Penny's Probably Powerful Phylometer™ and the rise of statistical phylogenetics

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Unlike many contributors to this issue in honour of David Penny's 70th birthday, I cannot trace my academic lineage to David. Instead, I see myself as the neighbour's kid, looking over the fence at the goings-on in 'Uncle' David's yard, and every so often, having the pleasure of an invite to come over and play. I did receive a pat on the back from 'Uncle' David back in 1990 when I was a PhD student and he was the New Zealand examiner of my doctoral dissertation, a tome with the rather soporific title 'The Principles and Methods of Phylogenetic Systematics, and its Application to the Taxonomy of the Pronocephalidae Looss 1902 (Platyhelminthes: Digenea)' (Rodrigo 1990; I cite it here, because it is unlikely to get cited anywhere else). David wrote some kind words and awarded me a pass, although I am not altogether convinced that he actually read my thesis!

Looking back, I think the paper by David and colleagues that had the greatest impact on my work was their test of the theory of evolution using the congruence of phylogenetic trees of 11 species and five different biomolecules (Penny et al. 1982). The idea was simplicity itself: evolution predicts that independent genetic sequences obtained from related species should bear the imprint of the same evolutionary history. We can recover this imprint by building phylogenetic trees that depict the pattern of evolutionary relationships amongst species. Therefore, if we have two or more sequence trees, the similarity of their topologies provide corroboration for evolution. The real insight in the paper by Penny et al was to recognise that we can elevate what had been, up to this point, a simple inspection of phylogenies (for similarities and differences) to the level of a rigorous hypothesis test. This heralded the beginning of statistical phylogenetics – amongst the quasi-philosophical debates between cladists and pheneticists, between cladists and transformed cladists, between cladists and everyone else, this paper stood out as a beacon of common sense and rigour. Whereas others may have anticipated the possibility that phylogenetics is amenable to hypothesis tests (e.g. Cavender 1978), Penny et al. showed how such tests can be formulated and the power of their application. In a sense, these tests are 'phylometric', because they are based on phylogenetic measures of evidential support – hence the title of this paper.

In this particular test, as with any statistical test, one begins with a statistic. In this case, the statistic is the distance between any two phylogenetic trees, and Penny et al. used the Robinson–Foulds distance (Robinson & Foulds 1981). Once a statistic is available, we need to know the distribution of that statistic under the null model or hypothesis. Penny et al. used a null hypothesis that effectively said that the distance between two independent trees was a function of chance. It is possible to generate the null distribution of the Robinson–Foulds distance by building pairs of random trees and computing their distances. Their results showed that the distances between trees of the sequences that Penny et al. used were highly unlikely to have been obtained if the null hypothesis was true. Consequently, these sequences must share some part of their history.

In 1993, my colleagues and I added our own modest 'widget' to the Penny Phylometer™ (Rodrigo et al. 1993). Amongst biologists, frequently the aim is not to test whether any two phylogenies are more similar than expected by chance, but rather to ask whether the differences between two trees are a consequence of sampling. In this case, the null hypothesis is that both trees are (sample) estimates of the same underlying history. As with Penny et al. (1982), the test statistic was the Robinson-Foulds metric, and the procedure to generate the null distribution is based on a randomization procedure, the phylogenetic bootstrap (Felsenstein 1985). Readers are directed to the paper by Rodrigo et al. (1993) for the details.

In 1989, Penny and colleagues again applied the Phylometer™, this time to viruses from space. Sir Fred Hoyle, the
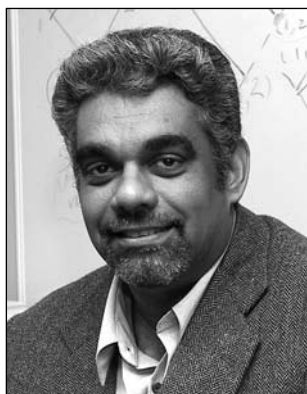
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distinguished British astronomer, and his colleague, Chandra Wickramasinghe were strong proponents of the theory of panspermia – that life had originated elsewhere and had spread throughout the universe. They had made the claim on several occasions (e.g. Hoyle & Wickramasinghe 1986) that it is quite reasonable to suggest that the earth is constantly bombarded by viruses and bacteria from space. Henderson *et al*. (1989) tested this hypothesis in several different ways using real data. In one example, they examined the phylogeny of Human Influenza Virus, arguing that under a multi-comet model in which influenza viruses are introduced repeatedly, there would be no reason that viruses from two successive epidemics would be more closely related. In contrast, if influenza viruses were part of a continuing and evolving history, then we would expect that successive epidemics would yield closely related viruses. By reconstructing the phylogeny of influenza viruses, and applying the appropriate statistical tests, Henderson *et al*. (1989) were able to reject the multi-comet theory (and indeed, other 'viruses-from-space' theories) in favour of a more earthbound explanation.

A digression at this point: in 1999, I was involved in a proposal to develop the first multidisciplinary astrobiology course at the University of Washington. The course brought together astronomers, oceanographers, paleontologists, microbiologists and evolutionary biologists. The proposal was funded by the National Science Foundation, and I was asked to give one of the first seminars. I spoke about the Henderson *et al*. (1989) rebuttal of Hoyle & Wickramasinghe (1986). After the seminar, one of the astronomers in the audience asked whether this rebuttal was general knowledge amongst biologists. It seemed that Hoyle and Wickramasinghe were still presenting their theory as a plausible alternative at conferences and in print. Wickramasinghe continues to be a proponent of panspermia (e.g. Wickramasinghe *et al.* 2003a), and in a paper published in *The Lancet*, no less, suggested that SARS may have an extraterrestrial origin (Wickramasinghe *et al.* 2003b).

Every pioneer must expect to face challenges and I think that David relishes the opportunity to argue the point. So allow me to raise a challenge here by revisiting Penny *et al.* (1982). This paper claimed to be a test of evolution, but the question has to be asked: what if the trees of the five proteins were no more similar than expected due to chance? Would we have rejected evolution as a valid scientific fact? I think not – after all, every biologist knows (and it has become ever more apparent) that genes and proteins are exchanged regularly, so that many histories are better represented as networks than as trees. Showing that the trees were no more similar than expected owing to chance would have been a fascinating biological conundrum that would have encouraged biologists to search for better *evolutionary* explanations. It would never have prompted a re-evaluation, let alone a rejection, of evolution *per se*.

If the intent of the Phylometer™ is to evaluate the weight of evidence in support of (or against) evolution versus some non-scientific credo like Creationism or Intelligent Design, then it must fail, because the latter are simply not empirically rational hypotheses. Phylometric tests work well when the alternatives are well-defined empirically, as in the 'viruses-from-space' example.

None of this, of course, detracts from the significant contributions that David Penny and his colleagues have made to this whole area of statistical phylogenetics. After 1982, phylogenetic trees were no longer grist for the pseudo-intellectual mill of the cladists, but were scientific tools that whittled away the collection of plausible hypotheses that explain our past, leaving only the few that withstand the rigours of critical testing.

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