Neutral cage interactions in *Mus macedonicus* (Rodentia: Muridae): an aggressive mouse?

Daniel Frynta¹ & Jovana Čiháková²

¹Department of Zoology, Charles University, Viničná 7, CZ–128 44 Praha 2, Czech Republic
²Department of Parasitology, Charles University, Viničná 7, CZ–128 44 Praha 2, Czech Republic

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Abstract. Intrasexual encounters between captive wild-born mice were video-recorded and subsequently analysed. Both sexes performed highly agonistic interactions in which upright postures were dominant behavioural elements. The categories of agonistic behaviour associated with attacking, fighting or chasing were more represented in male-male interactions than in those female-female. On the other hand, females spent more time by introductory behaviour and also by amicable behaviour. In spite of the above facts, the high level of female aggression in *Mus macedonicus* should be emphasized.

Agonistic behaviour, female aggression, behavioral ecology, wild mice, *Mus macedonicus*

INTRODUCTION

*Mus macedonicus* Petrov et Ružič, 1983, is a strictly outdoor species of mouse inhabiting the south of the Balkan Peninsula (e. g., Orsini et al. 1983, Bonhomme et al. 1984, Vohralík & Sofianidou 1987, 1992), as well as the large territories of the Middle East (e. g., Kratochvíl 1986, Auffray et al. 1990, Orlov et al. 1992). Despite its large distribution area it was recognized as a distinct species first in 1980s, and therefore, only a few studies concerning the population biology (e. g., Vohralík et al. 1996) and behaviour (Frynta et al. 1992, Frynta 1994) of this species have been published.

According to our experience with captive *M. macedonicus*, they seem to be unusually aggressive. It is nearly impossible to establish a stable group consisting of a few unfamiliar individuals. Interactions of unfamiliar mice are regularly accompanied with strong violence. Due to this phenomenon, it is usually difficult even to form a new breeding pair. Our attempts to establish heterosexual pairs, e. g., 50 pairs in September 1988, resulted frequently in serious wounding or even death of the subordinate individual. Sometimes, e. g., stimulated by cage cleaning, the aggression appeared after the seemingly peaceful period.

The aims of this study were 1) to assess the level of intraspecific aggression in *M. macedonicus*, and 2) to compare behavioural patterns performed in male-male and female-female encounters. The study is part of a research program involving comparative studies of aggressive behaviour in Palaearctic murids (Frynta et al. 1995, Čiháková & Frynta 1996, Munclinger 1996). For this purpose, a simple procedure of dyadic encounters in neutral cage permitting quantification of the behavioural elements in a standardised test was adopted. This procedure is widely used in the studies of rodent behaviour, and despite some limitations (see the Discussion) it remains a useful tool for comparative studies (e. g., Montgomery 1978: *Apodemus sylvaticus* versus *A. flavicollis*, Cassaing 1990: *Mus domesticus*, *M. spretus* and *M. spicilegus*).
MATERIALS AND METHODS

Experimental animals were captured near the Krumovo village (SE Bulgaria) in September 1988. They were initially housed in heterosexual pairs. However, strong fights resulted in separation or even death of the subordinate animal. Each standard cage (42×22×22 cm) contained sawdust bedding, nesting material (hay) and shelters. Ad libitum water and food (DOS2b mouse and rat breeder diet, wheat etc.) were provided.

18 males and 8 females of *Mus macedonicus* were included in the investigations. At the time of testing, they were adult (overwintered) and sexually mature, males with testes in scrotal position. Despite their sexual maturity, females were actually neither pregnant nor lactant.

Encounters between mice were carried out in a 50×30×35 cm glass cage. The cage was divided by a thick card partition into two equal parts. During testing, the cage was illuminated by a single 40 W red light bulb suspended in the distance of approximately 1.5 metres. Mice were tested during the dark phase of their light-dark cycle (LD 14:8). At the beginning of each experimental session, two mice were placed in the pen, on the opposite sides of the partition, and left for five minutes. The central partition was then removed and video recording by a single VHS-camera started. The video camera was stopped at the end of the session, i.e., ten minutes after the moment when one or both animals paid attention to the other for the first time. After each session the cage was thoroughly cleaned using 96% ethanol.

In total, 18 male-male and 8 female-female encounters were performed. Each animal was tested with different opponents two times. Repeated testing of the same individual occurred no earlier than 24 hours after the preceding test. No effect of multiple testing was evident.

| Tab. 1. Mean duration (in seconds) of different behavioural categories. n = number of dyads. Means are calculated per encounter. Asterisks indicate significant comparisons (P<0.05) |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Females n = 8 | Males n = 18 | Mann-Whitney |
| threat-attack | 29.5 | 70.87 | 0.0225* |
| chase | 0.00 | 8.18 | 0.0323* |
| roll-over fight | 1.85 | 10.57 | 0.0083* |
| upright postures | 215.12 | 297.41 | 0.4873 |
| ambivalent | 29.06 | 49.54 | 0.1484 |
| avoid-retreat | 16.91 | 35.06 | 0.4205 |
| flee-jump-freeze | 1.65 | 13.72 | 0.0343* |
| submissive | 0.00 | 5.22 | 0.3674 |
| AGONISTIC | 323.84 | 490.54 | 0.0902 |
| (S. E.) | (67.95) | (48.52) | |
| attend | 11.04 | 25.11 | 0.0955 |
| approach | 41.55 | 38.81 | 0.5597 |
| nose | 133.69 | 43.38 | 0.0709 |
| INTRODUCTORY | 186.28 | 107.29 | 0.0486* |
| (S. E.) | (43.31) | (12.76) | |
| mutual groom | 13.23 | 0.97 | 0.0826 |
| body contact | 19.04 | 0.09 | 0.0068* |
| AMICABLE | 32.26 | 1.07 | 0.0427* |
| (S. E.) | (16.48) | (0.91) | |
| self groom | 122.46 | 123.04 | 0.7180 |
| crouch-sit | 259.55 | 162.37 | 0.1735 |
| INDIVIDUAL | 382.01 | 285.42 | 0.2323 |
| (S. E.) | (65.91) | (33.93) | |
| loco-explore | 200.03 | 223.95 | 0.4874 |
| rear-jump | 75.59 | 91.73 | 0.4204 |
| EXPLORATORY | 275.61 | 315.68 | 0.5229 |
| S. E. | (31.56) | (30.07) | |
We distinguished 33 behavioural elements adopted with minor changes from Gurnell (1977) and Montgomery (1978), for description see Čiháková & Frynta (1996), which were summarised into 17 categories (Roman numerals) and 5 functional blocks:
(A) Agonistic: (I) Threat-attack, (II) Chase, (III) Roll-over fight, (IV) Upright postures (Box, Neutral upright, Defensive upright and/or threat), (V) Ambivalent (to-from and/or tail-rattle), (VI) Avoid-recoilt, (VII) Flee-freeze (including jump-avoid), (VIII) Submissive.
(B) Introductory: (I) Attend, (II) Approach, (III) Nose.
(C) Amicable: (I) Mutual groom, (II) Body contact (i.e., lie on, crawl under/over, block)
(D) Individual: (I) Self groom, (II) Croach-sit.
(E) Exploratory: (I) Loco-explore, (II) Rear-jump.
Video records of the encounters were subsequently observed and analysed. The observed behavioural elements were quantified using the computer program package ACTIVITIES (Vrba & Donát 1993). Data on total duration of each of these elements for a particular session and animal were used as the primary data for further analysis. Duration was expressed in seconds.
The data obtained in males and females were compared using the two-tailed Mann-Whitney test.

RESULTS

Both sexes spent considerable proportion of time by agonistic behaviour (Fig. 1, Table 1). In a typical case mutual upright postures (mostly defensive) of both opponents were predominant. Also the representation of ambivalent behaviour was high. There were only few chase-flee sequences, most attacks resulted in a defence, but not fleeing. Sexual differences were not apparent. However, agonistic behaviour was the most represented functional block of behaviour in males (41% of time), whereas females spent slightly, but not significantly (P=0.09), less time (27%) by this behaviour. The situation becomes more clear when the individual behavioural categories are compared: roll-over-fight (P=0.0083), threat-attack (P=0.0225), chase (P=0.0323) and flee-jump-freeze (P=0.0343) were significantly more represented in male-male interactions than in those female-female.

Fig. 1. Percentage of time spent in different types of behaviour in male-male (right) and female-female (left) encounters of *Mus macedonicus.*
Significant sexual differences were found also in two other functional blocks of behaviour. Females spent more time by introductory behaviour (P=0.0486) and also by amicable behaviour (P=0.0427), which was poorly represented in females (3% of time), but nearly absent in males (less than 0.1%).

**DISCUSSION**

Male aggression has a considerable effect on population structure in house mice (Crowcroft & Rowe 1963, Zegeren 1980). However, in contrast to the extensive literature about commensal mice, there are only a few studies dealing with the social behaviour in European species of aboriginal mice (Cassaing & Croset 1985, Sokolov et al. 1990, Hurst et al. 1994, 1996). Comparative studies showed high variation in the level of aggression among mice species (Zegeren & Oortmersen 1981, Cassaing 1990, Munclinger 1996). It may indicate corresponding variation in social and possibly also mating systems.

Behavioural patterns displayed by male *Mus macedonicus* in our experiments suggest high level of aggression, e. g., they spent more than two times more time by agonistic behaviour, when compared with *Mus domesticus* in similar experiments performed by Munclinger (1996).

Surprisingly, not only males, but also females of *Mus macedonicus* were highly aggressive. The phenomenon of female aggression was repeatedly described in house mice (e. g., Hood 1988, Brain & Parmigiani 1990, Parmigiani & Palanza 1994). However, female aggression towards conspecifics (especially adult females) is restricted either to the lactation period and pup defence or to the cohabitation with a territorial male (Brain & Parmigiani l. c.). Therefore, most studies performed in both commensal and aboriginal species of the genus *Mus* (e. g., Cassaing 1990, Munclinger 1996) reported only low levels of female-female aggression in the neutral-cage conditions.

Considering the extreme male aggression in *Mus macedonicus*, female aggression in this species can be simply explained by the genetic correlation of this character between sexes (Hood & Cairns 1988). No matter to its cause, however, the high representation of agonistic behaviour in female-female encounters probably interacts with social and/or mating system in this species.

The relevance of simple laboratory experiments to natural situation is a matter of discussion. It is obvious that behavioural interactions are situation specific. The results of laboratory studies of aggressive behaviour are dependent upon the presence of scent marks, rearing conditions, prior experience and other environmental factors (e. g., Hood & Cairns 1989, Brain & Parmigiani 1990, Hurst 1993). On the other hand, as clearly demonstrated in laboratory mice, a considerable component of this behaviour is inherited and can be altered by an artificial selection (Cairns et al. 1983, Gariépy et al. 1988, Cairns et al. 1990).

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