chiral centres, and cation- and anion-binding sites, and substitutions that modulate PNA solubility. It is also conceivable that the polyamide backbone can be modified to generate PNAs that recognize left-handed rather than right-handed DNA, as well as tertiary interactions in RNA.

Further improvements in the biological potency of PNAs could result from conjugating them with ligands known to facilitate permeation into cells; indeed, for the near future, one can envisage PNA analogues that are tailor-made to help tackle specific biological questions at the cellular level. But the main hopes for this technology are for drug therapies — success there will depend not only on efficacy, but on cost. □

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EVOLUTIONARY BIOLOGY

Bacterial tick-tock

Paul H. Harvey and Robert M. May

THE expanding catalogues of molecular sequence data have greatly extended our knowledge of the phylogenetic relations among prokaryotic organisms[1]. It is, however, often difficult to assign a clear chronology to prokaryotic evolution; molecular methods are better at giving us the topology of phylogenetic trees than specific branch lengths. New work by Nancy Moran and others[2] focuses on various species of bacteria that are internal symbionts of aphids, and shows how information about the dated phylogenies of the insect hosts can be used to calibrate the molecular clocks of bacteria.

For multicellular plants and for animals — especially vertebrates — fossil evidence enables us to construct phylogenetic trees with fairly reliable dates on the nodes, and chronological lengths on the branches. Such chronologies can then be combined with molecular sequence data to estimate rates of base-pair substitution. Conversely, the substitution rates thus estimated can be used to assign tentative dates to phylogenetic events for which there is no fossil evidence. But, because prokaryotic fossils cannot usually be associated with modern organisms, there are few such direct calibrations of rates of molecular evolution among bacteria.

Boundaries

Ochman and Wilson[3] have sought to infer the rates at which molecular clocks tick for bacteria, by using ecological arguments. They propose that large-scale ecological events, which can be roughly dated, can put upper and lower boundaries to the origins of bacterial lineages associated with particular habitats. For instance, those bacteria associated with the nodules of leguminous plants must have diverged from related bacterial taxa after the origin of land plants, but before the origin of legumes. Using palaeobotanical evidence, Ochman and Wilson date the common ancestor of the *Rhizobium* genus (in nodules) and the *Agrobacterium* (not in nodules) at between 415 million years and 110 million years (Myr) ago. Inferences of this kind clearly give very rough estimates of ancestral ages. Moreover, even these rough estimates depend on the assumption that modern bacterial taxa are lineal descendants of the original occupants of a new ecological niche, which is not necessarily true.

It would be nice to have more accurate ways of dating nodes and branch-lengths in bacterial evolutionary trees. For one thing, the consequent estimates of substitution rates would help in reconstructing phylogenies for prokaryotes, and elucidating the underlying mode and tempo of evolution[4,5]. For another, such chronologies would allow us to test ideas about how generation times and other factors may affect substitution rates among bacteria, causing the molecular clock to tick faster along some branches than others[6,7].

Moran *et al.*[2] estimate such dates for a group of bacteria mutualistically associated with aphids. These endosymbiotic bacteria live within specialized aphid cells, are passed by maternal inheritance (vertical transmission) from aphid to aphid, and are essential for the growth and reproduction of their hosts[8]. Analysis of the 16S ribosomal DNA (rDNA) sequence has established that the bacteria are a monophyletic group or distinctive clade within the Proteobacteria[9]. Their phylogeny has a one-to-one correspondence with that of their diverse group of aphid host species[10,11]. This precise concordance implies a long-term cospeciation of bacteria and aphids, resulting from vertical inheritance from an ancestral aphid in which the initial infection occurred.

There is a fairly good fossil record for the aphids, which allows their phylogenetic tree to be dated. The evidence for synchronous radiation of the aphid's endosymbionts thus enables consequent dates to be assigned to divergence events among the bacteria. Moran and colleagues' analyses of rDNA sequences show that rates of base substitution are similar for all the different endosymbiotic lineages, and (from calibration against aphid dates) that they correspond to a roughly constant rate of 0.01 to 0.02 base substitutions per site per 50 Myr. These estimates of rates are amongst the best yet available for prokaryotes. The estimated range lies slightly above the earlier, and much rougher, estimate (0.01 substitutions per site per 50 Myr) made by Ochman and Wilson. Moran and colleagues' finding of approximate constancy in rates of base substitution is further supported by their observation that each of their 12 aphid endosymbiont species is distanced from *Escherichia coli* — one of the most closely related bacteria for which the 16S rDNA sequence is available — by about the same number of base substitutions (to within 10 per cent).

Divergence

Most of the aphid dates (10 of the 12 species) are based directly on fossils. The other two are melaphidine species (*Melaphis rhois* and *Schlectendalia chinensis*), one from America and the other from Asia. By using the roughly constant ticking of the rDNA molecular clock of the associated endosymbiotic bacteria, Moran *et al.* estimate that the American and Asian melaphidines diverged around 50–70 Myr ago. This is in cheering agreement with an earlier minimum estimate of 48 Myr ago, based on purely biogeographical considerations[12]. Overall, this bacterial clock indicates that the minimum age of this mutualistic association is around 160–280 Myr. And this, in turn, is a good estimate of the age of the common ancestor of modern Aphidoidea, or the aphid Eve as it were.

More generally, the consistency between the findings of Moran *et al.* and the earlier estimates encourages us to believe that rates of 16S rDNA base substitution may be sufficiently constant, "even among organisms with very different lifestyles, that molecular clocks may be widely useful for reconstructing the evolutionary history of prokaryotes"[2]. □

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1. Woese, C. R. *Microbiol. Rev.* **51**, 221–271 (1987).
2. Moran, N. A., Munson, M. A., Baumann, P. & Ishikawa, H. *Proc. R. Soc.* B**253**, 167–171 (1993).
3. Ochman, H. & Wilson, A. C. *J. molec. Evol.* **26**, 74–86 (1987).
4. Simpson, G. G. *Tempo and Mode in Evolution* (Columbia Univ. Press, New York, 1944).
5. Nee, S., Mooers, A. O. & Harvey, P. H. *Proc. natn. Acad. Sci. U.S.A.* **89**, 8322–8326 (1992).
6. O'hUigin, C. & Li, W. *J. molec. Evol.* **35**, 377–384 (1992).
7. Gillespie, J. H. *Ecology* **56**, 493–495 (1975).
8. Douglas, A. E. *Biol. Rev.* **64**, 409–434 (1989).
9. Munson, M. A., Baumann, P. & Kinsey, M. G. *Int. J. syst. Bact.* **41**, 566–568 (1991).
10. Munson, M. A. *et al. J. Bact.* **173**, 6321–6324 (1991).
11. Baumann, P. *et al. ASM News* **59**, 21–24 (1993).
12. Moran, N. A. *Science* **245**, 173–175 (1989).