

## Social interactions within and between two distant populations of house mouse

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**Abstract.** We studied agonistic behaviour of male and female house mice from two distant populations (Bohemia and Eastern Turkey) each belonging to a different subspecies (*Mus musculus musculus* and *Mus musculus domesticus* respectively). Two hundred dyadic interactions in a neutral cage were performed following a balanced design. The effects of sex, population, testing order, and their mutual interactions on the neutral cage behaviour were evaluated. In both populations, male-male interactions were more agonistic than the female-female ones. Males from Turkey (*M. m. domesticus*) were much more agonistic than *M. musculus* males from Bohemia (*M. m. musculus*). They exhibited considerably more agonistic interactions not only in male-male but also in male-female encounters. Moreover, males from Turkey were regularly the winners of both interpopulational and intersexual encounters. Interactions between mice of different populations showed that mice discriminated individuals from the other population as opponents or even sexual partners, however, females spent more time sniffing males belonging to their own population.

**Key words:** agonistic behaviour, aggression, species recognition, wild mouse, hybrid zone

### Introduction

Genetic studies have recently indicated substantial differences between species and subspecies of house mice (cf. Boursot et al. 1993). Consequently, behavioural discrimination between house mice populations has recently received considerable attention. Most effort was devoted to chromosomal races (Ganem & Searle 1996, Ganem 1998) and subspecies/species, e.g., *M. m. musculus* and *M. m. domesticus* (Laukaitis et al. 1997, Christophe & Baudoïn 1998) forming natural hybrid zones. These studies have shown behavioural discrimination between the studied forms in populations from areas adjacent to the contact (hybrid) zone.

However, our previous study failed to demonstrate discrimination between distant populations (from Turkey and Bohemia) of house mice belonging to two different subspecies. In the study we performed a set of experiments in which the tested mice were allowed to choose between odours of their conspecifics (Munclinger & Frynta 1997). The aim of this study was to test the same populations under different experimental design allowing not only olfactory communication, but also direct contact between the studied animals. We selected a standardized neutral-cage procedure in order to compare social interactions within and between studied populations. The following questions were investigated: 1, Are there any differences between the study populations and/or sexes in their social behaviour (e.g. aggression and social investigation). 2 Who is the winner of interspecific encounters? 3, Does a mouse behave differently when it interacts with a mouse of the opposite sex belonging to its own and another population?

## Material and Methods

Eighty adult, socially experienced, commensal house mice from the following two geographical regions (20 males and 20 females from each region) were used in the investigations (The mice from one geographical region are referred as one population).

1, Bohemia (Czech Republic). Wild born individuals captured in wooden live traps in different localities of central Bohemia (villages: Černošice, Soutice, Satalice) during the winter 1993-1994. They were kept in the laboratory for at least two months prior to testing. This group were *M. m. musculus*.

2, Eastern Turkey. Studied mice were adults (more than 3 months old) of the first, second and third generation born in laboratory. Two pairs of ancestral individuals were captured in the towns Van (one male and two females) and Dogubayazit (one male) in August 1992. Both localities are situated in the easternmost part of Turkey about 130 km apart. These individuals are most likely to be *M. m. domesticus*. They are long-tailed, dark coloured, commensal mice with the standard house mouse karyotype ( $2n=40$ ).

All animals were kept under an artificial 12 L: 12 D light cycle and individually housed in plastic cages 30 x 15 x 15 centimeters in size. Water and food (mouse and rat breeder diet, wheat etc.) were provided ad libitum. Each cage contained sawdust bedding, nesting material (paper) and shelters.

The encounters between mice were carried out in a 50 x 30 x 35 cm glass cage. The cage was divided into two equal parts by a thick card partition. During testing, the cage was illuminated by a single 40 W red light bulb. The mice were tested during the dark phase of their light-dark cycle. 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.

The experiments were performed in July 1993. In total, 200 dyadic encounters (20 encounters for every combination of population and sex) were performed. Dyads consisting of individuals belonging to the same and different populations are further referred to as conspecific and heterospecific, respectively. Each animal was tested with different opponents five times (i.e., with the conspecific of the opposite sex, heterospecific of the same sex, the heterospecific of the opposite sex, and twice with the conspecific of the same sex). Repeated tests on the same individual occurred at least 24 hours apart. In order to rule out possible effects of sequence bias and repeated testing the experiments followed a strictly balanced design.

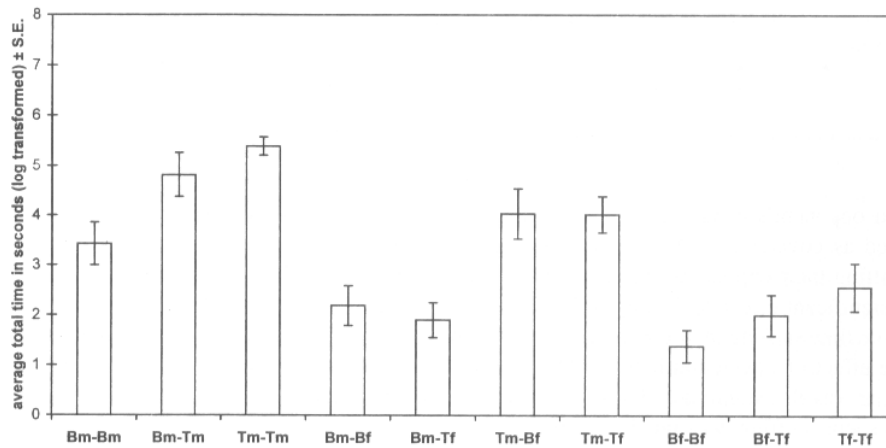
The video records of the encounters were subsequently observed, and the duration of behavioural elements was quantified using the computer program package ACTIVITIES (V r b a & D o n á t 1993). We used the standard catalogue of 34 behavioural elements for the purpose of data collection (see Č i h á k o v á & F r y n t a 1996 for details). The following three variables were derived from the primary data and used in further analyses: 1) Agonistic behaviour - total time spent by agonistic elements of behaviour (i.e., Threat, Aggressive upright, Attack, Chase, Roll-over fight, Neutral upright, Box, To-fro (repeated approach and avoidance), Tail rattling and defensive elements - see below, scores recorded in each member of a dyad were summed), 2) Defensive behaviour - sum of time intervals spent by defensive elements (i.e., Defensive upright, Defensive threat, Avoid, Retreat, Flee, Jump

avoid, Freeze, and Submissive posture), 3) Sniffing the opponent - sum of time intervals spent by sniffing or following the opponent (elements: Nose-nose, Nose-anal, Nose-body and Follow).

Statgraphics v. 5.0 was used for statistical testing. The data were either log-transformed and further treated by analyses of variance/covariance or compared by non-parametric statistics (Mann-Whitney U-test or Wilcoxon matched-pair tests).

## Results

The analysis of variance computed on log-transformed data revealed that the time spent by agonistic behaviour was considerably affected both by the Testing order ( $F = 8.4$ ,  $p < 0.0001$ ) and the Type of dyad ( $F = 13.3$ ,  $p < 0.0001$ ) (Fig. 1, Table 1). No effect of interactions of the above factors was found ( $F = 0.89$ ,  $p = 0.648$ ). Dyads of different types were further compared by Tukey tests (Table 2). This procedure revealed that: 1) Mice from Turkey spent more time in agonistic behaviour during male-male dyads than males from Bohemia, 2) Male-male dyads were more agonistic than female-female dyads of corresponding population, 3) Male-female dyads in which a male from Turkey participated were more agonistic than male-female dyads involving a male from Bohemia.



**Fig. 1.** Average total time spend by agonistic behaviour. Key: Bm - males from Bohemia, Tm - males from Turkey, Bf - females from Bohemia, Tf - females from Turkey.

Whatever the population and/or sex of their opponents was, the male mice from Turkey were the regular winners of the encounters. In matched-pair comparisons they spent less time exhibiting defensive behaviour than their opponents, i.e., male mice from Bohemia ( $Z = 3.76$ ,  $p = 0.0002$ ), females from Bohemia ( $Z = 3.26$ ,  $p = 0.001$ ), and females from Turkey ( $Z = 2.92$ ,  $p = 0.003$ ). The mice from Turkey were also the winners of the female-female encounters with the mice from Bohemia ( $Z = 2.66$ ,  $p = 0.008$ ).

In male-female interactions the time spent in sniffing the opponent was used as a measure of social investigatory behaviour. This behaviour is strongly affected by the social behaviour of the opponent. Therefore, we performed ANOVA on log transformed data in which the factors were Species of the subject, Testing order and Conspecific or Heterospecific dyad,

**Table 1.** Mean duration (in seconds) of different behavioural categories. Means are calculated per encounter. Key: Bm - males from Bohemia, Tm - males from Turkey, Bf - females from Bohemia, Tf - females from Turkey.

	Bm-Bm	Bm-Tm	Tm-Tm	Bm-Bf	Bm-Tf	Tm-Bf	Tm-Tf	Bf-Bf	Bf-Tf	Tf-Tf
Agonistic behaviour	89.9	291.1	281.5	30.7	16.6	178.8	113.1	10.8	33.5	53.7
Defensive behaviour	36.4	117.7	70.2	15.1	8.5	67.8	35.3	4.4	14.0	14.6
Sniffing the opponent	226.5	84.5	141.9	291.4	309.3	174.0	253.1	287.1	285.6	356.6

**Table 2.** Agonistic behaviour, results of Tukey tests (see text for more details). Key: Bm - males from Bohemia, Tm - males from Turkey, Bf - females from Bohemia, Tf - females from Turkey, \*  $p < 0.05$ .

Bm-Bm										
*	Bm-Tm									
	*	Tm-Tm								
	*	*	Bm-Bf							
		*	*	Bm-Tf						
			*	*	Tm-Bf					
					*	Tm-Tf				
*	*	*			*	*	Bf-Bf			
	*	*						Bf-Tf		
	*	*								Tf-Tf

and opponent's behaviour (Time spent by aggressive behaviour and Social sniffing) were used as covariates. The results showed that the females from Bohemia spent more time sniffing their opponents than the females from Turkey ( $F = 17.33$ ,  $p = 0.0001$ ). There was a significant effect of Testing order ( $F = 4.71$ ,  $p = 0.002$ ). Interestingly, the females spent more time sniffing their conspecific males than heterospecific ones ( $F = 8.57$ ,  $p = 0.005$ ), and the effect of interaction between the conspecific/heterospecific type of a dyad and Testing order was also significant ( $F = 4.71$ ,  $p = 0.002$ ). On the contrary, no effect of the above factors on social sniffing was found in males.

Despite the fact that Sexual behaviour was not recorded frequently (in six interactions only), mounting attempts were observed even in dyads involving mice from different populations. At least one copulation was recorded in each type of male-female dyads.

## Discussion

We found considerable differences between the studied populations. Male mice from Turkey were without doubt more aggressive than mice from Bohemia and this difference determined the behavioural pattern expressed in neutral cage encounters. The presence of a male from Turkey in a dyad regularly led to a highly agonistic interaction, irrespective of the sex, population and behaviour of the other participant. The above results support the idea that *M. m. domesticus* males are more aggressive than *M. m. musculus* as previously proved by authors who studied mice populations in the north-western part of Europe (Hunt & Selander 1973, Thuesen 1977, Van Zegeren & Van Oortmerssen 1981).

We found that *M. m. musculus* males from Bohemia were the losers of most male-male encounters. It supports the earlier observations that *M. m. musculus* males are not only less aggressive, but also the losers when confronted with *M. m. domesticus* males. Consequently, "peaceful" *M. m. musculus* males may be easily displaced by the more aggressive *M. m. domesticus* intruders (Zegeeren & Oortmerten 1981). Some phenomena described in a hybrid zone between *M. m. musculus* and *M. m. domesticus*, e.g., asymmetric introgression with longer tails of introgression on the *M. m. musculus* side (e.g., Boursot et al. 1993), may be explained by the above behavioural mechanism.

Female aggression is highly context specific (e.g., Brain & Parmigiani 1990, Palanza et al. 1996). As expected in neutral cage condition (Parmigiani & Palanza 1994), we found only limited aggression in female-female encounters. Nevertheless, as in males, the mice from Turkey were the winners of female-female interactions. It can be explained by genetic correlation between male and female aggression demonstrated in laboratory mice (e.g., Hood 1988, Hood & Cairns 1988).

The studied populations differed also in their females attitude to males. The females from Bohemia spent more time sniffing males. We suppose that females from Turkey are adapted to high level of male aggression and consequently minimize their direct interactions with unfamiliar males. We did not find such difference in our experiments based on olfaction (Munclinger & Frynta 1997). Females displayed low interest in odours of males in our olfactory preference tests. They did not preferred scented boxes over non-scented controls. A close proximity of a male is probably a stronger signal than scented sawdust bedding.

The occurrence of fighting and copulations between mice from different populations suggest that males recognize opponents from the other population as rivals and/or sexual partners. In contrast to results of Christophe & Baudoin (1998), no sign of subspecies discrimination was found in males. On the other hand, our data suggest that females discriminate between males of different origin. However, the discrimination did not result in a strong mating barrier and females allowed heterospecific males to copulate in laboratory experiments. Although female mice play a more active role in mate selection in natural conditions than in the laboratory (Hurst 1986), limited power of pre-mating isolation between *M. m. musculus* and *M. m. domesticus* is evident even from the occurrence of natural hybrids in all contact territories. It contrasts with stronger pre-mating isolation mechanisms between *M. musculus* and *M. spicilegus* (e.g., Kotenkova et al. 1989, Sokolov et al. 1990).

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