



## The meek inherit the earth: less aggressive wild mice are more successful in challenging situations

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Numerous studies have shown an association between aggressiveness and several other behavioural traits. For example, more aggressive animals were bold and active explorers tending to form persistent routines whereas less aggressive animals were shy, careful but more flexible. While the former are thought to be more successful under stable conditions the latter should have advantages in more dynamic situations. These differences can apply not only to individuals but also to populations, species or groups of species with important implications to species distributions and speciation rates. Here we utilized the Morris water task (MWT) to investigate how two subspecies, *Mus musculus musculus* and *M. m. domesticus*, known to differ in aggressiveness, cope with stressful situations. We found that less aggressive *musculus* males performed significantly better in solving the MWT than more aggressive *domesticus* males. This suggests that *M. m. musculus* is more flexible and could be more successful under stressful and/or dynamic situations typical of dispersal bouts. It seems plausible that this difference may have had an influence on the secondary contact between *musculus* and *domesticus* populations in the past and perhaps still can affect the dynamics of the European hybrid zone between the subspecies. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **113**, 310–319.

**ADDITIONAL KEYWORDS:** behavioural strategies – dispersal – house mouse – Morris water task – *Mus musculus domesticus* – *Mus musculus musculus* – stress.

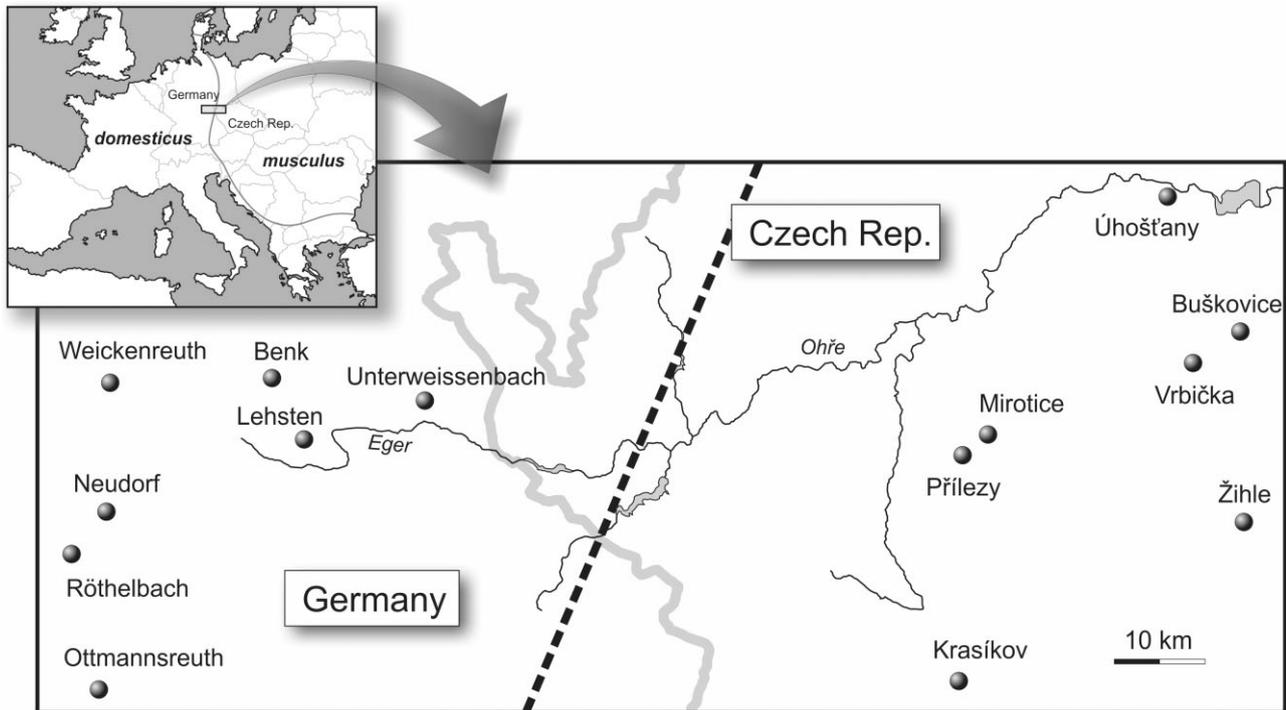
### INTRODUCTION

The house mouse (*Mus musculus* Linnaeus, 1758) has become one of the most important models in many fields of biomedical and evolutionary studies (Berry, 1981; Foster, Small & Fox, 1981; Moriwaki, Shiroishi & Yonekawa, 1994; Britton-Davidian & Searle, 2005; Fox *et al.*, 2007; Macholán *et al.*, 2012). While these studies have mostly used ‘classical’ inbred strains growing attention has recently been paid to wild mice or laboratory strains derived from wild-captured individuals. There are several subspecies of the house mouse with adjacent ranges and varying degree of intergradation between them. The best studied inter-

subspecific contact zone is between *M. m. musculus* and *M. m. domesticus* in Europe (Fig. 1; see Baird & Macholán, 2012, for review). This secondary hybrid zone was shown to be a tension zone (Payseur, Krenz & Nachman, 2004; Raufaste *et al.*, 2005; Macholán *et al.*, 2007) maintained by dispersal and selection against hybrids (Key, 1968; Barton & Hewitt, 1985). Therefore, dispersal is a key parameter affecting its dynamics.

Dispersal itself is influenced by various extrinsic and intrinsic forces, including behavioural traits and social contexts (Lidicker & Stenseth, 1992). For example, in fish as well as in birds, it was shown that one of key factors related to dispersal – exploration – was correlated with aggressiveness: more active (i.e., ‘fast’ and/or ‘far’) explorers were more bold, aggressive and neophilic than less active (‘slow’) explorers

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**Figure 1.** The Czech–Bavarian transect across the house mouse hybrid zone (dashed line) in Europe. Dots indicate localities where wild mice were trapped. On the upper left panel is the position of the zone in a global scale with the study area indicated by shaded rectangle.

(Verbeek, Drent & Wiepkema, 1994; Verbeek, Boon & Drent, 1996; Rehage & Sih, 2004). Both aggressiveness and propensity for exploration has been subject of many studies focused on suites of behavioural traits designated as personality, temperament, coping styles or behavioural syndromes (Sih, Bell & Johnson, 2004; Conrad *et al.*, 2011). A behavioural syndrome or coping style that has only recently received attention from behavioural ecologists is described as proactive/reactive behaviour (Koolhaas *et al.*, 1999, 2010). Proactive animals are characterized by higher levels of aggressiveness, tend to be bold, actively explore their environment, and readily form persistent routines whereas reactive individuals are shy and less aggressive, often respond to the emerged situation by freezing or avoidance, and adjust cautiously to changes in environment (Koolhaas *et al.*, 1999, 2010; Sih *et al.*, 2004). Importantly, coping strategies or behavioural syndromes are not limited to individuals but apply also to population and species (or even groups of species) with important implication to population/species distributions and rates of speciation (Sih *et al.*, 2004; Conrad *et al.*, 2011).

Differences in the level of aggressiveness were found also between the two house mouse subspecies: *M. m. domesticus* males and females were found to be more aggressive than *M. m. musculus* (Thuesen,

1977; van Zegeren & van Oortmerssen, 1981; Frynta *et al.*, 2005; Piálek *et al.*, 2008; Ďureje, Vošlajerová Bímová & Piálek, 2011). As the former are less tolerant towards subordinate individuals (van Zegeren & van Oortmerssen, 1981) we can expect the social pressure to be stronger within *M. m. domesticus* populations, potentially causing higher emigration rate. Indeed, during motivation tests mice of the *domesticus* origin revealed a higher propensity to move from the home cage across a water barrier to a target box (Hiadlovská *et al.*, 2012). Moreover, in another voluntary-based experiment these mice were more bold in open field exploration (Hiadlovská *et al.*, 2013).

Considering these behavioural characteristics, one should expect *M. m. domesticus* to have a competitive advantage over *M. m. musculus*, potentially resulting in movement from *domesticus* range to *musculus* range. This advantage can be further strengthened by lower choosiness of *M. m. domesticus*. When tested with diverse odour signals, *musculus* females and males preferred significantly more mates of their own subspecies, whereas *domesticus* females and males did not discriminate between consubspecific and heterosubspecific mates (Smadja, Catalan & Ganem, 2004; Bímová, Karn & Piálek, 2005; Bímová *et al.*, 2009; Vošlajerová Bímová *et al.*, 2011; Ganem, 2012).

However, recent studies from southern Bavaria (Wang *et al.*, 2011) and the Czech Republic/north-western Bavaria (Macholán *et al.*, 2011) found evidence of the zone movement in the opposite direction, i.e. from east (*musculus* range) to west (*domesticus* range). This suggests there must be forces that at least counterbalance the competitive advantage of *domesticus* males. Here we tested this prediction using wild and wild-derived animals of both subspecies. As in house mouse populations are males the predominant among dispersers (Lidicker, 1976; van Zegeren, 1980), we only employed males in our experiments. The first experimental group consisted of F<sub>1</sub> hybrids between two consubspecific, wild-derived inbred strains. These strains have been proven as suitable surrogates of the two subspecies in various behavioural studies (Piálek *et al.*, 2008; Bímová *et al.*, 2009; Ďureje *et al.*, 2011; Vošlajerová Bímová *et al.*, 2011; Hiadlovská *et al.*, 2013). The reason for utilizing F<sub>1</sub> hybrids was an endeavour to avoid the effect of inbreeding. On the other hand, F<sub>1</sub> hybrids between two inbred strains can show a sort of heterotic effect affecting experimental results. To minimize this pitfall and to capture variation present in natural populations we tested also males of the first-generation offspring of wild-captured mice born in captivity. Such animals are often considered a best possible compromise (Garland & Stephen, 1991). To test how the two subspecies perform under stressful conditions related to the travelling phase of the dispersal process (Lidicker & Stenseth, 1992; Bullock, Kenward & Hails, 2002), we carried out a slightly modified version of the Morris water task (MWT) test (Morris, 1981, 1984) to approximate the natural situation as much as possible. As an adversely stimulated procedure, the MWT tests the ability of an animal to cope with a stressful situation brought about by placing the animal into water and forcing it to find a rescue platform in unfamiliar environment – a biologically realistic component of dispersal. If the house mouse subspecies differ in their strategies of coping with stressful conditions according to the proactive-reactive axis paradigm, we expect the more aggressive subspecies to be ‘penalized’ by behavioural rigidity compared to the less aggressive yet more flexible subspecies. Such differences in behavioural strategies may have been important upon secondary contact between the subspecies in the past and still be potentially affecting the dynamics of the established hybrid zone.

## MATERIAL AND METHODS

### THE MORRIS WATER TASK SETUP

The basic procedure of the MWT is that an animal is placed in a large circular water pool with an invisible

platform that allows it to escape the water. Typically, visual cues are available and by repeating the process, the animal learns to find the platform by these cues, and reach it in significantly shorter time than by chance (Morris, 1984; Terry, 2009). In this study, the water maze was a circular vat made of white plastic, 60 cm in diameter and 100 cm high. Before each experiment, it was filled with 20 °C tap water to a depth of 13 cm. During orientation blocks (see Experimental design), a 4.2 × 4.2 cm rectangle escape platform was located 10 cm from the wall, 0.5 cm below the water surface. The position of the maze in the experimental room, room equipment (ceiling lighting, furniture) and the location of the escape platform remained unchanged during the whole experimental period. To make the platform invisible the water was stained with a non-toxic white tempera dye (Koh-i-noor 162793, České Budějovice, Czech Republic). To further aid orientation, four symbols (☺, ♠, ♥, ♦) were placed on the maze wall, each approximately 4 cm above the water level, in the centre of each quadrant. All experiments were recorded with a digital video camera located above the maze.

### PILOT EXPERIMENT

To check efficacy of our version of the MWT, we first carried out a pilot study using two standard laboratory strains. For their good performance in MWTs (D’Hooge & De Deyn, 2001; Wahlsten, Cooper & Crabbe, 2005), we employed males of the C57BL/6J strain whereas C3H J<sup>-1</sup> males were used as negative controls since this strain is unable to orient by visual cues due to homozygosity for the retinal degeneration allele *Pde6b<sup>rd1</sup>* (The Jackson Laboratory, 2002; Brooks *et al.*, 2005). The C57BL/6J and C3H J<sup>-1</sup> males were purchased from ANLAB (Brno, Czech Republic) and VELAZ (Prague, Czech Republic), respectively. All the animals were weaned at 20 days, isolated at 55 days, and tested at 91–95 days of age.

### TESTED ANIMALS

Four groups of males were used for the experiments. The first two groups consisted of reciprocal F<sub>1</sub> hybrids between consubspecific wild-derived strains, BUSNA and STUF (derived from *M. m. musculus* populations) and STRA and Schweben (derived from *M. m. domesticus* populations), respectively. For details on BUSNA, STUF and STRA see Piálek *et al.* (2008) The fourth strain is a lineage established with a pair captured in Schweben, central Germany, and kept in the Institute of Vertebrate Biology – these mice represented the 10<sup>th</sup> to 12<sup>th</sup> generation of brother–sister mating, thus, strictly speaking, these mice were not

**Table 1.** Numbers of tested  $G_1$  mice with localities of their origin (see also Figure 1)

Locality	<i>M. m. domesticus</i>		Locality	<i>M. m. musculus</i>	
	Coordinates	<i>N</i>		Coordinates	<i>N</i>
Benk	50° 11' N, 11° 52' E	2	Buškovice	50° 13' N, 13° 22' E	2
Lehsten	50° 07' N, 11° 55' E	2	Krasíkov	49° 53' N, 12° 56' E	2
Neudorf	50° 02' N, 11° 39' E	2	Mirovice	50° 07' N, 13° 00' E	2
Ottmannsreuth	49° 53' N, 11° 37' E	2	Přílezy	50° 06' N, 12° 57' E	2
Röthelbach	49° 59' N, 11° 35' E	2	Úhošťany	50° 21' N, 13° 16' E	2
Unterweissenbach	50° 09' N, 12° 06' E	2	Vrbice	50° 09' N, 13° 14' E	2
Weickenreuth	50° 10' N, 11° 40' E	1	Vrbička	50° 11' N, 13° 18' E	2
			Žihle	50° 03' N, 13° 22' E	2

fully inbred. We used eight individuals of each reciprocal cross, i.e. 16 males per consubspecific group (we checked for the effect of cross direction, none was detected). The third and fourth groups comprised the laboratory-bred first-generation offspring of wild-trapped *M. m. musculus* and *M. m. domesticus* mice. In all cases the parental pairs were sampled at the same locality well outside the hybrid zone (*musculus*: at least 36 km east of the zone centre, with mean distance 49 km; *domesticus*: at least 22 km west of the centre, mean 41 km) (Fig. 1), with the subspecies status confirmed as described in Macholán *et al.* (2007). The offspring are hereafter referred to as  $G_1$  *musculus* (16 males) and  $G_1$  *domesticus* (13 males), respectively (Table 1). All individuals were weaned at 20 days of age with siblings of the same sex, isolated at 55 days, and tested at 90–133 days of age.

#### EXPERIMENTAL DESIGN

All mice were kept in polycarbonate cages (16 × 28 cm, 15 cm high) under the light: dark regime 14:10 (light starting at 06 am) and temperature 20 °C. Pelleted food (ST1, VELAZ, Prague, Czech Republic) and water were provided *ad libitum*. Sawdust bedding and nesting material (shredded paper) were changed every fifth day. Prior to the experiment, each male was weighed and examined for his health condition. The experiments were performed in two basic classes of tests: swimming performance block and orientation block. The mice tested in the pilot experiment only underwent the orientation block whereas all other mice were subjected to both. The swimming performance block consisted of a single trial, carried out between 9:00 h and 12:00 h. During the trial, both the platform and orientation symbols were absent and the water was kept clear. The animal was placed in the centre of the maze and rescued after 2 min.

In the orientation block, each mouse was subjected to four successive trials, separated by at least 20 min

periods, the first trial starting at least 4 h after the swimming performance block. Each trial was initiated by releasing a tested individual nearby the maze wall in a randomly chosen quadrant (not including the quadrant with the escape platform). The same starting locations were not used in consecutive trials. The animal was allowed to search the maze for a maximum of 60 s. When it reached the platform, it was left there for 10–20 s and then carefully removed from the vat and returned to its cage. If the male did not succeed he was guided to or put on the platform, left there for 20 s (gently restrained if necessary to avoid his jumping back to the water) and then returned to his cage.

All the work with mice was done in the breeding facility of the Institute of Vertebrate Biology, ASCR, in Studenec, which has been licensed for keeping small mammals according to the Czech law (Licenses no. 3245/2003–2010; and 227203/2011-MZE-17214 2011–2016). Procedures were approved by the Ethical Commission of the Institute of Vertebrate Biology, ASCR. The animal experiments followed the Institutional and National Committees for Animal Welfare protocols, and live mice were handled by authorized persons only (License No. CZ 00548).

#### DATA COLLECTION

We analyzed the digital recordings using the MTS program (Modular Tracking System; designed and provided by M. Kučera). With this software, we assessed the length of the path covered by the tested male during one performance block trial. By projecting a concentric circle of 50 cm in diameter onto the water surface, we could also evaluate the distribution of the animal's movement within the maze. In this way we estimated the proportion of the path travelled by the male in the 'open water' (i.e. within the inner circle) *vs.* the path covered within the outer annulus (i.e. 5 cm wide marginal strip). In the latter case mice

moved in close proximity to the wall, having tactile contact with it by their paws, flank or tail, thus displaying thigmotaxis.

#### DATA ANALYSES

We tested the laboratory strains, F<sub>1</sub> and G<sub>1</sub> groups separately using the same statistical procedures. In the orientation block, we calculated the success rate as the proportion of trials in which an individual reached the platform within the given period (60 s). The mean success rate was compared between the subspecies (or strains – in case of pilot experiment) using a generalized linear model (GLM) with the logit link and binomial distribution of errors. The distribution of success between the trials was visualized by an interaction plot, showing the success each of group and trial. Here expectation for a given trial is the proportion of successful trials in the respective group and binomial confidence interval was calculated using the Clopper–Pearson method (Clopper & Pearson, 1934).

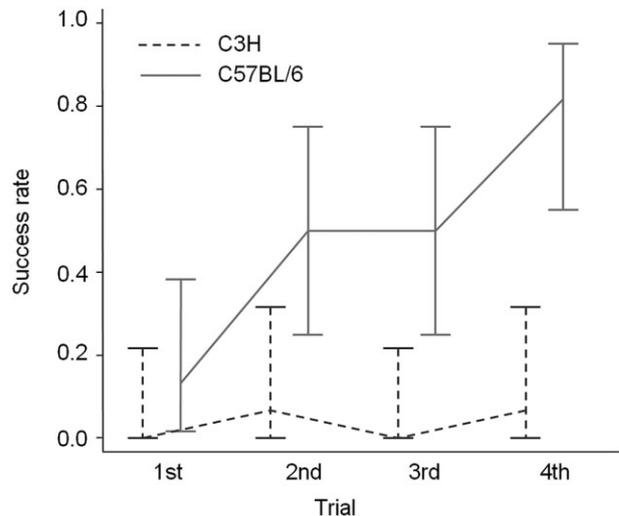
Differences in swimming performance between subspecies were tested with a general linear model comparing the total path length covered during the performance block trials. Individual body mass was included as a covariate. The model was then simplified by retaining only factors that significantly improved the fit according to F test ( $P_F \leq 0.05$ ) and Akaike Information Criterion ( $\Delta AIC \geq 10$ ).

Within each group we characterized spatial distribution of males' movements during the performance block by fitting standardized major axis (SMA) regression between path lengths in the inner and outer part of the maze (Warton *et al.*, 2006). We then used the Wald statistic to test whether two regression lines were shifted along their common slope. Given the slope of regression lines was always undistinguishable from  $-1$  (at 0.05 significance level), a shift of lines indicates a difference in the distribution of activity while controlling for differences in total path length travelled.

#### RESULTS

The pilot experiment confirmed differences in orientation ability between the classical laboratory strains. Using GLMs, mean success rates were significantly higher for the C57BL/6 strain compared with the C3H strain (0.48 vs. 0.03;  $z = 4.43$ ,  $P < 0.001$ ). In addition, as presented in Figure 2, C57BL/6 males showed a clear and significant trend towards the higher success rate between trials whereas C3H males' success remained constant.

Both (BUSNA  $\times$  STUF)F<sub>1</sub> and G<sub>1</sub> *musculus* males had a considerably higher mean success rate (0.33 and 0.42, respectively) compared to (STRA  $\times$  Schweben)F<sub>1</sub>



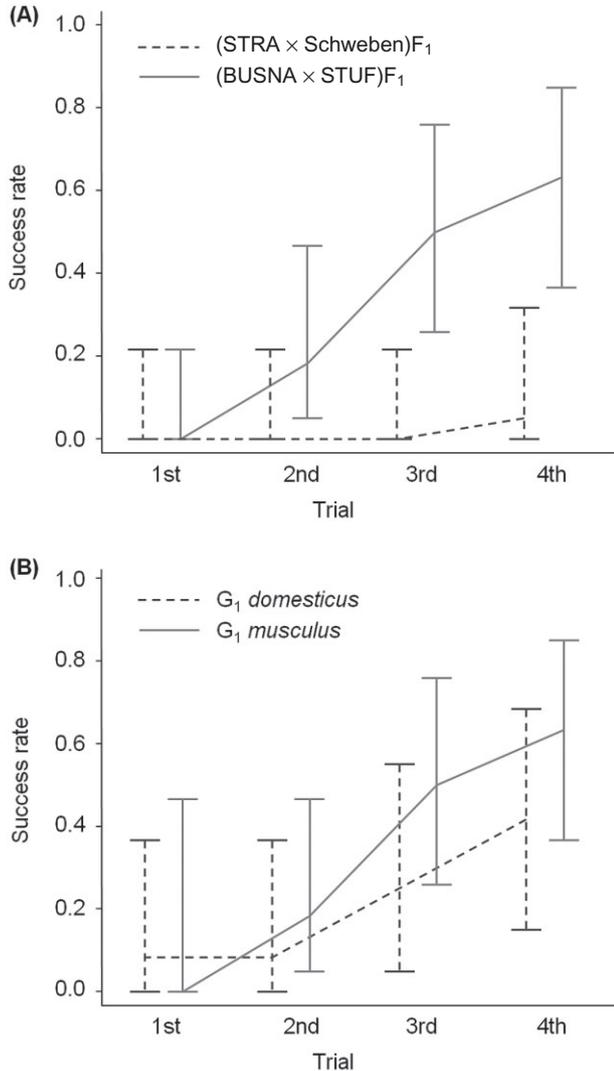
**Figure 2.** Success rates in two standard laboratory strains used in a pilot study. Plotted are means for the orientation block trials and their confidence limits for each combination of strain and trial.

and G<sub>1</sub> *domesticus* (0.02 and 0.19, respectively), see also Figure 3. In both cases the difference was significant (GLM, F<sub>1</sub>:  $z = 3.29$ ,  $P = 0.001$ ; G<sub>1</sub>:  $z = 2.58$ ,  $P = 0.010$ ). Although the effect size was ten times higher in F<sub>1</sub> than in G<sub>1</sub> males (odds ratio in favour of *musculus* = 30.77 and 3.06, respectively), the difference was not significant ( $\chi^2$ ,  $df = 1$ ,  $P = 0.103$ ).

Individual body mass had no significant effect on total path length in either F<sub>1</sub> or G<sub>1</sub> mice as removing this parameter from the model did not result in significantly decreased fit (F<sub>1</sub>:  $P_F = 0.252$ ,  $\Delta AIC = 0.853$ ; G<sub>1</sub>:  $P_F = 0.757$ ,  $\Delta AIC = 3.354$ ). Likewise, comparison of the reduced and full model revealed the effect of subspecies as non-significant in both groups of mice (F<sub>1</sub>:  $P_F = 0.732$ ,  $\Delta AIC = 1.872$ ; G<sub>1</sub>:  $P_F = 0.834$ ,  $\Delta AIC = 1.952$ ). Thus, no difference between the subspecies was found in the total length of the path the mice travelled in the water (Fig. 4). However, the (STRA  $\times$  Schweben)F<sub>1</sub> males covered a significantly longer path within the outer marginal strip of the water maze than the (BUSNA  $\times$  STUF)F<sub>1</sub> males (Wald statistic = 36.39,  $P < 0.001$ ) (Fig. 5A) whereas G<sub>1</sub> males showed no difference (Wald statistic = 0.00,  $P = 0.974$ ) (Fig. 5B).

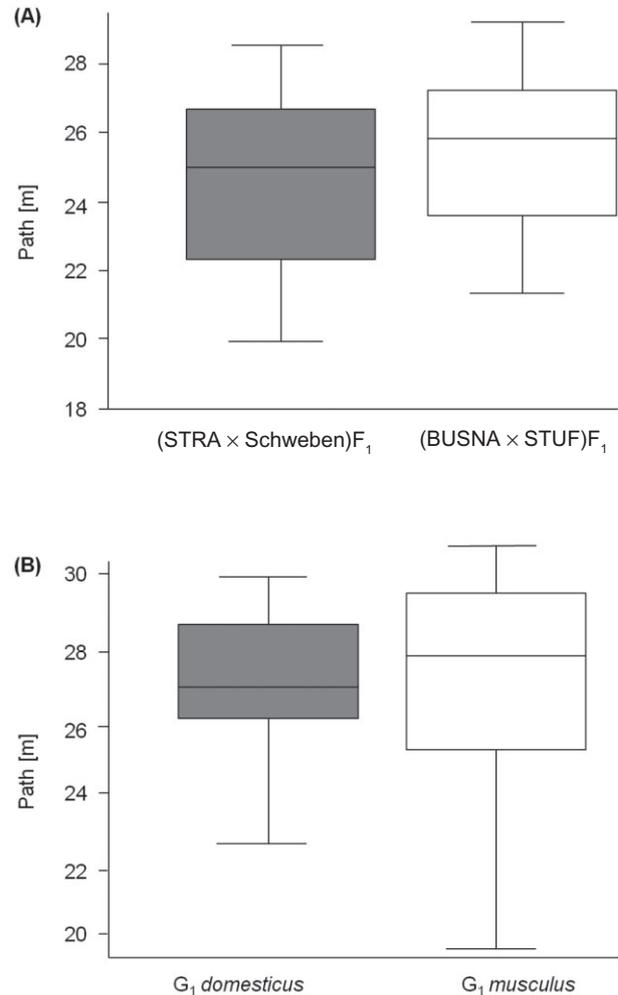
#### DISCUSSION

In this study we tested whether two house mouse subspecies (*M. m. musculus* and *M. m. domesticus*) differ in their strategies of coping with an unfamiliar or stressful situation using the Morris water maze. This is one of the classical behavioural tests which has been frequently modified with respect to size and



**Figure 3.** Success rates in four groups of experimental animals. (A) Consubspecific hybrids between wild-derived strains. (B) First-generation offspring of wild-captured mice. Plotted are means for the orientation block trials and their confidence limits for each combination of group and trial.

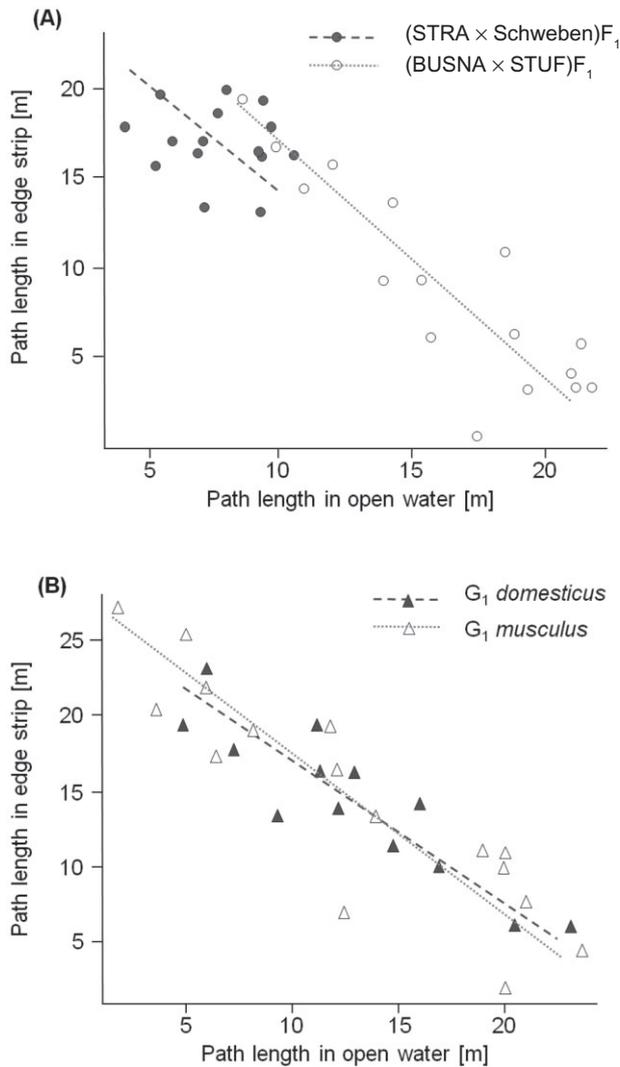
shape of the maze or platform, trial length, arrangement of trials etc. (Wolfer & Lipp, 2000; D’Hooge & De Deyn, 2001; Wahlsten *et al.*, 2005). Since in our experiment, we focused on the *initial* phase of MWT, when mice begin to cope with completely new situation, we subjected the tested mice only to four trials, during which the animals are expected to suffer from strongest stress. Before the actual experiment we carried out a pilot study using two standard laboratory strains, C57BL/6 and C3H. Because of homozygosity for the retinal degeneration allele *Pde6b<sup>rd1</sup>* causing blindness by weaning age, C3H mice were able to find the platform only by chance even



**Figure 4.** Path lengths covered during the performance blocks averaged across the four experimental groups. (A) Consubspecific hybrids between wild-derived strains. (B) First-generation offspring of wild-captured mice. The box-and-whisker plots show medians, upper and lower quartiles and a path range.

during repeated trials, whereas C57BL/6 mice displayed a significant improvement in orientation during later trials (Fig. 2). Thus we consider this approach to be sufficient for detecting initial difference between good and bad MWT performers.

Besides the coping style, numerous other parameters such as sex, age, nutrition state or exposure to infection were proven to have influence on the MWT performance (D’Hooge & De Deyn, 2001). However, the influence of these factors was suppressed in the present study by employing individuals of the same sex, age, nutrition, health status, and life experience. On the other hand, the swimming speed of tested males could have been affected by differences in body mass (Owen *et al.*, 1997). The two subspecies are



**Figure 5.** SMA regression lines fitting the proportion between individual path lengths in the inner circle and the outer annulus (marginal strip) during the performance block. (A) Conspecific hybrids between wild-derived strains. (B) First-generation offspring of wild-captured mice. Dots indicate individual path lengths and lines SMA regressions.

known to differ in size, with *M. m. domesticus* being usually bigger (Sage, 1981; van Zegeren & van Oortmerssen, 1981). Similar size differences were also found between our experimental groups (stronger for  $F_1$  than for  $G_1$  mice – see Supporting Information). However, the influence of this parameter on swimming performance was shown to be negligible in the present study.

As earlier experiments revealed *M. m. domesticus* to be more ardent swimmers than *M. m. musculus* (Brubaker, 1970; Hiadlovská *et al.*, 2012), we tested swimming by measuring the total path the mice

covered during a two-minute period (swimming performance block). As this time was the same for all mice any differences in the path necessarily reflected differences in average swimming speed. The resulting mean paths were almost identical for representatives of the two subspecies within the  $F_1$  and  $G_1$  group, respectively (Fig. 4). Thus we can conclude that differences between *musculus* and *domesticus* males we found can be attributed to differences in strategies of coping with the MWT.

In previous studies, mice were reported to react to being placed in water by motionless floating (Wolfer *et al.*, 1998). However, this behaviour, typical of inbred strains, was not observed during our trials. Other instinctive reaction is to swim along the maze wall known as thigmotaxis (Wolfer *et al.*, 1998). This behaviour was significantly more expressed in  $F_1$  males derived from two *domesticus* inbred strains than in males derived from *musculus* strains. This seems to contradict results of previous exploration studies showing stronger thigmotactic movement in *musculus* males (Hiadlovská *et al.*, 2013). However, in the cited study of Hiadlovská *et al.* (2013) entering the open field arena was voluntary, so we expect that situation to be much less anxiety-determined. During the MWT, animals are forced to ‘explore’ the water maze, and under such circumstances, excessive thigmotaxis is considered as an indication of increased anxiety, and, more importantly, of low behavioural flexibility (Wolfer *et al.*, 1998; Wolfer & Lipp, 2000). Thus our results suggest higher behavioural flexibility in *musculus* derived  $F_1$  males. On the other hand, *musculus* and *domesticus*  $G_1$  males revealed almost the same swimming strategy. This difference between performance of the  $F_1$ 's and  $G_1$ 's may be caused by higher variation in behavioural response in wild mice compared to laboratory strains (Festing & Lovell, 1981; Austand, 2002) as shown in Figure 5. Another possible explanation may be a different reaction of the *musculus* vs. *domesticus* lineages to inbreeding. This effect may have been accompanied by the increased response variation in the *musculus* derived  $F_1$  males (Fig. 5A). Nevertheless exact causation of the discrepancy remains unclear.

In the orientation blocks, both  $F_1$  and  $G_1$  *musculus* males were considerably more successful than *domesticus* males. In particular, (STRA  $\times$  Schweben) $F_1$  strikingly performed almost as poorly as blind C3H mice in reaching the platform (cf. Figure 2 vs. 3A). Although we did not check visual ability of experimental animals by genetic or histological methods, neither our experience during testing or previous behavioural studies (Piálek *et al.*, 2008; Dureje *et al.*, 2011; Hiadlovská *et al.*, 2013) nor reports from routine manipulations indicated any visual deficiencies. We

thus reject the possibility that our *domesticus* mice failed in the MWT due to poor sight. Although both subspecies were equally capable swimmers in the Morris maze, the pattern of the swimming activity could have potentially influenced the MWT performance. For example, the *domesticus*-derived (STRA × Schweben)F<sub>1</sub> males who preferred swimming along the wall might have simply missed the platform. However, we did not find differences in latency to and/or frequency of contacts with the platform between the subspecies (data in Supporting Information). Typically, during the second or third trial, *musculus* males started to climb on the platform after one or two initial contacts whereas *domesticus* males kept crossing it without stopping their movement. Similarly, Benus, Koolhaas & van Oortmerssen (1987) found more aggressive mice to be unable to register small adjustments in the maze, running blindly across, while less aggressive mice spent more time exploring them. The same behavioural rigidity of more aggressive males was also recorded both in non-social experiments (reversal learning test) and during social (e.g. dyadic) interactions (Benus *et al.*, 1990). The better performance in finding the platform we revealed in less aggressive *M. m. musculus* suggests higher behavioural flexibility of this subspecies in comparison with *M. m. domesticus*, in agreement with predictions of the proactive-reactive axis concept (Koolhaas *et al.*, 1999, 2010; Sih *et al.*, 2004).

How are the differences in behavioural strategies related to dispersal? As evidenced by a number of studies (Thuesen, 1977; van Zegeren & van Oortmerssen, 1981; Frynta *et al.*, 2005; Ďureje *et al.*, 2011) *M. m. domesticus* is characterized by more violent interactions between population members, especially between males, than *M. m. musculus*. Within *M. m. domesticus* populations we thus expect stronger social pressure and hence higher emigration rate. As reported by Hiadlovská *et al.* (2012, 2013), *domesticus* males showed higher activity in open field exploration and propensity to overcome a water barrier. However, readiness to explore or/and emigrate is only one step in the dispersal process (Lidicker & Stenseth, 1992). Leaving the natal area is followed by a phase of travelling (transfer). During these dynamic and risky conditions (Errington, 1946), more flexible mice are likely to be favoured by natural selection (Benus *et al.*, 1991). As we observed during our MWT experiments, less aggressive animals seem to cope better with stressful situations. They also pay more attention to relatively inconspicuous changes in environment (Benus *et al.*, 1987; Sih *et al.*, 2004) and show more secure-seeking behaviour (Hiadlovská *et al.*, 2013). The number of immigration events depends on both the rate of dispersal and on the probability of survival to successful

deme establishment. Hence *musculus* mice may be less likely to succumb to dangers related with dispersal to a new territory.

How can (differences in) behavioural flexibility affect the dynamics of secondary contact? At present, the European house mouse hybrid zone consists of a mixture of multiple filial and backcross generation hybrids where F<sub>1</sub> hybrids are missing or extremely rare (Macholán *et al.*, 2007; Albrechtová *et al.*, 2012). Although we have no information about behavioural strategies of natural mouse hybrids it is reasonable to assume that behaviour of these individuals is somehow mixed, i.e. encounters following pure parental strategies do not occur in the zone. Nevertheless, first, parental populations of the two subspecies once *must* have met and second, as suggested in Introduction we may expect a mechanism outweighing an obvious competitive advantage of *domesticus* males over *musculus* ones rendered by their higher aggressiveness (Thuesen, 1977; van Zegeren & van Oortmerssen, 1981; Frynta *et al.*, 2005; Ďureje *et al.*, 2011), more active exploration strategy in an open field arena (Hiadlovská *et al.*, 2013), higher willingness to swim (Brubaker, 1970; Hiadlovská *et al.*, 2012), and lower choosiness of sexual partner (Smadja *et al.*, 2004; Bímová, Karn & Piálek, 2005; Bímová *et al.*, 2009; Vošlajerová Bímová *et al.*, 2011; Ganem, 2012). We believe that such counterbalancing mechanisms can result from higher flexibility of *M. m. musculus* under stressful conditions likely to be experienced in natural settings.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Body mass differences.

**Figure S2.** Latency of the first contact with platform in consecutive trials.

**Figure S3.** Frequency of contact with platform in consecutive trials.