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The mouse hybrid zone in Central Europe: from morphology to molecules

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Abstract. Despite the long-term study of the house mouse hybrid zone in Europe knowledge of its course in some areas is still rather vague. Comparisons of different portions of the zone showed some common patterns, however, several discordances were also revealed, the most remarkable being introgression of the Y chromosome. We sampled mice along the presumed course of the secondary contact zone between two subspecies, *Mus musculus musculus* and *M. m. domesticus*, from Schleswig-Holstein to southern Bavaria, in order to localize more precisely its position. A second aim was to reveal whether introgression shows some general rules obscured until now by studies of geographically isolated transects of the zone. We employed maternally (mtDNA), paternally (Y), and biparentally inherited markers and related their introgression patterns to the hybrid index (*HI*) based on five X-linked loci. While transition of autosomal loci across the zone was congruent with changes in *HI*, mtDNA showed bidirectional introgression with alien alleles occurring far behind the zone. Finally, the Y chromosome displayed asymmetric unidirectional introgression of the *musculus* type into *domesticus* background. We discuss evolutionary forces shaping these patterns.

Key words: geography, introgression, mtDNA, *Mus musculus musculus*, *Mus musculus domesticus*, X chromosome, Y chromosome

Prologue: "Go west, young man!"

It was in the early 1990's that Jan Zima asked the second author (MM), then his Ph.D. student, to take a short visit to the westernmost tip of Bohemia (Czech Republic; Fig. 1A) and collect some house mice. The idea behind this simple plan was that the western subspecies of the house mouse, *Mus musculus domesticus*, could have been occurring in that area. At that time, only the eastern subspecies, *M. m. musculus*, was known from this part of Europe. Mid-June, when the first trial came about, is not a very convenient season for trapping mice and so it is not surprising that the catch was not stunning – not more than three individuals were captured. However, protein electrophoresis showed all these mice to be hybrids and in autumn of the same year more house mice were collected and the results of the pilot analysis confirmed. The presence of a contact zone between the two subspecies in western Bohemia was thus proven (Macholán & Zima 1994). Later a Ph.D. student at the Charles University in Prague, Pavel Munclinger, joined the crew, however, it was only after the last author (JP) had returned from his postdoc stay in Edinburgh and substituted Jan Zima, that the project gained significant momentum. A research network of three teams (Brno, Studenec, Prague) was set up and as years passed by, this basic web has been expanding. The last (but not least) momentum was gained by capitalizing on the inference skills of SJEB – so, like in the famous Dumas's novel, three "mousketeers" (or "mouse consortium" as SJEB put it) became four.

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Of course, the idea that the hybrid zone runs through western Bohemia did not appear out of thin air. Zimmermann (1949) was the first who pictured ranges of both subspecies in Europe and suggested the occurrence of presumed hybrids in some populations. However, Zimmermann's picture could only be approximate, assuming only *M. m. musculus* to occur in the Czech Republic (Czechoslovakia at that time). The distribution of the two subspecies in Central Europe was made clearer through a morphological study of 424 skins and 369 skulls of mice from Bavaria (southeastern Germany; Fig. 1), suggesting presence of *M. m. domesticus* in areas close the Czech-Bavarian border (Kraft 1985). Finally, some populations from eastern Bavaria appeared to harbour Robertsonian fusions, i.e. chromosomal rearrangements typical for *M. m. domesticus* but virtually absent in *M. m. musculus* (see Zima & Macholán 1989 and Zima et al. 1990 for the only exception), thus corroborating Kraft's results gained from morphology of *M. musculus* in that region (R. Hübner, pers. comm.). Occurrence of the western subspecies in Bohemia became a reasonable idea and Jan seized the opportunity.

Mouse hybrid zone studies in Europe

Certainly the contact between ranges of the two taxa is not limited to Central Europe. The house mouse hybrid zone (HMHZ) is more than 2500 km long and runs from Scandinavia to the Black Sea. Following the pioneering works of Zimmermann (1949) from Central Europe and Degerbøl (1949) and Ursin (1952) from the Jutland peninsula, this zone has been studied in Denmark, northern Germany (Schleswig-Holstein), eastern Germany (Saxony), north-eastern Bavaria and western Bohemia, southern Bavaria and north-western Austria (Oberösterreich), and eastern Bulgaria (see Baird & Macholán 2012 for a recent review). These studies, among other things, have led to a better understanding the course of the zone, however, its position in some parts of Europe remains only vaguely defined.

Over the course of time, the number of mice analyzed and markers employed has been increasing. This progress has led to some insight into the HMHZ dynamics and genetic architecture of barriers against admixture. It was shown that carefully designed sampling in two-dimensional space, using an explicit cline model, and rigorous statistical treatment is essential for hybrid zone studies (Macholán et al. 2007, Baird & Macholán 2012). It was also argued that hybrids are disadvantaged through reduction of fertility rather than lowered viability (Baird & Macholán 2012, Baird et al. 2012) and that the incomplete postzygotic isolation is reinforced through assortative mating (Vošlajerová Bímová et al. 2011). Along with increasing sampling size and the number of markers assayed, several comparisons of geographically separated transects were carried out (Teeter et al. 2010, Dufková et al. 2011, Wang et al. 2011, Janoušek et al. 2012). These studies revealed some common patterns, for example, that the X chromosome appears to harbour more genetic incompatibilities than autosomes (Tucker et al. 1992, Dod et al. 1993, Macholán et al. 2007, Janoušek et al. 2012), these loci being localized mostly in the central part of the X (Payseur et al. 2004, Teeter et al. 2010, Dufková et al. 2011, Macholán et al. 2011, Janoušek et al. 2012). On the other hand, introgression of some markers has been shown to differ between various portions of the HMHZ. The two most notable examples are mitochondrial DNA (mtDNA) and the Y chromosome. In the former case, only a limited gene flow across the zone was revealed in Denmark (Vanlerberghe et al. 1988b) whereas its introgression is less steep and resembles introgression of most autosomal loci in Central Europe (Božíková et al. 2005). The difference in the Y chromosome introgression patterns is even more striking: introgression of this chromosome has been shown to be absent in Denmark (Vanlerberghe et al. 1986, Dod et al. 2005) and Bavaria (Tucker et al. 1992) in stark contrast with massive introgression of *musculus* Y into *M. m. domesticus* territory further north in the western part of the Czech Republic (Munclinger et al. 2002, Macholán et al. 2008). Due to a similar, but less pronounced introgression of mtDNA and an associated distortion of the sex ratio it was suggested that the Y introgression pattern is due to genetic conflict between elements on the X and Y chromosomes (Macholán et al. 2008), and subsequent work on X marker introgression patterns seems to support this hypothesis (Macholán et al. 2011).

There has been some disagreement over whether the architecture of the species barrier in the HMHZ differs from place to place (cf. Teeter et al. 2010 vs. Macholán et al. 2011, Baird et al. 2012, Baird & Macholán 2012). For clarity, here when we refer to the architecture of the species barrier we follow a succinct definition suggested by Nichols: "the nature of the network of pleiotropic effects that causes the barrier to gene flow between the taxa" (R. Nichols pers. comm.). Certainly, when many loci are visualised, there are differences in introgression patterns in different transects. However, stochastic effects on loci not involved in the barrier itself are only to be expected. An explanation of the

nature of these discrepancies requires consideration and proper weighting of many of factors that may contribute to stochastic patterns, e.g., colonization history, population dynamics, local climatic and geographic conditions, as well as the possibility of spatial differences in selection against hybrids. We should also take into account human influences such as differences in sampling and analytical design or genotyping error. Obviously, the number of HMHZ studied is still too low to get a consensual view, and insight from more parts of the HMHZ, optimally from the central region along the whole contact, would be informative. However, comparisons between transects are often difficult due to differences in sampling and markers scored and data from large portions of the HMHZ are still lacking.

In this study we conducted a survey of mouse populations along the presumed course of the HMHZ from Schleswig-Holstein to southern Bavaria to at least partially fill this gap. First we wished to investigate genetic structure of mice from hitherto unsampled areas in order to localize more precisely the position of the zone. Second we wanted to reveal whether introgression shows some general rules obscured until now by individual transect observations. We do not cover regions in Denmark, where the hybrid zone has been precisely localized using molecular markers and is known to have complicated colonization history (Dod et al. 1993, 2005, Prager et al. 1993, 1996, Raufaste et al. 2005), and also the region south east of the Alps in which HMHZ position was specified through an analysis of skull characters (Macholán et al. 2003, see also Baird & Macholán 2012 and references therein).

Material and Methods

To estimate the course of HMHZ we genotyped 136 mice at 45 localities in Germany, Czech Republic and Poland (approximately 47°52′-53°54′ N and 9°28′-15°30′ E). This dataset was supplemented with published data from various parts of Europe. The whole genetic dataset comprised 270 localities and 3704 mice. Finally, we also employed published morphological data of Zimmermann (1949) and Kraft (1985), collected from 40 and 24 localities, respectively. For localities surveyed in this study, we scored four sets of biallelic diagnostic markers fixed for alternative alleles in *M. m. musculus* and *M. m. domesticus*. The first set consisted of a single marker on the Y chromosome, namely the presences/absence of an insert in the *Zfy2* gene (Nagamine et al. 1992, Boissinot & Boursot 1997). This insertion was scored following the protocol described in Orth et al. (1996). The second set included a *BamH* I restriction site in the mitochondrial *Nd1* gene shown to discriminate between the subspecies (Munclinger et al. 2002, Božíková et al. 2005) The third set consisted of five X-linked SINE and/or LINE insertions chosen to be distributed along the whole chromosome: *XL1_332L07* (abbreviated as *X332*), *X65C* (*X65*), *XB2_347N11* (*X347*), *Btk*, and *Syap1* (Munclinger et al. 2002, Macholán et al. 2011). The fourth set of markers included autosomal loci consisting of allozymes (Ferris et al. 1983, Tucker et al. 1992, Prager et al. 1993, 1996, Macholán et al. 2008, Piálek et al. 2008) or single nucleotide polymorphisms (Teeter et al. 2008, 2010).

Published genotype data for autosomal, X- and Y-linked markers and mtDNA were obtained from the following regions and references: Schleswig-Holstein (Ferris et al. 1983, Prager et al. 1993, 1996), north-western Poland (Ferris et al. 1983, Prager et al. 1996), the "Saxony transect" (a linear transect stretched from Thuringia in the west through Saxony-Anhalt and Saxony to south-western Poland in the east) (Teeter et al. 2010), the "Czech transect" (a two-dimensional sampling area in north-eastern Bavaria and western Bohemia) (Božíková et al. 2005, Macholán et al. 2008), south-eastern Czech Republic (Piálek et al. 2008), and the "Bavarian transect" (a linear transect from southern Bavaria to Oberösterreich, Upper Austria; Tucker et al. 1992, Payseur et al. 2004, 2005, Božíková et al. 2005, Teeter et al. 2008, 2010). A list of localities, their coordinates and population scores for autosomal, X- and Y-linked markers and mtDNA are in Supplementary Table S1.

Hybrid indices (*HIs*) were calculated to summarize data from multiple loci scored at autosomes and the X chromosome. They are expressed as the mean frequencies of the *musculus* alleles over all loci and individuals scored in a locality and designed as HI_X for the X-linked and HI_{AA} for the autosomal markers.

Results and Discussion

Position of the HMHZ in Central Europe

Frequencies of the X- and Y-linked markers display roughly the same geographic distribution as morphological data (Fig. 1A-B and D, respectively). Contrary to these data sets, in northern and southern regions of the study area *M. m. domesticus* mtDNA is found markedly eastward within an otherwise *M. m. musculus* genetic background (Fig. 1C). This inconsistency challenges the mtDNA diagnostic marker as the most relevant trait for delimiting distribution ranges of the mouse subspecies. In

the following, because of the higher effective population size of the X chromosome relative to the Y chromosome and stronger selection on X-linked loci in comparison to autosomal loci (for references see Introduction), we chose HI_X for delineating the course of the HMHZ in the region under study.

Starting from the Baltic Sea, the zone runs between Weitendorf and Reinstorf (localities #14, #36 in Fig. 1A), in the direction from Wismar to Schwerin (Lake Schweriner). Then it turns south-west between Lalendorf and Groß Wokern (#37 and #38, both *M. m. musculus*) and Lübz and Suckow (#15 and #16, both *M. m. domesticus*) to Lake Müritz and then it turns south to Berlin. Although our sampling on the *M. m. domesticus* side does not allow us to precisely describe its course here, morphological data suggest that this subspecies is present in Berlin (#311 in Fig. 1D). Mice sampled in Hohenstein ($N = 4$, #42 in Fig. 1A), located about 25 km from the eastern suburbs of Berlin, are *M. m. musculus* at all five X-linked markers. South of Berlin the zone runs between Elsterwerda (#44) and Lauchhammer (#43) in the west (both prevailingly *M. m. musculus*) and Kreischau and Mehderitzsch (#82 and #81, both predominantly *M. m. domesticus*, Teeter et al. 2010) with the centre close to Zschirla (#80, $HI_A \approx 0.70$, $HI_X \approx 0.66$) between Leipzig and Chemnitz (Teeter et al. 2010). Further to the south, the zone runs through the westernmost tip of the Czech Republic (Munclinger et al. 2002, Božíková et al. 2005, Macholán et al. 2007, 2008, 2011, Dufková et al. 2011, Wang et al. 2011).

The position of the HMHZ in Bavaria is not as easy to detect as in northern Germany. The zone seems to bend south-eastwards where the River Wondreb/Odrava crosses the Czech-German border and then follows the state border where the contact between mouse subspecies is hampered by presence of the Oberpfälzer Wald/Český les Mts. Stronger gene flow can be surmised in the gap between this mountain range and the more south-eastern Bayerischer Wald/Šumava Mts., along the River Kouba/Chambach lowland. This river is the tributary of the River Regen which flows into the River Danube in Regensburg where occurrence of *M. m. musculus* was assumed by Zimmermann (1949) and Kraft (1985). Both authors reported morphologically *musculus*-like mice some 25 km north of this city (Parsberg/Oberpfalz, Amberg, Neumarkt, Burglengenfeld, localities # 277-281 in Fig. 1D), whereas our genetic data from mice collected from nearby localities (Gebenbach #26, Pessensricht #27, Frechetsfeld #28, Unterkatzbach, Fig. 1A) suggest presence of *M. m. domesticus* in this region. Further south, the HMHZ turns to Munich. Based on the genetic data gathered from the Bavarian transect (Sage et al. 1986b, Tucker et al. 1992, Payseur et al. 2004, Teeter et al. 2008, 2010, Wang et al. 2011) it was suggested that the River Isar forms a barrier between the subspecies (Sage et al. 1986b, 1993). Our sampling at four localities along both banks of this river south of Munich suggests a position of the zone east of the Isar; the HMHZ is thus likely to bend to the south-east in Munich with the presumed centre between Palnkam (#33) and the River Inn.

The *M. m. musculus* populations from the north-eastern Schleswig-Holstein (Prager et al. 1993, 1996, 1997, localities #4-8 in Fig. 1B, C) seem to have disjunct distribution. We are unable to infer whether this isolate was a part of the continual range of consubspecifics detected here further to the east (#36 in Fig. 1A) and separated from it by invasion of *M. m. domesticus* (represented by localities #14 in Fig. 1A, #13 in Fig. 1D) or is related to *M. m. musculus* populations in Fehmarn Isl. (Prager et al. 1993, 1996, 1997, localities #1-3 in Fig. 1B, C) and Sweden/Norway (Jones et al. 2010).

The length of the MHMZ covered in this study is about 850 km. Along its course, the zone runs through different environments. From a perspective point of view it would be interesting to find if the zone is solely maintained by migration of parental taxa in the zone and selection against hybrids or whether and to what extent the zone interacts with geographic barriers posed by mountains, riverine systems or artificial structures such as autoroutes. For example, in southern Germany such a barrier forming a step in gene frequency was suggested to occur across the floodplain of the River Isar (Sage et al. 1986b, 1993). While this river can reduce gene flow in the region north of Munich, our data suggest this may not be the case for the region south of Munich, where *M. m. domesticus* is present on both banks of this river. On the other hand, we did not find any support in our data for association of the HMHZ centre with a previously suggested geographic barrier in northern Germany, where the zone was suggested to be associated with the River Elbe (Zimmermann 1949). Rather it seems that this river transverses the HMHZ in Saxony and is far away from the zone in other places.

Introgression varies among different parts of the mouse genome

Since position of the HMHZ was approximated from X-linked markers, we used HI_X as the proxy to which introgression patterns at the autosomal, Y-linked and mtDNA markers are compared. Each of these

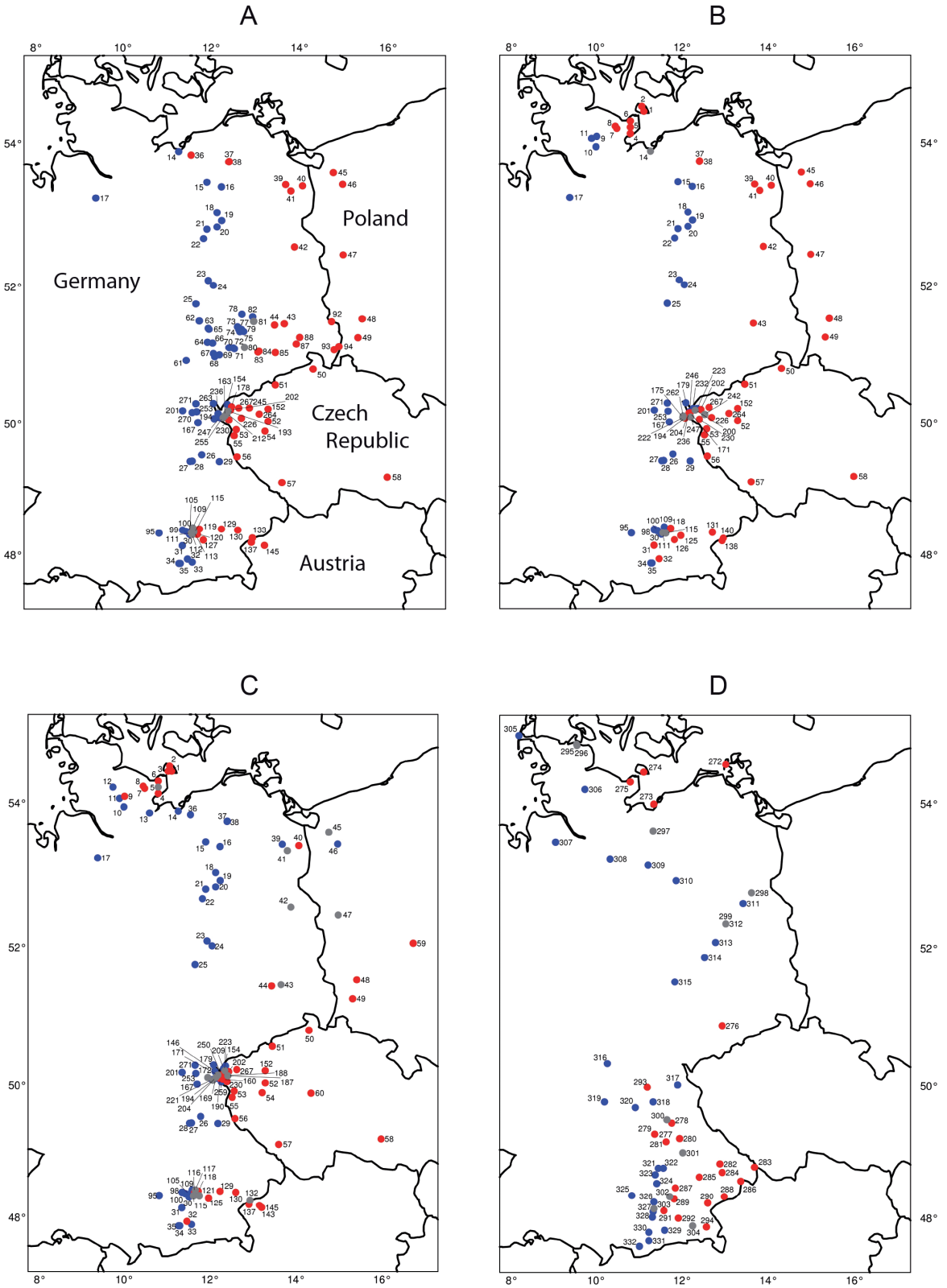

Fig. 1. *Map of the study area with house mouse localities sampled and analysed for X-linked markers (A), Y-linked markers (B), mtDNA (C), and morphological traits (D). The symbols are:* ● – M. m. domesticus *(defined as* HI ≤ *0.2),* ● *– polymorphic/heterozygous populations (0.2 <* HI *< 0.8) and* ● – M. m. musculus *(*HI ≥ *0.8), except panel D, in which classification of these categories were taken from original studies (Zimmermann 1949, Kraft 1985) and their designation of mice to either category was accepted (with the same symbols for* domesticus*, hybrids and* musculus *as in panels A-C).*

biparental, paternal and maternal markers displays a unique pattern of introgression in the concordance plots (Fig. 2).

Autosomal loci. Changes in frequencies at autosomal loci are strongly correlated with HI_X (linear fit, Pearson's $r = 0.97$, 93.9 % of the variation explained). This concerted transition of gene frequency along the diagonal is termed here as the symmetric bidirectional pattern of introgression (Fig. 2A). On the contrary, introgression of the haploid loci deviates from this pattern and cannot be modelled as a linear fit (lack of fit gives $P = 0.02$ for the Y-linked loci and $P = < 0.0001$ for the mtDNA loci).

Y chromosome. Unidirectional asymmetric introgression characterises introgression of the Y-linked loci. Out of 224 populations analysed for both X a Y loci, there are 11 cases, when the *domesticus* Y was found in the *musculus* background (Fig. 2B). Does it mean that the unidirectional asymmetric introgression documented for the Y chromosome does not hold?

Due to continual sampling in the Czech transect within 1991-2011, we obtained some evidence that these cases represent rather results of long-distance migration events of *M. m. domesticus* into *M. m. musculus* territory. Introgressed alleles and haplotypes are expected to persist there for limited time, but disappear later due to colonization from neighbouring parental demes. For example, two samples were analysed from Rudolec, a village about 12 km east of the zone centre. Both samples contained the *domesticus* Y in 1999 (frequencies of *musculus* Y: 0.33 and 0.4). From 2000 up to 2003, when last mice were sampled at the localities, only the *musculus* Y was detected at both sampling sites,

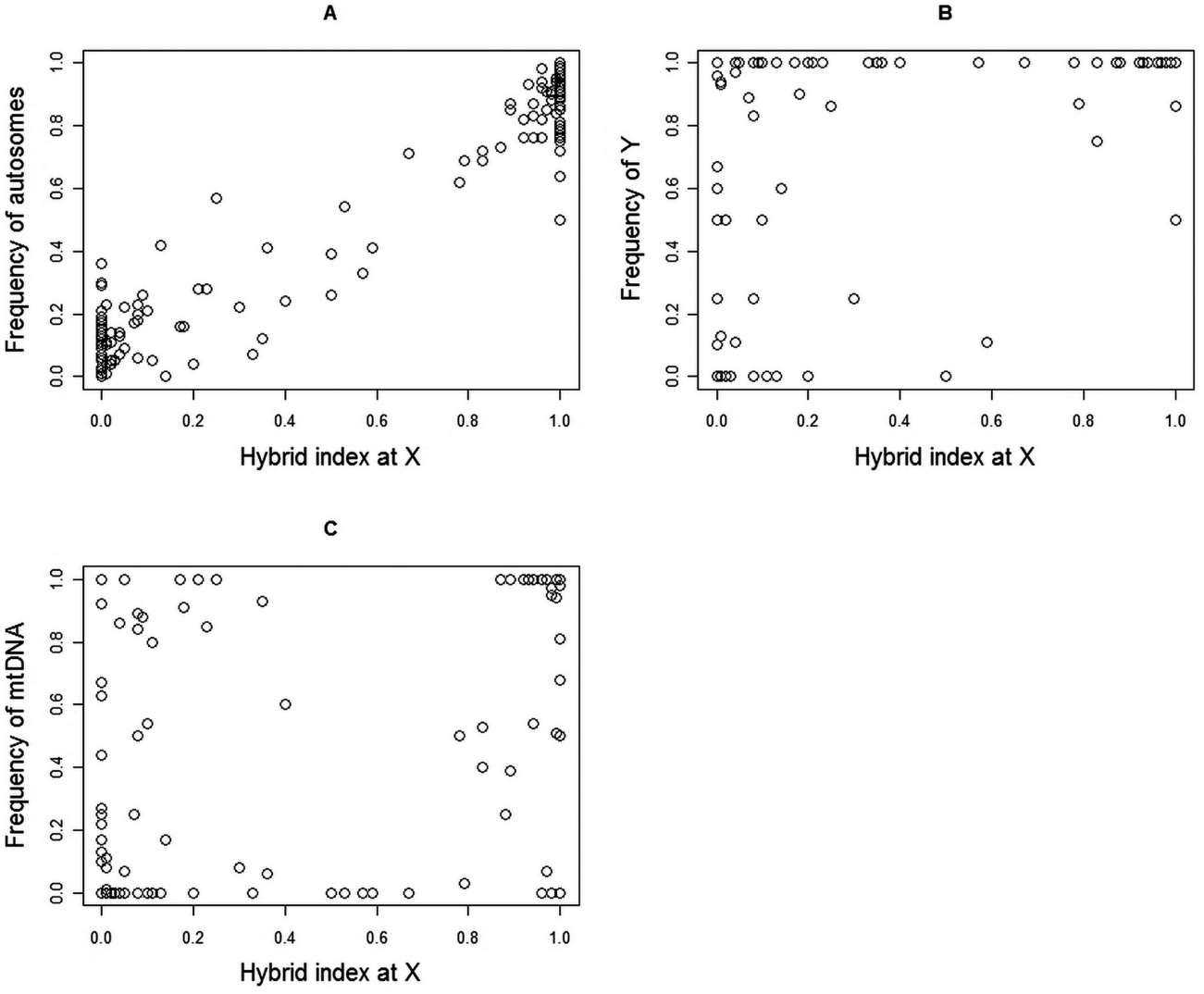

Fig. 2. *Frequencies at autosomal (A), Y-linked (B) and mtDNA loci (C) plotted against hybrid index based on five X-linked loci. Parental* M. m. domesticus *is in the left bottom corner (coordinates: 0,0) and parental* M. m. musculus *in right upper corner (1,1).*

increasing over-year average frequencies within the localities to 0.5 and 0.86, respectively (Macholán et al. 2008, Fig. 2B). Although we cannot make such inference from other populations because they were sampled only once, the rarity of such atypical findings (4.9 %) makes introgression of *domesticus* Y rather exceptional and does not break the rule of the unidirectional asymmetric introgression for the Y chromosome. On the contrary, data from other segments of the HMHZ are in conformity with this empirical rule. In Transcaucasia and north-eastern Iran the presence of two mtDNA lineages indicates that these are regions of admixture between the mouse subspecies; however, in both regions only the *musculus* Y chromosome was detected (Boissinot & Boursot 1997). Of the 34 males trapped on the *domesticus* side in the Danish transect, six (18 %) had *musculus* Y chromosomes (Dod et al. 1993). Similarly, Jones et al. (2010) found the *musculus* Y chromosome to be widespread in otherwise *M. m. domesticus*-type populations in Norway.

mtDNA. Bidirectional asymmetric introgression characterises mtDNA (Fig. 2C). Alien allelic variants can introgress into either mouse genome, although the findings of *domesticus* mtDNA in *M. m. musculus* populations seem to be more prevalent (Fig. 2C). While introgression at autosomal and Y-linked loci is mostly limited to the HMHZ, mtDNA can be found far away from its centre. This phenomenon is not locally restricted. For example, locality Parlino in north-western Poland (#46 in Fig. 1C) with the *domesticus* type of mtDNA is 80 km from Lindhorst, Uckerland (#39 in Fig. 1A) which is still within the *musculus* range. Likewise, Długoszyn (#47 in Fig. 1C) with *domesticus* mtDNA is about 100 km east of Berlin, and the presence of *domesticus* mtDNA within *M. m. musculus* territory was also reported from southern Bavaria by Božíková et al. (2005) (cf. Simbach, #132 in Fig. 1C, ca. 90 km east of the River Isar). Introgression of *musculus* mtDNA onto *domesticus* background seems to be more restricted, up to 25 km behind the zone centre in the Czech transect (Macholán et al. 2008).

Why do we see different introgression patterns?

Differences in concordance and symmetry observed in pairwise comparison of introgression between four sets of markers can reflect variation in effective population sizes between the marker types, recombination rates, and effects of stochastic or systematic factors.

The effective population size decreases from autosomal loci (N_e), to X-linked loci (¾ N_e) to mtDNA and Y-linked loci (¼ N_e). Hence the markers will be differentially subject to drift, with corresponding consequences for probability of fixation of alternative variants (see Polechová & Barton 2011). Another important factor potentially responsible for differences in introgression patterns can be recombination. Among the markers scored in this study, only those on the autosome and X can recombine away from their flanking background. When two individuals, each of different mouse subspecies, will meet and mate, recombination at each subsequent generation will break parental combinations. If both marker sets are neutral, they will spread across the hybrid zone, segregate in Mendelian fashion and occupy any place with coordinates given by their frequencies. Obviously, this is not the picture observed in Fig. 2A. Instead we see a nearly linear relationship between the two types of loci. This observation can be partly ascribed to the fact that diagnostic loci were chosen in all the studies covered in this paper (see Material and Methods). By definition, diagnostic loci are fixed for alternative alleles, and thus we cannot expect their segregation in localities geographically distant from the hybrid zone centre. However, such localities will all be located either in the bottom left corner in Fig. 2A (coordinates with *HI*s (0,0) defining parental *M. m. domesticus*) or in upper right corner (1,1) defining parental *M. m. musculus*. Thus we can conclude that both types of loci, summarised in terms of *HIs*, interact only within the HMHZ and are not able to introgress into *musculus* or *domesticus* genomes.

A more appropriate explanation of the lack of introgression of markers depicted in Fig. 2A is based on the tension zone model. According to this model the zone is maintained by a dynamic equilibrium between influx of parental gene combinations into the zone and selection against hybrids and recombinants (Barton & Hewitt 1985). Selection has been evidenced in the HMHZ both on the phenotype level (Britton-Davidian et al. 2005, Turner et al. 2012, Albrechtová et al. 2012) and genetic level (Raufaste et al. 2005, Macholán et al. 2007, 2011, Janoušek et al. 2012, see Baird & Macholán 2012 for recent review). These authors have come to agreement that the HMHZ is maintained through weak selection acting on many loci, rather than strong selection affecting a single or a few loci. In the tension zone model the selected loci will not behave independently resulting in strong effective selection (Barton & Gale 1993). As a result allele frequencies at the selected loci will change in a concerted way as observed in Fig. 2A. Consequently, the presence of recombination and selection can

largely explain the concordance in frequency changes between autosomal and X-linked loci.

MtDNA and most of the Y chromosome do not recombine and, in addition, have uniparental inheritance. From the population-genetic point of view, we consider two possible scenarios of mouse dispersal in a hybrid zone to explain their different introgression. Both scenarios start with colonization of new habitats by a female from one source and a male from another source subspecies. While recombination at autosomes and the X chromosome will create a wide range of hybrids with HI between 0 and 1, both haploid chromosomes will be in the absence of gene influx always fixed for their alternatives depending on their maternal or paternal origin. Consequently, their frequencies will tend to be positioned along the horizontal axes with fixed *musculus* or *domesticus* alleles in Fig. 2B, C, just depending on the maternal or paternal source of the haploid marker/locus.

Polymorphism at mtDNA and Y chromosomes observed along the vertical axes in Fig. 2B, C can only be detected under two alternative scenarios, in which a deme is invaded by mice carrying advantageous gene variants. First, suppose an advantageous allele enters a deme of the other subspecies. Its spread will initially be delayed due to incompatibilities brought about by loci at the same and/or other chromosomes (Piálek & Barton 1997). However, the positively selected locus will ultimately recombine and/or segregate away from counterselected loci, cross the zone and spread through the other subspecies' range. The spread can be stopped by the presence of either a physical barrier or other incompatible allele(s) at the same locus. Fixation of *musculus* mtDNA and Y variants on the *domesticus* genetic background is indicated in Fig. 2 B, C by positions of localities with $HI_{(mtDNA,\ Y)} = 1$ and $HI_X = 0$. Increasing frequencies at both mtDNA and Y loci along vertical axis at $HI_X = 0$ are suggestive of remnants of the spread of advantageous *musculus* alleles to *domesticus* background from low frequencies until fixation. The symmetric gradient of *domesticus* mtDNA at $HI_X = 1$ (Fig. 2C) then documents the spread *domesticus* mtDNA into *musculus* populations. Although the pattern shown in Fig. 2C is drawn from the Czech transect (Božíková et al. 2005) it is not unique for this transect as it was also observed in Bulgaria (Vanlerberghe et al. 1988a). Interestingly, in Fig. 2B, C most populations are positioned within a space out of the selection domain discussed above for autosomal and X-linked loci. So far this argument was used as if selection operates directly on any mtDNA- or Y-linked locus. The observed introgression can suggest positive selection at other loci and hitch-hiking with sex-subspecific traits (see, e.g. Macholán et al. 2008) or positive epistatic or pleiotropic interactions.

An alternative to the spread-of-advantageous-alleles model is past movement of the HMHZ leaving salient of alien allele(s) that locally persist as footprints of previous distribution (Macholán et al. 2011). While the majority of the alien alleles will be replaced by gene flow from neighbouring populations coupled with genetic drift (Polechová & Barton 2011), slow removal of non-native alleles can indicate some selective advantage, frequency-dependent selection or preferential mate choice for that marker. Both scenarios are grounded on assumption that the loci are fixed for alternative alleles in each subspecies. However, as argued by Vošlajerová Bímová et al. (2011) some loci thought to be diagnostic may represent ancestral polymorphism and caution must be taken before interpretation of results.

No studies focusing on effects of mtDNA on house mouse phenotype are known; thus only genotyping other loci in mice from localities with introgressed mtDNA can help to decipher whether this chromosome is experiencing positive selection either as a whole or due to a specific locus mutation. On the contrary, we found evidence that sperm count and motility were more favourable in *domesticus* males harbouring introgressed Y *musculus* than the same males with native Y chromosome (Albrechtová et al. 2012). Improvement of sperm quality due to asymmetric replacement of the Y chromosome is in agreement with the pattern of introgression envisaged in Fig. 2C.

Conclusion

Sampling localities along an 850 km stretch of the HMHZ in Central Europe allowed us to identify different patterns of introgression experienced by autosome, mtDNA- and Y-linked loci. Our evidence supports the idea that asymmetric unidirectional introgression of the Y *musculus* type is driven positively through its outperforming the Y *domesticus* type. The causative mechanisms underlying bidirectional asymmetric introgression of mtDNA are less clear; three scenarios to explain the present distribution of the mtDNA variants are selective advantage, zone movement and ancestral polymorphism. Further mtDNA genotyping should allow these alternatives to be distinguished.

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Supplementary online materials

Supplementary Table S1. *List of localities, coordinates and average frequencies at autosomal (HIAA), X-linked (HIX), Y-linked and mtDNA loci (Excel file; URL: http://www.ivb.cz/folia/download/dureje_et_al._supplementary_table_s1.xlsx).*