Aggression in reciprocal crosses of two subspecies of wild house mouse

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A b s t r a c t . We studied reciprocal hybrids of *Mus musculus musculus* and *M. m. domesticus*. These two subspecies of house mouse were found to differ in their social behaviour, the former being less aggressive than the latter. The paternal effect on aggression (observed repeatedly in laboratory mice) was not found. However, F_2 generation mice were less aggressive than those from the F_1 generation, and the maternal effect was also significant in a homogenous test set.

Introduction

Aggression is an important component of mouse social behaviour. The level of aggression is species specific and may vary, even between populations or strains (B r a i n & P a r m i g i a n i 1990, G a n e m & S e a r l e 1996, S l u y t e r et al. 1996b). At least in part, these differences are determined by genotype, as has been made clear from model experiments on laboratory mice (C a i r n s et al. 1983, S l u y t e r et al. 1996b). It has been repeatedly demonstrated that artificial selection has a strong and immediate effect on measures of aggression recorded in standardised tests (H o o d 1988a, H o o d 1988b, S l u y t e r et al. 1996b). Special attention has been paid to paternal effects, which have been repeatedly reported in studies quantifying mouse aggression. In their study dealing with reciprocal crosses of strains DBA/1/Bg and C57BL/10/Bg, S e l m a n o f f et al. (1975) hypothesised that the Y chromosome was responsible for the variation observed in mouse aggression. Y chromosome correlates on aggression were subsequently shown in several inbred strains and in lines bidirectionally selected for attack latency (see review by C a r l i e r et al. 1990). Moreover, some comparisons of congenic strains confirmed the Y chromosome effect on aggression - usually the more aggressive hybrid has the Y of the more aggressive strain (G u i l l o t et al. 1995, C a r l i e r et al. 1990, M o h a n a n & M a x s o n 1998).

Clear, fixed Y chromosome differences have been found between the subspecies *Mus musculus musculus* L., 1758 and *Mus musculus domesticus* Schwarz & Schwarz, 1943 (B o i s s i n o t & B o u r s o t 1997) and it is of interest that these subspecies also differ in their social behaviour. The phenomenon whereby *M. m. domesticus* males are more aggressive than those of *M. m. musculus* has been shown by authors studying mouse populations from the north-western part of Europe (T h u e s e n 1977, Z e g e r e n & O o r t m e r s e n 1981). We have also demonstrated the same phenomenon by comparing the social behaviour of house mice from two distant populations (i.e. Bohemia and eastern Turkey), each of which belong to different subspecies (M u n c l i n g e r & F r y n t a 2000). Male mice from Turkey (*M. m. domesticus*) were undoubtedly more aggressive than Bohemian mice (*M. m. musculus*).

In this paper we have attempted to demonstrate the differences between reciprocal F_1 males from crosses of two subspecies of wild house mouse to prove the paternal effect on aggression; this is the obligatory first step for testing the Y chromosome effect. In addition, we also compared their descendants (F_2 hybrids) to randomise the effects of autosomal genes.

Material and Methods

Parental generation mice were born in the laboratory (about 4th - 6th out-bred generation in captivity). *Mus m. musculus* stock were derived from 20 males and 20 females captured in the Bohemian villages of Černošice, Soutice and Satalice (Czech Republic) during winter 1993-1994. *Mus m. domesticus* were descendants of eight pairs of mice captured in the vicinity of the town of Kilkis, Kilkis District (Greece) in May 1995. Reciprocal hybrids of the above populations were used as experimental subjects. Twenty males of each combination of generation (F_1 and F_2) and cross direction, i.e. female *M. m. domesticus* x male *M. m. musculus* (hereafter MdMmF$_1$ males) and female *M. m. musculus* x male *M. m. domesticus* (hereafter MmMdF$_1$ males) were used. Experimental mice were adult (i.e. at least two months old), socially experienced males housed in heterosexual pairs (i.e. together with an adult female of the identical filial generation and cross direction).

All animals were kept under an artificial 12 L: 12 D light cycle and housed in pairs in 30 x 15 x 15 cm plastic cages. Water and food (ST1 mouse and rat breeder diet, wheat etc.) were provided ad libitum. Each cage contained sawdust bedding, nesting material (paper) and shelters.

The experiments were performed in January-March 1999. A standard neutral cage procedure was used. Encounters between mice were carried out in a 50 x 30 x 35 cm glass cage, divided into two equal parts by a thick card partition. During testing, the cage was illuminated by a single 40 W red light bulb. 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 commenced. The video camera was stopped at the end of the session (ten minutes after the moment at which one or both animals paid attention to the other for the first time). The cage was thoroughly cleaned using 96% ethanol after each session. Repeated tests on the same individual were undertaken at a minimum interval of 24 hours.

In the first set we tested mice in homogenous dyads, i.e. with opponents of the same generation and cross direction. In this set 120 dyadic encounters were staged (i.e. 30 encounters for every combination of generation and cross direction). Each animal was tested with different opponents three times.

In the second set, the same individuals were tested in heterogeneous dyads consisting of individuals belonging to the same generation, but different cross direction. 20 and 17 heterogeneous dyads were performed on the F_1 and F_2 generations, respectively.

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). S e l m a n o f f et al. (1976) and M a x s o n et al. (1979) suggested the "any aggression" score as being a more robust and reliable index of fighting behaviour than any single

agonistic act. Therefore, only the following four variables were derived from the primary data and used in further analyses:

1. Aggressive behaviour – the sum of the time intervals spent in the following elements of aggressive behaviour: Threat, Aggressive upright, Attack, Chase, Roll-over fight.

2. Defensive behaviour – the sum of the time intervals spent by defensive elements: Defensive upright, Defensive threat, Avoid, Retreat, Flee, Jump avoid, Freeze, Submissive posture.

3. Agonistic behaviour – the total time spent in aggressive, defensive, and neutral (Neutral upright, Box, To-fro - repeated approach and avoidance, Tail rattling) elements of behaviour. We believe this variable is the best indicator of hostile motivation in interacting mice. This well-defined and continuous variable shows nearly log-normal distribution, so we used it as first choice in our testing.

4. Attack latency – latency of the first attack expressed in seconds. This variable was highly correlated with time spent in agonistic behaviour (Spearman's $r = -0.78$, $n = 157$).

The behaviour of both interacting animals in a particular dyad is obviously intercorrelated. Thus, in the first set of experiments dealing with comparisons of homogenous dyads we summed the scores of agonistic and aggressive behaviour recorded in both members of a single dyad. These were log-transformed and further treated by analyses of variance/covariance (Statgraphics v. 5.0). The scores for defensive behaviour as well as attack latency were treated by non-parametric statistics. Defensive behaviour was used solely for comparisons within heterogeneous dyads and analysed with Wilcoxon matched-pair tests.

Results

The mean time spent in agonistic behaviour in the first set of experiments is shown in Fig. 1. The proportion of time spent in agonistic behaviour by MdMmF$_1$ males (F$_1$ generation = 22%, F$_2$ = 12%) was higher than in MmMdF$_1$ males (F$_1$ generation = 11%, F$_2$ = 4%).
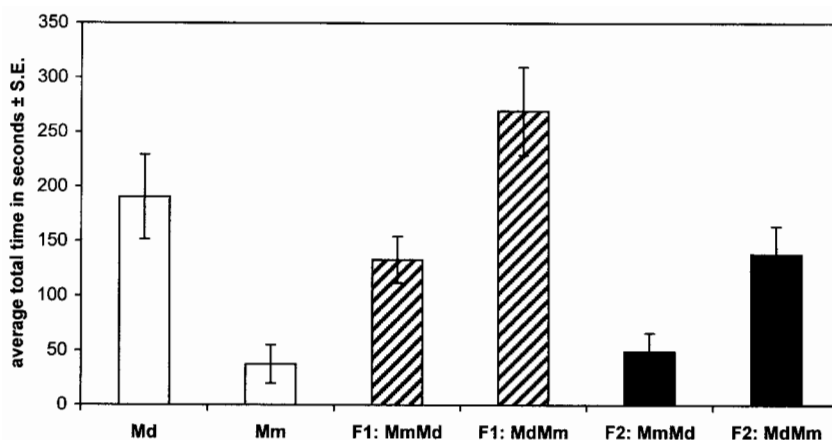

Fig.1. Average total time spend in agonistic behaviour by homogenous dyads. The results of parental population tests are shown for comparison. Key: Md – *M. m. domesticus* males, Mm - *M. m. musculus* males, F1: MdMm, F1: MmMd, F2: MdMm, F2: MmMd MdMm – different generations and types of hybrids, see Material and Methods for more details.

Analysis of variance, as controlled for body weight (covariate F = 8.59, p = 0.0041), revealed significant differences in the time spent in agonistic behaviour between reciprocal hybrids (F = 18.27, p < 0.0001) and between the F_1 and F_2 generations (F = 6.51, p = 0.0121). The effect of interaction between the above factors was also important (F = 5.63, p = 0.0193). The differences between reciprocal hybrids were also supported when aggressive behaviour was treated separately (F = 3.99, p = 0.0482).

Attack latency was not affected by the cross direction in the F_1 generation (Mann-Whitney test: z = -1.29, p = 0.1977) and only a marginal effect was found in the F_2 (z = -2.05, p = 0.0401). Attack latency was considerably longer in both $MmMdF_1$ and $MdMmF_1$ hybrids in the F_2 than in the F_1 generation (cross direction pooled: z = -3.84, p = 0.0001; average rank = 72.0 and 49.0, respectively).

Unlike homogenous set tests, further experiments involving both reciprocal hybrids in a single dyad enabled matched pair comparisons. No effect of reciprocal hybridisation was found, either on the time spent in defensive behaviour (Wilcoxon matched-pair tests: z = 0.6185, p = 0.5362, n = 37 dyads, F_1 and F_2 generations pooled) or on attack latency (z = 0.28, p = 0.7776). The F_1 generation also remained more agonistic than the F_2 generation in this experiment (ANOVA: F = 5.79, p = 0.0215).

Discussion

We did not find any paternal effect on agonistic behaviour in male mice; moreover, we found a significant maternal effect. Surprisingly, the more aggressive hybrid did not carry the Y chromosome of the more aggressive subspecies.

Studies carried out on laboratory mice have not led to consensus on how the Y chromosome determines the level of aggression in males. R o u b e r t o u x et al. (1994) failed to demonstrate the effect of the non-pseudoautosomal region of the Y on mouse aggression. Other studies have suggested influences from the pseudoautosomal region, genetic background and the maternal environment (C a r l i e r et al. 1991, R o u b e r t o u x et al. 1994). However, there is some evidence that artificial selection for male aggression leads to a correlated response in female aggression (H o o d & C a i r n s 1988). This finding provides indirect evidence for the effect of genes not located on the Y chromosome, because the Y chromosome is absent in females.

To simulate natural conditions we kept mice in pairs, and the offspring had equal chances to be influenced by their fathers and their mothers. In spite of this, we found a significant maternal effect in the first set of experiments. We have no cross-fostering data to demonstrate that the differences between reciprocal hybrids are of genetic nature, and therefore prenatal and/or postnatal environmental effect cannot be excluded. Such environmental effects were previously revealed by studies of laboratory mice (C a r l i e r et al. 1991, but see S l u y t e r et al. 1995, 1996a).

It should be noted that aggression is a context-specific phenomenon (e.g. S u c h o m e l o v á & F r y n t a 2000) and therefore details of experimental procedure can considerably influence results. In order to reduce stress, we studied mice in a standard neutral cage environment, i.e. outside the context of territoriality in which mice fight more seriously (G r a y & H u r s t 1995), and we avoided the use of inbred laboratory mice as standard opponents. We suppose that this design is simply not applicable in wild mouse studies. Moreover, G u i l l o t et al. (1995) showed that the standard opponent test is less

sensitive to parental (Y chromosome) effects than homogenous set tests. Nevertheless, social behaviour is determined by both interacting animals, so it is not surprising that the results of our first and second set were not the same. Unlike the total time spent in agonistic behaviour, attack latency was found to be only slightly effected by cross direction – even though these two variables were highly correlated. This may suggest that the total time spent in agonistic behaviour is a more sensitive indicator of hostility than attack latency is.

Despite differences between reciprocal hybrids, all the F_1 hybrids were highly aggressive and their mean scores for agonistic behaviour (201 s) even exceeded those previously recorded in the more aggressive (*M. m. domesticus*) parental population (191 s). We suggest that the fact that the F_1 hybrids consistently exhibited more agonistic behaviour than the F_2 in both experimental sets may be explained by recombination of the loci affecting agonistic behaviour.

We conclude that paternal effects are not easily demonstrable in our wild mice crosses. The differences observed between reciprocal hybrids, as well as between F_1 and F_2 generations, in their social behaviour cannot be attributed to the Y chromosome as a single factor, and the possible effects of genes on other chromosomes, hybrid advantage, and even non-genetic maternal effects cannot be rejected.

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