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## GENETIC DISTANCE BETWEEN THREE SPECIES OF THE GENUS *Apodemus* Kaup, 1829

**ABSTRACT:** In this paper allozyme variation at 11 loci of six different enzymes (ICD, E.C. 1.1.1.42; LDH, E. C. 1.1.1.27; SOD, E. C. 1.15.1.1; MDH, E. C. 1.1.1.37; ME, E. C. 1.1.1.37; 6-PGD, E. C. 1.1.1.44) obtained by the method of starch gel electrophoresis were studied. Individuals of three species of genus *Apodemus* (*A. flavicollis* (Melc.), *A. sylvaticus* (L.), and *A. agrarius* (Pall.)) were trapped at Doroslovo (Vojvodina). Genetic identity (I) and genetic distance (D) were calculated on the basis of allelic frequency while genetic relationships between three species of the genus *Apodemus* were presented on the UPGMA dendrogram. Low genetic distance (D=0.20) was obtained between *A. sylvaticus* and *A. flavicollis*, whereas high between *A. agrarius* and *A. flavicollis* (D=1.02) and between *A. agrarius* and *A. sylvaticus* (D=1.20).

**KEY WORDS:** *Apodemus*, genetic distance, isozymes.

### INTRODUCTION

Genus *Apodemus* Kaup, 1829 is represented with five species, namely: *Apodemus agrarius* (Pallas, 1771); *A. flavicollis* (Melchior, 1834); *A. mystacinus* (Denford and Alston, 1877); *A. sylvaticus* (Linne, 1758); and *A. microps* Kratochvil and Rosicky, 1952 in Europe (Corbet and Oveden, 1980). In Vojvodina however, four species (*A. flavicollis*, *A. sylvaticus*, *A. agrarius*, and *A. microps*) were found (Petrov, 1979).

*Apodemus flavicollis* (Melchior, 1834): large, yellow-reddish complete collar or wide spot on the breast, is a genuine forest edge mouse sometimes occurring outside the forest (Petrov, 1992). *Apodemus sylvaticus* (Linne, 1758) is usually found together with *A. flavicollis* at forest edge and only exceptionally deep in the forest, open spaces, and clearings (Petrov, 1992). Its morphological characteristics are the following: small in size and elongated pectoral spot without collar. *Apodemus agrarius* (Pallas, 1771) usually occurs on lower terrains in marshes and near rivers and ponds of the Pannonian Plains. Most often it may be found around bushes, in reeds, under tall weeds, and in willow graves (Petrov, 1992).

In spite of increasing number of various molecular techniques isozyme electrophoresis is still a widely applied and valuable tool in the field of biochemical systematics. Protein electrophoresis is proved to be a powerful technique in solving problems at the species and subspecies levels (Filippucci, 1989).

According to A y e s h a et al. (1986), *Apodemus flavicollis* and *A. sylvaticus* from Yugoslavia are not distinguishable on the basis of the alleles of *Ldh-1*, *Ldh-2*, *Gpd*, *Gpi-1*, *Gpi-2*, *Mdh-1*, *Mdh-2*, *Hk-3*, *Got-1*, *Got-2*, *Sordh*, *Sod-1*, *Sod-3* loci. Their genetic distance to *A. agrarius* is great when other parameters are considered.

The aim of this paper is to establish genetic distance between three species of the genus *Apodemus* on the basis of allelic isozyme variability.

## MATERIAL AND METHODS

Individuals of three species of the genus *Apodemus* were trapped in 1991 at Doroslovo (Vojvodina). Traps were placed in different habitats and in different plant communities: oakhorbeam forest (Doroslovo forest), planted poplar forest along the DTD canal, on willow groves and meadow vegetation along the riverbed of the Mostonga. Traps were also placed on marsh terrains with meadowsteppe vegetation, uncultivated land characterized by ruderal vegetation, on fields with alfalfa, plowed fields, and in the village. The number of trapped specimens were: *A. flavicollis* (37), *A. sylvaticus* (6), and *A. agrarius* (26).

Tissues samples from liver were homogenised in distilled water (1:1 w/v) at 4°C, and centrifuged for three minutes at 6000 rpm. The clear supernatant was absorbed to filter paper wicks (4x6 mm) and inserted into gel. Horizontal starch gel (12%) electrophoresis was performed using the following buffer systems:

### A) TCE, pH 7

1. isocitrate dehydrogenase (ICD, E. C. 1.1.1.42)
2. lactate dehydrogenase (LDH, E. C. 1.1.1.27)
3. superoxid dismutase (SOD, E. C. 1.15.1.1.)

### B) LiOH, pH 8,1

1. malate dehydrogenase (MDH, E. C. 1.1.1.37)
2. malic enzyme (ME, E. C. 1.1.1.37)
3. 6-phosphogluconate dehydrogenase (6-PGD, E. C. 1.1.1.44)

After electrophoresis the gels were sliced and stained according to S e l a n - d e r et al. (1971). Allozymes were designated numerically according to their mobility (100% = faster mobility).

Genetic identity (I), genetic distance (D) and UPGMA dendrogram were calculated according to N e i (1972).

## RESULTS AND DISCUSSION

Six isozymes, namely isocitrate dehydrogenase (ICD), lactate dehydrogenase (LDH), superoxid dismutase (SOD), malate dehydrogenase (MDH), malic enzyme (ME), and 6-phosphogluconate dehydrogenase (6-PGD) controlled by 11 loci were analyzed. Three loci (*Mdh-2*, *Icd-2* i *Sod-2*) were monomorphous in all the examined species of the genus *Apodemus* (Tab. 1).

Tab. 1 – Allelic frequencies of species of the genus *Apodemus*

LOCUS	ALLELE	ALLELIC FREQUENCY		
		<i>A. flavicollis</i>	<i>A. Agrarius</i>	<i>A. sylvaticus</i>
<i>Ldh-1</i>	100	0.20		
	90	0.05		0.33
	78	0.37		
	70		0.33	
	65			0.33
	60		0.33	
	54	0.37		
	45			0.33
40		0.33		
<i>Ldh-2</i>	36	0.50		
	24			0.50
	20		0.50	
	18	0.50		0.50
<i>Mdh-1</i>	100	1		1
	78		1	
<i>Mdh-2</i>	5	0.50	0.50	0.50
	3	0.50	0.50	0.50
<i>6-Pgdh</i>	40	0.21		0.30
	36	0.57	0.70	0.70
	33	0.22	0.30	
<i>Me-1</i>	80	1		1
	40		1	
<i>Me-2</i>	30			1
	20	0.64		
	18		1	
	16	0.36		
<i>Icd-1</i>	100		0.33	
	96		0.33	
	94	0.33		0.33
	90		0.33	
	85	0.33		0.33
	65	0.33		0.33
<i>Icd-2</i>	3	1	1	1
<i>Sod-1</i>	60		1	
	49	0.60		0.70
	32	0.40		0.30
<i>Sod-2</i>	3	1	1	1

*A. flavicollis* and *A. sylvaticus* were characterized by the same alleles in *Mdh-1*, *Me-1*, and *Sod-1* loci while the allele frequency was different only in the *Sod-1* locus. *A. flavicollis* and *A. agrarius*, as well as *A. sylvaticus* and *A. agrarius* possessed different alleles in investigated polymorphous loci.

From the allele frequencies at 11 loci studied Nei's values of genetic identity (I) and distance (D) were calculated among species of genus *Apodemus* (Tab. 2). The value of genetic distance between *A. flavicollis* and *A. sylvaticus* was evidently lower (D=0.20), than between *A. flavicollis* and *A. agrarius* (D=1.02), as well as between *A. sylvaticus* and *A. agrarius* (D=1.20). UPGMA dendrogram summarising the genetic relationships found is presented in Fig. 1.

Tab. 2. - Values of genetic identity (Nei's I; above the diagonal) and distance (Nei's D; below the diagonal), between three species of the genus *Apodemus*

	<i>A. flavicollis</i>	<i>A. sylvaticus</i>	<i>A. agrarius</i>
<i>A. flavicollis</i>		0.82	0.36
<i>A. sylvaticus</i>	0.20		0.29
<i>A. agrarius</i>	1.02	1.20	

Value of genetic identity calculated on the basis of the allelic variability of isozymes between *Apodemus flavicollis* and *A. sylvaticus* was smaller than in *Apodemus agrarius* (Gemeke, 1980); Ayesha et al., 1986; Filippucci et al., 1989). This results were confirmed by the cytological analyses (Vujošević et al., 1984). Our results also showed that species *A. flavicollis* and *A. sylvaticus* were genetically closer when compared with *A. agrarius*.

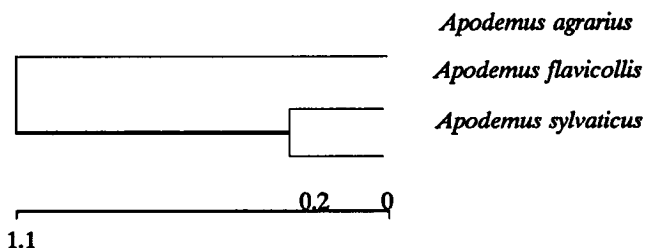


Fig. 1. - UPGMA dendrogram summarizing the genetic relationships among three species of the genus *Apodemus*

Enzyme polymorphism in our study, however, was somewhat higher than in papers reported by Ayesha et al. (1986) and Filippucci et al. (1989), and may be explained by tissue specificity (Vapa et al., 1992), greater number of individuals analyzed in this study, as well as by different methodology used.

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## ГЕНЕТИЧКА УДАЉЕНОСТ ИЗМЕЂУ ТРИ ВРСТЕ РОДА *Apodemus* Кауп, 1829.

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### Резиме

У раду је испитивана алозимска варијабилност 11 локуса 6 различитих ензима (ICD, Е.С. 1.1.1.42; LDH, Е. С. 1.1.1.27; SOD, Е. С. 1.15.1.1; MDH, Е. С. 1.1.1.37; ME, Е. С. 1.1.1.37; 6-PGD, Е. С. 1.1.1.44) методом електрофорезе на скробном гелу. Примерци три врсте рода *Apodemus* (*A. flavicollis*, (Meis.) *A. sylvaticus* (L.) и *A. agrarius* (Pall.)) изловљени су на локалитету Дорослово (Војводина). На бази аелне фреквенције израчуната је генетичка блискост (I) и генетичка удаљеност (D), док су генетички односи између три врсте рода *Apodemus* приказани на UPGMA-дендрограму. Генетичка удаљеност између врста *A. flavicollis* и *A. sylvaticus* је била знатно нижа ( $D=0,20$ ), у односу на вредности генетичке удаљености између врста *A. flavicollis* и *A. agrarius* ( $D=1,02$ ) и *A. sylvaticus* и *A. agrarius* ( $D=1,20$ ).