Genome Size in Conodonts (Chordata): Inferred Variations During 270 Million Years

S. Conway Morris and E. Harper

DNA is too unstable to be preserved during fossilization, but it still seems possible to infer the genome content of fossils because in every group of organisms investigated cell size is proportional to quantity of DNA. Accordingly, information on macroevolutionary trends in genome size through millions of years is potentially available. This survey of inferred variation in genome content in fossils is based on measurements of epithelial cells in extinct conodonts over a period of 270 million years. Why genome size varies so widely amongst living organisms is a subject of continuing debate. Paleontology offers a distinct temporal perspective, but lack of data on conodont paleoecology make the proposed adaptive explanations for genome variation difficult to test.

The genome content of eukaryotes ranges across about five orders of magnitude (about 10^{-3} to 10^{2} pg per nucleus) (*1*), but shows little correlation with organismal complexity. In many species the amount of DNA is far in excess of immediate coding requirements. In many, but not all, species variation in genome content seems to be limited, and closely related taxa may have widely different quantities of DNA. This divorce between genome value and either the evolutionary grade or physiological complexity of an organism is referred to as the C-value paradox. It has attracted wide attention, but there is lack of agreement whether varying DNA values have an adaptive significance (*2–4*). Correlations between genome content and factors such as rates of development (*5–9*) and latitudinal distributions (*10*) are contrasted against hypotheses for "selfish" (*11, 12*), "junk" (*13*), or "ignorant" (*14*) DNA. Substantial changes in genome content have been inferred from phylogenies (*9, 15, 16*), whereas within lineages more specialized forms tend to have reduced amounts of DNA (*6, 17*). A consistent and reliable correlation with genome content, however, is that of cell size (*1, 3, 18*). This relation holds for groups as different as angiosperms, amphibians, and protozoans, although there is no specific value of DNA quantity for a given cell volume in all organisms. Variations in fossil material may be inferred (*19, 20*) because cell walls and imprints are commonly preserved, although direct evidence is limited (*21, 22*). In prospect is the possibility of investigating changes in genome size through millions of years and on a macroevolutionary scale encompassing the origin of major groups and the effects of extinctions.

S. Conway Morris, Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, United Kingdom.
E. Harper, Department of Earth Sciences, Open University, Milton Keynes MK7 6AA, United Kingdom.

Conodonts are known almost exclusively from the denticulate feeding apparatuses composed of a series of phosphatic elements. Associated soft parts are exceptionally rare, but recent discoveries from the Carboniferous indicate a conodont affinity with agnathan chordates (*23*). Conodont elements grew by centrifugal secretion of fine lamellae, and a widely accepted hypothesis is that the secretory epithelium that mantled each element retracted to permit the animal to pursue its predatory activities (*24*). A variety of surface ornamentation is known, amongst which a polygonal ultrastructure (Fig. 1) has been documented in all but one (Hibbardellacea) of the ten conodont superfamilies. These polygons, which are especially frequent on platform elements, are believed to represent imprints of the secretory epithelium (*25*). Accordingly the dimensions of surface polygons are taken to provide a guide to cell size of the overlying tissue layer (*26*). Even though the third dimension of these cells is not known, calculations suggest that given the overall mean size range (~4 to 18 μm), volumetric increase could only have been avoided if the cells became impossibly thin. Indeed, even had the cells maintained a constant height then the volumetric differences would have been more than an order of magnitude.

Dimensions of about 8580 surface polygons (450 elements, mostly platforms) in 193 species and subspecies (53 genera, about 23% of total generic diversity) of conodonts, representing almost the entire stratigraphic range of this group, were obtained (*27*). Bivariate comparisons between polygon size and element length were made for each geological period (Ordovician to Triassic) and also for the three superfamilies with good generic representation (Prioniodontacea, Polygnathacea, and Gondolellacea). In general correlations were statistically insignificant, although weakly significant values were obtained for the Devonian and Triassic periods, and for the Polygnathacea.

A consistent lower limit on cell size (about 4 μm) appears to have persisted for most of the history of the conodonts, although from the Permian onward this value seldom falls below about 6 μm (Fig. 2). Epithelial cell dimensions, and thus inferred genome content, show consistently low values for the Ordovician. Small cell sizes also predominate in the Silurian and early Devonian, although data are too scanty to draw reliable conclusions. Thereafter, there is a dramatic increase in many taxa, with some polygons averaging more than 18 μm across. However, during most of the Carboniferous there is some evidence for an overall decline in cell size, although the paucity of data for the interval must make this a preliminary conclusion. This possible trend is then reversed so that during the Permian and Triassic cell values once again show a wide variation.

Although cell size and genome content are positively correlated, determining the likely amount of DNA in each conodont species is not straightforward. Data on genome size in epithelial cells comparable to those responsible for conodont element secretion, such as the enamel-secreting ameloblasts of vertebrates, appear not to be available. In chordates determinations of genome size are based largely on erythrocytes and lymphocytes, and a simple extrapolation to other cell types may not be justifiable. Nevertheless, compared with the available data (*28*) on the relatively small genomes of agnathans, which are probably most closely related to conodonts (*23*), then the small cells (volume taken as 144 μm^3, 4 by 6 by 6 μm) would have had correspondingly reduced genomes (~1 pg). The largest cells are calculated to have had genomes of between about 6 and 17 pg, the value chosen depending on whether the unknown third dimension remained unchanged or increased isometrically [volumes taken as 1512 μm^3 (18 by 14 by 6 μm) or 4536 μm^3 (18 by 14 by 18 μm) respectively]. However, living agnathans are regarded as specialized, and their correspondingly reduced genomes (*17*) may jeopardize useful comparison with conodonts. If, therefore, the correlation between cell volume and genome size in a wider variety of vertebrates (*28*) is taken as the guide for estimation, then in principle the genome content of conodonts might have had a wider range of between about 1 and 150 pg.

The trend (Fig. 2) described here bears on many aspects of the C-value paradox. The small genomic values inferred for the earliest conodonts in our sample parallels the case in early dipnoans (*21*), rhipidistians, and amphibians (*22*). Equally striking, the protracted interval of inferred genomic stasis for at

least the first 50 million years of conodont history, in the face of concurrent generic diversification, reinforces the conclusion that genomic size need bear no simple relation to phenotypic diversity. Contrary to some suggestions (*29*), therefore, in both conodonts and fossil lungfish (*21*), the relatively low quantities of DNA do not seem to have limited evolutionary potential. It has been speculated that the amount of DNA in an evolving lineage is controlled by fluctuations in the relative proportions of coding to noncoding DNA (*6, 17*). However, given this early episode of genomic stasis, such a system may be difficult to extrapolate through tens of millions of years unless some extrinsic factor served to promote small genome size.

Thereafter, the greater degree of variation in inferred genome size for much of conodont history shows no obvious correlation with diversity, although as the data are almost entirely based on platform elements the possibility exists that nonplatform-bearing species exhibit different trends. Note, however, that even when conodonts were entering their final end-Triassic decline (*30*), inferred genome values remained widely variable and there is no evidence that their extinction was linked to increasingly small genome size (*17*).

If additional DNA was prevented from accumulating in the initial Ordovician diversification, evidently this was not precluded amongst some younger species in which inferred genome size is substantially larger. However, no persistent trend of inexorable accumulation has been identified in any lineages; rather, in at least the upper Devonian *Palmatolepis* clade (*31*), no consistent size increase or decrease through time is apparent. Nevertheless, attempts to link inferred genome size to adaptive features of conodonts are largely frustrated by the existing lack of relevant paleoecological information. It is clear, however, that no correlation can be demonstrated between cell size and morphological complexity of the conodont elements. Thus, though little is known yet of relationships between trophic specialization and element (especially platforms) architecture, inferred genome size is unlikely to be linked immediately to this aspect of ecology. Given the proposed link between genome size and metabolic rates, including oxygen consumption (*18*), links between inferred genome values and occupation by different conodont species of variably oxygenated environments might be predicted. However, our data indicate no obvious correlation between conodont cell size and host sediments inferred to have accumulated under different oxygen regimes, although postmortem introductions may confuse an original pattern. A search for correlations between cell size and position upon onshore to offshore transects was also made because some teleost fish show a positive correlation between genome content and depth of habitation (*32*). Paucity of relevant data made this difficult to test, but no convincing trends emerged. On a broader scale, plotting of inferred genome sizes of various taxa on paleogeographic maps for given intervals also failed to reveal any systematic pattern. Only in the Ordovician do conodonts show a wide latitudinal distribution (*33*), but at this time near-polar Baltic and Avalonian

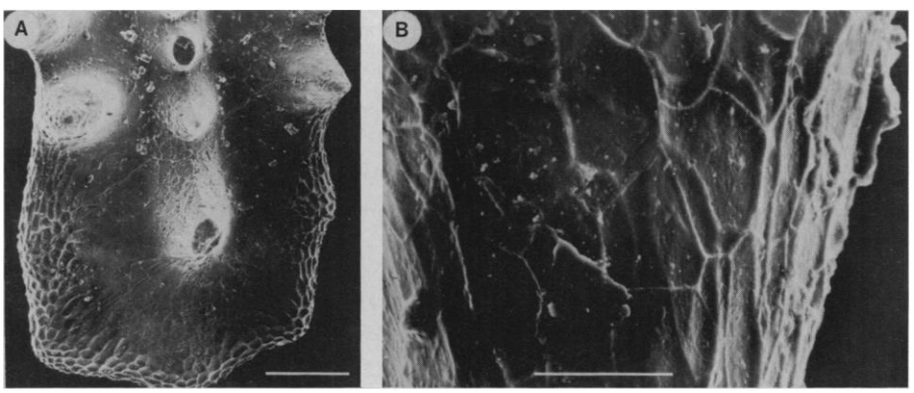

Fig. 1. Polygonal pattern representing imprint of epithelial cells on the oral surface of the platform element of *Epigondolella* spp. from the Triassic of British Columbia. (**A**) Posterior section of element; scale bar, 500 μm. (**B**) Detail of imprints; scale bar 10 μm.

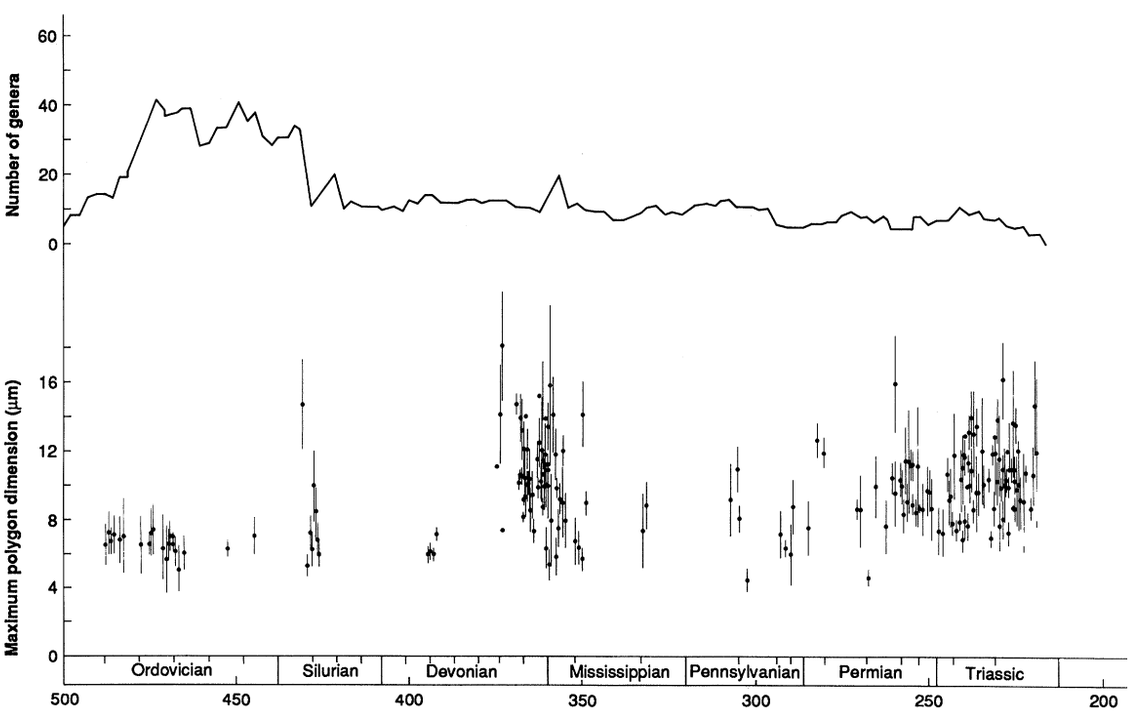

Fig. 2. Variation in size of epithelial cells of conodonts from the Ordovician to the Triassic. Each point represents the mean of the maximum dimensions in a single species or subspecies, the error bar being 1 SD. The number of polygonal imprints that could be measured varied widely from species to species. In a few taxa only a single cell was available, but usually between 5 and 50 cells per element could be measured. In most species only one or two elements with measurable polygons have been illustrated, but occasionally the total reaches five or six. Upper line represents variation in generic diversity of conodonts [data from (*35*)].

elements have comparable cell sizes to those of warm, shallow waters of equatorial Laurentia and Gondwana (Australia). Thereafter, conodonts appear restricted to within 40° of the paleoequator (*33*), and no link between cell size and latitudinal position is evident.

At present, therefore, variations in inferred genome size lack an obvious adaptive explanation. Suggestions that variation in genome size is linked to paedomorphosis (*5, 9, 18*) indicate one avenue of inquiry, but little is now known of heterochronous processes in conodonts (*34*). Investigations of cell size in the fossil record of conodonts and other groups where comparable data are potentially available (for example, brachiopods, arthropods, vertebrates, and vascular plants) can be used to test further adaptive explanations of changes in inferred genome size.

REFERENCES AND NOTES

1. B. J. Shuter *et al.*, *Am. Nat.* **122**, 26 (1983).
2. T. Cavalier-Smith, *Annu. Rev. Biophys. Bioeng.* **11**, 273 (1982).
3. ———, in *Evolution of Genome Size*, T. Cavalier-Smith, Ed. (Wiley, Chichester, 1985), pp. 105–184.
4. M. D. Bennett, *New Phytol.* **106** (suppl.), 177 (1987).
5. O. B. Goin *et al.*, *Copeia* **1968**, 532 (1968).
6. K. Bachmann, O. B. Goin, C. J. Goin, *Brookhaven Symp. Biol.* **23**, 419 (1972).
7. M. D. Bennett, *Proc. R. Soc. London* **B181**, 109 (1972).
8. H. A. Horner and H. C. Macgregor, *J. Cell Sci.* **63**, 135 (1983).
9. S. K. Sessions and A. Larson, *Evolution* **41**, 1239 (1987).
10. M. D. Bennett, *Envir. Exp. Bot.* **16**, 93 (1976).
11. L. E. Orgel *et al.*, *Nature* **288**, 645 (1980).
12. W. F. Doolittle, in *Genome Evolution*, G. A. Dover and R. B. Flavell, Eds. (Academic Press, London, 1982), pp. 3–28.
13. S. Ohno, *Brookhaven Symp. Biol.* **23**, 366 (1972).
14. G. Dover, *Nature* **285**, 618 (1980).
15. K. Bachmann and H. J. Price, *Chromosoma* **61**, 267 (1977).
16. S. W. Sherwood and J. L. Patton, *ibid.* **85**, 163 (1982).
17. R. Hinegardner, in *Molecular Evolution*, F. J. Ayala, Ed. (Sinauer, Sunderland, MA, 1976), pp. 179–199.
18. E. Olmo and A. Morescalchi, *Experientia* **31**, 804 (1975).
19. M. Vialli and G. Sacchi Vialli, *Rendiconti Istituto Lombardo Sci. Lett.* **B103**, 234 (1969).
20. B. Runnegar, *Palaeontology* **29**, 1 (1986).
21. K. S. Thomson, *J. Exp. Zool.* **180**, 363 (1972).
22. ——— and K. Muraszko, *ibid.* **205**, 315 (1978).
23. R. J. Aldridge *et al.*, *Lethaia* **19**, 279 (1986).
24. S. Bengtson, *ibid.* **9**, 185 (1976).
25. R. W. Pierce and R. L. Langenheim, *Geol. Soc. Am. Bull.* **81**, 3225 (1970).
26. For many species several elements were available for measurement. Despite the wide variety of sources, measurements of the same species illustrated by different authors usually showed good agreement. In low magnification illustrations the number of epithelial polygons per unit length was measured. Illustrations at higher magnifications allowed more reliable estimates of size frequency and variation to be obtained. Because polygons tend to be somewhat elongate, here the maximum dimension was used.
27. More than 110 separate articles and papers were consulted; full details of this data source will be provided elsewhere. In addition, H. Armstrong, R. L. Austin, R. D. Burnett, J. Long, A. D. McCracken, G. S. Nowlan, and J. E. Repetski provided photographs of conodonts from which we derived cell measurements.
28. E. Olmo, *Basic Appl. Histochem.* **27**, 227 (1983).
29. H. J. Price, *Bot. Rev.* **42**, 27 (1976).
30. D. L. Clark, *J. Paleontol.* **57**, 652 (1983).
31. D. L. Clark *et al.*, *Treatise on Invertebrate Paleontology*, part W. Miscellanea. Supplement 2, Conodonta. (Geological Society of America, Boulder, CO, and University of Kansas, Lawrence, 1981).
32. A. W. Ebeling *et al.*, *Am. Nat.* **105**, 549 (1971).
33. W. C. Sweet, *J. Paleontol.* **59**, 485 (1985).
34. T. W. Broadhead and R. McComb, *Fossils Strata* **15** 149 (1983).
35. R. J. Aldridge, *Syst. Assoc. Spec.* **34**, 231 (1988).

36. R. J. Aldridge, M. D. Bennett, and G. Dover are thanked for reviewing the manuscript and helpful discussions, while a number of investigators (*27*) generously made available unpublished photographs and stratigraphic data. We thank M. Orchard for photographs for Fig. 1, S. Ripper for drafting Fig. 2, and S. Last for typing the manuscript. We acknowledge financial support from Gonville and Caius College for E. H. Cambridge Earth Sciences Publication 1263.

14 March 1988; accepted 1 July 1988

Technical Comments

Do Short-Term Tests Predict Rodent Carcinogenicity?

Long-term rodent studies are expensive ($1 to $2 million) and time-consuming (3 to 4 years), so there is great interest in being able to predict their results with the use of short-term tests (STTs). This interest is particularly keen since a battery of such tests costs approximately $10,000. Tennant *et al.* (*1*) examined the results of the rodent test and genetic toxicity tests for 73 compounds recently tested by the National Cancer Institute and concluded that, of the four STTs examined, a single test, the Ames *Salmonella*-microsome test, is 60% concordant with the rodent test and that any one of the other three tests do not help in predicting rodent carcinogenicity. One might conclude that the other three tests are superfluous; either they lack additional information or they are not cost-effective.

The conclusion of Tennant *et al.* appears to be based largely on looking at pairs of STTs. A rearrangement of their results, Table 1, shows a good correlation between the number of STT positives and the probability of a positive rodent result. When all four STTs were positive, the rodent test was positive about 80% of the time. In only three instances were all STTs positive and the rodent test negative.

Even with no STT positives, the rodent test was positive about 40% of the time. What are the possible explanations? Nongenetic mechanisms of carcinogenicity might be operable. These are likely because the rodent tests are conducted at maximal doses, which might be expected to upset hormonal or other physiological balances. It is also likely that some of the positive rodent results are statistical false-positives. There are many types of spontaneous tumors; for rats and mice, males and females, there can be 40 to 100 statistical tests for each compound. In multiple testing situations, Gill (*2*) has argued that one should expect positive results by chance alone. Haseman *et al.* (*3*), in examining paired control groups from 18 studies, found one or more statistically significant differences between the control groups 44% of the time. In six of the 18 studies, there were two or more statistically significant results. Also, Brown and Fears (*4*) estimated that false-positive results could be expected 30% of the time in long-term rodent tests.

Figure 1 summarizes the above points: one might expect 30 to 44% of the rodent results to be false-positives; the rodent-STT regression line predicts about a 40% nongenetic or statistical false-positive rate. Even with four STT positives, one should expect about 20% of the rodent tests to be negative.

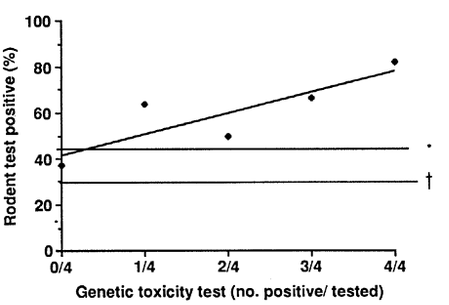

Fig. 1. Positive results in rodent tests versus positive results in genetic toxicity tests. *Estimated false-positive rate (*3*). †Estimated false-positive rate (*4*) ($y = 41.5 + 3.7x$, $P < 0.007$).