*Symmetry: Culture and Science*
*Vol. 4, No. 2, 1993, 151-159*

# ASYMMETRY IN THE DEVEVELOPMENT OF THE *CAENORHABDITIS ELEGANS* AND ITS EVOLUTIONARY IMPLICATIONS

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**Abstract:** *The spatiotemporal unfolding of the* Caenorhabditis elegans *has been reconstructed by computer. Real developmental parameters have been estimated for the organization of temporal and spatial asymmetries. A temporal asymmetry in cellular birth-death processes, and spatial asymmetries in nuclei and in the distribution of cell division axes have been established. These can affect the morphogenesis and the relative evolutionary invariance of the worm.*

## 1. THE PROBLEM

The primary aim of this project is to construct a model for a star worm, representing the minimally essential explanatory characteristics of a small multicellular developing organism from zygote state in a tractable form. As it is well known, there exist models for *Drosophila* development, but, strangely, models are lacking for a simpler worm. There are many reasons for constructing a model for a small animal such as *C. elegans*. We list some of them.

A current tendency in modelling developing organisms is to increase the number of model parameters, making them irrealistic, untractable, using sometimes more than ten or twenty 'in principle measurable' quantities. There is a chance to reverse this tendency in the following way. First, we have put the whole cell lineage of *C. elegans* into computer. By extracting real, estimated developmental parameters from the real cell lineage, it is possible to incorporate data into models to make them more realistic, than in the numerical solutions of complicated developmental models. In other words, our expectation is that using more realistic developmental parameters instead of pure, ad hoc numbers for numerical simulations, we get more realistic representations for developing organisms.

There are many unresolved questions, which may be answered or approximated from a different angle on the basis of estimated or computed properties of the real cell lineage.

What is the origin of the 'wormness' or the worm body plan? Sydney Brenner (cf. Lewin, 1984) says that in the beginning of development there is a noisy worm, becoming progressively refined, more worm-like later. This problem can be investigated using cell lineage.

What is a reconstructed morphogenetic field? The heritage of experimental embryology (Hamburger, 1988) has not given a clear answer. The possibility of the reconstruction of a deformation field describing the individual cell displacements (or some marker of it represented by the nuclei) may clarify important morphogenetic problems. If there exists an underlying morphogenetic field generating spatiotemporal unfolding of the cell lineage, then we can have more insight into the nature of the spatial and temporal organization of cell lineage.

What are the competitive and cooperative connections between sublineages? (Cf. theories of Buss, 1987 and Arthur, 1988 on the evolution of development, and their criticism). Induction and lateral inhibition have been shown to control complex intercellular signalling and pattern formation (Horvitz and Steinberg, 1991), without establishing the relative cell division rates in the whole set of sublineages.

How is the cell lineage organized into cell layers or shell structures? The study of the mechanical and mechanochemical basis of morphogenesis (D'Arcy Thompson, 1917; Huzella, 1933; Wainwright *et al.*, 1976; Odell *et al.*, 1981; Newman and Comper, 1990) can be extended to the investigation of a whole developing multicellular organism. The folding of cell layers, the dynamic cell patterns, the interactions of generic and genetic mechanisms should reflect the whole developmental history in the language of cell lineages.

## 2. THE DATA

Three classes of data were measured on the basis of Sulston *et al.* (1983, Figs. 3, 8a, b, c, and Appendix) and Sulston *et al.* (1988, Appendix 3, Part A: Figs. 1, 2, 3, 4, 5, 6, 7, and 8). The measured data from the drawings were as follows:

1) The distances between the nuclei of the cells in three dimensions. We know that these 'data' represent only neighbourhood relations. However, for the estimation of the relative displacements the positions of the nuclei provide sufficient information.

2) The lifetime or duration of the individual cells.

3) Finally, the largest and the smallest measures (diameters) of the nuclei.

The most relevant spatial information is the neighbourhood relations. The estimated error of the measurements is 10 $\mu$m, which is negligible as compared to the uncertainty of the original data presented in the two Sulston *et al.* papers.

In the computation some inconsistencies were found among measurements coming from different drawings (sometimes in positions, sometimes in lineages); the origin is still unknown. For safety, these data were omitted from analysis, affecting cca. 20 per cent of the points. The computations on the cell lineage have been done until $L1$ stage, containing some 550 cells.

## 3. COMPUTATIONS ON THE *C. ELEGANS* CELL LINEAGE

Up to now two classes of computations have been done. The first concerns cellular birth-death processes. Figure 1 shows a temporal asymmetry in the kinetics of birth-death processes. There is a maximum of lifetime of cells around the tenth generation. Note that cells surviving the $L1$ stage have been omitted.

Figure 2 shows the directions in cell cleavage. Obviously, the relative angles of cell division planes (either between neighbouring or ancestor and decdendant cells) must play a very important role in morphogenesis. For these angles a definite statement exists in the literature (Hyman and White, 1987), that "there are two different patterns of cleavage during early embryogenesis": in one class of cells the division axes keep their original directions in successive divisions, while in the other the pattern of successive division planes is orthogonal. We test this observation in the total sample of data mentioned above.

Let us introduce some notations. We have a grandmother cell, with two daughters, connected by a line. Both mother cells end up in two-two daughters, connected by a pair of lines. The angle between any of these two lines and the preceding one will be denoted by $\theta_i$, where $i$ stands for the pairs of the granddaughters. Then in the whole sample $\theta$ has a distribution $f(\theta)$, normalized to unity.

The zero hypothesis is that the cleavage directions in generation $x+1$ are independent of those for the ancestors in generation $x$. Then $f(\theta)$ is spherically symmetric.

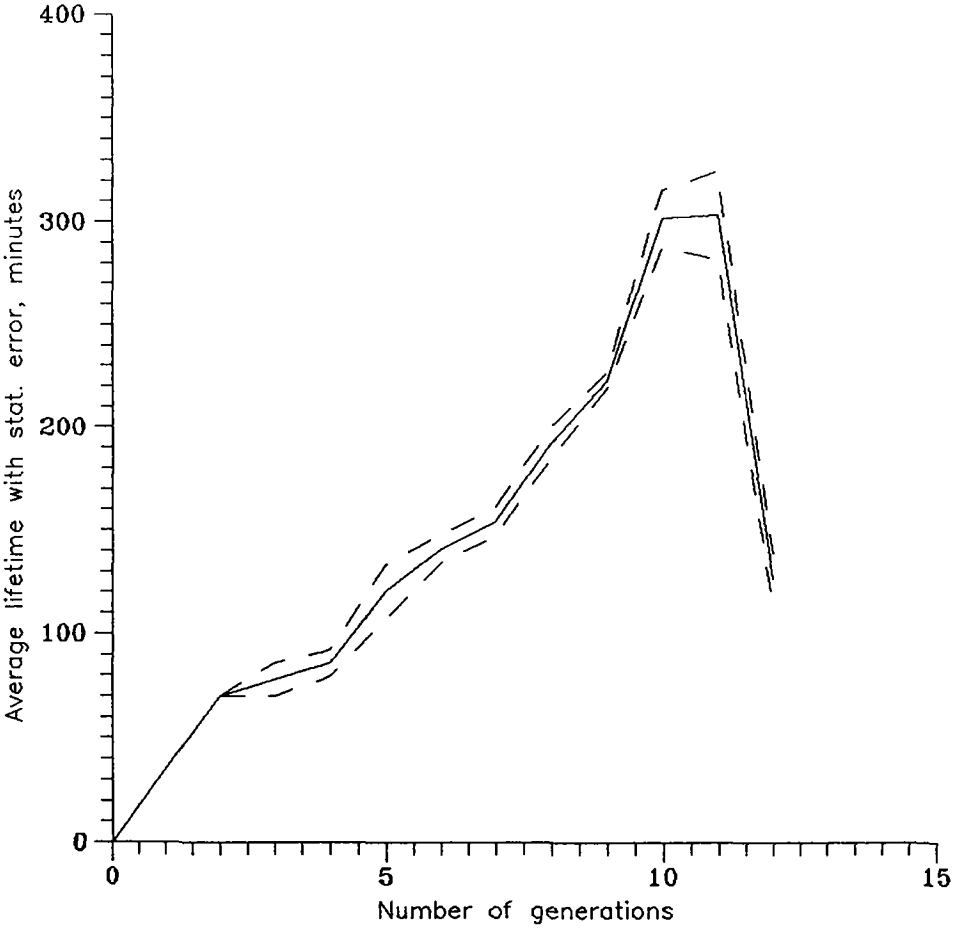


**Figure 1:** Average lifetime (solid) with statistic error (dashed) in different cell generations. Cells surviving stage $L1$ are omitted.

If so, then

$$<\cos^2\theta> = 1/3 \tag{3.1}$$

where $< >$ stands for expectation value. In our sample $N = 78$. (Note that $\theta$ involves three generations.) In this sample

$$<\cos^2\theta> = 0.425 \pm 0.024 \tag{3.2}$$

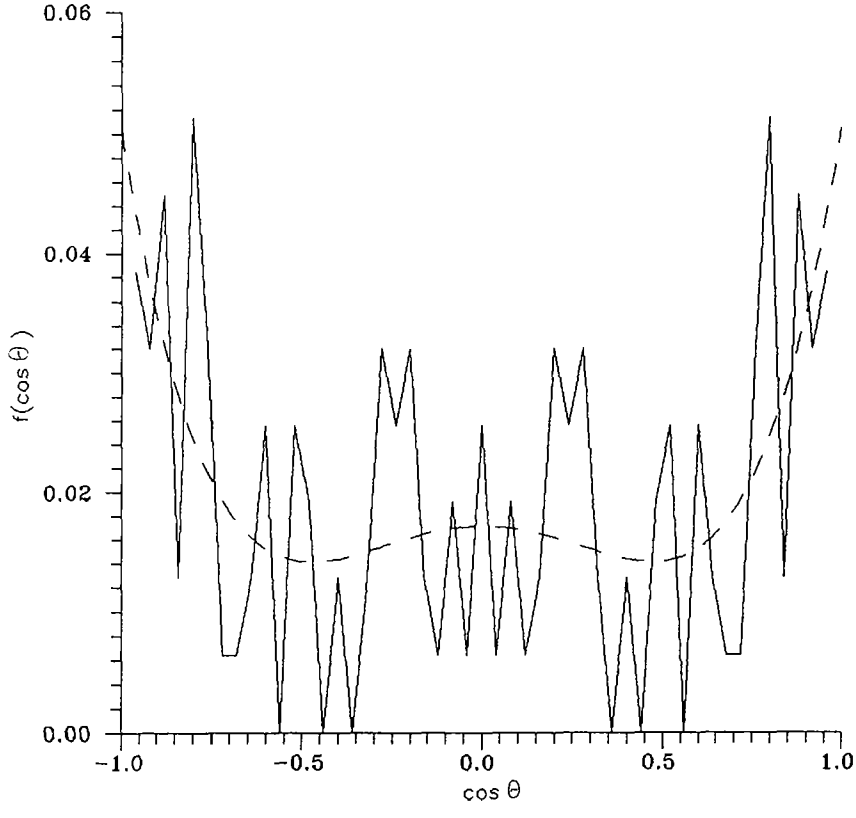


**Figure 2:** Distribution of the cosine between cleavage directions of mother cell and daughter cells. Dashed: quartic fit.

that is the distribution is not spherical above $3\sigma$ level, and there is a tendency for parallel axes.

However, this does not tell too much about the claimed orthogonal family. So let us see the whole distribution (although for $N = 78$ substantial statistical fluctuations are expected). That is shown on Figure 2. (The distribution is an even function, since no line has any directionality.) The dashed line is the best quartic fit, for a curve having a peak in the center $\theta = 90°$. Note the very slight central peak, completely negligible compared to the ends at 0° and 180°.

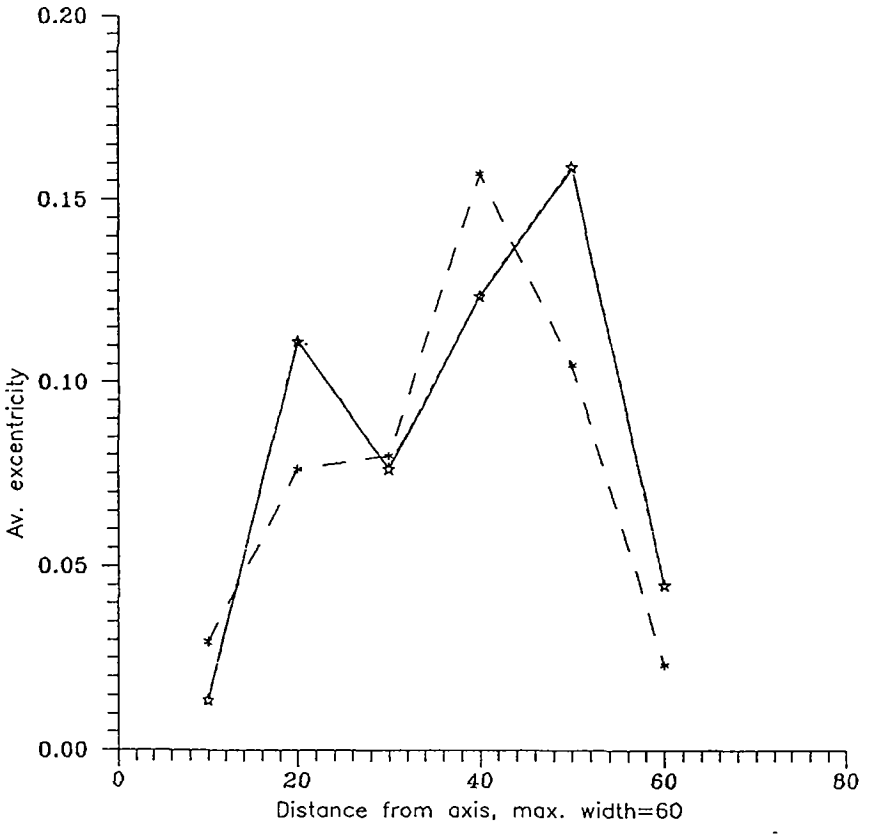


**Figure 3:** Average excentricity of nuclei at different distances from the anterior-posterior axis. Solid: dorsal, dashed: ventral sides.

Finally, Figure 3 is about the symmetry of the individual nuclei. Obviously by a pure mechanical influence of a fluid-like medium poorly structured deformable bodies are expected to remain spherical, due to Pascal's Law of hydrostatics. Figure 3 shows the average excentricities in 6 layers, successive from the main axis of the body in stage $L1$, separately for dorsal and ventral sides. Statistic errors are not displayed; they may be substantial, however the figure shows a definite tendency, therefore for the present we may regard the curves established. Moderate excentricities can be observed in different layers.

## 4. EVOLUTIONARY CONSEQUENCES

There are at least four possible evolutionary relevances of this project, related to the mechanical or other design of the worm.

The first is the exploration of developmental constraints restricting phenotypic variation in worm evolution. The distribution of cell division axes described in this paper is a developmental constraint on form and symmetry (cf. Wood, 1991).

Secondly, when a worm model has been constructed, there is a chance for the exploration of the possible domains of developmental pathways or morphologies by the perturbation of the model.

The third possibility is the comparative analysis between the worm developmental characteristics and the developmental properties of other species' organisms in a quantitative way (cf. Conklin, 1905; Wilson, 1892, 1925; Morris *et al*, 1989; Brown and Wolpert, 1990; Davidson, 1990). A resolvable problem is that whether or not there exists and underlying (generic) qualitative similarity in different developmental dynamics or body plans, from which the developmental diversity can be derived.

The fourth option is to extract and to catalogue the developmental rules and their relations in terms of revealing correlations between a variety of developmental properties, within and between different temporal and spatial developmental domains. This can help in organizing the accumulating worm data, without too much sign of discovery of developmental regularities (cf. Horder, 1989; Holliday, 1990; Wolpert, 1990; Molnár, 1990).

## 5. CONCLUSIONS

In Figure 1 one can observe a definite and statistically significant pattern of change of lifetimes of cell generations. Until the tenth generation a continuous decrease of division rate is seen; after that the lifetime starts to decrease but this may quite be the consequence of separation of two classes of cells of different fates, since we ignored here the ones surviving for adult life. A possible explanation of this temporal asymmetry of the cell lineage is that increasing cell density implies shortage of resources.

As told above, Figure 2 does *not* show an expressed family of cell with orthogonally patterned division history. However note the wide central plateau. Therefore it seems that the second family, if recognisable at all, is characterized not by orthogonal planes but by a tendency of large plane rotations between successive divisions. Observe that a cell has no way to detect a *right* angle rotation. Anyway, the predominance of parallel divisions seems to be able to contribute to the elongation of the animal during its development, ('Wormness').

As for Figure 3, a tendency for moderate excentricity is suggested. This may have various origins, e.g., the cell structure. However, serious differences are seen in different layers. So cell interaction effects cannot be excluded. While the hydrodynamic stress is isotropic, at large pressures spontaneous symmetry breaking is not

unknown in the compression of deformable media. Of course, further studies are needed to separate the possible intracellular (e.g., cytoskeleton-induced) and intercellular causes of the temporal and spatial asymmetries discussed.

## 6. ACKNOWLEDGEMENT

I. Molnár thanks Alan Coulson, Robin Holliday, Tim J. Horder, Stuart A. Kauffman, and Robert Waterston for the helpful discussions and/or suggestions on the *C. elegans* or other development, modelling, or comparative analysis.

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