Whole-genome analyses resolve early branches in the tree of life of modern birds

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To better determine the history of modern birds, we performed a genome-scale phylogenetic analysis of 48 species representing all orders of Neovaves using phylogenomic methods created to handle genome-scale data. We recovered a highly resolved tree that confirms previously controversial sister or close relationships. We identified the first divergence in Neovaves, two groups we named Passerea and Columbidea, representing independent lineages of diverse and convergently evolved land and water bird species. Among Passerea, we infer the common ancestor of core landbirds to have been an apex predator and confirm independent gains of vocal learning. Among Columbidea, we identify pigeons and flamingoes as belonging to sister clades. Even with whole genomes, some of the earliest branches in Neovaves proved challenging to resolve, which was best explained by massive protein-coding sequence convergence and high levels of incomplete lineage sorting that occurred during a rapid radiation after the Cretaceous-Paleogene mass extinction event about 66 million years ago.

The diversification of species is not always gradual but can occur in rapid radiations, especially after major environmental changes (1, 2). For example, the rapid radiation of birds (19) occurred in response to the Cretaceous-Paleogene (K-Pg) mass extinction event about 66 million years ago (Ma). However, other nuclear (9–12) and mitochondrial (13, 14) DNA studies propose an earlier, more gradual diversification, beginning within the Cretaceous 80 to 125 Ma. This debate is con-

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they proposed several large new clades, including a waterbird clade containing taxa such as penguins, pelicans, and loons, as well as a landbird clade containing woodpeckers, birds of prey, parrots, and songbirds. Despite these efforts, the relationships among the deepest branches within Neaves; the positions of a number of chronically challenging taxa such as shorebirds, mousebirds, owls, and the enigmatic hoatzin; and the identification of the first divergence of Neaves [proposed to have given rise to two equally large clades designated Metaves and Coronaves (25)] remain unresolved.

Although some of the findings of the initial multi-gene studies (8, 37) have since been corroborated with larger sequence (26–28) or transposable element (TE) insertion data sets (29), other proposed clades were not supported (27, 28). Furthermore, complete mitochondrial genome analyses recover different relationships (14, 16) and fail to support higher landbird monophyly [but see (30)]. Some of the differences among studies could arise from gene tree incongruence, possibly due to incomplete lineage sorting (ILS) of those genes (34, 35). We test this hypothesis through phylogenetic analysis on 48 avian genomes we collected or assembled, representing all commonly accepted extant neognath orders (36, 37) and two palaearcogas, with several nonavian reptiles and human as outgroups.

**Species choice, computational developments, and total evidence nucleotide data set**

We chose species representing all neovian orders according to different classifications (see supplementary materials section 1 (SM1)). These include groups that have been challenging to place within the avian tree, such as the hoatzin, cuckoo-roller, nightjars, mousebirds, mesites, and seriemas (table S1). We also included species postulated to descend from deep nodes in their orders to break up potentially long branches, such as the kea for songbirds (Passeriformes). We included vocal-learning species (oscine songbirds, hummingbirds, and parrots), used as models for spoken language in humans (38), and their proposed closest vocal-nonlearning relatives (suboscines, swifts, falcons, and/or cuckoos, depending on the tree) to help resolve differences in trees that lead to different conclusions on their independent gains (15, 17, 18, 26, 29, 38, 39). The resulting data set consisted of 45 avian genomes sequenced in part for this project (48 when including previously published species (40–42)) and three nonavian reptiles (American alligator, green sea turtle, and green anole lizard (439)) (table S1), with details reported (44–42).

We were confronted with computational challenges not previously encountered in smaller-scale phylogenetic studies. Differently annotated genomes complicated the identification of orthologs, and the size of the data matrix made it impossible to use many standard phylogenetic tools. To address these challenges, we generated a uniform reannotation of the protein-coding genes for all avian genomes based on synteny in chicken and zebra finch (SM2). We found that the SATé iterative alignment program (53, 54) yielded more reliable alignments than other algorithms for large-scale data, and we developed alignment-filtering algorithms to remove unaligned and incorrectly overaligned sequences (SM3). We developed ExAML, a computationally more efficient version of the maximum likelihood program RAxML.
for estimating species trees from genome-scale concatenated sequence alignments (SM4) (55–57). We also developed a statistical binning approach that improves multispecies coalescent analyses for handling gene trees with low phylogenetic signal to infer a species tree (SM5) (58). These computationally intensive analyses were conducted on more than 9 supercomputer centers and required the equivalent of >400 years of computing using a single processor (SM3 and SM4).

From these efforts, we identified a high-quality orthologous gene set across avian species, consisting of exons from 8251 syntenic protein-coding genes (~40% of the proteome), introns from 2516 of these genes, and a nonoverlapping set of 3769 ultraconserved elements (UCEs) with ~1000 bp of flanking sequences. This total evidence nucleotide data set comprised ~41.8 million bp (table S3 and SM4), representing ~3.5% of an average avian genome.

A genome-scale avian phylogeny

Total evidence nucleotide tree

The total evidence nucleotide alignment partitioned by data type (introns, UCEs, and first and second exon codon positions; third positions excluded as described later) analyzed with ExaML under the GTR+GAMMA model of sequence evolution (SM4) resulted in a highly resolved total evidence nucleotide tree (TENT) (Fig. 1 and fig. S1). The three recognized major groupings within extant birds—Palaeognathae, Galloanseres, and Neoaves (the latter two united in the infraclass Neognathae)—were recovered with full (100%) bootstrap support (BS). The tree revealed the first divergence within extant Neoaves, resulting in two fully supported, reciprocally monophyletic sister clades that we named Passerea (after its most speciose group Passeriformes) and Columbea (after its most speciose group Columbiformes) (Fig. 1; see SM6 for rationale of clade names).

Within Passerea, the TENT strongly confirmed the monophyly of two large closely related clades that we refer to as core landbirds (Telluraves) and core waterbirds (Aequornithia) (8, 16, 17, 27, 36, 59);
we use the term “core” instead of “higher” to prevent interpretation that these groups are more advanced or more recently evolved than other birds. Within core landbirds, we found 100% BS for a previously more weakly supported clade (Australaves) containing seriema (historically placed in Gruiformes), falcons (historically grouped with other diurnal birds of prey), parrots (historically difficult to place), and Passeriformes and a sister clade (Afroaves) containing Accipitriformes birds of prey, owls, mousebirds, woodpeckers, and bee eaters, among others (Fig. 1) (8, 17, 26, 29, 60).

Core waterbirds were sister to a fully supported clade (Phaethontimorphae) containing tropicbirds and the sunbittern (Fig. 1) (27, 28). We did not include Phaethontimorphae in the core waterbirds because their relationship had relatively low 70% BS, although their aquatic (tropicbirds)

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**Fig. 2. Metatable analysis of species trees.** Results for different genomic partitions, methods, and data types are consistent with or contradict clades in our TENT ExaML, TENT MP-EST*, and exon-only trees and previous studies of morphology (15), DNA-DNA hybridization (24), mitochondrial genes (14), and nuclear genes (17). Letters (A to DD and a to e) denote clade nodes highlighted in Fig. 3, A and B, of the ExaML and MP-EST* TENT trees. Each column represents a species tree; each row represents a potential clade. Blue-green signifies the monophyly of a clade, and shades show the level of its BS (0 to 100%). Red, rejection of a clade; white, missing data. We used a 95% cut-off (instead of a standard 75%) for strong rejection due to higher support values with genome-scale data. The threshold for the mitochondrial study was set to 99% due to Bayesian posterior probabilities yielding higher values than BS. An expanded metatable showing partitioned ExaML, unbinned MP-EST, and additional codon tree analyses is shown in fig. S2.
Comparisons of TENT with previous studies

The TENT contradicted some relationships in avian phylogenies generated from morphological characters (15), DNA-DNA hybridization (24), and mitochondrial genomes (14, 18) (Figs. 2, fig. S2, and Fig. 3A versus fig. S3, A to C). For example, our Falconiformes excluded the previously included eagles and New World vultures (now in Accipitriformes); our Coraciiformes was more narrowly delineated and excluded hornbills and cuckoo-rollers; our Pelicaniformes excluded tropicbirds; and our Gruiformes excluded seriesms, bustards, the sunbittern, and mesites. The TENT did not fully support the view based on one gene (β-fibrinogen) that the first divergence in Neovaves resulted in two equally large Metaves and Coronaves radiations (25). However, all Columbea species in the TENT were in the previously defined Metaves, supporting the hypothesis of two parallel radiations of birds with convergent adaptations (25).

The TENT was most congruent with past (8, 17) and more recent (27, 28) smaller-scale multilocus nuclear trees (Figs. 2 and 3A and fig. S3D), although most congruence was limited to the core landbirds and core waterbirds. Within the former, we recovered Australas and Afroaves (60), although with a different branching order in our tree; our taxon sampling is insufficient to address the biogeographic justification of their names. The TENT recovered a number of groups not present in these previous trees, and even for those present, the TENT had higher BS (Fig. 2). Absence of nonavian outgroups in our TENT above was not responsible for variation with past studies because we recovered the same topology when including outgroups (Fig. 2 and fig. S4, A and B), despite the outgroups having only ~30% orthologous sequences in the TENT alignment (e.g., fig. S21; SM3).

More data are responsible for resolving early branches of the tree

Despite the many fully supported (100% BS) relationships in the TENT, lower support was obtained for 9 of the 45 internal branches (although still within the high 70 to 96% BS range). Almost all were at deep divergences within the Neoaves, after the Columbea and Passerea divergence and before the ordinal divergences (Fig. 1 and fig. S1). The monophyly of each of the superorders, however, had 100% BS. The presence of these lower BS values is in contrast to the expectation that genome-scale alignments would result in complete phylogenetic resolution (34, 35, 67).

However, consistent with this hypothesis, we found that most relationships that had less than 100% BS with the full TENT data exhibited a steady increase in support with an increase in random subsets of the TENT data (Fig. 2 and fig. S5). The placement of the Phaethontimorphae (sunbittern and tropicbirds) and hoatzin changed when smaller (25 to 50%) amounts of data were analyzed. Further exploring data amount, we used the assembled ~11-billion-bp chicken genome (46) as a reference to generate a 922-million-bp MULTIZ alignment of putatively orthologous genome regions across all species, comprising ~30% of an average assembled avian genome and corresponding to the maximal orthologous sequence obtainable across all orders under our homology criteria (SM4). We ran ExaML on the alignment for ~42 CPU years, with 20 maximum likelihood searches on distinct starting trees and 50 bootstrap replicates before reaching our convergence criterion (SM4) on a whole-genome tree (WGT). Notably, all runs resulted in one of two trees: one identical to the TENT topology (fig. S4C) and a second almost identical to the TENT (fig. S4D).

This latter tree differed from the TENT by local shifts in five branches, all cladades that had less than 100% BS in the TENT (fig. S4, A and D). Given the relatively minor differences between the second WGT and the TENT, together they corroborate the majority of relationships in the avian tree of life. Although the WGT has more data (table S3), the orthology (SM2) and alignment (SM3) qualities are higher for the TENT, and thus we consider the TENT more reliable.

Noncoding data contribute more to the TENT topology

We sought to determine if different genomic partitions contribute differently to the TENT and found that ExaML trees using only introns or UCEs from the TENT data were largely congruent with the TENT and WGTs for branches that had strong support (BS > 75%) in the intron and UCE trees (Figs. 2; 4, A and B; and 5B). However, the intron tree, and even more so the UCE tree, had lower resolution than the TENT (Fig. 5A), mostly on deep branches (Fig. 4, A and B), consistent with fewer data leading to lower resolution on deeper branches. For the intron tree, some lower-resolution branches had local shifts, but they matched those found in the second WGT or the 25 to 75% data subsets of the TENT; an exception was Phaethontimorphae, which had local shifts, but they matched those found in the second WGT and the TENT, together they corroborate the majority of relationships in the avian tree of life. To test this hypothesis, we compared the distribution of gene trees that have strong conflict (>75% BS) with branches of the ExaML TENT. We focused on introns because they had greater gene tree resolution (higher average BS) than exons or UCEs (fig. S24 and SM4).

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Multispecies coalescent approach informs a species tree similar to the TENT

To determine if ILS affected the concatenated tree analyses, we explored whether a multispecies coalescent model leads to a different tree topology. Multispecies coalescent methods estimate the species tree from a set of gene trees and are statistically consistent when discordance among gene trees results from ILS (64, 65). However, the inferred species tree can have low resolution (BS) and be less topologically accurate when the input gene trees are poorly resolved (33, 66), a problem that many of our genes faced (SM4). Thus, we developed a statistical binning technique that first groups genes into sets based on phylogenetic similarities, from each set estimates a supergene tree, and uses them in the maximum pseudolikelihood estimation of the species tree
(MP-EST) multispecies coalescent approach (67) to infer a species tree (SM5) (58). This approach produced more accurate estimated species trees compared with MP-EST applied to unbinned gene data sets that have low phylogenetic signal (i.e., figs. S2 and S9; SM7) (58). It produced a highly resolved binned MP-EST (MP-EST*) TENT tree that was highly congruent with the ExaML TENT (Fig. 3, A and B). There were only local shifts of five clades, nearly all on lower-support (<100% BS) branches of the ExaML and MP-EST* TENTs (Fig. 3, A and B). The monophyly of Afroaves was the only case of 100% BS in the ExaML TENT that conflicted with the MP-EST* TENT tree and involved a local shift in

**Fig. 3. Evidence of ILS.** (A) Cladogram of ExaML TENT avian species tree, annotated for nodes from Fig. 2 (letters), for branches with less than 100% BS without and with (parentheses) third codon positions, for strong (>75% BS) intron gene tree incongruence and congruence, and for indel congruence on all branches (except the root). Thin branch lines represent those not present in the MP-EST* TENT of (B). (Inset) ExaML branch lengths in substitution units (expanded view in fig. S7). Color coding of branches and species is as in Fig. 1. (B) Cladogram of MP-EST* TENT species tree, annotated similarly as in the ExaML TENT in (A). Thin branch lines represent those not present in the ExaML TENT of (A). (C) Percent of intron gene trees rejecting (>75% BS) branches in the ExaML TENT species tree relative to branch lengths. Letters denote nodes in (A) that either have less than 100% support or are different from the MP-EST* TENT in (B). (D) Percent of intron gene trees supporting (>75% BS) branches in the ExaML TENT species tree relative to branch lengths. (E) Indel hemiplasy [the inverse of percent of retention index (RI) = 1.0 indels that support the branch; see SM9] correlated with ExaML TENT branch length (log transformed). (F) Indel hemiplasy correlated with ExaML and MP-EST TENT internal branch divergence times in millions of years (log transformed). Plotting with internal branch times was necessary to compare both trees (SM9). (G) TE hemiplasy with owls among the core landbirds. Line color, shared TE tree topology; line thickness, relative proportion of TEs that support a specific topology (total numbers shown in the owl node). Circles at end of lines indicate loss of the TE allele in that species after ILS, as the sequence assembly contains an empty TE insertion site (SM10). Only topologies with two or more TEs are shown. (H) TE hemiplasy with songbirds among the core landbirds.

**A** ExaML TENT

**B** MP-EST* TENT

**C** Intragen gene tree incongruence

**D** Intragen gene tree congruence

**E** Indel hemiplasy vs branch length

**F** Indel hemiplasy vs time

**G** TE hemiplasy owl

**H** TE hemiplasy songbirds
Fig. 4. Species trees inferred from concatenation of different genomic partitions. (A) Intron tree. (B) UCE tree. (C) Exon c12 tree. (D) Exon c123 tree. The tree with the highest likelihood for each ExaML analysis is shown. Color coding of branches and species is as in Fig. 1 and fig. S1. Thick branches denote those present in the ExaML TENT. Numbers give the percent of BS.
**Fig. 5. Comparisons of total support among species trees and gene trees.**

(A) Average BS across all branches of species trees from varying input data as in Fig. 2, ordered left to right from lowest to highest values. (B) Numbers of incompatible branches (out of 45 internal), at different support thresholds, with the ExaML TENT tree, ordered left to right from most to least compatible (expanded analysis in fig. S6). (C) Analyses of intron, exon, and UCE gene tree congruence and incongruence with nodes in the ExaML TENT, MP-EST* TENT, and other species trees. Names and letters for clades are as in Figs. 2 and 3. "Missing" denotes the case in which an ortholog is not present for any taxa or is present for only one taxon, and hence monophyly cannot be determined. "Partially missing" indicates the case in which some taxa are missing but at least two of the taxa are present, and thus we can still categorize it as either monophyletic or not. For further details, see SM7.
the owl with mousebirds and Accipitrimorphae birds of prey. Two branches with <100% BS in the ExaML TENT increased to 100% in the MP-EST* TENT, including Phaethontimorphae with core waterbirds. The intron trees supported some branches more in the ExaML and some more in the MP-EST* TENT (Fig. 3, A and B). Nevertheless, the overall topology of both trees was very similar, including the basal Columba and Passeerea divergence.

All estimates of gene trees differ from our candidate species trees

No single intron, exon, or UCE locus from our TENT data set had an estimated topology identical to the ExaML TENT or MP-EST* TENT (fig. S10, A and B). The top three loci (all introns) with the closest inferred topologies differed from the two versions of the TENT on more than 20 to 30% of their branches. Average topological distance with the ExaML species tree was 63% for the introns, 66% for the UCEs, and 80% for the exons. To test whether our total evidence data set missed some genes with the TENT topologies, we constructed a more comprehensive collection of genes trees with phylomedB, which assigns orthology using maximum likelihood analyses (http://phylomedb.org) (see SM8 and (68)). For ~13,000 (low-coverage genomes) to ~18,000 (high-coverage genomes) annotated genes across avian species (44), phylomedB inferred orthologs for 94.58% of them and these agreed with the syntenic-based orthology of the 8251 protein-coding genes of the TENT by 93%. This more complete set of protein-coding genes still did not have a single estimated gene tree that was fully congruent with the ExaML or MP-EST* TENT trees (fig. S10, C and D), and there was overall low congruence with the species trees (http://tol.cgenomics.org/birds_v1) (fig. S11, A and B). The conflicting nodes largely reflected branches with low statistical support (approximate likelihood ratio test <0.95), which primarily corresponded to the short successive deep branches of Neoeaves. These findings can be explained by both a low amount of phylogenetic signal in individual loci (figs. S24 to S26 and SM4) and a high amount of ILS during the neovadian radiation.

Indels suggest a high degree of ILS at the earliest branches of the Neoeaves tree

We further assessed ILS using insertions and deletions (indels) (69), because they have less homoplasy (convergence) than single nucleotides (SNMs), and unlike gene trees, indels can be examined as discrete characters mapped to a reference tree without the added inference of constructing trees from them. We scored 5.7 million indels from the TENT alignment, of which 24% were shared by two or more taxa (table S3). We found indel incongruence on all branches of the ExaML TENT, as measured inversely by the percent of the indel characters uniquely defining each branch (Fig. 3A, red numbers; SM9). Like the gene trees, there appeared to be a successive decrease in the percentage of indels that supported deeper branches of each major clade (Fig. 3A). Most branches with the highest levels of indel incongruence belonged to the shortest and deepest ones that made local shifts in analyses, with the two branches joining mousebirds and owls exhibiting the highest indel incongruence and the shortest internal branch lengths in the ExaML TENT (Fig. 3A and fig. S7). Consistent with these findings, indel incongruence was inversely correlated with internal branch length, and branch length explained 87% ($R^2$) of the variation in the percentage of nonhomoplasious indels defining each branch (Fig. 3E). The correlation of indel incongruence versus branch time was similar for both ExaML and MP-EST* TENT trees (Fig. 3F).

Indel incongruence is not due to the indels supporting another species tree, as applying ExaML on indels from the total evidence alignment as binary data produced a total evidence indel tree that was largely congruent with the ExaML TENT and MP-EST* TENT for all but one node with a local shift of pigeon within Columbea (fig. S12). Homoplasy due to convergence is thought to be positively correlated with branch length (i.e., long branch attraction (70)). The only known source of incongruence that is inversely correlated with internal branch length is hemiplasy (differential inheritance of poly- morphic alleles) (64, 71). Because hemiplasy is a hallmark of ILS and 87% of the variation in indel incongruence is explained by branch length, our indel findings suggest high levels of ILS during the basal radiation of Neoeaves, with comparable support for the ExaML or MP-EST* versions of the TENT.

Transposable elements with higher ILS in the deepest branch of core landbirds with owls

We tested for a signature of ILS in TE insertions, which have extremely low homoplasy because independent insertions into the same location in a genome are rare (SM10) (72, 73). We focused on the owl because its position exhibited one of the strongest incongruencies among the species tree results. Of 3671 barn owl long terminal repeat TE insertion loci orthologous in all of the bird genomes, 61 were informative for owls among core landbirds and showed two dominant exclusive TE topologies: (i) an owl + Accipitrimorphae topology, as seen in the MP-EST* TENT; and (ii) an owl + Coracimorphae topology that excludes mousebird, as seen in the UCE tree (Fig. 3G compared to Figs. 3B and 4B). Nine other topologies (4 with one, 3 with another, and 3 for the remainder) plus seriemas, with no alternative to-
in the GTR + GAMMA model of sequence evolution, the third codon position exhibited a much stronger variation (fig. S15B). Reducing this variation by RY recoding of purines (R) and pyrimidines (T) on the third codon position (SM4) made the c123 tree topology more similar to the c12 topology (fig. 2 and fig. S14D). These results demonstrate that the third codon position exerts a strong influence on the protein-coding-tree topology, overriding signals from the first and second codon positions. They also suggest that a signal in the third codon position could also be associated with convergent life traits.

**Heterogeneous protein-coding genes associated with life history traits**

We further investigated the source of the conflict in the protein-coding genes (SM11) and found that trees using all codon positions from the 10% most compositionally homogeneous (low-variance) exons \( (n = 830) \) were more congruent with the c12 tree and, thus, more similar to the TENT than to the c123 tree (figs. S2 and 6A; cladograms in fig. S16, A to C). Conversely, trees using all codon positions from the 10% most compositionally heterogeneous (high-variance) genes \( (n = 830) \) were more congruent with the exon c123 and c3 trees (figs. 2 and 6B and fig. S16, B and D). The branch lengths of the high-variance exon tree showed a strong positive correlation with GC content and a negative correlation with the average body mass of species, seen at a much lesser magnitude in the low-variance exon tree (fig. 6, A to D). The correlations for the high-variance genes were also stronger on the third codon position (fig. S17, A and B) \( (75, 76) \). In addition, the genomic positions of the high-variance genes were skewed toward the ends of the chomosomes, whereas the positions of the low-variance genes were skewed toward the center (fig. 6, E and F, and fig. S17, C and D). Although the available introns of these genes had significant correlations among GC content and body mass and among GC content and chromosome position, they exhibited less heterogeneity overall (fig. S17, A to D) and yielded trees that were much more congruent with each other and with the TENT (figs. S2 and S17, E and F). An ExaML TENT tree that included the third codon position \( (TENT + c3) \) was identical in topology to the ExaML TENT without the third codon position and had increased support for six of the nine branches that had less than 100% BS (fig. S1 versus fig. S16, also Figs. 3A and 5A).

These results suggest that in the context of protein-coding data only, high-base compositional heterogeneity and life history have a strong impact on incongruence with the species tree, and thus are not suitable for generating a highly resolved phylogeny. However, in the context of large amounts of noncoding genomic data, the phylogenetic signal in the exon data adds support to the species tree.

**Dating the radiation of Neoaves**

The generation of a well-resolved avian phylogeny allowed us to address the timing of avian diversification. To estimate the avian timetree with genomic-scale data, we used first and second codon positions from 1156 clock-like exon genes (which do not strongly exhibit the above protein-coding compositional bias), calibrated with 19 conservatively chosen avian fossils (plus nonavian outgroups) as minimum bounds for lineage ages (with a maximum-bound age constraint of 59.6 Ma for Neornithes), in a Bayesian autocorrelated relaxed clock method using MCMCTREE \( (77) \) on the fixed ExaML TENT topology (SM12).

Our results suggest that after the Palaeognathae and Neognathae divergence about 100 Ma in the Late Cretaceous, the Palaeognathae diverged into their two stem lineages \( (ratites and tinamous \( 11,75) \approx 84 \text{ Ma}, \) and the Neognathae diverged into their stem lineages \( (Gallinaceous and Neoaves) \approx 88 \text{ Ma} \) (fig. 1). Although the 95% credibility interval for the ostrich-tinamous divergence is broad, its lower bound is consistent with the fossil record \( (79) \). In contrast, both the earliest divergence within Gallorostrales and an explosive diversification within Neovaves were dated to occur around the K-Pg boundary, with 95% credibility intervals spanning 6.5 million years, on average. In particular, the most basal divergences within Neoaves \( (Columbea, Pussera, and two more) \) occurred before the K-Pg transition \( (67 \text{ to } 69 \text{ Ma}) \) and all others after, with nearly all ordinal divergences completed by 50 Ma (fig. 1, dashed line). The estimated age for the basal split of Passeriformes, representing \( \approx 60\% \) of all living \( \approx 10,400 \) avian species, was around 39 Ma. These divergence times conflict with some previous studies based on nuclear \( (9-12) \) and mitochondrial \( (33, 34) \) DNA but are consistent with the fossil record \( (80) \), including the identification of Vagatisia arai, a very Late Cretaceous \( (66 \text{ to } 68 \text{ Ma}) \) stem-anseriform fossil \( (80, 81) \), and the dearth of verifiable Neoaves fossils in the Late Cretaceous \( (5) \). These findings were similar regardless of the specific tree from this study we dated or whether we used a later minimum age \( (86.5 \text{ Ma}) \) for Neornithes (table S16; more discussion on dating in SM12).

**Discussion**

Our study is an example of the extraordinary amount of genomic sequence data required to produce a highly supported phylogeny spanning a rapid radiation. The conflict we observe with other data types \( (14, 15, 24) \) can no longer be considered to be due to error from smaller amounts of sequence data \( (8, 17) \) nor to differences in concatenation versus coalescence methods \( (27, 28) \). The absence of a single gene tree identical to the avian species tree is consistent with studies in yeast \( (82) \), indicating that phylogenetic studies based on one or several genes, especially for rapid radiations, will probably be insufficient. The major sources of the gene tree incongruence we find are low-resolution gene trees and substantial ILS during the rapid radiation. It is possible that some of the deep branches of the species tree are in the anomaly zone \( (63) \), although the gene tree support is not high enough to confidently test this idea. It is also possible that some gene and local species tree incongruence could reflect ancient hybridization during the radiation, but distinguishing between this and other sources of hemiplasy \( (83) \) would require more complete assemblies, genes without mis-

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*Note:* The text continues with further discussion and findings related to avian diversification, including the timing of the radiation of Neoaves, the role of genomic data in resolving conflicts with fossil records, and the implications for understanding the evolutionary history of birds.
Fig. 6. Life history incongruence in protein-coding trees. (A) Species tree inferred from low–base composition variance exons (n = 830 genes) graphed with branch length, third codon position GC (GC3) content (heatmap), and log of body mass (numbers on branches). (B) Species tree inferred from high–base composition variance exons (n = 830 genes), graphed similarly as in (A). The %GC3 scale is higher and ~10 times wider for the high-variance genes, and the branch lengths are ~3 times longer [black scales at the bottom of (A) and (B)]. Color coding of species’ names is as in Fig. 1. Cladograms of trees in (A) and (B) are in figs. S16, A and B. (C and D) Correlations of branch length with GC content (C) and body mass (D) of the low-variance and high-variance exons. Correlations were still significant after independent contrast analyses for phylogenetic relationships (SM11). (E and F) Relative chromosome positions of the low-variance (E) and high-variance (F) exons normalized between 0 and 1 for all chicken chromosomes and separated into 100 bins (bars). The height of each bar represents the number of genes in that specific relative location. The two distributions in (E) and (F) are significantly different (P < 2.2 × 10−16, Wilcoxon rank sum test on grouped values). For further details, see SM11.
broadly, the Columbea and Passerea clades appear to have many ecologically driven convergent traits that have led previous studies to group them into supposed monophyletic taxa (8, 17, 23). These convergences include the footpropelled diving trait of grebes in Columbea with loons and cor- morants (25) in Passerea, the wading-feeding trait of flamingos in Columbea with ibises and egrets (24, 99) in Passerea, and pigeons and sandgrouse in Columbea with shorebirds (killdeer) in Passerea (24). These long-known trait and morphology alliances suggest that some of the traditional nonmonophyletic trait associations are based on polyphylytic assemblages.

In conclusion, our genome-scale analysis supports the hypothesis of a rapid radiation of diverse species occurring within a relatively short period of time (36 lineages within 10 to 15 million years; Fig. 1) during the K-Pg transition, with many interordinal divergences in the 1- to 3-million-year range. This rate of divergence is consistent with modern speciation rates, but it is notable that so many lineages from a single stem lineage survived extinction. Subsequent ecological diversification of surviving lineages is consistent with a proliferation of the earliest fossil stem representatives of most modern orders by the latest Paleocene to Eocene. Our finding is broadly consistent with recent estimates for placental mammals (100), but see SM12 (101) and thus supports the hypothesis that the K-Pg transition was associated with a rapid species radiation caused by a release of ecological niches following the environmental destruction and species extinctions linked to an asteroid impact (2, 4, 5, 102).

REFERENCES AND NOTES
Whole-genome analyses resolve early branches in the tree of life of modern birds
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