

## TECHNICAL RESPONSE

## AVIAN GENOMICS

# Response to Comment on “Whole-genome analyses resolve early branches in the tree of life of modern birds”

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Mitchell *et al.* argue that divergence-time estimates for our avian phylogeny were too young because of an “inappropriate” maximum age constraint for the most recent common ancestor of modern birds and that, as a result, most modern bird orders diverged before the Cretaceous-Paleogene mass extinction event 66 million years ago instead of after. However, their interpretations of the fossil record and timetrees are incorrect.

It is well known that changing the prior maximum age constraint in a Bayesian relaxed clock analysis can change estimates of species divergence times (1). Jarvis *et al.* (2) estimated the rapid diversification of Neoaves to be near the Cretaceous-Paleogene (K-Pg) boundary based on data-rich analyses, including first and second codon

positions of 1156 clocklike nuclear genes, 19 internal fossil calibrations, and a maximum age constraint of 99.6 million years ago (Ma) on the most recent common ancestor (MRCA) of Neornithes (modern birds) based on the Early-Late Cretaceous boundary. Mitchell *et al.* (3) claim that our 99.6-Ma constraint was “inappropriate.” Their contention is, instead, that a maximum constraint at 117.5 Ma—well into the Early Cretaceous (~145 to 99.6 Ma)—is “more appropriate,” and upon applying it, they unsurprisingly found that many divergences moved deeper in time, thus implying a major radiation of Neoaves in the Late Cretaceous (~99.6 to 66 Ma) before the 66-Ma K-Pg mass extinction event. Evaluating Mitchell *et al.*'s arguments requires answers to two questions: (i) is the maximum age constraint they used more appropriate than that used by Jarvis *et al.* (2) and (ii) does the use of different constraints for the MRCA of Neornithes substantially alter conclusions regarding timing of the neoavian radiation?

Addressing question (i), although Mitchell *et al.* claim that their 117.5-Ma prior is more appropriate, in fact it is a calibration product of the Early Cretaceous (110 Ma) fossil *Gansus yumenensis* (4) and a prior probability density chosen by Jetz *et al.* (5), rather than direct fossil evidence. Further, *Gansus* is an ornithurine (4) lying far outside other Mesozoic fossils more closely related to Neornithes (6, 7) and thus is an inappropriately old constraint for dating modern birds. Consequently, the empirical basis for their calibration is not well justified.

Mitchell *et al.* criticize our use of the empirical fossil record on the grounds that the record is taphonomically biased across space and time due to poor preservation of Southern Hemisphere Late Cretaceous terrestrial avian fossils, which they infer represent the most basal neornithines. Although

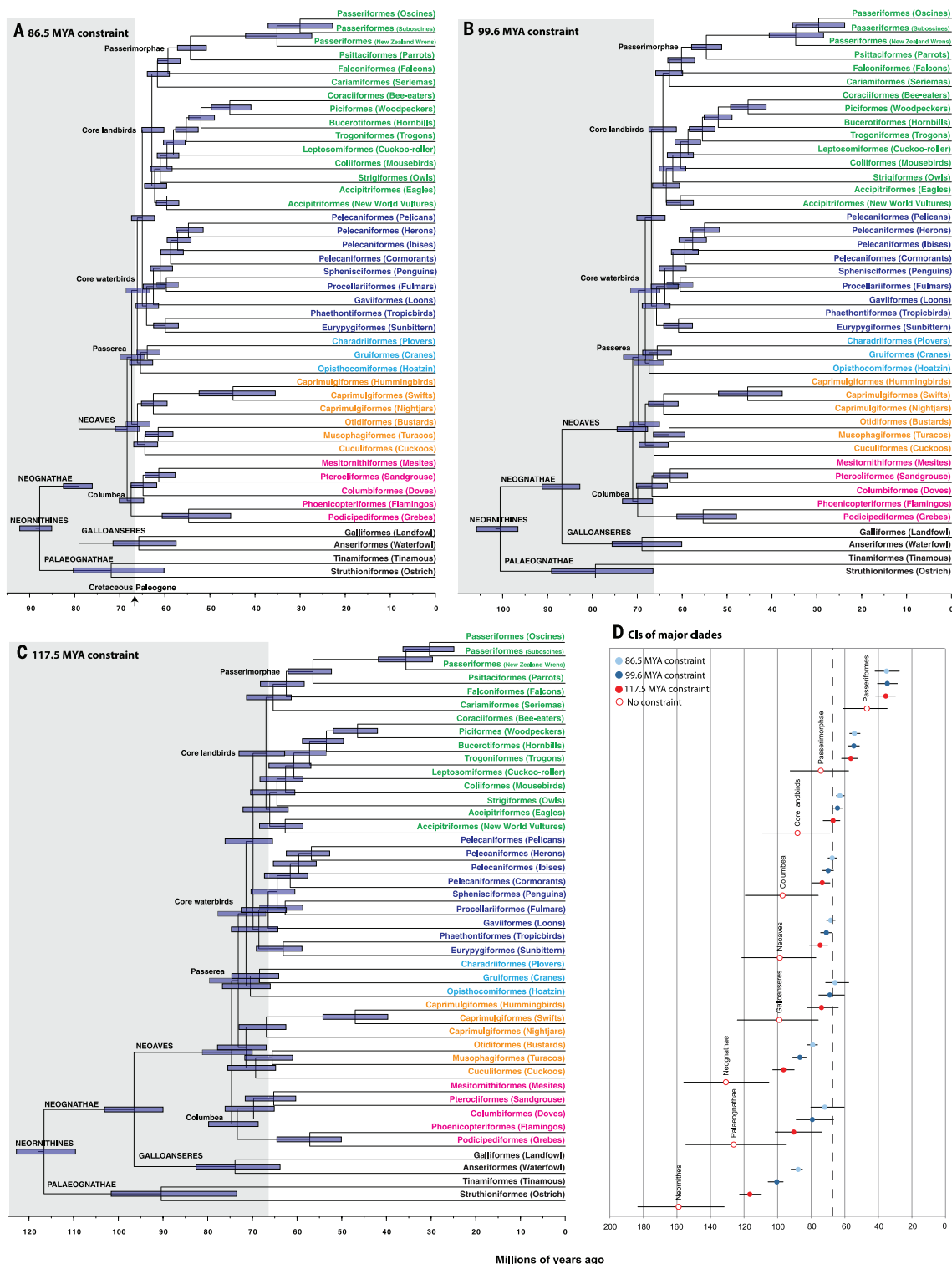
the Northern Hemisphere is better sampled than the Southern Hemisphere (8), small terrestrial neornithine birds ecologically equivalent to terrestrial neornithines are conspicuous in the Late Cretaceous avifaunas from Argentina (9) and Madagascar (10), whereas neornithines are conspicuously absent. Moreover, based on their survey of the avian fossil record, Fountaine *et al.* (11) conclude that “it is unlikely that the modern clades would have remained independently cryptic throughout [the Late Cretaceous].”

Mitchell *et al.* also largely dismiss the Early Cretaceous fossil record as uninformative with respect to the origin of neornithines, but then call attention to several fossils from the beginning of the Late Cretaceous to bolster their argument for an Early Cretaceous origin. We believe, however, that their interpretations of these fossils are faulty. Mitchell *et al.* refer to *Austinornis* at 85 Ma and cite Myers (12). However, Myers (12) does not mention *Austinornis* or an 85-Ma age for it. Clarke (13), on the other hand, while noting that *Austinornis lentus* has one character that suggests it might be a stem-galliform, stresses that this fossil should not be used in dating analyses (it lacked 99% of the characters in her matrix). Mitchell *et al.* raise the issue of a putative neornithine fossil from Patagonia ~83 to 94 Ma (14). However, the neornithine relationships of this fragmentary fossil are uncertain (15), and even if it were a neornithine, there would be no inconsistency with our results. Our results are consistent with the most convincing pre-K-Pg Neornithes fossil to date, *Vegavis*, a very Late Cretaceous (66 to 68 Ma) (16) inferred stem-anseriform (2). In contrast, the diverse and well-preserved Jehol Biota of China, which provides a window into a ~130 to 120 Ma Early Cretaceous period (17), has never yielded a neornithine fossil. The issue is not whether fossils assignable to the avian crown group (Neornithes) will be found in the Early to Late Cretaceous but that, with the exception of *Vegavis*, they have not been documented. The fossil record does not support the presence of a diverse Neornithine avifauna in the Early or Late Cretaceous, and especially not of Neoaves, the focus of Jarvis *et al.* (2).

To address question (ii), we examined the sensitivity of our relaxed clock analyses to different maximum age constraints for Neornithes in more detail. We note that even younger age constraints have been proposed (18), which Jarvis *et al.* (2) examined. Comparing results from all three proposed maximum constraints of 86.5 (18), 99.6 (2), and 117.5 Ma (3), among the 37 divergences of major ordinal lineages in Neoaves, only 1 to 4 out of 37 (3 to 11%), 5 to 9 out of 37 (14 to 24%) and 10 to 15 out of 37 (31 to 41%), respectively, predate the K-Pg boundary (Fig. 1, A to C) (mean dates). The exact numbers of divergences pre-K-Pg vary slightly depending on parameters and stochasticity of software dating methods (see figure legend) and the exact date used for the K-Pg, since so many divergences are near it. Importantly, the 95% credibility intervals (CI) showed that few divergences were exclusively pre-K-Pg boundary (0, 8, and 14%) under all three constraints, whereas the vast majority of CIs overlapped with the

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**Fig. 1 Genome-scale avian timetrees with 95% CIs and different maximum age constraints for Neornithes. (A to C)** Timetrees using the 86.5, 99.6, and 117.5 Ma maximum constraints, respectively, on exons of 1156 clocklike genes and the most recent version of the MCMCTREE program (20). The 95% CIs (blue bars) in (A) and (B) are slightly narrower and some divergences moved backward or forward in time by approximately one million years compared with those in Jarvis *et al.* (2), as a result of stochastic MCMC sampling effects and using an updated version of the software. However, the overall results are similar and all lie within the previously defined 95% CIs. Branches with

multiple species in the same suborder have been collapsed, and the reptilian outgroups (lizard, turtle, and alligator) are not shown. We do not show the four extinct lineages included in the Mitchell *et al.* (3) tree because they are not directly used in the Bayesian dating analysis. Order/suborder names are color coded according to large monophyletic groups. (D) Comparison of dates and 95% CIs for key avian divergences using the different maximum constraints. There are no dramatic shifts in dates beyond the Neornithes, Palaeognathae, and Neognathae splits, unless one eliminates the maximum age constraint for Neornithes altogether. MYA, million years ago.

K-Pg (24, 32, and 57%) or were exclusively post-K-Pg (76, 60, and 30%) (Fig. 1, A to D). Thus, most divergences are very near the K-Pg boundary, not many millions of years before. Removing the maximum age constraint altogether results in much earlier divergence-time estimates (Fig. 1D). However, such removal is difficult to justify given the absence of Early Cretaceous neornithine fossils. Inappropriately, Mitchell *et al.* try to contradict our conclusions using divergence dates for all Neornithes in the tree, when our claim was only about Neoaves (2). Moreover, they collapsed the branches of nearly all post-K-Pg ordinal divergences into deeper ones, making it appear that most divergences were pre-K-Pg.

In summary, our previous conclusion that Neoaves underwent a rapid radiation near the K-Pg boundary is well founded. Although the estimated dates of the origins of Neornithes and Neognathae do vary with the choice of maximum age constraints, the divergence times for Neoaves are much less sensitive. Improvements to relaxed clock methods may further reduce error due to model fit, but the interpretation of the fossil record will remain the most important component of relaxed clock analyses (19). The extremely short branch

lengths at the base of Neoaves, along with the evidence for discordance among gene trees due to incomplete lineage sorting [figure 3 in Jarvis *et al.* (2)], support what many previous analyses have found, that Neoaves underwent a very rapid radiation near the K-Pg boundary. Such rapid radiations are often associated with ecological drivers. The K-Pg Chicxulub asteroid impact with global ecological changes and mass extinctions around 66 Ma is consistent with its being one potential driver.

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