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Mitochondrial DNA in the hybrid zone between *Mus musculus musculus* and *Mus musculus domesticus*: a comparison of two transects

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We studied mtDNA introgression across the contact zone between *Mus musculus musculus* and *M. m. domesticus* in two independent transects in the Czech Republic and Bavaria, Germany. A total of 1270 mice from 98 localities in the Czech transect and 456 mice from 41 localities in the Bavarian transect were examined for presence or absence of a *Bam*HI restriction site in the *mt-Nd1* gene. Using this simple mtDNA marker, variants that belonged to the *M. m. domesticus* lineage (presence of restriction site) could be unequivocally distinguished from those belonging to the *M. m. musculus* lineage (absence of restriction site). The extent of introgression of mtDNA, three autosomal allozymes and the X chromosome was compared. The introgression of X markers was more limited than was that of the allozymes and mtDNA. In the Czech transect, the centre for the mtDNA cline was shifted about 3.6 km to the west relative to the X chromosome cline, with asymmetric introgression from *M. m. musculus* to *M. m. domesticus*. Interestingly, in the Bavarian transect, the centre of the mtDNA cline was shifted about 10.9 km to the east relative to the X chromosome cline, with asymmetric introgression from *M. m. domesticus* to *M. m. musculus,* opposite in direction to that observed in the Czech transect. *Biological Journal of the Linnean Society*, 2005, **84**, 363–378.

ADDITIONAL KEYWORDS: allozyme – cline – gene flow – house mouse – introgression – mtDNA – RFLP – SNP.

INTRODUCTION

Animal mitochondrial DNA (mtDNA) differs from nuclear DNA in that it exists in thousands of copies per cell, it is inherited mostly maternally, it evolves quickly and it lacks recombination (Avise, 1994; Birky, 2001; Ballard & Whitlock, 2004; see also e.g. Eyre-Walker & Awadalla, 2001 and Burzyñski *et al*., 2003 for evidence of infrequent mtDNA recombination; Gyllensten *et al*., 1991 for evidence of paternal inheritance). Mitochondrial genomes have generally been considered to be selectively neutral and not closely linked to genes responsible for maintaining reproductive isolation (Barton & Jones, 1983), although the

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neutrality of mtDNA has recently been challenged (William, Ballard & Kreitman, 1995; Rand, 2001; Gemmell, Metcalf & Allendorf, 2004). Since the mitochondrial genes are less closely linked, on average, with nuclear genes than nuclear genes are with each other (Barton & Jones, 1983; Barton & Hewitt, 1985), the mitochondrial genome has the potential to overcome boundaries of populations or even species more easily than do nuclear genes, which can be more closely linked to loci under selection on the hybrid background. The smaller effective population size of mtDNA compared with nuclear loci may facilitate this process. Therefore, even a very small amount of immigration is sufficient to establish a neutral mitochondrial haplotype in a foreign population (Takahata & Slatkin, 1984). However, mtDNA introgression may arise not only by chance due to the low effective population size of mtDNA, but also by selection due to local adaptation of mitochondria, or by selective introgression following mutational meltdown in small populations (Ballard & Whitlock, 2004). Interspecific mitochondrial introgression or complete transfer, accompanied by little or no nuclear introgression, has been shown to occur in a number of taxa, for example in *Triturus* (Babik, Szymura & Rafiński, 2003), *Oreochromis* (Rognon & Guyomard, 2003), *Dendroica* (Rohwer, Bermingham & Wood, 2001), *Thomomys* (Ruedi, Smith & Patton, 1997), *Microtus* (Jaarola, Tegelström & Fredga, 1997), *Salvelinus* (Wilson & Bernatchez, 1998) and *Drosophila* (William & Ballard, 2000) (see also Avise (1994: 260) and Arnold (1997: 52–56, 58–63, 162–172) for a review of older literature).

Hybrid zones, defined as regions where genetically distinct populations meet and produce hybrids (Barton & Hewitt, 1985), offer an excellent tool with which to investigate levels of gene flow and to compare the involvement of diverse parts of the genome in forming reproductive barriers between incipient species. If a mitochondrial gene, even a single differentiated codon, was under epistatic selection, the entire mtDNA genome would be involved, due to lack of recombination. Only a small number of studies have shown tight coincidence and concordance between mtDNA and allozyme clines (e.g. Szymura, Uzzell & Spolsky, 2000; Dasmahapatra *et al*., 2002). These cases seem to be the exception, however. As it turns out, mtDNA clines in hybrid zones are often discordant with those inferred from nuclear loci. Such discordance may be caused either by introgression through stable hybrid zones, or the occasional founder effect (Barton & Hewitt, 1989) as, for example, likely occurred in house mice *Mus musculus* in Scandinavia (Gyllensten & Wilson, 1987; Prager *et al*., 1993; see also below).

Hybrid zones in *M. musculus* provide an excellent opportunity to study congruence between nuclear and mitochondrial patterns of introgression. Two commensal *M. musculus* taxa (here considered as subspecies (Auffray *et al*., 1990)) occur in Europe: *M. m. domesticus* Schwarz & Schwarz, 1943, in the west and south, and *M. m. musculus* Linnaeus, 1758, in the north and east. In the area of secondary contact there is a narrow zone of hybridization. This hybrid zone stretches across the Jutland Peninsula and from the Baltic coast of East Holstein, through central Europe and the Balkan Peninsula to the Bulgarian Black Sea coast (see Boursot *et al*., 1993 and Sage, Atchley & Capanna, 1993 for reviews and Macholán, Kryštufek & Vohralík, 2003 for the position of the zone in the Balkans). Consistent with the prediction that mtDNA has the potential to flow across contact zones more rapidly than do nuclear genes, introgression of mitochondrial genomes has been shown previously in three transects across the *M. m. musculus*/*M. m. domesticus* hybrid zone (encircled regions in Fig. 1).

An interesting pattern of mtDNA introgression is found in the south–north transect through the Danish part of the hybrid zone. In this region the southern populations of *M. m. domesticus* have two mtDNA variants (S1 and S2) and the northern *M. m. musculus* populations possess a third variant (N), also of *M. m. domesticus* origin (Ferris *et al*., 1983; Vanlerberghe *et al*., 1986, 1988b; Gyllensten & Wilson, 1987). Gyllensten & Wilson (1987) showed that the introgression of *M. m. domesticus* mtDNA extends into Sweden for at least 750 km beyond the Danish hybrid zone. The results of Vanlerberghe *et al*. (1988b) suggested that there is a steep cline between the southern and northern types of *M. m. domesticus* mtDNA across the hybrid zone compared with the ten autosomal loci. However, when a larger sample was analysed the degree of introgression was found to be much more extensive than originally thought (B. Dod & P. Boursot, unpubl. data).

Similarly, all 104 mice from 12 localities across the East Holstein hybrid zone were found to carry *M. m. domesticus* mtDNA (Prager *et al*., 1993). In the eastern part of the Holstein transect, where the mice have nearly pure, or largely pure, *M. m. musculus* nuclear genomes, 86% of the animals have mtDNA belonging to the clade which includes Swedish, Finnish and northern Danish mice (variant N). On the *M. m. domesticus* side of the Holstein hybrid zone, 61% of mice carry this type of mtDNA. In contrast, as shown by Vanlerberghe *et al*. (1988b), only 1% of the *M. m. domesticus* mice in south Jutland carry the N type of mtDNA. Gyllensten & Wilson (1987) suggested that northern Denmark, Sweden and Finland were colonized from the East Holstein Peninsula and invoked a series of founder and island-hopping events when mouse populations were sparse. The colonizing

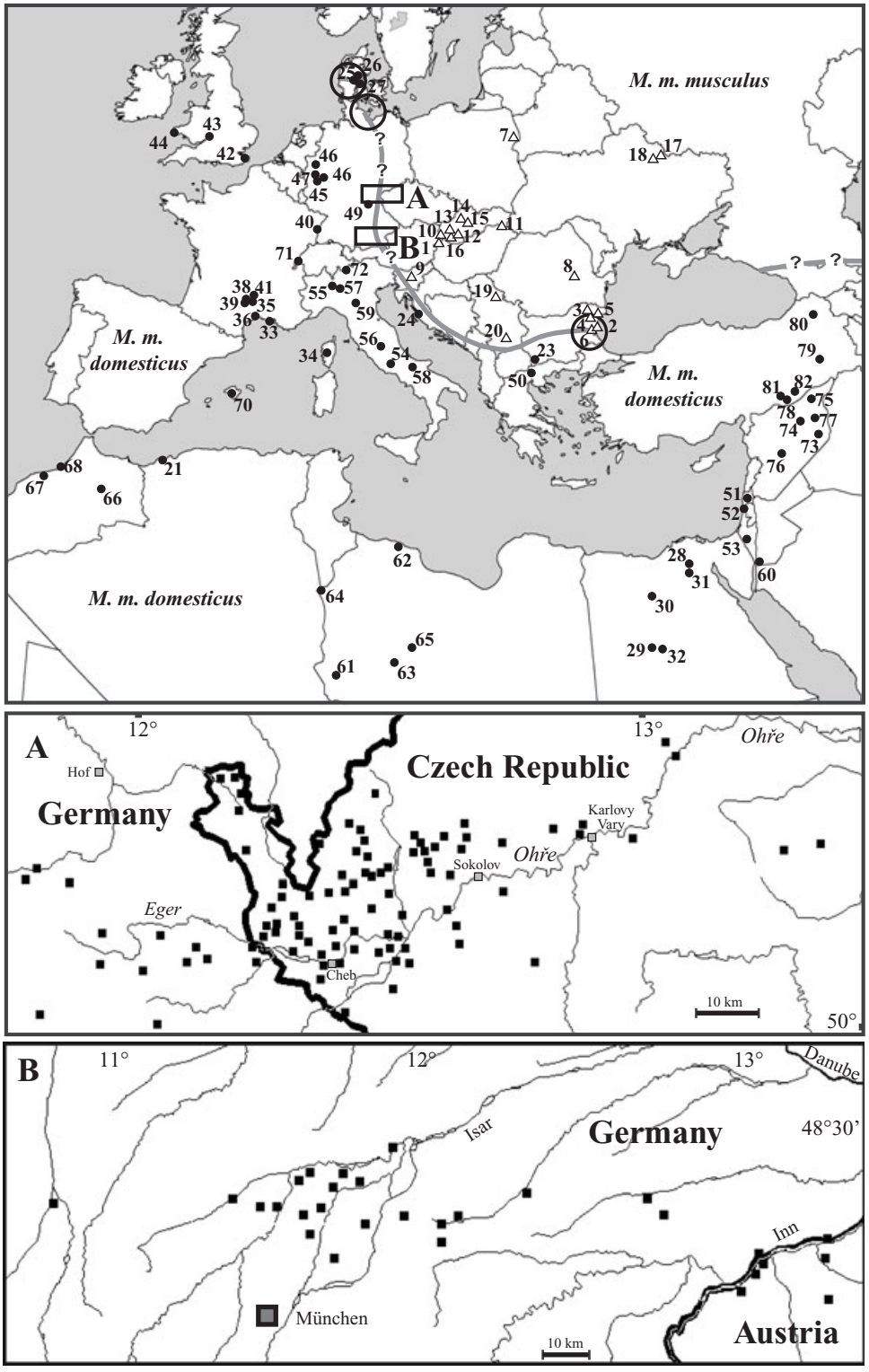

Figure 1. Geographical position of the *Mus musculus musculus*/*M. m. domesticus* hybrid zone, previously studied transects (open circles) and localities used for testing the *Bam*HI marker (numbered as in Appendix 1). Presence of *M. m. domesticus* (●) and *M. m. musculus* (△) mtDNA types are indicated. The two transects covered in this study (A and B) are enlarged below the main map.

females were probably hybrids carrying *M. m. domesticus* mtDNA and predominantly *M. m. musculus* nuclear DNA. This hypothesis, based on mtDNA restriction analyses, was further supported by mtDNA sequencing of Scandinavian mice (Prager *et al*., 1993).

In the Bulgarian hybrid zone transect, mtDNA introgression seems to occur in the opposite direction, from the range of *M. m. musculus* into the range of *M. m. domesticus*. While 25% of scored individuals had *M. m. musculus* mtDNA on a *M. m. domesticus* genetic background, only 2% had the opposite combination (Vanlerberghe *et al*., 1988a). The distribution of mtDNA types in Caucasus, where only 65 mice from 11 localities were studied, seemed rather chaotic (Orth *et al*., 1996). However, given the small number of mice analysed it is difficult to say whether the differences between allozymes and mtDNA introgression are really significant. Also, the extent of nuclear gene introgression in Caucasus seems to be higher than it is in other transects in Europe. It should also be noted that the mouse populations south of Caucasus are less differentiated (Din *et al*., 1996), and the situation is also complicated by the fact that the centre of the zone has not yet been determined.

Summarizing our understanding of mtDNA behaviour in the hybrid zone between *M. m. musculus* and *M. m. domesticus*, we see that our knowledge is restricted to the northern and southern ends of the hybrid zone, and that mtDNA behaviour varies considerably among various transects. There is little published data available on mtDNA introgression in the central part of the hybrid zone. To date, only two studies have been published with information on mtDNA introgression in this region of the *M. musculus* hybrid zone, and neither of these focused specifically on the hybrid zone. Prager, Tichy & Sage (1996) studied 97 mice from four localities from a transect in the vicinity of Bavaria and found evidence of long-distance mtDNA introgression (about 100 km eastwards from the centre of the zone). However, Munclinger *et al*. (2002) did not find any signs of introgression of *M. m. domesticus* mtDNA into the range of *M. m. musculus* in their examination of 410 mice from 49 localities in the Czech Republic and Slovakia, as well as adjacent areas of north-eastern Bavaria and southern Thuringia.

In this study we analysed and compared the Bavarian and the Czech transects across the hybrid zone in more detail. Specifically, we asked whether the pattern of mtDNA behaviour could change over a rather limited geographical distance. Since the movement of the X chromosome has been shown to be severely limited in four independent transects and the X chromosome is assumed to be under strong selection in the hybrid zone (Tucker *et al*., 1992; Dod *et al*., 1993; see also Dod *et al*., 2005, this issue; Payseur & Nachman, 2005, this issue), the X chromosome clines were taken as a baseline for comparison of widths and centre positions of mtDNA clines between transects. Data for presumably selectively neutral allozymes were also included for purposes of comparison (see also Raufaste *et al*., 2005, this issue).

MATERIAL AND METHODS

Animals

The commensal mice used in this study were collected in two independent transects approximately 180 km apart: a transect in the westernmost part of the Czech Republic and adjacent areas of north-east Bavaria (herein called the Czech transect) and a transect in southern Germany and western Austria (herein called the Bavarian transect). The two-dimensional Czech transect was approximately 110 km long and 40 km wide and consisted of 98 localities. Mice were collected during the years 1992–2002. A detailed description of the Czech transect will be provided elsewhere (M. Macholán *et al*., unpubl. data). The Bavarian transect was sampled as a linear west–east transect over 180 km across the hybrid zone and consisted of 41 localities. Mice were collected in 1984–85 and 1992. Most of the samples analysed in the German transect were the same as those in Tucker *et al*. (1992). The positions of transects and localities are indicated in Figure 1.

DNA isolation

DNA was isolated from frozen or ethanol-preserved tissues using proteinase K digestion and subsequent extraction with phenol–chloroform and ethanol precipitation (Hoelzel & Green, 1992).

mtDNA markers

Twenty-five mice from 19 localities from the Czech transect and 15 mice from 12 allopatric populations were used in a pilot study to identify mtDNA markers useful in studying the hybrid zone. Four restriction sites (*Bam*HI, *Eco*RI, *Hpa*I, *Xba*I) given in Boursot *et al*. (1996) and one (*Dra*I) in Orth *et al*. (1996) assumed to differentiate *M. m. domesticus* and *M. m. musculus* mtDNA were examined. Primers flanking the restriction sites were found using the programme Primer3 (Rozen & Skaletsky, 2000). Nucleotide positions of the fragments studied, primer sequences and relevant restriction enzymes are given in Table 1. Bayona-Bafaluy *et al*. (2003) demonstrated that the mouse mtDNA sequence frequently used as a reference (Bibb *et al*., 1981) contains errors. However, we used the positions according to Bibb *et al*. (1981) to

Table 1. Characteristics of mtDNA fragments used to test for fixed differences between *Mus musculus musculus* and *M. m. domesticus*

Position and length of fragment	Restriction enzyme	Position of restriction site	Primers	Gene region
3270–3699, 430 bp	*Bam*HI	3565	5′-TTACTTCTGCCAGCCTGACC-3′ 5′-ATGGTGGTACTCCCGCTGTA-3′	*mt Nd1*
3815–4240, 426 bp	*Eco*RI	4013	5′-TCTCCGTGCTACCTAAACACC-3′ 5′-GCGAGGCCTAGTTTTATGGA-3′	*mt-Nd2*
8395–8817, 423 bp	*Hpa*I	8637	5′-AGCAGTCCGGCTTACAGCTA-3′ 5′-GTGGCCTTGGTAGGTTCCTT-3′	*mt-Nd3*
9366–9783, 418 bp	*Xba*I	9652	5′-CGTCTCCATTTATTGATGAGGAT-3′ 5′-TTGTGTTCATTCATATGCTAGGC-3′	*mt-Tp*
15 372–15 837, 466 bp	*Dra*I	15589	5′-CACCACCAGCACCCAAAAGCT-3′ 5′-TAAGGGGGAACGTATGAGGCG-3′	*mt-Co3*

Nucleotide positions are according to Bibb *et al*. (1981) but see Bayona-Bafaluy *et al*. (2003).

allow easy comparison with the literature cited above. All the mtDNA fragments were amplified under the same PCR conditions: 1.5 mM $MgCl_2$ and 200 μM each dNTP. Reactions were performed using an MJ thermal cycler as follows: 95 °C for 3 min and 35 cycles at 95, 55 and 72 °C, each for 30 s. The PCR products were digested with the restriction endonuclease and subsequently analysed on 2% agarose gels. On the basis of the pilot study (see Results) the *Bam*HI restriction site was selected as a reliable marker and used to examine 1270 mice in the Czech transect and 456 mice in the Bavarian transect.

Nuclear markers

The following markers were used to compare the mitochondrial and nuclear genomes in the two transects. For the X chromosome, we used a B1 insertion in the *Btk* gene (Munclinger, Boursot & Dod, 2003), and RFLP markers X1 (DXSmh141) at 7.8 cM and X2 (DXWas31) at 68.0 cM (Tucker *et al*., 1992). For the autosomes, we used three enzyme markers located on three different chromosomes: *Np, Es1, Mpi* (M. Macholán *et al*., unpubl. data; Tucker *et al*., 1992). The sample size for the X chromosome and allozyme data in the Bavarian transect was slightly smaller than that for the mtDNA screening (see Tucker *et al*., 1992 for details).

Data analysis

Hybrid zone analysis has been developed primarily for fitting clines on two dimensions defined by a distance parameter of each population along a linear transect through a hybrid zone, and the allele frequency of a particular marker in the corresponding population. These conditions were met in the Bavarian transect, where the sampling design followed a linear arrangement of the localities going from west to east (Fig. 1). Hence, the locality distances were computed as a distance from the westernmost locality, Augsburg.

Due to the sampling design adopted in the Czech transect, each locality could be characterized unequivocally in three-dimensional space. Geographical coordinates defined the first two dimensions (with the *x*-axis and *y*-axis running in the west–east and south–north direction, respectively). The third dimension (*z*-axis) was the average frequency of the *M. m. musculus* alleles calculated over six autosomal allozymes located on six different chromosomes *Idh*, *Gdh*, *Sod1*, *Np*, *Est1* and *Mpi* (M. Macholán *et al.*, unpubl. data). This frequency (hereafter refered to as HI6) ranged from 0 for a pure *M. m. domesticus* to 1 for a pure *M. m. musculus*. To reduce space dimensionality we used the Spline module to fit a surface to the *xyz* coordinate data based on the cubic spline smoothing procedure. The stiffness parameter, which determines the degree to which the fitted curve depends on local configurations of the analysed values, was set to 0.25. The position of the centre of the hybrid zone was defined as the isocline with an HI6 frequency of 0.5. The distance (DIST) of a given locality was calculated as a perpendicular (the shortest) distance to the 0.5 isocline.

Cline fitting in three-dimensional space was performed using Statistica, version 6.0 (StatSoft Inc., 2001). The frequency of *M. m. musculus* alleles for each locality and each marker was computed and the hyperbolic tangential (tanh) equation

$$P_i = \left(1 + \tanh\left[\frac{2(x_i - c)}{w}\right]\right)\Big/2$$

(Szymura & Barton, 1986, 1991; Porter *et al*., 1997) was applied to approximate clines; P_i is the expected frequency of the *musculus* alleles, x_i is the distance of

the *i*th population along the transect, *c* is centre and *w* is width (all in kilometres). Maximum likelihood was used to fit the data to the hybrid zone model following the approach described by Szymura & Barton (1986, 1991). Clines were approximated using a computer program developed by J. Piálek which incorporated the Metropolis algorithm (Press *et al*., 1989) and followed the method detailed by Porter *et al*. (1997). It differed from Porter's method in that it used sample frequencies instead of individual genotypes to fit data. The statistics used to maximize the tanh model for sample frequencies was

$$G = \sum_i \left[p_i \ln\left(\frac{p_i}{P_i}\right) + (1-p_i)\ln\left(\frac{1-p_i}{1-P_i}\right)\right],$$

where p_i and P_i are the observed and expected frequencies at the *i*th population, respectively. The estimates of the two parameters *c* and *w* were further used for comparisons of mtDNA and autosomal and X chromosome data.

We developed this model of data fitting to keep the estimates of cline shape as simple as possible (only two parameters, *c* and *w* were estimated). The model produced an S-shaped curve and represented in our opinion the most robust model estimate, in that it was minimally dependent on local conditions and therefore represented the most suitable model for comparison between different transects of the hybrid zone. Other techniques estimating more parameters to characterize cline shape (ClineFit by Porter *et al*., 1997; Analyse by N. Barton & S. Baird http://helios.bto.ed.ac.uk/evolgen/Mac/Analyse/) might reflect locally different inferences between selection and geographical barriers or epistatic interactions. The contribution of these factors would be difficult to explain without detailed knowledge of local conditions and would therefore make a direct comparison among transects more difficult. For this reason such models were not considered in this study.

Comparison of mtDNA, autosomal loci and the X chromosome

The cline characteristics for presumably selectively neutral allozymes and for markers on X chromosomes that are assumed to be under strong selection (Tucker *et al*., 1992; Dod *et al*., 1993) were employed to estimate the degree of mitochondrial gene flow in the hybrid zone.

The X chromosome clines were taken as a baseline for comparison of widths and centre positions of mtDNA clines between transects. To allow easy comparison of the two transects, the distance of the centres for *Btk* and X2 (X chromosome markers) were set to 0 km and the other values were recalculated accordingly (Figs 2, 3). In other words, the *Btk* and X2 clines were forced to coincide. Therefore, positive values of distances indicated the position of a given locality on the *M. m. musculus* side of the hybrid zone and negative values indicated the *M. m. domesticus* side according to X chromosome markers.

RESULTS

Verification of the potential mtDNA markers

Only two of the five mtDNA markers scored in the panel of wild mouse DNA showed fixed differences

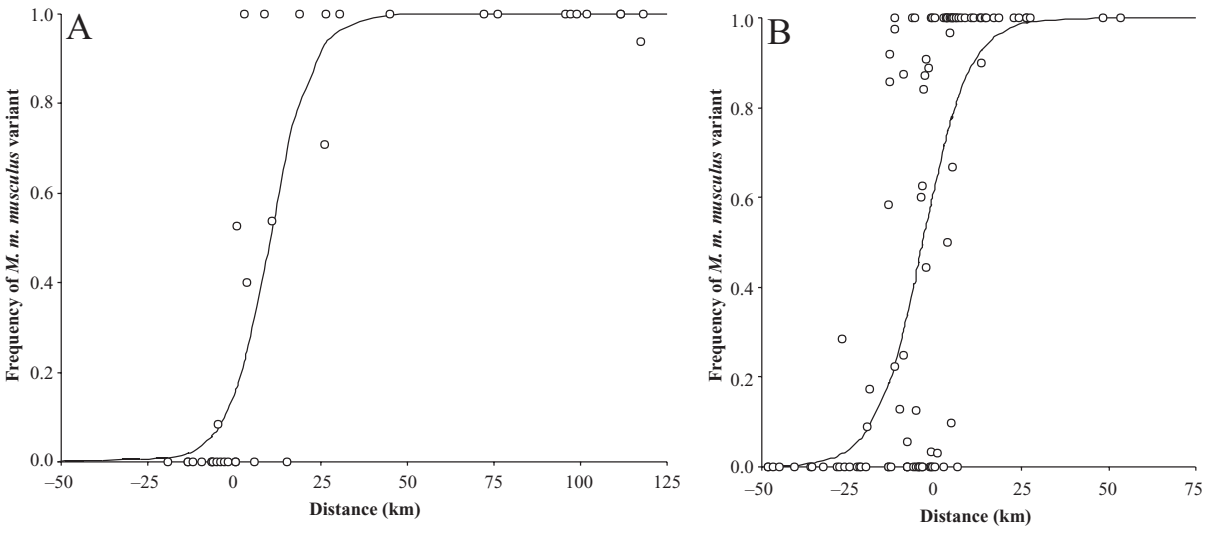

Figure 2. Frequency of *Mus musculus musculus* mtDNA (○) and the model fitted to these data in the Bavarian (A) and the Czech (B) transects. Localities Simbach and Ranshofen-Holfinger were not included in the analysis of the Bavarian transect. The zero distance along both transects refers to the centres of the clines for *Btk* and X2 (X chromosome markers).

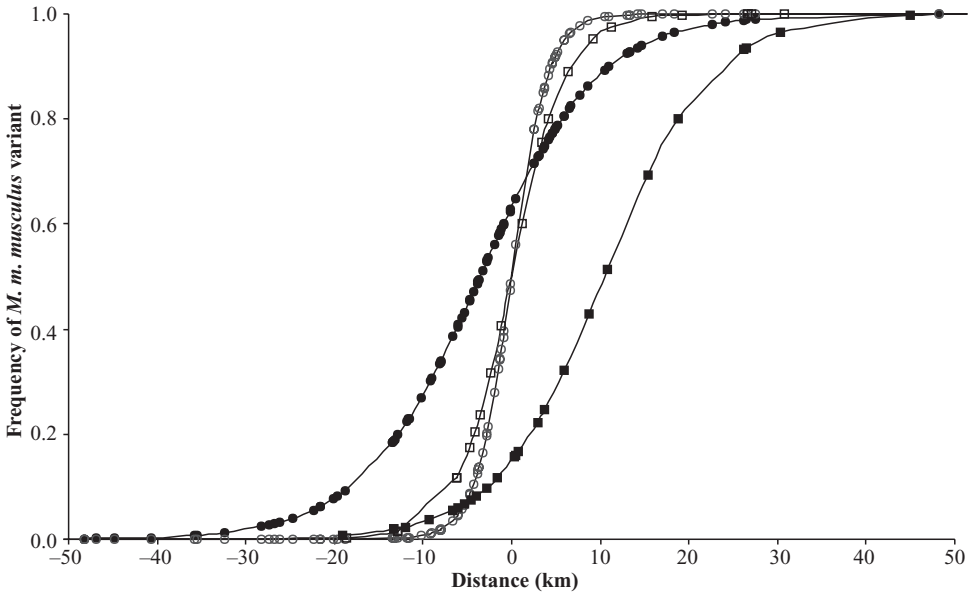

Figure 3. Comparison of the Czech (circles) and Bavarian (squares) transects (fitted clines only; for original data see Appendices 2 and 3). Clines for X chromosome markers (*Btk* and X2, open symbols) and mtDNA (filled symbols) are compared in the central parts of both transects. To ease comparison, the cline centres for the X chromosome markers were set to 0 km for both transects.

between *M. m. musculus* and *M. m. domesticus*: restriction sites 3565 for *Bam*HI and 9562 for *Xba*I (Table 2). The other restriction sites did not appear as diagnostic markers for the following reasons. The restriction site of *Eco*RI was found to be present in both *M. m. musculus* and *M. m. domesticus* (in contrast with, e.g. Yonekawa *et al*., 1982 or Vanlerberghe *et al*., 1988b). Sequencing of two samples (*M. m. musculus* from locality Boučí and *M. m. domesticus* from locality Straas) confirmed the presence of the restriction site in both subspecies. (All sequences produced during the pilot study have been deposited in the GenBank database, accession numbers AY 394055–AY 394061). The restriction sites of *Hpa*I and *Dra*I were not fixed in one subspecies and missing in the other one. In three *M. m. domesticus* mice from two localities we also found a new unpublished haplotype using *Hpa*I. There was an additional restriction site at position 8731. With additional screening (Table 3), localities possessing only one, two or even all three of the haplotypes were found. The localities possessing the two *Hpa*I restriction sites were not obviously clustered geographically.

As the presence/absence of the *Bam*HI restriction site was easy to score and had high repeatability, we chose it as the best marker to screen the mtDNA of our hybrid zone samples. Before using this marker to investigate the pattern of mtDNA transition across the hybrid zone, we further quantified its reliability. We analysed 144 animals from 43 allopatric populations (for numbers of scored animals and geographical position of each locality see Appendix 1 and Fig. 1). Published data (Yonekawa *et al*., 1982; Boursot *et al*., 1996; Munclinger *et al*., 2002) for 54 samples from 34 localities were also included. All scored samples from areas occupied by *M. m. musculus* (Marshall & Sage, 1981) had the *M. m. musculus* type of mtDNA (absence of restriction site), whereas samples from areas occupied by *M. m. domesticus* had the *M. m. domesticus* type of mtDNA (presence of restriction site). We also sequenced two animals from allopatric populations near the hybrid zone (Neuenreuth and Nová Ves u Sokolova) and two animals from a locality in the centre of the hybrid zone (Nový Drahov). We found that absence of the restriction site was caused by a synonymous transition at position 3656 (G instead of A in the *M. m. musculus* mtDNA fragment). Finally, mice from localities where a discrepancy between mtDNA and allozyme data occurred in the Czech transect were also screened for the *Xba*I restriction site. No inconsistency was observed between the two markers.

mtDNA INTROGRESSION ACROSS THE HYBRID ZONE

Figures 2 and 3 show the predicted frequencies of the *M. m. musculus* alleles of mtDNA, *Btk* and X2, in different localities along the Czech and the Bavarian transects. In the Bavarian transect two localities were excluded for reasons given below. Actual frequencies are given in Appendices 2 and 3.

Table 2. The suitability of potential mtDNA markers in *Mus musculus*: observed and expected results obtained for the five tested restriction enzymes

					Observed (expected) presence of restriction site				
Locality	Latitude	Longitude	Country	*N*	*Bam*HI	*Eco*RI	*Hpa*I	*Xba*I	*Dra*I
M. m. domesticus									
Aš	50°12′	12°12′	CZE	2	+ (+)	+ (+)	– (+)	– (–)	– (–)
Dolní Luby	50°14′	12°25′	CZE	2	+ (+)	+ (+)	+ +(+)	– (–)	– (–)
Doubí	50°07′	12°24′	CZE	2	+ (+)	+ (+)	– (+)	– (–)	– (–)
Essen	51°27′	07°00′	GER	2	+ (+)	+ (+)	– (+)	– (–)	– (–)
Grasseman	50°01′	11°47′	GER	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Hohenberg	50°05′	12°13′	GER	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Kleinwerdern	50°00′	12°01′	GER	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Mierhof	50°04′	11°54′	GER	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Neuenreuth	50°05′	12°07′	GER	1	+ (+)	+ (+)	– (+)	– (–)	+ (–)
Röslau	50°04′	11°59′	GER	1	+ (+)	+ (+)	+ +(+)	– (–)	– (–)
Straas	50°10′	11°45′	GER	2	+ (+)	+ (+)	– (+)	– (–)	– (–)
Thierstein	50°06′	12°06′	GER	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Waldau	50°02′	11°31′	GER	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Cave	41°49′	12°55′	ITA	2	+ (+)	+ (+)	+ (+)	– (–)	+ (–)
Massegros	44°18′	03°10′	FRA	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
Rupite	41°27′	23°16′	BUL	1	+ (+)	+ (+)	– (+)	– (–)	– (–)
M. m. musculus									
Anenská Ves	50°12′	12°33′	CZE	2	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Boučí	50°14′	12°35′	CZE	2	– (–)	+ (–)	– (–)	+ (+)	– (+)
Brno	49°12′	16°37′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Dlouhé Mosty	50°06′	12°22′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Dolní Nivy	50°14′	12°38′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Kostelní Bříza	50°06′	12°37′	CZE	2	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Křižovatka	50°11′	12°23′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Kulířov	49°23′	16°50′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Necpaly	48°59′	18°58′	SVK	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Nový Kostel	50°13′	12°25′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Obilná	50°5′	12°28′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Stráž nad Ohří	50°20′	13°02′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Vimperk	49°03′	13°46′	CZE	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Terca	45°31′	26°31′	ROM	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)
Šušara	44°56′	21°07′	YUG	1	– (–)	+ (–)	– (–)	+ (+)	+ (+)

Determination of the subspecies status in localities close to the hybrid zone was based on six allozyme loci (M. Macholán *et al.*, unpubl. data). Presence of a restriction site is represented by plus (+), absence of a restriction site is marked as minus (–). The haplotype with two restriction sites for *Hpa*I is marked as (++). BUL, Bulgaria; CZE, Czech Republic; FRA, France; GER, Germany; ITA, Italy; ROM, Romania; SVK, Slovakia; YUG, the former Yugoslavia.

The changes in the haplotype frequencies of mtDNA resulted in a wide cline, suggesting that the movement of mtDNA across the zone was not restricted by selection or other factors. The X chromosome clines had a surprisingly similar shape in both transects (Fig. 3). The shape of the mtDNA cline was similar to the cline of autosomal allozymes but differed from the steep cline for X chromosome markers (see Table 4 for cline parameters and confidence intervals). Cline width, which correlated with the level of selection, was nearly the same for mtDNA and all three allozymes in the Czech transect. Cline width for *Mpi* in the Bavarian transect was narrower than were those for the other allozymes and mtDNA.

Apparent non-coincidence of mtDNA and X clines was found (Table 4, Fig. 3). The mtDNA cline centre was shifted about 3.6 km to the west in the Czech transect and 30.3 km to the east in the Bavarian transect. This large difference in the position of the centre in Bavaria was caused by long-distance intro-

Table 3. Additional screening of presence/absence of *Hpa*I restriction sites in the mtDNA of *Mus musculus*

Locality	Country	N	Haplotype 1	Haplotype 2	Haplotype 3
M. m. domesticus					
Aš	CZE	9	9		
Čirá	CZE	3			3
Dolní Luby	CZE	11	1	6	4
Dolní Pelhřimov	CZE	2	2		
Hranice	CZE	10	5	5	
Krásňany	CZE	2	2		
Lužná	CZE	12	12		
Milhostov	CZE	7	3	4	
Neuenreuth	CZE	10	8		2
Skalka (Hazlov)	CZE	2	2		
Smrčina	CZE	10	10		
Svatý Kříž	CZE	6	10		
Straas	GER	13	13		
Pomaylors	FRA	3	3		
St. Saturnin-de-Lenne	FRA	2	2		
Cave	ITA	1		1	
Casablanca	MOR	1	1		
M. m. musculus					
Libořezy	CZE	2	2		
Vimperk	CZE	2	2		

Haplotype 1: restriction site for *Hpa*I is not present; haplotype 2: presence of one restriction site at position 8637; haplotype 3: presence of two restriction sites at positions 8637 and 8731. Positions are according to Bibb *et al*. (1981). CZE, Czech Republic; FRA, France; GER, Germany; ITA, Italy; MOR, Morocco.

Table 4. mtDNA, allozyme and X chromosome markers cline parameters for the Czech and Bavarian transects of the hybrid zone between *Mus musculus musculus* and *M. m. domesticus*

	Czech transect		Bavarian transect	
	Centre (km)	Width (km)	Centre (km)	Width (km)
mtDNA	–3.57	26.57	30.34	100.30
	(–3.92; –3.22)	(26.35; 27.79)	(30.16; 30.52)	(99.89; 100.71)
mtDNA (40)			20.31 (20.17; 20.45)	61.86 (61.48; 62.24)
mtDNA (39)			10.93 (10.85; 11.02)	24.02 (23.85; 24.19)
Btk	0	8.07		
	(–0.13; 0.13)	(7.81; 8.32)		
X1			0.49 (0.47; 0.51)	5.91 (5.88; 5.95)
X2			0 (–0.04; 0.04)	12.23 (12.10; 12.36)
Es1	–1.25	21.04	5.15	22.33
	(–1.38; –1.11)	(20.50; 21.57)	(4.95; 5.35)	(21.92; 22.74)
Mpi	0.79	21.50	0.79	9.91
	(0.61; 0.97)	(20.90; 22.10)	(0.76; 0.81)	(9.85; 9.98)
Np	0.59	33.29	7.47	29.72
	(0.37; 0.81)	(32.55; 34.04)	(7.38; 7.57)	(29.29; 30.14)

To allow easy comparison, the cline centres for *Btk* and X2 (X chromosome markers) were set to 0 km and the other values were recalculated accordingly. The locality Ranshofen-Holfinger was not included in the mtDNA (40) cline. The localities Ranshofen-Holfinger and Simbach were not included in the mtDNA (39) cline. Confidence intervals are given in parentheses under each centre and width value.

gression of *M. m. domesticus* mtDNA. If the outlying localities with unexpected frequencies (localities Simbach 150.2 km from Augsburg, frequency of *M. m. musculus* mtDNA 0.4, and Ranshofen-Holfinger 152.8 km from Augsburg, frequency of *M. m. musculus* mtDNA 0) were not included in the analysis, the difference between positions of mtDNA and X clines centres was markedly smaller. The exclusion of locality Ranshofen-Holfinger (mtDNA 40) changed the difference between the two centres to 20.3 km, and the exclusion of both localities (mtDNA 39) reduced it to 10.9 km.

DISCUSSION

The selection of proper markers with fixed alternative alleles in the parental taxa is an important precondition of hybrid zone studies. In spite of extensive knowledge of the mouse genome the number of such markers is very limited for wild mice. Given this fact, the simple subspecies-specific markers we used can be valuable in unequivocally distinguishing between *M. m. musculus* and *M. m. domesticus* mtDNA in a variety of studies. The fact that only two of five presumed mtDNA markers showed fixed differences between *M. m. musculus* and *M. m. domesticus* underscores the importance of sampling a large number of allopatric populations in pilot experiments preceding the screening of individuals from the hybrid zone. Some inaccuracy may also arise when the positions of the restriction sites are deduced from restriction maps of total mtDNA. As a consequence the relatively small fragments amplified by PCR may not contain the expected restriction site.

The width of mtDNA clines in our study fell into the range of allozymes but was larger than that of the X markers, as we might predict for a part of the genome that is not under strong selection in the hybrid zone. Moreover, the centres of mtDNA clines were displaced far outside the main clusters of clines. Therefore, we can assume that mtDNA is unlikely to be directly involved in systems responsible for reproductive isolation of the two mouse taxa. Surprisingly, the mtDNA introgression in Bavaria was opposite in direction to that observed in the Czech transect. Although considerable differences between transects across the mouse hybrid zone have been shown previously, the fact that geographically very close transects can show quite different results is of interest. This finding corroborates the view that mitochondrial genes often have the potential to pass through boundaries of differentiated taxa more easily than do nuclear genes. There are several interacting factors that may account for the observed behaviour of mtDNA in the hybrid zone, and it is difficult to distinguish between them. Introgression may arise due to the low effective population size of mtDNA and hence its greater sensitivity to random effects such as drift or founder effect. Additionally, the presumed neutrality of mtDNA, and/or weaker linkage to genes under strong selection in the hybrid zone could influence the introgression of mtDNA in the hybrid zone. However, it should be mentioned that the presumed neutrality of mouse mtDNA has been challenged by Nachman, Boyer & Aquadro (1994) and also that the assumption concerning the lower effective population size of mtDNA can be questioned. The effective population size is also affected by the distribution of reproductive success (Ballard & Whitlock, 2004). This holds true especially in species with strong sexual selection and therefore a small effective number of males, which is probably the case in *M. musculus*, with only dominant males being expected to reproduce. Under such circumstances the rate of drift in nuclear genes is not necessarily lower than that for mitochondrial ones (Ballard & Whitlock, 2004).

At two localities in the Bavarian transect surprisingly long distance introgression of *M. m. domesticus* mtDNA into the *M. m. musculus* range (about 100 km) was observed. As the neighbouring localities did not carry the *M. m. domesticus* variant of mtDNA it seems likely that human-mediated transport of mice may account for such results. The influence of such long-distance transport was also visible at the *Es1* locus, where *M. m. domesticus* alleles were also found at the same two localities. Such long-distance introgression due to human activities has been demonstrated repeatedly in mice (e.g. Orth *et al*., 1998; see also Pocock, Hauffe & Searle, 2005, this issue).

Although stochastic processes appear to be the major factor influencing the behaviour of mtDNA in the hybrid zone between *M. m. musculus* and *M. m. domesticus*, we cannot fully exclude nonrandom association between cytoplasmic and nuclear genes (cytonuclear disequilibria) as a factor influencing local variation in the transects studied. In future work we would like to investigate these alternatives using exact tests for cytonuclear disequilibria that have been developed recently (Asmussen & Basten, 1994; Basten & Asmussen, 1997).

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REFERENCES

Arnold ML. 1997. *Natural hybridization and evolution*. New York and Oxford: Oxford University Press.

Asmussen MA, Basten CJ. 1994. Sampling theory for cytonuclear disequilibria. *Genetics* **138:** 1351–1363.

Auffray J-C, Marshall JT, Thaler L, Bonhomme F. 1990. Focus on the nomenclature of European species of *Mus*. *Mouse Genome* **88:** 7–8.

Avise JC. 1994. *Molecular markers: natural history and evolution*. New York: Chapman & Hall.

Babik W, Szymura JM, Rafiński J. 2003. Nuclear markers, mitochondrial DNA and male secondary sexual traits variation in a newt hybrid zone (*Triturus vulgaris* × *T. montandoni*). *Molecular Ecology* **12:** 1913–1930.

Ballard JWO, Whitlock MC. 2004. The incomplete natural history of mitochondria. *Molecular Ecology* **13:** 729–744.

Barton NH, Hewitt GM. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16:** 113–148.

Barton NH, Hewitt GM. 1989. Adaptation, speciation and hybrid zones. *Nature* **341:** 497–503.

Barton NH, Jones JS. 1983. Mitochondrial DNA: new clues about evolution. *Nature* **306:** 317–318.

Basten CJ, Asmussen MA. 1997. The exact test for cytonuclear disequilibria. *Genetics* **146:** 1165–1171.

Bayona-Bafaluy MP, Acín-Pérez R, Mullikin JC, Park JS, Moreno-Loshuertos R, Hu P, Pérez-Martos A, Fernández-Silva P, Bay Y, Enríquez JA. 2003. Revisiting the mouse mitochondrial DNA sequence. *Nucleic Acids Research* **31:** 5349–5355.

Bibb MJ, van Etten RA, Wright CT, Walberg MW, Clayton DA. 1981. Sequence and gene organization of mouse mitochondrial DNA. *Cell* **26:** 167–180.

Birky CV Jr. 2001. The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. *Annual Review of Genetics* **35:** 125–148.

Boursot P, Auffray J-C, Britton-Davidian J, Bonhomme F. 1993. The evolution of house mice. *Annual Review of Ecology and Systematics* **24:** 119–152.

Boursot P, Din W, Anand R, Darviche D, Dod B, Deimling FV, Talwar GP, Bonhomme F. 1996. Origin and radiation of the house mouse: mitochondrial DNA phylogeny. *Journal of Evolutionary Biology* **9:** 391–415.

Burzyński A, Zbawicka M, Skibinski DOF, Wenne R. 2003. Evidence for recombination of mtDNA in the marine mussel *Mytilus trossulus* from the Baltic. *Molecular Biology and Evolution* **20:** 388–392.

Dasmahapatra KK, Blum MJ, Aiello A, Hackwell S, Davies N, Bermingham EP, Mallett T. 2002. Inferences from a rapidly moving hybrid zone. *Evolution* **56:** 741–753.

Din W, Anand R, Boursot P, Darviche D, Dod B, Jouvin-Marche E, Orth A, Talwar GP, Cazenave P-A, Bonhomme F. 1996. Origin and radiation of the house mouse: clues from nuclear genes. *Journal of Evolutionary Biology* **9:** 519–539.

Dod B, Jermiin LS, Boursot P, Chapman VM, Nielsen JT, Bonhomme F. 1993. Counterselection on sex chromosomes in the *Mus musculus* European hybrid zone. *Journal of Evolutionary Biology* **6:** 529–546.

Dod B, Smadja C, Karn RC, Boursot P. 2005. Testing for selection on the androgen-binding protein in the Danish mouse hybrid zone. *Biological Journal of the Linnean Society* **84:** 447–459.

Eyre-Walker A, Awadalla P. 2001. Does human mtDNA recombine? *Journal of Molecular Evolution* **53:** 430–435.

Ferris SD, Sage RD, Huang CM, Nielsen JT, Ritte U, Wilson AC. 1983. Flow of mitochondrial DNA across a species boundary. *Proceedings of the National Academy of Sciences, USA* **80:** 2290–2294.

Gemmell NJ, Metcalf VJ, Allendorf FW. 2004. Mother's curse: the effect of mtDNA on individual fitness and population viability. *Trends in Ecology and Evolution* **19:** 238–244.

Gyllensten U, Wharton D, Josefsson A, Wilson AC. 1991. Paternal inheritance of mitochondrial DNA in mice. *Nature* **352:** 255–257.

Gyllensten U, Wilson AC. 1987. Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. *Genetical Research* **49:** 25–29.

Hoelzel AR, Green A. 1992. Analysis of population-level variation by sequencing PCR-amplified DNA. In: Hoelzel AR, ed. *Molecular genetic analysis of populations. A practical approach*. New York: Oxford University Press, 159–280.

Jaarola M, Tegelström H, Fredga K. 1997. A contact zone with noncoincident clines for sex-specific markers in the field vole (*Microtus agrestis*). *Evolution* **51:** 241–249.

Macholán M, Kryštufek B, Vohralík V. 2003. The location of the *Mus musculus* / *M. domesticus* hybrid zone in the Balkans: clues from morphology. *Acta Theriologica* **48:** 177–188.

Marshall JT, Sage RD. 1981. The taxonomy of the house mouse. *Symposia of the Zoological Society of London* **47:** 15–25.

Munclinger P, Boursot P, Dod B. 2003. B1 insertions as easy markers for mouse population studies. *Mammalian Genome* **14:** 359–366.

Munclinger P, Božíková E, Šugerková M, Piálek J, Macholán M. 2002. Genetic variation in house mice (*Mus*, Muridae, Rodentia) from the Czech and Slovak Republics. *Folia Zoologica* 51: 81–92.

Nachman MW, Boyer SN, Aquadro CF. 1994. Nonneutral evolution at the mitochondrial NADH dehydrogenase subuit 3 gene in mice. *Proceedings of the National Academy of Sciences, USA* **91:** 6364–6368.

Orth A, Adama T, Din W, Bonhomme F. 1998. Hybridiza-

tion naturelle entre deux sous-espèces de souris domestique, *Mus musculus domesticus* et *Mus musculus castaneus*, près du lac Casitas (Californie). *Genome* **41:** 104–110.

Orth A, Lyapunova E, Kandaurov A, Boissinot S, Boursot P, Vorontsov N, Bonhomme F. 1996. L'espèce polytypique *Mus musculus* in Transcaucasie. *Comptes Rendus de l'Académie des Sciences – Series III – Sciences de la Vie* **319:** 435–441.

Payseur BA, Nachman MW. 2005. The genomics of speciation: investigating the molecular correlates of X chromosome introgression across the hybrid zone between *Mus domesticus* and *Mus musculus*. *Biological Journal of the Linnean Society* **84:** 523–534.

Pocock MJO, Hauffe HC, Searle JB. 2005. Dispersal in house mice. *Biological Journal of the Linnean Society* **84:** 565–583.

Porter AH, Wenger R, Geiger H, Scholl A, Shapiro AM. 1997. The *Pontia daplidice–edusa* hybrid zone in northwestern Italy. *Evolution* **51:** 1561–1573.

Prager EM, Sage RD, Gyllensten U, Thomas WK, Hübner R, Jones CS, Noble L, Searle JB, Wilson AC. 1993. Mitochondrial DNA sequence diversity and the colonisation of Scandinavia by house mice from East Holstein. *Biological Journal of the Linnean Society* **50:** 85–122.

Prager EM, Tichy H, Sage RD. 1996. Mitochondrial DNA sequence variation in the Eastern house mouse, *Mus musculus*: Comparison with other house mouse mice and report of 75-bp tandem repeat. *Genetics* **143:** 427–446.

Press WH, Flannery BP, Teukolsky SA, Vetterling WT. 1989. *Numerical recipes in Pascal*. Cambridge: Cambridge University Press.

Rand DM. 2001. The units of selection on mitochondrial DNA. *Annual Review of Ecology and Systematics* **32:** 415–448.

Raufaste N, Orth A, Belkhir K, Senet D, Smadja C, Baird SJE, Bonhomme F, Dod B, Boursot P. 2005. Inferences of selection and migration in the Danish house mouse hybrid zone. *Biological Journal of the Linnean Society* **84:** 593–616.

Rognon X, Guyomard R. 2003. Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *Oreochromis niloticus* in West Africa. *Molecular Ecology* **12:** 435–445.

Rohwer S, Bermingham E, Wood C. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution* **55:** 405–422.

Rozen SS, Skaletsky HJ. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, eds. *Bioinformatics methods and protocols: methods in molecular biology*. New York and Totowa: Humana Press, 365–386.

Ruedi M, Smith MF, Patton JL. 1997. Phylogenetic evidence of mitochondrial DNA introgression among pocket gophers in New Mexico (family Geomyidae). *Molecular Ecology* **6:** 453–462.

Sage RD, Atchley WR, Capanna E. 1993. House mice as models in systematic biology. *Systematic Biology* **42:** 523–561.

StatSoft Inc. 2001. *STATISTICA: data analysis software system*. Tulsa, OK: StatSoft.

Szymura JM, Barton NH. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Crackow in southern Poland. *Evolution* **45:** 1141–1159.

Szymura JM, Barton NH. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparison between transects and between loci. *Evolution* **45:** 237–261.

Szymura JM, Uzzell T, Spolsky C. 2000. Mitochondrial DNA variation in the hybridizing fire-bellied toads, *Bombina bombina* and *Bombina variegata*. *Molecular Ecology* **9:** 891–899.

Takahata N, Slatkin M. 1984. Mitochondrial gene flow. *Proceedings of the National Academy of Sciences, USA* **81:** 1764–1767.

Tucker PK, Sage RD, Warner J, Wilson AC, Eicher EM. 1992. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. *Evolution* **46:** 1146–1163.

Vanlerberghe F, Boursot P, Catalan J, Gerasimov S, Bonhomme F, Botev BA, Thaler L. 1988a. Analyse génétique de la zone d'hybridation entre les deux sous-espèces de souris *Mus musculus domesticus* et *Mus musculus musculus* en Bulgarie. *Genome* **30:** 427–437.

Vanlerberghe F, Boursot P, Nielsen JT, Bonhomme F. 1988b. A steep cline for mitochondrial DNA in Danish mice. *Genetical Research* **52:** 185–193.

Vanlerberghe F, Dod B, Boursot P, Bellis M, Bonhomme F. 1986. Absence of Y-chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. *Genetical Research* **48:** 191–197.

William J, Ballard O. 2000. When one is not enough: Introgression of mitochondrial DNA in *Drosophila*. *Molecular Biology and Evolution* **17:** 1126–1130.

William J, Ballard O, Kreitman M. 1995. Is mitochondrial DNA a strictly neutral marker? *Trends in Ecology and Evolution* **10:** 485–488.

Wilson CC, Bernatchez L. 1998. The ghost of hybrid past: fixation of arctic charr (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). *Molecular Ecology* **7:** 127–132.

Yonekawa H, Moriwaki K, Gotoh O, Miyashita N, Migita S, Bonhomme F, Hjorth JP, Petras ML, Tagashira Y. 1982. Origins of laboratory mice deduced from restriction patterns of mitochondrial DNA. *Differentiation* **22:** 222–226.

APPENDIX 1

Allopatric populations of *Mus musculus musculus* and *M. m. domesticus* used for testing the *Bam*HI marker

No.	Locality	N	mtDNA	Country	No.	Locality	N	mtDNA	Country
1	Illmitz [1]	1	1	AST	42	Canterbury	1	0	GBR
2	Bania [1]	1	1	BUL	43	Gloucester	4	0	GBR
3	Gal Toshevo [1]	1	1	BUL	44	Skokholm [2]	1	0	GBR
4	Kranevo [1]	1	1	BUL	45	Bonn	12	0	GER
5	Sokolovo [1]	1	1	BUL	46	Essen	2	0	GER
6	Vlas [1]	1	1	BUL	47	Cologne	5	0	GER
7	Białowieża [1]	1	1	POL	48	Neunkirchen	2	0	GER
8	Terka	1	1	ROM	49	Waldau	1	0	GER
9	Ljubljana [1]	1	1	SLO	50	Kilkis	5	0	GRE
10	Bratislava	1	1	SVK	51	Afula [1]	1	0	ISR
11	Hraň [3]	7	1	SVK	52	Kefar Galim [1]	1	0	ISR
12	Topol'čany	6	1	SVK	53	Sede Boger [1]	1	0	ISR
13	Jurský Šůr	3	1	SVK	54	Cave	1	0	ITA
14	Necpaly [3]	8	1	SVK	55	Cislago [1]	1	0	ITA
15	Senica	1	1	SVK	56	Cittaducale [2]	1	0	ITA
16	Zemianska Ol'ča [3]	8	1	SVK	57	Milano [2]	1	0	ITA
17	Charkov	3	1	UKR	58	Molize [2]	1	0	ITA
18	Ljubotin, Charkov	1	1	UKR	59	Orcetto [1]	1	0	ITA
19	Šušara	1	1	YUG	60	Akaba	1	0	JOR
20	Kosovo	1	1	YUG	61	Al Awaynat	1	0	LIB
21	Oran [1]	1	0	ALG	62	Al Qusbat	3	0	LIB
22	Belair	1	0	AUS	63	Gabroon	4	0	LIB
23	Rupite	1	0	BUL	64	Ghadamis	5	0	LIB
24	Zadar [2]	1	0	CRO	65	Sabha	3	0	LIB
25	Bredsten [1]	1	0	DAN	66	Azrou [1]	1	0	MOR
26	Hov [1]	1	0	DAN	67	Azemmour 1]	1	0	MOR
27	Odis [1]	1	0	DAN	68	Casablanca	1	0	MOR
28	Cairo [1]	1	0	EGY	69	Napier	4	0	NZL
29	Mut (oasis Dakhla)	1	0	EGY	70	Majorca [1]	1	0	SPA
30	oasis Baharija	1	0	EGY	71	Bassins	3	0	SWI
31	Sakara	1	0	EGY	72	Poschiavo [2]	1	0	SWI
32	Tunaida (oasis Dakhla)	2	0	EGY	73	Dura Europos	2	0	SYR
33	Grau du Roi [1]	1	0	FRA	74	Halabijah	2	0	SYR
34	Corsica [1]	1	0	FRA	75	Hasake	1	0	SYR
35	Massegros	2	0	FRA	76	Palmyra	2	0	SYR
36	Montpellier [1]	1	0	FRA	77	Tell Sheik Hamad	1	0	SYR
37	Montpellier	38	0	FRA	78	Harran	2	0	TUR
38	near Severac	3	0	FRA	79	Narlidere	2	0	TUR
39	Pomayrols	10	0	FRA	80	Sirbasan	1	0	TUR
40	Saasenheim [1]	1	0	FRA	81	Top Dagi	1	0	TUR
41	St. Saturnin-de-Lenne	2	0	FRA	82	Yenice	4	0	TUR

Number of animals scored and average frequency of a *Mus musculus musculus* allele are given for each locality. Published data [(1) Boursot *et al*., 1996; (2) Yonekawa *et al*., 1982; (3) Munclinger *et al*., 2002] are also included. Localities are numbered as in Figure 1. ALG, Algeria; AST, Austria; AUS, Australia; BUL, Bulgaria; CRO, Croatia; DAN, Denmark; EGY, Egypt; FRA, France; GER, Germany; ISR, Israel; ITA, Italy; JOR, Jordan; LIB, Libya; MOR, Morocco; NZL, New Zealand; POL, Poland; ROM, Romania; SLO, Slovenia; SPA, Spain; SVK, Slovakia; SWI, Switzerland; SYR, Syria; TUR, Turkey; UKR, Ukraine; YUG, the former Yugoslavia.

APPENDIX 2

Observed frequencies of *Mus musculus* alleles in the Czech transect

Locality, country	Distance	mtDNA (*N*)	*Btk* (*N*)	*Es1* (*N*)	*Mpi* (*N*)	*Np* (*N*)
Straas 1, GER	–48.3	0 (20)	0 (21)	0 (22)	0 (22)	0 (22)
Straas 2, GER	–48.2	0 (30)	0.012 (30)	0 (30)	0 (30)	0 (30)
Münchberg, GER	–46.9	0 (3)	0 (3)	0 (2)	0 (2)	0 (2)
Grassemann, GER	–44.9	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Benk, GER	–40.7	0 (14)	0 (15)	0 (13)	0 (14)	0.036 (14)
Meierhof, GER	–35.8	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Lehsten, GER	–35.5	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
Roeslau, GER	–32.5	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
Kleinwendern, GER	–28.3	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Trojmezí, CZE	–27.4	0 (3)	0 (3)	0.167 (3)	0 (2)	0.667 (3)
Hebanz, GER	–26.8	0.286 (8)	0 (9)	0.071 (8)	0.286 (8)	0.071 (8)
Hranice, CZE	–26.2	0 (18)	0 (18)	0.353 (18)	0.222 (18)	0 (18)
Krásňany, CZE	–24.7	0 (7)	0 (7)	0.083 (7)	0 (7)	0 (7)
Smrčina, CZE	–22.4	0 (14)	0 (13)	0.231 (13)	0 (14)	0 (14)
Höchstädt, GER	–21.6	0 (2)	0 (1)	0 (1)	0 (1)	0 (1)
Thierstein, GER	–21.6	0 (10)	0 (14)	0.107 (14)	0 (14)	0 (14)
Neuenreuth, GER	–20	0 (33)	0 (33)	0.088 (34)	0.035 (33)	0.07 (33)
Neuenreuth8, GER	–19.7	0.088 (30)	0 (32)	0.083 (30)	0.145 (31)	0.242 (31)
Aš, CZE	–18.8	0.174 (23)	0 (21)	0.025 (20)	0.022 (23)	0 (23)
Libá 1, CZE	–13.4	0.583 (12)	0.133 (12)	0.1 (10)	0.1 (10)	0.25 (10)
Hammermühle, GER	–13.4	0 (5)	0.2 (5)	0 (5)	0 (5)	0.1 (5)
Hohenberg, GER	–13.3	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Polná, CZE	–13.3	0.92 (25)	0 (25)	0.239 (23)	0.08 (25)	0.62 (25)
Libá 2, CZE	–13.2	0.857 (14)	0.056 (12)	0.107 (14)	0.036 (14)	0.464 (14)
Skalka (Hazlov), CZE	–12.8	0 14	0 (14)	0.038 (13)	0.219 (16)	0.2 (15)
Hazlov, CZE	–11.8	0.222 (9)	0 (9)	0 (9)	0.167 (9)	0.167 (9)
Hůrka 1, CZE	–11.6	0.974 (35)	0.25 (37)	0.17 (44)	0.047 (43)	0.08 (44)
Hůrka 2, CZE	–11.6	1 (4)	0.167 (4)	0.25 (4)	0 (4)	0.25 (4)
Plesná, CZE	–10.2	0.129 (62)	0.009 (69)	0.155 (116)	0.098 (138)	0.086 (140)
Poustka 2, CZE	–9.2	0.25 (20)	0.067 (20)	0.048 (21)	0.412 (17)	0.238 (21)
Poustka 1, CZE	–9	0.875 (16)	0.091 (16)	0.063 (16)	0.563 (16)	0.313 (16)
Vojtanov, CZE	–8.1	0 (1)	0 (1)	0.5 (1)	0 (1)	1 (1)
Lužná, CZE	–8.1	0.056 (54)	0.049 (58)	0.25 (66)	0.189 (65)	0.53 (65)
Luby, CZE	–8	0 (4)	0 (4)	0.333 (3)	0 (4)	0 (4)
Čirá, CZE	–8	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
Klest, CZE	–6.6	1 (2)	0 (2)	0.25 (2)	0 (2)	0 (2)
Dolní Luby, CZE	–6.1	0 (10)	0 (10)	0.35 (10)	0.05 (10)	0 (10)
Starý Rybník, CZE	–6	1 (8)	0.3 (7)	0.278 (9)	0.111 (9)	0.188 (8)
Křižovatka, CZE	–5.7	0.125 (8)	0 (8)	0 (8)	0 (8)	0.188 (8)
Skalka (Cheb), CZE	–5.4	0 (4)	0 (4)	0 (4)	0.375 (4)	0.875 (4)
Spálená, CZE	–4.8	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Dolní Pelhřimov, CZE	–4.8	0 (6)	0.125 (6)	0.333 (6)	0.417 (6)	0.75 (6)
Božetín, CZE	–4.3	0 (1)	0 (1)	0.5 (2)	0.5 (2)	0.5 (2)
Střížov, CZE	–3.9	0.6 (5)	0.4 (6)	0.286 (7)	0.429 (7)	0.143 (7)
Nový Kostel, CZE	–3.8	0.625 (8)	0 (8)	0.125 (8)	0 (8)	0.125 (8)
Svatý Kříž, CZE	–3.7	0 (10)	0 (8)	0.778 (9)	0 (10)	0.35 (10)
Suchá, CZE	–3.7	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
Horní Ves, CZE	–3.3	0.842 (19)	0.077 (19)	0.079 (19)	0.441 (17)	0.289 (19)
Dlouhé Mosty, CZE	–2.8	0.872 (39)	0.207 (37)	0.355 (31)	0.474 (39)	0.423 (39)
Nový Drahov, CZE	–2.8	0.444 (18)	0 (18)	0.222 (18)	0 (18)	0.056 (18)
Nová Ves (Cheb), CZE	–2.6	0.909 22	0.176 (22)	0.591 (22)	0.071 (21)	0.045 (22)
Dolnice, CZE	–1.9	0.889 (27)	0.079 (29)	0.133 (30)	0.113 (31)	0.258 (31)
Starý Hrozňatov, CZE	–1.5	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)

APPENDIX 2 *Continued*

Locality, country	Distance	mtDNA (*N*)	*Btk* (*N*)	*Es1* (*N*)	*Mpi* (*N*)	*Np* (*N*)
Mlýnek 2, CZE	–1.3	1 (5)	0 (5)	0 (5)	0.2 (5)	0.3 (5)
Doubí, CZE	–1.3	0.032 (29)	0.31 (29)	0.352 (27)	0.4 (25)	0.037 (27)
Mlýnek 1, CZE	–1.1	0 (5)	0.4 (5)	0 (1)	0.5 (1)	0 (1)
Děvín, CZE	–1	0 (1)	0 (1)	0 (1)	0.5 (1)	1 (1)
Jindřichov, CZE	–0.8	1 (18)	0.25 (18)	0.912 (17)	0.235 (17)	0.471 (17)
Kopanina 1, CZE	–0.2	1 (13)	0 (13)	0.615 (13)	0.056 (9)	0 (12)
Kopanina 2, CZE	–0.1	0 (2)	0 (2)	0.5 (2)	0.25 (2)	0 (2)
Milhostov, CZE	0.5	0.03 (28)	0.804 (33)	0.643 (28)	0.796 (27)	0.5 (28)
Hluboká, CZE	2.6	0 (1)	1 (1)	0.5 (1)	0 (1)	0.5 (1)
Krajková 1, CZE	2.6	1 (5)	1 (5)	1 (5)	1 (5)	0.1 (5)
Krajková 2, CZE	3	1 (9)	1 (9)	1 (9)	1 (9)	0.5 (9)
Dolina, CZE	3.1	1 (22)	0.939 (22)	0.9 (20)	1 (21)	0.65 (20)
Krajková 3, CZE	3.5	1 (15)	1 (15)	1 (15)	0.933 (15)	0.467 (15)
Nebanice 1, CZE	3.6	0.5 (6)	0.778 (6)	0.75 (4)	0.5 (4)	0.875 (4)
Nebanice 3, CZE	3.7	1 (9)	0.917 (9)	0.722 (9)	0.778 (9)	1 (9)
Nebanice 2, CZE	4.1	1 (13)	1 (14)	0.893 (14)	0.679 (14)	0.786 (14)
Obilná, CZE	4.3	0.968 (58)	0.979 (62)	0.75 (58)	0.974 (58)	1 (58)
Kaceřov 2, CZE	4.5	0.098 (40)	0.985 (39)	0.879 (33)	0.355 (38)	0.654 (39)
Anenská Ves, CZE	4.6	1 (12)	1 (12)	0.727 (11)	0.955 (11)	0.773 (11)
Kaceřov 1, CZE	4.8	0.667 (18)	1 (20)	1 (9)	0.632 (19)	0.921 (19)
Boučí, CZE	4.8	1 (17)	1 (17)	0.933 (15)	0.867 (15)	1 (15)
Hřebeny, CZE	5	1 (1)	1 (1)	1 (1)	0 (1)	1 (1)
Mostov, CZE	5.2	1 (31)	1 (31)	0.912 (34)	0.955 (33)	0.985 (34)
Chotíkov, CZE	5.9	1 (13)	1 (13)	0.792 (12)	0.875 (12)	0.792 (12)
Okrouhlá, CZE	5.9	1 (1)	1 (1)	1 (1)	1 (1)	0 (1)
Lipoltov, CZE	6.5	0 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Habartov, CZE	6.7	1 (1)	1 (1)	1 (1)	1 (1)	0.5 (1)
Dolní Nivy, CZE	7.7	1 (4)	1 (4)	0.75 (4)	1 (4)	1 (4)
Lomnice, CZE	8.6	1 (7)	1 (7)	0.5 (7)	1 (7)	1 (7)
Svatava, CZE	10.6	1 (1)	1 (1)	1 (1)	1 (1)	0.5 (1)
Hlavno, CZE	11	1 (6)	1 (6)	1 (6)	0.417 (6)	1 (6)
Rudolec 1, CZE	13.1	1 (10)	1 (9)	1 (6)	0.75 (8)	0.813 (8)
Rudolec 2, CZE	13.3	0.9 (10)	1 (9)	0.917 (6)	1 (8)	1 (8)
Vintířov, CZE	13.3	1 (4)	1 (4)	1 (2)	1 (2)	1 (2)
Kostelní Bříza, CZE	14.3	1 (24)	1 (24)	1 (24)	0.875 (24)	1 (24)
Nová Role, CZE	14.6	1 (1)	1 (1)	1 (1)	1 (1)	0 (1)
Staré Sedlo, CZE	17	1 (13)	1 (11)	1 (13)	1 (13)	1 (13)
Počerny, CZE	18.4	1 (11)	1 (15)	1 (17)	1 (17)	0.794 (17)
Dalovice, CZE	22.7	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Osvinov, CZE	24.1	1 (3)	1 (3)	1 (3)	0.833 (3)	1 (3)
Nová Ves (Sokolor), CZE	26.2	1 (63)	1 (64)	1 (55)	1 (55)	1 (55)
Stráž nad Ohří, CZE	26.4	1 (4)	1 (4)	1 (4)	1 (4)	1 (4)
Sedlečko (Karlovy Vary), CZE	27.6	1 (9)	1 (9)	1 (10)	1 (10)	1 (10)
Podbořanský Rohozec, CZE	48.2	1 (5)	1 (6)	1 (4)	1 (4)	1 (4)
Buškovice, CZE	53.6	1 (27)	1 (27)	0.933 (15)	1 (15)	1 (15)

Numbers in parentheses represent sample sizes for specific loci. CZE, Czech Republic; GER, Germany.

APPENDIX 3

Observed frequencies of *Mus musculus* alleles in the Bavarian transect

Locality, state	Distance	mtDNA (*N*)	X1	X2	*Es1*	*Mpi*	*Np*
Augsburg (quail), GER	0	0 (11)	0	0	0	0	0
Augsburg (camel), GER	0	0 (8)	0	0	0	0	0
Ebersbach, GER	37.1	0 (5)	0	0	0	0	0
Kammerberg-Zandt, GER	43	0 (5)	0	0	0	0	0
Kammerberg-Hartl, GER	43	0 (4)	0	0	0	0	0
Bachenhausen, GER	44.2	0 (1)					
Appercha, GER	47	0 (21)					
Gesselthausen-Ziigletrumm, GER	49.6	0 (42)	0	0	0.054	0	0
Gesselthausen-Warta, GER	49.6	0 (66)	0	0	0.033	0	0
Giesenbach, GER	50.2	0 (3)					
Eberspoint, GER	51	0 (11)	0	0	0.278	0.278	0.056
Massenhausen/Neufahrn, GER	51.6	0.083 (12)	0	0	0	0.083	0.208
Giggenhausen/Neufahrn, GER	52.2	0 (1)	0	0	0	0	0
Neufahrn bei Freising, GER	53.4	0 (73)	0.454	0.44	0.352	0.444	0.191
Thalhausen, GER	54.6	0 (1)	0	1	0	0	0
Pulling-Petryszak, GER	56.6	0 (7)					
Pulling-Appels, GER	56.7	0 (2)					
Achering, GER	57	0.526 (16)	0.767	1	0.9	0.912	0.765
Freising, GER	59.2	1 (7)	1	1	0.3	1	0.5
Tuntenhausen, GER	60	0.4 (5)	0.714	0.714	0.375	0.5	0.9
Gut Wildschwaig, GER	62.2	0 (4)	1	1	0.75	0.75	0.5
Rudlfing, GER	65	1 (24)	1	0.706	0.892	1	0.587
Schwaig, GER	67	0.538 (13)	1	0.714	1	1	0.846
Dornhaselbach, GER	71.6	0 (2)	1	1	0.75	1	0.75
Tittenkofen, GER	75	1 (2)	1	1	0.5	1	0.75
Sonnendorf, GER	82.4	0.708 (27)	1	1	1	1	0.955
Hogersdorf, GER	82.8	1 (1)	1	1	1	1	0.5
Atting, GER	86.6	1 (2)	1	1	1	1	1
Brundl, GER	101.2	1 (13)					
Attenham, GER	128.2	1 (9)					
Mitterskirchen, GER	132.2	1 (1)	1	1	1	1	1
Simbach, GER	140.9	0.389 (21)	1	1	0.567	1	1
Konigsaich, AST	151.8	1 (2)	1	1	1	1	1
Ranshofen-Holfinger, AST	143.5	0 (5)	1	1	0.875	1	1
Ranshofen-Penias, AST	153.4	1 (1)	1	1	1	1	1
Braunau; 26 Laabstrasse, AST	155.2	1 (3)	1	1	1	1	0.833
Nofing, AST	158.2	1 (6)	1	1	1	1	1
Aufhausen, GER	167.8	1 (2)					
Rodham, AST	168	1 (3)					
Leitham-Fuchs, AST	173.8	0.938 (12)					
Leitham-Hubinger, AST	174.2	1 (2)					

Numbers in parentheses represent sample sizes for mtDNA. For sample sizes of other markers see Tucker *et al*. (1992). GER, Germany; AST, Austria.