**DataBase Figure1 | Correlation between the body size and the relative egg size among Palaeognathae**. The vertical axis indicates the egg weight (EW) of Palaeognathae (grams). The horizontal axis indicates the body mass (BM) of the Palaeognathae (grams). The regression line (Log10EW=0.6688×log10BM-0.2174, R2=0.983) was estimated from extant Palaeognathae excluding kiwi (Apterygidae). The dashed lines indicate 95% confidence interval. Aepyornithidae and Apterygidae were indicated by orange dots. The common ancestor of them was indicated by a red squared dot. Dinornithidae was indicated by the pale green dots. Other paleognaths were indicated by pale blue dots. The illustrations of Aves were drawn by Mr. Takashi Oda.

**DataBase Figure2| Correlation between the body weight and the mitochondrial nucleotide substitution rate in the Aves.** The vertical axis indicates the body weight (grams in log scale) and the horizontal axis indicates the mitochondrial nucleotide substitution rate (% per site per 1000 million years). The regression line (Log10BM = −0.0208x + 4.213, PP>0.99, R² = 0.3636) was estimated from the extant Aves. The flightless birds were indicated by blue dots (ratites) and the deep blue dot (penguin). The volant birds were indicated by grey dots (tinamous), red dots (Galloanserae), pale orange dots (Aequorlitornithes except for penguin), yellow dots (Apodiform), and orange dots (Australaves).. The ancestral paleognaths were indicated by yellow dots, and their body weights were estimated by this regression line based on their estimated substitution rates.

**DataBase Figure3 | Comparison of the fossil calibration strategies.** (A) Divergence time estimation among the crown Aves with the reptilian outgroups. The time tree coloured in black was estimated by using the fossil calibrations used in this study (see Supplemental Table S2: basic calibrations), but the root of the crown Aves was assumed to be younger than 86.5Ma. The time tree coloured in red was estimated by using the following calibration strategy: The calibrations within the neognaths (nodes 24-26: see Supplemental Figure S1 and Table S2) were limited to the minimum boundaries only. There was no calibration within the paleognaths. The calibrations within the outgroups as well as the split between Crocodilia and Aves were the same as Supplemental Table S2. The first split of the crown Aves was constrained between 66Ma and 86.5 Ma. (B) Divergence time estimation among the crown Aves without the reptilian outgroups. The time trees coloured in black and red are the same as panel A, but the outgroups were not used. The root ages of the crown Aves (dashed lines) and of the crown Palaeognathae (chaine lines) were compared between the rooted tree and unrooted trees. (C) The prior distributions of the divergence times concerning the first splits of the crown Aves, the crown paleognaths, and the notopaleognaths used for this study (Supplemental Table S2: basic calibrations. There is no assumption on the root age of crown Aves), as well as the red coloured time tree shown in the panel A. Note the effect of the rooting issue and the calibration strategy is especially remarkable in the estimates within the Palaeognathae.

**DataBase Figure4 |** **Fossil Palaeognathae in Paleocene and Neogene.** The approximate locations of fossil records of Palaeognathae are shown. Triangles indicate volant paleognaths and squares indicate flightless ratites. Oospecies (species known only from eggs) are not indicated. The colour of the symbol indicates geological age: red (Paleocene), orange (Eocene), yellow (Oligocene), green (Miocene), and blue (Pliocene). The numbers beside the symbols indicate the species. **Paleocene:**1*.* Parris and Hope’s lithornid fossil, 2*. Lithornis celetius,* 3*. Fissuravis weigelti,* 4*. Remiornis heberti,* 5*. Diogenornis fragilis*. **Eocene:** 6*. Lithornis plebius,* 7*. Lithornis promiscuus ,* 8*. Lithornis nasi,* 9*.* *Lithornis hookeri.* 10*. Pseudocrypturus cercanaxius,* 11. *Paracathartes howardae,* 12. *Palaeotis,* 13. *Eleutherornis helveticus,* 14. *Proceriavis martini,* 15. *Eremopezus eocaenus,* **Oligocene:**16. *Lithornis vulturinus,* 17. *Emuarius guljaruba,* 18. *Emuarius gidju,* 19.Tambussi *et al.*’s ratite fossil. **Miocene:**20. *Struthio coppensi,* 21. *Struthio* sp.,22. *Struthio linxiaensis,* 23. *Struthio orlovi,* 24.Bertelli & Chiappe’s Tinamidae fossil,25. *Crypturellus reai,* 26. Tennyson *et al.*’s Dinornithiformes fossil*,* 27. *Proapteryx micromeros*. **Pliocene:**28. *Struthio wimani,* 29. *Struthio brachydactylus,* 30. *Struthio dmanisensis,* 31. *Struthio asiaticus,* 32. *Dromaius ocypus,* 33. *Nothura* sp.,34. *Eudromia* sp.Detailed information on these fossils is summarized in “DataBaseTable1”. The maps of the Paleocene (56 Ma) and Miocene (23 Ma) were downloaded from the Palaeomap project (<http://www.scotese.com/>).

**DataBase Figure5 | Patterns of DNA fragmentation and nucleotide misincorporation in the mitochondrial sequence reads.** The base composition in the first and last 10 nucleotides of the MiSeq reads identified as mitochondrial sequences are shown in a grey frame. The base composition in the 10 nucleotides upstream and downstream of the reads in the reconstructed mitochondrial genomes arealso shown. Each circle represents the average base frequency per nucleotide position. Frequencies of all base substitutions and indels observed between the MiSeq reads and the reconstructed mitochondrial genomes are shown as red (C to T), blue (G to A) and grey lines

**DataBase Figure6 | The effects on the divergence time estimates of Aves by the setting of the partition.** The time tree with the red coloured branches was based on the simpler partitions (15 partitions model), and tree with the black coloured branches was based on the optimized partitions (248 partitions model: the 95% CI of the estimated times are also shown on the nodes)

**DataBase Figure7 | The effects on the divergence time estimates of Aves with fast evolving genes.** The timings (and their 95% CI) of the first splits within the crown Aves and within the Palaeognathae were shown. The vertical axis indicates the divergence times in Ma. The horizontal axis indicates the different data sets used for these estimations. “15” indicates the whole partitions (empirical simpler 15 partitions) used in this study. “14” indicates the estimates based on the 14 partitions (the mitochondrial 3rd codon positions were excluded from the whole 15 partitions). “11a” indicates the estimates based on the 11 partitions (the mitochondrial genomes were excluded). “11b” indicates the estimates based on the 11 partitions (the 3rd codon positions of the nuclear and the mitochondrial protein coding genes were excluded). “9” indicates the estimates based on the 9 partitions (introns and the 3rd codon positions of the nuclear and the mitochondrial protein coding genes were excluded). “6” indicates the estimates based on the 6 partitions (introns and the 3rd codon positions of the nuclear genes as well as the mitochondrial genomes were excluded).