

Genome-wide architecture of reproductive isolation in a naturally occurring hybrid zone between *Mus musculus musculus* and *M. m. domesticus*

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Abstract

Studies of a hybrid zone between two house mouse subspecies (*Mus musculus musculus* and *M. m. domesticus*) along with studies using laboratory crosses reveal a large role for the X chromosome and multiple autosomal regions in reproductive isolation as a consequence of disrupted epistasis in hybrids. One limitation of previous work has been that most of the identified genomic regions have been large. The goal here is to detect and characterize precise genomic regions underlying reproductive isolation. We surveyed 1401 markers evenly spaced across the genome in 679 mice collected from two different transects. Comparisons between transects provide a means for identifying common patterns that likely reflect intrinsic incompatibilities. We used a genomic cline approach to identify patterns that correspond to epistasis. From both transects, we identified contiguous regions on the X chromosome in which markers were inferred to be involved in epistatic interactions. We then searched for autosomal regions showing the same patterns and found they constitute about 5% of autosomal markers. We discovered substantial overlap between these candidate regions underlying reproductive isolation and QTL for hybrid sterility identified in laboratory crosses. Analysis of gene content in these regions suggests a key role for several mechanisms, including the regulation of transcription, sexual conflict and sexual selection operating at both the postmating prezygotic and postzygotic stages of reproductive isolation. Taken together, these results indicate that speciation in two recently diverged (c. 0.5 Ma) house mouse subspecies is complex, involving many genes dispersed throughout the genome and associated with distinct functions.

Keywords: genomics/proteomics, hybridization, mammals, speciation

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Introduction

A crucial step in speciation according to the biological species concept (Dobzhansky 1937; Mayr 1942) is the

formation of reproductive barriers between emerging independently evolving lineages. Postzygotic reproductive barriers such as hybrid sterility and inviability are often caused by Bateson–Dobzhansky–Muller incompatibilities (BDMIs) (Bateson 1909; Dobzhansky 1936; Muller 1940, 1942; Coyne & Orr 2004). The BDMI model is based on the accumulation of mutations in allopatric populations that cause incompatibilities between interacting

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genes in hybrid genomes when the populations come together. Another common phenomenon associated with postzygotic isolation is the observation that the X chromosome plays a disproportionately large role in hybrid sterility, termed the large X-effect (Charlesworth *et al.* 1987; Coyne & Orr 1989; Masly & Presgraves 2007). The large X-effect seems to be a consequence of a higher density of BDMIs on the X chromosome than on the autosomes, although the underlying cause of this higher density is still unclear and may be due to faster evolution of the X, sex-ratio meiotic drive or the disruption of gene regulation on the X during spermatogenesis (Presgraves 2008). BDMIs between the X chromosome and the autosomes also provide an explanation for Haldane's rule (Muller 1940, 1942; Turelli & Orr 1995; Zeng 1996), a phenomenon whereby the heterogametic sex is the sex that suffers from reduced viability or fertility in hybrid genomes (Haldane 1922; Orr 1997). Haldane's rule together with the large X-effect may apply more generally to taxa with differentiated sex chromosomes (Coyne & Orr 2004).

Most of the pioneering work on the genetic basis of reproductive isolation has been performed on fruit flies of the genus *Drosophila* (Coyne & Orr 2004). The experiments support a prominent role of the X chromosome in the formation of postzygotic reproductive barriers (Coyne & Orr 1989). However, the importance of autosomal factors in the formation of reproductive barriers has also been confirmed (True *et al.* 1996; Orr & Irving 2001; Tao *et al.* 2003; Masly & Presgraves 2007; Lu *et al.* 2010).

The importance of the X chromosome in the formation of a reproductive barrier has also been documented in laboratory crosses of mouse strains (Oka *et al.* 2004, 2007; Storchová *et al.* 2004; Good *et al.* 2008a,b, 2010). Several QTL studies identified the central portion of the X chromosome as contributing to hybrid male sterility (Oka *et al.* 2004, 2007; Storchová *et al.* 2004). In addition, Good *et al.* (2008b) and White *et al.* (2011) identified more proximal parts of the X chromosome as contributing to hybrid male sterility. Moreover, the data of Good *et al.* (2008b) suggest that multiple regions of the X appear to be associated with hybrid male sterility, emphasizing the importance of the X as a whole. More recently, Good *et al.* (2010) reported extensive overexpression of X-linked genes in the testes of sterile but not fertile hybrid mice and suggested that sterility might be due to a general disruption of X chromosome gene expression during male meiosis.

Despite the importance of the X chromosome, a significant contribution of autosomes to hybrid male sterility has also been suggested in mice. It has been known for a long time that chromosome 17 harbours gene(s) involved in hybrid male sterility (Forejt & Iványi 1974;

Forejt 1996; Vyskočilová *et al.* 2005, 2009; White *et al.* 2011), and the first hybrid sterility gene (*Prdm9*) was positionally cloned to the proximal part of chromosome 17 (Mihola *et al.* 2009). However, it is clear from mapping studies that other autosomes also contribute to hybrid sterility (Oka *et al.* 2007; White *et al.* 2011). In addition, consomic strains carrying each *Mus musculus musculus* chromosome on a largely *M. m. domesticus* background showed reduced fertility for nearly every autosome (Gregorová *et al.* 2008). Although the male and female effects of fertility were not explored separately in this study, the consomic strains demonstrate that genes underlying hybrid sterility map to most autosomes. The genetic basis of hybrid sterility in mice is clearly complex, and laboratory crosses may be inefficient in identifying specific genes because the genomic regions identified in mapping studies tend to be quite large (Storchová *et al.* 2004; Good *et al.* 2008a; Vyskočilová *et al.* 2009; White *et al.* 2011).

From this perspective, hybrid zones, narrow regions where two genetically diversified populations meet, may be more appropriate for the study of the genetic basis of reproductive isolation (Payseur 2010). Barton & Hewitt (1985) argued that most naturally occurring hybrid zones are tension zones maintained by the dispersal of parental types and selection against hybrids as a result of incompatibilities between co-adapted parental genomes. From multilocus cline theory (Barton 1983; Barton & Gale 1993), it is known that unless the strength of selection (s) exceeds the rate of recombination (r) between loci (described by the coupling coefficient: $\theta = s/r$), different parts of chromosomes behave partially independently enabling neutral loci to escape the influence of loci under selection, thus making it possible to distinguish regions associated with negatively selected reproductive barriers from neutral regions. In instances where hybrids represent complex mosaics of parental genomes because of multigenerational backcrossing and intercrossing, it is possible to distinguish regions under selection on a much finer scale than is possible with laboratory crosses (Rieseberg & Buerkle 2002; Buerkle & Lexer 2008; Payseur 2010; White *et al.* 2011; Baird & Macholán 2012).

The two house mouse subspecies (*M. m. musculus* and *M. m. domesticus*) which diverged from a common ancestor approximately 500 000 BP (Boursot *et al.* 1996; Suzuki *et al.* 2004; Chevret *et al.* 2005; Salcedo *et al.* 2007; Geraldès *et al.* 2008, 2011; Duvaux *et al.* 2011) form a narrow hybrid zone that extends from Bulgaria to Denmark (Boursot *et al.* 1993; Macholán *et al.* 2003). Recently, hybrid mice have been reported also from Norway (Jones *et al.* 2010). The house mouse hybrid zone is a tension zone as suggested by Barton & Hewitt (1985) and confirmed by others (Payseur *et al.* 2004;

Raufaste *et al.* 2005; Macholán *et al.* 2007). Reduced fertility of hybrid males probably plays the most important role in maintaining the tension zone as it has been demonstrated in laboratory crosses (Forejt & Iványi 1974; Forejt 1996; Storchová *et al.* 2004; Britton-Davidian *et al.* 2005; Vyskočilová *et al.* 2005, 2009; Good *et al.* 2008a,b, 2010; Míhola *et al.* 2009; White *et al.* 2011) and was recently reported directly from the centre of the hybrid zone (Turner *et al.* 2012). In addition, increased parasite loads in hybrids have been suggested (Sage *et al.* 1986; Moulia *et al.* 1993); however, recently, the opposite has been reported from a different part of the hybrid zone (Baird *et al.* 2012; Gouy de Bellocq *et al.* 2012). Also, hybrid female sterility has been proposed to contribute to hybrid failure (Britton-Davidian *et al.* 2005).

Multiple studies using cline theory (Nagylaki 1975; Barton 1979, Barton 1983, Barton & Hewitt 1985; Barton & Gale 1993; Gompert & Buerkle 2009, 2010) have demonstrated selection acting on X-linked markers in the house mouse (Tucker *et al.* 1992; Dod *et al.* 1993; Macholán *et al.* 2007, 2008; Teeter *et al.* 2008). Other studies (Payseur *et al.* 2004; Dufková *et al.* 2011; Macholán *et al.* 2011) have identified the central part of the X chromosome as a particularly important region harbouring loci involved in reproductive isolation, in accord with the results of laboratory crosses (Oka *et al.* 2004; Storchová *et al.* 2004). Teeter *et al.* (2008) found some autosomal regions to be potentially contributing to reproductive isolation, including a few that are in linkage disequilibrium with X-linked markers. However, that study used fairly sparse marker coverage, so the extent to which the X chromosome interacts epistatically with different autosomal regions is still unclear.

Here, we present a whole genome analysis of hybrid mice collected from two previously studied transects across the hybrid zone (Wang *et al.* 2011). One transect is located in southern Bavaria and Austria (BV), and the second transect is located 200 km to the north in Bavaria and the Czech Republic (CZ). By comparing the patterns of introgression in two transects, we identified common patterns that are likely to reflect intrinsic genetic incompatibilities. We surveyed 1401 SNP markers (Wang *et al.* 2011) across the genome and analysed these data using a genomic cline approach (Gompert & Buerkle 2009, 2010). This approach compares individual loci to the average pattern for all loci and assesses whether loci are neutral or under selection. Here, we focus on those loci inferred to be involved in epistasis based on their non-neutral patterns of introgression, following Gompert & Buerkle (2009). First, we compared introgression patterns between pairs of markers on the X chromosome and identified adjacent markers with similar epistatic patterns of introgression in both transects. We then compared these regions with autosomal

markers to identify candidate BDMIs. We found that these regions harbour genes involved in a number of distinct processes including transcriptional regulation, sexual conflict and sexual selection.

Materials and methods

Mice

House mice were collected from the CZ and BV transects (Wang *et al.* 2011). Because X-linked markers are haploid in males, X-autosome comparisons are only possible using data from females. The BV transect is located 200 km south of the CZ transect. It is one-dimensional extending 176 km in a linear east–west direction from south-central Bavaria, Germany, to north-western Austria (Tucker *et al.* 1992; Payseur *et al.* 2004; Božíková *et al.* 2005). A total of 254 females of 432 total mice collected from 41 localities in three different years (1984, 1985 and 1992) were used. The CZ is two-dimensional and is 130 km long × 50 km wide, extending from north-eastern Bavaria, Germany, to western Bohemia, Czech Republic (Božíková *et al.* 2005; Macholán *et al.* 2007, 2008, 2011). A total of 425 females of 869 mice collected from 79 localities between 1991 and 2003, with >95.5% collected since 1997, were used.

SNP development and genotyping

A previously published data set of 1401 SNPs, diagnostic for the two subspecies and providing reliable genotyping calls (Wang *et al.* 2011), was used. The data matrix consisted of 1316 autosomal and 85 X-linked SNP markers. The median and mean gap sizes between two SNPs are approximately 1.86 Mb. However, several gaps are present owing either to the lack of coverage in the Mouse Diversity Array (Yang *et al.* 2009), from which markers were selected, or by human factors. For a detailed description of SNPs used and the matrix of genotypes, see Wang *et al.* (2011).

Genomic clines

To describe the behaviour of SNP markers from across the genome, we started with genomic cline analysis using the Introgress program (Gompert & Buerkle 2009, 2010). The analysis uses a multinomial regression approach to characterize the behaviour of genetic markers along a genomic admixture gradient represented by individual hybrid index scores estimated using a previously published method (Buerkle 2005). The behaviour of a marker is represented as a probability of observing a particular genotype given the hybrid index (Gompert & Buerkle 2009, 2010) and the likelihood of this model is

compared to the likelihood of observing the data under a neutral model. Following Gompert & Buerkle (2009), four alternative patterns of selection are considered including overdominance (in which heterozygous genotypes are observed more than expected), underdominance (in which heterozygous genotypes are observed less than expected), directional selection (in which individual clines are shifted relative to the genome average) or epistasis (defined in this context as interactions between a single locus and the genomic background and characterized by steep homozygote clines and high and sharp heterozygote clines). Here, we focus particularly on those loci inferred to be involved in epistatic interactions. Hybrid indexes were based on 1316 autosomal loci (Wang *et al.* 2011). To obtain *P*-values estimates, the data were permuted 1000 times. The two significance levels ($P_{\text{introgress}} \leq 0.01$ and $P_{\text{introgress}} \leq 0.001$) were used to distinguish markers with patterns significantly differ from the neutral prediction.

Overall strategy

Previous work has shown that the demographic dynamics of this hybrid zone are quite different in these two transects (Wang *et al.* 2011), making it difficult to compare patterns directly. Because we know that the X chromosome underlies reproductive isolation between these taxa and because we were interested in identifying markers involved in BDMIs, we began by identifying regions on the X chromosome that show evidence of epistasis and where adjacent markers are not significantly different from one another, termed X-linked uniform regions (XURs; see details below). We then compared XURs between transects and identified those with similar patterns of introgression. Subsequently, these were compared to autosomal regions to identify those autosomal regions that may be important in reproductive isolation.

Comparison of introgression models between pairs of markers

The genomic cline approach was modified to compare introgression models between pairs of markers. Introgression models between pairs of X-linked markers were compared to identify adjacent markers on the X with similar patterns of introgression (described below). The mode of selection for all markers from each region was identified using results from the original genomic cline analysis.

A log-likelihood ratio of introgression models (M_{m_1} and M_{m_2}) for a pair of markers (m_1 and m_2) was used as a measure of similarity between their models. Models M_{m_1} and M_{m_2} were inferred by the approach imple-

mented in the original version of INTROGRESS (Gompert & Buerkle 2009, 2010), and a log-likelihood ratio of the M_{m_1} and M_{m_2} models given the marker m_1 data (D_{m_1}) was obtained as follows:

$$\Delta LL = \ln \frac{L(M_{m_1}|D_{m_1})}{L(M_{m_2}|D_{m_1})}$$

For the comparison of X-linked markers, markers m_1 and m_2 were assigned arbitrarily, while for the X-linked vs. autosomal markers, m_2 was assigned to X-linked markers. A significance test for the two model comparison was conducted by the permutation of the m_2 data. Genotypes for each individual were chosen according to assigned weights represented by conditional probabilities of genotypes given hybrid index as inferred by the approach implemented in the original version of the Introgress program (Gompert & Buerkle 2009, 2010). *P*-value estimates were then obtained by comparing log-likelihood ratio of two marker models (M_{m_1} and M_{m_2}) with the distribution of log-likelihood ratios for M_{m_2} and models based on 1000 times permuted m_2 data. The two significance levels ($P_{\text{pairwise}} \leq 0.01$ and $P_{\text{pairwise}} \leq 0.001$) were used to distinguish whether the behaviour of the two markers significantly differ from each other. All computations were conducted in the R statistical environment (R Development Core Team 2005) using a modification of the INTROGRESS package (Gompert & Buerkle 2009, 2010).

Characterizing the structure of X chromosome introgression

To identify adjacent regions exhibiting similar patterns of introgression along the X chromosome, log-likelihood ratios and *P*-values for pairs of markers along their physical position on the chromosome were plotted. The regions of uniform introgression, XURs, were identified using a *P*-value cut-off >0.01 for pairs of markers in each transect separately. This cut-off provides a conservative estimate, thus minimizing false positives when comparing X-linked markers to autosomes. The overlap between XURs in the two transects were subsequently defined as shared X-linked uniform regions (SXURs). However, only XURs comprising markers with non-neutral patterns of introgression obtained by the original version of the Introgress program (Gompert & Buerkle 2009) were used.

Distribution of autosomal markers within and between chromosomes and between transects

Autosomal markers exhibiting similar patterns to X-linked markers were plotted along their physical position. Adjacent markers were pooled into regions,

and the distribution of the region sizes was tested against a distribution of region sizes based on the permutations of marker positions. The procedure was repeated 1000 times. The aim of this approach was to test whether markers putatively under selection clustered more often than expected by chance alone.

Significance of over/under enrichment was also tested using a hypergeometric distribution as suggested by Boyle *et al.* (2004). Bonferroni correction was used to treat for multiple comparisons. Results of these two tests were then compared between the two transects.

To reveal shared regions harbouring candidate loci associated with reproductive isolation, a comparison of identified markers between the two transects was conducted. As any overlap might have arisen by chance alone, the significance of the overlap was tested. The positions of identified regions were permuted taking into account the structure of the autosomal genome (i.e. varying size of chromosomes and regions harbouring candidate loci). This procedure was repeated 1000 times with the number of overlapping markers recorded each time. *P*-value estimates were obtained by comparing the observed number of markers shared between the two transects against the distribution of simulated overlaps.

Gene enrichment in regions exhibiting epistasis

Gene content was characterized for both X-linked and autosomal SNP markers identified as exhibiting epistasis with gene content restricted to within 1 Mb of the marker or in cases when an adjacent marker was closer than 1 Mb, to within the total distance between the two markers. Genes were downloaded from the Ensembl website (<http://www.ensembl.org>) using the BioMart tool (<http://www.biomart.org>). Only genes identified as protein-coding genes [protein-coding genes, IG_(C/D/J/V)] were included for further analysis. BioMart was also used to assign Ensembl Ids to Gene Ontology (GO) term names (Ashburner *et al.* 2000). GO terms with <50 genes in the whole genome (X, autosomes) were excluded from further analyses. The enrichment of particular GO terms within the identified regions was characterized. The 'biological process' and 'molecular function' domains were tested separately in each transect for autosomal regions only. The GO term enrichment was tested for X chromosomal and autosomal regions for cases in which markers overlapped between transects and then for autosomal regions for each transect separately. A hypergeometric distribution as suggested by Boyle *et al.* (2004) was used to identify significantly enriched GO terms. As a threshold of significance, a *q*-value representing a false discovery rate (FDR) provided by *qvalue* R package (Storey & Tibshirani 2003) was used.

Results

The pattern of introgression on the X chromosome

We characterized the patterns of introgression for 85 markers along the X chromosome in the CZ and BV transects. With the exception of a 10.5-Mb gap located between positions 23 and 33.5 Mb along the chromosome, the markers were approximately evenly spaced.

We used the Introgress program (see Material and Methods) to assess the prevailing pattern of introgression along the X chromosome in both transects and compared it to the neutral prediction based on the averaged introgression of 1316 autosomal markers (Fig. 1). Epistasis of varying strength (see Gompert & Buerkle 2009) is inferred to be a prevailing non-neutral pattern in both transects. The average genomic cline over all X-linked markers exhibits overabundance of *M. m. musculus* genotypes with respect to the null model prediction (autosomal average) in the BV transect, while the opposite is true for the CZ transect.

To compare the patterns of introgression along the X chromosome, the log-likelihood ratios and *P*-values estimates were calculated for all pairs of X-linked markers using the modified version of the Introgress program (see Material and Methods). When log-likelihood ratios were plotted against physical map position, a distinct pattern of introgression emerged (Fig. 2a). The whole X chromosome was partitioned into several large regions, interestingly, in a similar way in both transects. The only difference was found to be in the degree of independence these regions exhibited across the length of the chromosome. Chromosomal regions in the BV transect introgressed in a more similar way than in the CZ transect. To find regions along the X with similar patterns of introgression, XURs were defined. They are represented by adjacent markers with a homogeneous pattern of introgression for which the log-likelihood ratio between them did not exceed the sampling error (*P*-value >0.01) (Figs 2b and 3). These included 19 and 16 XURs in the BV and the CZ transects, respectively (Fig. 3, Table S1a,b, Supporting information). The sizes of XURs varied from 0.7 to 17.0 Mb in the BV transect and from 1.4 to 14 Mb in the CZ transect and they included from 2 to 9 (BV) and 2 to 8 (CZ) SNP markers, respectively. Almost all of the markers from within XURs exhibited introgression patterns significantly deviated from neutral prediction as provided by the Introgress program (Gompert & Buerkle 2009). The only exceptions were XUR 2 and 3 in the CZ transect. Assuming a common reproductive basis for these patterns across hybrid populations, we then identified overlapping XURs between transects, defining them as SXURs. We identified 10 SXURs comprising 29 X-linked

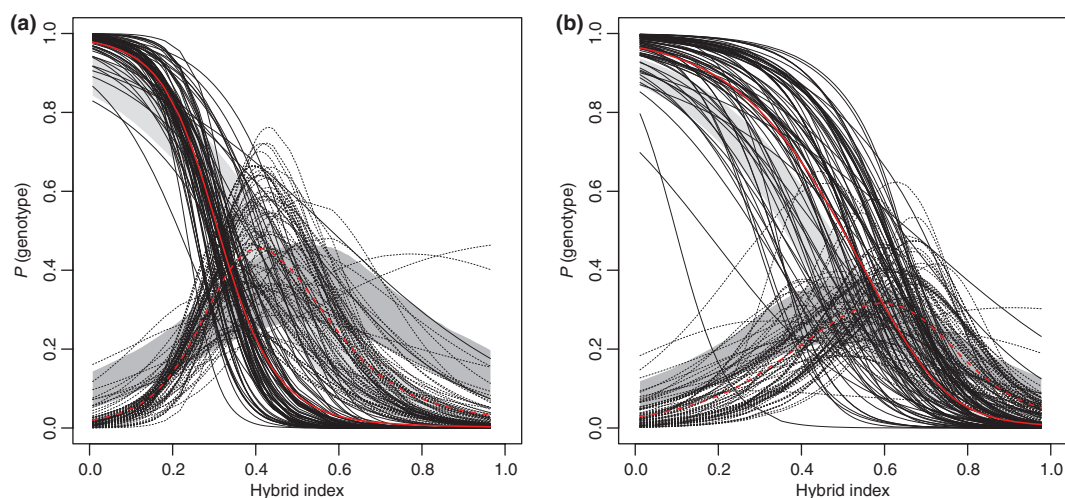


Fig. 1 Genotype probabilities given hybrid index (genomic clines) for 85 X-linked markers as provided by the Introgress program (Gompert & Buerkle 2009) for (a) BV and (b) CZ transect (solid lines: homozygotes for *M. m. domesticus* alleles; dashed lines: heterozygotes). Null expectations (light grey: homozygotes for *M. m. domesticus* alleles; dark grey: heterozygotes) are based on the permutation of autosomal data (1316 markers). Hybrid index used from Wang *et al.* (2011). Red lines represent average genomic clines for X-linked markers.

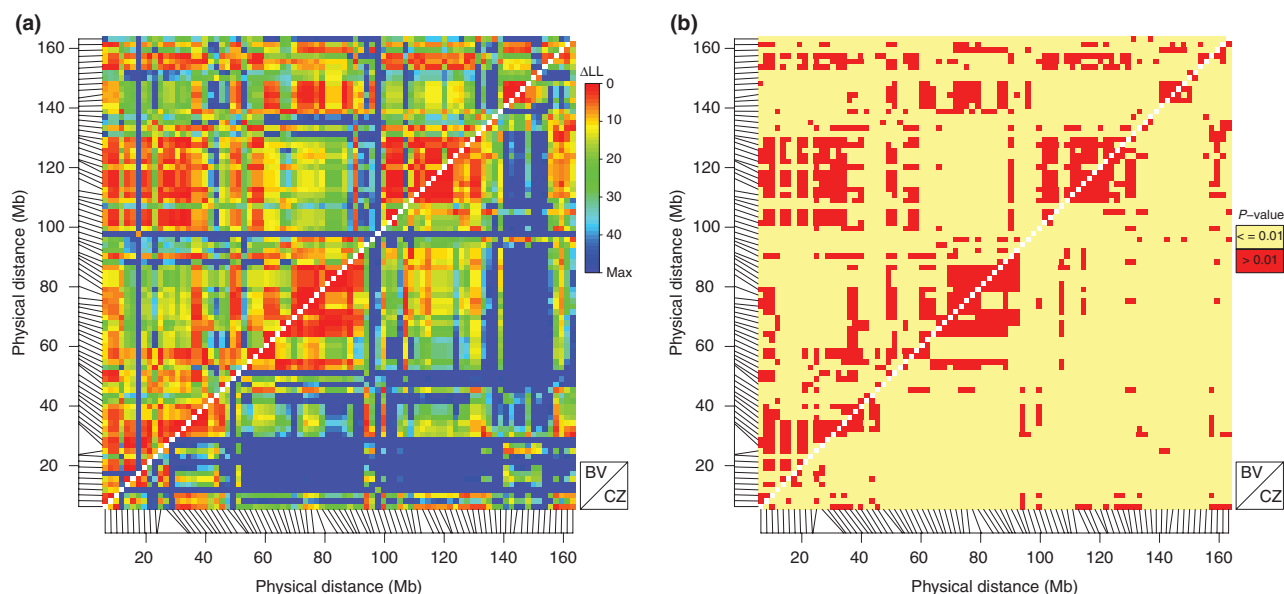


Fig. 2 Comparison of introgression models for pairs of markers plotted along their physical positions on the X chromosome (BV: above diagonal; CZ: below diagonal). (a) log-likelihood ratios (ΔLL) for pairs of markers. The more similar behaviour of the two markers the lower ΔLL . (b) statistical significance of pairwise comparisons (red: behaviour of the two markers is not significantly different). Plotted in the R statistical environment (<http://www.r-project.org/>) using modified version of LDHEATMAP package (Shin *et al.* 2006).

markers (Fig. 3, Table 1). The identified SXURs were based on the overlap between transect-specific XURs. The size of these groups ranged from 1.2 to 10.1 Mb and included from 2 to 5 markers.

Epistasis was identified as the predominant non-neutral pattern for individual markers within the SXURs

with variation in the strength of the effect between regions and transects (Figs 4 and S1a,b, Supporting information). All markers from within introgress groups in BV transect had their genomic clines shifted into *M. m. domesticus* range with respect to the neutral prediction. In the CZ transect, markers tended to show the

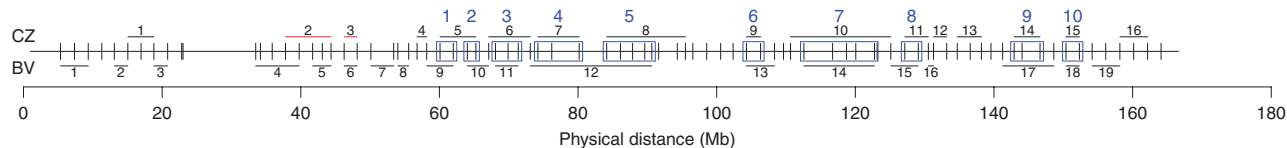


Fig. 3 The X-linked uniform regions (XURs) were defined based on the P -value estimates for the pairwise comparisons of X-linked markers in each transect separately (black lines). Red lines signify regions containing markers *not* significantly different from neutral expectations based on the permutation of autosomal data (1316 markers). The overlapping regions between XURs are considered as shared X-linked uniform regions (SXURs) (blue rectangles).

Table 1 Shared X chromosome linked uniform regions (SXURs) with candidate epistatically interacting loci as identified from both transects

Region ID	No markers	Begin (bp)	End (bp)	Size (Mb)	Marker positions	Marker name
1	2	60 091 526	62 000 232	1.909	28, 29	X-60091526, X-62000232
2	2	64 029 949	65 262 708	1.233	30, 31	X-64029949, X-65262708
3	3	68 083 956	71 371 693	3.288	33, 34, 35	X-68083956, X-69903086, X-71371693
4	3	74 210 508	80 161 976	5.951	37, 38, 39	X-74210508, X-76207917, X-80161976
5	5	84 132 877	90 640 869	6.508	40, 41, 42, 43, 44	X-84132877, X-86116001, X-87868682, X-89621357, X-90640869
6	2	104 292 926	106 332 092	2.039	52, 53	X-104292926, X-106332092
7	5	112 587 884	122 771 038	10.183	57, 58, 59, 60, 61	X-112587884, X-116719686, X-118730961, X-120066236, X-122771038
8	2	127 137 209	129 088 513	1.951	64, 65	X-127137209, X-129088513
9	3	142 906 662	146 641 816	3.735	74, 75, 76	X-142906662, X-144055466, X-146641816
10	2	150 391 903	152 318 392	1.926	78, 79	X-150391903, X-152318392

opposite trend with the exception of two regions located in the distal part of the X chromosome.

Analysis of epistasis for autosomes

The log-likelihood ratios and P -value estimates for all X-autosomal pairs were calculated, and all pairwise comparisons having P -values above two different thresholds (P -value_{pairwise} > 0.01, 0.001) were considered. Subsequently, the introgression of these markers was tested against a neutral model as provided by the original version of the INTROGRESS program (Gompert & Buerkle 2009, 2010) to exclude those markers with introgression models that are not significantly different from the neutral expectation (P -value_{introgress} ≤ 0.01, 0.001). Using the more conservative cut-off (P -value_{pairwise} > 0.01 and P -value_{introgress} ≤ 0.01), we identified 204 and 237 autosomal markers showing epistasis in the BV and CZ transects, respectively (Fig. S2, Supporting information; Table 2). Using a less conservative cut-off (P -value_{pairwise} > 0.001 and P -value_{introgress} ≤ 0.001), we identified 246 and 329 autosomal markers in BV and CZ transects, respectively (Fig. 5, Table 2). The introgression patterns of all identified markers were plotted using the original version of the INTROGRESS program (Fig. S3a,b, Supporting information).

Comparison of introgression between the BV and CZ transects

The autosomal markers under epistasis were compared between the two transects. Using a more conservative cut-off as defined above, we identified 39 markers under putative epistasis in both transects (~3% of markers), and using the less conservative cut-off, we found 69 markers under putative epistasis (~5% of markers; Table 2). Because these markers might randomly overlap between the two transects, we simulated the data to estimate the probability of obtaining the same overlap by chance. The overlap between the two transects based on the more conservative cut-off was not significantly different from the random overlap (P -value = 0.251); however, the overlap based on the less conservative cut-off was significantly different (P -value = 0.036).

Distribution of autosomal markers under epistasis across the genome

To characterize the genome-wide architecture of reproductive isolation, the clustering of markers under putative epistasis was explored via permutations. The output of the permutation study indicated that the

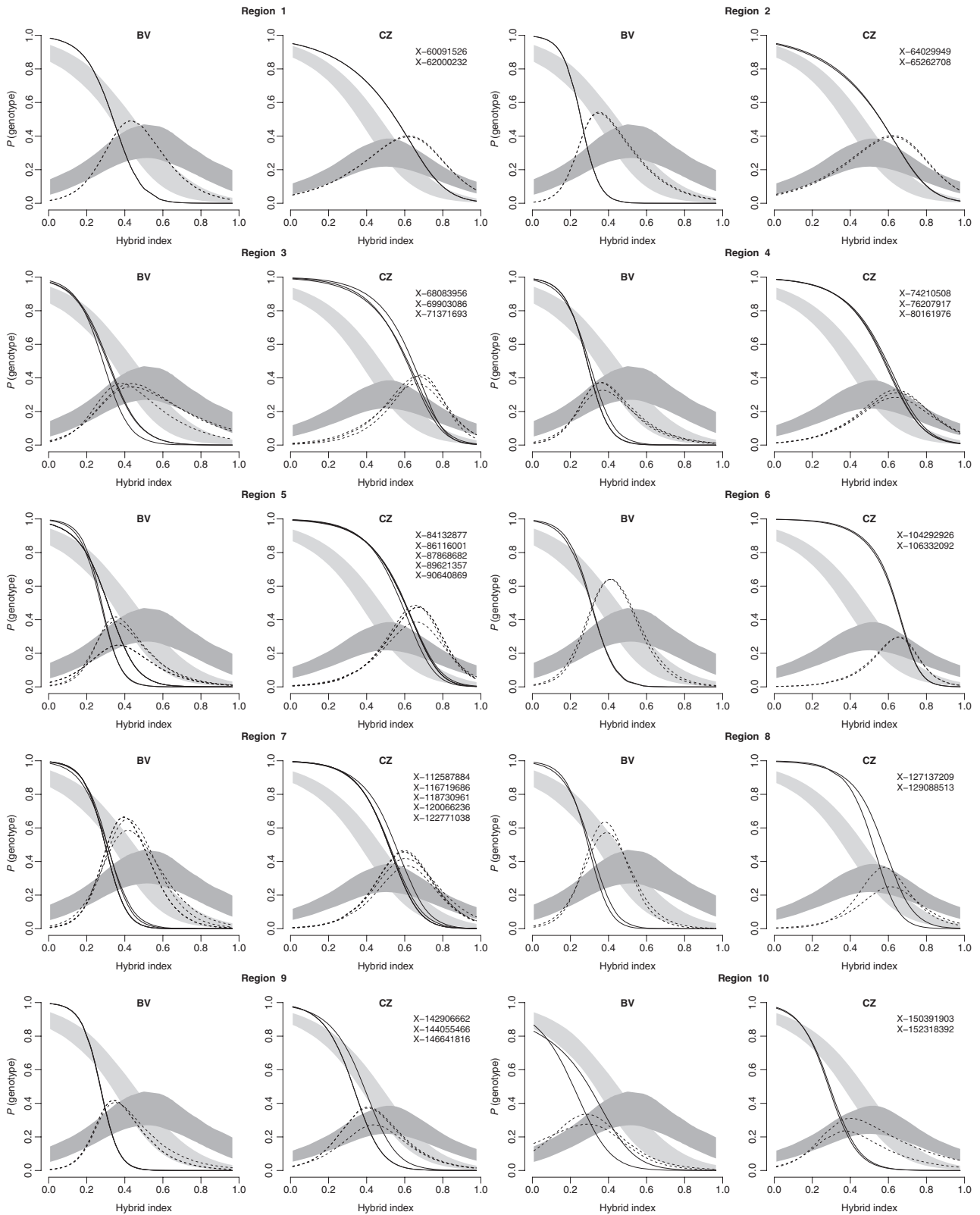


Fig. 4 Genomic clines for 29 X-linked markers from within the 10 SXURs plotted for each transect separately. Probability of genotype (*M. m. domesticus* homozygotes—solid line; heterozygotes—dashed line) plotted on hybrid index. Null expectations (light grey: homozygotes for *M. m. domesticus* alleles; dark grey: heterozygotes) are based on the permutation of autosomal data (1316 markers). Hybrid index used from Wang *et al.* (2011). Numbers correspond to the SXURs numbers in Fig. 3.

Table 2 Number of autosomal candidate epistatically interacting markers under two different cut-offs

Transect	0.01*		0.001**	
	No markers	%	No markers	%
BV	204	15.50	246	18.69
CZ	237	18.01	329	25.00
BV and CZ	39	2.96	69	5.24

* $P\text{-value}_{\text{pairwise}} > 0.01$ and $P\text{-value}_{\text{introgress}} \leq 0.01$.

** $P\text{-value}_{\text{pairwise}} > 0.001$ and $P\text{-value}_{\text{introgress}} \leq 0.001$.

identified markers were grouped more often than expected by chance alone (Fig. S4a,b, Supporting information). We identified 143 (BV) and 162 (CZ) regions for the more conservative cut-off ($P\text{-value}_{\text{pairwise}} > 0.01$ and $P\text{-value}_{\text{introgress}} \leq 0.01$) and 163 (BV) and 203 (CZ) regions for the less conservative cut-off ($P\text{-value}_{\text{pairwise}} > 0.001$ and $P\text{-value}_{\text{introgress}} \leq 0.001$), respectively. The largest fraction of all regions was represented by single markers in both transects (~68%) and the fraction of regions comprising two or more markers decreased steeply with increasing number of markers per region. The largest regions identified spanned up to 11 Mb (BV) and 9 Mb (CZ), respectively. There were no differences in the distribution of sizes between transects. In our comparison between transects, we identified 59 overlapping regions exhibiting epistasis using the less conservative cut-off ($P\text{-value}_{\text{pairwise}} > 0.001$ and $P\text{-value}_{\text{introgress}} \leq 0.001$). Forty-nine of these are composed of a single marker.

We also conducted an analysis of marker distribution between chromosomes for each transect separately and then compared the results. When autosomes were tested for over- and under-abundance of identified markers, two chromosomes, chr. 15 in BV transect (less conservative cut-off) and chr. 8 in CZ transect (more conservative cut-off) were found to have an under-abundance and over-abundance, respectively. For over-

lapping markers exhibiting epistasis in the two transects, there was no significant enrichment except for chromosome 11 using the less conservative cut-off ($P\text{-value}_{\text{pairwise}} > 0.001$ and $P\text{-value}_{\text{introgress}} \leq 0.001$).

Gene enrichment in regions exhibiting epistasis

Gene content was characterized for X-linked and autosomal SNP markers identified as exhibiting epistasis, with attention to those regions identified in both transects (Table S3, Supporting information). Using Gene Ontology terms (Ashburner *et al.* 2000) under the domain 'molecular function', several groups were consistently enriched in all four analyses (BV and CZ including X, BV and CZ excluding X, BV and CZ, separately). The first group included terms involved in DNA binding and regulation of transcription. The group was represented by several related terms (GO:0003700, GO:0003677, GO:0003676, GO:0043565, GO:0016779). The second group included terms involved in the regulation of peptidase activity (GO:0004252, GO:0030414, GO:0016779), and the third group included chemokine and cytokine activity (GO:0008009, GO:0005125). Other GO terms were specific for the overlap between transects and included various terms involved in metal ion binding, electron carrier activity, energetic metabolism and/or a combination of these groups. Under the domain 'biological process', the group common to both transects included terms associated with transcription and its regulation represented by two GO terms (GO:0006355 and GO:0006351). Interestingly, in the case where we included the X chromosome in the analysis, two additional biological processes terms, skeletal muscle tissue development (GO:0007519) and DNA recombination (GO:0006310), appeared to be significant. It is also worth noting that one BV-specific group included terms involved in response to bacterium and viruses (GO:0006952, GO:0042742, GO:0009615). All the identified GO terms were found to be significant at an FDR ≤ 0.1 .

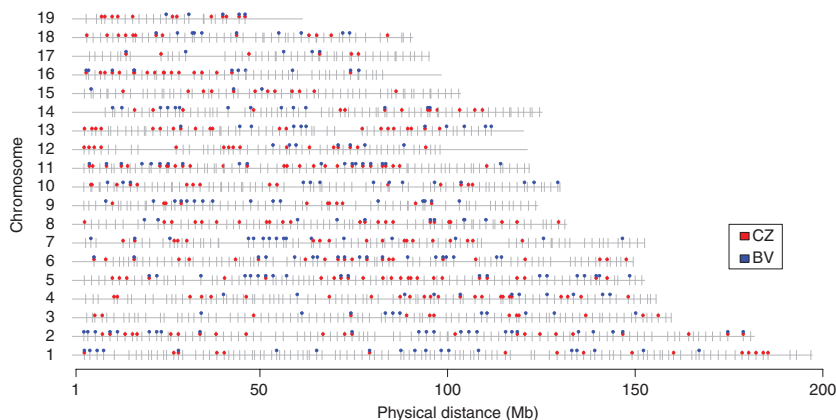


Fig. 5 Genome location of autosomal markers. Grey ticks depict all of the 1316 autosomal markers. Blue and red circles depict candidate epistatically interacting markers in BV and CZ transect, respectively ($P\text{-value}_{\text{pairwise}} > 0.001$ and $P\text{-value}_{\text{introgress}} \leq 0.001$).

Discussion

We surveyed 1401 SNPs approximately evenly spaced along the genome in two transects of the house mouse hybrid zone to identify regions of the X chromosome and autosomes that may be involved in BDMIs. Given our concerns that differences in hybrid zone movement between these transects (Wang *et al.* 2011) might obscure similarities between transects, we devised a method that does not rely on a direct comparison of introgression models between transects. We assessed the introgression along the X chromosome to identify regions exhibiting epistasis in both transects. Markers from these regions were subsequently used to reveal autosomal markers under putative epistasis common to both transects.

In general, genotypic patterns across a hybrid zone may be due to various forces including selection acting on intrinsic incompatibilities, selection in particular environments, drift, migration or demographic processes such as local extinction or colonization of populations across the landscape. Many of these processes may operate differently in different transects. Thus, comparisons of 'signatures of selection' among transects provide one means of distinguishing selection that is owing to intrinsic incompatibilities from selection that may be specific to a particular environment or false positives that may result from stochastic processes. We argue that common pattern of epistasis (as defined by Gompert & Buerkle 2009) observed in both transects is caused by selection against intrinsic incompatibilities responsible for reproductive isolation.

X chromosomal introgression

Most markers along the entire X chromosome were inferred to be involved in epistatic interactions in both transects, thus emphasizing the importance of interactions between X-linked loci and the genomic background as a cause of reproductive isolation. These patterns are consistent with the large X-effect (Charlesworth *et al.* 1987; Coyne & Orr 1989; Masly & Presgraves 2007) and with findings from house mice laboratory crosses (Storchová *et al.* 2004; Oka *et al.* 2004, Britton-Davidian *et al.* 2005, Good *et al.* 2008a, 2010). While these patterns of X-linked introgression are similar across the two transects, we detected a difference in the position of X-linked genomic clines with respect to the neutral prediction. This is likely due to differences in hybrid zone movement between the two transects (Wang *et al.* 2011), although we cannot fully discount other processes.

The identified regions common in both transects were detected in the central and distal portions of the X chromosome. This is in accord with previous findings from

the hybrid zone as well as from laboratory crosses. Payseur *et al.* (2004), Macholán *et al.* (2011) and Dufková *et al.* (2011) identified reduced gene flow in a central portion of the X chromosome in the BV and the CZ transects, respectively. In addition, laboratory crosses between wild-derived inbred strains suggested several loci along the X chromosome are associated with various sterility phenotypes. Recently, lowered testis weight, smaller sperm counts and reduced sperm velocity have been reported from the hybrid zone (Turner *et al.* 2012). Storchová *et al.* (2004) and Vyskočilová *et al.* (2009) found the central portion of the X to be associated with testis weight as well as sperm count phenotypes. In this part of the X, approximately between 60 and 80 Mb, Storchová *et al.* (2004) obtained the strongest signal. This region corresponds to five of the SXURs (1, 2, 3, 4 and 5) identified in our study (Fig. 3). Oka *et al.* (2004) also implicated the central portion of the X. However, in their study, this region was associated with a sperm head morphology phenotype. Recently, Good *et al.* (2011) reported five candidate genes potentially involved in reproductive isolation from the central portion of the X chromosome. All are involved in male reproduction, and all have elevated rates of evolution. However, only two of them overlapped with regions we identified in the hybrid zone (SXUR 4: *Pbsn* and SXUR 5: *Tsga8*). The distal part of the X was found to be associated with either testis weight (Storchová *et al.* 2004) or testis weight and sperm counts (Oka *et al.* 2004; Vyskočilová *et al.* 2009). We identified two large regions comprising three (6, 7 and 8) and two (9 and 10) SXURs, respectively, which correspond with these previous findings. However, some recent studies found that the proximal part of the X is associated with testis weight and/or sperm counts, while the central and distal parts are associated with sperm head morphology (Good *et al.* 2008b; White *et al.* 2011). This discrepancy might be due to the fact that these studies crossed different inbred strains. Because *M. m. musculus* and *M. m. domesticus* have been isolated for only a short time (*c.* 0.5 Ma), alleles associated with reproductive isolation may not be fixed across populations. This was shown by Vyskočilová *et al.* (2005, 2009) who found a polymorphism in hybrid sterility in *M. m. musculus* populations. The fact that some wild-derived inbred strains are mosaics of *M. m. musculus* and *M. m. domesticus* genomes (Yang *et al.* 2011) might also contribute to the discrepancy in results between various crosses. Alternatively, all of these studies may be limited by their inability to precisely map the QTL of interest.

Autosomal introgression

We identified 69 autosomal markers that (i) showed similar patterns of introgression with the SXUR's,

(ii) were inferred to be involved in epistatic interactions and (iii) were shared between the BV and CZ transect. These regions, which constitute ~5% of the autosomal markers, are good candidates for BDMIs underlying reproductive isolation.

A total of 69 markers shared between transects is a relatively small subset of the markers identified in the two transects separately (246 in BV and 329 in CZ), highlighting the importance of comparing transects for filtering out potential 'false positives'. However, the lack of a more extensive overlap between the two transects may be due to polymorphisms associated with hybrid sterility (Forejt & Iványi 1974; Britton-Davidian *et al.* 2005; Vyskočilová *et al.* 2005, 2009; Good *et al.* 2008) or stochastic processes (Gompert & Buerkle 2009, 2011; Dufková *et al.* 2011; Polechová & Barton 2011). In addition, the density of markers may not be fine enough to cover the entire genome. As markers in close proximity may exhibit different behaviour in a hybrid zone (e.g. Macholán *et al.* 2011), we might have missed some of the incompatibility loci, thus underestimating the number of regions involved in reproductive isolation.

Incompatibility loci were shown to be primarily equally distributed along the autosomal genome as there were no significant differences in the abundance of identified markers between chromosomes consistent over the two transects. The only exception was chromosome 11 where we found significant enrichment for markers shared between transects. The predominantly equal distribution of incompatibility loci is in agreement with other laboratory studies (Gregorová *et al.* 2008; White *et al.* 2011) and suggests that incompatibilities gradually accumulated as a function of time and strength of selection which is consistent with an allopatric model (Barton & Charlesworth 1984; Geraldès *et al.* 2008, 2011; Nosil *et al.* 2009; Duvaux *et al.* 2011).

Gene content of the regions under putative epistasis

The gene content of X-linked and autosomal regions under putative epistasis was characterized. We were particularly interested in which GO biological processes and molecular functions were associated with genes from within these regions. Among all enriched categories, one of the most important findings was the involvement of a group of genes associated with DNA binding, transcription and its regulation, thus emphasizing the role of between-loci interactions (e.g. transcription factors) as suggested by the BDMIs. A second group involved genes with peptidase inhibitory activity that was recently related to sexual conflict and sexual selection in the female reproductive tract (Dean *et al.* 2008, 2009, 2011; Dorus *et al.* 2010). Interestingly, three

out of four rapidly evolving genes (*Serpine2*, *Spink3*, *Spinkl*) identified in the seminal vesicles and subsequently referenced to proteolytic inhibitory activity by Dean *et al.* (2009), fall into epistatically interacting regions. Other studies found proteolytic inhibitory activity enriched in rapidly evolving genes functioning mostly on the outer surface of sperm (Dorus *et al.* 2010; Dean *et al.* 2011). Thus, these observations are consistent with a role for sexual conflict in reproductive isolation. The next GO term, chemotaxis, was enriched in the overlap of the two transects. The ability of sperm to find their way through the female reproductive tract is key to successful reproduction (Clark *et al.* 2006). From this perspective, chemotaxis may play a very important role in reproduction and may be under strong selection as well. The selective force operating at this stage may be sperm competition when females undergo multiple mating (Dean *et al.* 2006; Dean & Nachman 2009).

Finally, genes involved in defence response to bacteria and viruses were identified in the BV transect. Clark *et al.* (2006) suggested a role for resistance against various pathogens and female immune system in sperm. Our findings on defence response are also in accord with Dean *et al.* (2008) who found the same biological processes (defence response, defence response to bacterium) enriched in mouse epididymal proteins.

Our GO analyses do not suggest the involvement of intraspecific individual recognition mechanisms, which would be in accord with previous analyses (Laukaitis *et al.* 1997; Talley *et al.* 2001; Smadja & Ganem 2002, 2005, 2008; Smadja *et al.* 2004; Cheetham *et al.* 2007; Stopková *et al.* 2007; Ganem *et al.* 2008; Thom *et al.* 2008; Vošlajerová Bímová *et al.* 2011) including MUPs (Cheetham *et al.* 2007; Stopková *et al.* 2007; Thom *et al.* 2008) and ABPs (Karn & Dlouhy 1991; Laukaitis *et al.* 1997; Talley *et al.* 2001). The reason for this failure may be simply that genes involved in intraspecific recognition have not been annotated or their role in reproductive isolation is not large enough to be captured by the approach used. Also, there is a possibility that these genes fall within several gaps where there is no marker coverage.

Comparing autosomal regions exhibiting epistasis to other studies

We compared autosomal regions identified in the hybrid zone to be under epistasis to regions and/or genes identified in previous studies. We focus only on the shared regions under epistasis between the two transects as they are most likely to harbour loci involved in reproductive isolation (Fig. 6, Table S2, Supporting information).

Several studies focused on the association of hybrid sterility phenotypes with particular markers in the

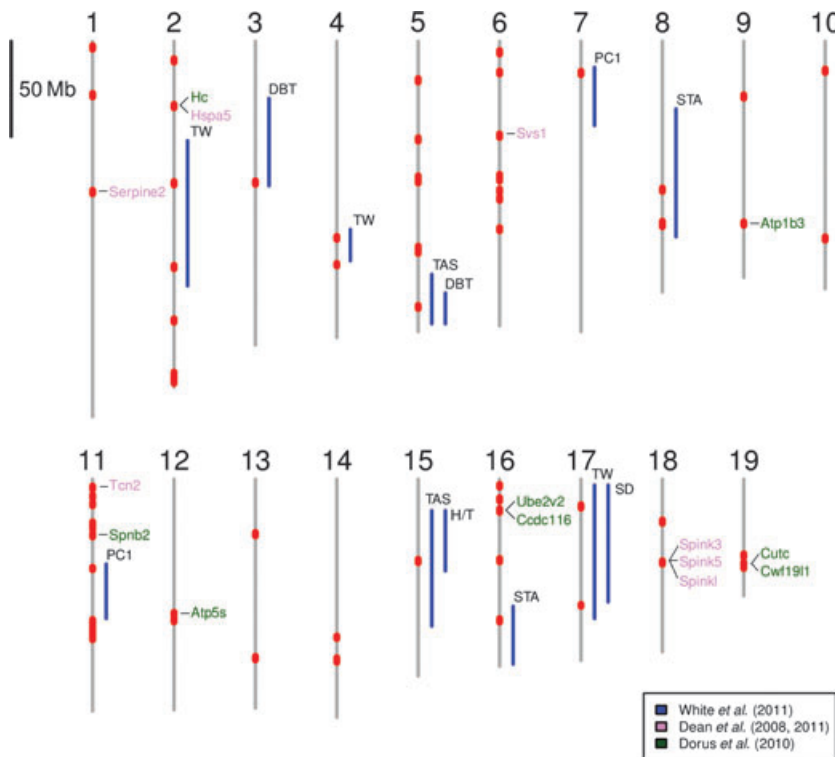


Fig. 6 Candidate autosomal regions associated with reproductive isolation in the house mouse. Shared regions between the two transects exhibiting epistasis (this study) are shown in red. The QTL regions associated with hybrid sterility phenotypes (White *et al.* 2011) are represented as blue lines. Phenotype abbreviations, TW, relative testis weight; SD, sperm density; PC1, sperm head morphology; STA, seminiferous tubule area; DBT, distal bent tail; TAS, total abnormal sperm; H/T, headless/tailess correspond to those in White *et al.* (2011). Genes identified in Dean *et al.* (2008, 2011) are coloured in violet and those identified by Dorus *et al.* (2010) are coloured in green.

genome using QTL analysis (Mihola *et al.* 2009; Vyskočilová *et al.* 2009; White *et al.* 2011). White *et al.* (2011) recently mapped traits underlying hybrid male sterility in a large F2 mapping population. In general, the identified QTL were in wide genomic intervals. Nonetheless, the results of White *et al.* (2011) provide a useful comparison to the present study, because similar hybrid male sterility phenotypes are observed in the hybrid zone (Turner *et al.* 2012). Several markers identified in overlapping regions between the two transects were found to fall within five QTL regions associated with relative testis weight or sperm density phenotypes (2-74558017, 2-118591908, 4-103390028, 17-14320574 and 17-65932862). Other markers (3-74249497, 5-139601762, 7-16643634, 8-77982361, 8-95400455, 8-96505302, 11-46639287, 15-42990323 and 16-74152290) fall within nine QTL regions associated with sperm morphology. Although QTL regions sometimes span large portions of a chromosome making an assessment on the overlap with our data difficult, for certain cases, the overlap is very precise and/or markers identified reside very close to regions with the highest likelihood (4-103390028, 5-139601762, 7-16643634, 8-77982361, 8-95400455, 8-96505302, 16-74152290). Although an autosomal region involved in hybrid male sterility was found to be on chromosome 17 (Forejt & Iványi 1974; Forejt 1996) and, recently, a particular gene (*Prdm9*—Chr17:15682083-15701318) was identified (Mihola *et al.* 2009), we found no correspondence with our data from the hybrid zone. This is in contrast to Vyskočilová *et al.* (2009) and White *et al.*

(2011) who found an association between the proximal portion of chromosome 17 and sperm counts and relative testis weight. Given the large span of this QTL in both studies, it is possible that a different region (i.e. marker 17-14320574) is involved.

Also, we found several genes (*Atp1b3*, *Atp5s*, *Ccdc116*, *Cutc*, *Cwf191*, *Hc*, *Hspa5*, *Serpine2*, *Spink3*, *Spink5*, *Spink1*, *Spnb2*, *Svs1*, *Tcn2*, *Ube2v2*) in the regions of overlap whose products are known to be involved in male reproduction (Dean *et al.* 2009a, 2011; Dorus *et al.* 2010). Although the study of Harr (2006) who searched for genomic regions of high differentiation between house mouse subspecies suffered from potential methodological biases (Boursot & Belkhir 2006), two autosomal regions clearly emerged. One of them in the proximal region of chromosome 2 correlates with our results based on the two-transect comparison. However, the second of them on chromosome 10 does not appear to overlap with any of our markers under putative epistasis.

Although there is always a possibility that correspondence between our data and previous studies is by chance, in some cases, the overlap is very precise, thus reinforcing the evidence for epistasis involving these particular regions. Agreement between our data and other studies and our ability to more finely map regions exhibiting epistasis demonstrate the utility of using naturally occurring hybrid populations exhibiting multi-generational backcrossing and intercrossing to explore the genetic basis of reproductive isolation.

Indeed, our analysis of gene content in regions exhibiting epistasis implicates a key role in reproductive isolation for several mechanisms, involving the regulation of transcription, sexual conflict and sexual selection operating at both the postmating prezygotic (e.g. genes expressed on the outer surface of the sperm) and postzygotic (e.g. candidate loci associated with hybrid male sterility) stages of isolation. These findings, together with information that epistatically interacting loci are distributed throughout the genome, provide strong evidence that reproductive isolation in emerging house mouse species is polygenic, complex and likely resulted from the accumulation of incompatibilities between *M. m. musculus* and *M. m. domesticus* during the relatively short time period in which these populations diverged in allopatry.

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This work resulted from collaboration among several research groups who use naturally occurring hybrid populations to understand the genetic underpinnings of reproductive isolation in house mice.

Data accessibility

The genotype data matrix is available from <http://hdl.handle.net/2027.42/83674>.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Genomic clines for X-linked markers from each of the 19 and 16 XURs in BV (a) and CZ (b) transect, respectively. Probability of genotype (*M. m. domesticus* homozygotes—solid line; heterozygotes—dashed line) plotted on hybrid index. Null expectations (light grey: homozygotes for *M. m. domesticus* alleles; dark grey: heterozygotes) are based on the permutation of autosomal data (1316 markers). Hybrid index is taken from Wang *et al.* (2011). Numbers correspond to the SXURs numbers in Fig. 3.

Fig. S2 Genome location of autosomal markers. Grey ticks depict all of the 1316 autosomal markers. Blue and red circles depict candidate epistatically interacting markers in BV and CZ transect, respectively ($P\text{-value}_{\text{pairwise}} > 0.01$ and $P\text{-value}_{\text{introgress}} \leq 0.01$).

Fig. S3 Genomic clines for identified autosomal markers by region in the BV (a) and CZ (b) transect, respectively. Probability of genotype (*M. m. domesticus* homozygotes—solid line; heterozygotes—dashed line) plotted on hybrid index. Null expectations (light grey: homozygotes for *M. m. domesticus* alleles; dark grey: heterozygotes) are based on the permutation of autosomal data (1316 markers). Hybrid index is from Wang *et al.* (2011).

Fig. S4 Observed numbers of epistatically interacting markers per autosomal region identified using P value thresholds of (a) $P\text{-value}_{\text{pairwise}} > 0.001$ and $P\text{-value}_{\text{introgress}} \leq 0.001$, and (b) $P\text{-value}_{\text{pairwise}} > 0.01$ and $P\text{-value}_{\text{introgress}} \leq 0.01$ plotted by size category (red dots) against the distribution of regions based on the permutation of markers assuming their mutual independence (black dots, 95% CI). The size of the regions is defined by the number markers comprising them.

Table S1 X chromosome-linked uniformed regions identified for the BV transect (a) and CZ transect (b).

Table S2 The list of autosomal regions exhibiting epistasis and associated published reports linking hybrid sterility phenotypes and/or rapidly evolving genes in both transects (CZ&BV) or in BV and CZ, individually.

Table S3 Results of the GO enrichment analysis. GO terms found on $\text{FDR} \leq 0.1$ for transect specific autosomal regions exhibiting epistasis (BV, CZ) and for the overlap of the two transects including or excluding the X chromosome.

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