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ARTICLE

Sperm quality, aggressiveness and generation turnover may facilitate unidirectional Y chromosome introgression across the European house mouse hybrid zone

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Abstract

The widespread and locally massive introgression of Y chromosomes of the eastern house mouse (*Mus musculus musculus*) into the range of the western subspecies (*M. m. domesticus*) in Central Europe calls for an explanation of its underlying mechanisms. Given the paternal inheritance pattern, obvious candidates for traits mediating the introgression are characters associated with sperm quantity and quality. We can also expect traits such as size, aggression or the length of generation cycles to facilitate the spread. We have created two consomic strains carrying the non-recombining region of the Y chromosome of the opposite subspecies, allowing us to study introgression in both directions, something impossible in nature due to the unidirectionality of introgression. We analyzed several traits potentially related to male fitness. Transmission of the *domesticus* Y onto the *musculus* background had negative effects on all studied traits. Likewise, *domesticus* males possessing the *musculus* Y had, on average, smaller body and testes and lower sperm count than the parental strain. However, the same consomic males tended to produce less- dissociated sperm heads, to win more dyadic encounters, and to have shorter generation cycles than pure *domesticus* males. These data suggest that the *domesticus* Y is disadvantageous on the *musculus* background, while introgression in the opposite direction can confer a recognizable, though not always significant, selective advantage. Our results are thus congruent with the unidirectional *musculus* → *domesticus* Y chromosome introgression in Central Europe. In addition to some previous studies, they show this to be a multifaceted phenomenon demanding a multidisciplinary approach.

Introduction

According to the classical Dobzhansky–Muller model (Dobzhansky 1936; Muller 1942), when a population is split by a geographic barrier, different alleles are fixed in the two subpopulations due to selection and/or random drift. As a result, the diverged genomes can be incompatible when mixed during secondary contact, leading to reduced viability or fertility of hybrids. Irrespective of the causes of hybrid viability and/or fertility disruption, gene flow of sex-associated markers across a hybrid zone is expected to be hampered. This seems to be the case in many secondary contact zones studied so far (Storchová et al. 2010; Beysard and Heckel 2013; Carneiro et al. 2013; Maroja et al. 2015), including the hybrid zone between two house mouse subspecies, *Mus musculus musculus* and *M. m. domesticus*, in Europe (Macholán et al. 2007, 2011; Baird and Macholán 2012).

The European house mouse hybrid zone is a more than 2500-km long belt of hybrid populations running from

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Scandinavia to the Black Sea coast (Boursot et al. 1993; Macholán et al. 2003; Baird and Macholán 2012). This zone is a complex mix of multigeneration hybrids and backcrosses, essentially without F1 individuals, and with the lowest fitness in the zone centre (Raufaste et al. 2005; Macholán et al. 2007). In agreement with predictions of the large X-effect principle (Charlesworth et al. 1987; Coyne and Orr 1989; Coyne 1992), the mouse X chromosome harbours more genes under strong counterselection within the zone than do the autosomes (Dod et al. 1993; Macholán et al. 2007, 2008, 2011; Teeter et al. 2008).

House mouse hybrids, just as those of all other mammals (Presgraves 2008), confirm yet another principle known as Haldane's rule, which states that when one sex is missing, rare or sterile in hybrids, it is the heterogametic sex (Haldane 1922). This rule has been proven both in laboratory crosses (Forejt and Ivanyi 1975; for review see Forejt et al. 2012; Oka and Shiroishi 2012) and natural populations (Baird and Macholán 2012). There is now compelling empirical evidence that Haldane's rule can be associated with an arms race between genes involved in genomic conflict (Frank 1991; Hurst and Pomiankowski 1991; Tao et al. 2001; Orr and Irving 2005; Orr et al. 2007; Phadnis and Orr 2009; Meiklejohn and Tao 2010; Presgraves 2010; Crespi and Nosil 2013; Patten 2018). Obvious battlefields for such arms races are sex chromosomes that differ in their transmission mode and hence compete over sex ratio (Burt and Trivers 2006; O'Neill and O'Neill 2018; Patten 2018). Accordingly, we should expect extremely strong selection against the transition of Y chromosomes across the hybrid zone.

Very limited Y introgression was indeed reported from Bulgaria (Vanlerberghe et al. 1986), Denmark (Vanlerberghe et al. 1986; Raufaste et al. 2005; Dod et al. 2005) and south-eastern Bavaria (Tucker et al. 1992). However, Munclinger et al. (2002) found *M. m. musculus* Y chromosomes deep in *M. m. domesticus* territory on the border between north-eastern Bavaria (Germany) and western Bohemia (Czech Republic). This finding motivated a larger-scale study across the Czech–Bavarian portion of the hybrid zone that revealed massive unidirectional *musculus* → *domesticus* Y introgression extending up to tens of kilometres behind the zone centre. It was also shown that it is coupled with sex ratio differences, indicating intragenomic conflict between sex chromosomes (Macholán et al. 2008). Moreover, the presence of *musculus* Ys within the *domesticus* range in western Norway (Jones et al. 2010) and the results of an extensive study over a large area from the Baltic Sea to the northern slopes of the Alps (Ďureje et al. 2012; Macholán et al. 2019) suggest that this phenomenon is rather widespread in Central Europe.

While molecular studies of samples representing large areas can characterize the extent and directionality of introgression, only studies of underlying factors can shed light on its adaptive nature. Given that the Y chromosome is inherited exclusively paternally, obvious candidates of traits mediating the spread of *musculus* Y chromosomes are characters associated with sperm quantity and quality. For example, Albrechtová et al. (2012) analyzed two sperm traits, sperm count and sperm motility, in males collected across the Czech–Bavarian portion of the mouse hybrid zone. This study revealed that (i) both sperm count and motility were significantly reduced in hybrids relative to additive expectations, and (ii) in males of predominantly *M. m. domesticus* genetic background possessing introgressed *M. m. musculus* Ys, the sperm counts were higher than in *M. m. domesticus* males with their own, consubspecific, Y chromosomes (Albrechtová et al. 2012). However, sperm count and motility traits are likely to be just two facets of the whole story. Indeed, we can expect the Y introgression to be driven by an interplay of multiple traits. For example, in species like the house mouse, males seizing social dominance within the deme leave more descendants than their inferior counterparts. Higher rank is achieved through male–male contests, and so the ability to win these encounters is an important component of male fitness (Anderson and Hill 1965; Singleton and Hay 1983). The fighting success can be approximated by the level of aggression, which can be, in turn, correlated with body size (Brenner 1989).

Since Y chromosome introgression across the house mouse hybrid zone is unidirectional (Munclinger et al. 2002; Macholán et al. 2008, 2019; Ďureje et al. 2012), *musculus* males with introgressed *domesticus* Ys are very rare in nature. Therefore, fitness consequences of the transition of Y chromosomes onto heterosubspecific genetic backgrounds in both directions can only be tested under laboratory conditions. For this purpose, we have created two substitution (consomic) strains carrying Y chromosomes (precisely the non-recombining region of the Y) of the other subspecies. This approach has yet another advantage in that introgressed Ys appear on pure and homogeneous background. We focused on several traits that can potentially be related to male fitness: size (body length and weight), testis weight, sperm quantity and quality, aggression and generation turnover. The latter trait simply measures the rate of generation increase over time. We assume that individuals with faster reproductive generation cycles have a selective advantage over those with slower reproduction. Therefore, if mice bearing the introgressed Y chromosome are faster-reproducing, the Y will be spreading across non-introgressed populations. Our data suggest that whereas the *domesticus* Y is disadvantageous on the *musculus* background, introgression in the opposite direction may confer a selective advantage. Our results are thus in accordance with the unidirectional *musculus* → *domesticus*

introgression of the Y chromosome observed in Central Europe.

Materials and methods

Mice

As surrogates of wild *M. m. musculus* and *M. m. domesticus*, we used two wild-derived inbred strains, BULS (hereinafter nicknamed MUS for simplicity) and STRA (hereinafter nicknamed DOM), respectively. The former is derived from a pair captured in Buškovice, ~60 km east of the hybrid zone centre, whereas the latter originates from Straas, ~45 km west of the zone centre (Piálek et al. 2008). These strains were used for creating consomic lineages possessing the non-recombining part of the Y chromosome (non-pseudoautosomal region, Y^{NPAR}) of the other strain. The Y^{NPAR} transfer has started with production of (DOM × MUS)F1 and (MUS × DOM)F1 males. These males were then backcrossed at least six times with females of the maternal strain to purify the genetic background of the recipient line (for simplicity we call MUS and DOM backgrounds subspecific merely to reflect the fact that the strains represent *musculus* and *domesticus* subspecies, respectively). The resulting males with the DOM (STRA) genetic background and substituted Y^{NPAR} of the MUS (BULS) origin were termed STRA.BULS-ChrY.NPAR (hereinafter abbreviated as DOM.Y^m), and the males from the reciprocal type of backcrossing were termed BULS. STRA- ChrY.NPAR (hereinafter MUS.Y^d). Both the parental and consomic strains were created and maintained in the breeding facility of the Institute of Vertebrate Biology of the Czech Academy of Sciences in Studenec.

We used two sets of males for phenotypic scoring. The first group, designated for analysis of morphometric and sperm-related traits and generation turnover, consisted mostly of fathers directly employed for production and maintenance of the four inbred strains. The second set comprised males put aside for tests of aggressiveness. All mice were housed in polypropylene cages (16 × 28 × 15 cm), provided with sawdust bedding, under the following constant conditions: light-to-dark photoperiod 14:10, temperature 22 °C, food (Standard mouse pellet ST-1, Velaz, Czech Republic) and tap water available ad libitum. Parental males were isolated from pregnant females at least 2 days before parturition. To eliminate unequal distribution of maternal investment and within-litter competition, litters were culled to six pups, preferentially with equal sex ratio, and the progeny were weaned at 20 days of age. The Institute of Vertebrate Biology breeding facility has been licensed (227203/2011-MZE-17214) for keeping small mammals according to Czech law since 2000.

Molecular analyses

DNA was isolated from a piece of spleen or tail using the DNeasy® 96 Tissue Kit (QIAGEN) following the manufacturer's instructions. Genetic background of the recipient strain was checked for contamination of donor strain alleles in 40 mice destined for behavioural experiments. The probability that there are traces of the donor genome in the recipient background after six generations of backcrossing is 0.0078. To check for contamination, we used a panel of 25 microsatellite loci (see Supplementary Material online). No traces of donor genome, except Y chromosome, were detected. The PCR conditions and protocols for the microsatellites were published in Piálek et al. (2008) and Kawałko et al. (2009).

Y chromosomes were typed using an 18-bp deletion in the *Zfy2* gene that is present in *M. m. musculus* and absent in *M. m. domesticus* (Boissinot and Boursot 1997; Munclinger et al. 2002) to confirm the presence of the expected Y type in the substitution strains. No traces of introgression of foreign alleles were detected in any of these strains (data not shown). This suggests that the consomic males had sufficiently pure genetic background. Screening of *Zfy2* confirmed that the DOM.Y^m strain harboured the *musculus* Y^{NPAR}, whereas the *domesticus* Y^{NPAR} type was present in the MUS.Y^d strain.

Sperm and body size-related traits

In total, we investigated 46 males of MUS (generation G12–17 of brother–sister mating), 54 of DOM (G12–18), 37 of MUS.Y^d (BC6–13 of backcrossing) and 49 of DOM.Y^m (BC6–19). All the animals were sacrificed by cervical dislocation at 134 days of age (see below), weighed, measured and dissected for molecular analyses. As body-related variables, we measured body weight and length. Both testes were weighed individually using analytical balances with precision of 0.0001 g, and both values were averaged. Spermatozoa were released from the whole left epididymis to 2 ml of 1% sodium citrate, and the number of sperm heads was then counted in 10 squares of the Bürker chamber using an Olympus CX41 microscope under 200× magnification (for details see Vyskočilová et al. 2005). The mean value was then used as a representative of the individual's sperm count. The proportion of dissociated sperm heads (DSH) was estimated from three squares. This variable was treated as a binomial, with heads classified either as joined to or dissociated from the tail. The proportions of DSH were transformed using the arcsine transformation to render them concordant with a normal distribution. Since body weight (BW), body length (BL), testis weight (TW), sperm count (SC) and transformed DSH data did not reveal significant deviations from normality with the

Kolmogorov–Smirnov test at the 5% level, we could treat them using univariate and multivariate parametric procedures.

As described in the following section, we weighed males tested in dyadic encounters for aggressiveness five times during the interval from 70 to 134 days (the last measurement being immediately before sacrifice). Therefore, we used the repeated measures multivariate analysis of variance (MANOVA) to evaluate potential differences in BW gain between the four groups, reflecting both the influence of genetic background and Y substitution type.

The body and sperm-related traits were then analyzed using principal component analysis (PCA) and redundancy analysis (RDA) with a single explanatory variable: Strain (MUS, MUS.Y^d, DOM and DOM.Y^m) and four response variables: BL, TW, SC and DSH. Strain was used as a supplementary variable in PCA. Since body weight of males tested in dyadic encounters was already analyzed with the repeated measure MANOVA as described above, and it was strongly correlated with body length ($R = 0.9240$; $P \ll 0.001$), it was not included in RDA and PCA. All the variables subjected to PCA were divided by their standard deviations to control for differences in variance between them. In the case of RDA, we tested multivariate normality as Mardia's multivariate kurtosis and skewness (kurtosis = 5.742, $P = 0.219$, skewness = 16.596, $P = 0.083$). To meet normality of residuals, the response variables were separately transformed using the Canoco flexible log-transformation formula (ter Braak and Šmilauer 2012). Significance was tested using Monte Carlo permutation of the observed data. The programme Canoco 5 (ter Braak and Šmilauer 2012; Šmilauer and Lepš 2014) was used for RDA. Multivariate differences between the groups (strains) were tested using Mahalanobis distances rendered by MANOVA. Then we partitioned the data to individual parameters and tested them using a series of the Tukey HSD post hoc tests. All these statistical procedures, except RDA, were performed using the Statistica 13.5 package (TIBCO Software Inc. 2018).

Behavioural experiments

Fighting ability is generally measured in dyadic encounters where tested males are confronted with standard opponents (Scott 1942; Ginsburg and Allee 1942). Since attack behaviour is a result of an interaction between genotype and the conditions under which the mice are tested (Roubertoux et al. 2005; Maxson 2009; Maxson et al. 2013), we adopted an approach consisting of four successive dyadic interactions involving two different experimental setups and two types of opponents. This complex design allowed us to assess the fighting ability of tested males during a long-term experiment controlling for their experience with outcomes of previous aggressive interactions (Lagerspetz and Lagerspetz 1971; Corridi et al. 1993). To eliminate the effects of social environment potentially affecting offensive behaviour (Parmigiani et al. 1989; Sluyter et al. 1994; Le Roy et al. 1999) the experimental males ('testees') were isolated in separate cages for at least 10 days prior to the first test, without any previous sexual interaction. We used isolated unfamiliar males of parental DOM (STRA, aggressive) and MUS (BULS, nonaggressive) strains as opponents in all the tests.

We tested 20 males of each strain, two parental and two consomic, i.e. 80 males in total, each male being subjected to four tests (see below). During the experiments, the MUS males were at generations G13–G14 and DOM at G14 of brother–sister mating, MUS.Y^d at the 6–8th generation of backcrossing (BC6–8) and DOM.Y^m at BC6–10. Males of each of the four strains were divided randomly into two equally sized groups ($n = 10$), each of which was assigned randomly to start dyadic encounters either with a DOM or a MUS opponent. Each male was tested first under neutral conditions (neutral cage test 1, NC1) and 10 days later under asymmetric conditions (resident–intruder test 1, RI1) with a first opponent type. This series of trials was repeated after 1 month; however, this time, the tested males were introduced to the opposite MUS/DOM opponent than during the first trial series (NC2 and RI2).

The same opponent was never used twice against the same testee. The opponents were isolated at least 5 days before experiments, marked by dorsal fur cut and tested no more than three times with at least 10-day intervals between the tests. The neutral arena test was performed in a new clean cage at 70 and 110 days of testees' age. Males were simultaneously introduced from the opposite sides of the arena. All experiments were video-recorded for 6 min following the first contact between the males (for details see Piálek et al. 2008; Ďureje et al. 2011). At the end of the trial, the opponent was removed from the cage, and the testee was left in the same cage for another 10 days to establish his home territory. The cage was not cleaned during this time. The resident–intruder tests were then carried out at 80 and 120 days of age. An opponent was introduced into a resident's cage, and the observation lasted for 6 min as described above. All video records were analyzed using the Observer software (http://www.noldus.com/). All males were weighed immediately before each test; the last (fifth) measurement was recorded 2 weeks after the last test when the males were sacrificed (see above). All tests were performed in a transparent Perspex cage (39 × 24 × 23 cm) with a transparent lid and sawdust bedding on the floor.

Based on the analyses of offensive/defensive behaviour of both tested and opponent males in each trial, males were assigned either to the 'Winner' (W) or 'Loser' (L) category.

The winner was defined as the male who won the encounter through displaying aggressive postures, attacking and chasing the opponent, whereas the loser lost the encounter by displaying submissive postures, or was attacked/chased by the opponent (van Oortmerssen 1971; Čiháková and Frynta 1996; Koolhaas et al. 2013). When no aggressive interaction between the males was observed during the whole duration of the test, we defined the trial result as 'No Fight' (NF category).

The Y substitution effect was estimated by tracking changes of aggressive behaviour along the series of successive tests from NC1 to RI2, where parental and consomic males were evaluated with a total score of aggressiveness. This composite score was simply the sum of individual scores across all four tests where losing the fight was given 0, winning was given 2 and 'No Fight' was scored as 1. Hence, the overall fighting ability ranged from 0 (for a male who lost all encounters) to 8 (when the male won all encounters). The Wilcoxon and Kruskal–Wallis non-parametric test were used to evaluate the effects of the genome and Y type on the fighting performance of testees between the parental and the respective consomic strain. All the behavioural statistical analyses were performed using the JMP statistical package (http://www.jmp.com).

Generation turnover

Keeping parental and consomic strains for a time interval over ~3 years allowed us to test the influence of Y chromosome transmission on the rate of generation increase over time—a variable we call generation turnover (GT). We assumed a linear increase of generations in time modelled as $GT_i \sim a_i + b_i$*time, where $a_i + b_i$ stands for the intercept and slope of the ith group of males, respectively. For analysis, we utilised records of birth dates of the first litter per individual females from 177 MUS (G11–20), 24 DOM.Y^m (BC6–19), 23 DOM.Y^m (BC6–13) and 153 DOM (G10–22) litters (377 births in total). As we were interested in slopes of a generation increase in time expressed in days, we first adjusted all datasets to start from G1 by subtracting minimum generation. Then we adjusted the time-serial data to start at day 0 on the timescale (abscissa, subtraction of minimum date) and 0 on the generation rate scale (ordinate, subtraction of individual y intercepts). Subsequently, the data for each group were fitted with a linear model, and slopes of these lines were then tested with an analysis of covariance (ANCOVA) in the R statistical environment (RStudio Team 2015). Confidence intervals were constructed as described in Crawley (2013), and pairwise comparison of generation turnover slopes was analyzed using the *emmeans* package (https://cran.r-project.org/web/packages/emmeans/index.html) accounting for interaction between GT and time.

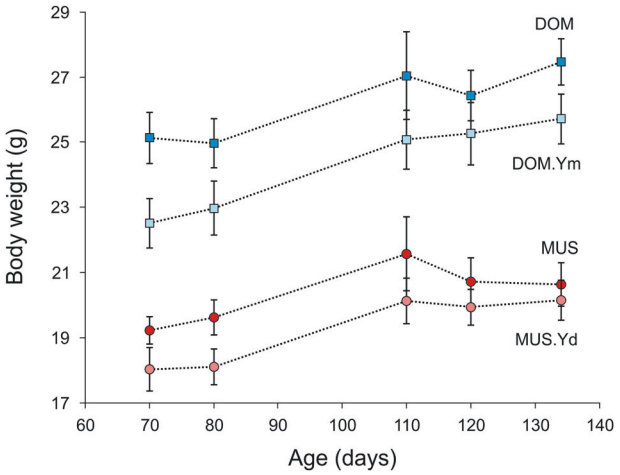

Fig. 1 Mean body weights plotted against age. The mice were weighed at 70 days (neutral cage test 1), 80 days (resident–intruder test 1), 110 days (NC2 test), 120 days (RI2 test) and 134 days when they were sacrificed. Vertical lines show 95% confidence intervals.

Results

Body and sperm variables

The body weights of all tested males varied with increasing age from 70 (NC1) until 134 days (time of dissection) (Fig. 1). Repeated measures done by the MANOVA test ascribed most variation, summed along the whole age span, to the genetic background ($F_{(1,74)} = 281.89$, $P \ll 0.001$). This reflects the fact that the DOM males were on average heavier by 5.50 g than the MUS males across all age periods. Among the four strains, the interaction between the genome and the Y type was significant ($F_{(1,74)} = 22.39$, $P \ll 0.001$); however, no significant effect of the Y chromosome itself was observed ($F_{(1,74)} = 1.99$, $P = 0.160$). As expected, body weight was found to change as a function of age ($F_{(4,71)} = 75.79$, $P \ll 0.001$). While no interactions between age and the Y types were detected ($F_{(4,71)} = 1.15$, $P = 0.340$), the interactions between age and genomes ($F_{(4,71)} = 2.60$, $P = 0.040$) and between age, genome and the Y type were significant at the 5% level ($F_{(4,71)} = 2.79$, $P = 0.030$).

Due to the significant effect of subspecific genetic background on temporal changes of body weight, we subsequently tested the effect of the Y separately within the DOM + DOM.Y^m and MUS + MUS.Y^d group, respectively. Males of the two parental strains were on average heavier by 1–2 g than males of corresponding consomic strains, and this effect was highly significant in both cases (DOM vs. DOM.Y^m: $F_{(1,37)} = 13.73$, $P < 0.001$; MUS vs. MUS.Y^d: $F_{(1,37)} = 8.80$, $P < 0.005$). The differences between body mass in the former pair of strains remained unchanged along the whole age span from NC1 to dissection (repeated measures' MANOVA, $F_{(4,34)} = 3.02$,

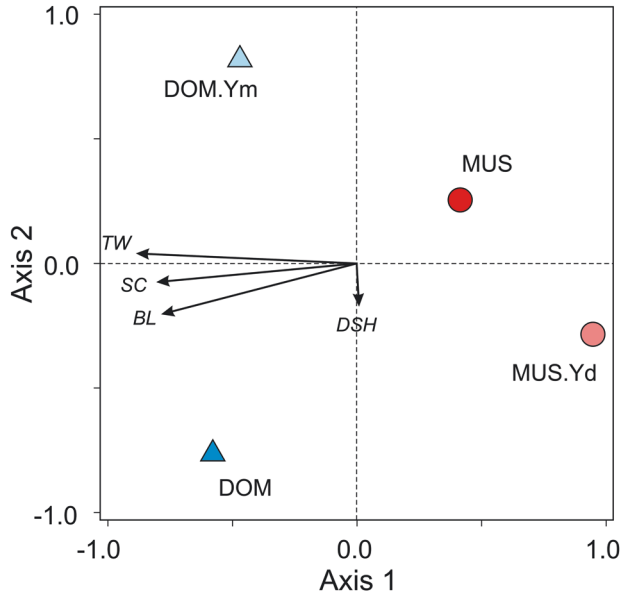

Fig. 2 Results of RDA. TW testis weight, SC sperm count, BL body length, DSH proportion of dissociated sperm heads (pseudocanonical correlation with the first and second axis = 87 and 24%, respectively).

$P = 0.030$), whereas those in the latter pair were not significant ($F_{(4,34)} = 1.44$, $P = 0.240$). However, the MUS.Y^d consomics were lighter by 6.2% than MUS males at 70 days of age, although the difference was only 2.4% at the age of dissection (Fig. 1). Thus, both types of Y chromosome substitution resulted in a decrease in body weight during the whole period under study.

The results of RDA and PCA were very similar in partitioning variance according to male groups (Fig. 2 and S1, Supplementary Material online). The first axis separates two groups according to the subspecies' genetic backgrounds (DOM + DOM.Y^m vs. MUS + MUS.Y^d). This discrimination is dominated by BL, TW and SC, suggesting that males with the STRA (*domesticus*) background are, on average, bigger and have larger testes with more sperm than males with the BULS (*musculus*) background (the pseudocanonical correlation with the first axis = 87%). The second axis discriminates between MUS vs. MUSY^d and especially between DOM vs. DOM.Y^m according to the Y chromosome type, the main response variable being the proportion of deformed sperm heads (the pseudocanonical correlation with the second axis = 24%). Altogether, all the explanatory variables accounted for 58.53% of the total variance (the adjusted explained variation = 57.84%). While the first axis discriminates predominately between different backgrounds, it also shows that the Y *domesticus* chromosome transmission onto the *musculus* background has a notably stronger effect than the reciprocal transfer. More importantly, although the proportion of sperm dissociations (DSH) has much smaller influence than BL, TW and SC, the second axis clearly demonstrates the opposite effect of the Y chromosome substitution (Fig. 2). The transfer of *domesticus* Y chromosome is correlated with the increase in DSH, whereas the transmission in the opposite direction results in transgressive segregation where the DOM.Y^m males appear to have the lowest proportion of aberrant spermatozoa (DSH) in their sperm across all strains (cf. Figs. 2 and 3d). These results suggest that introgression of Y chromosome from the other subspecies results in a statistically significant decrease in body size (DOM vs. DOM.Y^m), testis size (MUS vs. MUS.Y^d) and lower sperm count (both contrasts). At the same time, transfer of the MUS Y chromosome onto the STRA background has a positive effect on sperm head development, whereas the reciprocal introgression has a negative effect. MANOVA revealed highly significant differences between all the strains (Wilks' lambda = 0.119, $F_{(12,\ 474)} = 48.77$, $P \ll 0.001$), and also squared Mahalanobis distances between centroids of all four groups were highly significant ($P \ll 0.001$).

Subsequently, we analyzed each variable separately using the Tukey HSD test. We focused on differences between pairs of male groups sharing the subspecies-specific genetic background (i.e. MUS vs. MUS.Y^d, and DOM vs. DOM.Y^m). For body length (BL), testis weight (TW) and sperm count (SC), both consomic strains revealed lower mean values relative to the corresponding parental strains (Fig. 3a–c, Table S1; Supplementary Material online), corroborating the results of repeatedly measured body weight as well as RDA. On the other hand, while not significant at the 5% level ($P = 0.109$) due to high variance, there was a notable positive effect of transferring the *musculus* Y chromosome onto the *domesticus* background in DSH (Fig. 3d).

Aggressiveness

The results of the dyadic encounters differed quite consistently according to the genetic background of the tested males: irrespective of the Y type they carried and the test condition, when males with the MUS genetic background were tested against DOM (STRA) opponents, they lost 78 of 80 (97.5%) of their encounters across all experimental designs (Fig. S2, Supplementary Material online). Conversely, again irrespective of the Y type, when males with the DOM genetic background were tested against MUS (BULS) opponents, they won 73 of 80 (91.3%) of their encounters (Fig. S2).

The composite scores over all four fighting trials revealed that males from the four strains differed from each other in their fighting abilities (Kruskal–Wallis $\chi^2_{(3)} = 59.22$, $P \ll 0.001$). The strain's genetic background was the most significant factor (Fig. 4). Within the group of males with the same background, substitution of the Y led to opposite effects on fighting ability. A negative effect was

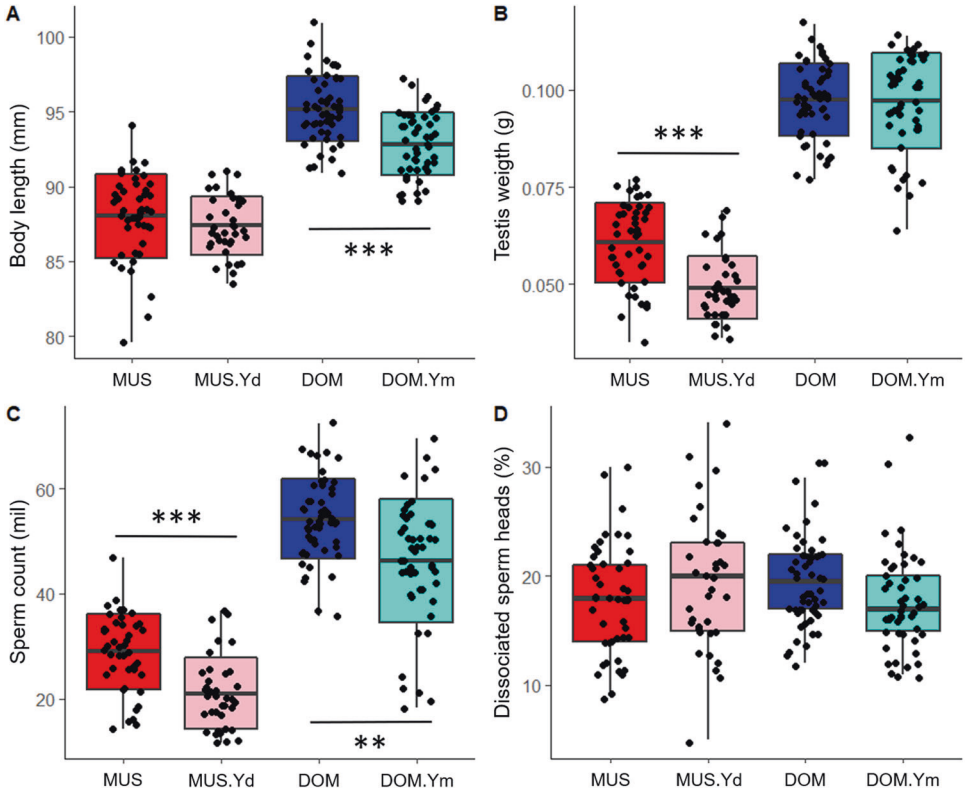

Fig. 3 Comparisons between strains differing in Y chromosome types (i.e. parental vs. consomic). BL body length (**a**), TW testis weight (**b**), SC sperm count (**c**), DSH proportion of dissociated sperm heads (**d**). The crossbars and the boxes show medians and the range between the 1st and 3rd quartile, respectively; the whiskers span the 1.5 interquartile ranges. Black dots represent individual values; $^{**}P < 0.01$; $^{***}P < 0.001$.

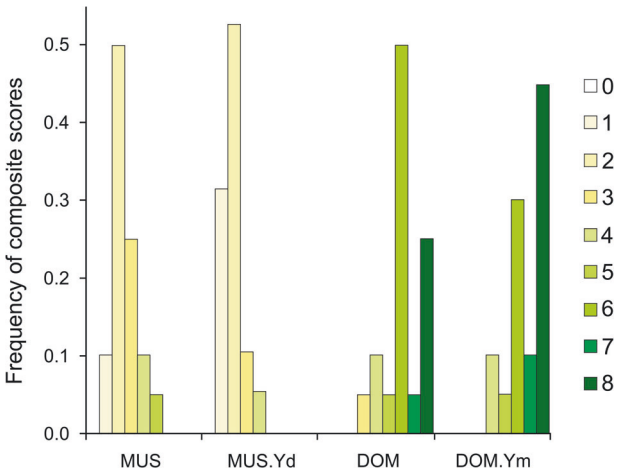

Fig. 4 Frequencies of the composite scores summed over all four dyadic encounters differ between the MUS and DOM genetic backgrounds. The legend to the right of the chart indicates the fighting ability, ranging from the least aggressive (light yellow) to the most aggressive (dark green) males.

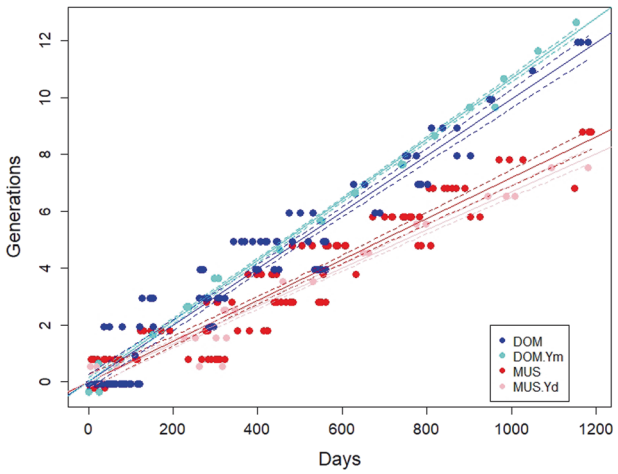

Fig. 5 Rates of increase of generations in time of the four male groups. Data points are rescaled to start from zero (for details, see the main text; see also Tables S2, Supplementary Material online).

observed in MUS.Y^d males, which gained only 75% of the composite scores of MUS males, this drop being significant at the 5% level (Wilcoxon test: $Z = -2.02$, $P = 0.04$). By contrast, a positive effect of the Y substitution was revealed in the DOM.Y^m males who won by almost 10% more encounters than the DOM males though this difference was not significant (Wilcoxon test: $Z = 1.36$, $P = 0.17$).

Generation turnover

We found a significantly faster generation turnover in males of the *domesticus* genetic background (DOM, DOM.Y^m) relative to males of the *musculus* background (MUS, MUS.Y^d) ($F_{(3,369)} = 4533$, $P \ll 0.001$, Fig. 5). By contrast, we found no significant effect of Y chromosome (DOM: $P = 0.126$; MUS: $P = 0.047$; Bonferroni-adjusted $\alpha = 0.0125$). Similarly, although pairwise comparisons of all males'

groups computed using estimated marginal means revealed significant contrasts between the DOM and MUS backgrounds, the effects of Y chromosomes within each background appeared non-significant (Table S2, Supplementary Material online).

Discussion

Although Y chromosome introgression is not a completely unknown phenomenon in mammals (e.g. *Odocoileus*: Cathey et al. 1998; *Spermophilus*: Ermakov et al. 2006; *Macaca*: Bonhomme et al. 2009; *Canis*: Wheeldon et al. 2013; *Papio*: Chiou 2017), the introgression of *M. m. musculus* Ys into *M. m. domesticus* territory in Central Europe (Macholán et al. 2008, 2019; Ďureje et al. 2012) has important evolutionary implications and thus raises questions about its underlying mechanisms. In this study, instead of analyzing natural hybrids of heterogeneous genetic background, we partitioned the effects of Y-linked genes by producing two consomic inbred strains, one derived from a *M. m. musculus* strain and possessing the *domesticus* Y^{NPAR} (BULS.STRA-ChrY, in this paper MUS.Y^d in short) and the other derived from a *M. m. domesticus* strain and carrying *musculus* Y^{NPAR} (STRA.BULS-ChrY, DOM.Y^m in short). These substitution strains were then compared with the respective parental strains BULS (MUS) and STRA (DOM). This approach is especially helpful in cases where one of the hybrid types (here *musculus* males with introgressed *domesticus* Y chromosomes) is missing or extremely rare in nature. We focused on sperm count (SC) and occurrence of dissociated sperm heads (DSH), as well as other phenotypic traits that can contribute to fitness differences between the strains: body weight at five subsequent time points of the males' life span, body length, testis weight, fighting success and generation turnover.

We found that transmission of *domesticus* (STRA) Y^{NPAR} onto *musculus* (BULS) genetic background resulted in deterioration of *all* the traits under study. In particular, the MUS.Y^d males tended to be smaller, with smaller testes (both absolutely and relatively) and with less sperm. In addition, these males appeared to be less successful in dyadic encounters with the *musculus* strain and to have slower generation turnover. Although not all these detrimental effects were statistically significant, we found *no* case of an opposite outcome in these males. These results are consistent with the strong barrier to *M. m. domesticus* Y chromosome introgression found in the European house mouse hybrid zone (Vanlerberghe et al. 1986; Tucker et al. 1992; Dod et al. 2005; Macholán et al. 2019) and in agreement with Haldane's rule. By contrast, the consequences of transmission of *musculus* (BULS) Y^{NPAR} onto *domesticus* genetic background were less unequivocal. While the consomic males were, on average, significantly smaller and produced less sperm than males of the parental strain (DOM), their testis weights were significantly higher, and the frequency of dissociated sperm heads was (non-significantly) lower. In addition, the DOM.Y^m males were most successful in dyadic encounters of all four strains. Finally, although the substitution of the Y^d chromosome resulted in a slower turnover, transfer of the Y^m had a non-significant positive effect, leading to the fastest turnover of all the strains.

The connection between sperm-associated traits and fitness seems rather straightforward. For example, Albrechtová et al. (2012) and Turner et al. (2012) found lower sperm count and its motility in males from the hybrid zone centre. These hybrids are known, based on indirect genetic evidence (Raufaste et al. 2005; Macholán et al. 2007), to have reduced fitness relative to parental populations. However, Albrechtová et al. (2012) showed that in males of predominantly *domesticus* genetic background possessing introgressed *musculus* Y chromosomes, sperm count was even higher than in *domesticus* males with their own (consubspecific) Y chromosomes. This observation appears to be consistent with the spread of *musculus* Y chromosomes across and far behind the zone centre. Interestingly, here we found a significant decrease in sperm count not only in MUS.Y^d males but also in DOM.Y^m individuals. This finding either suggests that the Y alone is not sufficient for the SC rescue in *domesticus* males with introgressed *musculus* Y chromosomes, or represents another piece of evidence on polymorphism of the Y-linked effects on sperm count recently detected by Martincová et al. (2019a).

Another important sperm-related trait is the frequency of spermatozoa with dissociated tails (DSH). In this paper, we confirmed the results of Martincová et al. (2019b) who found higher DSH in males carrying *domesticus* Y chromosomes relative to those possessing *musculus* Ys. More importantly, we found a marked, though non-significant, improvement of sperm development in DOM.Y^m males who displayed similar, or even a slightly lower, DSH than that of the donor MUS strain (Fig. S2D, Supplementary Material online). A substantial increase in the occurrence of tailless spermatozoa in a sperm batch may have deleterious consequences for a male's fitness. While a male with a moderate or even high proportion of deformed spermatozoa can still be able to fertilize a female or a few females, his reproductive success might be reduced when the number of females is higher, or if females can mate with multiple males, which is the case for both house mouse subspecies (Dean et al. 2006; Manser et al. 2011; Thonhauser et al. 2014; own unpublished data).

Sperm can also be disqualified by head deformations resulting from aberrant development. For example, in vivo fertilization experiments demonstrated that the uterus

junction acts as a barrier preventing deformed sperm reaching the eggs (Krzanowska 1974; Nestor and Handel 1984). Martincová et al. (2019a) found increased occurrence of abnormal sperm heads (ASH) in F1 hybrids between females of wild *musculus*-derived inbred strains (*musculus* WDS, 12 strains) and males of *domesticus* WDS (16 strains), in agreement with our results for dissociated sperm. However, the authors reported the same effect also for F1s of reciprocal crosses, in contradiction to our DSH data revealing the opposite trend (Fig. S2D, Supplementary Material online). However, it is not clear how the data on laboratory F1 hybrids are relevant to the mouse hybrid zone as these individuals are absent or extremely rare in nature (Macholán et al. 2007, 2019). It should be also noted that, like in sperm count, the causes of increased occurrence of abnormal sperm heads are likely to have a more complex genetic basis. This is suggested both by the low proportion of total variance in this trait explained by the Y chromosome and by great differences in ASH between various recombinant inbred strains sharing the same Y (Martincová et al. 2019b).

The spread of Y chromosomes can also be facilitated by an increased ability of their bearers to outcompete rival males. Many studies consistently found *M. m. domesticus* to be more aggressive than *M. m. musculus* (Thuesen 1977; van Zegeren and van Oortmerssen 1981; Volfová et al. 2002; Frynta et al. 2005). This difference is retained during the inbreeding process (Piálek et al. 2008; Ďureje et al. 2011; this study) and cannot be explained by postnatal maternal effects (Ďureje et al. 2011). The level of aggressiveness is usually tested using various measures, the most frequent being attack latency and frequency of attacks. However, it has been shown that aggression is a complex behaviour (for reviews, see Maxson 2009; and Maxson et al. 2013) consisting of diverse components (e.g. tail rattling, chase, sideways and upright posture and attack), which can be controlled by different genes. Moreover, differences have been found between offensive vs. defensive aggression or between male–male, female–male or female–female offense, and depend on the way aggression is defined and/or quantified (Catlett 1961; Guillot and Chapouthier 1996; Roubertoux et al. 2005; Maxon et al. 2013). More importantly, we believe that it is more crucial for a male to finally win (or lose) a confrontation, than how quickly he starts to fight or how many times he attacks the opponent. For this reason, we measured the total score of wins/losses across four types of encounters involving the neutral cage test and resident–intruder test. While MUS.Y^d males performed significantly worse than MUS males, transmission of Y^m chromosome onto the DOM genetic background resulted in 10% increase in fighting success, despite the fact that these males were smaller, and the outcome of male–male interactions was correlated with body weight (Bartoš and Brain 1986; Hilakivi-Clarke and Lister 1992). The fact that substitution of the Y leads to changes in the level of aggressiveness is consistent with the notion that this chromosome harbours genes involved in aggressive behaviour: if it was determined predominately by autosomal loci, we should expect the same levels of aggressiveness in consomics as in the parental strains.

Relevance to the house mouse hybrid zone

Throughout this paper, we have repeatedly pointed to the fact that our results are in agreement with the unidirectional *musculus* Y introgression. However, it may be argued that the traits under study cannot, *per se*, explain this phenomenon. For example, although higher aggressiveness of the DOM.Y^m males relative to the DOM males can yield an advantage of the former over the latter, at the same time, DOM males are more aggressive than the MUS (and even more so than the MUS.Y^d) males. So, following the same logic, we should expect *M. m. domesticus* males to spread in expense of *M. m. musculus* males in areas without the *musculus* Y introgression, a phenomenon for which there is no evidence. Nevertheless, we saw that, while the transmission of the *domesticus* Y chromosome resulted in negative effects in *all* the traits, the reciprocal transfer had positive effects not only on fighting performance but also on the proportion of dissociated sperm heads and generation turnover. Although the improvements were not always significant, the trend is apparent. In agreement with Martincová et al. (2019b), our results suggest that the *musculus* Y introgression involves several mechanisms and genes, most probably including autosomal loci. On the other hand, we must be aware that we tested just two Y chromosomes and backgrounds, whereas there are multiple Y chromosome variants within and around the zone, though not all of them were found to introgress (Macholán et al. 2019). Since this genetic Y-linked polymorphism has consequences on phenotypic variation of fitness-related traits (Martincová et al. 2019b), the picture is likely to be more complex than can be deciphered with the consomic strains.

Another potential objection to our study may be that it does not distinguish between the pseudoautosomal (Y^{PAR}) and non-recombining (Y^{NPAR}) part of Y chromosome. The reason for this drawback is the difficulty to find suitable diagnostic markers in the Y^{PAR} segment. Nevertheless, the number of functional genes in the Y^{PAR} is extremely low (Soh et al. 2014; Morgan and Pardo-Manuel de Villena 2017), and involvement of this region in Y chromosome introgression is thus unlikely. It could also be argued that the two inbred strains used here as surrogates of both mouse subspecies cannot capture the whole variability of wild populations. This argument is correct. However, if nothing else, we at least show that the spread of the *musculus* Y

chromosome into *domesticus* territory is a multifaceted phenomenon, and that its study should require a multidisciplinary approach.

Data availability

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.brv15dv6w.

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Author contributions BVB and JP conceived and supervised the project. BVB and ĽĎ carried out the behavioural experiments. JP and ĽĎ dissected the animals and prepared samples for molecular analyses and JP and IM assessed sperm count and the proportion of dissociated sperm heads. Statistical analyses were performed by BVB, JP, MM and KB. The paper was written by MM, JP and BVB.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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