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Features of the evolutionary transition/transversion bias of birds and mammals by the CYTB gene

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Differences in the nature of the transition/transversion bias of birds and mammals, as well as families with small and large body-sized species, are proven by the example of the CYTB gene. It was that for birds compared to mammals, as well as in families of small birds and mammals compared to larger ones, the frequency of transversions is significantly higher and the frequency of transitions is lower. This leads to a decrease in the transition/transversion bias and a decrease in the rate of its evolutionary compensation. The possible cause of this phenomenon is the greater intensity of individual metabolism and the resulting increase in mutation rates in birds and small species. Exceptions are extremely small species that are characterized by a state of hypothermia. The high level of metabolism and mutability explains the richness of bird species, as well as the highest activity of speciation in small organisms. In addition, the transition/transversion bias should be considered as a reliable integral indicator of individual metabolic intensity at the family level.

Keywords: *transition/transversion bias, intensity of metabolism, mutations rate, speciation, molecular evolution.*

The answer to the question of the connection between the intensity of metabolism and the pace of the mutational process is relevant for the formation of theoretical foundations of mutagenesis, also has an impact on carcinogenesis and can play an important role in the understanding of evolutionary and genetic phenomena mechanisms. A definite link between the rate of metabolism, intensity of mutational process and scale of nucleotide substitutions in diverging phyla is quite expected [1, 2]. Attempts to prove this position were made at the level of population studies. It is known that populations of small body-sized species have a higher level of genetic polymorphism than the large body-sized species [3, 4]. However, the answer to the question of what is the cause of the phenomenon — the different intensity of metabolism and, consequently, mutability or the difference in population sizes of small and large animals, has not yet been collected. Additional prob-

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lems involve direct estimates of nucleotide substitution accumulation rates in divergent groups of organisms that differ in metabolic intensity, such as birds and mammals or small and large organisms. The reasons for the complexities are the ambiguity of systematics, various durations of reproductive cycles and insufficient integrity of paleontological records.

Of particular interest is the evolutionary study of the transition/transversion bias phenomenon and its evolutionary compensation. The essence of this occurrence is that at the level of spontaneous mutational process and early stages of speciation, the frequency of transitions significantly exceeds the frequency of transversions; however, at the later stages of speciation the ratio of frequencies equalizes; subsequently, at the genus level individual DNA markers may even demonstrate a predominance of transversions [5—7]. In connection with the necessity of an answer to the question of whether metabolism and mutational process are related, a comparative analysis considering the nature of transition/transversion bias in birds and mammals and in families with large and small species, where metabolic rates differ, is relevant.

The nucleotide sequences of the CYTB gene taken from GenBank cover the main and most studied taxonomic groups of birds and mammals and serve as material for comparative study. In this work, mostly complete sequences from 1140 to 1143 bp were taken. In some cases, sequences with lengths of at least 1000 bp were used. In the latter case, alignment was performed. For this, we used programs BioEdit (v7.2.5) and MEGA X [8] using the ClustalW algorithm [9] (Hall, 1999).

Nucleotide substitutions are classified at the intraspecific, species, and genus levels of divergence in accordance with the taxonomy used when the CYTB gene sequence was entered into GenBank. The calculations did not take into account taxonomic incidents that require revisions for their corrections. Moreover, to solve the problems and purposes of this work, taxonomic unambiguity is not a decisive factor.

Table 1. **The values of the ts/tv-index at different levels of nucleotide substitutions in birds and mammals**

Nucleotide substitution classes	Birds			Mammals			t
	M	SE	N	M	SE	N	
0-0,02	13,2	3,85	57	11,0	1,18	57	0,55
0,02-0,04	10,8	0,73	57	12,1	1,14	56	0,96
0,04-0,06	8,1	0,19	60	13,6	2,70	57	2,03
0,06-0,08	5,8	0,08	59	9,4	0,78	57	4,59
0,08-0,10	4,0	0,04	60	7,4	0,45	56	7,53
0,10-0,12	2,9	0,02	60	6,0	0,46	56	6,73
0,12-0,14	2,1	0,01	57	4,1	0,26	57	7,69
0,14-0,16	1,7	0,004	52	3,0	0,20	56	6,50
0,16-0,18	1,5	0,002	39	2,3	0,15	54	5,33
0,18-0,20	1,4	0,005	26	2,0	0,11	52	5,45
0,20-0,22	1,1	0,005	10	1,6	0,10	31	4,99
0,22-0,24		—		1,6	0,13	19	—
0,24-0,26		—		1,3	0,22	7	—

Note. M — arithmetic means values; SE — their standard error; N — number of values for each class of substitutions; t — Student's t-test.
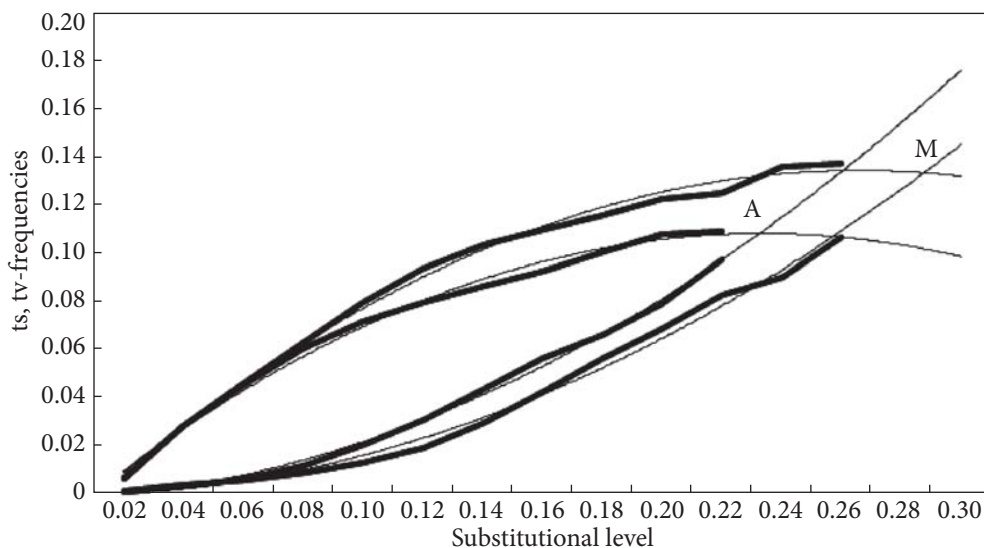
Fig. 1. Changes in frequencies of transitions (ts) and transversions (tv) depending on the total level of nucleotide substitutions for birds (A) and mammals (M) at the family level. The approximation is performed by a polynomial function

The frequencies of nucleotide substitution types were calculated directly by pairwise comparison of the sequences under study. Pairwise values of genetic distances were calculated using the MEGA program (v 11.0.11) [10].

For birds, 4168 CYTB gene sequences were involved, representing 3257 species belonging to 60 family taxa and 24 orders, respectively. For mammals, 3,540 sequences from 2,254 species belonging to 57 families and 12 orders were analyzed. As a rule, complete nucleotide sequences were used. As an exception, sequences consisting of at least 800 nucleotides were taken. The level of families was taken as a benchmark. For micromammals, the maximum divergence at this taxonomic level reaches 22—26% of nucleotide substitutions, for macromammals 14—20%, for birds 12—20%. Therefore, in order to achieve a single ranking of micromammals within Muridae, Cricetidae, Soricidae, Vespertilionidae, the analysis was carried out at the level of subfamilies, and within Murinae even at the level of tribes. For macromammals in the case of superfamily Hominoidea and suborders Odontoceti, Mysticeti and order Perissodactyla, the families were combined during the bias calculations. Birds were also grouped together. This concerned the superfamilies Sylvioidea and Emberizioidea, as well as the orders Coraciiformes and Suiliformes.

The evaluation of the transition/transversion bias was carried out using the ts/tv-index, which reflects the ratio of the transitions and transversions frequencies. The decimal logarithm of the geometric mean value between the lowest and highest body weight in grams was used as an integrated measure of size at the family level.

A comparison of ts/tv-index values at different levels of genetic differentiation in birds and mammals (Table 1) proves the fact of a clear transition/transversion bias and the fact of its evolutionary compensation. The maximum values of the ts/tv-index at the level of 11.9 occur at the intraspecific level (range of nucleotide substitutions 0—0.04), at early stages of speciation (0.04—0.08) the index decreases to 9.2, and at the species level (0.08—0.16) decreases more than twice, amounting 3.91, and at the generic level (over 0.16) it makes only 1.65.
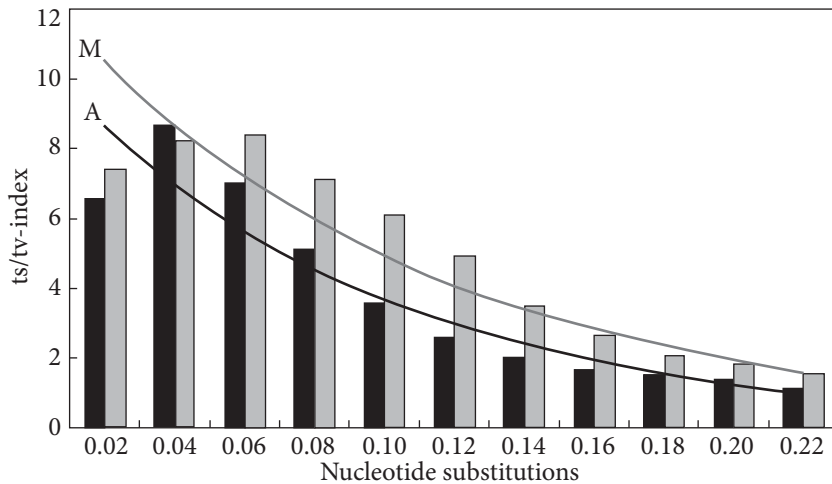
Fig. 2. Changes in average values of ts/tv-index for each class of nucleotide substitutions in birds (A) and mammals (M) at the family level
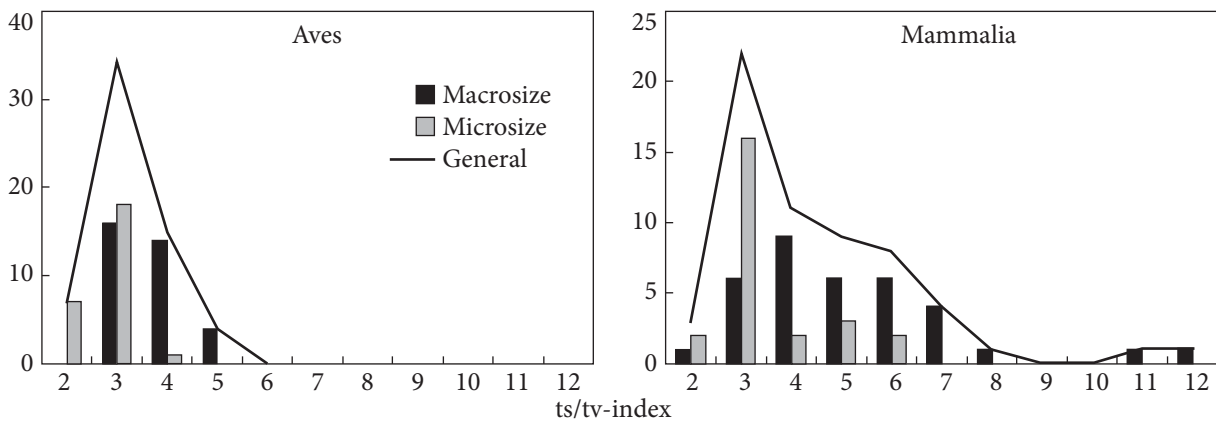

Fig. 3. Distribution of the accumulated average values of ts/tv-index in the range of nucleotide substitutions from 0 to 0.16. Note: Macrosize — families with large body species with relative mass more than 1.5; Microsize — families with small body species with relative mass less than 1.5; General distribution for the class

The reason for this trend is the rapid growth of transition frequencies against the background of minimal changes in transversion frequencies during the early stages of speciation. At the final stages of speciation, the opposite trend is observed: a slowdown in growth of the frequency of transitions and a leap in the frequency of transversions (Fig. 1).

In general, the transition bias is more pronounced in mammals than in birds (Tables 1, 2, Figs. 2, 3). The reason for this is an anticipatory increase in the frequency of transitions and, on the contrary, a lag in the frequency of transversions (see Fig. 1). Moreover, at the early stages of evolutionary divergence (range 0—0.04) there are no differences between birds and mammals, in the range 0.06—0.08 the differences are minimal, reaching a maximum at the species level (0.08—0.16), and at the generic level (0.18—0.22) they slightly decrease.

If we proceed from the approximation model, the bifurcation point (complete equalization of transition and transversion frequencies) for birds should be in the range of 0.22—0.24 nucleotide substitutions, and in mammals it is in the range of 0.28—0.30 (see Fig. 1), obviously proving higher rates of mutation in bird species.

Similar trends in the transition/transversion bias found at the level of interclass comparisons occur within classes and are associated with the body weight of representatives of different families (see Table 2). In general, it turns out that large mammals and birds families are likely to have higher rates of transition/transversion bias compared to smaller animals. They have a higher frequency of transitions and lower frequency of transversions, correspondingly a higher value of the ts/tv-index and a lower contribution of transversions. Thus, the average values of the ts/tv-index in micromammals (representatives of Myomorpha, Soricidae, Chiroptera) with geometric mean logarithmic body mass of up to 1.5 is 2.39 (SE = 0.094), and in nominal macromammals with a mass of more than 1.5 have $M_{ts/tv}$ = 3.22 (SE = 0.12; t = 5.53; $P < 0.001$). A similar tendency is observed for birds: families with small-sized species with a conditional mass of up to 1.5 have $M_{ts/tv}$ = 1.47 (SE = 0.069), while large-sized species, respectively, have $M_{ts/tv}$ = 3.63 (SE = 0.21 t = 9.78; $P < 0.001$). At the same time, the smallest micromammals (Soricinae, Crocidurinae, Myosoricinae, Sminthidae, Vespertilioninae, Myotinae, Kerivoulinae, Murininae, Hipposerididae, Molossiidae) have a ts/tv-index of 4.9 (min—max = 3.7—6.4), which is above average even for macromammals. A similar trend is observed in birds. In families with smallest species (Aegithalidae, Phyloscopidae, Trochilidae) the ts/tv-index ratio is on average 2.64, which is significantly higher than the average level of small birds.

Therefore, the evolutionary and divergent events occuring at the molecular level in birds and mammals, as well as in families consisting of large and small species, are ambiguous. The differences are related to the higher frequency of transitions and the relative scarcity of transversions in mammals and large organisms. This leads to the presence of a much higher transition/transversion bias and its slow evolutionary compensation compared to birds and small species.

Table 2. **Statistical parameters of characteristics of transition/transversion bias in birds and mammals: arithmetic mean values (M), their standard errors (SE), correlation coefficients (r) between the bias indicators and logarithmic geometric mean body mass at the family level**

Classes	Parameters	Characteristics of transition/transversion bias						
		Σts 0-0,16	Σtv 0-0,16	Σts/Σtv 0-0,16	Σtv,% 0-0,16	ts/tv 0,08-0,10	ts/tv 0,14-016	ts/tv 0,18-0,20
Mammals	M	0,53	0,12	5,04	0,18	7,35	3,15	2,11
	SE	0,004	0,004	0,266	0,006	0,387	0,203	0,108
	r	0,51	−0,57	0,57	−0,57	0,45	0,35	0,48
	N	57	57	57	57	57	57	55
Birds	M	0,47	0,17	2,92	0,26	4,15	1,80	1,43
	SE	0,005	0,005	0,122	0,008	0,266	0,10	0,07
	r	0,57	−0,60	0,62	−0,60	0,44	0,56	0,25
	N	60	60	60	60	60	60	32
	t	10,82	9,64	7,78	9,97	7,17	6,24	5,42

Note. Σts — sum of the accumulated average frequencies of transitions; Σtv — sum of the accumulated average frequencies of transversions; Σts/Σtv — value of the ts/tv index at the level of accumulated average frequencies of transitions and transversions; Σtv, % — share of transversions at the level of accumulated average frequencies. Numbers show the ranges of nucleotide substitutions. t is the value of Student's t-test when comparing the average values for birds and mammals.

There are two explanations for this phenomenon. Firstly, the distribution of frequencies of transitions and transversions is initially close to the model of random processes, but for some reason a certain shortage of transversions occurs due to the effect of natural selection in mammals compared to birds and in large species compared to small species. Secondly, the differences are due to the peculiarity of the mutation process in organisms of different sizes, as well as in birds and mammals, which is caused by different intensity of individual metabolism. It is well known [11, 12] that birds compared to mammals, and small organisms compared to larger ones, have higher body temperatures and, accordingly, higher intensity of metabolism. Consequently, it can be assumed that as a result of an increase in the rate of chemical reactions, this leads to an increase in the number of errors, including in DNA synthesis, which is accompanied by a relative increase in the number of transversions.

In this regard, the fact that in the families of extremely small species the nature of the transition/transversion bias is the same as in families of large birds and mammals seems quite natural. The reason is the propensity of extremely small homeothermal animals to hypothermia, when body temperature and the intensity of metabolism drop dramatically. This means that the average daily, and in the case of hibernation, the average annual intensity of metabolism is quite moderate.

It can be assumed that the differences in metabolic rate of small and large species determine not only the differences in the levels of genetic polymorphism of their populations, but also the intensity of speciation. As a result, the maximum species richness falls within the range of optimal [13], but not extremely low abundance species.

The obtained result may have an important applications in evolutionary bioenergetics, as it is clear that the degree of transition/transversion bias is a reflection of the average level of metabolism at the family level, and such estimates are important when conducting comparative studies.

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ОСОБЛИВОСТІ ЕВОЛЮЦІЙНОГО ТРАНЗИЦІЙНО-ТРАНСВЕРСИВНОГО ЗСУВУ У ПТАХІВ ТА ССАВЦІВ ЗА ГЕНОМ CYTB

Відмінності характеру транзитивно-трансверсивного зсуву у птахів і ссавців, а також родин з дрібними і великими за розмірами видами доведено на прикладі гена CYTB. Встановлено, що у птахів порівняно із ссавцями, а також у родинах дрібних птахів і ссавців порівняно з великими за розмірами значно вища частота трансверсій і нижча частота транзицій. Це спричинює зменшення транзитивно-трансверсивного зсуву і зниження темпів його еволюційної компенсації. Причиною феномена, очевидно, є більша інтенсивність індивідуального обміну речовин і зумовлене цим підвищення темпів мутування у птахів і дрібних видів. Винятком є екстремально дрібні види, яким властивий стан гіпотермії. Високий рівень метаболізму і мутабільності пояснює видове багатство птахів, а також найбільшу активність видоутворення у дрібних за розміром організмів. Крім того, транзитивно-трансверсивний зсув слід розглядати як надійний інтегрований показник інтенсивності індивідуального метаболізму.

Ключові слова: *транзитивно-трансверсивний зсув, інтенсивність метаболізму, мутаційний процес, видоутворення, молекулярна еволюція.*

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