Trajectories of Cell Volume Distributions during the Growth Cycle of *Tetrahymena*

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An empirical method is introduced to study changes in volume distributions of populations of cells as they progress through the batch growth cycle. Batch cultures of the ciliate protozoan *Tetrahymena* follow a simple circular growth trajectory when analysed by this method.

INTRODUCTION

The study of volume distributions of cells grown in laboratory culture is important for quantitative understanding of the nature of the morphological and physiological changes which occur in growing populations of cells (Cameron, 1973; Craigie & Cavalier-Smith, 1982). Several attempts have been made to model the volume distributions of cells, either at single points in the asexual growth cycle (as in cells growing exponentially in continuous culture), or at different phases during the batch growth of large populations (Koch & Schaechter, 1962; Powell, 1964; Roberts, 1980). These modelling attempts, based on simple assumptions about the binary fission process, have been successful only in very limited situations, such as the hypothetical state of balanced growth when the volume distribution has reached a steady state (Campbell, 1957; Hamburger & Zeuthen, 1957; Leick, 1967; Johnson, 1968; Cameron, 1973). Cell volume distributions of single-celled organisms such as the ciliate protozoan *Tetrahymena* do not follow a single family of curves, such as the log-normal or gamma distributions, as the cells progress through the growth cycle, even though during the middle of the cycle exponentially growing cells have log-normally distributed volumes (Cameron, 1973). Rather, the shape and character of the distributions change in different phases of the growth cycle. Furthermore, there are practical problems in obtaining good estimates of the dispersion parameters even of mid-exponential distributions (Roberts, 1980). I report here a simple, purely empirical method for studying changes in shape of cell volume distributions that does not require knowledge of the exact distributional forms or their parameters.

METHODS

Cells of *Tetrahymena elliotti*, strain L1630/1c of the Culture Centre of Algae and Protozoa, Cambridge, were grown at 27–29 °C in axenic culture on a nutrient medium of proteose peptone (Oxoid; 20 g l⁻¹) and yeast extract (Oxoid; 0.1 g l⁻¹) in distilled water. In order to assess inherent variations in cell size distributions, three subclones of this amicronucleate, and therefore asexually reproducing, clonal strain of *T. elliotti* (formerly *T. pyriformis* GL, phenoset B: Nanney & McCoy, 1976) were grown in shaken batch cultures and sampled periodically. One subclone (clone 1) was further subdivided (but not recloned) as an additional replicate. In addition, similar batch cultures of other *Tetrahymena* strains were used for comparative purposes. These were: the two different mating types of *T. thermophila* (formerly *T. pyriformis*, syngen 1), strains 30007 and 30008 of the American Type Culture Collection, Rockville, Md., U.S.A. (grown at 26 °C and 24 °C, respectively); *T. pyriformis*, syngen 5 strain UM30 (grown at 25 °C); *T. corlissi* strain 1–2 (grown at 25 °C); and *T. vorax* strain TETRA 2 (grown at 25 °C).

For all cultures 1 ml of late-stationary phase (240 h) test tube stock culture was inoculated into 200 ml of sterile medium in 500 ml conical flasks on a reciprocal shaker. Samples (10 ml) were diluted with 10 ml of freshly made sterile-filtered saline (6.5 g NaCl l⁻¹) and immediately counted and sized with a Coulter counter (model Fn) and Channelizer (model C1000) with 100 channels (Coulter Electronics, Harpenden), using a probe with 200 μm
orifice diameter and uniform settings throughout (amplification, 1; aperture current, 128; window width, 100; basal threshold, 4; sample volume, 0.5 ml). The midpoint of channel 1 was 112 μm² and each channel was 220 μm² wide. As hydrodynamic focusing was not used, there was a uniform systematic bias in the volume determination from this apparatus (Roberts, 1980), but this should not have affected purely comparative analyses.

Because each volume distribution consisted of 100 points, each giving the frequency of counts in a certain size interval, a method was needed to parsimoniously explain this 100-dimensional variability in an analytical space of lesser dimensionality. Principal components analysis was used for this purpose, especially because of the high serial correlation among adjacent channel counts.

Whenever data can be represented as a number of points in multidimensional space, where each dimension is a measurement of some attribute of the individual data point, it is useful to seek a representation of lesser dimensionality. This may be accomplished, for example, by discarding certain attributes and combining others. Principal components analysis provides a unique and rigorous way to accomplish this, and it has been found to be useful in a broad variety of applications. In this method, a matrix is formed which consists of either the correlations or the covariances between each pair of measured attributes. This matrix forms the basis for subsequent analysis, whereby the latent roots (eigenvalues) and vectors of this dispersion matrix are calculated by solving a simple matrix equation using standard iterative procedures (Tatsuoka, 1971; Gates & Berger, 1974). This representation has the advantage of providing a uniquely determined, repeatable representation of the data, in which the derived components are well-defined linear combinations of the original variables.

By examining the correlations between the original variables (in this case, channel counts) and the new component axes, it is possible to interpret the relative contribution of the variables to the particular linear combination of these which constitutes each of the principal components. Indeed, when the correlation matrix is the basis of the analysis, the coefficients in that linear combination are just the desired correlations themselves (Tatsuoka, 1971). Often, one set of variables will show high positive correlations with a principal axis, while another set will show high negative correlations; in that case, the component is said to represent a contrast between the two sets of variables. Other variables may show very low and effectively zero contributions to an axis.

The major axes of variation in the data are found uniquely and in such a way that the first principal axis accounts for most of the variation in the data set (as measured by the sum of the diagonal elements of the original matrix), while the second axis is orthogonal (i.e., uncorrelated and independent) to the first and accounts for the next largest contribution to the original variation, and so on. In this way, the data are rigidly rotated so that most of the useful variation along the original 100 variable axes may be accounted for in only a few dimensions, each of which is a unique linear combination of the original variables. The great amount of redundancy in the full 100-channel distribution can thus be reduced to a representation in which each point corresponds to an entire volume distribution, as before, but where there are only a few axes.

RESULTS AND DISCUSSION

Typical variations in the shapes of cell volume distributions for axenically grown batch cultures of Tetrahymena are shown in Fig. 1 (based on clone 1A). The mean cell volume decreased as the culture passed from early-exponential phase (when there were few cells and the distribution was broad and necessarily more irregular) towards late-exponential phase and into stationary phase, when there was a narrow and more uniform distribution of small cell volumes (corresponding to the thin, elongate, and starved condition of the cells). Figure 2 gives the exponential segment of the growth curves for all subclones. These subclones had growth rates of 0.109, 0.153, 0.138 and 0.154 h⁻¹, respectively.

Principal components analysis was done for the subclonal experiment (see Fig. 3). Component I accounted for 64% of the variance in the original data, while component II accounted for another 21%. Thus, 85% of the original variation was along the first two principal component axes, while 90% of that total variation was accounted for by three principal axes; hence, very little information was lost by the procedure, which started with 100, albeit highly correlated, axes. Furthermore, the two-dimensional representation did not distort the three-dimensional picture very much: the top, beginning end of the band of points was elevated above the plane of the paper, while the left, terminal end lay below.

The composition (loading pattern) of the first component indicated a contrast between the magnitudes of the first third of the size channel distribution (i.e. the relative number of smaller volume cells) with the remainder, while the second component contrasted the middle channels with the two ends. Loosely speaking, this means that distributions with high peaks in the early channels (e.g., stationary phase phase cultures with many small cells) lay at the left of component I,
Growth trajectories in *Tetrahymena*

Fig. 1. Relative frequency distributions of cell volumes during batch culture of *T. ellioti*. The distributions illustrated are taken from the data for clone 1A at 22 h (●), sample size, N = 1310 cells and mean cell volume, MCV = 9440 μm³; at 46 h (□), N = 19605, MCV = 8979 μm³; at 65 h (▲), N = 61281, MCV = 7893 μm³; at 165 h (○), N = 143508, MCV = 4707 μm³. Superpositions along the baseline are indicated by the last-occurring symbol.

Fig. 2. Exponential segment of batch growth curve for four subclones of *T. ellioti*: clone 1A (●), clone 1B (▲), clone 2 (■), clone 3 (▲). Volume distributions for three samples of subclone 1A (○) are shown in Fig. 1.
Fig. 3. Principal component representation of volume distribution trajectory for four subclones of *T. elliotti* during axenic batch growth: clones 1A (●), 1B (▼), 2 (□) and 3 (▲). The analysis was based on the correlation matrix among the channel counts for the relative frequency distributions. Each clone was sampled at 18, 20, 22, 40, 42, 44, 46, 63, 65, and 165 h. Some of these times are labelled for subclone 1A. The volume distributions corresponding to certain samples (○) of that subclone are given in Fig. 1. All of the subclonal trajectories begin at 18 h in the upper left, circle clockwise during exponential growth, and terminate at the left at 165 h. Also shown is a fermenter culture (■) of the same strain that progressed, also clockwise, from 17 h to 65 h. This culture was obtained by aerating and stirring a batch culture at 25 °C.

while distributions dominated by larger cells were toward the right. Along component II, the contrast was between the relative importance in the distributions of medium-sized cells and those at the extremes of size.

It was now possible to visualize the complex changes in shape of the volume distributions in a simple manner. As the culture progressed through the growth cycle, the volume distributions followed an approximately circular trajectory around distribution space. Clearly, there was more variation in the initial stages of growth than in the mid-exponential portion of the curve. This was due to the small numbers of cells present in early batch growth and their different lag times to division. Thus, the delay in clone 2 was evident in the figure. By 22 h it had not yet advanced to the same stage as the other clones, even though it was of comparable density (see Fig. 2), but by the time of the next sampling, at 40 h, it had caught up.

All cultures ended their trajectories at a similar point in stationary phase, at 165 h. Because the inoculum for each subclone was derived from a late-stationary phase culture, it is likely that the circle would have been closed if the cultures had been either followed longer or sampled earlier.

Replicates followed very similar trajectories (Fig. 3), and thus it was possible, given only a single sample from a culture grown under identical conditions, to determine from the position of its volume distribution along the trajectory, what phase of the growth cycle the culture was in. This required only the determination of the volume distribution in the sample and the calculation of at most a few linear combination formulae derived from the original analysis of similar cultures.

Furthermore, the effect of various factors on, for example, the rate of progression of a culture through the growth cycle, could be determined once the reference conditions of growth had been analysed. New conditions could simply be projected into the reference space to see their effect. For example, it was clear from the fermenter trajectory (Fig. 3) that aeration led to a much more rapid progression through the growth cycle, with cell volume distributions being already in a late-exponential part of trajectory space when first sampled at 17 h, and already in stationary phase by 65 h, when shaken batch cultures were still in exponential growth. The method could also be used to compare, at a glance, the power of general theoretical models of cell size distributions to match actual growth trajectories over the whole cycle.
Growth trajectories in Tetrahymena

The method outlined was quite robust in practice. In the example shown in Fig. 3, virtually identical results could be obtained by using, e.g., 100, 50, or 33 channel dimensions. This property was the result of the high serial correlation of counts in adjacent size channels. Figure 4, based on 50 channels (obtained by combining adjacent pairs of channel counts), shows the results of enlarging the reference analysis to include several other strains of Tetrahymena, sampled at various times during axenic batch growth. In this analysis, the first component accounted for 64% of the total variation, while the second accounted for 20%. Compositions by channel number were similar to the analysis of Fig. 3. This plot demonstrated that the basic circular trajectory of the original data was retained, that other strains of the same species complex fell along the same path, but that a species of quite different biology, T. vorax, lay off that trajectory. The latter species, for example, undergoes transformation from a typical small-mouthed (microstome) form to a large-mouthed (macrostome) carnivorous form (Buhse & Cameron, 1968).

The present survey supports the hypothesis that all the members of the 'Tetrahymena pyriformis' complex of syngens (Corliss, 1973), or biological species, and the several related amicronucleate strains will follow the same trajectory under the same conditions of cell growth, while members of the other subgenera (and particularly the macrostome-forming species) will show different patterns of change in cell volume distributions during the growth cycle. Confirmation of this hypothesis would require more samples of entire growth trajectories for the different species.

The stability of the indicated trajectories needs to be examined with respect to different conditions of batch growth, in media containing bacteria as well as in simple nutrients, and in chemostat cultures. For example, it is expected that different temperature regimes would only speed the progress of a culture through the trajectory and not alter greatly the trajectory itself, whereas cultures containing bacteria should follow a different set of trajectories. As noted above, the method also provides a convenient way of studying the effects of toxicological and other agents on the normal growth trajectories of a given strain. Cells in different morphological and physiological states can achieve similar densities and growth rates, and thus appear to be at the same state of the growth cycle. The analysis of growth by means of cell volume trajectories provides a more sensitive technique for detecting subtle differences in the condition of cultures, and is applicable to chemostat as well as to batch cultures.
Previous morphometric work with *Tetrahymena* strains has shown that the average multivariate cell structure during comparable phases of the growth cycle differs characteristically among different strains (Gates & Berger, 1974), that such differences are temporally stable (Gates & Berger, 1976), and that cell morphologies in principal component space follow different trajectories for growth with bacteria as opposed to axenic growth (Taylor et al., 1976). Those studies involved measurements of large numbers of individual silver-stained specimens, which were difficult and tedious to perform, but showed that much greater variation existed in the distributions of linear cell dimensions than in the distributions of cell volumes. It may be that cell size, which is an integrated, composite property of the entire set of cell dimensions, is under tighter control developmentally as well as genetically, and is more subject to the regulating influences of natural selection. Indeed, it is known that there is a good correlation between cell size and cell growth rate, at least over a broad range of cell types (Taylor & Shuter, 1981). The method proposed here should enable a more detailed study of this question, within a single cell type, to be undertaken. Furthermore, by identifying precisely the various substages of the growth cycle, such trajectory analyses will permit a finer dissection of the nature of morphological changes which occur in populations of cells during the growth cycle.

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REFERENCES


