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Transgressive segregation in a behavioural trait? Explorative strategies in two house mouse subspecies and their hybrids

ZUZANA HIADLOVSKÁ[1,2]*, BARBORA VOŠLAJEROVÁ BÍMOVÁ[1,3], ONDŘEJ MIKULA[1,3], JAROSLAV PIÁLEK[3]† and MILOŠ MACHOLÁN[1]

[1]*Laboratory of Mammalian Evolutionary Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, Czech Republic*
[2]*Institute of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno, Czech Republic*
[3]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic*

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Hybrid zones between genetically diverged populations are widespread among animals and plants. Their dynamics usually depend on selection against admixture and dispersal of parental forms in the zone. Although indirect estimates of selection have been the target of many studies, dispersal has been neglected. In this study we carried out open field experiments to test whether males of two house mouse subspecies, *Mus musculus musculus* and *Mus musculus domesticus*, differ in their propensity to disperse and in their character of exploration. We tested wild-caught males and males of two wild-derived inbred strains. In addition, we examined reciprocal F_1 crosses to test the prediction that these hybrids display intermediate behaviours. We revealed that *M. m. musculus* males were less hesitant to enter the experimental arena than were *M. m. domesticus* males, but once inside the arena their movements were more timid. F_1 males differed from both parental strains, with longer latencies to enter the arena, but explored the arena in a similar fashion as the *M. m. domesticus* males, thus displaying transgressive behavioural phenotypes. These results contribute to our knowledge of behavioural divergence between the mouse subspecies, and add a new facet to the study of speciation. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **108**, 225–235.

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INTRODUCTION

With the increasing accumulation of molecular data it now appears that natural hybridization, resulting in gene flow between different taxa, is more widespread than previously thought, with potentially important implications for speciation and biodiversity research, as well as for conservation strategies (Barton, 1992; for review see Mallet, 2005; Macholán, 2012). Mixing parental genomes brings new combinations of alleles, and so the phenotypes of hybrids may vary considerably. Often novel phenotypes that are not intermediate between parental traits are observed in hybrids. This phenomenon, known as transgressive segregation, has been evidenced in a number of taxa (Rieseberg, Archer & Wayne, 1999; Rieseberg *et al.*, 2003), and may result in the successful colonization of habitats unoccupied by parental taxa, or may even contribute to the origin of a new species (Nolte & Sheets, 2005; Nolte *et al.*, 2005; Mallet, 2007; Stelkens & Seehausen, 2009).

Most frequently, hybridization is confined to more or less localized hybrid zones. As convincingly argued by Barton & Hewitt (1985, 1989), hybrid zones that are independent of gene flow are very rare (see also Baird & Macholán, 2012, for review), and hence

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*Corresponding author. E-mail: 328868@mail.muni.cz
†Current address: Department of Population Biology, Institute of Vertebrate Biology, ASCR, Studenec, Czech Republic

studies of the dispersal of animals are of prime interest. This is especially true for the most common type of hybrid zone, i.e. the 'tension zone' (Key, 1968), which is maintained by the balance between dispersal and selection against admixture, rather than by adaptation to different local habitats.

Whereas consequences of gene flow can be traced by looking for genetic footprints (Barton, 1992), factors affecting dispersion are less well known. Conventionally, three phases of dispersal are distinguished: leaving (emigration); travelling (transfer); and arriving (immigration) (Lidicker & Stenseth, 1992). Obviously leaving a familiar home range and exploring unknown areas is a very important phase (Errington, 1946). It depends on many factors such as sex, age, health status, population density, food and water availability, and habitat structure (Andreassen, Stenseth & Ims, 2002; Singleton & Krebs, 2007). When exploring, animals often avoid open areas. If they are exposed to unprotected places they may perform a typical fear-related behaviour, such as thigmotaxis, i.e. moving towards solid objects (Warne, 1947; Brown, 1953; Randall, 1999; Augustsson, Dahlborn & Meyerson, 2005), and frequent retreats to secure places between excursions (Choleris *et al.*, 2001; Augustsson & Meyerson, 2004; Latham & Mason, 2004).

One of the best-studied tension zones is the zone of secondary contact of two house mouse subspecies, *Mus musculus musculus* Linnaeus, 1758, and *Mus musculus domesticus* Schwarz & Schwarz, 1943. In Europe, the zone extends from Scandinavia to the Black Sea coast (for a review, see Baird & Macholán, 2012). *Mus musculus* populations are usually structured into small demes, typically comprising one dominant male and between three and six reproducing females, with juveniles (Crowcroft, 1955; Reimer & Petras, 1967; Berry & Jakobson, 1974; Singleton, 1983). Dominance is established through male–male contests, and the winner gains a well-defined territory and sires most of the litters in the deme (DeFries & McClean, 1970; Oakeshott, 1974; Singleton & Hay, 1983), but success in excluding intruders and tolerance of subordinate males may vary (Crowcroft, 1966; Reimer & Petras, 1967; Lidicker, 1976; Hurst, 1987). This suggests an important role of aggression in a male's life history. According to the social subordination hypothesis (Gaines & McClenaghan, 1980), aggression is the main force shaping the dispersal pattern of a population (Gray & Hurst, 1997): in mammals, *M. musculus*, emigrants are predominately subordinate males that have reached maturity. These males either stay on the periphery of the defended area (Singleton & Hay, 1983) or leave their natal deme in response to social pressure (Crowcroft, 1955; Brandt, 1992; Sandnabba, 1997; Pocock, Hauffe & Searle, 2005). Most of the dispersing males attempt to establish their own home ranges elsewhere (Anderson & Hill, 1965; van Zegeren, 1980), often in vacant areas. Conversely, females leave their demes less frequently (van Zegeren, 1980), making males a natural target of experiments focused on explorative strategies. On the other hand, females are supposed to be more often tolerated by unfamiliar dominant males, and hence to more easily enter an established non-native deme. This would increase the effective migration rate of females. However, an experiment conducted by Jones *et al*. (1995), introducing a group of mice from one Scottish island onto another, showed that nuclear markers spread throughout the colonized island, whereas mitochondrial DNA (mtDNA) did not, suggesting a more successful dispersal of males compared with females.

A number of studies of mouse dispersal have been published (for a review, see Sage, 1981; Kotenkova & Bulatova, 1994; Pocock *et al*., 2005); however, most of these studies have focused on one subspecies only (*M. m. domesticus*), and a comparison between the subspecies is lacking. Wild *M. m. domesticus* males (Hunt & Selander, 1973; Thuesen, 1977; van Zegeren & van Oortmerssen, 1981; Munclinger & Frynta, 2000; Frynta *et al*., 2005), as well as their wild-derived inbred representatives (Piálek *et al*., 2008; Ďureje, Vošlajerová Bímová & Piálek, 2011), have repeatedly been proven to be more aggressive than *M. m. musculus* males. Given the relationship between exploration and aggression, we can expect to find differences in exploratory strategies between the two subspecies, with more aggressive *M. m. domesticus* males being more eager to disperse and bolder in exploration than *M. m. musculus* males. An important insight into potential differences in the propensity to disperse and explore unfamiliar areas can be achieved through properly designed behavioural experiments, such as the open field test, elevated plus test, and related methods.

In this study, we used the open field test to estimate exploratory strategies in wild males sampled from allopatric populations of the two subspecies, as well as males from two wild-derived inbred strains. In addition, we scored reciprocal F_1 hybrids between the two strains. Specifically, we tested whether: (1) the two subspecies differ if their exploratory behaviour; and (2) F_1 hybrids between the inbred strains display an intermediate phenotype, i.e. the values of individual parameters quantifying the exploratory behaviour are equidistant from both parental strains. We found that *M. m. musculus* and *M. m. domesticus* males indeed use different strategies of exploration, whereas hybrid males are characterized by transgressive behavioural phenotypes that are either similar to one of the parental strains or are significantly different from both strains.

MATERIAL AND METHODS

TESTED ANIMALS

The experiments were carried out using six groups of mice. The first two experimental groups consisted of 14 *M. m. domesticus* and 14 *M. m. musculus* males, respectively, sampled from wild populations well outside the central European portion of the hybrid zone (Fig. 1; Table 1). To ensure independence between the samples, we used no more than two males per locality. Mice were captured from September to October 2009–2011 using wooden traps, and were then transported to the breeding facility of the Institute of Vertebrate Biology in Studenec. The subspecies status was confirmed with six autosomal and five X-linked markers that are diagnostic for the subspecies, as described in Macholán *et al.* (2007).

Another two groups comprised mice from two wild-derived inbred strains representing both subspecies: 'Straas Aggressive', derived from *M. m. domesticus* (STRA), and 'Buškovice Non-Aggressive', derived from *M. m. musculus* (BUSNA), see Piálek *et al.* (2008) for details. The two parental strains have been established and are currently maintained in the breeding facility in Studenec (Piálek *et al.*, 2008). We tested 30 males of the 24th–30th generation of brother-sister mating, with 15 of each strain. Finally, F_1 hybrids have been produced by crossing STRA females with BUSNA males [(ST × BU)F_1] and vice versa [(BU × ST)F_1], and 15 males from each cross direction were tested.

All mice were kept in Perspex cages (16 × 28 × 15 cm) under a 14-h light/10-h dark photoperiod. Pelleted food (ST1, VELAZ, Prague, Czech Republic) and water were available *ad libitum*. Sawdust bedding and nesting material was changed every fifth day. Wild males were tested at least 30 days after capture, and prior to testing each male was isolated in a separate cage for at least 14 days. The parental strains and F_1 hybrid males were weaned at 20 days of age with siblings of the same sex, isolated at 55 days, and tested at 85–97 days of age.

EXPERIMENTAL DESIGN

To examine the exploration activity we carried out a standard open field test. A circular arena, 100 cm in diameter, with 50 cm high walls, was constructed of white plastic. The floor was divided into three sections with two concentric circles: the central circle, inner annulus, and outer annulus. The diameters of the circles were 30 and 90 cm, respectively. The arena was connected with a glass tube (5 cm in diameter, 10 cm long) to a Perspex habituation box (35 × 25 × 13 cm) (Fig. 2). Between trials, the floor, walls, and connecting tube were thoroughly cleaned with 96% ethanol and paper towels. The habituation box was cleaned using a solution of $NaClO_4$ (< 5%) and then thoroughly rinsed with tap water after each test.

All tests were performed during the light phase of the day, from 09:00 to 14:00 h. Before each trial the male to be tested was placed in the habituation box and allowed to habituate for at least 15 min. After habituation, the box was connected to the arena by a glass tube, and the sliding door was lifted so that the animal could enter the arena and move freely between the arena and the box.

Each trial lasted 10 min and was video-recorded and processed using MODULAR TRACKING SOFTWARE (custom designed and purchased from M. Kučera). The observed time interval started when the animal approached the opening of the arena for the first time and attempted to enter it. The animal's first actual entrance (FAE) was defined as all four paws touching the floor. We termed the 'delay' between the start of the trial (first attempt to enter) and FAE as the *latency to enter* parameter. If FAE did not happen during the whole trial the animal was scored with a penalty of 600 s.

Movement patterns during the first 3 min after FAE were described by the following parameters: *total time* spent in the arena; number of *box entrances*, when the animal left the arena and returned to the habituation box; and by the spatial distribution of the animal's activity within the arena. This distribution was calculated as the *proportions of total time* spent within one of the three arena sections. For some mice, the latency-to-enter phase was longer than 7 min, making the remaining time of recorded behaviour shorter than 3 min, and data about their behaviour after FAE are therefore missing.

STATISTICAL ANALYSES

Group 'medians' were estimated using the Hodges–Lehmann estimator (Hodges & Lehmann, 1963) for univariate variables (*latency to enter*, *total time*, and *box entrances*) and compared with non-parametric rank tests (Zar, 1999). The inbreds and their hybrids were first compared with Kruskal–Wallis ANOVA testing for overall differences in the three variables among the experimental groups (see above). Where the differences were significant, the Wilcoxon rank sum test was applied to perform three a priori defined comparisons: between STRA and BUSNA; between these strains and their F_1 hybrids; and between F_1 hybrids of the two reciprocal crosses. Wild-captured *M. m. musculus* and *M. m. domesticus* males were compared using a series of Wilcoxon rank sum tests.

The *proportions of total time* spent in the three sections, by definition, sum to unity, which has two

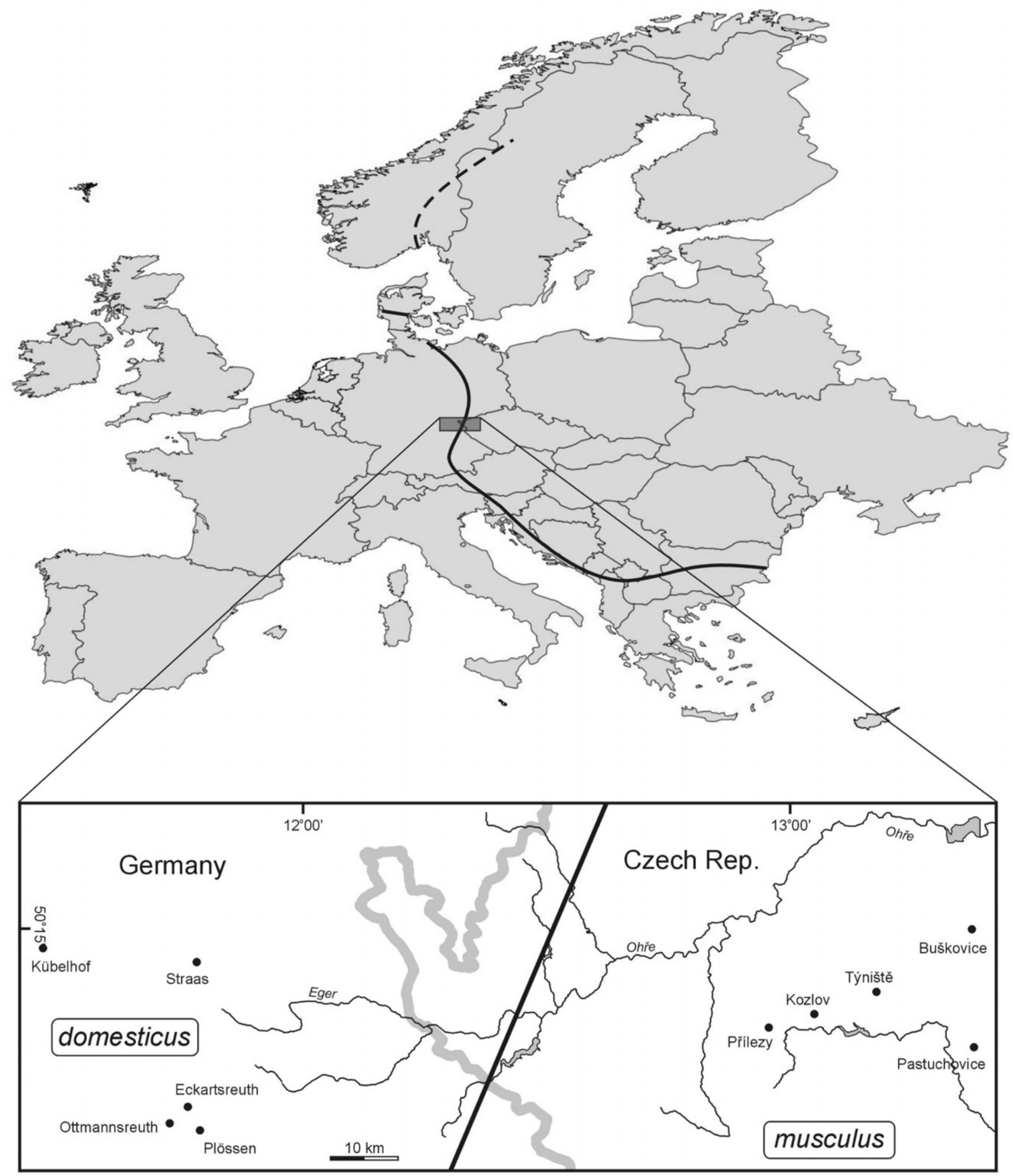

Figure 1. The position of the *Mus musculus musculus–Mus musculus domesticus* hybrid zone in Europe. The dashed line indicates the tentative position of the zone in Norway, after Jones *et al*. (2010). Below, detailed map of the study area, showing the sites from which wild-captured males were sampled (including localities where the founder mice of the BUSNA and STRA inbred strains were originally trapped). The solid line in the bottom panel indicates the approximate course of the zone defined in Macholán *et al*. (2007).

Table 1. The origin and numbers of tested wild mice, with geographic coordinates of the localities sampled (see also Fig. 1)

Locality	*Mus musculus domesticus* Coordinates	*N*	Locality	*Mus musculus musculus* Coordinates	*N*
Benk	50°11′ N, 11°52′ E	1	Buškovice	50°13′ N, 13°22′ E	2
Eckartsreuth	49°56′ N, 11°47′ E	2	Kozlov	50°07′ N, 13°02′ E	1
Kübelhof	50°12′ N, 11°26′ E	2	Krasíkov	49°53′ N, 12°56′ E	1
Ottmannsreuth	49°53′ N, 11°37′ E	1	Pastuchovice	50°04′ N, 13°23′ E	2
Plössen	49°51′ N, 11°47′ E	2	Přehořov	50°02′ N, 13°23′ E	1
Rieglersreuth	50°08′ N, 11°45′ E	1	Přílezy	50°06′ N, 12°57′ E	2
Röthelbach	49°59′ N, 11°35′ E	2	Týniště	50°09′ N, 13°11′ E	2
Straas	50°11′ N, 11°46′ E	2	Vrbice	50°09′ N, 13°14′ E	1
Unterweissenbach	50°09′ N, 12°06′ E	1	Vrbička	50°11′ N, 13°18′ E	1
			Žihle	50°03′ N, 13°22′ E	1

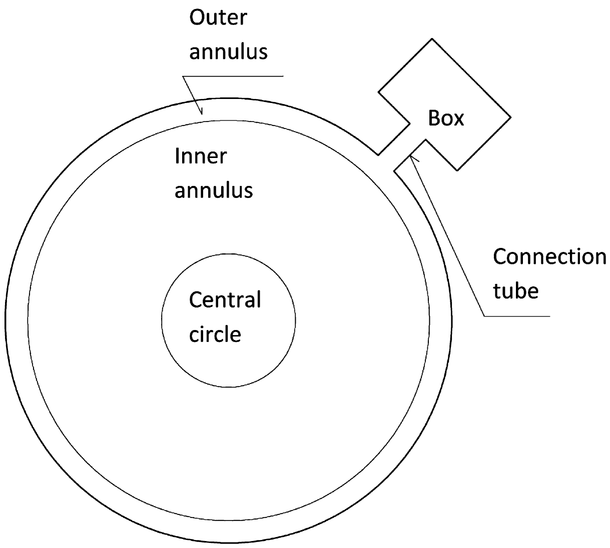

Figure 2. The open field arena design, with the habituation box attached.

consequences precluding their straightforward analysis. First, there are strong but spurious correlations among them. Second, the data matrix is rank deficient, as the third proportion can always be computed from the first two. An isometric log-ratio transformation (van den Boogaart, 2005) was therefore applied to individual observations to make their multivariate analysis valid. This is a two-step process: to eliminate spurious correlations, the measures for each individual are divided by their geometric means, and logarithms of these ratios are then taken (Aitchison, 1986). Rank deficiency is eliminated by projecting the matrix containing these log-ratios onto the appropriate orthonormal basis (Egozcue *et al*., 2003). Differences in the resulting isometric log-ratios were tested using a linear model, with group comparisons arranged as in non-parametric tests. Finally, group means of isometric log-ratios estimated by the linear model were transformed back to the original scale of three proportions.

All statistical procedures were performed using R 2.14.0 (R Development Core Team, 2011), with the packages ‘exactRankTests’ (Hothorn & Hornik, 2006) for Wilcoxon rank sum tests and ‘compositions’ (van den Boogaart, Tolosana & Bren, 2011) for the analysis of compositional data.

RESULTS

Wild *M. musculus* differed only in the *box entrance* parameter, showing that *M. m. musculus* males left the arena more often during the trial (Tables 2 and 3). Although differences in other variables were not significant, *M. m. domesticus* males tended to wait longer before making their first entrance but spent more time in the arena. They also had a lower tendency to creep along the wall (i.e. they spent less time in the outer annulus than *M. m. musculus* males) and withdrew less often to the habituation box.

Significant differences between the two wild-derived inbred strains were found in three variables: *latency to enter*; *total time*; and *proportions of total time* (Tables 2 and 3). Consistent with the results for wild mice, STRA males wavered longer before entering the arena, but then spent a proportionally longer time in the inner annulus than BUSNA males. The STRA males also displayed higher values of *total time*, although this difference was not significant ($P = 0.231$). Although the difference in the *box entrances* was not significant ($P = 0.106$), the BUSNA males revealed the same tendency as the wild males tested, i.e. more frequent retreats to the box.

Table 2. Estimates of central tendency and confidence limits for the four parameters studied. The estimators are Hodges–Lehmann 'medians' for univariate variables and means for the time proportions spent in the three sections

	Latency to enter (s)	*Total time* (s)	*Box entrances* (*N*)	Central (%)	Inner (%)	Outer (%)
Mus musculus musculus	5.5 (3.5–9.5)	125 (104.0–137.5)	3.8 (2.5–5.0)	1.5	13.8	84.7
Mus musculus domesticus	9.8 (6.0–46.5)	135 (119.0–150.5)	2.3 (1.5–3.0)	2.8	21.5	75.6
Inbreds	50.3 (23.0–122.5)	100.5 (85.0–114.5)	2.8 (2.0–4.0)	4.9	29.2	65.9
F_1	177.8 (110.5–312.0)	149.3 (136.5–159)	2.8 (2.0–3.5)	2.5	44.3	53.1
BUSNA	13.8 (8.0–111.5)	95.3 (75.0–107.0)	3.3 (2.0–4.5)	6.8	19.9	73.3
STRA	87.3 (50.0–259.5)	112 (83.0–135.0)	2.8 (1.5–4.0)	3.0	43.7	53.3
(BU × ST)F_1	164.8 (72.0–287.0)	141 (128.0–154.0)	3.3 (2.0–4.0)	3.8	47.7	48.5
(ST × BU)F_1	196.8 (96.5–368.0)	155.8 (134.0–171.0)	2.3 (1.5–3.0)	1.5	40.1	58.3

Table 3. Results of statistical tests

Variable		Wild *musculus* vs *domesticus*	All captive raised	Inbreds vs F_1	BUSNA vs STRA	(BU × ST)F_1 vs (ST × BU)F_1
Latency to enter the arena	Statistic	68.50	21.37	212.50	35.00	98.05
	P	0.113	*0.000*	*0.000*	*0.001*	0.573
Total time in the arena	Statistic	74.00	23.60	76.00	65.00	46.00
	P	0.280	*0.000*	*0.000*	0.231	0.145
Box entrances	Statistic	161.00	6.12	–	–	–
	P	*0.002*	0.106	–	–	–
Time proportions	Statistic	0.14	0.51	0.18	0.36	0.20
	P	0.1439	*0.000*	*0.009*	*0.005*	0.100

For univariate variables, the statistic is *W* from a Wilcoxon rank sum test (two-level comparisons) or *H* from a Kruskal–Wallis test (four-level comparisons). For the time proportions spent in the three circles, a Pillai's trace from a MANOVA was used. 'Inbreds' refer to a pooled group of BUSNA and STRA males; 'All captive raised' mice mean BUSNA, STRA, and both reciprocal F_1 hybrids. Significant values are in italics.

Remarkably, both reciprocal F_1 hybrids displayed exploration characteristics that were not intermediate between those of the parental inbred strains. Hybrids were more reluctant to enter the arena but once inside they spent longer there than both the BUSNA and STRA males (Fig. 3A). These differences were greater than the differences between the parental strains (Table 3). The movement of both types of F_1 hybrids within the arena was similar to the movement of the STRA males, i.e. they spent a larger proportion of time in the inner annulus (Fig. 3B; Table 3). Thus, the hybrids displayed either an exploration strategy very similar to one of their parents (STRA) or even stronger *M. m. domesticus*-like behaviour than the STRA males themselves.

DISCUSSION

Explorative strategies are very important for *M. Musculus*, characterized by neophilic social behaviour (Singleton & Krebs, 2007), with potential implications for mouse dispersal and the evolution of reproductive isolation. The open field experiments reported here revealed significant differences in male explorative strategies between two inbred strains derived from wild populations of *M. m. musculus* (BUSNA) and *M. m. domesticus* (STRA). Although the differences between wild-caught males were not significant for most of the behavioural parameters tested, they were in the same direction as differences between the inbred males. This corroborates the notion of the two inbred strains as suitable surrogates of both subspecies (Piálek *et al.*, 2008; Bímová *et al.*, 2009; Ďureje *et al.*, 2011; Vošlajerová Bímová *et al.*, 2011). Wild males displayed a shorter latency phase and spent a longer time in the arena than inbred males. At the same time, their movements within the arena were more 'cautious', as suggested by the longer time spent in the outer annulus. These differences may reflect their diverse social status and previous experience of wild males from their native habitats (Clément, Calatayud & Belzung, 2002). Another source of variation can be greater genetic variation in wild mice, which display a wider range of behavioural responses (Bímová, Karn &

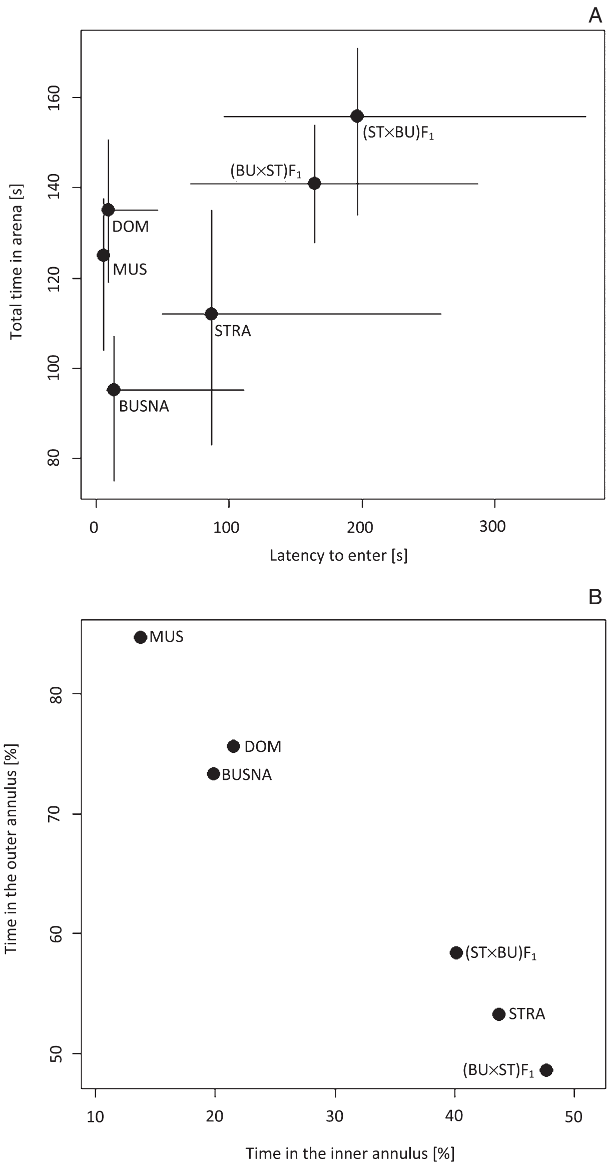

Figure 3. A, Hodges–Lehmann 'medians' of the *latency to enter* and *total time* spent in the arena, with confidence intervals. B, mean proportions of time spent in the inner and outer annulus. Differences in the time spent in the central circle are seen as deviations from the linear arrangement of the points.

Piálek, 2005). Indeed, when wild males of the first captive-born generation that have been raised under controlled conditions were subjected to the same experiments (preliminary data not shown here), they too displayed a notably longer latency to enter and time spent in the arena, as well as a lower tendency to thigmotaxis in *M. m. domesticus* males, compared with *M. m. musculus* males.

Compared with *M. m. musculus*-derived BUSNA males, *M. m. domesticus*-derived STRA males held back longer from entering an unfamiliar area, but once inside, they spent a longer time in exploration, with less frequent retreats to the shelter. As shown above, these differences are stronger than those between wild-caught males. This finding can be discussed in the context of agonistic behaviour preserved at different levels in the two strains (STRA, 'Straas Aggressive', and BUSNA, 'Buškovice Non-Aggressive'). Dispersal and agonistic behaviour was proposed to represent a single behavioural syndrome because of their co-occurrence during ontogenesis (Rusu & Krackow, 2005). According to Koolhaas *et al.* (1999), there are two coping styles in *M. musculus*: proactive, usually performed by aggressive individuals; and reactive, and typical for non-aggressive individuals. Similarly, Parmigiani *et al.* (1999) observed a higher level of anxiety and a lower tendency to explore in low-aggression mice. Other works reported no substantial difference in exploration between aggressive and non-aggressive individuals (Benus, Koolhaas & van Oortmerssen, 1992; de Boer, van der Vegt & Koolhaas, 2003), although some of the papers admit there can be a slight tendency of non-aggressive males to initiate fewer exploratory bouts (Benus *et al.*, 1992). On the contrary, in some studies aggressive mice were considered to be less explorative, possibly because of low behavioural flexibility (Benus, Koolhaas & van Oortmerssen, 1987) or a higher level of emotionality and anxiety (Guillot & Chapouthier, 1996; Hood & Quigley, 2008). In escape tests, which are very similar to the open field experiment used in the present study, aggressive mice displayed shorter latencies than moderately aggressive (Van Loo *et al.*, 2004) or non-aggressive (Coppens, de Boer & Koolhaas, 2010) mice. Hence, the results of previous studies are not consistent, probably because of the different experimental designs and animals used.

Behaviour in an unfamiliar area is another aspect of exploratory activity. Although the strains did not differ significantly in the time spent in the arena, the character of their movement differed: after entering the arena BUSNA males showed a tendency for thigmotaxis, manifested here by a proportionally longer time spent close to the arena wall, whereas STRA mice explored the space more boldly. This result is in agreement with the studies of Veenema *et al.* (2003) who found non-aggressive individuals to perform fewer movements in the open field relative to aggressive individuals. A higher level of thigmotaxis was also found in non-aggressive strains of rats (de Boer *et al.*, 2003).

From the results discussed above it appears that none of the subspecies can be considered as simply more explorative. Rather, they have adopted different strategies of exploration. Also, the relationship between aggression and exploratory behaviour patterns could have evolved differently in *M. m. musculus* than in *M. m. domesticus*. The latent phase before the first entrance to the arena can be viewed as the time of risk assessment. During this period the males tested were repeatedly sniffing around the opening and stretching out to the arena, demonstrating the 'stretched exploratory posture' (Bartolomucci *et al.*, 2004). Thus, the longer latencies of *M. m. domesticus* or *M. m. domesticus*-derived mice could be seen as an active screening of new surroundings. This may be related to the higher inter-male aggression present in *M. m. domesticus* populations (Thuesen, 1977; van Zegeren & van Oortmerssen, 1981; Munclinger & Frynta, 2000; Frynta *et al.*, 2005). Under such conditions a male may benefit from a prolonged and thorough ascertainment of the potential presence of other male(s) in an unfamiliar area. If the presence of a dominant male is detected, the newcomer may quickly retreat, hence avoiding the risk of a potentially harmful encounter. If the space is vacant, or a subordinate male is present, he may establish control over the area by following the 'bourgeois strategy' (i.e. play 'hawk' if you own the territory and 'dove' if you do not). The post-entrance higher 'self-confidence' of *M. m. domesticus* males may reflect this situation. In their experiment, Hood & Quigley (2008) observed a very similar aggression-dependent pattern: a long latency to enter the arena but a short latency to contact a novel object in more aggressive males, and the opposite trend in amicable males. Hence the observed differences in exploratory strategy might reflect the adaptation to different social milieu of the two subspecies.

Very interesting results were revealed in F_1 hybrids: males of both reciprocal crosses displayed behaviours that were either similar to one of the parental inbred strains (i.e. in their movement around the arena) or more extreme than either of the parental strains (i.e. their latency to enter and time spent in the arena). This phenomenon is called transgressive segregation, and it has been observed in many species of plants and animals (for a review, see Rieseberg *et al.*, 1999, 2003), including mice (Bateson & D'Udine, 1986; Hauffe & Searle, 1993; Alibert *et al.*, 1994; Renaud, Alibert & Auffray, 2009). As argued by Rieseberg *et al.* (1999), transgressive phenotypes are mostly brought about by the complementary action of additive alleles that are dispersed between the parental lines. Transgression is therefore observed more often in crosses of domesticated and/or inbred animals than in crosses of outbred, wild animals. Recombination between complementary genes should also result in a stronger effect in F_2 hybrids than in F_1 hybrids. This prediction was confirmed for the shape of the mouse mandible (Renaud *et al.*, 2009). Transgressive segregation for behavioural traits was observed in F_2 hybrids between the C57BL6/J and SEC/1 ReJ strains (Bateson & D'Udine, 1986). In that study, second-generation hybrids displayed significantly longer latency to contact an unknown object, and contacted it less often than F_1 hybrids, which were intermediate between the parental strains. On the contrary, F_1 hybrids between C57BL/6 and BALB/c mice showed higher activity than the parental strains (Le Pape & Lassalle, 1984).

The emergence of novel extreme phenotypes is considered an important factor with significant ecological and evolutionary consequences, manifested by the ability to colonize areas uninhabited by hybridizing parental taxa and the hybrid origin of a new species, respectively (Arnold, 1997; Rieseberg *et al.*, 1999; Nolte & Sheets, 2005; Nolte *et al.*, 2005; Mallet, 2007; Stelkens & Seehausen, 2009). Our data finding F_1 hybrids to be extremely prone to explore novel areas may fit with these predictions; however, the *M. musculus* hybrid zone is populated by many-generation hybrids, and no F_1 individuals have been documented so far (see Baird & Macholán, 2012, for review), so it is not clear how transgressive segregation could affect the evolutionary dynamics of the two subspecies.

In conclusion, it appears that mouse exploration represents a complex phenotype. Although inbred lineages cannot encompass the whole genetic variation present in natural populations, it is important that all differences (significant or not) between wild *M. m. musculus*/*M. m. domesticus* and inbred *M. m. musculus*/*M. m. domesticus*-derived mice had the same polarity. Thus it is tempting to conclude that there are genuine differences between the subspecies in their exploration of an unfamiliar environment behind these trends, with *M. m. domesticus* males adopting a longer risk assessment, followed by more 'self-confident' exploration, than *M. m. musculus* males. Again, it is difficult to see what consequences these differences can have for the evolution of *M. musculus* in general, and the hybrid zone dynamics in particular. Nor is there any evidence that these differences have evolved as an adaptive response to different ecological or social conditions of the two diverging taxa (or, alternatively, as a result of a random processes, or as a by-product of divergence in other traits). Nevertheless, the results presented in this paper can be seen as an important contribution to our knowledge of behavioural differentiation between the more aggressive *M. m. domesticus* and non-aggressive *M. m. musculus* subspecies (Ganem, 2012). As aggression is tightly connected with dispersal and exploratory behaviour, this work adds a new facet to the study of speciation.

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