

divergence dates not falling well within the Precambrian.

Is the conclusion drawn by Rokas *et al.* sound? At first glance, it appears so, but can their conclusion stand up to closer scrutiny? Accepting that the genes analyzed by the authors evolved without gene duplication and that the amino acids are aligned correctly, most phylogenetic methods assume that the evolutionary dynamics of the 12,060 amino acid sites are independently and identically distributed, and that they evolved under the same stationary, reversible, and homogeneous conditions (8). The assumptions arise from the need to render phylogenetic methods tractable and easy to use, and they are unlikely to be realistic. To account for the observation that the sites in a gene may evolve at different rates, some phylogenetic methods are able to model rate heterogeneity across sites using a Γ distribution (9). Rokas *et al.* used this approach for the whole alignment but did not consider that different parts of the alignment may require different Γ distributions. Nor did they consider that some sites may vary nonindependently (10) and that the distribution of variable sites may vary across lineages and through time, an issue that is notoriously difficult to resolve (11). A logical extension to the work would be to partition the alignment and estimate the evolutionary rates for different genes separately.

Violation of the assumed stationary, reversible, and homogeneous conditions may lead to compositional differences in the aligned amino acid sequences and hence to errors in phylogenetic estimates (12). Rokas *et al.* recognized this potential source of error but used a test that is known to be flawed, even though better tests are known (13). Furthermore, they chose a phylogenetic method that, while it accounts for compositional variation in the sequence alignment, is unsuitable: It assumes that the sites are independently and identically distributed, which they have already shown not to be the case. Moreover, they used a single Markov (probabilistic) model to analyze the alignment of amino acids, in effect using a "one size fits all" approach, where it would have been better to use several Markov models to capture gene-specific differences in the evolutionary processes (14).

Rokas *et al.* used nonparametric bootstrap and posterior probabilities to gauge support for the pattern and order of speciation events (branches in their phylogenetic tree). The former is widely recognized to be statistically unwise. Bootstrap values are estimates of the expected frequency with which speciation events (internal branches) occur in the optimal tree, using data constructed from the original alignment by sam-

pling sites with replacement (15). It is not a measure of accuracy or confidence, but of data consistency. Further, the increase in bootstrap value when more genes are included may be misleading, because longer sequences naturally tend to have higher bootstrap values (see the figure). The posterior probabilities of speciation events being correctly identified are also prone to error when the phylogenetic assumptions are violated in the sense described above.

In light of these concerns, are the conclusions of Rokas *et al.* justified? Should we ignore their study? Most certainly not, because they have produced a wealth of data and have shown that it might just be possible that the fossil record can be reconciled with molecular data. This, in itself, should be cause for celebration and an incentive to acquire sequence data from the remaining 26 animal phyla. Likewise, it should encourage development of methods that assess when data violate phylogenetic assumptions, and that cope with such data. To achieve these goals, we need to know more about the structure and function of gene products before we can develop models that appropriately address the early evolution of animals.

PHYSIOLOGY

The Tick-Tock of Aging?

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The relationship between organismal development and aging has long been a matter of intense debate. It seems natural to posit that developmental timing mechanisms that culminate in reproductive maturity continue to affect post-reproductive biology, with consequences for total organism life span. On the other hand, evolutionary theories of aging discount regulated aging per se, because the force of selection declines with age and drops precipitously after reproductive potential ends. In other words, a program that actively ages the organism is unlikely to be selected for in evolution. Instead, aging is thought to entail the passive stochastic accumulation of damage to molecules, cells, and organs, leading to loss of fertility and organismal demise. Therefore, the notion that regulated intrinsic biological timers control aging seems superficially untenable.

Just this possibility, however, has been raised by Boehm and Slack on page 1954 of this issue (1). They have found that compo-

References

1. C. Darwin, *The Origin of Species by Means of Natural Selection*. (John Murray, London, ed. 6, 1888), p. 313.
2. D. E. G. Briggs, R. A. Fortey, *Paleobiology* **32**, 594 (2005).
3. S. J. Gould, *Wonderful Life: The Burgess Shale and the Nature of History* (Hutchinson Radius, London, 1989).
4. E. J. Douzery, E. A. Snell, E. Bapteste, F. Delsuc, H. Philippe, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15386 (2004).
5. K. J. Peterson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 6536 (2004).
6. K. J. Peterson, N. J. Butterfield, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 9547 (2005).
7. A. Rokas, D. Krüger, S. B. Carroll, *Science* **310**, 1933 (2005).
8. V. Jayaswal, L. S. Jermini, J. Robinson, *Evol. Bioinformatics Online* **1**, 62 (2005).
9. Z. Yang, *Trends Ecol. Evol.* **11**, 367 (1996).
10. D. Penny, B. J. McComish, M. A. Charleston, M. D. Hendy, *J. Mol. Evol.* **53**, 711 (2001).
11. P. J. Lockhart *et al.*, *Mol. Biol. Evol.* **23**, 40 (2006).
12. S. Y. W. Ho, L. S. Jermini, *Syst. Biol.* **53**, 623 (2004).
13. L. S. Jermini, S. Y. W. Ho, F. Ababneh, J. Robinson, A. D. W. Larkum, *Syst. Biol.* **53**, 638 (2004).
14. M. Pagel, A. Meade, *Syst. Biol.* **53**, 571 (2004).
15. J. Felsenstein, *Evolution* **39**, 783 (1985).
16. J. W. Valentine, D. Jablonski, D. H. Erwin, *Development* **126**, 851 (1999).
17. A. Rambaut, N. C. Grassly, *Comp. Appl. Biosci.* **13**, 235 (1997).
18. D. L. Swofford, *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, 4.0 Beta (Sinauer, Sunderland, MA, 2002).

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nents of a nematode's (*Caenorhabditis elegans*) heterochronic circuit—namely the *lin-4* microRNA and its target, the nuclear protein encoded by *lin-14*—not only perturb developmental timing but also influence organismal life span. They do so by regulating insulin/IGF-1 (insulin-like growth factor-1) signaling, a cellular regulatory pathway whose modest decrease in activity leads to increased longevity across taxa (2).

Just as each cell in a developing organism has a positional identity that is determined by gradients of morphogens and hierarchies of transcription factor activity, cells also have a temporal identity dictated by regulatory signaling cascades. Pioneering work in *C. elegans* led to the discovery of the heterochronic loci (3), which constitute a regulatory circuit that confers temporal identity to the various tissues. These genes determine cellular programs of division, migration, and differentiation that are appropriate for a specific developmental stage. In addition, the interactions among these heterochronic loci ensure the proper succession of larval temporal fates. Normally, *C. elegans* develops to adulthood through four larval stages, L1 to L4. Worms with mutations in the heterochronic loci inappropriately express cel-

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