

<https://doi.org/10.15407/dopovidi2023.02.093>

UDC 575.83:599

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Evolutionary transition-transversion bias by the example of the cytb gene of palearctic Muridae (Rodentia) and Vespertilionidae (Chiroptera)

Presented by Corresponding Member of the NAS of Ukraine V.I. Kashuba

A comparative analysis of the features of the transition-transversion bias of the nucleotide sequence of the cytb gene in microbats (Vespertilionidae, Chiroptera) and mice (Muridae, Rodentia) shows both general regularities and certain family-level features. A common feature of the two families is the fact of the sharp predominance of transitions over transversions at the early stages of the evolutionary process, followed by the equalization of the ts/tv-displacement at the species and genus levels of divergences, as well as the fact that the increase in the frequency of transitions in phyletic lineages is gradual, and the transition is intermittent. At the same time, the levels of spontaneous mutations and evolutionary drift, as well as the rate of ts/tv drift compensation, are specific to families. These circumstances do not make it possible to obtain comparable estimates of divergence in different phyla and cause insurmountable difficulties in creating a universal formula for molecular clocks.

Keywords: evolutionary ts/tv rate bias, Muridae, Vespertilionidae, molecular evolution, molecular clock.

The pace of the spontaneous mutation process is one of the key questions in genetics, with several aspects remaining insufficiently studied, the evolutionary aspect in particular. Initially, it was believed that the rates of spontaneous mutations of homologous genes in different phyletic

Citation: Mezhzherin S.V., Morozov-Leonov S.Y., Zhalay O.I., Kokodiy S.V., Tereshchenko V.O., Rostovskaya O.V., Tsyba A.O. Evolutionary transition-transversion bias by the example of the cytb gene of palearctic Muridae (Rodentia) and Vespertilionidae (Chiroptera). *Dopov. Nac. akad. nauk Ukr.* 2023. No 2. P. 93–98. <https://doi.org/10.15407/dopovidi2023.02.093>

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lineages are stable and comparable [1]. However, currently, this postulate has become doubtful [2-5]. The reason for that is the fact that the rate and nature of mutations differ not only between phyletic groups but also within them at different levels of divergence. Currently, we are talking about evolutionary transition : transversion bias [6-8]. It is known that during spontaneous mutations, transitions ($A \leftrightarrow G$, $T \leftrightarrow C$ substitutions) many times exceed the frequency of transversions ($A \leftrightarrow C$, $A \leftrightarrow T$, $G \leftrightarrow C$, $G \leftrightarrow T$ substitutions) [9-10]. The tendency of the significant predominance of transitions persists even at the primary stages of speciation. In contrast, at the species level of divergence and higher the ratio of transitions and transversions equalizes.

Nevertheless, the proposition about the unevenness of molecular evolution remains insufficiently studied and substantiated. Notably, its pace is estimated by the ratio of genetic distances to taxonomic status, which is often subjective, or to paleontological data, where the incompleteness of the annals is usual. There is a need for clear evidence, which can be obtained through comparative genetic studies and is necessary for a final solution to the problem of uneven rates of molecular evolution.

For this purpose, a comparative analysis of the evolutionary bias was carried out based on the cytb gene of Palearctic species of the mouse family (Muridae, Rodentia) and microbats (Vespertilionidae, Chiroptera).

The object of the study was the sequences of the cytb gene presented in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). The work used 150 Muridae sequences belonging to 30 Palearctic taxa of the genera *Apodemus* s. l. (*Alsomys*, *Apodemus* s. str., *Sylvaemus*), *Micromys*, *Mus*, *Rattus*. *Microtus arvalis* and *Cricetus cricetus* of the Cricetidae family were used as outgroups. Furthermore, 207 sequences of 71 Palearctic species of the Vespertilionidae family (*Barbastella*, *Eptesicus*, *Hypsugo*, *Miotys*, *Murina*, *Nyctalus*, *Pipistrellus*, *Plecotus*, *Vesperilio*) were used. The sequences of *Hyposidores galeritus* (Rhinolophidae) and *Miniopterus schreibersi* (Miniopteridae) were used as outgroups.

In the two studied mammalian families, the evolutionary bias clearly takes place (Table). Both for mice and microbats transitions occur quite more frequently than transversions at the

Mean values (M) and standard deviations (SD) of frequencies of transitions (ts) and transversions (tv) obtained during pairwise comparisons of cytb nucleotide sequences at different levels of divergence and ranges of substitution frequencies in general in Muridae and Vespertilionidae

| Levels | Frequency ranges, % | Muridae | | | | Vespertilionidae | | | |
|--------------|---------------------|---------|--------|-------|--------|------------------|--------|-------|--------|
| | | ts, % | | tv, % | | ts, % | | tv, % | |
| | | M | SD | M | SD | M | SD | M | SD |
| Populational | 0-3 | 1,16 | 0,0046 | 0,19 | 0,0002 | 0,12 | 0,0001 | 0,01 | 8E-07 |
| Semispecies | 3-6 | 3,74 | 0,0030 | 0,67 | 0,0005 | 4,67 | 0,0072 | 0,27 | 0,0003 |
| Allopecies | 6-9 | 6,36 | 0,0047 | 1,31 | 0,0014 | 6,77 | 0,0073 | 0,63 | 0,0004 |
| Species | 9-14 | 9,03 | 0,0091 | 2,45 | 0,0020 | 10,84 | 0,0065 | 2,21 | 0,0051 |
| Subgenus | 14-17 | 9,71 | 0,0064 | 6,26 | 0,0098 | 12,36 | 0,0053 | 3,05 | 0,0051 |
| Genus | 17-20 | 9,99 | 0,0094 | 7,72 | 0,0037 | 12,63 | 0,0060 | 6,25 | 0,0074 |
| Family | 20-24 | 11,41 | 0,0075 | 10,28 | 0,0077 | 13,13 | 0,0107 | 8,14 | 0,0200 |

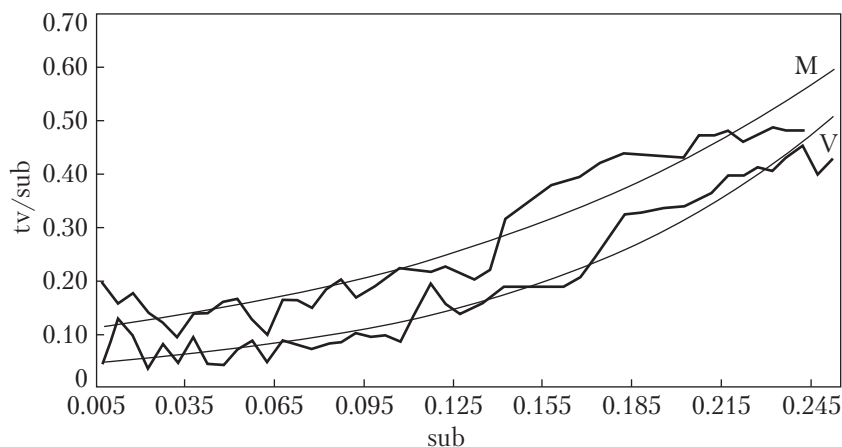


Fig. 1. Change in the relative frequency of transversions (tv/sub) depending on the total frequency of substitutions (sub): M – Muridae, V – Vespertilionidae

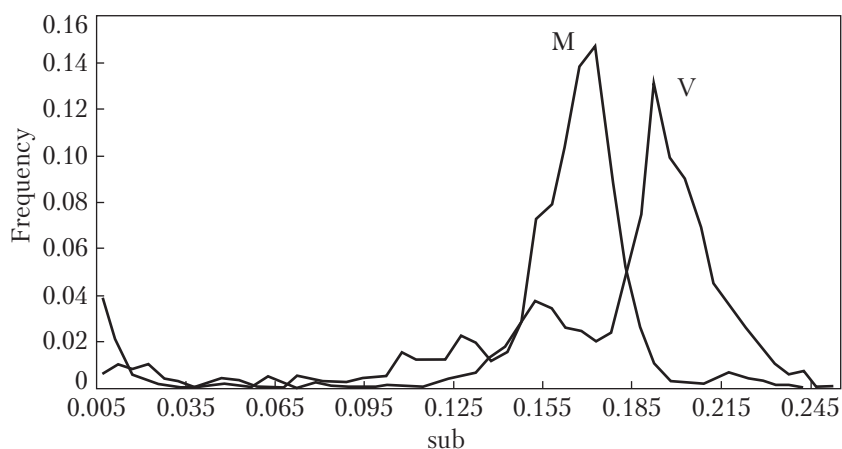


Fig. 2. Distribution of nucleotide substitutions obtained during pairwise comparisons in the phyletic lineages of Muridae (M) and Vespertilionidae (V)

levels of populations and subspecies. At the level of species divergence, the ratio of transitions and transversions evens out, although, except for interfamilial comparisons within the Muridae, transitions still slightly predominate.

At the same time, each family has its own characteristics of nucleotide substitutions. These are the following aspects: the level of spontaneous mutation process; average ts/tv bias for the family; the rate of compensation of the bias in the evolutionary lineage.

Intraspecific interpopulation variability should be considered as an indicator of the intensity of the spontaneous mutation process in this study (see Table). Its level is higher in Muridae than in Vespertilionidae. Thus, the average frequency of transversions in mice at the population level is 1.16 %, and in microbats 0.12 %, while the frequency of transitions is 0.19 and 0.01 %, respectively. This means that the total frequency of substitutions in mice is an order of magnitude higher than in microbats: 1.35 versus 0.13 %.

Differences between families are also distinctly revealed by the average level of ts/tv bias, which is more evident within Vespertilionidae at all stages of divergence (Fig. 1). At the intraspecific stages of the evolutionary process, the values of the ts/tv index in microbats are 12-17.3, at the species level they decrease to 4.9-10.7, and at the generic and higher levels of divergence, they

Fig. 3. Distribution of transitions (ts) obtained during pairwise comparisons in the phyletic lineages of Muridae (M) and Vespertilionidae (V)

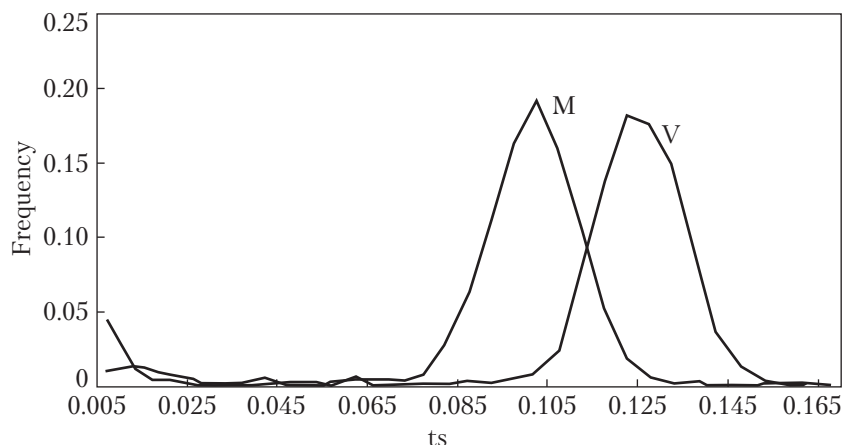
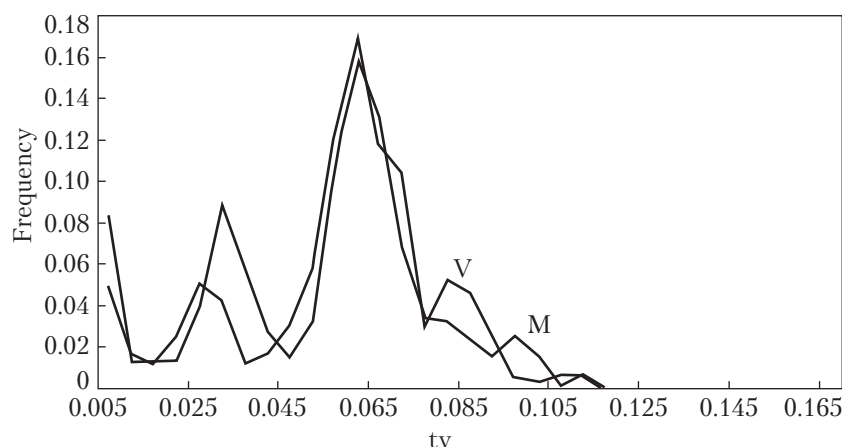


Fig. 4. Distribution of transversions (tv) obtained during pairwise comparisons in the phyletic lineages of Muridae (M) and Vespertilionidae (V)



are within 2-4.1. Whereas in mice, the value of the ts/tv-index is 5.6-6.1 at the intraspecific level, 3.7-4.9 at the species level, and 1.3-1.6 at the generic level.

The rates of saturation with different types of substitutions in the compared phyletic groups are also ambiguous. The evolutionary lineage of mice is characterized by a rapid saturation of transitions with a significant increase in the frequency of transversions, which leads to the leveling of the ts/tv index at the level of interfamily comparisons. For microbats the situation is different: saturation with transitions is evolutionarily belated with the frequency of transversions lagging behind. As a result, in bats, the frequency of transitions at the generic and family stages of evolution is higher than in mice, but the frequency of transversions is lower (see Table).

The ambiguity of the rate of compensation of the evolutionary bias in mice and microbats raises the question of the universality of evolutionary scenarios obtained by calculating nucleotide substitutions. Thus, the scenario based on the total number of substitutions is based on two non-synchronous peaks of values corresponding to species and genus levels of divergence in mice and microbats (Fig. 2). Moreover, for bats the peaks are biased towards larger values, which can be interpreted as a greater antiquity of this group of bats compared to Muridae. The distribution of transitions has one peak (Fig. 3), evidently biased in microbats towards large values compared

to mice, which means later saturation with transitions. The distribution of transversions has two peaks that coincide in mice and microbats (Fig. 4). Such ambiguity in the distribution of transitions and transversions is a piece of evidence that speciation is associated with different types of nucleotide substitutions. They should be correctly interpreted as “jumps” of transversion frequencies against the background of gradual transition saturation.

The evolutionary variability of the nucleotide composition has both general patterns and features specific to each of the families. The occasion of evolutionary bias and the fact that its compensation by different types of substitutions is quite independent should be attributed to general regularities. After all, at the first stages of speciation, the number of transitions increases sharply against the background of a certain inertness of transversions, while at the species and higher levels, on the contrary, there is a saturation of transitions while the frequency of transversions increases intermittently. Specific features include the ambiguity of the levels of mutational process and average bias, as well as the compensation speed of the ts/tv bias occurrence.

A comparative analysis of the variability of nucleotide sequences of the cytb gene in mice and microbats indicates the ambiguity of the nature of molecular evolution based on homologous features, both within and outside of the considered systematic groups. This means the impossibility of applying the universal formula of the molecular clock and building completely comparable phylogenetic schemes, which do not particularly match at the level of genera and above, even for relatively close systematic groups. Therefore, the evolutionary variability of molecular features is to some extent as ambiguous as the morphological ones. The differences are presented only by the absence of a modification component and the discrete nature of the variability of molecular features.

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Received 09.12.2022

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ЕВОЛЮЦІЙНИЙ ТРАНЗИТИВНО-ТРАНСВЕРСИВНИЙ ЗСУВ
НА ПРИКЛАДІ ГЕНА СУТВ ПАЛЕАРКТИЧНИХ MURIDAE (RODENTIA)
І VESPERTILIONIDAE (CHIROPTERA)

Порівняльний аналіз особливостей транзитивно-трансверсивного зсуву нуклеотидної послідовності гена суtv гладконосих (Vespertilionidae, Chiroptera) і мишачих (Muridae, Rodentia) показує як загальні закономірності, так і певні особливості родинного рівня. Загальними для двох родин є факт різкого переважання переходів над трансверсіями на ранніх етапах еволюційного процесу з наступним вирівнюванням ts/tv-зміщення на видовому та родовому рівнях дивергенцій, а також та обставина, що зростання частоти транзицій у філетичних рядах носить поступовий характер, а транзицій — стрибкоподібний. При цьому рівень спонтанного мутування та еволюційного зсуву, а також швидкість компенсації ts/tv зсуву є специфічними для родин. Ці обставини не дають можливості отримати порівнянні оцінки дивергенції в різних філумах і спричиняють непереборні труднощі для створення універсальної формули молекулярних годин.

Ключові слова: еволюційний ts/tv зсув, Muridae, Vespertilionidae, молекулярна еволюція, молекулярний годинник.