albumins. This reinforces the suggested elevation to familial status of the Phyllomedusinae. The average distance to Australian *Litoria* is 175 units. This indicates there is not a close phyletic relationship between *Phyllomedusa* and *Litoria* as suggested by *Bagnara* and *Ferris*[7]. Rather the fact that some species of *Litoria* and the phyllomedusines have similar, unusual melanosome structure and pigment may be due to convergence or to retention of an ancestral condition in these different phyletic lines. Tests with antisera to representative hylines show that Australian *Litoria* and American hylines diverged some 60 million years ago[14] whereas phyllomedusine frogs diverged from the ancestor giving rise to the hylines about 100–110 million years ago, long before the divergence of the Australian and American hylines. Phylogenetic analysis of the Hylidae[8] showed *Litoria* to be a member of the hyline assemblage of frogs and the Phyllomedusinae to be cladistically remote from the hyline species. This would suggest the unusal melanosome structure and pigments arose independently.

Additional studies with antisera to representative hylid and bufonid species indicated phyllomedusine and hylid albumins are more different from one another than are hylid and bufonid albumins-bufonid species belonging to a separate family. The average phyllomedusine – hyline distance is 170 immunological distance units; the average hyline – *Bufo* distance is 155 units and the average phyllomedusine – *Bufo* distance is 196 immunological distance units[15]. Therefore, at the molecular level, the Neotropical leaf frogs appear more distinct from hyline frogs than the latter are from bufonid species, members of an independent family. Thus the phyllomedusine frogs also deserve independent familial status in the superfamily Bufonoidea, along with the Hylidae and Bufonidae.

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[15] L. R. MAXSON, work in progress.

The Size Distribution of *Tetrahymena* in Relation to its Position in the Cell Cycle[1]

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Summary. *Tetrahymena* size distribution during the cell cycle was analyzed by means of radioautography with the aid of a sonic-digitizer, and a computer. The study demonstrates that as the organism ages and passes through the various cell cycle phases the volume distribution of the organisms in each phase remains lognormal.

The volume distribution of an exponentially proliferating *Tetrahymena pyriformis* population exhibits a typical pattern. Being skewed to the right the distribution is best described by the lognormal frequency function in which the logarithm of the volume is normally distributed. This observation has already been adequately documented by various methods. *Tetrahymena* shapes were measured microscopically (JAMES[2], SCHERBAUM et al.[3], SUMMERS[4]), or with the aid of a Coulter Counter (SCHMID[5,6]). These methods, however, do not furnish information upon the volume changes of the organism as it passes through the various phases of the life cycle. The present study demonstrates clearly that, even in the various cell cycle phases known as G_1, S and G_2, volume distributions in a logarithmically proliferating population are lognormal. To achieve this objective, a novel method for the study of cell shapes with a computerized digitizer was utilized.

Materials and methods. *Tetrahymena pyriformis* mating type I (WH_6) of Syngen I (American type culture collection) were grown axenically at 27 °C following the

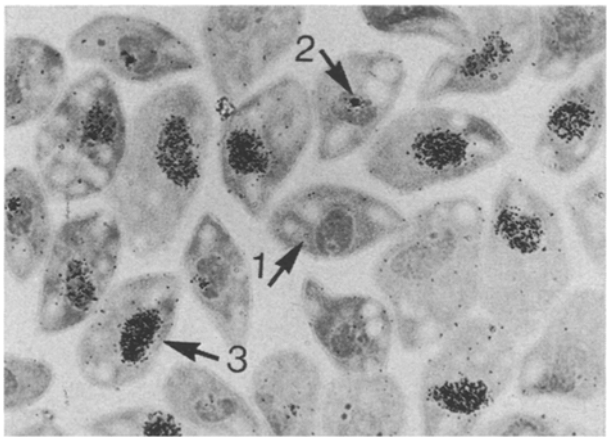

Fig. 1. Radioautogram of *Tetrahymena pyriformis* incubated with ^{3}H-thymidine for 10 min. 1. Nonlabelled cells. 2. Micronuclear labelled cells (S-1). 3. Macronuclear labelled cells (S-2). × 650.

method and medium as described by ELLIOTT et al.[7]. 24 h after the commencement of the logarithmic growth pattern, the organisms were incubated for 10 min in 5 μCi/ml thymidine-6-^{3}H (specific activity 27 Ci/m*M*, Amersham, England). The labelling was terminated by washing the organisms in nonradioactive medium. This was achieved by 3 successive 1-min centrifugations at 1000 rpm. Drops of nonradioactive medium containing the resuspended organisms were placed on subbed slides, air dried and fixed for 10 min with an alcohol-acetic acid fixative (1:3 v/v). The acid soluble fraction was then extracted by covering the slides for 5 min with ice cold 5% TCA and rinsed with 70% and 100% ethanol, and acetone.

[1] Acknowledgments. This work was supported by a research grant from Stiftung Volkswagenwerk No. 112273 to A. RON. The authors wish to acknowledge the technical help of Mrs. O. HOROVITZ and Miss S. URIELI, as well as the expert photomicrography of Mrs. E. SALOMON.

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[3] O. SCHERBAUM and E. ZEUTHEN, Expl Cell Res. *6*, 221 (1954).
[4] L. G. SUMMERS, J. Protozool. *10*, 288 (1963).
[5] P. SCHMID, Expl Cell Res. *45*, 460 (1967).
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The slides were then dipped into 1:1 diluted liquid radioautographic emulsion (Ilford K-5, England), stored in light tight boxes for 1 week, developed in Kodak D-19 developer for 3 min at 20 °C and fixed. The slides were then stained for 5 sec with toluidine blue (0.5% W/V). The organisms were photographed at 800 × magnification. The contours of each organism were traced from the pictures with the aid of a sonic digitizer (GRAF-PEN, Science Accesories Co.) and fed into a PDP-15/$_{20}$ computer in which the area enclosed by each contour was computed and stored. In this way, tracings of organisms in various phases of the cell cycle were obtained. The following criteria to determine the organism's position in the cell cycle were utilized. 1. Micronuclear labelling, S-1 (Figure 1). 2. Macronuclear labelling, S-2 (Figure 1). 3. Unlabelled cells, G-1 and G-2 (Figure 1). The area distribution of each group was computed and its shape evaluated. To study the shape of the distribution of all the organisms, the various 'phase' distributions were added up.

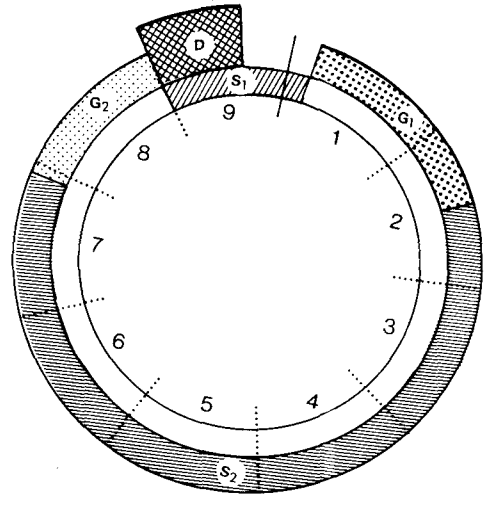

Fig. 2. The cell cycle of *Tetrahymena pyriformis*. The inner and the outer circles represent the micronuclear (S-1) and the macronuclear (S-2) cycle respectively. The numbers mark the hourly intervals. S-1, micronuclear DNA synthesis; S-2, macronuclear DNA synthesis; G-1, pre-macronuclear DNA synthetic phase; G-2, post-macronuclear DNA synthetic phase; D, cytokinesis.

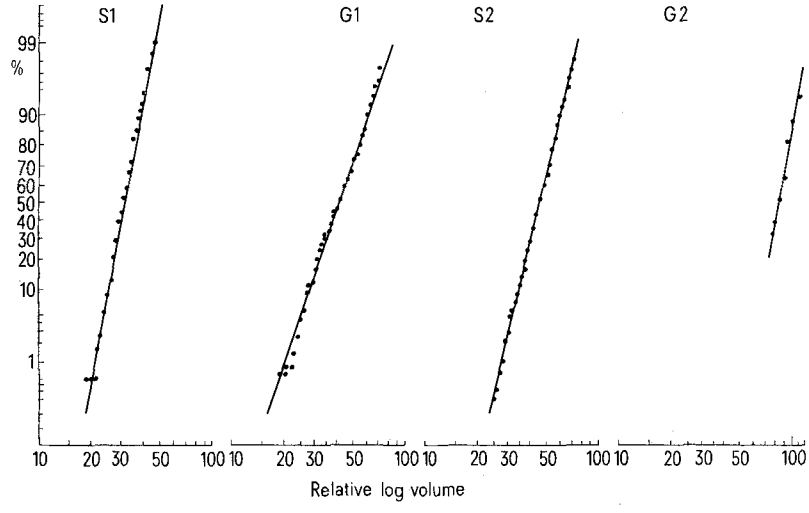

Fig. 3. Cumulative lognormal volume distribution of *Tetrahymena pyriformis* in various phases of the cell cycle. Experiment No. 1. S-1; S-2; G-1; G-2; explained in Figure 2.

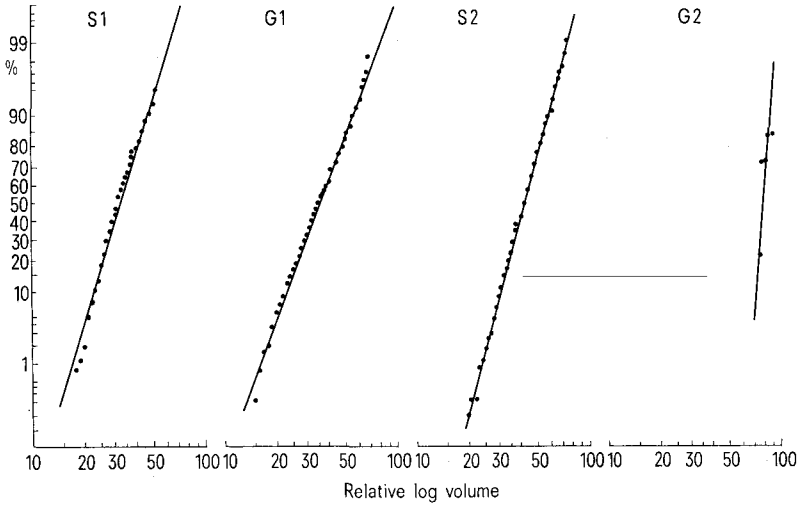

Fig. 4. Cumulative lognormal volume distribution of *Tetrahymena pyriformis* in various phases of the cell cycle. Experiment No. 2. S-1; S-2; G-1; G-2; explained in Figure 2.

Results. *Tetrahymena pyriformis* mating types exhibit 2 structurally differentiated nuclei: a diploid micronucleus and a polyploid macronucleus. Both synthesize DNA at different phases of the cell cycle which do not overlap in time. In the micronucleus DNA synthesis (S-1) occurs during cytokinesis, where it proceeds at a relatively fast rate, whereas in the macronucleus DNA synthesis (S-2) proceeds for about $5^1/_2$ h (Figure 2).

In the autoradiogram, the organisms in S-1 phase are easily distinguishable from those in the S-2 phase (Figure 1). The nonlabelled organisms belong either to the G-1 or to the G-2 phase group. The measurements were made upon 3000 organisms in logarithmic growth phase whose kinetic parameters are summarized in the Table. The S-1 and S-2 cells exhibit unimodal lognormal volume distributions (Figures 3 and 4). The mean volume of S-2 is greater than that of S-1 (Table). The nonlabelled cells volume exhibit a bimodal volume of distribution in which the 2 peaks were separated by a trough reaching the abscissa. Both distributions were then separated at the point of minimum of the trough, and studied separately (Figures 5 and 6). The majority of the nonlabelled cells belong to the distribution with the smaller mean value and belong therefore to the G-1 group. The minority are the G-2 cells. Both distributions are lognormal (Figures 3 and 4). The smaller peak representing the G-2 cells constitutes only 1% of the cell cycle which seems too small, since in other experiments the G-2 comprise a higher percentage of all cells (PRESCOTT et al.[8]). This apparent diminution of the G-2 cells resulted from the fact that the experiment had not been terminated abruptly. In order to achieve a uniform spreading out of the organisms on the slides, they were allowed to dry out slowly for 90 min. During this period most of them continued their procession through the cell cycle, divided and joined the G-1 cells, a process which led to a relative depletion of the G-2 cells. If we take this additional life span into account, the true G-2 fraction size, under our experimental condition, is about 12% (Figure 2).

The various distributions which were depicted separately are presented in Figures 5 and 6. Although all the distributions overlap, their means differ clearly (Table). The pooled distributions were found to be lognormal as well (Figure 7).

Kinetic parameters and log mean volumes of *Tetrahymena pyriformis* in various phases of the cell cycle (expressed in relative units)

Experiment No.	Phase	Cell No.	Cell (%)	Mean	Log mean	Log SD
1	S-1	193	16.02	31.5	1.363	0.0707
	G-1+G-2	376	31.20	42.0	1.500	0.1425
	S-2	636	52.78	45.5	1.530	0.0987
	Total cells	1205	100.00	31.3	1.495	0.1248
Measured G–2 fraction			1.3			
Estimated G–2 fraction			12.0			
2	S-1	273	14.94	32.5	1.384	0.1203
	G-1+G-2	545	29.83	36.0	1.439	0.1602
	S-2	1009	55.23	42.0	1.498	0.1217
	Total cells	1827	100.00	28.6	1.457	0.1417
Measured G-2 fraction			1.0			
Estimated G-2 fraction			12.0			

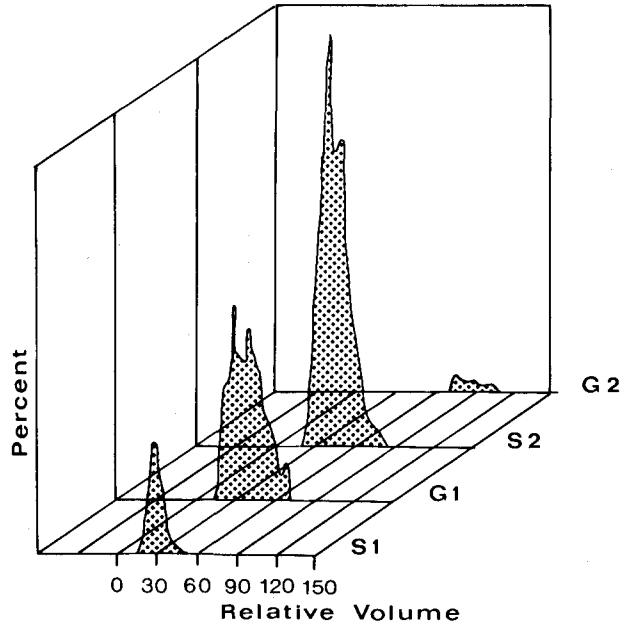

Fig. 5. Volume frequency distribution of *Tetrahymena pyriformis* in various cell cycle phases. Experiment No. 1. S-1; S-2; G-1; G-2; explained in Figure 2.

Discussion. In biology, the lognormal distribution function occurs relatively frequently (KOCH[9,10]), and has been used as a convenient distribution to describe various phenomena. In logarithmically proliferating organisms, the lognormality is directly associated with their exponential growth. SCHERBAUM et al.[11]), who studied the theoretical relationship between cell growth and their volume distribution in the *Tetrahymena*, arrived at the conclusion that even a linear volume growth in an exponentially proliferating cell population generates a lognormal volume distribution. The reason for this relationship is the age distribution of the organisms which is also exponential. In such a distribution, the number of young and small organisms is twice that of old and large organisms. The present study advances this argument even further. Since in each cell cycle phase the age distribution of the organisms is exponential, it seems natural that their volume distribution is lognormal (Figures 3 and 4).

In view of the present method in which the area of the organism was traced and computed, one is faced with the question as to whether the distributions so generated reflect the true shape volume distributions similar to those determined with the Coulter Counter. Actually each cell could be viewed as a cylinder whose base is the cell area 'S' and height the cell width '*h*'. Its volume V would be $V = hS$. Provided that h is constant, if S is distributed lognormally, hS has to be distributed lognormally also. By spreading the organism on subbed slides as described above, the variability of h is minimized and for all practical purposes could be viewed as constant. Since the distribution of all the organisms pooled together (Figure 7) exhibits a typical lognormal distribution, like any other *Tetrahymena* volume distribution measured electronically, the variability of h has to be low, otherwise the pooled distribution would deviate markedly from lognormality.

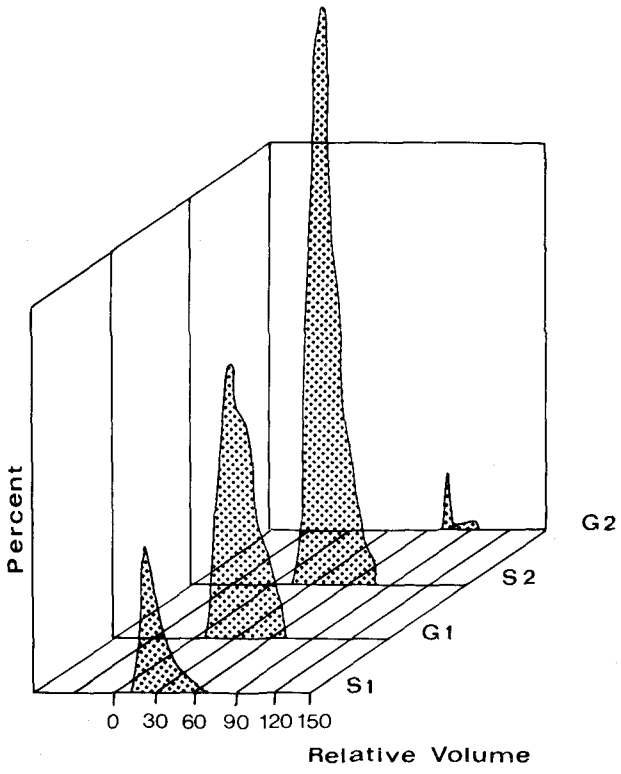

Fig. 6. Volume frequency distribution of *Tetrahymena pyriformis* in various cell cycle phases. Experiment No. 2. S-1; S-2; G-1; G-2; explained in Figure 2.

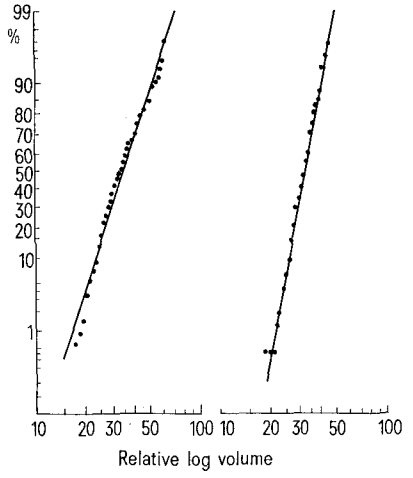

Fig. 7. Cumulative lognormal volume distribution of *Tetrahymena pyriformis*. Left figure: Experiment No. 1. Right figure: Experiment No. 2.

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Enhancement of Fe Absorption by Mn in Rice Roots (*Oryza sativa* L.)

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Summary. The rates of absorption of Fe by excized rice roots were measured in the absence and presence of different concentrations of $MnSO_4$. Fe absorption from 0.1 and 5 m*M* $FeSO_4$ was enhanced by $MnSO_4$ at concentrations above 0.1 and 5 m*M*, respectively.

Fe and Mn are micronutrients essential for plant growth, and because of their close chemical relationship, they play a significant role in their mutual absorption by plant roots. Mn absorption is generally inhibited by the presence of Fe[1,2], and there is evidence that Mn interferes with Fe utilization in chlorophyll synthesis rather than Fe trans-

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