Mutation rate is linked to diversification in birds

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How does genome evolution affect the rate of diversification of biological lineages? Recent studies have suggested that the overall rate of genome evolution is correlated with the rate of diversification. If true, this claim has important consequences for understanding the process of diversification, and implications for the use of DNA sequence data to reconstruct evolutionary history. However, the generality and cause of this relationship have not been established. Here, we test the relationship between the rate of molecular evolution and net diversification with a 19-gene, 17-kb DNA sequence dataset from 64 families of birds. We show that rates of molecular evolution are positively correlated to net diversification in birds. Using a 7.6-kb dataset of protein-coding DNA, we show that the synonymous substitution rate, and therefore the mutation rate, is correlated to net diversification. Further analysis shows that the link between mutation rates and net diversification is unlikely to be the indirect result of correlations with life-history variables that may influence both quantities, suggesting that there might be a causal link between mutation rates and net diversification.

avian | speciation | divergence | reproductive isolation | hybrid incompatibility

Diversification is the net result of the addition of species by speciation and the removal of species by extinction. Understanding the causes and consequences of diversification is central to evolutionary biology. Correlations between diversification, life history, and ecology are becoming increasingly well understood (e.g., refs. 1–4). However, the link between diversification and the rate of molecular evolution is still debated, with much of the attention focused on the role speciation plays in driving genetic change (5–13). Changes to specific genes have been linked to the development of reproductive isolation during species formation, and in some cases such genes have been shown to be under strong positive selection (reviewed in refs. 14 and 15). However, there is a growing body of evidence showing that diversification correlates positively with rates of DNA sequence evolution in "house-keeping" genes, which are associated with basic metabolic functions and, therefore, not expected to be directly involved in the process of diversification. These results raise the possibility that there is a general association between diversification and rates of genomic change.

Previous studies have noted that clades of flowering plants containing more species tend to have longer molecular branch lengths (6, 11), and that path lengths on molecular phylogenies from a range of taxa tend to be positively correlated to the number of nodes through which they pass (16). These results suggest that net diversification—the balance between speciation and extinction rates that gave rise to the extant diversity—is somehow linked to rates of DNA change over time.

One hypothesis put forward to explain the correlation between rates of molecular evolution and net diversification is that the process of speciation increases the rate of molecular evolution (10, 16). This hypothesis is based on the assumption that speciation is often associated with factors that have the potential to increase the rate of molecular evolution, such as adaptation to new environments and transient reductions in population size (reviewed in ref. 10). For example, if speciation occurs by the subdivision of populations, then lineages that speciate more frequently may have lower average population sizes. Because nonneutral substitution rates tend to be higher in smaller populations (17), lineages with high speciation rates might also have higher overall substitution rates.

However, there are other possible causal links between rates of molecular evolution and net diversification. For example, higher rates of molecular evolution may drive increases in net diversification if they cause increased speciation rates or decreased extinction rates. Both theory and empirical data suggest that speciation can occur more quickly in lineages with higher mutation rates if the development of reproductive isolation is mutation rate-dependent (18, 19). It has also been suggested that mutation rate is a key factor influencing extinction risk through the maintenance of genetic variation in populations (e.g., ref. 20). If this is the case, then higher mutation rates could be associated with decreased extinction rates, and thus with higher diversification rates.

Finally, rather than there being a direct causal relationship between rates of molecular evolution and net diversification, the correlation between the two may be caused by a third factor that influences both. For example, a previous study of plants suggested that environmental energy drives both rates of molecular evolution and net diversification, but that there is no direct link between the two (6).

Distinguishing between these hypotheses has important implications for understanding the roles of genetic change and population isolation in diversification, and in understanding the mechanistic basis of the correlation between diversification and the rate of molecular evolution. Previous studies have not been sufficient to determine the generality of the relationship between molecular change and diversification, nor have they tested the possible causes of this relationship. In addition, some studies have been criticized for failing to account for possible sources of error, such as the node-density effect, which could cause a spurious relationship between rates of molecular evolution and net diversification (5, 7, 12). It is therefore important that the relationship between rates of molecular evolution and net diversification is tested systematically on large datasets using methods that can detect the signatures of different evolutionary processes.

In this study, we use a sister-pairs approach to examine the relationships between rates of molecular evolution, net diversification, and life-history traits in birds. Each sister pair comprises two families of birds that share a common ancestor to the exclusion of all other families in the dataset (Fig. 1). By definition, each of the two lineages of the sister pair originated at the same time, so they have had the same amount of time to accumulate genetic differences or to accumulate species through the net effects of speciation and extinction. Therefore, any difference in the amount of molecular change accumulated along each lineage of the sister pair represents a difference in the rate of molecular evolution, and any difference in the species richness of these two sister lineages represents a difference in net diversification (6, 21). Phylogenetically independent sister pairs represent separate evolutionary instances of the association between molecular rates and net diversification, so we can use them as

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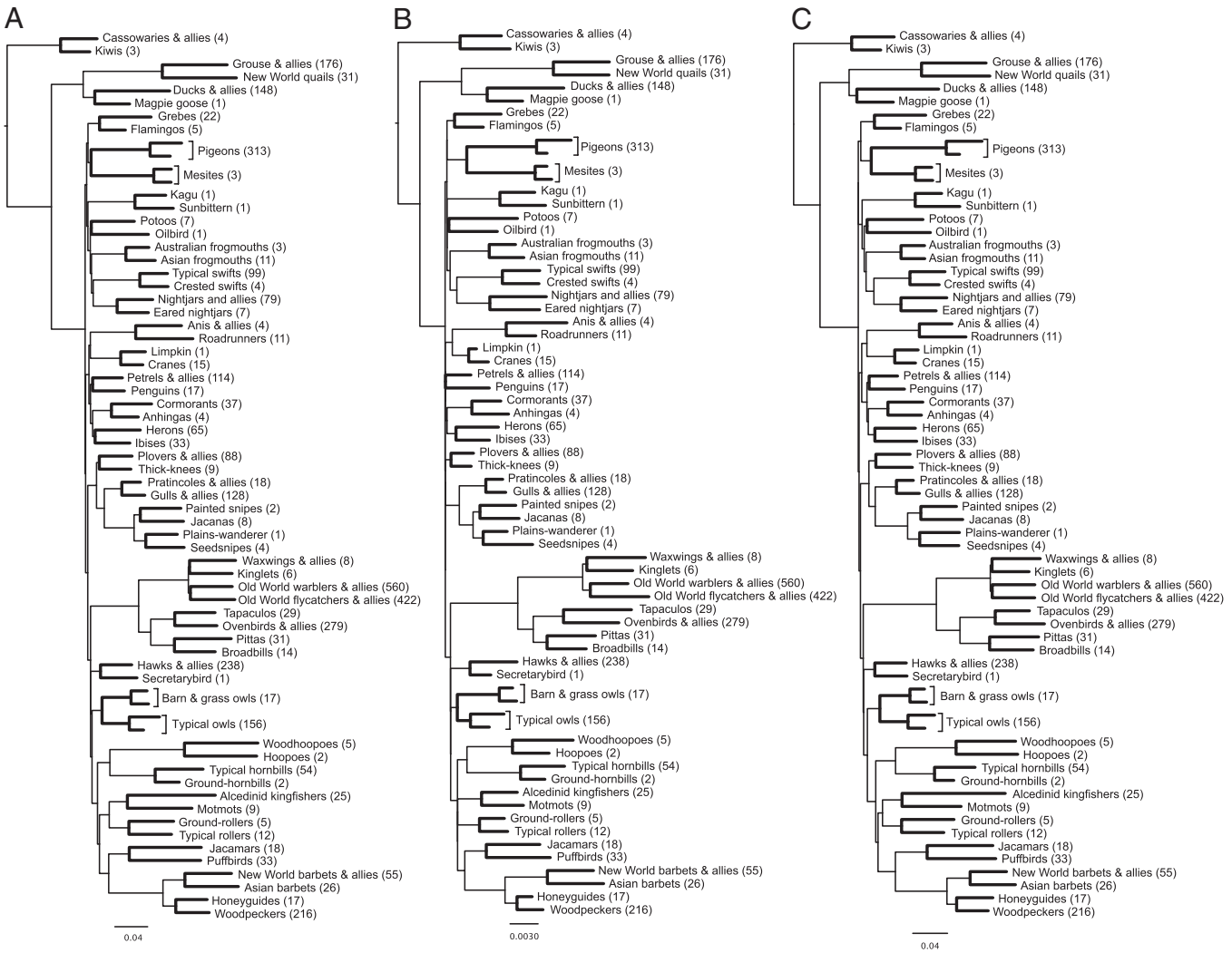

Fig. 1. Molecular phylogenies of the 32 phylogenetically independent pairs of families used in this analysis. The common name of each family and the number of species in that family (24, 25) are shown. Branch lengths used in the analysis are shown in bold. Branch lengths are proportional to (*A*) the total number of substitutions measured from the 17.5-kb dataset; (*B*) the number of nonsynonymous substitutions (*dN*) measured from the 7.5-kb protein-coding dataset; (*C*) the number of synonymous substitutions (*dS*) measured from the 7.5-kb protein-coding dataset.

datapoints in a statistical analysis to test for an association between molecular rates, net diversification, and life-history traits (22).

Using a large DNA sequence dataset (23) and estimates of the number of taxonomic species in families of birds (24, 25), we provide evidence for a positive relationship between the rate of molecular evolution and net diversification in families of birds. We use additional analyses of 17 nuclear protein-coding sequences and estimates of avian life-history characteristics to test hypothesized causes of the association between rates of molecular evolution and net diversification. By demonstrating a link between synonymous substitution rates and species richness in bird families, our results suggest that the link between molecular rates and net diversification in birds is mediated by the mutation rate. The results of further analyses show that variation in life-history traits cannot explain the observed correlations between molecular rates and net diversification, suggesting that higher genomic mutation rates may play a causal role in increasing the net diversification of bird families.

Results

There Is Significant Variation in Substitution Rates Among Bird Lineages. Fig. 1 shows that rates of molecular evolution vary even between closely related families of birds. For example, the ducks and allies (Anatidae) have molecular branch lengths more than twice as long as their sister family, the magpie goose (Anseranatidae) (Fig. 1), demonstrating a higher substitution rate in the duck lineage. We tested for the significance of the variation in rates of molecular evolution by comparing the likelihood of a molecular-clock model to that of a model in which rates of molecular evolution were free to vary among branches using the likelihood ratio test (*Methods*). We found highly significant variation in substitution rates for all partitions (introns, exons, and UTRs) and all measures of rates [total branch length, *dN* (nonsynonymous substitution rate) and *dS* (synonymous substitution rate)] used in this study (likelihood ratio test P value $<1 \times 10^{-100}$; see *SI Text* and Table S1 for full details). The branch-length estimates used in this study are much less than one substitution per site for all types of substitution measured (i.e., total, *dN*, and *dS* branch lengths), suggesting that substitutional saturation is unlikely to have limited our ability to accurately infer rates of molecular evolution (see *SI Text* and Figs. S1 and S2 for full details).

Net Diversification Is Positively Associated with Rates of Molecular Evolution in Birds. We measured clade size (the number of taxonomic species in each family) and molecular branch lengths for 64

bird families, which fall into 32 sister pairs (Fig. 1, and Dataset S1). We used these measures to calculate the difference in net diversification and the difference in the rate of molecular evolution between the two lineages of each sister pair (*Methods*). Each sister pair thus contributes a single datapoint to the analysis. Molecular branch lengths were estimated from a 19-gene, 17-kb partitioned supermatrix of introns, exons, and UTRs. These genes are sampled from across the genome, with loci from 15 of the 40 avian chromosomes (as assessed by homology to the chicken genome). To avoid the node-density effect, we deleted lineages so as to equalize the number of representatives per family (one species per family in all but two sister pairs) (Fig. 1). Linear regressions through the origin (21, 26, 27) reveal a significant positive association between differences in net diversification and differences in overall substitution rate ($P = 0.015, r^2 = 0.18$) (Fig. 1*A* and Fig. S5), indicative of a significant positive association between net diversification and rates of molecular evolution.

Net Diversification Is Associated with *dN* and *dS* but Not *dN/dS*. We used 7.6 kb of DNA data from 17 protein-coding genes to estimate differences in *dN*, *dS*, and ω (*dN*/*dS*) for each sister pair of families in our dataset (Dataset S1) (*Methods*). Because *dS* estimates the number of "silent" substitutions that do not change the amino acid sequence, it will be predominantly influenced by the mutation rate (28); *dN* estimates the number of substitutions that change the amino acid sequence, which is likely to include both neutral and nearly neutral changes. Because of this, *dN* is expected to be influenced both by the mutation rate and by population size (17). Therefore, ω is expected to be influenced by selection and effective population size, but not the mutation rate. Using linear regressions through zero (21, 26, 27), we found significant positive relationships between *dN* and net diversification ($P = 0.007, r^2 = 0.23$) (Fig. 1*B* and Fig. S5) and between *dS* and net diversification ($P = 0.010, r^2 = 0.19$) (Fig. 1*C* and Fig. S5). However, we found no evidence of an association between ω and net diversification ($P = 0.215, r^2 = 0.05$) (Fig. S5).

Body Size, Age at First Breeding, and Sexual Dichromatism Are Not Associated with Either Net Diversification or Rates of Molecular Evolution. Using linear regressions forced through zero (21, 26, 27), we found no significant associations between life-history traits (body size, age at first breeding, and sexual dichromatism) and net diversification, or between life-history traits and rates of molecular evolution (overall substitution rate, *dS*, *dN*, and ω) (Table S2 and Dataset S1). Full details of all of these analyses are presented in the *SI Text*.

Discussion

The results of this study show that rates of molecular evolution are significantly associated with net diversification (i.e., number of extant taxonomic species accrued since the origin of a clade) in families of birds (Fig. 1). These results are in accordance with previous studies of a range of other taxa, which have found significant correlations between rates of molecular evolution and net diversification (6, 9–11, 16).

Net Diversification Is Linked to Mutation Rate in Birds. Our analyses of *dN* (Fig. 1*B*) and *dS* (Fig. 1*C*) in relation to net diversification allow us to make some inference as to the possible mechanism behind the observed links between net diversification and rates of molecular evolution in birds. Synonymous mutations tend to have very small selective effects (of the order of 1×10^{-6}) (reviewed in ref. 29), and so are expected to behave as neutral mutations in lineages with effective population sizes smaller than about 10^6 (28, 30). Estimates of the effective population sizes of bird lineages vary, but the majority of estimates are at least an order of magnitude smaller than 10^6 (30–35). This finding suggests that the vast majority of synonymous mutations will behave as neutral mutations in bird lineages. The neutral substitution rate is driven solely by the rate at which neutral mutations are generated (28). Therefore, if synonymous mutations behave neutrally, differences in the synonymous substitution rate (*dS*) will be driven solely by differences in the underlying genomic mutation rate (28, 30). The significant association between *dS* and net diversification therefore suggests an association between mutation rates and diversification in birds. Increases in the mutation rate are also expected to increase the nonsynonymous substitution rate (36), so a link between mutation rate and net diversification may also explain the significant association between *dN* and net diversification (Fig. 1*B*).

Mutation rate could have a positive influence on net diversification if lineages with higher mutation rates are less likely to go extinct, more likely to speciate, or both. It is possible that higher mutation rates could be associated with lower extinction probabilities if increased mutation rates lead to the generation of a greater number of beneficial mutations (e.g., ref. 37), and thus allow more rapid adaptation, but the extent to which this would affect extinction rates in birds is questionable. A more likely hypothesis is that mutation rate could have a positive impact on the net speciation rate through the more rapid generation of differences between diverging lineages (e.g., refs. 38 and 39). These differences may cause observable differences between lineages, leading to recognition of a greater number of taxonomic species. Alternatively, a higher mutation rate could drive the evolution of new species by generating genetic differences that lead to hybrid incompatibility between lineages, whether or not these changes result in observable phenotypic differences.

When a population is subdivided, so that any mutation that arises in one population cannot be inherited by a member of the other population, the descendent populations will accumulate substitutions independently of one another. The two isolated subpopulations will gradually diverge, and eventually may become so different that they are unable to reproduce, either through the acquisition of phenotypic differences that create prezygotic reproductive isolation, or through the acquisition of genomic differences which create postzygotic reproductive isolation (or both). Previous studies in birds have shown that hybrid fitness is inversely proportional to the genetic distance between the hybrid's parents (19, 40, 41), and similar results have been reported in flies (42, 43), butterflies and moths (44), and frogs (45). Whatever the underlying cause of reproductive isolation, a higher rate of mutation would provide a greater amount of raw material for both drift and selection, and so could accelerate the development of separate species from two diverging populations (18).

Indirect Links with Life History Do Not Explain the Correlation Between Molecular Rates and Net Diversification. An indirect link between molecular rates and net diversification could arise if factors that correlate with substitution rates also scale with species richness of clades. There are a large number of factors that can affect mutation and substitution rates, leading to complex patterns of variation in the rate of molecular evolution between species (46). Comparative analyses have led to the detection of systematic patterns in the way that the rate of molecular evolution varies between species. For example, some studies have shown that rates of molecular evolution are related to body size and generation time in birds (47, 48). This generation time effect has been noted in a number of other taxa and is generally attributed to the influence of copy errors on the rate of molecular evolution (47, 49–51). It has also been suggested that families of birds with small body size or short generation times have higher net diversification (2, 52), although other studies have failed to find evidence for this link (e.g., refs. 3 and 53). We have shown that for our data, family-averaged values of age at first breeding or body size do not explain the observed variation in rate of molecular evolution, nor do they scale with net diversification (*Results*). These findings suggest that variation in body size and generation time cannot explain the links between rates of molecular evolution and net diversification observed here.

Net diversification in birds has been associated with a number of other traits, such as latitude, sexual dimorphism, geographic range size, dispersal, and feeding ecology, so it is pertinent to ask whether any of these could influence rates of molecular evolution. Some studies have shown that rates of avian diversification are higher at lower latitudes (4, 54, 55, but see ref. 56), but there is currently no evidence for a latitudinal gradient in rates of avian molecular evolution (57), although such a link has been suggested for other taxa (58, 59) and warrants further investigation.

Some studies have found that indicators of the strength of sexual selection are correlated with net diversification in birds (55, 60, 61), although other studies have found no evidence for this correlation (2, 62). It is possible that stronger sexual selection could also increase rates of molecular evolution through "male-driven evolution" (63). Males in species with multiple mating and strong sexual selection may need to produce more sperm. Because sperm are produced by continuous division of germ-line cells, the average number of cell divisions per sperm cell increases the more sperm is produced. Because mutations accumulate in the germ line with every copy, the increase in average number of cell divisions in the germ line of males that produce a lot of sperm could result in a higher neutral substitution rate (64). Thus, it is possible that sexual selection could drive increases in both net diversification and mutation rates, which could produce correlations such as those observed in this study. Sexual dichromatism is sometimes used as an indicator of the strength of sexual selection in bird taxa (e.g., ref. 60). However, we do not find a significant association between the proportion of species in a family that show sexual dichromatism and either molecular rates or net diversification (*Results*). This finding suggests that sexual selection cannot explain the links between rates of molecular evolution and net diversification observed here.

Several studies have connected geographic range size with net diversification in birds, but the direction of the relationship varies between studies (2, 55, 65, 66). Geographic range size could influence the effective population size of bird lineages, and so could also influence dN and ω (17). Similarly, dispersal rate could influence the effective population size. However, although these mechanisms could explain the observed correlation between dN and net diversification, they are unlikely to explain the observed correlation between dS and net diversification, as there is no obvious link between range size or dispersal rate and the mutation rate.

Feeding ecology has been linked to net diversification in birds (2), but we are unaware of any potential links between feeding ecology and mutation rates. Feeding ecology could potentially correlate with metabolic rate in birds (e.g., refs. 67 and 68), and the latter has been proposed as a driver of mutation rate (69, 70). However, studies that have examined multiple life-history variables have found no significant association between metabolic rate and rate of molecular evolution beyond its covariance with other life-history variables, such as body size, generation time, and longevity in birds (47, 71) and other animals (50, 71, 72).

Net Diversification and Effective Population Size. Some previous studies have explained the correlation between rates of molecular evolution and diversification as being primarily the result of population subdivision associated with speciation events (9, 10, 16). It has been proposed that speciation will often be accompanied by a reduction in population size, for example if a population is divided by some barrier to gene flow. If this is the case, then a lineage that has a higher speciation rate will undergo population size reductions more frequently, and this might lead to a lower long-term effective population size.

Reductions in effective population size (N_e) are expected to increase the fixation rate of nearly neutral mutations (those mutations with selective coefficients close to the reciprocal of N_e) across the genome (17, 73, 74). Because the proportion of nearly neutral mutations is expected to be higher for nonsynonymous than for synonymous mutations, reductions in N_e are expected to increase ω (17, 75). Despite the large size of our dataset (over 7.5-kb of protein-coding data), we do not find any evidence of a relationship between net diversification and ω (*Results*).

There are several possible explanations for the lack of evidence for a relationship between ω and net diversification in the present study: (*i*) diversification in birds is not significantly associated with reductions in N_e (40); (*ii*) diversification in birds does influence N_e but the effect of this on ω is overshadowed by other processes, such as periodic fluctuations in population size associated with environmental factors (e.g., refs. 76 and 77); (*iii*) diversification does influence N_e but the effect is too small to be detected here, or difficulties in estimating ω mask any signal (78–81). The results of the present study are not sufficient to distinguish between these possibilities; however, we note that previous studies have reported significant relationships between N_e and ω (e.g., ref. 82), even when ω is measured from relatively small amounts of data (e.g., refs. 83 and 84).

Conclusions

We have demonstrated a significant correlation between genome-wide rates of molecular evolution and the number of species in families of birds. This correlation holds when rates of molecular evolution are measured from only nonsynonymous or synonymous substitutions. Examination of our results suggests that the best explanation of the link between substitution rates and net diversification is that mutation rates influence the diversification of bird lineages. Clearly, the observation of a relationship between mutation rate and net diversification does not provide a full explanation of the process of diversification. For example, it was recently suggested that rates of diversification are partly determined by the rate of occurrence of rare events, such as the formation of isolating barriers between populations (85). The results of this study are not inconsistent with these mechanisms, but suggest that, once isolated, the probability of two populations diverging to become separate species may be partly determined by the underlying mutation rates in those populations. This theory raises the possibility that rate of molecular evolution could play a role in generating the variation in species richness among clades of birds (86), and provides a tantalizing insight into the accumulation of genetic differences between diversifying lineages.

Methods

Data. We removed the crocodylian outgroups from Hackett et al.'s (23) 52,383-bp alignment. We then created separate intron, exon, and UTR alignments, excluding the 4-bp exon 7 of TPM1, and all annotated inversions (as in ref. 23). To remove uncertain regions of the noncoding alignments, we used GBlocks (87) with the following parameters: minimum sequences for conserved/flank position: 86; maximum contiguous nonconserved positions: 10; minimum block length: 10; allow gap positions: with half. All alignments were checked by eye. The final 169-taxon, 17,438-bp alignment consisted of 7,560-bp exonic, 9,146-bp intronic, and 729-bp UTR DNA. Data and alignments are available from the authors.

Sister Pairs. Each tip on the published tree (23) was labeled at the family level following Sibley and Monroe (24, 25). This tree contained 111 out of 146 bird families (86%), with many families represented by more than one sequence. Phylogenetically independent sister pairs of families were chosen according to two criteria: (*i*) we chose only families whose representatives were monophyletic in our tree; (*ii*) each sister pair had to form a monophyletic pair to the exclusion of all other families in the tree. This process resulted in 32 phylogenetically independent sister pairs being selected (Fig. 1). Each sister pair comprises two families that share a common ancestor to the exclusion of all other families in the dataset, so have by definition have had equal amounts of time to accrue substitutions and undergo diversification. The number of taxonomic species in each family was determined from Sibley and Monroe (24, 25) (Fig. 1).

To avoid the node-density effect in subsequent branch-length estimation, we equalized the number of sequences representing each family of a sister pair by randomly deleting sequences from the family with more sequences. This procedure affected only 10 of the 32 sister pairs in the analysis, and will not produce any biases in the resulting data, as sequences were deleted without reference to their underlying rate of molecular evolution or clade size. This procedure resulted in 30 sister pairs in which each family was represented by

a single sequence, and a further two sister pairs in which each family was represented by two sequences (Fig. 1).

Life-History Traits. We collected published family-level averages of life-history data for as many of the families in our dataset as possible (Dataset S1). Data were collected for three life-history traits: body size (2), proportion of species in a family known to show sexual dichromatism (2), and age at first breeding (88). These traits were identified as potential correlates of both substitution rates and net diversification (*Discussion*), and have been included in studies of net diversification in birds (2, 3, 60, 88).

Molecular-Clock Tests. We tested for rate variation in the sequence data by comparing the likelihoods of a free-rates model (where each branch of the tree can take a different rate of evolution) to those of a molecular-clock model (where all branches of the tree share a single rate of evolution), on the phylogeny shown in Fig. 1. All molecular-clock tests were implemented in HyPhy v2.0 (89). Tests were performed on all data partitions (introns, exons, and UTRs) and on all measurements of substitution rate. We assessed the significance of the difference in likelihoods between models using the likelihood ratio test. Full details of these tests are given in the *SI Text*.

Total Branch-Length Estimation. Maximum-likelihood branch lengths were calculated using RAxML (90) on the phylogeny shown in Fig. 1, which is a pruned version of the phylogenetic tree from Hackett et al. (23). The data were separated into five partitions: first, second, and third codon sites, introns, and UTRs, and with each partition assigned a separate GTR+I+Γ substitution model. Branch lengths for each pair of families were then extracted from the resultant tree. For the four cases (representing two sister pairs) in which a family had more than one sequence in the alignment, the average branch length was calculated following the method of Barraclough and Savolainen (11).

Estimation of *dN* and *dS*. We calculated nonsynonymous (*dN*) and synonymous (*dS*) branch lengths from the 7,560-bp exonic alignment (see above) using two methods. First, we used CODEML4.1 (91), with codon frequencies estimated from the data (codonFreq = 2) and a separate *dN*/*dS* (ω) estimated for each branch of the tree (model = 1), and the tree fixed to the phylogeny in Fig. 1. Second, we used HyPhy v. 2.0 (89), using the MG94 model of codon evolution (92) with codon frequencies estimated from the data in a 3×4 matrix (i.e., the frequencies of each of the four bases was estimated for each of the three codon positions, and codon frequencies were estimated from that matrix). The MG94 model can be paired with any model of sequence evolution from the general time-reversible family (i.e., any special case of the GTR model). There are 203 such models, and we chose the most appropriate model using a custom written HyPhy batch-file (93), which fits all possible models to the data and chooses the best model using the Akaike Information Criterion. The selected model had four parameters, representing a special case of the GTR model in which $\theta_{AC} = \theta_{GT}$ and $\theta_{AG} = \theta_{CT}$. This model can be described by the following notation in HyPhy v2.0: MG94_3 $\times$ 4_012310. Family-average *dN* and *dS* branch lengths were calculated as above, and ω was calculated as the ratio of family-average *dN* to *dS* (Dataset S1). Both CODEML and HyPhy gave identical results with respect to testing correlations with net diversification. Because the models implemented in HyPhy give significantly higher likelihoods than those implemented in CODEML, we report those here.

Statistics. Molecular branch lengths, clade sizes, and life-history data were gathered for 64 families of birds, which fall into 32 monophyletic sister pairs (Fig. 1), where sister-pair relationships were determined using the most comprehensive recent phylogeny of avian taxa (23). For each sister pair, we calculated the difference in each variable between the two families of that sister pair (e.g., the difference in the branch lengths leading to Honeyguides and Woodpeckers) (Fig. 1). Because the two families of each sister pair are by definition the same age, each family has had the same amount of time to accrue substitutions. Thus, differences in families' branch lengths within a given sister pair reveal differences in rates of molecular evolution (e.g., the branch leading to the Woodpeckers is longer than the corresponding branch leading to the Honeyguides, so the Woodpeckers have had a greater net rate of molecular evolution). Similarly, differences in clade size represent differences in net diversification (e.g., the Woodpeckers have accumulated 216 species since they diverged from their common ancestor with the Honeyguides, who have accumulated only 17 species in the same time). We tested for associations between variables using linear regressions through the origin (94) of the differences in molecular branch lengths, clade-size, and life-history traits calculated from each sister pair (following the sister-pairs method, as described in ref. 22). Differences in clade size, molecular branch lengths, ω, body size, and age at first breeding were calculated as ln(B1) − ln(B2), where Bi represents the variable of interest for family *i*. Differences in sexual dichromatism were calculated without log transformation, because our measure of sexual dichromatism is a proportion, and log transformations are thus not appropriate. Diagnostic tests showed that these transformations were appropriate for removing phylogenetic signal from the data (27). We standardized all differences following the recommendations of Garland et al. (26) and Welch and Waxman (21). These standardizations serve to account for the potential confounding effects of the different amounts of time that sister pairs have had to diverge. Diagnostic tests showed that all standardized differences met the assumptions of the linear regression (21, 26, 27). Full explanations and results of these diagnostic tests, as well as details of the standardization factors used, are given in the *SI Text* and Figs. S3 and S4. We also performed all statistical tests on nonstandardized data, and the results do not differ.

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1. Moore BR, Donoghue MJ (2007) Correlates of diversification in the plant clade Dipsacales: Geographic movement and evolutionary innovations. *Am Nat* 170(Suppl 2):S28–S55.
2. Phillimore AB, Freckleton RP, Orme CD, Owens IP (2006) Ecology predicts large-scale patterns of phylogenetic diversification in birds. *Am Nat* 168:220–229.
3. Owens IPF, Bennett PM, Harvey PH (1999) Species richness among birds: Body size, life history, sexual selection or ecology? *Proc Biol Sci* 266:933–939.
4. Ricklefs RE (2006) Global variation in the diversification rate of passerine birds. *Ecology* 87:2468–2478.
5. Witt CC, Brumfield RT (2004) Comment on "Molecular phylogenies link rates of evolution and speciation" (I). *Science*, 303:173, author reply 173.
6. Davies TJ, Savolainen V, Chase MW, Moat J, Barraclough TG (2004) Environmental energy and evolutionary rates in flowering plants. *Proc Biol Sci* 271:2195–2200.
7. Hugall AF, Lee MS (2007) The likelihood node density effect and consequences for evolutionary studies of molecular rates. *Evolution* 61:2293–2307.
8. Venditti C, Pagel M (2008) Model misspecification not the node-density artifact. *Evolution* 62:2125–2126.
9. Webster AJ, Payne RJ, Pagel M (2003) Molecular phylogenies link rates of evolution and speciation. *Science* 301:478.
10. Venditti C, Pagel M (2009) Speciation as an active force in promoting genetic evolution. *Trends Ecol Evol* 25:14–20.
11. Barraclough TG, Savolainen V (2001) Evolutionary rates and species diversity in flowering plants. *Evolution* 55:677–683.
12. Brower AV (2004) Comment on "Molecular phylogenies link rates of evolution and speciation" (II). *Science*, 303:173, author reply 173.
13. Webster AJ, Payne RJ, Pagel M (2004) Response to comments on "Molecular phylogenies link rates of evolution and speciation." *Science* 303:173.
14. Orr HA, Masly JP, Presgraves DC (2004) Speciation genes. *Curr Opin Genet Dev* 14: 675–679.
15. Wu CI, Ting CT (2004) Genes and speciation. *Nat Rev Genet* 5:114–122.
16. Pagel M, Venditti C, Meade A (2006) Large punctuational contribution of speciation to evolutionary divergence at the molecular level. *Science* 314:119–121.
17. Ohta T (1992) The nearly neutral theory of molecular evolution. *Ann Rev Ecol Syst* 23: 263–286.
18. Orr HA, Turelli M (2001) The evolution of postzygotic isolation: Accumulating Dobzhansky-Muller incompatibilities. *Evolution* 55:1085–1094.
19. Price TD, Bouvier MM (2002) The evolution of F1 postzygotic incompatibilities in birds. *Evolution* 56:2083–2089.
20. Franklin IR, Frankham R (1998) How large must populations be to retain evolutionary potential? *Anim Conserv* 1:69–70.
21. Welch JJ, Waxman D (2008) Calculating independent contrasts for the comparative study of substitution rates. *J Theor Biol* 251:667–678.
22. Lanfear R, Welch JJ, Bromham L (2010) Watching the clock: Studying variation in rates of molecular evolution between species. *Trends Ecol Evol* 25:495–503.
23. Hackett SJ, et al. (2008) A phylogenomic study of birds reveals their evolutionary history. *Science* 320:1763–1768.
24. Sibley CG, Monroe BL (1990) *Distribution and Taxonomy of Birds of the World* (Yale University Press, New Haven).
25. Sibley CG, Monroe BL (1993) *Supplement to Distribution and Taxonomy of Birds of the World* (Yale University Press, New Haven, London).
26. Garland T, Harvey PH, Ives AR (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst Biol* 41:18–32.
27. Freckleton RP (2000) Phylogenetic tests of ecological and evolutionary hypotheses: Checking for phylogenetic independence. *Funct Ecol* 14:129–134.
28. Kimura M (1983) *The Neutral Theory of Molecular Evolution* (Cambridge University Press, Cambridge).
29. Chamary JV, Parmley JL, Hurst LD (2006) Hearing silence: Non-neutral evolution at synonymous sites in mammals. *Nat Rev Genet* 7:98–108.
30. Lynch M (2007) *The Origins of Genome Architecture* (Sinauer Associates, Sunderland, MA).

31. Jennings WB, Edwards SV (2005) Speciational history of Australian grass finches (Poephila) inferred from thirty gene trees. *Evolution* 59:2033–2047.
32. Mylecraine KA, Bulgin NL, Gibbs HL, Vickery PD, Perkins DW (2008) Limited genetic structure and evidence for dispersal among populations of the endangered Florida grasshopper sparrow, *Ammodramus savannarum floridanus*. *Conserv Genet* 9: 1633–1638.
33. Barrowclough GF, Shields GF (1984) Karyotypic evolution and long-term effective population sizes of birds. *Auk* 101:99–102.
34. Joseph L, et al. (2009) A tangled tale of two teal: Population history of the grey *Anas gracilis* and chestnut teal *A-castanea* of Australia. *J Avian Biol* 40:430–439.
35. Hansson B, Hasselquist D, Tarka M, Zehtindjiev P, Bensch S (2008) Postglacial colonisation patterns and the role of isolation and expansion in driving diversification in a passerine bird. *PLoS ONE* 3:e2794.
36. Yang Z, Nielsen R (1998) Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *J Mol Evol* 46:409–418.
37. Whitlock MC, Griswold CK, Peters AD (2003) Compensating for the meltdown: The critical effective size of a population with deleterious and compensatory mutations. *Ann Zool Fenn* 40:169–183.
38. Dobzhansky TG (1937) *Genetics and the Origin of Species* (Columbia University Press, New York).
39. Muller HJ (1942) Isolating mechanisms, evolution and temperature. *Biol Symp* 6: 71–125.
40. Price TD (2008) *Speciation in Birds* (Roberts and Company, Greenwood Village, Colorado).
41. Tubaro PL, Lijtmaer DA (2002) Hybridization patterns and the evolution of reproductive isolation in ducks. *Biol J Linn Soc Lond* 77:193–200.
42. Coyne JA, Orr HA (1997) ''Patterns of speciation in *Drosophila*'' revisited. *Evolution* 51:295–303.
43. Coyne JA, Orr HA (1989) Patterns of speciation in *Drosophila*. *Evolution* 43:362–381.
44. Presgraves DC (2002) Patterns of postzygotic isolation in Lepidoptera. *Evolution* 56: 1168–1183.
45. Sasa MM, Chippindale PT, Johnson NA (1998) Patterns of postzygotic isolation in frogs. *Evolution* 52:1811–1820.
46. Bromham L (2009) Why do species vary in their rate of molecular evolution? *Biol Lett* 5:401–404.
47. Mooers AO, Harvey PH (1994) Metabolic rate, generation time, and the rate of molecular evolution in birds. *Mol Phylogenet Evol* 3:344–350.
48. Galtier N, Blier PU, Nabholz B (2009) Inverse relationship between longevity and evolutionary rate of mitochondrial proteins in mammals and birds. *Mitochondrion* 9: 51–57.
49. Thomas JA, Welch JJ, Lanfear R, Bromham L (2010) A generation time effect on the rate of molecular evolution in invertebrates. *Mol Biol Evol* 27:1173–1180.
50. Bromham L, Rambaut A, Harvey PH (1996) Determinants of rate variation in mammalian DNA sequence evolution. *J Mol Evol* 43:610–621.
51. Smith SA, Donoghue MJ (2008) Rates of molecular evolution are linked to life history in flowering plants. *Science* 322:86–89.
52. Kochmer JP, Wagner RH (1988) Why are there so many kinds of passerine birds? Because they are small. A reply to Raikow. *Syst Zool* 37:68–69.
53. Orme CD, Isaac NJ, Purvis A (2002) Are most species small? Not within species-level phylogenies. *Proc Biol Sci* 269:1279–1287.
54. Cardillo M (1999) Latitude and rates of diversification in birds and butterflies. *Proc Biol Sci* 266:1221–1225.
55. Krüger O (2008) Engines of speciation: A comparative study in birds of prey. *J Evol Biol* 21:861–872.
56. Weir JT, Schluter D (2007) The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science* 315:1574–1576.
57. Bromham L, Cardillo M (2003) Testing the link between the latitudinal gradient in species richness and rates of molecular evolution. *J Evol Biol* 16:200–207.
58. Wright S, Keeling J, Gillman L (2006) The road from Santa Rosalia: A faster tempo of evolution in tropical climates. *Proc Natl Acad Sci USA* 103:7718–7722.
59. Gillman LN, Keeling DJ, Ross HA, Wright SD (2009) Latitude, elevation and the tempo of molecular evolution in mammals. *Proc Biol Sci* 276:3353–3359.
60. Barraclough TG, Harvey PH, Nee S (1995) Sexual selection and taxonomic diversity in passerine birds. *Proc Biol Sci* 259:211–215.
61. Seddon N, Merrill RM, Tobias JA (2008) Sexually selected traits predict patterns of species richness in a diverse clade of suboscine birds. *Am Nat* 171:620–631.
62. Morrow EH, Pitcher TE, Arnqvist G (2003) No evidence that sexual selection is an 'engine of speciation' in birds. *Ecol Lett* 6:228–234.
63. Ellegren H (2007) Characteristics, causes and evolutionary consequences of male-biased mutation. *Proc Biol Sci* 274:1–10.
64. Bartosch-Härlid A, Berlin S, Smith NGC, Møller AP, Ellegren H (2003) Life history and the male mutation bias. *Evolution* 57:2398–2406.
65. Cardillo M, Orme CDL, Owens IPF (2005) Testing for latitudinal bias in diversification rates: An example using New World birds. *Ecology* 86:2278–2287.
66. Phillimore AB, et al. (2007) Biogeographical basis of recent phenotypic divergence among birds: a global study of subspecies richness. *Evolution* 61:942–957.
67. Cooper CE, et al. (2002) Metabolic ecology of cockatoos in the south-west of Western Australia. *Aust J Ecol* 50:67–76.
68. Tieleman BI, Williams JB (2000) The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiol Biochem Zool* 73:461–479.
69. Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. *Proc Natl Acad Sci USA* 90:4087–4091.
70. Gillooly JF, Allen AP, West GB, Brown JH (2005) The rate of DNA evolution: Effects of body size and temperature on the molecular clock. *Proc Natl Acad Sci USA* 102: 140–145.
71. Lanfear R, Thomas JA, Welch JJ, Brey T, Bromham L (2007) Metabolic rate does not calibrate the molecular clock. *Proc Natl Acad Sci USA* 104:15388–15393.
72. Welch JJ, Bininda-Emonds OR, Bromham L (2008) Correlates of substitution rate variation in mammalian protein-coding sequences. *BMC Evol Biol* 8:53.
73. Woolfit M (2009) Effective population size and the rate and pattern of nucleotide substitutions. *Biol Lett* 5:417–420.
74. Charlesworth B (2009) Fundamental concepts in genetics: Effective population size and patterns of molecular evolution and variation. *Nat Rev Genet* 10:195–205.
75. Ota T (1972) Population size and rate of evolution. *J Mol Evol* 1:305–314.
76. Grant PR, Grant BR (1992) Demography and the genetically effective sizes of two populations of Darwin finches. *Ecology* 73:766–784.
77. Saether BE, Sutherland WJ, Engen S (2004) Climate influences on avian population dynamics. *Adv Ecol Sci* 35:185–209.
78. Eyre-Walker A (2006) The genomic rate of adaptive evolution. *Trends Ecol Evol* 21: 569–575.
79. Charlesworth J, Eyre-Walker A (2007) The other side of the nearly neutral theory, evidence of slightly advantageous back-mutations. *Proc Natl Acad Sci USA* 104: 16992–16997.
80. Bachtrog D (2008) Similar rates of protein adaptation in *Drosophila miranda* and *D. melanogaster*, two species with different current effective population sizes. *BMC Evol Biol* 8:334.
81. Axelsson E, et al. (2008) Natural selection in avian protein-coding genes expressed in brain. *Mol Ecol* 17:3008–3017.
82. Kosiol C, et al. (2008) Patterns of positive selection in six Mammalian genomes. *PLoS Genet* 4:e1000144.
83. Woolfit M, Bromham L (2003) Increased rates of sequence evolution in endosymbiotic bacteria and fungi with small effective population sizes. *Mol Biol Evol* 20:1545–1555.
84. Woolfit M, Bromham L (2005) Population size and molecular evolution on islands. *Proc Biol Sci* 272:2277–2282.
85. Venditti C, Meade A, Pagel M (2010) Phylogenies reveal new interpretation of speciation and the Red Queen. *Nature* 463:349–352.
86. Raikow RJ (1986) Why are there so many kinds of passerine birds? *Syst Zool* 35: 255–259.
87. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552.
88. Bennett PM, Owens IP (2002) *Evolutionary Ecology of Birds* (Oxford University Press, Oxford, United Kingdom).
89. Pond SL, Frost SD, Muse SV (2005) HyPhy: Hypothesis testing using phylogenies. *Bioinformatics* 21:676–679.
90. Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
91. Yang Z (2007) PAML 4: Phylogenetic analysis by maximum likelihood. *Mol Biol Evol* 24:1586–1591.
92. Muse SV, Gaut BS (1994) A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. *Mol Biol Evol* 11:715–724.
93. Ho SY, Lanfear R (2010) Improved characterisation of among-lineage rate variation in cetacean mitogenomes using codon-partitioned relaxed clocks. *Mitochondrial DNA* 21:138–146.
94. Harvey PH, Pagel MD (1991) *The Comparative Method in Evolutionary Biology* (Oxford University Press, Oxford, New York).