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# Maternal–foetal genomic conflict and speciation: no evidence for hybrid placental dysplasia in crosses between two house mouse subspecies

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## Abstract

Interspecific hybridization between closely related mammalian species, including various species of the genus *Mus*, is commonly associated with abnormal growth of the placenta and hybrid foetuses, a phenomenon known as hybrid placental dysplasia (HPD). The role of HPD in speciation is anticipated but still poorly understood. Here, we studied placental and foetal growth in $F_1$ crosses between four inbred mouse strains derived from two house mouse subspecies, *Mus musculus musculus* and *Mus musculus domesticus*. These subspecies are in the early stage of speciation and still hybridize in nature. In accordance with the maternal–foetal genomic conflict hypothesis, we found different parental influences on placental and foetal development, with placental weight most affected by the father's body weight and foetal weight by the mother's body weight. After removing the effects of parents' body weight, we did not find any significant differences in foetal or placental weights between intra-subspecific and inter-subspecific $F_1$ crosses. Nevertheless, we found that the variability in placental weight in inter-subspecific crosses is linked to the X chromosome, similarly as for HPD in interspecific mouse crosses. Our results suggest that maternal–foetal genomic conflict occurs in the house mouse system, but has not yet diverged sufficiently to cause abnormalities in placental and foetal growth in inter-subspecific crosses. HPD is thus unlikely to contribute to speciation in the house mouse system. However, we cannot rule out that it might have contributed to other speciation events in the genus *Mus*, where differences in the levels of polyandry exist between the species.

## Introduction

One approach towards understanding the mechanisms of speciation is to study the nature of reproductive barriers that contribute to genetic isolation between species (Coyne & Orr, 2004). The rate at which various forms of reproductive barriers evolve varies greatly among lineages. In mammals, hybrid inviability seems to arise especially quickly compared to other vertebrates (Wilson *et al.*, 1974; Fitzpatrick, 2004). It has been suggested that maternal–foetal genomic conflict over the allocation of maternal resources to developing foetuses could cause this rapid evolution of post-zygotic isolation in mammals (Zeh & Zeh, 2000; Elliot & Crespi, 2006).

Maternal–foetal genomic conflict arises in viviparous mammals, where offspring develop within the mother's body and obtain the necessary nutrients through the placenta (Zeh & Zeh, 2008). In such situations, the evolutionary interest of individual foetuses (or their fathers) is to maximize the nutrient transfer from the mother to foetuses, whereas mothers tend to limit supplying excessive nutrients to foetuses and allocate resources evenly among all her (present and possibly future) progeny (Haig, 1993). Maternal–foetal genomic conflict in mammals is mostly mediated by genomic imprinting, an epigenetic phenomenon, whereby some genes are monoallelically expressed according to the

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parent of origin (Barlow & Bartolomei, 2014). Imprinted genes contribute to diverse processes in development including the foetal growth (Peters, 2014). Maternal–foetal genomic conflict is predicted to be especially intense in polyandrous species where individual foetuses within a single brood or between subsequent broods have different fathers. Perpetual antagonistic coevolution between genes involved in maternal–foetal conflicts can cause the rapid divergence of those genes in different species. This can in turn result in increased or decreased placental and foetal growth in interspecific crosses, a phenomenon known as hybrid placental dysplasia (HPD). In the most extreme form, HPD may lead to the premature death of hybrid foetuses and can thus constitute a post-zygotic reproductive barrier.

Hybrid placental dysplasia and the related parent-of-origin growth phenotypes have been described in numerous mammalian interspecific crosses (Gray, 1971; Vrana, 2007; Wolf *et al.*, 2014; Brekke & Good, 2014), and it has thus been suggested that maternal–foetal genomic conflict might play a crucial role in mammalian speciation (Zeh & Zeh, 2000, 2008). This view is supported by the finding that the genes involved in pregnancy and in the control of foetal nutrient allocation are among the fastest evolving genes in the mammalian genome (Castillo-Davis *et al.*, 2004; Wildman, 2011). However, it should be noted that the majority of the known examples of HPD involve quite divergent species that do not hybridize in nature. It is thus unclear whether HPD constitutes a primary reproductive barrier contributing to speciation or arises as a result of post-speciation divergence.

The best studied examples of HPD occur in the rodent genera *Mus* (Zechner *et al.*, 1996) and *Peromyscus* (Rogers & Dawson, 1970). In the genus *Mus*, decreased placental size was found in crosses between *Mus musculus* females and the males of three other *Mus* species that occur in Europe, *Mus spretus*, *Mus macedonicus* and *Mus spicilegus*, whereas the opposite phenotype, increased placental size, was observed in the reciprocal crosses (Zechner *et al.*, 1996). Genetic studies revealed that the synergic action of multiple loci on the X chromosome causes the placental overgrowth (Zechner *et al.*, 1996; Hemberger *et al.*, 1999). It has been suggested that epigenetic modification of the X chromosome might be a proximate mechanism behind HPD in mice, although empirical evidence for this has not yet been found (Hemberger *et al.*, 1999, 2001; Schütt *et al.*, 2003). In addition, loss of imprinting at several loci has been observed in interspecific mouse hybrids from both reciprocal crosses (Shi *et al.*, 2004, 2005). Also in crosses between *Peromyscus maniculatus* and *Peromyscus polionotus*, a genomewide loss of imprinting with the contribution of an X-linked locus is responsible for parent-of-origin growth defects of $F_1$ hybrid foetuses and their placentas (Vrana *et al.*, 1998, 2000; Zechner *et al.*, 2004; Wiley *et al.*, 2008).

Here, we studied placental and foetal growth in hybrids between two house mouse subspecies, *Mus musculus domesticus* and *M. m. musculus*, which are in the early stage of speciation and still hybridize in nature (Boursot *et al.*, 1993; Duvaux *et al.*, 2011; Macholán *et al.*, 2012). These subspecies (sometimes referred to as species) diverged approximately 350 000 years ago (Geraldes *et al.*, 2011) and subsequently came into secondary contact along a narrow hybrid zone spanning across Europe (Payseur *et al.*, 2004; Macholán *et al.*, 2007, 2011; Wang *et al.*, 2011; Ďureje *et al.*, 2012; Janoušek *et al.*, 2012). Crosses between laboratory inbred strains derived from these subspecies show that, in accord with Haldane's rule, $F_1$ hybrid males are often sterile, whereas $F_1$ hybrid females are fertile (Forejt *et al.*, 2012). Reduced male fertility has also been observed in wild mice in the hybrid zone (Albrechtová *et al.*, 2012; Turner *et al.*, 2012). Furthermore, partial hybrid female sterility (Britton-Davidian *et al.*, 2005) and the reinforcement of mating-recognition systems (Smadja & Ganem, 2005; Vošlajerová Bímová *et al.*, 2011; Latour *et al.*, 2014) might contribute to reproductive isolation between these two subspecies. Interestingly, some part of the hybrid zone dynamics was suggested to be affected by genetic conflict (Macholán *et al.*, 2008), but the mechanism of this conflict remains unknown.

Here, we ask whether HPD occurs between *M. m. musculus* and *M. m. domesticus* subspecies and might thus contribute to their speciation. In addition, we assessed whether the genetic control of placental and foetal growth in house mouse inter-subspecific crosses is the same as for HPD observed in crosses between more distantly related *Mus* species.

## Materials and methods

### Animals and crosses

Four inbred or partially inbred mouse strains derived from two house mouse subspecies were used in this study: C57BL6/J (B6), SCHEST, STUS and PWD/Ph (PWD). B6 is a classical inbred strain predominantly of *M. m. domesticus* origin (Yang *et al.*, 2011) and was purchased from Velaz s.r.o (Lysolaje, Czech Republic). SCHEST mice were recently derived from a natural population of *M. m. domesticus* in Schweben, Central Germany (N: 50°26′, E: 9°35′), and were at the 7th–8th generation (G7–G8) of brother–sister mating. PWD is a wild-derived inbred strain, which was isolated from a natural population of *M. m. musculus* in Kunratice near Prague, the Czech Republic, and is maintained at the Department of Mouse Molecular Genetics, Institute of Molecular Genetics, Prague, the Czech Republic (Gregorová & Forejt, 2000). The second *M. m. musculus* representative was the STUS strain derived from a natural population in Studenec, the Czech Republic (Piálek *et al.*, 2008); these mice were at G22–G24

during the experiment. The SCHEST and STUS strains were maintained at the Research Facility Studenec, Institute of Vertebrate Biology, the Czech Republic. The SCHEST strain went to extinction at G14.

Crosses among the mouse strains were conducted at the Faculty of Science, Charles University in Prague, and the Research Facility Studenec. We carried out two types of $F_1$ crosses: inter-subspecific crosses (B6 × PWD, PWD × B6, SCHEST × PWD and PWD × SCHEST; females are always mentioned first) and intra-subspecific crosses (B6 × SCHEST, SCHEST × B6, PWD × STUS and STUS × PWD). Intra-subspecific crosses between two different inbred strains within each species are important for removing the effects of inbreeding depression. All inter-subspecific crosses were previously shown to yield sterile $F_1$ hybrid males (Gregorová & Forejt, 2000; Piálek *et al.*, 2008; J. Piálek, unpublished data). Furthermore, we performed two types of backcrosses where $F_1$ hybrid females (PWD × B6 or B6 × PWD) were mated with PWD or B6 males. Finally, we performed two intrastrain crosses (PWD × PWD and B6 × B6) as controls. In each cross, adult virgin females (2–10 months old) were mated with adult males overnight and then separated. Pregnant females were killed by cervical dislocation on embryonic day 18. Foetuses and placentas were dissected and weighed. A small sample of each foetus was collected for sex determination and genotyping.

The average male and female body weights of each strain to be used in the statistical analyses were determined based on 6–21 adult individuals 3–7 months old, with average age ranging between 133 and 156 days. The average body weights were larger for the *M. m. domesticus*-derived strains (26.79 and 25.95 g for B6, 28.57 and 28.17 g for SCHEST, for males and females, respectively) compared to the *M. m. musculus*-derived strains (17.72 and 17.46 g for PWD, 17.52 and 16.57 g for STUS, for males and females, respectively). Similar differences in body weight were observed also for wild animals of both subspecies as well as for other inbred strains derived from these subspecies (Piálek *et al.*, 2008; J. Piálek, unpublished data).

Mice were kept conventionally in the breeding facilities of the Institute of Vertebrate Biology AS CR in Studenec (license 6628/2008-10001) and Charles University in Prague (license 24773/2008-10001) in accordance with animal welfare regulations of the Czech Republic's Act for Experimental Work with Animals (Decree No. 207/2004 Sb., and the Acts Nos. 246/92 Sb., and 77/2004 Sb.), which are fully compatible with the corresponding EU standards.

### Sex determination and genotyping of mouse foetuses

DNA from mouse foetuses was isolated using a NaOH method as described in Storchová *et al.* (2004). Briefly, a small piece of tissue was added to 600 μL of 50 mM NaOH, heated to 95 °C for 90 min, vortexed and neutralized with 50 μL of 1 M Tris (pH 8). The sex of individual mouse foetuses was determined by multiplex PCR amplification using two sets of primers amplifying the male-specific *Sry* gene and the autosomal *IL3* gene serving as an internal control of PCR amplification. Primer sequences and PCR conditions are described in Lambert *et al.* (2000).

Genotyping of backcross individuals was performed using three polymorphic microsatellite markers, DXMit55, DXSR51 and DXMit197, lying in the proximal (7.4 Mbp or 3.3 cM), central (67.8 Mbp or 34.8 cM) and distal (151.5 Mpb or 69.4 cM) regions of the X chromosome, respectively. Marker positions were determined according to the mouse assembly GRCm38 and MGI mouse genetic map (Bult *et al.*, 2008). PCR amplification of microsatellite markers was performed as described in Storchová *et al.* (2004) and Bhattacharyya *et al.* (2014). Data on foetal and placental weights as well as all genotypes are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.3v321.

### Statistical analyses

To explore factors affecting foetal and placental weights in the $F_1$ crosses, we performed linear mixed-effects models with the following fixed effects: the cross type (i.e. inter-subspecific or intra-subspecific), the sex of individual foetuses and the average body weight of maternal and paternal strains. Litter identity (i.e. the litter to which the foetus belonged) was treated as a random effect in the models to control for correlations within litters. The linear mixed-effects models were fitted using the lme function in the R package nlme (R Core Team, 2014). To compare the effects of the average body weights of maternal and paternal strains on placental and foetal weights, we standardized all four variables across samples to obtain variables with zero mean and unit variance. This standardization provides effect sizes easily comparable among focal explanatory variables (Schielzeth, 2010).

### QTL analysis

R 3.1.0 (R Core Team, 2014) and its qtl package (Broman *et al.*, 2003; Broman & Sen, 2009) were used to perform QTL mapping. Standard interval mapping was implemented using the scanone function. Placental and foetal weights were modelled as continuous variables. Genotype probabilities between markers were calculated at a grid size of 5 cM and genotyping error rate of 5%. Significant ($P < 0.05$) and suggestive ($P < 0.63$) logarithms of the odds ratio (LOD) chromosome-wide thresholds were chosen as widely accepted cut-offs (Lander & Kruglyak, 1995). They were calculated by 20 000 permutations.

## Results

### Comparisons of placental and foetal weights in intra- and inter-subspecific $F_1$ crosses

We collected data on foetal and placental weights in four different intra-subspecific and four different inter-subspecific $F_1$ crosses. After excluding two litters consisting of only one foetus and five individual foetuses, which were considerably smaller than other foetuses within the litter and showed clear signs of resorption, our data set consisted of 272 foetuses belonging to 39 litters (Table 1). Two excluded litters with one foetus belonged in one case to intra-subspecific and in the second case to inter-subspecific crosses. All five excluded individual foetuses occurred in the PWD × B6 or PWD × SCHEST inter-subspecific crosses, that is where relatively small *M. musculus* female was mated with large *M. domesticus* male. Nevertheless, both these inter-subspecific crosses showed average litter sizes comparable to other crosses (Table 1). Moreover, the average litter sizes did not differ significantly between intra- and inter-subspecific $F_1$ crosses (*t*-test, $P < 0.05$), further suggesting that early staged spontaneous abortions are quite rare in our crosses.

A comparison of foetal and placental weights among different intra- and inter-subspecific $F_1$ crosses showed that intra-subspecific crosses between *M. m. domesticus* strains (i.e. B6 × SCHEST and SCHEST × B6) had larger foetuses and placentas than intra-subspecific crosses between *M. m. musculus* strains (i.e. PWD × STUS and STUS × PWD) (Fig. 1). This pattern is consistent with the fact that adult mice from the strains derived from *M. m. domesticus* are larger than those from *M. m. musculus* strains (see Materials and methods). The mean values of both foetal and placental weights for all inter-subspecific crosses (i.e. B6 × PWD, PWD × B6, SCHEST × PWD and PWD × SCHEST) were within the range of mean values observed in intra-subspecific crosses (Fig. 1). This demonstrates that $F_1$ crosses between the house mouse subspecies do not show abnormally sized foetuses or placentas as was described in crosses between different *Mus* species (Zechner *et al.*, 1996).

To reveal possible more subtle changes in foetal and placental growth between inter- and intra-subspecific $F_1$ crosses, we performed linear mixed-effect models with the random effect of litter identity and fixed effects of cross type (i.e. $F_1$ inter-subspecific or $F_1$ intra-subspecific), sex of individual foetuses and average weights of the parental strains. We found a significant effect of maternal strain weight on foetal weight ($F_{1,35} = 75.71$, $P < 0.0001$) as well as placental weight ($F_{1,35} = 12.27$, $P = 0.0013$). Paternal strain weight also had a significant effect both on foetal weight ($F_{1,35} = 33.33$, $P < 0.0001$) and on placental weight ($F_{1,35} = 69.06$, $P < 0.0001$). Interestingly, foetal weight was more strongly influenced by the mother's weight ($\beta = 0.72$, SE = 0.08) than the father's weight ($\beta = 0.48$, SE = 0.09). By contrast, placental weight was more affected by the father's weight ($\beta = 0.74$, SE = 0.09) than the mother's weight ($\beta = 0.33$, SE = 0.09). When the weights of parental strains were taken into account, we found no significant effect of cross type on either foetal weight ($F_{1,35} = 0.13$, $P = 0.7205$) or placental weight ($F_{1,35} = 1.50$, $P = 0.2288$). The sex of foetuses did not have a significant effect on placental weight ($F_{1,232} = 2.40$, $P = 0.1227$), but significantly affected foetal weight ($F_{1,232} = 7.44$, $P = 0.0069$). Male foetuses were consistently larger (difference between least square means = 0.09, SE = 0.03) in all inter-subspecific as well as in intra-subspecific crosses (Fig. 1).

We also checked whether there were any deviation from sex ratio parity in the eight analysed $F_1$ crosses (Table 1). In the total data set, we found slightly more male foetuses than female foetuses (143 males: 129 females), giving a sex ratio of 1.11. In individual $F_1$ crosses, the male : female sex ratios varied from 0.77 to 2.25, but were not significantly different from an equal sex ratio in any cross (chi-square test, $P > 0.05$).

### QTL analysis of placental and foetal weights in $BC_1$ generations

Theoretically, if HPD is caused by recessive incompatibilities, it could be masked in $F_1$ crosses, but could appear in backcross generations. To explore this possibility, we analysed placental and foetal weights in 89 foetuses

**Table 1** Numbers of analysed litters and foetuses, average litter sizes and male-to-female sex ratios in intra- and inter-subspecific $F_1$ crosses.

| Cross | Cross type | No. of litters | No. of foetuses | Average litter size | M : F sex ratio |
|---|---|---|---|---|---|
| B6 × SCHEST | Intra-subspecific | 5 | 33 | 6.6 | 0.94 |
| SCHEST × B6 | Intra-subspecific | 4 | 16 | 4.0 | 1.00 |
| PWD × STUS | Intra-subspecific | 5 | 46 | 9.2 | 1.09 |
| STUS × PWD | Intra-subspecific | 5 | 26 | 5.2 | 2.25 |
| B6 × PWD | Inter-subspecific | 5 | 39 | 7.8 | 0.77 |
| PWD × B6 | Inter-subspecific | 5 | 35 | 7.0 | 1.19 |
| SCHEST × PWD | Inter-subspecific | 5 | 38 | 7.6 | 1.11 |
| PWD × SCHEST | Inter-subspecific | 5 | 39 | 7.8 | 1.17 |

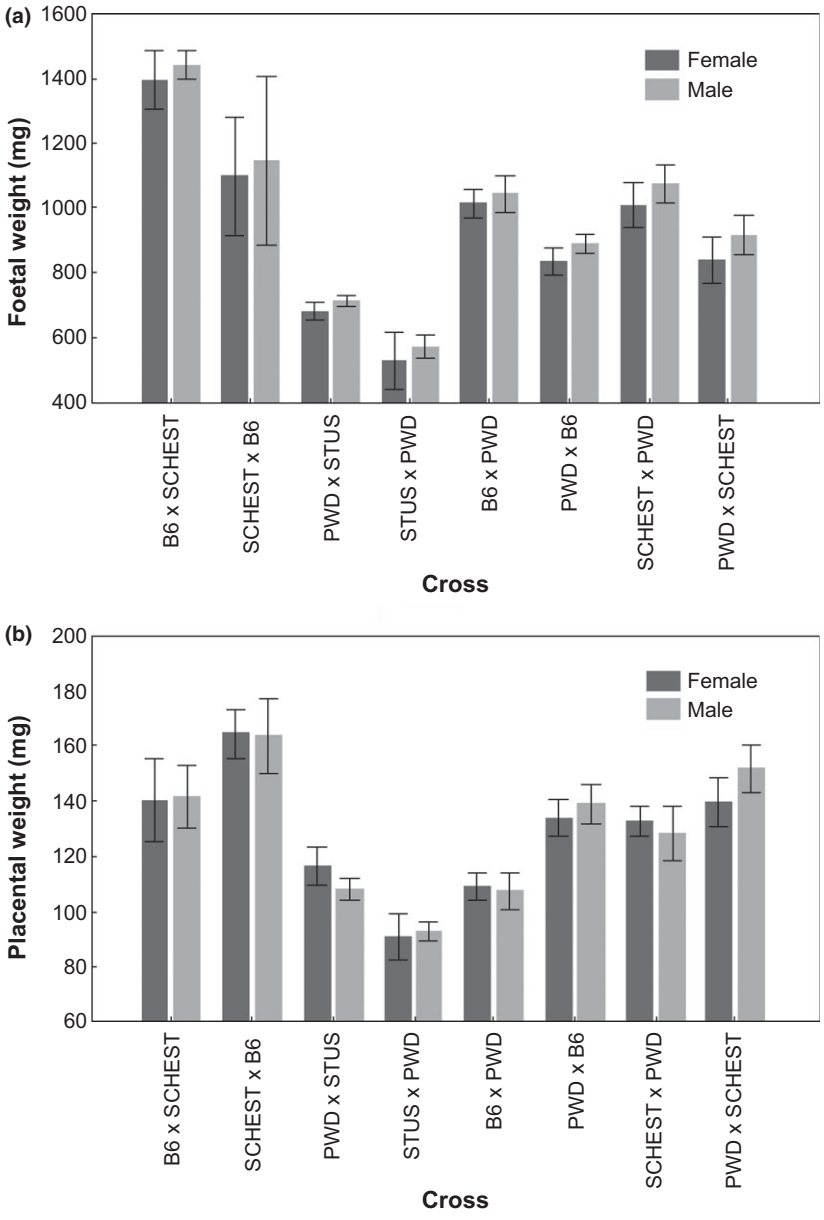



**Fig. 1** Mean foetal (a) and placental (b) weights in intra-subspecific and inter-subspecific $F_1$ crosses. Whiskers represent 0.95 confidence intervals.

belonging to ten litters in a (B6 × PWD) × B6 backcross and 107 foetuses belonging to 13 litters in a (B6 × PWD) × PWD backcross. As expected, the variance in placental and foetal weights in both types of backcrosses was higher than in $F_1$ crosses (B6 × PWD and PWD × B6) as well as in control intra-strain crosses (B6 × B6 and PWD × PWD). Nevertheless, placental as well as foetal weights in both types of backcrosses showed normal distributions and the vast majority of observed values were within the range of observed values in $F_1$ and intra-strain crosses, suggesting that HPD does not regularly occur in hybrid backcross generations.

Even though abnormally sized foetuses and placentas did not occur in hybrid backcross generations, the observed variability in foetal and placental weights could have a genetic basis. To study whether the X chromosome controls foetal and placental growth in inter-subspecific crosses, we genotyped 89 foetuses (49 males and 40 females) from the (B6 × PWD) × B6 backcross and 107 foetuses (50 males and 57 females) from the (B6 × PWD) × PWD backcross using three X-linked markers lying in the proximal, central and distal region of the X chromosome. We found no significant effect of the X chromosome genotype on the foetal weight in either type of backcross. QTL analysis for both sexes together, however, revealed a marginally significant effect of the X chromosome genotype on the placental weight in both types of backcrosses (Fig. 2).

This effect was mainly caused by the increased placental weight of male foetuses with PWD alleles on the X chromosome. In the (B6 × PWD) × B6 backcross, male foetuses with the PWD allele in the proximal and central marker showed significantly larger placentas than foetuses with B6 alleles (*t*-tests, $P = 0.027$, $P = 0.042$, respectively; Fig. 2a). In the (B6 × PWD) × PWD backcross, male foetuses with the PWD allele in the proximal and distal marker showed significantly larger placentas than foetuses with B6 alleles (*t*-tests, $P = 0.048$, $P = 0.020$, respectively; Fig. 2b). The female foetuses showed generally larger placentas when they had heterozygous PWD/B6 genotypes on the X chromosome than homozygous BB or PP genotypes, although the difference in placental weight for foetuses with different genotypes was not significant for any particular marker (*t*-tests, $P > 0.05$; Fig. 2).

## Discussion

Genomic conflicts can cause rapid divergent evolution and are thus assumed to play an important role in the origin of reproductive isolation and speciation (Crespi & Nosil, 2013). Here, we studied the role of maternal–foetal genomic conflict in the evolution of post-zygotic reproductive isolation between two recently diverged house mouse subspecies, *M. m. musculus* and *M. m. domesticus*.

Analysis of foetal and placental weights in eight inter- and intra-subspecific $F_1$ crosses showed that foetal weight was more affected by the mother's body weight than the father's body weight. By contrast, placental weight was more determined by the father's than the mother's body weight. To our knowledge, this is the first study where different maternal and paternal influences on foetal and placental development have been demonstrated in cross-breeding experiments. These parent-of-origin effects can explain very weak or no correlation between foetal and placental weights observed in interspecific mouse backcrosses (Kurz *et al.*, 1999). Our findings are also consistent with results of nuclear transplantation experiments in the house mouse. In these experiments, embryos with both sets of chromosomes inherited from the father (androgenetic embryos) or the mother (gynogenetic embryos) were created. Androgenetic embryos exhibit retarded embryonic development with well-developed extraembryonic membranes, whereas gynogenetic embryos are characterized by poorly developed extraembryonic membranes with a reasonably well-developed embryo proper (McGrath & Solter, 1984; Surani *et al.*, 1984). In chimeras with only some androgenetic and/or gynogenetic cells, androgenetic cells contribute strongly to the trophectoderm-derived tissues that form the outermost placental membrane, and only rarely contribute to any tissues of the embryo proper. By contrast, gynogenetic cells occur in all tissues of the embryo proper, but only rarely in the trophectoderm-derived tissues (Surani *et al.*, 1987, 1988; Thomson & Solter, 1988). This strong influence of the paternally inherited genome on placental development can be explained by the recently demonstrated over-representation of paternally expressed imprinted genes in the placenta (Wang *et al.*, 2013). Together, the observed differences in the function of maternal and paternal genomes in foetal and placental development lend support to the maternal–foetal genomic conflict hypothesis and suggest that this form of genomic conflict occurs in the house mouse subspecies.

When the effects of parental body weights were controlled for in our analyses, we observed no significant differences in foetal or placental weights between inter-subspecific and intra-subspecific $F_1$ crosses. This suggests that the genes involved in maternal–foetal conflict have not yet diverged sufficiently between the subspecies to cause HPD in $F_1$ crosses. However, based on our results, we cannot exclude that placentae in intra- and inter-subspecific crosses might slightly differ in their physiological functions or patters of gene expression. Abnormal placental and foetal development was also not observed in the $BC_1$ generation. We cannot, however, rule out that HPD may appear in some other hybrid generations, such as an $F_2$ cross, where recessive autosomal incompatibilities could be manifested. In fact, the overgrowth of foetuses and frequent death of females during delivery has been observed in one subconsomic mouse strain that has the proximal part of chromosome 10 from *M. m. musculus* (PWD strain) on the genetic background of *M. m. domesticus* origin (B6 strain) (Gregorová *et al.*, 2008). Unfortunately, placental size was not examined in this strain, and it is thus not clear whether the increased embryonic growth is the result of HPD or some other developmental incompatibilities. Furthermore, following the analogy with hybrid male sterility, where polymorphism in incompatibility genes has been recently demonstrated (Vyskočilová *et al.*, 2005, 2009; Good *et al.*, 2008a; Turner *et al.*, 2012), we cannot exclude that HPD may have evolved only recently and was not captured in the mouse strains used in this study. Finally, as the long-term inbreeding might affect epigenetic modifications of the genome as well as the intensity of maternal–foetal genomic conflict (Vergeer *et al.*, 2012), it would be desirable to supplement the results obtained in this study with studies on outbred animals sampled across wide ranges of both mouse subspecies.

Genetic analysis of the HPD in crosses between *M. spretus* and *M. musculus* has shown complex genetic control of this phenotype linked to the X chromosome (Zechner *et al.*, 1996; Hemberger *et al.*, 1999). In our study, we also revealed a marginally significant linkage of placental weight to the X chromosome in (B6 × PWD) × B6 and (B6 × PWD) × PWD backcrosses. This linkage was stronger in male foetuses, which

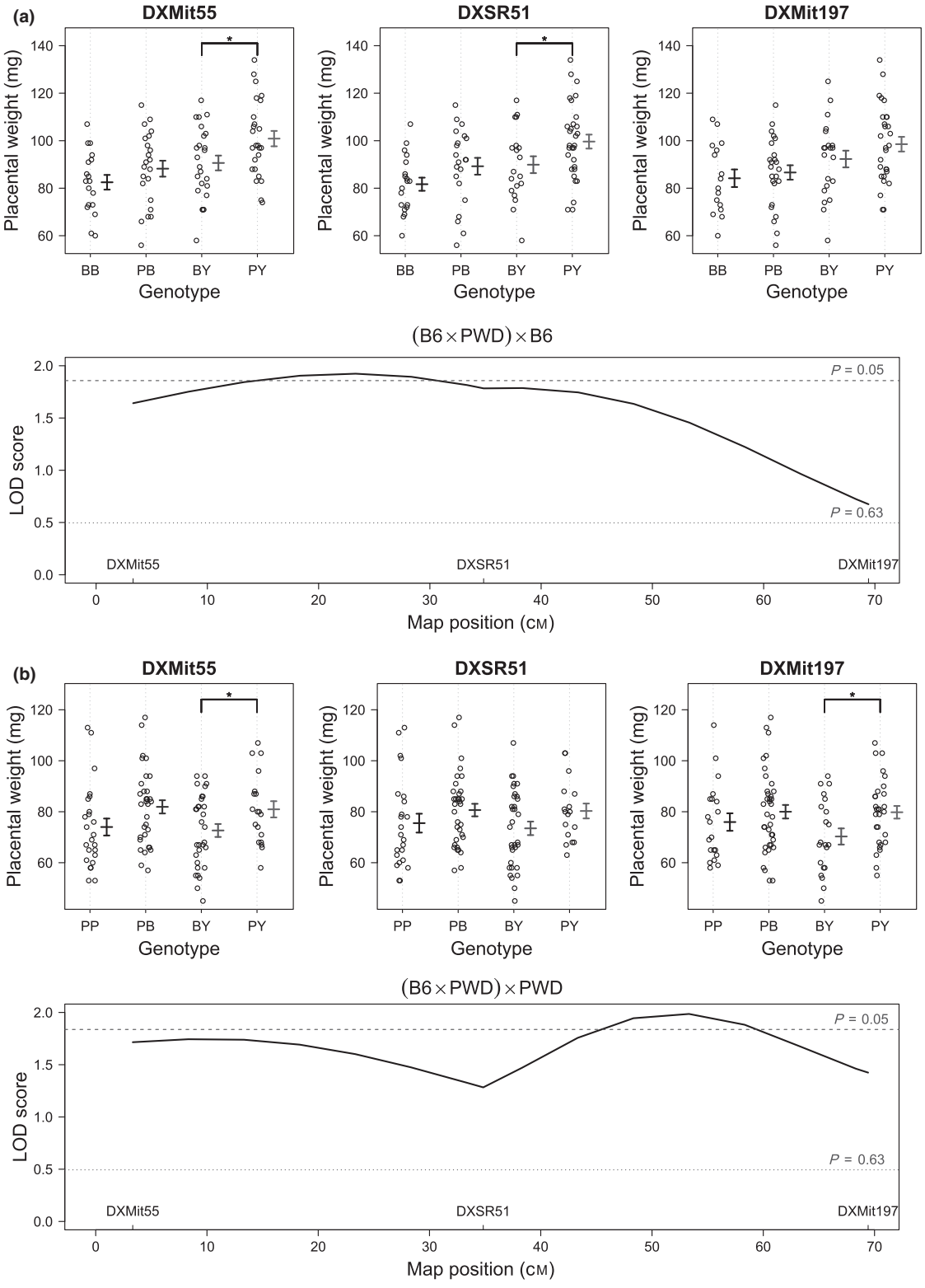
showed larger placentas when carrying the *M. m. musculus* (PWD) allele on the X chromosome. This sex difference in QTL effect is unexpected given the imprinted X chromosome inactivation, with gene expression occurring only from the maternally inherited X chromosome, in female placentas (Takagi & Sasaki, 1975). One possible explanation for this sex-biased QTL effect could be the escape from X chromosome inactivation, which has been demonstrated for some X-linked genes (Calabrese *et al.*, 2012). Alternatively, inactivation of the X chromosome in placenta might not be completely imprinted in inter-subspecific crosses. Although our QTL analysis was based on a small number of backcross individuals, and the low number of genetic markers used prevented us from precisely mapping the phenotype within the X chromosome, our results suggest that placental growth in hybrids between house mouse subspecies and hybrids between different *Mus* species might have a similar genetic basis. This further supports the view that maternal–foetal genomic conflict occurs in the house mouse system, but has not yet diverged sufficiently to cause abnormal phenotypes in inter-subspecific hybrids.

As we noted above, mating between PWD and B6 strains used in this study produces sterile hybrid males in the $F_1$ cross as well as in later backcrosses (Gregorová & Forejt, 2000; Storchová *et al.*, 2004; Bhattacharyya *et al.*, 2013) and interestingly, this phenotype was similarly mapped to the X-linked loci (Storchová *et al.*, 2004; Dzur-Gejdošová *et al.*, 2012; Bhattacharyya *et al.*, 2014). Remarkably, the genetic control of hybrid male sterility and HPD shows very similar features. The severity of both phenotypes is correlated with the length of the X chromosome region introgressed into the genetic background of different (sub)species (Hemberger *et al.*, 1999; Storchová *et al.*, 2004; Good *et al.*, 2008b; Oka & Shiroishi, 2012) and epigenetic dysregulation of the X chromosome has been suggested to be involved in both phenotypes, although empirical evidence for this in HPD has not yet been found (Hemberger *et al.*, 1999; Mihola *et al.*, 2009; Good *et al.*, 2010; Campbell *et al.*, 2013). However, it would be premature to deduce that similar molecular mechanisms might control both phenomena. Although our data do not rule out this interesting possibility, the observation that HPD does not occur in crosses between *M. m. musculus* and *M. m. domesticus* subspecies where hybrid male sterility has been observed suggests that both phenomena arise independently during evolution. In the *Mus* genus, HPD seems to arise in later stages of species divergence than hybrid male sterility and premating reproductive barriers.

Although our results show that abnormal placental growth is unlikely to significantly contribute to speciation in the house mouse subspecies, the question remains whether it might have contributed to some other speciation events in the genus *Mus*. According to the maternal–foetal genomic conflict hypothesis, HPD should evolve more rapidly in crosses between species that differ in levels of polyandry (Zeh & Zeh, 2000). Indeed, HPD observed in crosses between recently diverged *Peromyscus* species occurs in the cross between a monogamous species, *P. polionotus*, and a genetically polyandrous species, *P. maniculatus* (Wolff, 1989). The house mouse is socially polygynous (Wolff & Sherman, 2007) with a relatively high rate of extra pair paternity reaching 20–30% in both subspecies (Dean *et al.*, 2006; Manser *et al.*, 2011; Thonhauser *et al.*, 2014). Similar levels of genetic polyandry in both house mouse subspecies could explain why HPD evolves so slowly in this system. Unfortunately, our knowledge about the mating systems of the three other *Mus* species occurring in the Europe, *M. spretus*, *M. macedonicus* and *M. spicilegus*, which produce HPD in crosses with *M. musculus*, is limited. Several lines of evidence suggest that they could be mostly monogamous (Patris & Baudoin, 2000; Baudoin *et al.*, 2005; Cassaing & Isaac, 2007; Cassaing *et al.*, 2010). On the other hand, their high relative testes size compared to house mouse subspecies might indicate intensive sperm competition occurring in these species, contradicting their monogamy (Frynta *et al.*, 2009). Nevertheless, any changes in the levels of polyandry between the house mouse and other *Mus* species could result in faster evolution of HPD in their crosses, which might then play a role in speciation. Unfortunately, the species showing HPD in interspecific crosses are already separated by other prezygotic and/or post-zygotic barriers and do not regularly hybridize in nature (Auffray & Britton-Davidian, 2012; Suzuki & Aplin, 2012). The possibility that HPD acted as a primary reproductive barrier in at least some speciation events in the genus *Mus* is thus difficult to test.

Importantly, crosses between different house mouse subspecies were repeatedly used in studies of genomic imprinting in mammals (reviewed by Barlow & Bartolomei, 2014). If HPD occurred in these crosses, the results of such studies could be affected by more or less severe disruption of genomic imprinting as has been observed in crosses between different *Mus* and *Peromyscus* species (Vrana *et al.*, 1998; Shi *et al.*, 2004, 2005;

**Fig. 2** QTL analysis of placental weight in the backcrosses (B6 × PWD) × B6 (a) and (B6 × PWD) × PWD (b). Upper panel: placental mean weights ± SE are indicated for each marker and genotype. Comparisons of genotypes for each sex and marker were performed separately, and significance is indicated by star if unadjusted *P*-value < 0.05. Lower panel: single QTL scan for placental weight of both sexes together. Logarithms of the odds ratio (LOD) scores are plotted at 5-cM intervals. Significance ($P = 0.05$) and suggestive ($P = 0.63$) thresholds are indicated by the dashed and dotted line, respectively, and were derived from 20 000 permutations.


(a)
DXMit55
DXSR51
DXMit197
Placental weight (mg)
Genotype
BB
PB
BY
PY
(B6 × PWD) × B6
LOD score
P = 0.05
P = 0.63
DXMit55
DXSR51
DXMit197
Map position (cM)
(b)
PP
PB
BY
PY
(B6 × PWD) × PWD

Wiley *et al.*, 2008). Our results showing the absence of HPD in crosses between *M. m. musculus* and *M. m. domesticus* thus validate the use of this system as a general model for study of placental growth and parent-of-origin expression.

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