chromosome, for example, consists almost entirely of constitutive heterochromatin while chromosome 12 and the *X* chromosome both possess interstitial C-bands halfway along their long arms, and chromosome 1 has a small C-band at the distal tip of the short arm. These variations in constitutive heterochromatin content provide an additional means of identifying these chromosome pairs.

It is also common to find the C-bands differ in size between homologues. This is particularly true of chromosome pair No. 13, which showed a heteromorphism in at least 4 out of the 9 animals studied (e.g. Figure 3). More work is required to determine exactly how widespread this polymorphism is, and how much other variation there might be in the horse karyotype.

The Phylogenetic Status of Phyllomedusine Frogs (Hylidae) as Evidenced from Immunological Studies of their Serum Albumins[1]

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Summary. Based on immunological comparisons of the serum albumins of phyllomedusine frogs with both hyline and bufonid species, it is suggested that phyllomedusine frogs be erected to familial status within the superfamily Bufonoidea.

The Neotropical leaf frogs, Phyllomedusinae, consist of three genera; *Phyllomedusa* (31 species), *Agalychnis* (8 species), and the monotypic *Pachymedusa*. These frogs constitute a distinct phyletic line in the family Hylidae. All frogs tested in this subfamily are unique among other hylids in possessing large amounts of powerful bradykinin-like and physalaemin-like polypeptides in their skin[3]. All species in this subfamily are also characterized by having vertical pupils, diploid chromosome complements of 26, arboreality, and moderately ossified skulls (with or without the dermis co-ossified with the skull). All members deposit their eggs on vegetation over water, into which the hatchling tadpoles drop. The aquatic tadpoles are unique in having a sinistral spiracle lying ventrally on the midline[4]. Consideration of the above characteristics led DUELLMAN[5] to recognize these three genera as a separate hylid subfamily. DUELLMAN's decision has been confirmed by recent biochemical studies which have shown that at least some phyllomedusines, and no other hylids, excrete uric acid rather than ammonia and urea as do most anurans[6]. Additionally, further studies on phyletic affinities of phyllomedusine frogs have suggested these frogs may be closely related to at least some of the Australian hylids (*Litoria*). 2 of 5 species of *Litoria* tested were found to possess large fibrous melanosomes, containing a novel red pigment, which were formerly identified as unique to Neotropical leaf frogs[7].

My interest in phyllomedusine frogs arose during studies of albumin evolution in the anuran superfamily Bufonoidea[8]. These studies with albumins of both hylid and bufonid species suggested that the phyllomedusine frogs should be erected as a proper family, perhaps intermediate between the Hylidae and the Bufonidae. Indeed, earlier serological studies by CEI[9], involving precipitin tests with short term antisera to whole serum, led him to suggest the Phyllomedusinae might represent an independent phyletic branch arizing from some undifferentiated Hylid-Bufonoid stock.

Materials and methods. Antisera to pure albumin from a single specimen of *Phyllomedusa trinitatus*[10] was made in 4 male New Zealand white rabbits over a three-month immunization schedule. The individual antisera were tested for purity and pooled according to published procedures[11]. As sources of albumin, plasma or skeletal muscle preserved in a phenoxyethanol solution[12] were used. Microcomplement fixation studies with the albumins of representatives of all three phyllomedusine genera as well as with other hylid and bufonid species were performed. Results are reported as immunological distance units. For anuran albumins 1 unit of immunological distance between 2 species represents roughly one amino acid difference in the albumins of these 2 species[13].

Results and discussion. The Table summarizes the results of tests with antiserum to *P. trinitatus* albumin. All available species of *Phyllomedusa* form one immunological cluster with a range of 0–61 units. This is the order of magnitude seen between species of *Gastrotheca* (Hylidae: Amphignathodontinae), between North American *Hyla* species (Hylidae: Hylinae), and between species of *Bufo* (Bufonidae)[8]. *Pachymedusa dachnicolor* is of interest since, when first described by COPE in 1864, the species was placed in the genus *Agalychnis*. It led a spotty history of transfer from *Agalychnis* to *Phyllomedusa* until 1968 when DUELLMAN proposed its independent generic status. Immunologically *Pachymedusa* albumin appears more distant from *P. trinitatus* than any species of *Phyllomedusa* but not quite as distant as both species of *Agalychnis* available for this study. Thus at the molecular level *Pachymedusa*'s generic status is also justified. The

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[3] J. M. CEI and V. ERSPAMER, Copeia *74* (1966).

[4] W. E. DUELLMAN, *The Hylid Frogs of Middle America*, Monograph of the Museum of Natural History (University of Kansas 1970), vols. 1 and 2.

[5] W. E. DUELLMAN, Univ. Kansas Publ. Mus. nat. Hist. *18*, 3 (1968).

[6] V. H. SHOEMAKER and L. L. MCCLANAHAN, JR. J. comp. Physiol. B *100*, 331 (1975).

[7] J. T. BAGNARA and W. FERRIS, Copeia *83*, 592 (1975).

[8] L. R. MAXSON, Ph. D. thesis Univ. of California, Berkeley and California State University, San Diego (1973).

[9] J. M. CEI, Bull. Serol. Mus. *30*, 4 (1963).

[10] A generous gift of G. E. DREWRY.

[11] L. R. MAXSON and A. C. WILSON, Syst. Zool. *24*, 1 (1975).

[12] L. KARIG and A. C. WILSON, Biochem. Genet. *5*, 211 (1971).

[13] L. R. MAXSON and A. C. WILSON, Science *185*, 66 (1974).

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.

[14] L. R. MAXSON, V. M. SARICH and A. C. WILSON, Nature, Lond. *255*, 397 (1975).
[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