Fauna and Systematics Zoodiversity, 60(4): 313–326, 2026

DOI 10.15407/zoo2026.04.313
UDC 575.86:577.218:598.2

DIFFERENTIATION OF BIRD TAXA AT THE LEVEL OF TRANSITION-TRANSVERSION BIAS IN THE CYTB GENE SEQUENCIES

S. V. Mezhzherin *, S. Yu. Morozov-Leonov & S. Ye. Holodryha

I. I. Schmalhausen Institute of Zoology,
vul. B. Khmelnytskogo, 15, Kyiv, 01054 Ukraine

E-mail: smezhzherin@gmail.com
* Corresponding author

S. V. Mezhzherin (http://orcid.org/0000-0003-2905-5235)
S. Yu. Morozov-Leonov (https://orcid.org/0000-0003-1784-7753)
S. Ye. Holodryha (http://orcid.org/0009-0004-3450-4132)

urn:lsid:zoobank.org:pub:7F73D4F3-1197-4E5A-9940-C56451B99184

Differentiation of bird taxa at the level of transition-transversion bias in the CYTB **gene sequencies. Mezhzherin, S. V., Morozov-Leonov & S. Yu., Holodryha, S. Ye.** — The study of DNA marker variability provides robust insights into historical relationships, evolutionary rates, and the magnitude of genetic differentiation among taxa. This is particularly relevant for the youngest and most diverse group of vertebrates, traditionally classified as the class *Aves*. One of the core principles of molecular evolution theory is the transition–transversion bias, i. e., the paradoxical predominance of homotypic nucleotide substitutions (transitions) over heterotypic substitutions (transversions) during spontaneous mutational processes and speciation, a pattern that becomes progressively attenuated with increasing genetic divergence. This phenomenon exhibits group-specific features at higher taxonomic levels, allowing the identification of evolutionary characteristics unique to each lineage. The present study investigates patterns of evolutionary transition–transversion bias in birds using the mitochondrial CYTB gene as a model. The results demonstrate that the magnitude of the bias varies both along the evolutionary hierarchy depending on taxonomic level and among avian families. A predominance of transitions over transversions is observed at the population, species, genus, and family levels, whereas a slight predominance of transversions is detected at the order level. Temporal variation in transition–transversion ratios is driven by saltatory accumulation of transitions at the population, species, and genus levels against a background of relatively stable transversion frequencies. This pattern may be explained by genetic saturation effects or the peculiarities of the mutation process at

different levels of divergence. Within *Aves*, nucleotide substitution patterns are characterized by an increase in transversion frequency and a decrease in transition frequency, accompanied by a reduction in the overall transition–transversion bias in small-bodied species from evolutionarily young families, compared with more ancient, large-bodied lineages with lower metabolic rates. The increase in transversion frequency in small-bodied, evolutionarily progressive bird groups is associated with elevated metabolic rates, leading to increased cellular oxygen availability and, consequently, higher levels of oxidative DNA damage. Metabolic intensification in birds thus represents not only a driver of enhanced genetic diversity and evolutionary advancement, but also evidently results in an increased mutational load, which may ultimately contribute to the evolutionary extinction of historically young, small-bodied taxonomic groups.

Key words: molecular evolution, birds, transition-transversion bias, body size, metabolic rate.

Introduction

The class Aves represents the evolutionarily youngest group of vertebrates which has achieved remarkable biological success, one indicator of which is its exceptional species richness — the highest among extant tetrapods (more than 11, 000 species) (https://www.birdlife.org/birds/). A characteristic feature of the genetic divergence within avian taxa is the comparatively reduced magnitude of genetic differences relative to taxa of the same rank in comparison to other vertebrate groups. This unusual pattern was first identified through multilocus allozyme analysis (Avise et al., 1980; Avise & Aquadro, 1982) and was later confirmed using DNA markers (Kessler & Avise, 1985; Johns & Avise, 1998 Kartavtsev & Lee, 2006). Nevertheless, the scale of genetic divergence observed in birds is not unprecedented (Mezhzherin & Piontkovskaya, 1998), as it corresponds to that of macromammals, whose level of differentiation is generally lower than that of micromammals (Mezhzherin & Morozov-Leonov, 1995).

The genetic mechanisms underlying bird evolution remain an active area of research and ongoing debate (Zhang et al., 2014; Duchene et al., 2025). Their relatively low genetic differentiation may be attributed to two main factors: the paleontological youth of the class *Aves* and specific features of their molecular evolution. The first explanation appears plausible, given that from the perspective of modern phylogenetics, birds represent a clade equivalent to an order-level grouping within reptiles (Benton, 2015), which in theory should constrain the number of taxonomic subdivisions. Nevertheless, within Aves, taxonomic levels equivalent to those found within Theria are naturally distinguished, and the number of bird species much greater.

At the same time, although the genetic distances among corresponding taxonomic categories in birds are smaller than in mammals, the differences are not reduced strictly to one taxonomic rank. This apparent discrepancy could be explained by proposing that birds exhibit high mutation rates than mammals (Bergeron et al., 2023), enabling them to accumulate substantial genetic divergence over a shorter evolutionary timescale.

Birds represent a young and thus rapidly evolving lineage, characterized by the highest and most constant body temperature among vertebrates, accompanied by an exceptionally high metabolic rate (Clarke & Rothery, 2008). It is well established that elevated environmental temperatures accelerate the rate of spontaneous mutations, a phenomenon demonstrated in poikilothermic organisms (Lindgren, 1972; Chu et al., 2018). However, the notion that higher body temperatures and intensified metabolism in homeothermic animals lead to increased mutation rates (Martin & Palumbi, 1993) remains largely intuitive and has yet to be empirically confirmed.

One of the fundamental principles of molecular evolution theory is the phenomenon of transition-transversion bias (Li & Graur, 1991). This principle reflects the consistently higher frequency of transitions — homotypic nucleotide substitutions (A↔G, T↔C) compared to transversions — heterotypic substitutions (A↔T, A↔C, G↔T, G↔C) at the level of spontaneous mutations (Brown et al., 1982; Jukes, 1987; Collins et al., 1994; Duchene et al., 2025). However, as the phylogenetic distance between diverging lineages increases, there is a gradual shift from transition bias toward the accumulation of random nucleotide substitutions (Rosenberg et al., 2003; Mezhzherin et al., 2024 a). This shift is associated with differential rates of change in the frequencies of transitions and transversions along evolutionary lineages.

A comparative study has demonstrated that birds exhibit significantly higher rates of transversion accumulation and display smaller magnitudes of transition–transversion (ts/tv) bias compared to mammals (Mezhzherin & Morozov-Leonov, 2024). Furthermore, the extent of ts/tv bias is considerably lower in families composed of small-bodied species than in those including large-bodied species. Considering that birds have higher metabolic rates than mammals and that, within homeothermic species, smaller-bodied animals exhibit higher metabolic rates than larger-bodied ones (Kleiber, 1932; Schmidt-Nielsen, 1983; Clarke & Rothery, 2008). There is strong reason to suggest a positive correlation between metabolic rate and mutation rate in homeothermic vertebrates. This correlation may reflect distinct molecular mechanisms underlying transitions and transversions.

The relationship between energy flux, mutation rates, and the degree of evolutionary radiation has been a central focus in biology over recent decades (Martin & Palumbi, 1993; Allen et al., 2006; Gillooly et al., 2004; Gillooly & McCoy, 2007; Chu et al., 2018). In this context, the molecular evolution of birds is of particular interest, especially with regard to assessing the ratio of transition to transversion frequencies across major taxonomic levels in connection with two key factors: the paleontological age and body size. To explore these evolutionary patterns, a study was conducted on the CYTB gene, one of the most widely used genetic markers in vertebrate evolutionary genetics (Irwin et al., 1991; Johns & Avise, 1998; Kartavtsev, 2011).

Material and Methods

The study was based primarily on complete sequences of the CYTB gene obtained from the NCBI Nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/). Sequences shorter than 1000 base pairs were excluded. In total the study used sequences from 5,100 species representing 1,500 genera, 120 families, and 40 orders within the class Aves.

The analysis of evolutionary transition–transversion patterns was conducted across five levels of divergence. Their primary analysis and generalization were conducted according to the following algorithms.

Population level (with nucleotide substitution frequency ranging from 0 to 0.02) was assessed through pairwise comparisons of specimens from different localities. A total of 92 taxonomic groups of family rank were analyzed (Table S1) (https://doi.org/10.6084/m9.figshare.30022558).

Transition and transversion frequency analysis at the species and genera levels was performed in two ways, based on pairwise comparisons of nucleotide sequences (Table S2) (https://doi.org/10.6084/m9.figshare.30022558). The first approach is based on generalizing the results of pairwise comparisons of nucleotide sequences of species within the same genus and different genera within the same family, respectively. The second approach is based on average transition and transversion frequencies for each family at the species and genera levels of divergence. Cases of false taxonomic status, when substitution frequencies did not exceed 0.02, were generally not included in the calculations.

Features of accumulation of frequencies of transitions and transversions at the level of divergence of families and orders were evaluated through the comparisons between selected species of different families within the same order; and between species from different orders (Table S3) (https://doi.org/10.6084/m9.figshare.30022558).

The evaluation of transition–transversion bias (ts/tv bias) was based on a standard measure — the ratio of transition to transversion frequencies (ts/tv ratio).

The fossil record of birds was sourced from a widely accepted reference. We identified phyletic stages (clades) corresponding to the periods of establishment of the major avian orders (Benton, 2015): (i) divergence of Palaeognathae from Neognathae; (ii) divergence of Galloanserae from Neoaves; (iii) formation of the main groups within Neoaves excluding Passeriformes — such as the "Waterbird assemblage," "Swifts, pigeons, and parrots, …" and "Woodpeckers, tookans, trogons, …"; (iv) the emergence of the order Passeriformes. The distribution of orders among clades is presented in Table S4 (https://doi.org/10.6084/m9.figshare.30022558).

The paleontological histories of families, genera, and species were derived from G. Mayr (Mayr, 2009, 2017), with particular emphasis on the Corvidae family as a model taxon. The paleontological age of the bird families, genera and species was determined based on data taken from the Paleobiology Database (https://paleobiodb.org/). The paleontological age of these taxa was the age of the oldest series of finds identified in the database.

Body size was estimated at the family level by calculating the common logarithm of the geometric mean body mass (in grams), based on the minimum and maximum known species values within family presented in Table S4. The source of primary data was the free Avonet database (https://opentraits.org/datasets/avonet.html).

Sequence alignment was performed using BioEdit (v7.2.5) and the ClustalW algorithm (Abbott Laboratories, Green Oaks, Illinois, USA, 1999) (Hall, 1999). Pairwise genetic distances, individual distance matrices, and phenograms (based on the UPGMA algorithm), as well as sequence verification, were generated using MEGA software (v11.0.11) (Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, University Park, Pennsylvania, USA, 2021) (Tamura et al., 2021). Nucleotide substitution frequencies were calculated directly by analyzing all possible pairwise sequence comparisons.

Statistical processing of the data was carried out using the software packages STATISTICA 12.0 and PAST (Hammer et al., 2001).

Results

Transition-transversson bias in evolutionary row. Transition and transversion frequencies, as well as the ts/tv index vary within the class Aves depending on the taxonomic level of divergence (Tables S5–S6). At the population, species and generic levels transitions accumulate preferentially relative to transversions, which leads to a obvious transition-transversion bias, the degree of which decreases as the taxonomic level increases. At the family level, transition and transversion frequencies tend to equalize. Finally, at the order level, transversions predominate. These patterns in nucleotide substitution frequencies are accompanied by a decreasing transition-transversion bias with increasing taxonomic rank. Specifically, the mean ts/tv ratio decreases from 9.43 at the population level to 4.1 at the species level, 2.1 at the generic level, 1.1 at the family level, and 0.96 at the order level.

The distributions of transition frequencies across taxonomic divergence levels have a broad transgressions with close modal values excluding species level (Fig. 1). Consequently, the ratio of average transition frequencies across these four taxonomic levels is approximately 1 : 1.3 : 1.3 : 1.4. In contrast, the distributions of transversion frequencies are clearly distinct at all four divergence levels, uniformly spanning a wide range and displaying sharply different means in the ratio 1 : 2.5 : 4.8 : 5.9 (Fig. 2).

The average growth rate of the frequencies of transitions and transversions at the different taxonomical levels is not accompanied by a slowdown in the rate of accumulation of nucleotide substitutions in general (Fig. 3). This situation can be considered as evidence of the autonomy and non-substitute nature of transition and transversion in the evolutionary row of birds.

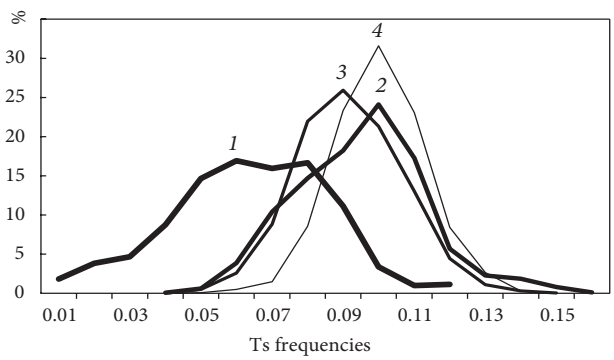

Fig. 1. Distributions of transition frequencies obtained from pairwise comparisons at four taxonomic levels of the birds: *1* — species; *2* — generic; *3* — family; *4* — order

Rates of nucleotide substitutions within an evolutionary row. The average nucleotide substitution frequency per million years in the evolutionary lineages of birds increases with decreasing paleontological age, reaching its maximum values at the species and intraspecific levels (Fig. 4). Generally, the rate of increase in transition substitution frequencies exceeds that of transversions; however, this trend is apparent only during the later stages of avian phylogeny. The aver-

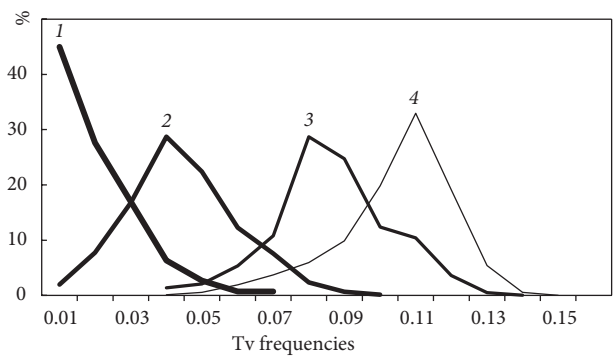

Fig. 2. Distributions of transversion frequencies obtained from pairwise comparisons at four taxonomic levels of the birds: *1* — species; *2* — generic; *3* — family; *4* — order

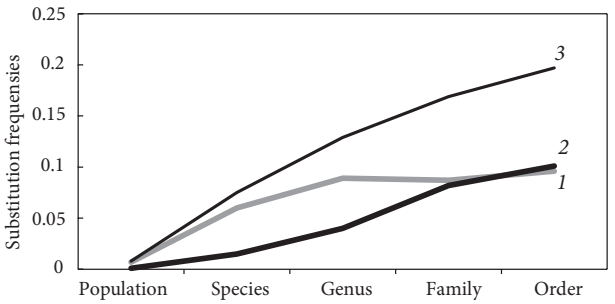

Fig. 3. Changes of transition and transversion mean frequencies against the background of the rates of accumulation of nucleotide substitutions at different levels of taxonomic differentiation: *1* — transition frequencies; *2* — transversion frequencies; *3* — summarized substitutions

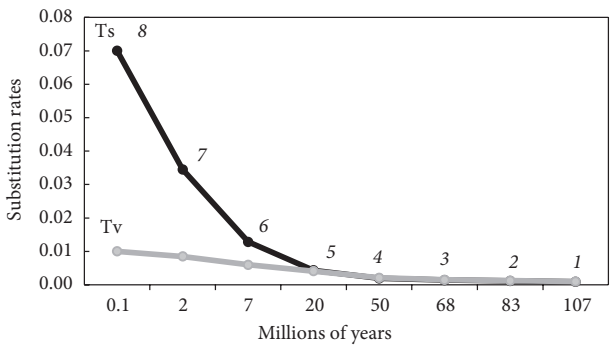

Fig. 4. Changes in the average frequencies of transitions and transversions, calculated for one million years, in the evolutionary series of birds: *1* — formation of Palaeognathae, *2* — formation of Galloanserae, *3* — formation of Neoaves order groups without Passeriformes, *4* — formation of Passeriformes, *5* — average age of families, *6* — average age of genera, *7* — average age of species, *8* — supposed maximum age of intraspecific divergence

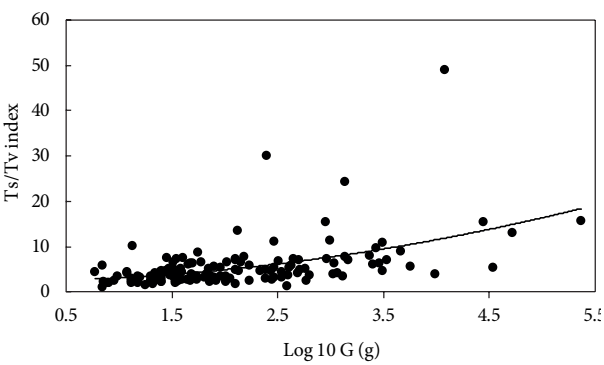

Fig. 5. Scatterplot of the effect of the body weight ($\log_{10}$ G (g)) on mean ts/tv—indices of the species differentiation calculated at the families level. The effect is significant at the highest level ($F = 6.8$; $df_1 = 7$; $df_2 = 137$; $p < 0.001$)

age nucleotide substitution frequency per million years for both transitions and transversions during the formation of the major orders Palaeognathae, Galloanserae, and Neoaves (50 to 107 million years ago) ranges from 0.1% to 0.2%. During the evolutionary radiation of Passeriformes (25 to 50 million years ago), the substitution rate increases to 0.2–0.4%. At the family level (10 to 20 million years ago), substitution frequencies fluctuate between 0.4% and 0.8%. At the genus level (3.5 to 7 million years ago), the transition rate rises sharply to 1.3–2.6%, while the transversion rate increases more modestly to 0.6–1.2%. For species-level divergence, with an average age of about 2 million years, the averaged transition rate reaches 3.5% per million years, compared to 0.9% for transversions. At the intraspecific level, these values reach approximately 7% and 1%, respectively. Thus, over a span of approximately 60 million years, the rate of transition accumulation increases approximately 35-fold when comparing the order and species levels, and up to 70-fold relative to the population level. In contrast, transversion rates increase only about 9 to 10 times over the same periods.

Comparison of transition-transversion bias of orders and families. Mean frequencies of transitions and transversion at the family level vary depending on the order and family identities (Table S4). Factors deter-

mining this variability are the mean family body size and the order group paleontological age. As shown by the results of rank correlation analysis (Table S7), there are a reliable relationships between nucleotide frequencies and above-mentioned biological parameters. At the species level negative correlations between these parameters and changes in frequencies of transversion as well as negative correlation between transitions and body weight are observed. At the generic level, there are positive correlations between age and transition frequencies and negative between body weight and transversion frequencies. Correlations of transversion frequencies with body size and paleontological age are clearly stronger than those of transitions, and body size variation correlates more closely with variability in transition and transversion frequencies than paleontological age. In principle, these trends are confirmed by ANOVA (Table S8) (https://doi.org/10.6084/ m9.figshare. 30022558).

The values of the transition–transversion index clearly depend both on mean body size at the family level (Figs 5–6) and on the paleontological age of the order-level group (Figs 7–8). These relationships represent positive correlations and are expressed at both the species and genus levels (Table S7).

Given the high correlation between body size and paleontological age ($r = 0.76$; $p = 2.9E^{-28}$), it can be assumed that the correlation of paleontological age with

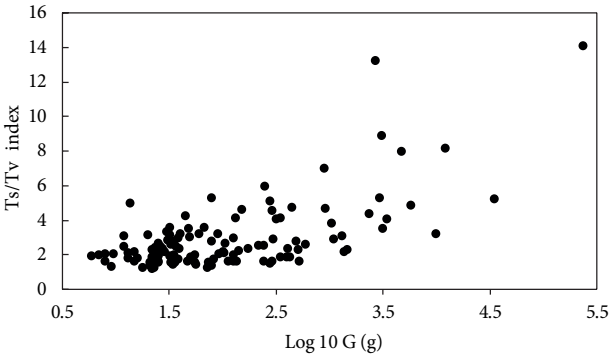

Fig. 6. Scatterplot of the effect of the body weight on mean ts/tv indices of the genus differentiation calculated at the families level. The effect is significant at the highest level ($F = 11.3$; $df_1 = 6$; $df_2 = 115$; $p < 0.001$)

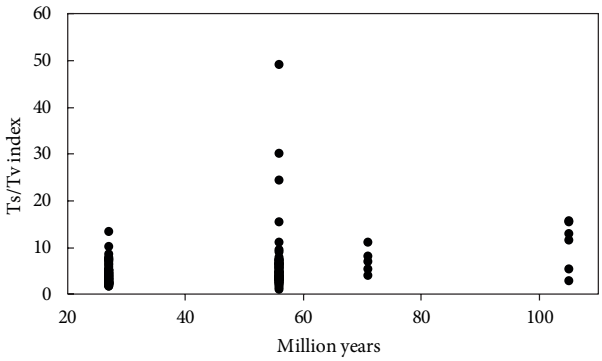

Fig. 7. Scatterplot of the effect of the order's paleontological age on mean ts/tv-indices of the species differentiation calculated at the families level. The effect is significant at the significant level ($F = 3.5$; $df_1 = 3$; $df_2 = 141$; $p = 0.016$)

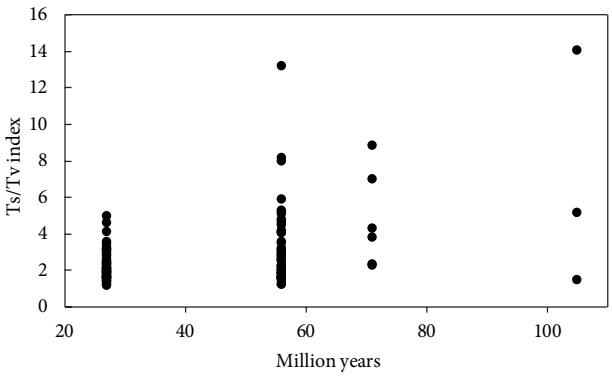

Fig. 8. Scatterplot of the effect of the order's palaeontological age on mean ts/tv-indices of the genus differentiation calculated at the families level. The effect is significant at the highest level ($F = 9.9$; $df_1 = 3$; $df_2 = 141$; $p < 0.001$)

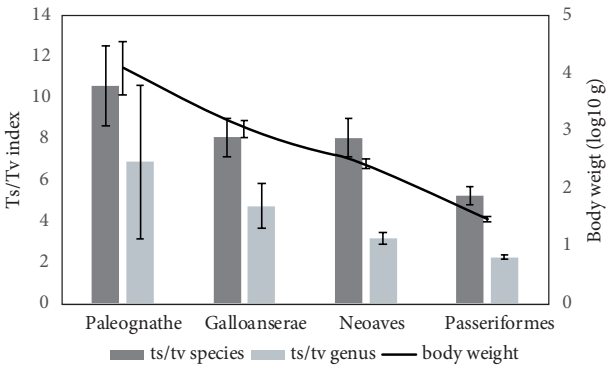

Fig. 9. Mean frequencies of ts/tv indices at the species and generic levels against the background mean body weight ($\log_{10}$ G (g)) in the four main evolutionary clades of recent birds. Note: calculations within Neoaves are implemented without Passeriformes. Bars represent standard errors

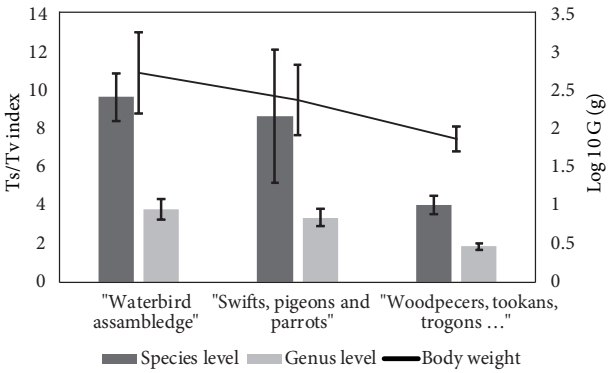

Fig. 10. Mean values of ts/tv indices at the species and genus levels and mean body weight ($\log_{10}$ G (g) at the family level of three Neoaves clades (after Benton, 2015) without Passeriformes. Note: bars represent standard errors

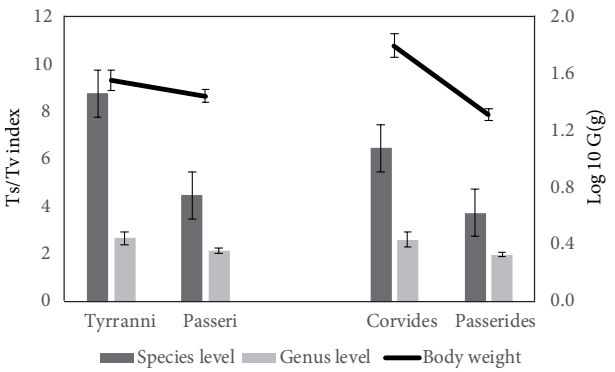

Fig. 11. Mean values of ts/tv indices at the species and generic levels and mean body weight ($\log_{10}$ G (g)) at the family level within Passeriformes. Note: bars represent standard errors

nucleotide substitution frequencies is mediated by body size. However, the quite probable combined effect of these two factors on the variation of nucleotide substitutions was confirmed by two-way ANOVA (Table S9) (Zenodo. doi.org) in all six possible comparisons in contrast to the one-way ANOVA, under which the effect of body size is significant in five cases, whereas the effect of paleontological age is significant in four cases (Table S8).

Differences in the rates of nucleotide substitution accumulation and the magnitude of transition–transversion bias at the species and genus levels are also evident within the evolutionary lineage of recent birds.

First, representatives of the ancient groups Palaeognathae and Galloanserae, which generally include the largest bird species, exhibit the lowest nucleotide substitution frequencies (Fig. 9) and the highest ts/tv ratios. Conversely, the youngest clade of small body sized birds, the order Passeriformes, shows the highest nucleotide substitution rates and the lowest ts/tv ratios. The average values calculated for families within the Neoaves clade, excluding Passeriformes, occupy an intermediate position.

Second, correlations between transversion frequencies and body weight are observed Neoaves without Passeriformes, which is attributable to the lower body weight and correspond-

ingly lower ts/tv index values in families belonging to the "Woodpekcers, toucans, trogons, …" clade (Fig. 10).

Nevertheless the trend of correlation between transversion frequencies and body weight has exclusions. The pattern within Passeriformes is less straightforward. Families within the suborder Tyranni exhibit the highest ts/tv index values among Passeriformes, despite having similar body sizes to those in the suborder Passeri (Fig. 11). The clade Corvides encompasses the largest species in this order; their ts/tv indices have a larger value than those of the smaller Passerides but comparable to those of Tyranni (Fig. 11).

Some uncertainty is also observed in certain interorder comparisons. The group of orders "Woodpekcers, toucans, trogons, …" includes birds with an average greater body weight ($M = 1.88 \pm 0.12$) than in the order Passeriformes ($M = 1.47 \pm 0.04$; $t_{st} = 3.36$, $p < 0.01$), however, both clades do not differ at the mean ts/tv index values at the species and genus levels (Figs 10–11).

Discussion

The analysis of nucleotide substitutions in the cytb gene across different taxonomic levels within the class Aves reveals several evolutionary trends related to differential rates of accumulation of transition and transversion frequencies as well as ts/tv indices. Notably, there is a pronounced evolutionary transition-transversion bias, manifested as a tenfold decrease in the ts/tv index values with increasing taxonomic rank. This pattern is driven by a marked acceleration in the accumulation rate of transitions relative to transversions at the genus and species levels. This trend is expected based on the results of previous studies (Mezhzherin & Morozov-Leonov, 2024).

Of particular interest is the sharp increase in the rate of accumulation of transversion frequencies at the genus divergence level and above, occurring against a background of a relatively stable rate of transition accumulation. A similar pattern has been reported for the evolutionary lineage of mammals (Mezhzherin et al., 2024), suggesting that this phenomenon should be interpreted more broadly than as a taxon-specific phenomenon. The underlying causes of this pattern can be interpreted by examining two alternative conceptual approaches.

The gradualistic approach treats this pattern as a consequence of genetic saturation (Philippe et al., 2011), whereby, with increasing levels of divergence, rare transversions progressively replace easily reversible transitions. The second one is punctuational in nature. It is based on the conceptually aligned theory of systemic mutations (Goldschmidt, 1940). It suggests that the formation of species- and genus-level taxa in birds is largely driven by spontaneous mutational processes, whereas taxonomic levels above the family are shaped by saltatory genome transformations, particularly affecting non-coding regions (Mezhzherin et al., 2024 a). The theoretical basis for this assumption lies in the fact that macroevolutionary stages leading to the emergence of orders, classes, and phyla involve transformations of embryogenesis, which can only be fixed through abrupt genomic reorganizations rather than through

the gradual accumulation of nucleotide substitutions. In contrast, at the species and genus levels, morphogenetic differences among taxa are determined by divergent trajectories of postnatal ontogeny.

In addition, there are several purely empirical arguments supporting this interpretation: (1) the rate of increase in transition frequencies exhibits a nonlinear pattern, which would not be expected under a strictly gradual process; (2) the increases in transition and transversion frequencies along evolutionary lineages occur independently, as the transversion shift observed at the genus and family levels in birds (Fig. 3) is not accompanied by a reduction in the total number of nucleotide substitutions, i. e., no decrease is observed in the number of sites undergoing substitution; (3) the ultimate outcome—characterized by saturation and relative stabilization of transition and transversion frequencies at the divergence levels corresponding to classes and phyla—is the attainment of an equilibrium state consistent with the model of random nucleotide substitutions (Rosenberg et al., 2003; Mezhzherin & Morozov-Leonov, 2024), rather than the displacement of transitions by transversions.

This latter approach is consistent with current studies of avian macroevolution. For example, the loss of flight capability in birds is evidently associated with changes in regulatory regions of genes involved in flight, rather than in protein-coding regions of the genome (Sackton et al., 2019). Moreover, genes located in close proximity to non-coding regions are enriched for signaling pathways involved in embryonic development (Yusuf et al., 2020). These observations support the idea that associations between genotype and phenotype exist at the genome-wide scale, and that changes in these associations occur over macroevolutionary timescales.

Regular changes in the patterns of nucleotide substitutions are observed within the evolutionary lineages of recent birds, associated with two main aspects. The first aspect concerns the relationship between the order paleontological age and nucleotide substitution parameters. At the species and genus levels, there are positive correlations between paleontological age and the magnitude of transition-transversion bias. At the species level, this is achieved through a decrease in transversion frequencies, whereas at the genus level, conversely, it is driven by an increase in transition frequencies in older taxonomic groups.

The second aspect is the relationship between body size and nucleotide substitution parameters. In this case, strictly trends are observed at two taxonomic levels: positive correlations between body size and substitution rates, driven primarily by a decrease in transversion frequencies.

The observed tendency for the increased rates of transversion accumulation in younger and smaller-sized avian evolutionary groups, accompanied by a corresponding decrease in the magnitude of the ts/tv bias, can be plausibly explained by elevated mutation rates associated with intensive metabolism. This is especially relevant for small bird species, which maintain the highest body temperatures among vertebrates (Clarke & Rothery, 2008).

While transitions largely arise from tautomeric shifts and thus represent predominantly spontaneous DNA changes (Löwdin, 1966), transversions are primarily caused by more specific mechanisms, notably DNA oxidation (Shigenaga et al., 1989). The extent of such oxidative damage correlates directly with the intracellular concentration of reactive

oxygen species, which is elevated in organisms with high metabolic rates (Martin & Palumbi, 1993). Therefore, this phenomenon should be particularly pronounced in small, evolutionarily advanced bird species, which is indeed the case.

Current perspectives on the relationship between metabolic rate and evolutionary variability of genetic parameters in birds remain contradictory (Nabholz et al., 2009; Wright et al., 2014). However, a pronounced positive correlation between body size and transversion frequency has been demonstrated not only in birds but also in mammals (Mezhzherin et al., 2024 b; Mezhzherin, Morozov-Leonov, 2024). These findings, together with the generally lower transition-transversion bias rates observed in birds compared to mammals (Mezhzherin, Morozov-Leonov, 2024), support the hypothesis of a direct link between metabolic rate and mutation rate in homeothermic organisms.

This situation leads to an intriguing conclusion: metabolic intensification in birds acts as a catalyst for evolutionary advancement, yet is simultaneously associated with an elevated mutation rate. This increase in mutation frequency suggests that point mutations may be largely neutral. Alternatively, the progressive metabolic intensification could impose constraints on the long-term persistence of small body-sized evolutionary young and rich in species groups.

This rather paradoxical hypothesis — that avian taxonomic groups with high speciation rates may also be prone to higher extinction risk — was previously proposed (Owens et al., 1999). Our results, based on an assessment of transition-transversion bias, which demonstrate an increased rate of mutational processes in young, evolutionarily progressive groups, provide support for this idea. Nevertheless, an alternative explanation remains quite plausible: nucleotide substitutions in lineages simply have little effect on fitness.

Conclusion

Therefore, analysis of transition and transversion accumulation across avian lineages reveals the following patterns.

The transition-transversion bias attenuates with ascending taxonomic rank.

This attenuation is driven by a marked increase in transition frequencies at the species and genus levels, while transversion rates remain relatively constant.

Evolutionarily recent, small-bodied bird families exhibit accelerated transversion accumulation, leading to a weaker bias. This is likely a consequence of elevated individual metabolic rates associated with rapid evolutionary diversification.

These observations raise important questions regarding the comparability of genetic differentiation estimates developed for different vertebrate classes and the feasibility of establishing a universal molecular clock scale even within a relatively narrow taxonomic group. Furthermore, comparisons of differentiation scales across taxonomic levels within a single large phylum may be more reliably conducted by examining transversion frequencies. However, the development of a universal scale for genetic differentiation among homologous taxa with varying metabolic rates remains challenging, given that increased metabolic rates may influence not only transversion frequencies but potentially transition frequencies as well.

Data availability statement. The dataset of our publication is available online at: https://doi.org/10.6084/m9.figshare.30022558

Funding. Financial support was received from National Academy of Sciences of Ukraine.

REFERENCES

Allen, A. P., Gillooly, J. F., Savage, V. M. & Brown J. H. 2006. Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences,* **103** (2), 9130–9135. https://doi.org/10.1073/pnas.0603587103

Avise, J. C. & Aquadro, C. F. 1982. A comparative summary of genetic distances in the vertebrates. *Evolutionary Biology*, M. K. Heght et al., eds. Plenum Press, New York, 151–185.

Avise, J. C., Patton, J. C. & Aquadro, C. F. 1980. Evolutionary genetics of birds. II. Conservative protein evolution in North American sparrows and relatives. *Systematic Zoology*, **29** (4), 323–334. https://doi.org/10.2307/2992339.

Benton, M. J. 2005. *Vertebrate palaeontology*. 3rd ed., Blackwell Publishing, USA, UK, Australia, 1–455.

Bergeron, L. A., Besenbacher, S., Zheng, J. et al. 2023. Evolution of the germline mutation rate across vertebrates. *Nature*, **615** (7951), 285–291 .http://doi.org/10.1038/s41586-023-05752-y

Brown, W. M., Prager, E. M., Wang, A. & Wilson, A. C. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution*, **18** (4), 225–239. http://doi.org/10.1007/BF01734101

Chu, X.-L., Zhang, B.-W., Zhang, Q.-G., Zhu, B.-R., Lin K. & Zhang D.-Y. 2018. Temperature responses of mutation rate and mutational spectrum in an Escherichia coli strain and the correlation with metabolic rate. *BMC Evolutionary Biology* **18** (126). https://doi.org/10.1186/s12862-018-1252-8

Clarke, A. & Rothery, P. 2008. Scaling of body temperature in mammals and birds. *Functional Ecology*, **22** (1), 58–67. https://doi.org/10.1111/j.1365-2435.2007.01341.x

Collins, D. W. & Jukes, T. H. 1994. Rates of transition and transversion in coding sequences since the human-rodent divergence. *Genomics*, **20** (3), 386–396. https://doi.org/10.1006/geno.1994.1192. PMID: 8034311

Duchene, D. A., Chowdhury, A.-A., Yang, J. et al. 2025. Drivers of avian genomic change revealed by evolutionary rate decomposition. *Nature*, **641** (8065), 1208–1216. https://doi.org/10.1038/s41586-025-08777-7

Gillooly, J. F., McCoy, M. W. & Allen, A. P. 2007. Effects of metabolic rate on protein evolution. *Biology Letters*, **3** (6), 655–660. https://doi.org/10.1098/rsbl.2007.0403

Gillooly, J. F., Allen, A. P., West, G. B. & Brown, J. H. 2004. The rate of DNA evolution: effects of body size and temperature on the molecular clock. *Proceedings of the National Academy of Sciences,* **102** (1), 140–145 https://doi.org/10.1073/pnas.0407735101

Goldschmidt R. The material basis of evolution. Yale University Press, 1940, 1–436.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence aligment editor and analysis program for Windows95/98 NT. *Nucleic Acid Symposium*, **41**, 95–98.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* **4** (1), 1–9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Irwin, D. M., Kocher, T. & Wilson, A. C. 1991. Evolution of the cytochrome-B gene of mammals. *Journal of Molecular Evolution*, **32** (2), 128–144. https://doi.org/10.1007/BF02515385

Johns, G. C. & Avise, J. C. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. *Molecular Biology and Evolution*, **15** (11), 1481–1490. https://doi.org/10.1093/oxfordjournals.molbev.a025875

Jukes, T. H. 1987. Transitions, transversions, and the molecular clock. *Journal of Molecular Evolution*, **26** (1–2), 87–98. https://doi.org/10.1007/BF02111284

Kartavtsev, Y. P. 2011. Divergence at Cyt-b and Co-1 mtDNA genes on different taxonomic levels and genetics of speciation in animals. *Mitochondrial DNA*, **22** (3), 55–65. https://doi.org/10.3109/19401736.2011.588215

Kartavtsev, Y. P. & Lee, J.-S. 2006. Analysis of nucleotide diversity at the cytochrome b and cytochrome oxidase 1 genes at the population, species, and genus levels. *Russian Journal of Genetics*, **42** (4), 341–362.

Kessler, L. G. & Avise, J. C. 1985. A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. *Molecular Biology and Evolution*, **2** (2), 109–125. https://doi.org/10.1093/oxfordjournals.molbev.a040339

King, M. C. & Wilson, A. C. 1975. Evolution at two levels in humans and chimpanzees. Science 188 (4184), 107–16. https://doi.org/10.1126/science.1090005

Kleiber, M. 1932. Body size and metabolism. *Hilgardia,* **6** (11), 315–353.

Li, W. H. & Graur, D. 1991. *Fundamentals of molecular evolution*. Sinauer Associates, Sunderland, MA, 1–284.

Lindgren, D. 1972. The temperature influence on the spontaneous mutation rate II. Investigation by the aid of waxy mutants. *Hereditas*, **70** (2), 165–178. https://doi.org/10.1111/j.1601-5223.1972.tb01378.x.

Löwdin, P. O. 1966. Quantum genetics and the aperiodic solid. *Advances in Quantum Chemistry*. **2**, 213–360. https://doi.org/10.1016/S0065-3276(08)60076-3.

Martin, A. P. & Palumbi, S. R. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences,* **90** (9), 4087–4091. https://doi.org/10.1073/pnas.90.9.4087.

Mayr, G. 2009. *Paleogene fossil birds*. Springer-Verlag, 1–261. https://doi.org/10.1007/978-3-540-89628-9.

Mayr, G. 2017. *Avian evolution. The fossil record of birds and its paleobiological significance*. John Wiley & Sons, Chichester, 1–306

Mezhzherin, S. V. & Morozov-Leonov, S. Yu. 1995. Genetic differentiation of mammalian taxa: assessment by biochemical gene markers. *Zhurnal Obshchei biologii,* **56** (1), 71–96 [In Russian].

Mezhzherin, S. V. & Piontkovskaya, E. A. 1998. Biochemical gene differentiation of bird taxa (Aves). Genetic differentiation of mammalian taxa: assessment by biochemical gene markers, *Zhurnal Obshchei biologii*, **59** (3), 229–248 [In Russian].

Mezhzherin, S. V. & Morozov-Leonov, S. Yu. 2024. Features of the evolutionary transition/transversion bias of birds and mammals by the CYTB gene. *Reports of the National Academy of Sciences of Ukraine*, 2, 68–74. https://doi.org/10.15407/dopovidi2024.02.068

Mezhzherin, S. V., Morozov-Leonov, S. Yu. & Holodryga, S. 2024 a. Genetic differentiation and evolutionary transition-transversion bias in Vertebrates by the example of the CYTB gene with emphasis on mammals and primates. *Cytology and Genetics*, **58** (6), 547–553. https://doi.org/10.3103/S0095452724060045

Mezhzherin, S. V., Morozov-Leonov, S. Yu., Rostovska, O. V., Tereshchenko, V.O. & Zhalay, E. I. 2024 b. Cryptic speciation and characteristics of the transition bias following an example of the cytb gene in Palearctic Mammals. *Zoodiversity*, **58** (1), 27–38. https://doi.org/10.15407/zoo2024.01.27

Nabholz, B., Glémin, S. & Galtier, N. 2009. The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, **9** (54). https://doi.org/10.1186/1471-2148-9-54.

Owens, I. P. F., Bennett, P. M. & Harvey, P. H. 1999. Species richness among birds: body size, life history, sexual selection, or ecology? *Proceedings of the Royal Society B. Biological sciences*, **266** (1422), 933–939. https://doi.org/10.1098/rspb.1999.0726

Philippe, H., Brinkmann, H., Lavrov, D. V., Littlewood, D. T., Manuel, M., Wörheide, G. & Baurain, D. 2011. Resolving difficult phylogenetic questions: why more sequences are not enough. *PLOS Biology*, **9** (3), e1000602. https://doi.org/10.1371/journal.pbio.1000602

Rosenberg, M. S., Subramanian, S. & Kumar, S. 2003. Patterns of transitional mutation biases within and among mammalian genomes. *Molecular Biology and Evolution*, 20 (6), 988–993. https://doi.org/10.1093/molbev/msg113

Sackton, T. B., Grayson, P., Cloutier, A. et al. 2019. Convergent regulatory evolution and loss of flight in paleognathous birds, *Science,* **364** (6435), 74–78. https://doi.org/10.1126/science.aat7244.

Shigenaga, M. K., Gimeno, C. J. & Ames, B. N. 1989. Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proceedings of the National Academy of Sciences*, 86 (24), 697–701. https://doi.org/10.1073/pnas.86.24.9697.

Tamura, K., Stecher, G. & Kumar, S. 2021. Version 11. *Molecular Biology and Evolution*, **38** (7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Wright, N. A., Gregory, T. R. & Witt, C. C. 2014. Metabolic "engines" of flight drive genome size reduction in birds. *Proceedings of the Royal Society B. Biological Sciences*, 281 (1779), 20132780. https://doi.org/10.1098/rspb.2013.2780

Yusuf, L., Heatley, M. C., Palmer, J., Barton, H. J., Cooney, C. R. & Gossmann, T. I. 2020. Noncoding regions underpin avian bill shape diversification at macroevolutionary scales. *Genome Research*, **30** (4), 553–565. https://doi.org/10.1101/gr.255752.119

Zhang, G., Li, C., Li, Q. et al. 2014. Comparative genomics reveals insights into avian genome evolution and adaptation. *Science*, **346** (6215), 1311–1320. https://doi.org/10.1126/science.1251385

Received 12 November 2025
Accepted 12 August 2026