Genetic variations in the genus *Apodemus* (*Rodentia: Muridae*) in Lithuania

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Apodemus agrarius, *A. flavicollis* and *A. uralensis* from different localities of Lithuania were analyzed by means of allozyme PAG electrophoresis and RAPD. Seven polymorphic allozyme loci were studied and the genetic diversity of *A. agrarius* and *A. flavicollis* was estimated according to the allelic distribution and mean heterozygosity per locus. Ten random primers were used for RAPD analysis. Four of them gave specific patterns and could be used as genetic markers for the species analyzed. The genetic similarity among the species varied from 0.632 to 0.875. This corresponds well with the genetic similarity (0.720) between *A. agrarius* and *A. flavicollis* based on allozyme data.

Key words: *Apodemus*, allozymes, RAPD, genetic variation

INTRODUCTION

The genus *Apodemus* (Kaup, 1829) consists of 21 currently recognized species. These species are widely distributed in the temperate zone of the Palearctic region, in Europe, Asia and Northern Africa. Four species of this genus are found in Lithuania [1].

The systematics and phylogeny within the genus are often discussed [2–4]. A number of new species have been described in the last years [5]. It was proposed to raise some subspecies to the species rank [4].

A traditional morphological approach is usually difficult because of a uniform external appearance and the common occurrence of phenotypic sibling species. That is why molecular analysis methods are often chosen. In our research, allozyme electrophoresis along with RAPD were used. RAPD is a multilocus technique, which allows obtaining information on the general polymorphism of a genome. In most cases the phylogenetic trees derived from the results of multilocus methods are expected to be more distinct, better reflecting the real phylogeny than the trees based on results of single-locus methods. Data of isozyme electrophoresis are believed to be of systematic value in mammals at the level of subspecies, species and closely related genera [6].

MATERIALS AND METHODS

Samples for allozyme electrophoresis of striped mice and yellow-necked mice were analysed using polyacrilamide gel electrophoresis, following Davis and Brewer [7, 8] with some modifications [9]. The enzymes screened were as follows (isozyme, abbreviation, E.C. number, and corresponding structural gene loci in parentheses): glucose-6-phosphate dehydrogenase (GPD, 1.1.1.49, *Gpd-1, Gpd-2*), non-specific esterase (EST; 3.1.1.-, *Est-1,-2,-3,-4*) and xanthine dehydrogenase (XDH; 1.2.3.2).

Genetic diversity was quantified by the mean number of alleles per locus, the mean observed heterozygosity averaged over all loci using the BIOSYS-2 software. Genetic distances corrected for small sample sizes were calculated according to Nei [10].

Genomic DNA was extracted from frozen liver of 10 *A. agrarius*, 30 *Apodemus flavicollis*, and 5 *A. uralensis,* using a Genomic DNA Purification Kit #KO512 (Fermentas, Lithuania). The isolated DNA was amplified [11] using an RTOH-180 kit (Fermentas, Lithuania) consisting of 10 random primers (ROTH-180-01 5'-GCACCCGACG-3', ROTH-180-02 5'-CGCCCAAGC-3', ROTH-180-03 5'-CCATGG CGCC-3', ROTH-180-04 5'-CGCCGATCC-3', ROTH-180-05 5'-ACCCCAGCCG-3', ROTH-180-06 5'-GCACGCCGGA-3', ROTH-180-07 5'-GCACGC CGGA-3', ROTH-180-08 5'-CGCCCTCAGC-3',

ROTH-180-09 5'-GCACGGTGGG-3', ROTH-180-10 5'-CGCCCTGGTC-3'). Four primers (ROTH-180-01, ROTH-180-04, ROTH-180-06, ROTH-180-08) revealing more distinct differences among species were chosen for further analysis. PCR products were sorted according to their size by electrophoresis in 2% agarose gel and stained with etidhium bromide [12]. Fragments were analysed using the TotallLab v.1.10 (Nonlinear Dynamics Limited, England) software. The relative amount of amplified DNA indicated by the relative intensity of electrophoretic bands was not considered. The obtained data were used to compute the genetic similarity of species ($D = 2N_{AB} / (N_A + N_B$, where D is genetic similarity, N_A and N_B are total numbers of fragments in individuals A and B, N_{AB} is the number of identical fragments in individuals A and B) [13]. STATISTICA for Windows (Stat Soft, 1995) was used to carry out a cluster analysis by the UPGMA method and to construct the dendrogram.

RESULTS AND DISCUSSION

Using PAG electrophoresis of 3 enzyme systems, 7 polymorphic loci in the genus *Apodemus* were analyzed. The loci and allele frequencies in two species of *Apodemus* are listed in Table 1. No differential diagnostic loci between *A. agrarius* and *A. flavicollis* were found. The number of alleles per loci varied from 2.0 (SE = ±0.4) in *A. agrarius* to 3.1 (SE = ±0.5) in *A. flavicollis.* The mean heterozygosity varied from 0.450 (SE = ±0.121) in *A. flavicollis* to 0.530 (SE = ±0.168) in *A. agrarius.* The genetic distance between *A. agrarius* and *A. flavicollis* was 0.315 and the genetic identity 0.730. This confirms the results of Mezhzherin [14].

DNA extracted from mice of the genus *Apodemus* was amplified using 10 random primers. The aplified DNA of *Clethrionomys glareolus* was used as an outgroup. Four primers producing polymorphic fragments were chosen for further analysis. Each of these primers provided a distinct pattern of amplified PCR fragments. The number of fragments and the amount of intraspecific polymorphism varied among the primers (Fig. 1). The differences

Table 1. **Allozyme loci and allele frequency in two species of the genus *Apodemus*. (N) – the number of individuals screened**

Species	Alleles	Locus						
		Gpd1	Gpd2	Est1	Est2	Est3	Est4	Xdh
A. agrarius	(N)	(4)	(6)	(6)	(6)	(6)	(6)	(5)
	a		0.167				0.417	
	b	1.000			0.083		0.083	0.600
	c		0.833	0.500	0.750	1.000	0.417	0.400
	d			0.500	0.167		0.083	
A. flavicollis	(N)	(11)	(11)	(19)	(20)	(18)	(17)	(20)
	a	0.091		0.105	0.150	0.389		
	b	0.909	0.727	0.237	0.200	0.278	0.382	0.300
	c		0.273	0.447	0.375	0.250	0.618	0.650
	d			0.211	0.275	0.056		0.050
	e					0.028		

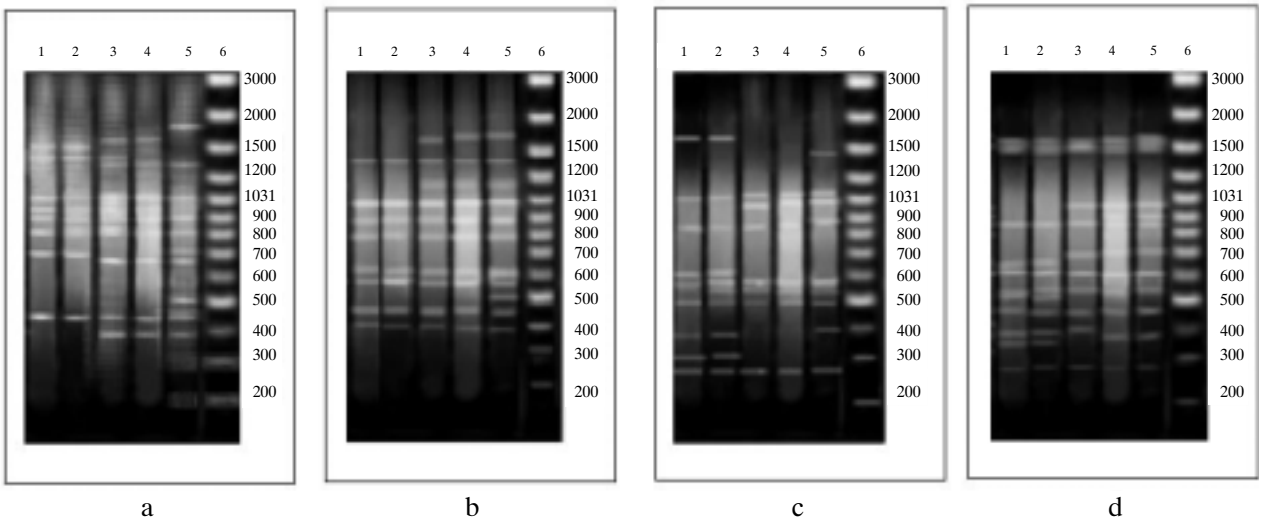

Fig. 1. Electrophoresis of amplified DNA fragments (*a* – ROTH-180-01, *b* – ROTH-180-04, *c* – ROTH-06, *d* – ROTH-180-08) in 2% agarose gel. *1, 2 – A. agrarius*; *3, 4 – A. flavicollis*; *5 – A. uralensis*, *6* – Gene Ruler™ 100bp DNA Lader Plus

Table 2. **Genetic similarity among mice species based on RAPD data**

Primer	Species	*A. agrarius*	*A. flavicollis*	*A. uralensis*
ROTH-180-01	*A. agrarius*	1.000	0.667	0.632
	A. flavicollis		1.000	0.762
	A. uralensis			1.000
ROTH-180-04	*A. agrarius*	1.000	0.737	0.737
	A. flavicollis		1.000	0.818
	A. uralensis			1.000
ROTH-180-06	*A. agrarius*	1.000	0.706	0.737
	A. flavicollis		1.000	0.875
	A. uralensis			1.000
ROTH-180-08	*A. agrarius*	1.000	0.762	0.727
	A. flavicollis		1.000	0.857
	A. uralensis			1.000
All primers	*A. agrarius*	1.000	0.720	0.709
	A. flavicollis		1.000	0.825
	A. uralensis			1.000

rius (D = 0.720) and confirmed the results of allozyme analysis. The RAPD analysis data were used to draw the dendrogram (Fig. 2). All the three analyzed *Apodemus* species form one cluster distant from the outgroup. The composition of this tree is in good agreement with the data derived from allozyme [15–21] and DNA [22, 23] analysis and with the current view on philogenetic relationships of the species studied.

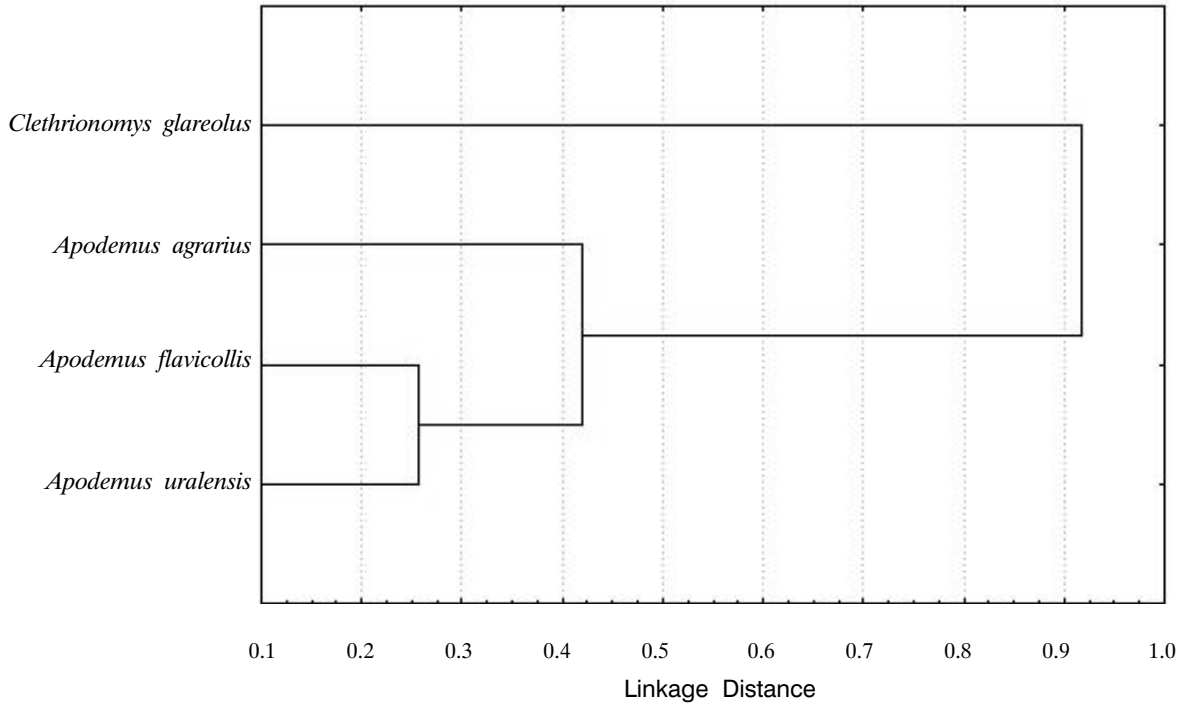

Fig. 2. Dendrogram showing genetic relations among the *Apodemus* species and outgroup based on RAPD data

among the species were usually distinct, suggesting the possibility of using the RAPD method for species identification.

Genetic similarity between the species based on RAPD data varied from 0.632 to 0.875 (Table 2). *A. uralensis* and *A. flavicollis* shared some bands that were not present in *A. agrarius*. The genetic similarity between *A. uralensis* and *A. flavicollis* was 0.825. The genetic similarity was lowest between *A. uralensis* and *A. agrarius* (D = 0.709). It was slightly higher between *A. flavicollis* and *A. agra-*

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APODEMUS (_RODENTIA: MURIDAE_) GENTIES PELINIŲ GRAUŽIKŲ GENETINĖ ĮVAIROVĖ LIETUVOJE

S a n t r a u k a

Apodemus agrarius, *A. flavicollis* ir *A. uralensis* iš skirtingų Lietuvos vietovių tirtos naudojant izofermentų elektroforezės poliakrilamidiniame gelyje ir atsitiktinai amplifikuotos polimorfinės DNR (AAPD) metodus. Nustatyti 7 polimorfiniai izofermentiniai lokusai, genetinė įvairovė vertinta alelių skaičiumi lokuse, vidutiniu heterozigotiškumu rūšiai. AAPD analizei naudota 10 atsitiktinių pradmenų, iš kurių keturi buvo specifiniai tirtoms rūšims. Genetinis panašumas tarp tirtų *Apodemus* genties rūšių kito nuo 0,632 iki 0,875. Tai atitiko genetinį panašumą (0,720) tarp *A. agrarius* ir *A. flavicollis*, nustatytą izofermentine analize.