

SOCIOSEXUAL BEHAVIOR OF FEMALE MOUND-BUILDING MICE, *MUS SPICILEGUS*, IN A FORCED-PAIRING EXPERIMENT

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ABSTRACT

The mound-building mouse, *Mus spicilegus*, is an outdoor species from south-eastern Europe. Socio-spatial associations in the field and behavioural experiments in the laboratory reveal a monogamous mating system. To insight into the pre-mating period, which gives to females the opportunity to evaluate the quality of males, we investigate the reaction of sexually naïve females to an unfamiliar social partner and behavioural responses of females and males, who had a partner and offspring to experimentally introduced conspecific intruders of the opposite sex. Sociosexual behavior of mice was studied in two groups of pairs: 1. pairs without sexual experience and 2. sexually experienced pairs. The mice used in this study originated from a stock of wild mound-building mice captured in Northern Bulgaria and bred in captivity for three generations. Each pair was placed in glass breeding cage (50 x 50 cm). During the first 24 h after the formation of the group, the interactions between the partners were observed using 24 h video surveillance. Next, the evolution of social relationships in the groups was followed by regular observations for 1 h three times a day till the birth of their first offspring. Interactions between sexually naïve mound-building mice were mainly agonistic, ranging from high aggressiveness to defensive postures. The overcome of the aggressiveness was registered between 3 and 5 day. In the groups where aggression was high, the latency to breed was prolonged. The results showed the occurrence of sharp conflicts when the partner was undesired. Pair-bonded males and females demonstrated intense attacks toward the unfamiliar partner. As a result, two females and one male were killed during the first day. This study confirms a strong pair bond in established breeding pairs in *M. spicilegus*.

Key words: Mus spicilegus, monogamy, pair bond, sociosexual behavior, selective aggression

Introduction

The mound-building mouse, *Mus spicilegus*, is an outdoor species from south-eastern Europe. In Bulgaria the distribution of *M. spicilegus* is restricted in the north by the Balkan mountain, where it inhabits mainly agroecosystems. In the beginning of autumn, mound-building mice build complex mounds and supply them with seeds. In these mounds, they spend the winter. In spring, mound-building mice leave the mound and begin to reproduce in agricultural fields [13, 18 and 20].

Socio-spatial associations in the field [19] as well as behavioural experiments in the laboratory reveal a monogamous mating system [1, 14 and 15]. According to [2, 9, 18 and 19] in the field females are first to settle and are then joined by males originating from surrounding populations. In *M.spicilegus* reproduction does not exceed 4 mo for overwintering individuals [7 and 13]. Therefore, as mentioned [4] mate choice is crucial in this species, because the chosen partner is likely to be the only one in their short reproductive life. According to [2 and 16] previous experiments showed that females are very intolerant towards unfamiliar adult mice, whether male or female, so males must overcome the intolerance of females in order to gain access to reproduction. To insight into the pre-mating period, which gives to females the opportunity to evaluate the quality of males, we investigate the female reaction to a unfamiliar social partner. Previous field studies on socio-spatial structure of *M.spicilegus* during breeding period in the region of Northern Bulgaria demonstrated that there are a lot of single female and male individuals in the population. It is not clear if breeding pairs remain together until one member dies, or the surviving partner does not pair with a new mate. In this connection we studied behavioural responses of females and males, who had a partner and offspring to experimentally introduced conspecific intruders of the opposite sex. We expected extent of intra-individual conflict if individuals are forced to mate with a new partner.

Material and methods

The experiments were carried out with fifteen females and 15 male mound-building mice *M. spicilegus*. The mice used in this study originated from a stock of two different local populations of wild mound-building mice captured in Northern Bulgaria and bred in captivity for three generations.

Sociosexual behavior of mice - the behaviour that surrounds copulation [21], was studied in two groups of pairs: pairs without sexual experience and sexually experienced pairs as follow: 1. Pairs without sexual experience (sexually naïve) - 10 females were paired with 10 unfamiliar and unrelated partners. Mice were 8 mo old virgins, which had not previously encountered the individual with whom they were paired. The pairs were allocated at random. Males and females used originated from unrelated parents. Male mice were identified by hair clipping; 2. Sexually experienced pairs – Five couples were formed by five females who had already mated with another, but their partner had died and they lived with their first litter and five males who had a partner and offspring, but for the goal of the experiment were removed from the cage and paired with the mentioned females. Thus experimentally were formed 15 couples of unfamiliar and unrelated partners. Each pair was placed in glass breeding cage (50 x 50 cm) with free access to food and water. Cages contained sawdust bedding, nest-box and nesting material (hay). During the first 24 h after the formation of the group, the interactions between the partners were observed using 24 h video surveillance. Next, the evolution of social relationships in the groups was followed by regular observations for 1 h three times a day till the birth of their first offspring. Recordings were made using an infrared night vision camera. They started at the beginning of the dark phase. Demonstrations of attack, bite, mutual fight, boxing, chase, threat, defensive posture, and anogenital- nose- head- body sniffing, allogrooming, crawl over, crawl under, follow, side by side, and huddling together were considered. Partner preference formation was inferred when male and female were observed for a long time in side-by-side contact. Latency to breed with the allocated male was measured as the number of days between the introduction of the male and the appearance of their first offspring. Cases of death, and other changes in the groups also were registered. The significance of differences between behavioral patterns demonstrated by pairs without sexual experience and sexually experienced pairs were estimated by Mann-Whitney *U* test. The investigation conformed to international requirements for ethical attitude towards animals [11].

Results and discussion

During the first 24 h after the formation of the groups behavioural interactions between sexually naïve mound-building mice were mainly agonistic, ranging from high aggressiveness to defensive postures. Females often performed threats and defensive postures in response to approaches of males, avoiding contact with them that provoked male aggressiveness. The most frequent agonistic events observed in the groups were attacks, threats, chases, defensive postures. The amicable contacts between individuals were significantly less than agonistic ones during the first twenty-four-hour period ($U = 77.5$, Mann-Whitney *U* test, $P < 0.05$, two-tailed test), Fig. 1.

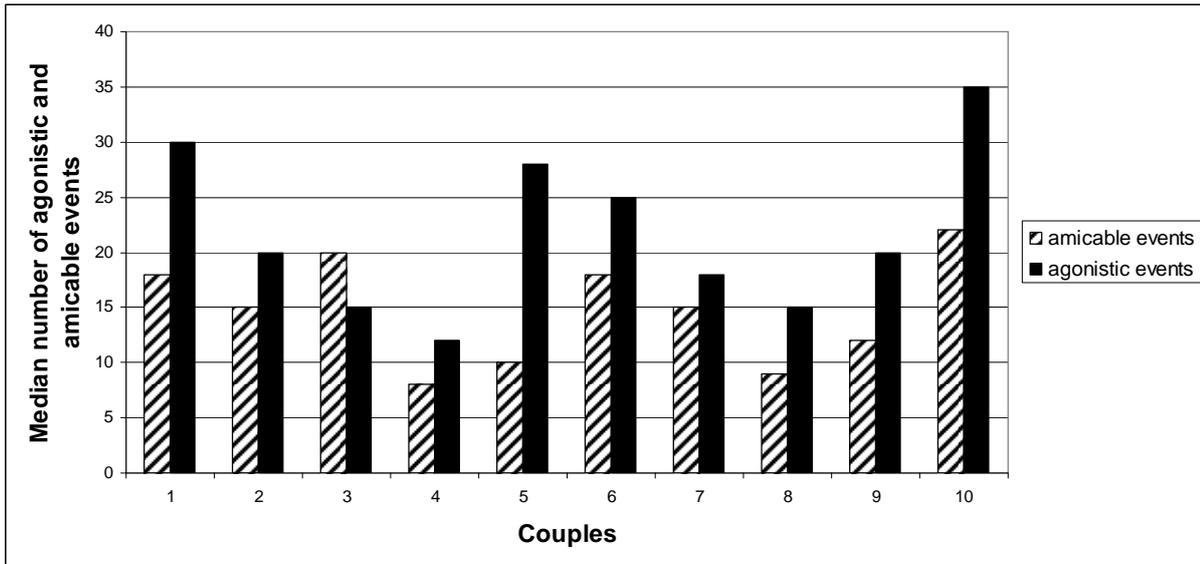


Fig 1. Median number of agonistic and amicable events recorded for 1 h in the groups of sexually naïve male and female during first twenty-four-hour period

These findings differ from those presented for other monogamous species for instance prairie vole, where sexually naïve voles are highly social and display nonselective affiliative behavior toward conspecifics [24]. Busquet et al. (2009) [4] suggest that in the field, *M. spicilegus* may need to engage in agonistic interactions so as to overcome the long-lasting social inhibition of reproduction in overwintering mounds. According to [6 and 21] events as attacks and chasing are observed frequently in rodents in similar situations and usually support mating. Our observation confirm aforementioned finding. After overcoming the aggressiveness, individuals spent a long time in side-by-side contact. In six of the groups the overcome of the aggressiveness was registered on 3 day, and the remaining groups on 4 and 5 day, respectively. According to [4] in this species gestation is 19 d long. In our experiment latency to breed with the allocated male (number of days between the introduction of the male and the appearance of their first offspring) ranged between 27 and 40 day. In the groups where aggression was high, the latency to breed was prolonged. Numbers of newborn mice in the groups varied between 3 and 5. Two couples remained infertile. Difference between pairs in latency to breed provided us with the possibility to correlate aggressive behaviour with reproduction. Moreover social stress is considered to have negative effects on reproduction [3 and 12]. It is reasonable to assume that males differed in their genetic, morphologic and physiological features, which reflect on behavioral response of females. According to [10 and 17] in a socially monogamous system free mate choice is severely constrained where most individuals are not available as social partners. Our experiment also demonstrated that in group of sexual experienced mice pairing with an unfamiliar partner elicited significantly higher agonistic behaviour than it was observed in the groups of sexual naïve mice ($U = 48$, Mann-Whitney U test, $P < 0.01$, two-tailed test). Median number of agonistic events between partners in the group of sexually experienced pairs was between 30 and 43. Pair-bonded males showed intense attack behaviors toward unfamiliar females. The females also displayed this behavioral pattern. In 3 of group fights were very persistent with strong aggression. As a result, two females and one male were killed during the first day. According to [14] postpartum oestrous females and oestrous females of *M. spicilegus* rarely copulated with the unfamiliar male. Female prefer the familiar male and to refrain from copulating with an unfamiliar male. Obviously, in result of cohabitation and mating with the previous partner, mice developed selective aggression toward unfamiliar conspecifics as it is reported for other monogamous species [22, 23 and 24]. Therefore, it could be

assumed that in mound-building mice the selective aggression also plays important role in maintaining the existing pair bond and limiting extra-pair copulations. According to [5, 8 and 24] in many monogamous species breeding pairs typically remain together until one member dies, and the surviving partner does not pair with a new mate. In the previous field studies on socio-spatial structure of *M.spicilegus* in the region of Northern Bulgaria during breeding period [19] found a lot of single female and male individuals. In this connection, the present study confirms a strong pair bond in established breeding pairs in *M. spicilegus*.

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