biology
letters

Hangin' on to our rocks 'n clocks: a reply to Brown *et al.*

Per G.P Ericson, Cajsa Lisa Anderson and Gerald Mayr

Biol. Lett. 2007 **3**, 260-261
doi: 10.1098/rsbl.2007.0103

biology letters
Biol. Lett. (2007) **3**, 260–261
doi:10.1098/rsbl.2007.0103
Published online 27 March 2007
Evolutionary biology

Reply

Hangin' on to our rocks 'n clocks: a reply to Brown *et al.*

Ericson *et al.* (2006) presented the first well-supported DNA phylogeny including representatives of all major groups of Neoaves and using 23 fossils to date it. The comment by Brown *et al.* (2007) highlights the methodological problems inherent in analyses of these data, i.e. that the choice of fossil constraints has a great impact on the age estimates and that different dating methods do not necessarily yield the same results.

It has been demonstrated that calibration points constitute the most important factor for obtaining stable and reasonable age estimates. All fossils used in our paper are from the Tertiary, as the phylogenetic assignment of Mesozoic neornithine taxa remain controversial and are based on few, highly homoplastic characters. Inclusion of *Vegavis*, suggested by Brown *et al.* (2007), has virtually no effect on the estimations of divergence dates; the 84 dated neoavian nodes become on an average only 0.1 Myr older (with a maximum increase of 1.2 Myr). Brown *et al.*'s (2007) claim that we have overlooked a recently described Paleocene penguin is wrong. This fossil was included (calibration point L), citing the original publication in which it was dated to 55–65 Myr. All age constraints in the study were taken from the mean geological dating, therefore setting the age of the penguin fossil to 60 Myr, not 55 Myr as stated by Brown *et al.* (2007) in their table (electronic supplementary material-3).

Brown *et al.* (2007) incorrectly supposed that we used a maximum constraint of 95 Myr for Neoaves in the PATHd8 analysis. Instead, the age of Neoaves was *estimated* as 95 Myr by PATHd8 using 22 fossils as minimum age constraints and one as a fixed age, as described in the electronic supplementary material. However, in order to facilitate a comparison of 'ghost ranges' between dating methods, we had to use a maximum age constraint on the root in order to perform the penalized likelihood (PL) analysis. Without the root being fixed, the PL analysis resulted in unreasonably old ages (e.g. 250 Myr for Neoaves). We regret this was not better explained in the paper and appreciate the opportunity to clarify this matter. Again, the maximum age for Neoaves given in our fig. 2 is an *unconstrained* estimate based on the age of the fossils—a maximum constraint was never imposed on the age of Neoaves in the PATHd8 analysis.

The underlying theme in Brown *et al.* (2007) is that they find an age of 95 Myr for Neoaves too young and claim that there is molecular evidence for an extensive Cretaceous radiation of the group. Indeed, our data suggest that Neoaves began to diversify in the Late Cretaceous. The difference is that we find no evidence, neither in our data nor in the literature, for an *extensive* pre-Tertiary radiation (previous molecular studies that rely on the same, highly uncertain fossil calibration point, we do not regard as reliable, see Graur & Martin 2004). The fossil record does not suggest an Early Cretaceous origin for Neoaves: not a single neornithine fossil bird is known from Early Cretaceous sites. All of the numerous and greatly diversified birds from the Chinese Jehol Biota, which have an age of approximately 120 Myr, and other Early Cretaceous sites include no taxa assignable to Neornithes, let alone Neoaves.

Brown *et al.* (2007) question our selection of the dating method. It is still an open question which molecular dating method is the best (or least bad). We can assess only the performance of a method by comparison of the obtained ages with the fossil record. A method which suggests large 'ghost intervals' for many nodes might be regarded as dubious. This is why we argue that the PATHd8 method provides more reliable estimates than PL—it is *not* that we chose to rely on the method which happens to give younger dates, as implied by Brown *et al.* (2007)!

It is not surprising that the results from Brown *et al.*'s (2007) multidivtime analysis indicate a more gradual diversification than our PATHd8 analysis. This has mainly to do with the properties of the different smoothing approaches of the two methods. The PATHd8 algorithm smoothes rates between sister groups, while the Bayesian implementation smoothes between mother and daughter lineages. From this follows that PATHd8 preserves more of the heterogeneity seen in the original phylogram, while multidivtime results in older crown group ages, and a smoother looking chronogram than the corresponding PATHd8 analysis.

Another issue is the way Bayesian methods handle lack of information. Since there is no information on evolutionary rates or divergence times in the branch lengths used as input, the posterior probabilities might be suspected to converge to the prior assumptions. What may constitute reasonable values for prior assumptions specifying rate at root node ('rtrate'), rate variation ('brownmean') and distribution of internal node ages ('minab') is highly unclear. Brown *et al.*'s (2007) use of 'preferred priors' means that the level of autocorrelation is arbitrarily chosen, which might affect the outcome.

Confidence intervals for r8s and PATHd8 analyses generated by bootstrapping of branch lengths, and credibility intervals obtained from multidivtime analyses, represent different approaches of evaluating how well the data support the hypothesis, and are not comparable. Furthermore, these intervals can never account for the error sources of real interest, errors in dating of fossil strata, fossil placement in phylogeny, topology, sequence data and so on.

The accompanying comment can be viewed at http://dx.doi.org/10.1098/rsbl.2006.0611.

Received 22 February 2007
Accepted 22 February 2007

In summary, the observed differences in estimated divergence times between our study and that of Brown *et al.* (2007) are not surprising, given the fact that Brown *et al.* (2007) used (i) a different DNA alignment (excluding 25% of the characters from our analysis which is likely to affect the relative branch lengths between taxa), (ii) different fossil calibrations (changing the age of some and changing the constraint status from fixed age to minimum age of one), and (iii) another dating method (parametric, with a different rate smoothing approach). With all these differences, one should expect the results to differ as well. We strongly disagree, however, that their divergence time estimates are any better than ours. In fact, compared to the large 'ghost ranges' and over-smoothed look of the chronogram of Brown *et al.* (2007), we believe that our results show a closer match to the original data. We thus maintain our opinion that the evidence for an *extensive* diversification of Neoaves in the Cretaceous indeed is weak.

Andrzej Elzanowski, Ulf S. Johansson and Thomas J. Parsons kindly suggested improvements of the text.

Per G. P. Ericson[1,*], Cajsa Lisa Anderson[2],
Gerald Mayr[3]
[1]*Department of Vertebrate Zoology and Molecular Systematics Laboratory, Swedish Museum of Natural History, PO Box 50007, 10405 Stockholm, Sweden*
[2]*Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden*
[3]*Sektion für Ornithologie, Forschungsinstitut Senckenberg, Senckenberganlage 25, 60325 Frankfurt am Main, Germany*
**per.ericson@nrm.se*

Brown, J. W., Payne, R. B. & Mindell, D. P. 2007 Nuclear DNA does not reconcile 'rocks' and 'clocks' in Neoaves: a comment on Ericson *et al. Biol. Lett.* **3**, 257–259. (doi:10.1098/rsbl.2006.0611)

Ericson, P. G. P. *et al.* 2006 Diversification of Neoaves: integration of molecular sequence data and fossils. *Biol. Lett.* **2**, 543–547. (doi:10.1098/rsbl.2006.0523)

Graur, D. & Martin, W. 2004 Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends Genet.* **20**, 80–86. (doi:10.1016/j.tig.2003.12.003)