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Olfactory preferences in two strains of wild mice, *Mus musculus musculus* and *Mus musculus domesticus*, and their hybrids

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ABSTRACT

We studied olfactory preferences of two strains of mice, *Mus musculus musculus* and *Mus musculus domesticus* (considered here to be subspecies), and their hybrids, to examine the possible role of odours as a behavioural, premating mechanism that could explain the characteristics of their natural hybrid zone. We used a choice test with the bedding material of animals of the opposite sex from the animal tested and from both subspecies. Male and female *M. m. domesticus* showed no preference either for their own subspecies' odours or for the other subspecies' odours. In contrast, *M. m. musculus* individuals and three types of hybrids (all the female hybrids and males from crosses between an *M. m. musculus* female and an *M. m. domesticus* male) sniffed for longer at materials from the *musculus* source than from the *domesticus* source. We interpreted the results as a preference for *musculus* odours. Differences between the two subspecies in their response towards consubspecific and heterosubspecific odours could explain the asymmetrical introgression observed in the hybrid zone.

In mammals, olfaction is now considered as one of the most important means of communication (Brown 1979). In rodents, in particular, it is well known that chemical signals play a role in social interactions (Brown 1985; Hurst 1989), territorial exclusion (Nyby et al. 1970; Hurst 1990), and mate selection (Mainardi et al. 1965; Lenington 1983). An animal can use odours to distinguish between members of its own species and a related species (Moore 1965; Doty 1973) and individuals of some species show a preferential response to odours of their own subspecies (Müller-Schwarze 1975).

We studied two strains of wild mice, *Mus musculus musculus* and *Mus musculus domesticus*, considered here to be subspecies as they present a continuum of natural interbreeding populations (Boursot et al. 1993). This taxonomic approach is also based on a molecular phylogeny, which uses several mitochondrial and nuclear DNA markers and combines different techniques (She et al. 1990). Some authors (Sage et al. 1993) still follow another approach and give species status to these two strains, based on more global genetic criteria. *Mus m. musculus* and *M. m. domesticus* interact along a natural hybrid zone (Hunt & Selander 1973; Thaler et al. 1981) that extends from Denmark to Bulgaria. Although an asymmetrical introgression is observed, this zone is relatively stable. Some traces of *M. m. domesticus* alleles can be found over a distance of at least 150 km from the contact point whereas the width of the cline of introgression for autosomal genes is limited to 40 km (Vanlerberghe et al. 1986).

The dynamics of this hybrid zone could be explained by different mechanisms such as genetic interactions (hybrid sterility) and/or precopulatory mechanisms against or in favour of these exchanges. Mate choice is one of these mechanisms and it is an important source of genetic exchange. Olfactory preferences may represent a premating, behavioural, sexual-isolating mechanism, and early olfactory experience may be a significant factor in the maintenance of species isolation when closely related species are sympatric (D'Udine & Alleva 1983).

In general, adult males and females are attracted to the odours of animals of the opposite sex (Rowe 1970; Keevin et al. 1981; Ferkin & Johnston 1995) and in some rodents, including mice, preference for the smells of other animals correlates well with mating preference (Yanaï & McClearn 1973; Huck & Banks 1980a, b; Newman & Halpin 1988; Egid & Brown 1989; Krackow & Matuschak 1991). This preference can be modified by kinship and familiarity (Barnard & Fitzsimons 1988), social status (Hayashi 1990; Drickamer 1992) and sociosexual experience (Albonetti & D'Udine 1986). Mice can distinguish urinary odours

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associated with major histocompatibility complex (MHC) haplotypes (H-2). Strains that are genetically identical, except for loci within the H-2, are usually used to test for odour preferences. Experiments with the CHR and the GBA congenic strains showed that female mice prefer the MHC dissimilar odour and tend to mate with males of a different MHC type to their own (Egid & Brown 1989). In contrast, no preference was shown by the males of these strains (Eklund et al. 1991).

In the present study we investigated the preferences of the two *Mus musculus* subspecies and their hybrids for odours of the two strains. Our aim was to examine the possible role of olfactory cues in mate choice and their influence on the characteristics of the hybrid zone. We used a choice test based on olfaction to find out if the two subspecies show a preference for their own strain and if hybrids show an intermediate pattern of response.

METHODS

Experimental Animals

The mice used were 16th and 17th generations from a wild *M. musculus domesticus* strain trapped in Denmark (Odis), and 7th–9th generation mice from a wild *M. musculus musculus* strain also from Denmark (Hov). We used first-generation hybrids between the two subspecies born in the laboratory. We noted hybrids as FdMm for crosses between an *M. m. domesticus* female and an *M. m. musculus* male and FmMd for crosses between an *M. m. musculus* female and an *M. m. domesticus* male.

Mice were reared by both parents until weaning at 21 days of age and males and females were kept separately in cages measuring 26 × 16 × 14 cm. Food (mice pellets, U.A.R. type AO4) and drink were provided ad libitum. The room was kept under constant conditions at 21–24°C, 35–40% relative humidity and a 14:10 h light:dark cycle (light on at 2200 hours and off at noon). Animals were isolated 15 days before testing. They were 3–5 months old and all were sexually naive. To avoid stressing the females, we did not control the stage of oestrus. Our results are expressed as individual means for 3 successive days of experiments, so include the behavioural variability shown during the sexual cycle. To prevent aggressive behaviour, we never rehoused males together after the experiments, but females sometimes were in the case of sisters. In general, tested animals were not kept isolated for long and were used for reproduction in the laboratory.

We used 10 *M. m. domesticus* males (mean body mass ± SD=22.28 ± 1.78 g) and nine females (18.61 ± 1.09 g), and nine *M. m. musculus* males (19.5 ± 1.43 g) and nine females (16.7 ± 1.93 g). We tested 10 FdMm males (24.78 ± 1.76 g) and 10 females (19.10 ± 1.98 g) and nine FmMd males (26.66 ± 1.84 g) and nine females (19.54 ± 1.59 g).

Materials

We carried out preference tests in a square central box (24 × 24 × 8.5 cm) which was connected to four boxes (20 × 15 × 7.5 cm) with glass tubes (10 cm long × 3.2 cm in diameter) at the middle of each side. Each of the peripheral boxes contained a petri dish (9 cm in diameter × 12 mm deep) with clean bedding material in two of them and soiled bedding from animals from each subspecies in the other two.

Test Procedure

We tested each mouse once daily for 3 days, during the dark phase (under dim red illumination). We placed the mouse in a nestbox under the central box and opened a trap door which led to the central box. After the mouse entered the central box, we closed the trap door and recorded its behaviour immediately for 15 min. We recorded with a Psion Organizer the time (s) spent sniffing for odours. The subject was considered sniffing when it sniffed intensely at the petri dishes with its nose in contact with or close to the soiled bedding.

The soiled bedding originated from individual cages where mice had lived for 2 weeks. Soiled bedding from different individuals was pooled and kept in a freezer (−15°C); it was defrosted 15 min before testing (ambient temperature). Each individual had the choice between two pools: one '*musculus*' and one '*domesticus*' from animals of the opposite sex (unrelated and unfamiliar with the test animal). The relative position of the two odour sources was reversed in successive tests. Between each test, we cleaned the apparatus with bleach and wiped it with 70% alcohol.

By using soiled bedding we tested only odour cues. There were no confounding factors such as interaction between individuals. Soiled bedding represents the entire odoriferous register of an animal; shavings, for example, are sniffed for longer than urine and faeces (Newman & Halpin 1988).

To avoid the effect of an individual odour source, we used pools for each subspecies. In this way, we tested preference for a subspecies odour and not for an individual odour. Moreover, by using odours from animals of the opposite sex, we tested not only a social, but also a sexual preference (see Introduction).

Statistical Analysis

We added together the data from the 3 days for each mouse, so each individual had a mean sniffing duration for the 3 days. To test for differences between the mean time spent sniffing at each petri dish, we used Wilcoxon signed-ranks tests. To compare males and females we used a Mann–Whitney *U* test.

RESULTS

The mice were attracted by the odour sources as they either did not sniff at the neutral ones or did so for just a few seconds (not exceeding a total of 7 s). Males, in general, spent more time sniffing at odour sources than females. In *M. m. domesticus* there was a significant difference as the total time ($\bar{X}$ ± SE) spent sniffing for males

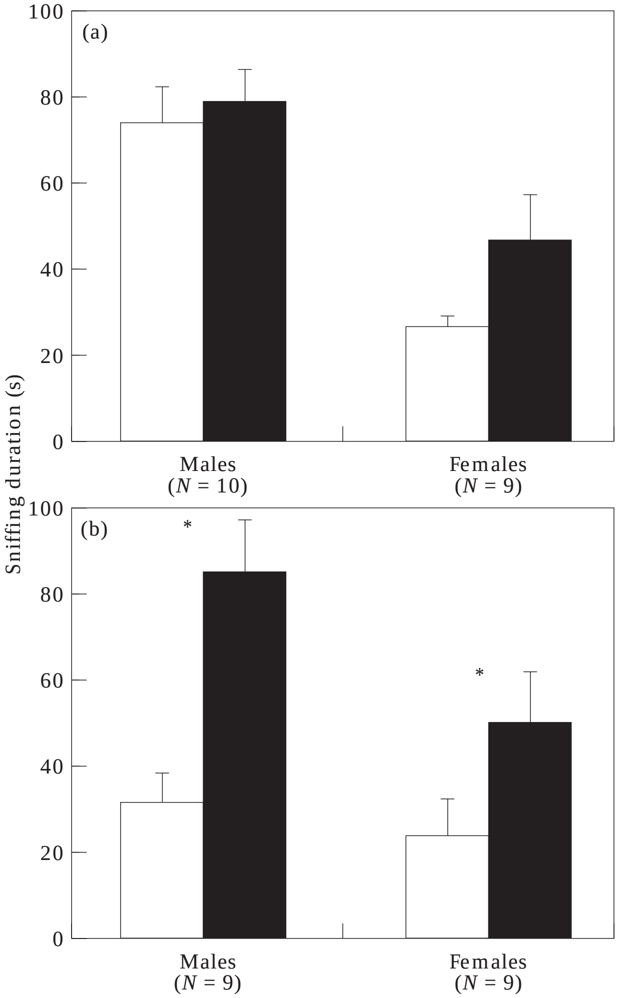

Figure 1. Time spent ($\bar{X}$±SE) by (a) *M. m. domesticus* and (b) *M. m. musculus* males and females sniffing at soiled bedding from *M. m. domesticus* (☐) and *M. m. musculus* (■) mice. *$P<0.01$, Wilcoxon signed-ranks test.

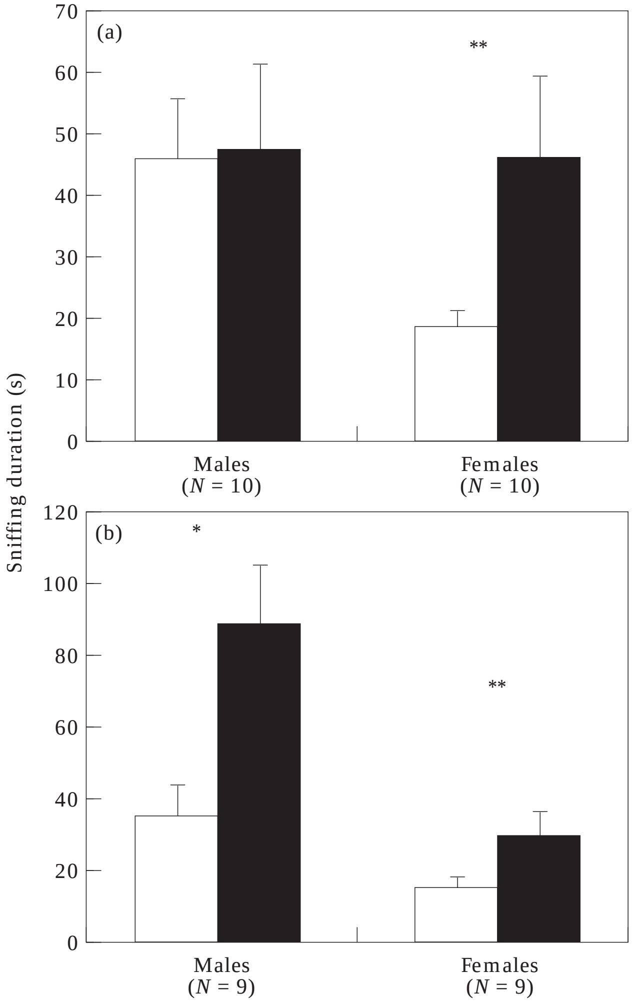

Figure 2. Time spent ($\bar{X}$±SE) by (a) FdMm hybrids and (b) FmMd hybrids sniffing at soiled bedding from *M. m. domesticus* (☐) and *M. m. musculus* (■) mice. *$P<0.05$; **$P<0.01$; Wilcoxon signed-ranks test.

was 156.38 ± 9.46 s and for females 74.18 ± 13.36 s (Mann–Whitney U test: $U=5$, $N_1=10$, $N_2=9$, $P=0.001$). There was no significant difference in *M. m. musculus* between males (116.7 ± 18.1 s) and females (74 ± 20.1 s; $U=22$, $N_1=N_2=9$, $P=0.10$). In hybrids FdMm no significant difference was observed between males (93.7 ± 21.7 s) and females (65.6 ± 14.6 s; $U=37$, $N_1=N_2=10$, $P=0.33$) and in hybrids FmMd males sniffed for longer (125.7 ± 19.7 s) than did females (46.99 ± 7.72 s; $U=9$, $N_1=N_2=9$, $P<0.01$).

Olfactory Preference of *M. m. domesticus*

Mus m. domesticus males and females did not spend different amounts of time sniffing at the two odour sources (Fig. 1a): males spent 74.6 ± 8.31 s sniffing at the *domesticus* source and 79.3 ± 8.04 s at the *musculus* one (Wilcoxon signed-ranks test: $T=19$, $N=10$, $P=0.43$) and females 26.8 ± 3.83 s and 47.33 ± 10.9 s, respectively ($T=7$, $N=9$, $P=0.074$). Hence, there was no preference for odours of one subspecies or the other.

Olfactory Preference of *M. m. musculus*

Males sniffed for longer at *M. m. musculus* female bedding material (85.6 ± 11.9 s) than at *domesticus* female odours (31.2 ± 7.01 s; Wilcoxon signed-ranks test: $T=0$, $N=9$, $P<0.01$; Fig. 1b). Females also sniffed for longer at *musculus* male odours (50.4 ± 12.2 s) than at *domesticus* ones (23.6 ± 8.58 s; $T=0$, $N=9$, $P<0.01$; Fig. 1b). *Mus m. musculus* individuals thus preferred the odours of individuals of the opposite sex from their own subspecies.

Olfactory Preference of Hybrids FdMm

Males spent the same time sniffing at *musculus* (47.8 ± 14.3 s) and *domesticus* bedding material (46 ± 9.72 s; Fig. 2a; $T=30$, $N=10$, $P=0.85$). Females, however, spent more time sniffing at *musculus* dishes (46.7 ± 13.44 s) than at *domesticus* ones (19 ± 2.81 s; $T=2$, $N=10$, $P<0.01$; Fig. 2a). Hence only the females showed a preference for an odour source and this was for the *musculus* one.

Olfactory Preference of Hybrids FmMd

Males sniffed for longer at *musculus* female bedding material (89.6 ± 16.95 s) than at *domesticus* bedding material (36.1 ± 8.37 s; T=3, N=9, P=0.02; Fig. 2b). Females also preferred *musculus* sources (30.8 ± 6.64 s) to *domesticus* ones (16.2 ± 2.94 s; T=0, N=9, P<0.01; Fig. 2b). The two sexes of FmMd hybrids thus both preferred the *musculus* odour.

DISCUSSION

This study shows the differential responses of the two subspecies towards conspecific odours. *Mus m. musculus* individuals showed a conservative pattern of choice, preferring the smell of their own subspecies, whereas *M. m. domesticus* individuals did not show any preference. Some authors have observed similar differences between laboratory strains of mice. D'Udine & Partridge (1981) tested the preferences of the two strains C57 and SEC: SEC males and females preferred the smell of SEC to the smell of C57 whereas C57 males and females showed no significant preference.

In the present study *M. m. domesticus* individuals did not prefer either odour source, and they seemed to be equally interested in the two subspecies' odours. One might argue that they were not able to distinguish between the two odour sources; however, another study based on a paradigm of habituation-discrimination (unpublished data) showed that *M. m. domesticus* males and females do discriminate between the two subspecies' odours. So they were able to make a choice but they simply did not show any preference. This is of interest if we consider that *M. m. domesticus* alleles were found far into the range of *M. m. musculus*. So, their lack of preference for an odour could allow them to copulate with individuals from both subspecies.

In contrast, *M. m. musculus* males and females significantly preferred their own subspecies' odour as they spent more time sniffing at *musculus* dishes. This attraction could also be associated with an avoidance of the heterosubspecific odour. For example, *M. m. musculus* individuals that have cohabited with midday jirds, *Meriones meridianus*, avoid the jird's odour when it is paired with a clean stimulus (Krasnov & Khokhlova 1996). This avoidance is correlated with cohabitation between the two species and the fact that jirds are considered as dominant competitors. We presented the two odour sources simultaneously, which did not permit us to distinguish clearly between avoidance of the heterosubspecific odour and indifference to it, but the experiment clearly demonstrated a preference for the *musculus* odour as the mouse had the possibility of going into neutral zones.

The differential response of *M. m. musculus* individuals towards the two odour sources may play a role in the maintenance of relative reproductive isolation. We can hypothesize that male and female *musculus* individuals show a sexual preference for partners from their own sub-species, remain in their territories and avoid those belonging to *domesticus* mice. The role of olfactory cues as in ethological isolating mechanisms has already been demonstrated in lemmings by Huck & Banks (1980a, b) and in mice by Bowers & Alexander (1967) and Cox (1984).

In hybrids, we observed a preference for the smell of *M. m. musculus* individuals except for FdMm males which did not show any preference. This preference for *musculus* partners could also contribute to the dilution of *M. m. musculus* alleles, by favouring crosses between hybrids and *musculus* individuals.

One might argue that preference for *musculus* odours could have been just a response to a stronger odour, but we used, for the two sources, reinforced odours from 2-week-old soiled bedding. So both signals were strong and, as not all the animals preferred the *musculus* odour, we cannot say that this source is a stronger signal than the other.

The genetic origin of an individual and the presence of odours during and before the weaning period are two parameters that could influence adult olfactory preferences. Both these factors are involved here since we used 'pure' individuals of the two subspecies that were reared by their two parents, and two types of hybrids that were reared by a father and a mother from different subspecies.

Individual experience before weaning plays a role in olfactory preference and the development of adult sexual preferences. Early olfactory experience could come from the parental environment, the mother in most cases but sometimes both the mother and the father. Littermates could also be a source of olfactory experience. Huck & Banks (1980a), by using cross-fostering, showed the central role the mother plays in the development of species-typical olfactory preferences in lemmings, with a stronger effect on young males.

Mainardi (1963a, b) showed, in constrast, the role of the father in the development of olfactory preference in females of two subspecies of mice, *Mus musculus domesticus* (he used two different strains) and *Mus musculus bactrianus*. Female *M. m. domesticus* reared with their mother and father preferred to mate with males of strains different from their own but they preferred a male of the same subspecies when they had to choose between males of the two subspecies. These preferences did not appear if the females were reared in the absence of their father. Other studies (Mainardi et al. 1965; Alleva et al. 1981), using artificially scented parents during the weaning period, have also shown the role of early olfactory experience in the future sexual preference of the offspring.

In our experiment, animals were reared by both parents. It seemed that an *M. m. musculus* mother and father induced a preference for *musculus* odours and in the case of hybrids, with an intermediate genetic pattern, one parent of the subspecies *musculus* was enough to induce the preference, except in the case of the FdMm hybrid males. In contrast, two *M. m. domesticus* parents did not induce a preference for the *domesticus* subspecies.

These results have to be confirmed by cross-fostering experiments between the two subspecies, in order to draw conclusions about the respective roles of genetics and early learning in the differential responses to odours observed. It seems, in any case, that these differences favour the extension of the *domesticus* range into that of *musculus* as genetic studies on the hybrid zone have led us to expect (see Introduction).

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