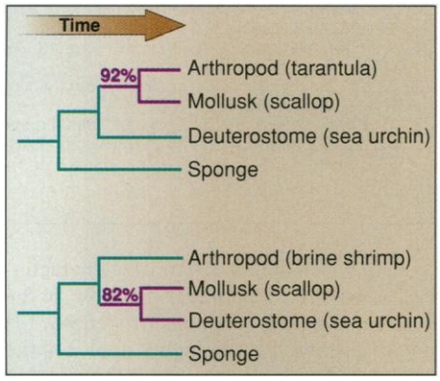
EVOLUTION

The Coming of Age of Molecular Systematics

Laura E. Maley and Charles R. Marshall

Before 1988 our view of the relationships among the approximately 33 plus major groups of living animals (phyla) was based on detailed analyses of morphology and development. Advances in molecular biology have greatly added to the arsenal of features that can be examined. Of these, most important have been gene sequences, particularly of the 18S ribosomal RNA (rRNA) gene (*1*). Animal relationships derived from these new molecular data sometimes are very different from those implied by older, classical evaluations of morphology (*2–10*). Reconciling these differences is a central challenge for evolutionary biologists at present. Growing evidence suggests that phylogenies of animal phyla constructed by the analysis of 18S rRNA sequences may not be as accurate as originally thought.

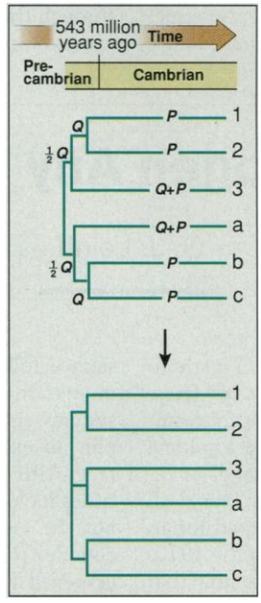


The problem with brine shrimp. Different representative species, in this case brine shrimp or tarantula for the arthropods, yield wildly different inferred relationships among phyla. Both trees have strong bootstrap support (percentage at node). The brine shrimp has long been known to produce artifactual groupings, but this was only confirmed with other sequences, which points to the importance of having data from many species from each phylum.

Inaccuracies may occur in molecular phylogenies for a variety of reasons (*11*). Prior to analysis, the sequences of corresponding genes from each animal must be placed in register (aligned) with each other so that homologous sites within each sequence can be compared. However, sequence divergences may be sufficiently large that unambiguous alignments cannot be achieved, and different alignments may lead to different inferred relationships. Additionally, the data are often sufficiently noisy that there may be a lack of strong statistical support for important groupings.

Two even more pressing problems must be faced when interpreting DNA phylogenies. First, empirical study shows that the degree of support for a relationship between two groups may be affected profoundly by the particular species chosen to represent each group (*12*). In extreme cases the inferred relationships between groups may change when different representative species are used (see figure at left). One solution to this problem is to increase the number of species analyzed, although for many phyla genes from only a few species have been sequenced. A disadvantage of this approach is that increasing the number of species dramatically increases the computation time required to find the best tree to represent the relationships among phyla (for five species there are just 15 possible unrooted trees, for 50 species there are 3×10^{74} possible trees, an impossibly large number of trees to evaluate).

The second problem is that of statistical inconsistency—the disconcerting situation where, as the amount of data analyzed increases, so does the apparent statistical support for an incorrect phylogenetic tree. In molecular analyses, species sharing the same nucleotides at the same sites in a gene are grouped together under the parsimonious assumption that those species share those nucleotides because they shared a recent common ancestor. However, unrelated species may share one of the four possible nucleotides at a site by chance alone (such similarities are termed homoplasies). The number of homoplasies increases with the total amount of evolution (that is, nucleotide changes) that has occurred between species. In some cases the accumulation of these chance events between distantly related species will overwhelm the similarities present due to the shared ancestry of more closely related taxa (*13*). This problem of homoplasious similarity swamping out the true phylogenetic signal is particularly acute when the true evolutionary tree has long branches (many changes) in proximity to very short branches (few changes), even if there is no rate variation among the lineages; the long branches will artificially group together, or attract each other (*14*, *15*) (see figure below), a problem exacerbated when the rate of evolution varies along the gene (*16*).



Long branch attraction for animals. When long branches on evolutionary trees are in close proximity to short internode branches as is likely the case for the animal phyla (upper tree), maximum parsimony, a widely used method for recovering evolutionary trees, will recover the wrong tree (lower tree). *P* and *Q* are probabilities of observing a change on a branch. The wrong tree will be recovered when $Q < P^2$, assuming binary characters [Modified from (*14*)]

Unfortunately, the conditions for long branch attraction are all in place for 18S rRNA analyses of animal relationships; it appears that the majority of the animal phyla diverged from each other relatively quickly a long time ago (*17*) (the true evolutionary tree probably has relatively short internal branches and long peripheral branches) (see figure at left) and that there is significant (almost three orders of magnitude) site-to-site rate variation across the 18S rRNA gene (*16*). Long branch attraction can be reduced by eliminating species that have unusually fast rates of evolution (*3*, *10*, *18*), but this by no means solves the problem, and demonstrating that with more data the degree of statistical support for a grouping increases [for example, (*18*)] does not mean that that grouping is correct.

With explicit models of DNA sequence evolution it is sometimes possible to prevent long branch attraction. The critical question is whether current models of 18S rRNA evolution are sufficiently accurate to successfully compensate for long branch attraction between the animal phyla. Without knowing the correct tree ahead of time,

L. E. Maley is in the Department of Earth and Space Sciences, University of California, Los Angeles, CA 90095–1567, USA. C. R. Marshall is in the Department of Earth and Space Sciences, Molecular Biology Institute, and the Institute of Geophysics and Planetary Physics, University of California, Los Angeles, CA 90095–1567, USA. E-mail: marshall@ess.ucla.edu

this question will be hard to answer. However, current models of DNA substitution usually fit the data poorly (*19*), and a 12S rRNA study (*20*) indicates that the most sophisticated methods of modeling site-to-site variation do not always give the correct tree, leaving open the possibility that these methods may also fail to prevent long branch attraction in 18S rRNA phylogenies of the animal phyla.

The amino acid sequences of proteins may be more immune to the problems of long branch attraction than the nucleotide sequences of 18S rRNA, and protein-coding genes constitute a much larger proportion of the genome than RNA-coding genes. Thus, it is likely that protein sequences will become a major source of data for inferring phylum-level relationships, especially with the growing number of animal genome projects.

Given the probable rapid divergence of most of the animal phyla, the complexities of 18S rRNA sequence evolution, and the problem of taxon sampling, it is difficult to have confidence in 18S rRNA trees in the absence of corroborating evidence. Fortunately, morphological and 18S rRNA phylogenies usually agree in their coarse structure. For example, there is agreement in the basal position of the diploblastic animals (which include jellyfish and corals), the grouping of the echinoderms, the hemichordates, and chordates; and the close relationship of the major protostome phyla such as mollusks, arthropods, and annelids (the "true" worms). But there are frequent minor and sometimes major disagreements, such as in the position of the lophophorate phyla (or even whether they are each other's closest relatives) (*3*). In cases of disagreement, it is an open question as to which (if either) interpretation is correct.

To be confident in our hypotheses of relations among the animal phyla we need to gather more DNA sequences, especially from undersampled phyla; develop better methods of DNA analysis on the basis of more realistic models of DNA evolution (*21*); and develop independent data sets using morphological, developmental, and other molecular data (*4*, *7*) to corroborate or falsify specific hypotheses or to combine in total-evidence analyses (*22*). Work is currently under way on all these fronts, which promise more secure hypotheses of the relationships among the animal phyla and, through them, a better understanding of the causes of major morphological innovation.

References and Notes

1. K. G. Field *et al.*, *Science* **239**, 748 (1988).
2. See http://phylogeny.arizona.edu/tree/eukaryotes/animals/animals.html for earlier references.
3. A.-M. A. Aguinaldo *et al.*, *Nature* **387**, 489 (1997).
4. G. Balavoine, *C. R. Acad. Sci. Ser. III Sci. Vie* **320**, 83 (1997).
5. K. M. Halanych *et al.*, *Science* **267**, 1641 (1995).
6. L. Y. Mackey *et al.*, *J. Mol. Evol.* **42**, 552 (1996).
7. M. E. Siddall, D. S. Martin, D. Bridge, S. S. Desser, D. K. Cone, *J. Parasitol.* **81**, 961 (1995).
8. M. J. Telford and P. W. Holland, *Mol. Biol. Evol.* **10**, 660 (1993).
9. J. M. Turbeville, J. R. Schulz, R. A. Raff, *ibid.* **11**, 648 (1994).
10. S. Carranza, J. Baguñà, M. Riutort, *ibid.* **14**, 485 (1997).
11. C. R. Marshall; *Comp. Sci. Stat.* **29**, 218 (1997).
12. G. Lecointre, H. Philippe, H. L. V. Lê, H. L. Guyader, *Mol. Phylogenet. Evol.* **2**, 205 (1993).
13. J. Felsenstein, *Syst. Zool.* **27**, 401 (1978).
14. M. D. Hendy and D. Penny, *ibid.* **38**, 297 (1989).
15. J. Kim, *ibid.* **45**, 363 (1996).
16. Y. Van de Peer, J.-M. Neefs, P. D. Rijk, R. De Wachter, *J. Mol. Evol.* **37**, 221 (1993).
17. S. Conway Morris, *Curr. Biol.* **7**, R71 (1997).
18. H. Philippe, A. Chenuil, A. Adoutte, *Dev. Suppl.*, 15 (1994).
19. J. P. Huelsenbeck and B. Rannala, *Science* **276**, 227 (1997).
20. J. Sullivan, K. E. Holsinger, C. Simon, *Mol. Biol. Evol.* **12**, 988 (1995).
21. J. Felsenstein and G. A. Churchill, *ibid.* **13**, 93 (1996).
22. A. de Queiroz, M. J. Donoghue, J. Kim, *Annu. Rev. Ecol. Syst.* **26**, 657 (1995).

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CHEMISTRY

Fixing Nitrogen Any Which Way

G. J. Leigh

This issue of *Science* contains (*1*) a stimulating report by Nishibayashi *et al.* (page 540) on the conversion of dinitrogen to ammonia. This report begins to show a gradual intertwining of many diverse strands of research into dinitrogen reactivity. This is all the more ironic in that the big expansion in nitrogen fixation research during the 1970s and 1980s has moved into reverse now that the direct economic return has been judged to be disappointing.

There are at least four different kinds of reactivity of dinitrogen described in the literature. Not all are fully defined, and some are very far from being mechanistically understood. The oldest in research terms is the Haber synthesis (*2*). This operates at high temperatures and pressures and uses a promoted metallic iron catalyst. The reaction appears to occur by chemisorption of both dihydrogen and dinitrogen on the catalyst, surface, followed by stepwise assembly of ammonia from these atoms. Highly reduced systems, such as a mixture of a metal halide plus an excess of a Grignard reagent, that react with dinitrogen to form ill-defined nitrides have been recognized for many years, but the clean splitting of dinitrogen by a complex to form a nitrido complex has been achieved only recently, by Cummins and his collaborators (*3*). In contrast, splitting of dihydrogen by metal complexes to form metal hydrides has long been known. As yet, no simple coordination compound can perform these two functions simultaneously, which is why metal complexes that are Haber-type catalysts are unknown. Chemists have comforted themselves with the thought that a metal surface can do things that complexes cannot do. In any case, there is little likelihood of developing a Haber catalyst that is as easy to prepare and as stable mechanically and chemically as metallic iron.

Biological catalysis of nitrogen fixation has provoked a great deal of speculation, some of it well founded. The now-characterized iron-molybdenum-sulfur cluster at the heart of the molybdenum nitrogenases (*4*) might appear to be a biological analog of the Haber catalyst, at least as far as the splitting of dinitrogen is concerned. In fact this is unlikely. No metal-sulfur cluster has yet been shown to react with dinitrogen. In any case, the reaction catalyzed by nitrogenases in biological systems fundamentally involves dinitrogen and water (plus an energy input) rather than dinitrogen and dihydrogen (plus an output of energy) as in the Haber process.

It is now generally accepted that the most efficient biological fixation by molybdenum nitrogenases involves the following stoichiometry:

$$N_2 + 8H^+ + 8 \text{ electrons} \rightarrow 2\,NH_3 + H_2 \quad (1)$$

The reasons for the production of dihydrogen are not clear. In addition, it seems that two molecules of adenosine 5′-triphospate (ATP) are hydrolyzed for the transfer of each electron, 16 in all for a single catalytic cycle. However, non-molybdenum nitrogenases exhibit different stoichiometries and that in any case the protein binding the cluster seems to be a necessary component of the nitrogenase system. The isolated cluster cannot fix nitrogen.

Now much of this dogma has been thrown into doubt. Although it was noted

The author is in the School of Chemistry, Physics, and Environmental Science, University of Sussex, Brighton BN1 9QJ, UK. E-mail: kafc1@maila.central.susx.ac.uk