Belg. J. Zool., 130 (Supplement 1): 115-120 November 2000

Strategies in open field behaviour of *Mus spicilegus* and *Mus musculus musculus*

Daniela Marinova Simeonovska-Nikolova

Faculty of Biology, "St. Kliment Ochridski",
The University of Sofia, 8 "Dragan Tzankov" blvd., 1421 Sofia, Bulgaria

ABSTRACT. The behaviour of *Mus spicilegus* and *Mus musculus musculus* was studied in a 15-minute open field test. A total of 12 behavioural events were recognized for both species. The dynamics and patterns of association of these behavioural events were analyzed. Significant sex-specific and species-specific differences were found in the dynamics of exploratory activity and also in some events of emotionality. Male and female *M. spicilegus* and *M. m. musculus* showed different patterns of behaviour in the experiment. Males demonstrated lower emotionality and appeared to explore the environment more actively than females. *M. m. musculus* showed higher exploratory activity than *M. spicilegus*.

KEY WORDS: exploratory behaviour, emotionality, behavioural strategies, *Mus spicilegus* and *Mus musculus musculus*.

INTRODUCTION

Mus spicilegus Petenyi 1882 and *Mus musculus musculus* Linnaeus 1758 are closely related species, which belong to different groups: outdoor mice and synanthropic mice. The distribution of *Mus spicilegus* and *M. m. musculus* in Bulgaria is restricted to the north of the Stara Planina Mountains. *M. m. musculus* is semi-synanthropic. *M. spicilegus* inhabits mainly agroecosystems. The populations of both species are sympatric during the spring-autumn period. At the end of autumn *M. m. musculus* comes back to houses, where it spends the winter. In autumn, *M. spicilegus* builds mounds of seeds and a complex of underground tunnels and nest cameras, which it uses in winter.

The differences in open field behaviour of many rodent species are interpreted on the basis of their ecological strategies and adaptation (FRYNTA, 1994). The behaviour of *M. spicilegus* has not yet been studied well. Only a few studies on the exploratory behaviour of *M. spicilegus* and *M. m. musculus* have been known so far (MESHKOVA et al., 1986; SOKOLOV et al., 1990; KOTENKOVA et al., 1994). According to KOTENKOVA et al. (1994) the behaviour of outdoor *musculus* is similar to that of outdoor forms of *spretus* and *spicilegus*, but SOKOLOV et al. (1990) showed that *M. spicilegus* is not as ecologically flexible as the synanthropic species. Therefore, more comparative data are needed to make a more precise comparison between the species of genus *Mus*.

In this context, the aim of the present paper is to study and compare the patterns of behaviour of *M. spicilegus* and *M. m. musculus* in an open field test.

MATERIAL AND METHODS

A total of 85 individuals of *M. spicilegus* and 58 individuals of *M. m. musculus* were tested. Adult and sexually mature animals were used in the test. Females were neither pregnant nor lactating. The mice tested had an average weight of 14.3±0.2 g for *M. spicilegus* and 14.8±0.2g for *M. m. musculus*. Both species were captured from wild populations in the same region of Northern Bulgaria (latitude 43° 22' N, longitude 24° 23' E). *M. spicilegus* and *M. m. musculus* were regularly captured and studied from spring to autumn for the period 1990-1993. The animals captured were individually housed in standard laboratory rodent cages for at least one week before the beginning of the experiments. The mice were kept in the laboratory at a temperature of 20°C±2°C, and in natural daylight. The animals were fed a mixed seed diet supplemented with carrots, apples and provided with water a*d libitum.*

Each animal was individually tested for 15 min (three series of 5 min intervals each) in the open field experi-

Corresponding author: D.M. Simeonovska-Nikolova, e-mail: mammals@sf.icn.bg

ment. The experiments were performed in a glass terrarium (50x50x40 cm). The bottom was demarcated into 10 squares of equal dimensions (10x10cm) by painted dark lines. Mice were tested during their active phase in the morning and in the evening (METCHEVA & GERASIMOV, 1994). The data from the open field test were registered in protocols by shorthand.

The skulls of mice tested were measured craniometrically for species identification by special keys for craniometrical determination (GERASIMOV et al. 1990).

A total of 12 behavioural events were established in the experimental groups of *M. spicilegus* and *M. m. musculus* tested by the open field method: ambulation (the animals move actively within the experimental arena), rearing (the animals rear up on hind legs), jumping (the animals perform jumps), self-grooming (self-grooming is predominantly demonstrated as washing), defecation (self-explanatory), climbing (the animals attempted to climb the walls), gnawing (the animals gnaw at the walls, corners and the floor of the experimental arena), digging (the animals perform digging movements with hind legs), touching and moving of feces (the animals touch or move feces with their muzzles), urination (self-explanatory), standing still (the animals stop moving for a short time) and turning (the animals turn around themselves).

Horizontal activity (ambulation) was measured by the number of squares visited. Significance of differences between the mean scores of the observed behavioural events was estimated by the Mann-Whitney test. Principal coordinate analysis based on matrices of Pearson's product moment correlation was used to study the patterns of association among events of behaviour in male and female animals (LEGENDRE & LEGENDRE, 1983; JONGMAN et al., 1987). Then the correlation matrices were used in factor analysis with varimax rotation.

RESULTS

Ambulation of females of both species remained lower than that of males (Table 1, Table 2). Ambulation of male *M. m. musculus* was higher than that of male *M. spicilegus* during the first two time intervals ($P<0.001$). At the 1st and 3rd min interval the ambulation of female *M. m. musculus* was also significantly higher than that of female *M. spicilegus* ($P<0.05$) and ($P<0.01$), respectively. The rearing of male and female *M. spicilegus* was significantly higher than that of male and female *M. m. musculus* during 2nd and 3rd min intervals ($P<0.01$). The mean scores of self-grooming decreased significantly ($P<0.05$) both in male and female *M. spicilegus*, but increased significantly ($P<0.01$) both in male and female *M. m. musculus*.

TABLE 1

Mean scores (M), standard error (SE), significance of difference between sexes (P) for the behavioural events observed in *M.spicilegus* and proportion (%) of individuals performing the act.

Events	code	males (n=54)			females (n=31)			
		M	SE	%	M	SE	%	P
ambulation 5 min	Am	75.3	4.3	100	76.9	6.1	100	
ambulation 10 min		79.8	3.8		67.5	4.1		<0.05
ambulation 15 min		65.4	3.6		48.8	3.7		<0.01
rearing 5 min	Re	29.4	2.2	100	30.1	2.4	100	
rearing 10 min		40.4	2.3		36.7	1.9		
rearing 15 min		35.3	2.3		29.4	2.4		
jumping 5 min	Jm	0.1	0.1	43	0.0	0.0	29	
jumping 10 min		0.7	0.2		0.3	0.1		
jumping 15 min		1.8	0.6		0.7	0.3		
self-grooming 5 min	Gr	2.9	0.2	100	2.4	0.2		
self-grooming 10 min		1.4	0.1		1.5	0.1		
self-grooming 15 min		1.2	0.1		0.9	0.2		<0.05
gnawing	Gn	2.5	0.4	69	0.9	0.2	41	<0.01
standing still	St	1.1	0.2	48	1.2	0.3	58	
climbing	Cl	0.9	0.2	39	1.1	0.2	54	
defecation	Df	3.5	0.2	100	3.5	0.3	100	
touching	Tch	0.6	0.2	27	0.7	0.2	35	
digging	Dg	0.04	0.03	14	0.4	0.1	32	<0.05
urination	Ur	0.4	0.1	30	0.8	0.2	48	<0.05
turning	Tr	0.3	0.1	24	0.7	0.1	48	<0.05

TABLE 2

Mean scores (M), standard error (SE), significance of difference between sexes (P) for the behavioural events observed in *M.m.musculus* and proportion (%) of individuals performing the act.

Events	code	males (n=54)			females (n=31)			
		M	SE	%	M	SE	%	P
ambulation 5 min	Am	126.9	7.6	100	97.0	7.5	100	
ambulation 10 min		111.5	5.0		80.2	6.1		
ambulation 15 min		83.2	8.3		71.5	6.4		<0.001
rearing 5 min	Re	30.3	2.4	100	31.1	2.5	100	
rearing 10 min		24.5	2.0		20.1	1.0		
rearing 15 min		17.8	1.6		16.1	1.8		
jumping 5 min	Jm	12.0	4.2	90.3	2.8	0.7	74	
jumping 10 min		10.1	3.3		6.4	2.0		
jumping 15 min		9.6	3.8		4.4	1.7		
self-grooming 5 min	Gr	1.0	0.2	100	1.0	0.2	100	
self-grooming 10 min		1.7	0.2		2.0	0.2		
self-grooming 15 min		2.9	0.2		1.4	0.2		
gnawing	Gn	10.1	1.2	100	6.4	0.7	100	<0.01
standing still	St	3.1	0.3	100	5.6	0.5	100	<0.001
climbing	Cl	11.3	1.3	100	7.3	1.2	99.5	<0.01
defecation	Df	3.6	0.5	90.3	3.3	0.3	96.2	
touching	Tch	0.4	0.2	16.1	0.1	0.1	11.1	
digging	Dg	5.0	0.6	93.5	2.1	0.5	77.7	<0.001
urination	Ur	0.3	0.1	25.8	0.1	0.1	11.1	
turning	Tr	0.6	0.1	54.8	0.7	0.3	40.7	

Jumping was performed by almost all male and female *M. m. musculus* during the test, while it seemed to be a rear behavioural act performed by *M. spicilegus*. The pooled mean score (for 15 min) of this behavioural event was significantly ($P<0.05$) greater in males of *M. spicilegus* than in females of the same species. The counts of jumping of male and female *M. m. musculus* remained significantly higher than those of *M. spicilegus* ($P<0.01$).

Significant differences between male and female *M. spicilegus* were established in the mean scores of gnawing, digging, turning and urination (Table 1). A significantly ($P<0.05$) larger proportion of males than females performed gnawing during the experiment, while significantly ($P<0.05$) fewer males than females performed turning. Significant differences between male and female *M. m. musculus* were established in the mean scores of standing still, climbing and digging (Table 2). The counts of gnawing, standing still, climbing and digging of male and female *M. m. musculus* were significantly higher than those of both male and female *M. spicilegus* ($P<0.001$). Only urination had significantly higher mean scores ($P<0.01$) in female *M. spicilegus* in comparison with female *M. m. musculus*.

The above-mentioned sex-specific and species-specific differences in some of the behavioural events suggested that more general differences existed between the open field behaviour of *M. spicilegus* and that of *M. m. musculus*. To check this hypothesis a principal coordinate analysis, based on Pearson's product moment correlation coefficient of behaviour events, was used. Ambulation and rearing in male *M. spicilegus* are strongly correlated (Fig.1 A). A gradient from events associated with emotionality (left part of the diagram) towards exploratory behaviour (right part of the diagram) can be traced. Such events as self-grooming, standing still, and events representing mixed behavioural activity, tend to cluster near to the origin of the diagram. A different pattern of association between the different events is demonstrated in female *M. spicilegus* (Fig. 1 B). In this case behavioural events seem to be more diffusely associated.

Ordination diagrams of male *M. m. musculus* (Fig. 2 A) show that ambulation and rearing are connected with the 1st factor. The cluster between climbing-gnawing-digging is well expressed, therefore, the exploratory behaviour is related to the escape behaviour. The events: urination and touching and moving of feces are located at the top end of the diagram. A diffusion of behavioural events of female *M. m. musculus* is observed (Fig.2 B), with the exception of the group of events associated with exploratory and escape behaviour (lower right part of diagram).

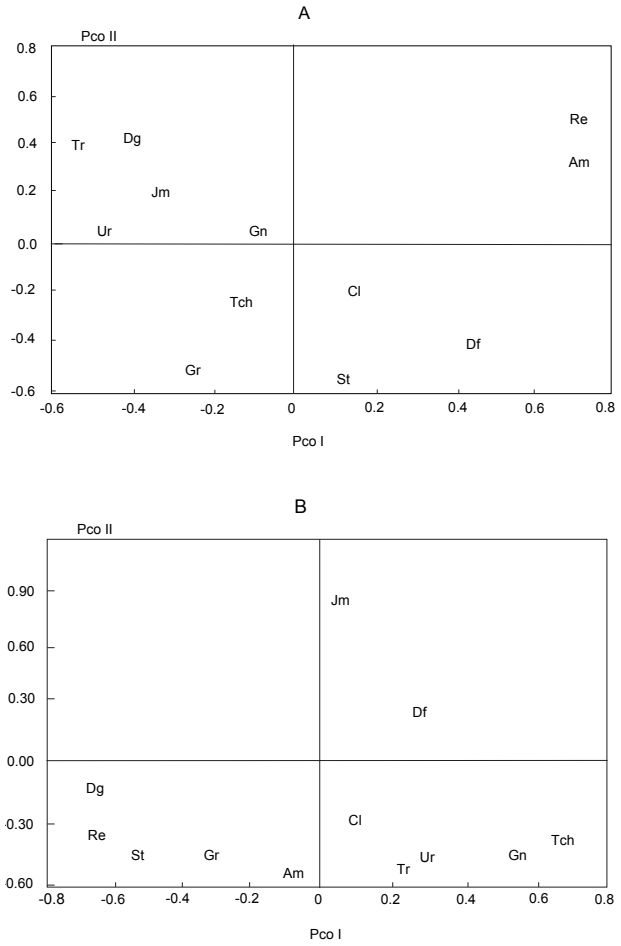

Fig. 1. – Strategies in open field behaviour of Mus spicilegus and Mus musculus musculus.

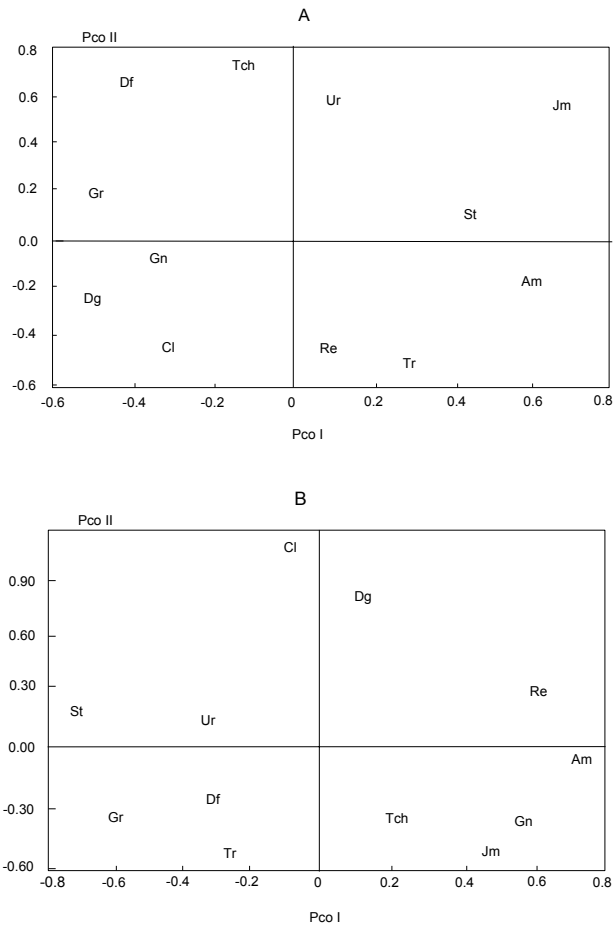

Fig. 2. – Strategies in open field behaviour of Mus spicilegus and Mus musculus musculus.

Moreover, a factor analysis on the same correlation matrices was carried out in order to analyze further the pattern of association between behavioural events. This will help in the search of the mechanism that is responsible for sex- and species-specific differences in behaviour. The results of the varimax rotation of the factor matrices for male and female *M. spicilegus* showed that the first factor of male *M. spicilegus* was well correlated with ambulation and rearing. Thus, it was associated with exploratory activity (Table 3). The second factor seemed to be associated with emotionality. The third factor could be associated with marking activity and the last factor with escape behaviour. The factor solution of female *M. spicilegus* was clearly different from that of males and that was difficult to interpret. Ambulation, rearing, jumping and touching and moving feces had a high contribution to sev-

TABLE 3

Varimax factor analysis, based of Pearson's correlation coefficient of the behavioural events in males and females of *Mus spicilegus*. (*Events contributing to at least 25% from the variance in each factor).

	males				**females**			
factor	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
variances %	19.3	15.4	12.1	10.9	25.6	16.1	11.6	11.1
cumulative %	19.3	34.7	46.7	57.6	25.6	42.2	53.3	64.9
ambulation	0.8*	-0.2	0.08	-0.05	0.4	0.6*	0.01	0.5*
rearing	0.9*	-0.07	-0.02	-0.005	0.2	0.4	0.6*	0.4
jumping	-0.02	0.1	0.3	0.7*	-0.4	-0.5*	0.04	0.3
self-grooming	-0.5	-0.3	0.4	0.2	-0.2	0.8*	0.04	0.3
gnawing	-0.04	-0.03	-0.1	0.8*	0.2	0.4	-0.7*	0.4
standing still	-0.3	-0.3	-0.03	-0.3	-0.07	0.6*	0.3	0.03
climbing	-0.2	0.07	-0.4	-0.1	0.6*	-0.02	0.07	0.2
defecation	0.02	-0.6*	0.2	-0.2	0.07	-0.2	-0.1	0.8*
touching	-0.1	-0.1	0.7*	-0.3	0.4	-0.02	-0.6*	-0.09
digging	-0.01	0.8*	0.04	-0.1	0.2	0.1	0.7*	-0.07
turning	-0.04	0.7*	0.5	0.1	0.9*	0.07	0.04	-0.02
urination	-0.09	0.2	0.7*	0.2	0.7*	0.2	-0.09	0.08

eral factors. Only, the fourth factor seemed to be associated with defecation.

The first factor of male *M. m. musculus* seemed to be associated with exploratory behaviour and tendency to escape (Table 4). The events associated with the second factor showed that it could be linked to a greater extent with exploratory activity. The third and the last factors were probably associated with emotionality. The factor solution of female *M. m.musculus* showed that ambulation, rearing, jumping and gnawing were related to exploratory behaviour and closely associated with the 1st factor. Climbing and digging had high contributions to the 2nd factor, therefore, it was most likely associated with exploratory behaviour and tendency to escape. The 3rd factor was probably associated with emotionality.

TABLE 4

Varimax factor analysis, based of Pearson's correlation coefficient of the behavioural events in males and females of *Mus musculus musculus*. (*Events contributing to at least 25% from the variance in each factor).

	males			females			
factor	**1**	**2**	**3**	**4**	**1**	**2**	**3**
variances %	27.6	24.7	18.7	14.9	50.7	20.3	14.0
cumulative %	27.6	52.3	71.0	85.8	50.7	71.0	85.0
ambulation	-0.02	0.8	-0.2	0.3	0.9*	0.1	0.07
rearing	0.5*	0.4*	0.2	0.1	0.7*	0.3	-0.001
jumping	-0.6*	0.3	-0.06	-0.04	0.7*	-0.1	-0.001
self-grooming	0.1	-0.6*	0.2	0.6*	-0.3	-0.4*	0.7*
gnawing	0.8*	0.07	-0.1	-0.3	0.8*	-0.06	0.08
standing still	-0.02	0.002	0.6*	-0.02	-0.8*	-0.1	0.3
climbing	0.5*	-0.3	0.4*	0.3	-0.2	0.8*	-0.04
defecation	0.1	-0.3	-0.2	-0.08	-0.1	-0.08	-0.3
touching	0.008	-0.2	-0.08	-0.4*	0.3	-0.04	-0.2
digging	0.6*	-0.06	-0.1	0.1	-0.04	0.6*	0.4*
turning	0.1	0.2	0.9*	0.3	-0.08	-0.2	0.04
urination	-0.007	-0.02	0.002	-0.3	-0.08	0.005	0.09

DISCUSSION

The sex and species differences in occurrence and patterns of association between behavioural events suggested that there were differences in the general strategies of exploring a new environment used by males and females of *M. spicilegus* and *M. m. musculus.* Males of *M. spicilegus* and *M. m. musculus* demonstrated higher horizontal activity (ambulation) than females in the experiment. According to MOTA (1987) high ambulation scores were usually seen as indicative of reduced fear in the animals and active environment exploration. Pooled mean scores of some events as jumping and gnawing were also significantly greater in males. On the other hand, events such as urination, turning, standing, usually associated with emotionality were significantly more frequent in females. Thus, males of *M. spicilegus* and *M. m. musculus* seemed to explore the environment more actively and maybe more efficiently than females. Exploratory activity in females, especially in females of *M. spicilegus*, appeared to be affected by emotionality to a higher degree. Ambulation and rearing were correlated well in male *M. spicilegus* as well as in female *M. m. musculus*. So it was neither a sex-specific nor species-specific correlation of these behavioural patterns. However, the behaviour of female *M. spicilegus* in the test seemed to be more influenced by emotionality in comparison with that of female *M. m. musculus*. The mean scores of urination and turning were greater in female *M. spicilegus* and the correlation between defecation and ambulation was better expressed. An emotional reactivity as reflected by plasma corticosteron was found to be higher in feral populations of *M. domesticus* compared with commensal populations of this species (FRYNTA, 1994).

M. m. musculus demonstrated higher exploratory activity than *M. spicilegus*. Moreover, *M. m. musculus* performed significantly more jumping, climbing and gnawing compared with *M. spicilegus*. The results showed that these actions were directed to active exploration of the surroundings and tendency to escape and probably they were connected with the commensal way of life. The relatively high proportion of climbing and jumping behaviour distinguished *M. m. domesticus* from *M. spretus* (MOTA, 1987). Similar differences in exploratory behaviour were recorded between *Mus macedonicus* and *Mus musculus* (FRYNTA et al., 1994). According to KOTENKOVA et al. (1994) the differences in exploratory behaviour of outdoor and synanthropic forms of mice demonstrated the importance of living conditions in the formation of stereotypes of exploratory behaviour during evolution. Therefore, presumably the commensal way of life of *M. m. musculus* contributed to the faster orientation and adaptation to new and unknown environments and situations.

Rearing was significantly higher in *M. spicilegus* than in *M. m. musculus*, a behaviour most likely related to the open field habitat in which they lived. BRUELL (1969) noted that the urination was an act of emotionality, but also connected with marking behaviour of mice in natural conditions. According to ARCHER (1973) defecation in new surroundings contributed to easier orientation of the animals on the basis of olfactory stimuli. The higher scores of urination of female *M. spicilegus* and the correlation between urination and touching and moving feces established for male *M. spicilegus*, suggested that marking activity had significant importance in orientation and exploration of the surroundings of this species. SOKOLOV

et al. (1990) also established difference in marking activity between *M. spicilegus* and *M. m. musculus*. According to SOKOLOV et al. (1990) the marking activity was higher in *M. spicilegus*.

The presence of sex-specific and species-specific differences in the patterns of open field behaviour probably is connected with the different adaptation and ecological strategies of *M. spicilegus* and *M. m. musculus*, resulting from the way of life of each sex and species in the populations. However, some further studies on movements, migrations and social interactions between both species are needed to improve the knowledge of their ecological adaptation and relationships in populations.

REFERENCES

ARCHER, J. (1973). Test for emotionality in rat and mice: A review. *Anim. Behav.*, 21: 205-235.

BRUELL, J.H. (1969). Genetics and adaptive significance of emotional defecation in mice. *Ann.N. Y. Acad. Sc.*, 159: 825-830.

GERASIMOV, S., H. NICOLOV, V. MICHAILOVA, J. AUFFRAY & F. BONHOMME (1990). Morphometric stepwise discriminant analysis of the five genetically determined European taxa of the genus *Mus*. *Biol. Jour. of the Lin. Soc.*, 41: 47-64

FRYNTA, D. (1994). Exploratory behaviour in 12 palaearctic mice species (*Rodentia*: *Muridae*). A comparative study using "free exploration" tests. *Acta Soc. Zool. Bohem.*, 57: 173-182.

JONGMAN R.H., C.J.F. BRAAKTER & O.F.R. VAN TONGEREN (1987). *Data analysis in community and landscape ecology*. Wageningen, Centre for Agricultural Publishing and Documentation, 299 p.

LEGENDRE L. & P. LEGENDRE (1983). *Numerical Ecology*. Elsevier Scientific Publishing Company.

KOTENKOVA E., N.N. MESHKOVA & N. ZAGORUIKO (1994). Exploratory behaviour in synanthropic and outdoor mice of superspecies complex Mus musculus. *Pol. Ecol. Studies*, 20, 4-3: 375-381.

MESHKOVA N.N., E.V. KOTENKOVA & S.I. LJALJUCHINA (1986). Behaviour of house mouse and mound-building mouse in the new environment. *Zool. Jour.*, 65: 123-133. (In Russian with summary in English).

METCHEVA R., S. GERASIMOV (1994). Comparative thermo-regulation characteristics of the four taxa of the mouse, *Mus musculus musculus* (L., 1958), *Mus musculus domesticus* (Schwars & Schwars, 1943), *Mus spicilegus* (Petenyi, 1882) and *Mus macedonicus* (Petrov & Puzic, 1983). *Ecologia*, Bulgarian Academy of Sciences, 26: 106-120.

MOTA, P.G. (1987). Behavioural analysis of two rodent species from different ecological habitats. A model for understanding the evolution of social behaviour. House Mouse Aggression. In: BRAIN P. et al. (eds.), Harwood Acad. Publishers: 69-86.

SOKOLOV, V.A., A.V. KOTENKOVA & S.I. LJALJUCHINA (1990). *Biologija domovoj i kurganchikovoj mysej (The biology of the house mouse and mound-building mouse)*. Moscow, Nauka, 207 pp. (In Russian).