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ATTRACTION–AVOIDANCE RESPONSE OF MOUND-BUILDING MOUSE, *MUS SPICILEGUS* IN AGROECOSYSTEMS TO CONSPECIFIC AND HETEROSPECIFIC ODOURS

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Abstract

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The behavioral responses of male mound-building mice *Mus spicilegus* to odours from males of their own species and odors from males of another species – house mouse *Mus musculus musculus* and yellow-necked mouse *Apodemus flavicollis* were studied in an attraction–avoidance test in order to clarify the role of odour in their relationships in wild populations in agricultural land. The experiments were carried out in the breeding season. *M. spicilegus* males showed attraction to conspecific odours from the same sex, but indifference to heterospecific odours of male *M. m. musculus*, and avoidance to odours of male *A. flavicollis*. The behavioural responses to conspecific and heterospecific odours are discussed in terms of attractiveness of odour cues, according to their significance in social relationships and spacing behaviour.

Key words: behavioural response, odour discrimination, olfactory cues, Mus, Apodemus, competition

Introduction

Rodents often colonise agricultural lands. In the region of North Bulgaria the mound-building mouse, *Mus spicilegus* is a species adapted to agroecosystems. At the beginning of autumn, mound-building mice build complex mounds and supply them with seeds. In these mounds, they spend the winter (Figure1). In spring, mound-building mice leave the mound and begin to reproduce in agricultural fields (Orsini et al., 1983; Milishnikov et al., 1998; Sokolov et al., 1998; Simeonovska-Nikolova, 2007a; Poteaux et al., 2008). In the region *M. spicilegus* is sympatric with *Mus musculus musculus* during the spring-autumn period (Orsini et al., 1983; Bonhomme et al., 1984; Sokolov et al., 1998). At the beginning of autumn *M. m. musculus* returns to houses, where it spends the winter. Acording to Sokolov et al. (1998) and Dobson, Baudoin (2002) from spring to autumn, mound-building mice and house mice may be found in the same habitats, but little is known about their interspecific relationships. Besides, *M. spicilegus* and wood mice *Apodemus flavicollis* and/or *Apodemus sylvaticus* are often dominant species in communities of small mammals in agroecosystems of North Bulgaria. Often wood mice are found close to mounds of *M. spicilegus*, which suggests that some eco-ethological mechanisms exist in their natural populations. A number of recent studies demonstrated that in Bulgaria *A. flavicollis* is more common than *A. sylvaticus* (Minkova and Popov, 2002; Popov, 2000, 2007, 2015). According to Peshev et al. (2004) the breeding of the yellow-necked mouse in Bulgaria occurs from March to the end of October. Responses to conspecific and heterospecific odours can provide insight into the socio-ecology of species even when little is known about the relationships of the animals in nature.

Odour communication is widespread in the animal world. With regard to small rodents olfactory cues are the most important cues used for conspecific, kin, sex, reproductive and social status recognition (Brown, 1979; Cox, 1984, 1989; Halpin, 1986; Hurst, 1989, 1990; Laukaitis et

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Fig.1. Mound of *Mus spicilegus*

al., 1997; Stopka and Macdonald, 1999; Talley et al., 2001; Musolf, 2002; Bimova et al., 2005). Individual recognition also forms the basis of territorial behaviour, identifying the individual or the group, defending resources such as mates, food or nest sites and allowing the detection of intruders and the rejection of unfamiliar animals (Brennan and Kendrick, 2006). Evidence from studies with adult rodents indicates that individual recognition enables distinctions between familiar individuals irrespective of relatedness and a separate mechanism enables discriminations based on genetic relatedness without prior familiarity (Todrank et al., 2005). Using a habituation–dishabituating procedure, Gouat et al. (1998) found that *M. spicilegus* are able to discriminate the olfactory signatures of two mound-building mouse males. Moreover, Colombelli-Negrel and Gouat (2006) demonstrated that mound-building mice are able both to perceive the change in diet and to identify the chemical signature of the donor. However, the significance of the chemical signals as mechanisms which control intra-and interspecific relationships of small mammals and contribute to their co-existence in wild populations is less studied. Furthermore, the use of wild rodents in experimental research is also very limited. In this connection, the behavioral responses of male mound-building mice *M. spicilegus* to odours from males of their own species and odours from males of another species – house mouse *M. m. musculus* and yellow-necked mouse *A. flavicollis* were studied in an attraction–avoidance test in order to clarify the role of odour in their relationships in wild populations. Having in mind that the population trend of *M. spicilegus* is decreasing (Coroiu et al., 2008), knowledge of behavioural mechanisms underlying the inter- and intraspecific spatial interaction of *M. spicilegus* could be relevant to its preservation.

Materials and Methods

Study animals and Housing Conditions

The mound-building mice were captured from two agroecosystems in the region of north Bulgaria (43°21′N, 24°15′E) and (43°19′N, 24°13′E) in late autumn of 2008 and early spring of 2009. The distance between the two locations is about 4 km. The mice were caught from mounds using Sherman live-traps, placed around mounds, near the entrance holes to the mounds. Having in mind that *M. spicilegus* is quite sedentary, occupying relatively small home ranges (Simeonovska-Nikolova, 2007a), the distance between the two locations, in which *M. spicilegus* were captured is large enough to allow the mice to know each other, i.e. they could be considered as unfamiliar. Trapping *M. spicilegus* individuals from mounds in late autumn and early spring, as well as the distance of the agroecosystem from settlements (3–4 km away from the closest village around) minimizes to a great extent the possibility of capturing the house mice *M. m. musculus,* with which the mound-building mice have morphological similarity. *M. m. musculus* were captured from rural houses in the spring of 2009. Along with this, yellow-necked mice *A. flavicollis* were captured in this period from agroecosystems in the same region, but in different locations from those of *M. spicilegus.* Thus the experimental mound-building mice had never encountered any *M. m. musculus* or *A. flavicollis* before testing. After the end of the experiments, the species determination of *A. flavicollis* was confirmed by cranial measurements using of a morphometrical key presented by Popov (1993) for discrimination between *A. sylvaticus* and *A. flavicollis* in Bulgaria.

The present experiment was carried out in the spring-summer period of 2009. Only male individuals were used. Ten male *M. spicilegus*, captured from the first agroecosystem were used as subjects and 10 males, captured from the second ones as donors. Eight male *A. flavicollis* and nine male *M. m. musculus* were used as donors. All mice used as subjects and donors were adults. The age of each animal was determined on the base of its body mass and reproductive status (Gouat at al., 2003; Simeonovska-Nikolova, 2007a; Cockel and Ruf, 2001; Zgrabszyńska and Piłacińska, 2002). The animals were individually housed in laboratory rodent cages for at least 2 weeks prior to the beginning of the experiments. Each species was placed in a separate laboratory room. The floor of each cage was covered with sawdust. The mice were maintained at natural ambient temperatures, 18–20°C and 13–14 h of light/day during the experiments. They were fed a mixed seed diet supplemented with carrots and apples, and provided with water ad libitum.

The apparatus was a tunnel T-maze, made of transparent Plexiglas (Simeonovska-Nikolova, 2007b). The initial runway ended in a T-junction (choice point) where the mouse had to turn to the left or the right in order to continue through the maze and go into the goal box. The equal construction and dimensions of the start box and the goal boxes (16.5 × 8 × 6 cm) allowed for their exchange and thus, the disturbance of the animals by handling was limited. During the habituation phase, the animal ran through the maze eight times without any stimulus present. The direction that it turned at the choice point was recorded. When left turn : right turn ratios were 4:4, 3:5 or 5:3, it was considered that the animal did not show an initial side preference and the experiment continued. The bedding material from individual cages where mice from both species had lived for at least 2 weeks was used as an odor source. The stimulus was placed in one small dish on the floor, 5 cm to the left or to the right of the junction (choice point). After the animal detected the stimulus by sniffing or touching it with its nose, the mouse was allowed to complete eight more trials. The direction which the animal turned to (toward the stimulus or to the opposite side) was recorded for these eight trails. Turn ratios of 4:4, 3:5 or 5:3 showed indifference, 2:6, 1:7, and 0:8 showed avoidance, and 6:2, 7:1, and 8:0 showed attraction. During these eight consecutive trials the same odour donor was used. To avoid any side preference, the position of the stimulus was changed for each trial. The tunnel system was placed on a glass floor, which facilitated its cleaning. The floor was cleaned between each trial. The experiments in which the mouse remained immobile in the apparatus or where it showed an initial side preference during the habituation phase were not taken into account. Tests were performed in the morning and in the evening. The research conformed to the international requirements for ethical attitude towards animals.

Data analysis

The response of mice to conspecific and heterospecific odors was assessed by subtracting the number of turns away from the stimulus from the number of turns toward the stimulus for each animal and then determining whether the mean difference for each male, female, and male–female group was significantly different from zero, using a paired t-test at $P < 0.05–0.001$. Significantly negative differences indicated avoidance of the stimulus, whilst significantly positive differences indicated attraction toward the stimulus. The insignificant differences were considered as indifference. The described method for assessing behavioral responses to olfactory stimuli used in this experiment was borrowed from other similar research on rodents (Heth and Todrank, 1995; Heth et al., 1996). The data were analyzed using Statistica version 7.0 statistical software.

Results

In the attraction-avoidance test male *M. spicilegus* showed an attraction to unfamiliar same-sex conspecific donors ($t = 5.4$, $P = 0.0004$, paired t test, $df = 9$), (Table 1).

The response of male *M. spicilegus* to odors from male *A. flavicollis* and male *M. m. musculus* was different. Male *M. spicilegus* were indifferent to odors from male *M. m. musculus* ($t = –1.35$, $P = 0.2$, paired t test, $df = 9$), but exhibited avoidance to odors from male *A. flavicollis* ($t = –2.33$, $P = 0.04$, paired t test, $df = 9$), (Table 1).

Discussion

The results in the present study showed that male *M. spicilegus* can discriminate same-sex conspecifics and herospecifics odours. Conspecific and heterospecific odor discrimination was reported in laboratory and field studies of various other rodents (Doty, 1972, 1973; Nevo et al., 1976; Stoddart, 1986; Heth et al., 1996; Christophe and Baudoin, 1998; Gouat et al., 1998; Maslak and Gouat, 2002).

Male *M. spicilegus* showed an attraction to odours of unfamiliar same-sex conspecific donors. Previous studies on the social organization of *M. spicilegus* demonstrated that

Table 1

Category of responses of male *M. spicilegus* to conspecific and heterospecific odors from the same sex in the breeding season

Subject	Donors		
M. spicilegus	*M. spicilegus*	*M. m. musculus*	*A. flavicollis*
	Attraction	Indifference	Avoidance
t	t = 5.4***	t = –1.35	t = –2.33*
$N_{attraction:indifference:avoidance}$	7 : 3 : 0	0 : 8: 2	1 : 3 : 6
Mean diff. ± SE	4.6 ± 0.85	–1.4 ± 1.03	–3.6 ± 1.54

Note: The significance of differences revealed by a paired t-test is shown: P < 0.05*,
P < 0.001***; $N_{attraction:indifference:avoidance}$ – Number of animals displaying attraction: indifference: avoidance; Mean diff. ± SE – Mean difference ± SE in the number of turns toward the stimulus minus the number of turns away from the stimulus

it is monogamous in the breeding period and both male and females individuals are aggressive to unfamiliar, but more tolerant towards familiar individuals (Simeonovska-Nikolova, 2008; Patris et al., 2002; Poteaux et al., 2008). In addition, Simeonovska-Nikolova (2012) found that male aggressiveness increased according to distance and territorial males respond less aggressively to their immediate neighbours than to distant ones. On this basis it could be suggested that the odour response of male *M. spicilegus* to odours of unfamiliar same-sex conspecific donors could be connected with the presence of intraspecific competition. It could be suggested that male *M. spicilegus* showed attraction to unfamiliar same-sex conspecific donors because they possibly accepted the latter as dispersing individuals, which searched for breeding territories. However, the interpretation of the behavioral response of male *M. spicilegus* to heterospecific odours is a little different. *M. spicilegus* and *M. m. musculus* were captured in different habitats and a possible reason the male *M. spicilegus* to be indifferent to the odour of male *M. m. musculus* is that mound-building mice had never encountered any *M. m. musculus*. However, male *M. spicilegus* showed the reaction of avoidance toward the odour of male *A. flavicollis*, which it had never experienced either. Hence, it could be considered that the odour of male *M. m. musculus* is of no significance to *M. spicilegus* males, i.e. *M. spicilegus* do not perceive *M. m. musculus* as a competitor.

Conclusion

Male *M. spicilegus* respond to same-sex conspecific and heterospecific odours in a way which seems to correspond to their social life in the breeding period. *M. spicilegus* males were indifferent to heterospecific odors of male *M. m. musculus*, which suggests that *M. spicilegus* do not perceive *M. m. musculus* as competitor. Probably the avoidance response of mound-building mice to heterospecific odours could possibly serve as a spacing mechanism, or a means to avoid aggressive encounters between syntopic species in natural habitats.

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