RELATIONS BETWEEN DISTANT POPULATIONS OF MUS MUSCULUS SENSU LATO: IS THERE ANY ODOUR-BASED DISCRIMINATION?

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Abstract

We tested the hypothesis that commensal mice behave differently when exposed to odours of males belonging to different species/subspecies. Simple choice tests were performed between House mice from Bohemia (belonging to pure Mus musculus musculus) and those from Eastern Turkey inhabiting the region of House mouse origin. No clear preferences were found. On the other hand, males of both commensal populations significantly discriminated between odours of commensal mice and controls (odour of aboriginal species or odourless substrate). The results are discussed in the context of hybrid zone processes found in commensal mice.

Key words: odour-based discrimination, species recognition, house mouse, commensal mouse

Introduction

House mice (Mus musculus sensu lato) are a proper model of speciation processes (S a g e e t al. 1993). Recently, most attention has been devoted to genetic or morphological differentiation between those closely related species (e.g., Bonhomme et al. 1986, She et al. 1990, Britton-Davidian 1990, Gerasimov et al. 1990, Boursot et al. 1993, 1996, Macholán 1996a, 1996b, 1996c, Prager et al. 1996).

According to the recognition species concept, which has been widely discussed in current evolutionary biology (P a t t e r s o n 1985, 1993), changes in recognition mechanisms may precede the genetic divergence. Therefore, there is a good reason to look for the behavioural discrimination between distant populations of rapidly evolving species such as the house mouse. Unfortunately, information about behavioural discrimination between House mice populations and/or species is still limited to studies concerning well established species. This is the case of behavioural discrimination resulting in effective precopulatory isolation mechanisms between aboriginal and commensal mice which was demonstrated repeatedly (S k o l o v e t al. 1984, 1990, K o t e n k o v a e t al. 1989, F r y n t a e t al. 1992, K o t e n k o v a 1994). Studies on behavioural discrimination between individual populations are fairly exceptional. This phenomenon was described among chromosomal races of M. domesticus in Scotland (G a n e m & S e a r l e 1996). Similarly, C o x (1984, 1989) reported strong preferences for odours of conspecifics belonging to subject’s own „deme“ in American populations of M. domesticus (when compared with odours of
neighbouring deme). Therefore, there is no doubt about abilities of commensal mice to assess population of an unfamiliar animal according to its odour. Moreover, olfactory cues are a powerful source of information enabling mice to discriminate even between genotypes (Beauchamp et al. 1988, Lennington et al. 1988, 1994, Egid & Brown 1989, Potts et al. 1991, Manning et al. 1992) and individuals with different social status (Hurst 1993). In spite of this fact, due to natural hybridisation between subspecies/species of commensal mice (e.g., between M. musculus and M. domesticus), the possibility of behavioural discrimination between these taxa has not been taken into account.

This study was performed to test behavioural discrimination based on olfaction between pure M. musculus and commensal mice from Eastern Turkey (M. cf. domesticus), i.e. mice from the territory of the Middle East in which the differentiation of commensal mice most probably started (Auffray 1990, Bourson et al. 1993). We tested responses to males odours that have been demonstrated to play a substantial role in communication among House mouse (Hurst 1990a, 1993).

Material and Methods

Eighty adult, socially experienced, commensal M. musculus sensu lato from the two following geographical regions (20 males and 20 females from each region) were included in the investigations.

Firstly, wild-born individuals were captured with the use of wooden live traps in different localities of central Bohemia (villages: Černošice, Soutice, Satalice), Czech Republic, during the winter of 1993 - 1994. The animals were maintained in the laboratory for at least two months before testing. The populations from which the test individuals were obtained belong to M. musculus sensu stricto.

Secondly, two pairs of ancestral individuals were captured in the towns Van (one male and two females) and Dogubayazit (one male) in Eastern Turkey during August 1992. Both localities are situated in the Easternmost part of Turkey about 150 km apart. Animals used in the study originate from the first, second and third generations born in laboratory. Until now a precise taxonomic determination of the studied mice has not been completely clear. Because the animals we used are long tailed commensal mice with the standard house mouse caryotype (2n = 40), they apparently belong to M. musculus sensu lato. In addition, laboratory-born M. spretus (strain SMF derived from Montpellier, France) and M. spicilegus (strain ZYP derived from animals captured in Pancevo, Yugoslavia) housed in pairs were used as odour sources only in choice tests. These strains were obtained from University of Montpellier II, France.

All animals were kept under an artificial 12 L: 12 D light cycle and individually housed in plastic cages 30 x 15 x 15 cm in size (T2 produced by FgÚ AV ČR). Ad libitum water and food (ST1 VELAZ mouse and rat breeder diet, wheat and apples) were provided. Each cage contained sawdust bedding, nesting material (paper) and plastic shelters (8 x 8 x 7 cm in size). Tests were performed during the first half of the dark phase of photoperiod.

Experiments were performed in plexiglass cages 38 x 22 x 22 cm. Two plexiglass containers (10 cm in diameter, 8 cm high, aperture 3 cm) were placed on the floor of the cage, one to the left and another to the right side (about 5 cm
from the wall). The aperture of each container was oriented to the opposite lateral side of the cage. Sawdust bedding (approximately 25 ml) from the cage in which a given male was housed was placed into containers as the odour source. It will be further referred as scented substrate.

Each tested animal from a given population and of a given sex was given a choice between two odours (obtained from animals used as odour sources). The left/right position of odours was regularly changed in a balanced design. All the procedures were performed blind.

At the beginning of each experimental session, the animal to be tested was placed in a small plexiglass transport container, transferred to an experimental cage and immediately released exactly in the centre of its floor. For the next 10 minutes, the animal was observed under red light illumination of low intensity (40 W red bulb) and the time spent in the left and/or right container was recorded using computer programme ACTIVITIES (Vrba & Donát 1993). After each session, the cage and both containers were thoroughly washed with 96% ethanol.

Three experimental sets were performed successively with each of the 80 experimental animals (20 of each population and sex). In the first set, the animals were allowed to choose between odours of males from Bohemia and Eastern Turkey. In the next two sets, we tested the choice between the odours of male from distant population (Eastern Turkey for animals from Bohemia and vice versa) and a control. Pure woodshavings were used as a control in the second set, whereas in the final (third) set, male odours of *M. spretus* or *M. spicilegus* were used.

The total amounts of time spent in containers scented with different scents were compared by non-parametric statistics (Wilcoxon test). STATGRAPHICS 5.0 was used for all statistical treatments. In order to test differences between responses to odours of *M. spretus* and *M. spicilegus*, we performed multifactor ANOVA on logit transformed time scores.

![Graph](image.png)

**Fig. 1.** Results of the first experimental set: mean time in seconds (+/- standard error) spent in containers during pair-choice tests. Responses to odours of males from Bohemia and Turkey are compared (referred as own vs distant populations). Asterisks indicate significant comparisons. Explanations: * p < 0.05, ** p < 0.01, *** p < 0.001.
Results

No apparent discrimination between odours of own population and odours of the other commensal population was found (Fig. 1, p > 0.1). The only exception was a weakly significant preference for male odours from the subject’s own population ($Z = 0.959$, $p = 0.049$) exhibited by females from the Turkey population. Males of both populations consistently preferred (Turkey $Z = 3.491$, 

Fig. 2. Results of the second experimental set: mean time in seconds (+/- standard error) spent in containers during pair-choice tests. Responses to odours of males from distant population (Bohemia or Turkey) and non-scented control are compared. Asterisks indicate significant comparisons. Explanations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 3. Results of the third experimental set: mean time in seconds (+/- standard error) spent in containers during pair-choice tests. Responses to odours of males from distant population (Bohemia or Turkey) and a control, i.e., the odour of aboriginal species ($M. spretus$ or $M. spicilegus$), are compared. Asterisks indicate significant comparisons. Explanation: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
p<0.001; Bohemia Z = 2.707, p = 0.007; Fig. 2) scented boxes over non-scented controls in the second set. No such preference was found in females (p>0.1). Animals from all experimental groups tended to spend more time in the boxes scented with male odours of aboriginal mice species than in the boxes scented with odours of the other commensal population, although this was significant only in males from Bohemia (Z = 3.117, p = 0.002; Fig. 3). No differences in response to odours of *M. spretus* and *M. spicilegus* were observed, nor for sex, recipient species and other interactions were found.

We can summarize that females unlike males generally displayed no preference for/against male odours when compared with controls. They also spent more time outside the boxes than males did (ANOVA: F = 15.6, d.f. = 1, p<0.001).

**Discussion**

In our experiments, females displayed lower interest in odours of unfamiliar males (when compared with controls) than males did. This phenomenon was also reported by Hurst (1990b). Therefore, despite a single significant comparison in the first set, only the results concerning male preferences will be discussed further.

Males of both populations behaved in a similar manner. The response to the odour of unfamiliar male was clearly demonstrated. They paid much more attention to scented than odourless boxes. At least one group of males (Bohemia population) also showed significant discrimination between odours of commensal and aboriginal species. Such discrimination has been repeatedly reported in literature (Sokolov et al. 1984, 1990, Kotenko et al. 1989, Frynta et al. 1992, Kotenkova 1994). The above authors found preferences for the subject’s own form of mice, however, our results showed the inverse preference. We suppose that this result can be interpreted in terms of novelty seeking and habituation to odours of commensal mice, which were used as stimuli in the first two sets of our experimental design.

In contrast to the above results, no signs of discrimination between odours of mice from Turkey and Bohemia were recorded. This result was surprising because the populations studied were mutually much more distant than those in which the odour-based discrimination was reported, e.g., populations of *M. domesticus* in Manitoba and Winnipeg regions (Cox 1984). However, as emphasized by Hurst et al. (1994), results obtained in an olfactometer where the tested animal is confronted with stimuli resembling a close proximity of the donor animal are not fully comparable with the results of the other odour preference tests.

On the other hand, our findings do not contradict current knowledge about patterns of isolation between commensal mice, i.e. in contrast to the obvious difference between behavioural responses to odours of aboriginal and commensal mice, there is no discrimination between two commensal forms. The absence of discrimination between these forms is in accordance with the ineffective precopulatory isolation manifested by the considerable gene flow through the hybrid zones between individual forms of commensal mice (Boursot et al. 1993, Tucker et al. 1992).
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**LITERATURE**


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