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NEWS AND COMMENTARY

Phylogenetic analyses using AFLPs

Deeper AFLPs

J Graves

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Amplified fragment length polymorphisms (AFLPs) are a highly versatile method of genotyping a large number of loci for short start-up times and low costs. AFLPs are produced by cutting DNA into fragments with restriction enzymes and then attaching short synthesized sections of DNA to the ends of the fragments. A subset of the fragments is then amplified by PCR using synthesized DNA complementary to the adaptor and part of the restriction site. The numerous amplified fragments (>100 for each pair of primers) are visualized and classified by length. AFLPs detect polymorphism in different genomic regions simultaneously. They have been extensively used in studies of population structure, to identify hybrids and in elucidating shallow phylogenies, especially in plants, fungi and bacteria, but rarely in animals (Bensch and Åkesson, 2005). However, AFLPs have long been thought unsuitable for phylogenies deeper than subspecies or closely related species. The paper by Dasmahapatra *et al*. (2009) in this issue strongly challenges this view.

Dasmahapatra *et al*. used AFLPs to construct a phylogeny of pinnipeds and found strong support using genetic distance measures for basal divergences, including the ancient divergence of true seals (*Phocidae*) and the sister relationship of the walrus (*Odobenidae*) to the eared seals (*Otariidae*). These same divisions in the seals have been found using both mitochondrial DNA and nuclear DNA sequences, and dated at 23 ± 1.36 and 18 ± 1.4 Mya, respectively (Higdon *et al*., 2007). Other relationships were similar to the results of the earlier published pinniped phylogenies using mitochondrial DNA sequences, nuclear sequences, or both.

The problem with using AFLPs for phylogenetic analysis is that AFLP bands of the same length in two species will frequently not be homologous. Furthermore, the frequency of shared homologous bands decreases with time. Althoff *et al*. (2007) compared simulated AFLPs from the genomes of *Drospohila melanogaster* and *D. simulans*. They found that only 59% (26/44) of bands shared between *D. melanogaster* and *D. simulans* were homologous when the estimated divergence time is 6 Mya. The increase in non-homologous shared fragments with time since divergence is so high that phylogenetic signal is lost too quickly to be used in constructing even interspecific phylogenies.

Why the difference in results? Althoff *et al*. generated fragments from 50 to 500 bp long in their simulation, among these they found that the homology of bands was increased by excluding smaller fragments. Dasmahapatra *et al*. used only longer bands. They followed a protocol that excluded loci showing widely variable levels of amplification across the species and they only used loci that amplified consistently, giving sharp bands with minimal size variation across the entire data set. They found 310 AFLP loci 100–350 bp in length that were polymorphic and could be scored unambiguously.

They compared the average pairwise interspecific AFLP Jaccard distances with pairwise percentage sequence differences of the mitochondrial DNA (cytb) for the same species and found a strong linear relationship between them ($r = 0.87$). They also found a similar correlation with average nuclear sequence divergences ($r = 0.89$). They argue that these strong correlations between the measures reflect a similarity in estimated divergence times, that is, AFLP markers are evolving in a reasonably clock-like manner and that, therefore, AFLPs are very suitable for phylogenetic analyses. An AFLP clock has very recently been demonstrated for shallow divergences as well (<13 000 years; Kropf *et al*., 2009).

The study also highlights another problem with using AFLPs; the phylogenetic methodologies using these fragments are not well developed. Dasmahapatra *et al*. used distance measures based on Jaccard and Nei-Li genetic distances. Most phylogenetic studies use sequence data and construct phylogenies using parsimony, maximum likelihood or Bayesian methods. Development of these for AFLP data has only started recently (Luo *et al*., 2007). The loss of an AFLP band is treated as having a common origin in parsimony, but it can occur independently in different lineages—and becomes more likely with increasing time. Both maximum likelihood and Bayesian methods require a model of evolution, but to date, methods using the pattern of molecular marker loss and gain have been constructed for restriction fragment patterns and are inadequate for AFLPs (Felsenstein, 2004). Dasmahapatra *et al*. have shown that AFLPs are useful for deep phylogenies; now we need to interest theoreticians in designing methods specifically for AFLP data.

J Graves is at the School of Biology, University of St Andrews, Harold Mitchell Building, St Andrews, Fife KY16 9TH, UK.

e-mail: jag@st-andrews.ac.uk

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