Genetic variation in house mice (*Mus*, Muridae, Rodentia) from the Czech and Slovak Republics

Pavel MUNCLINGER[1], Eva BOŽÍKOVÁ[1], Monika ŠUGERKOVÁ[2,3], Jaroslav PIÁLEK[4] and Miloš MACHOLÁN[2]

[1] *Biodiversity Research Group, Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic; e-mail: muncling@natur.cuni.cz*

[2] *Institute of Animal Physiology and Genetics, AS CR, Veveří 97, 602 00 Brno, Czech Republic; e-mail: macholan@iach.cz*

[3] *Department of Zoology and Ecology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; e- mail: sugerkova@centrum.cz*

[4] *Institute of Vertebrate Biology, AS CR, Studenec 122, 675 02 Koněšín, Czech Republic; e-mail: jpialek@brno.cas.cz*

A b s t r a c t. Genetic variation among populations of commensal house mice was studied across the territories of the Czech and Slovak Republics and in some adjacent areas of Germany. We used six diagnostic allozyme loci (*Es-2, Gpd-1, Idh-1, Mpi, Np, Sod-1*) and the following molecular markers: B1 insertion in the *Btk* gene (X chromosome), *Zfy2* 18-bp deletion (Y chromosome), *Bam*H I restriction site in the *mt-Nd1* gene (mtDNA) and *Hba-4ps* 16-bp insertion (diagnosing the presence of *t* haplotypes). In total, 544 individuals taken from 49 localities were examined. Almost the entire territories of the Czech Republic and Slovakia were found to be occupied by *Mus musculus*, the only exception being the westernmost parts of the Czech Republic, where *M. musculus* meets the range of *M. domesticus* and forms a narrow belt of hybrid populations. Despite this, *domesticus*-type alleles of some allozyme markers (notably *Es-2*) were also found at sites well within the range of *M. musculus*, either tens or hundreds of kilometres behind the hybrid zone. This provides evidence of either: (1) introgression of some markers into the species' genome due to free gene flow through the zone, or (2) human-mediated long-distance migrations, or (3) incomplete lineage sorting. Conversely, variants of molecular markers typical for *M. domesticus* in *Btk*, *Zfy2* and *mt-Nd1* were only found in the westernmost populations studied. *t* haplotypes were quite frequent in some populations, irrespective of whether *M. domesticus*, *M. musculus* or their hybrids, yet no *t*/*t* homozygotes were found. The mean frequency of *t*/+ heterozygotes found within the study populations was 13%.

Key words: house mouse, *Mus musculus*, *M. domesticus*, genetic variation, allozymes, sex chromosomes, mtDNA, *t* haplotype

Introduction

Notwithstanding taxonomic issues (e.g. A u f f r a y et al. (1990), M u s s e r & C a r l e t o n (1993), S a g e et al. (1993), D i n et al. (1996), M i t c h e l l - J o n e s et al. (1999)) there is general agreement that two commensal house mice taxa occur in Europe (here considered as distinct species): *Mus domesticus* Schwarz et Schwarz, 1943 (C o r b e t 1988) in the west and south, and *M. musculus* L., 1758 in the north and east. Where their ranges abut, a narrow belt of hybrid populations is formed. 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 B o u r s o t et al. 1993, S a g e et al. 1993).

Some sections of the *Mus* hybrid zone have been studied thoroughly, e.g. in Denmark (H u n t & S e l a n d e r 1973, V a n l e r b e r g h e et al. 1986, 1988b, D o d et al. 1993), East Holstein (P r a g e r et al. 1993, 1997), Bulgaria (B o u r s o t et al. 1984, V a n l e r b e r g h e et al. 1986, 1988a) and southern Germany (S a g e et al. 1986b,

Tucker et al. 1992) (for mtDNA see also Sage et al. 1990, Prager et al. 1996). Despite this, information from a major portion of the zone is scarce, so the precise ranges of the two *Mus* taxa are not well understood. This is particularly apparent in Central Europe. Here, apart from data from a transect across the hybrid zone in southern Germany and Austria (Sage et al. 1986a,b, Tucker et al. 1992, Prager et al. 1996), mtDNA data from Germany (Sage et al. 1990) and morphological data from Bavaria (Kraft 1984), little (at best) is known from the vast area of the former East Germany (DDR). In the Czech Republic (Czechia), the results of a pilot study on the identity of house mice from the western part of Czechia were presented by Macholán & Zima (1994), who provided evidence for the presence of *M. domesticus* in the westernmost parts of the country; this was later corroborated by morphological studies (Lazarová 1999). So, despite growing data on the position and dynamics of the *M. domesticus*/*M. musculus* hybrid zone in Europe, its precise position in Czechia remains undetermined.

This paper focuses on the following: (i) evaluation of the suitability of various molecular markers for diagnosis of the two taxa [We selected markers on sex chromosomes and a marker for mtDNA as well, since this part of mouse genome has been shown to behave differently when compared with allozymes in some parts of the hybrid zone (see references in discussion)] and demonstration of their utility in surveys of the Czech part of the hybrid zone; (ii) to extend the study of Macholán & Zima (1994) to a larger area of former Czechoslovakia (i.e. the current Czech and Slovak republics) and adjacent areas of Germany, thus increasing both the number of animals investigated, and the markers used, to obtain more data on the distribution of the two taxa in this part of Europe; (iii) although *t* haplotypes were studied extensively in *M. domesticus*, only limited information is available on their frequency in *M. musculus* populations (see Ruvinsky et al. 1991, and references therein) so we decided to type the mice studied for the presence of *t* haplotypes.

Material and Methods

Mice

Depending on the marker used, between 215 and 544 mice were studied. These were collected from 49 sites scattered across Czechia, Slovakia and adjacent areas of NE Bavaria and S Thuringia. The list of localities and numbers of animals examined are given in Table 1 (see also Figs 1, 2 for site locations).

Allozymes

Altogether, 544 individuals were studied. Kidneys were dissected from freshly killed animals (in some cases, animals dead for a few hours were also successfully scored) and stored at -80 °C until processed. Portions of tissue were crushed in homogenisation buffer (Pasteur et al. 1988) and centrifuged at 10,000 RPM for five minutes. Standard horizontal starch gel electrophoresis (Harris & Hopkinson 1976, Pasteur et al. 1988) was then carried out. Six presumptive enzymatic loci, considered diagnostic (or nearly so) between *M. domesticus* and *M. musculus* (Bonhomme et al. 1984, Milishnikov & Rafiev 1990, Tucker et al. 1992, Dod et al. 1993) were scored: Isocitrate dehydrogenase-1 (E.C. 1.1.1.42; *Idh1*, Chromosome 1), Glucose dehydrogenase-1 (E. C. 1.1.1.47; *Gpd-1*, Chr. 4), Superoxide dismutase-1 (E.C. 1.15.1.1; *Sod-1*, Chr. 16), Nucleoside phosphorylase (E.C. 2.4.2.1; *Np*, Chr. 14), Esterase-2 (E.C. 3.1.1.1; *Es-2*, Chr. 8) and Mannose phosphate isomerase (E.C. 5.3.1.8; *Mpi*, Chr. 9).

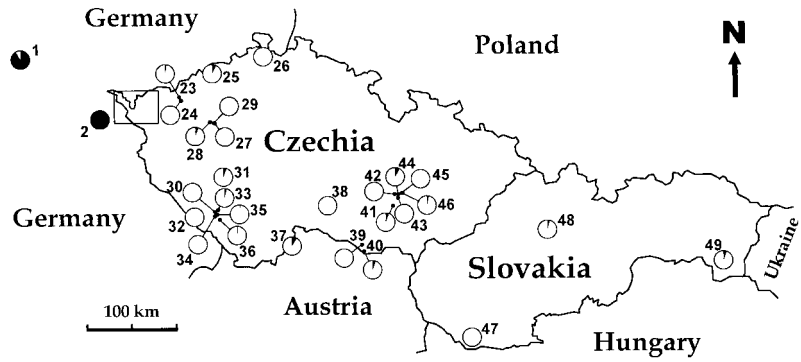

Fig. 1. Map of the study localities in the Czech and Slovak Republics. For each site, a pie diagram is shown depicting the values of the hybrid index HI6, i.e. frequencies of *domesticus* alleles averaged across six allozyme loci (HI6) per site (black sections). The rectangle indicates the area where hybridisation occurs between the two taxa (shown in detail in Fig. 2). Numbers of localities are the same as in Table 1.

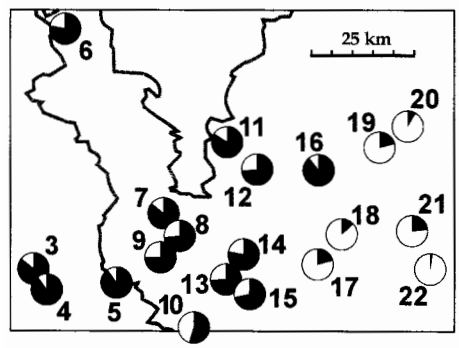

Fig. 2. Detail of the westernmost part of Czech Republic and adjacent areas of NE Bavaria. Pie diagrams depict frequencies of *domesticus* alleles averaged across six allozyme loci (HI6) at each site (black sections). An abrupt transition between the two taxa is apparent from the picture. Numbers of sites are as in Table 1.

Laboratory mice of the inbred C57BL/6J strain were used as standards and run alongside unknown samples on the same gel. This strain is autosomally *M. domesticus*, though its Y chromosome is of *M. musculus* origin (N a g a m i n e et al. 1992). Since, in all but a single case, only two alleles were shown in the study material, the alleles were designated "m" (*musculus*-like) or "d" (*domesticus*-like). In Nucleoside phosphorylase, one fast allele was present in *M. domesticus* (Np^{100}) whereas two alleles were found in *M. musculus*, slow (Np^{70} = m_1) and medium (Np^{90} = m_2); for simplicity, the last two alleles were considered as a single *musculus* allele throughout this paper. A simple hybrid index was calculated from the allozyme data, given as a frequency of *domesticus* alleles averaged across all alleles within individual populations. Two hybrid indices were used, one based on all six loci scored (HI6), and another one excluding *Es-2* (HI5). In addition, frequencies of *domesticus* alleles were computed for each locus.

D N A i s o l a t i o n

DNA was isolated from frozen or ethanol-preserved tissues using proteinase K digestion and subsequent extraction with phenol-chloroform and ethanol precipitation (H o e l z e l & G r e e n 1992).

X chromosome

Primers 5'-AATGGGCTAGCGTAGTGCAG-3' and 5'-AGGGGACGTACACTCAGCTTT-3' were designed to flank the B1 insertion in the Bruton agammaglobulinemia tyrosine kinase gene (*Btk*; GeneBank U58105.1, B1 is located at positions 77329–77464) on the X chromosome. The insertion appears to be fixed in *M. domesticus* populations but is absent in *M. musculus* (Munclinger et al. unpubl.). PCR conditions were 2 mM $MgCl_2$, 200 µM concentration of dNTPs, 0.5 u Taq polymerase (Promega) and 0.5 µM concentration of each primer. The 10× Buffer diluted 1:10 has a final composition of 10mM Tris-HCl, 50 mM KCl and 0,1% Triton® X-100. Reactions were performed using an MJ thermal cycler under the following conditions: after an initial three minute incubation at 95 °C, 35 cycles were performed at 95 °C, 62 °C, and 72 °C, each step for 45 s, with a final extension for ten min at 72 °C. PCR products were run on 2% agarose gels. A 342-bp fragment can be observed when the insertion was present, whereas a 206-bp fragment indicated the absence of the insertion. Both fragments are visible in heterozygotes, although the 342-bp band is slightly weaker.

Y chromosome

Male mice were typed for the presence or absence of an 18-bp deletion, located within the last exon of the Zinc finger protein 2, Y-linked gene (*Zfy2*) using the method given in Orth et al. (1996). The deletion was found to be fixed in *M. musculus* and absent in *M. domesticus* (Nagamine et al. 1992, Boissinot & Boursot 1997). Electrophoresis was run on a mixture of 3% NuSieve and 0.8% Serva agarose gels.

Mitochondrial DNA (mtDNA)

A 430-bp mtDNA fragment was amplified using the primers 5'-TTACTTCTGCCAGCCTGACC-3' (positions 3270–3289 in the complete mtDNA; Bibb et al. 1981) and 5'-ATGGTGGTACTCCCGCTGTA-3' (positions 3699-3680 in the complete mtDNA; Bibb

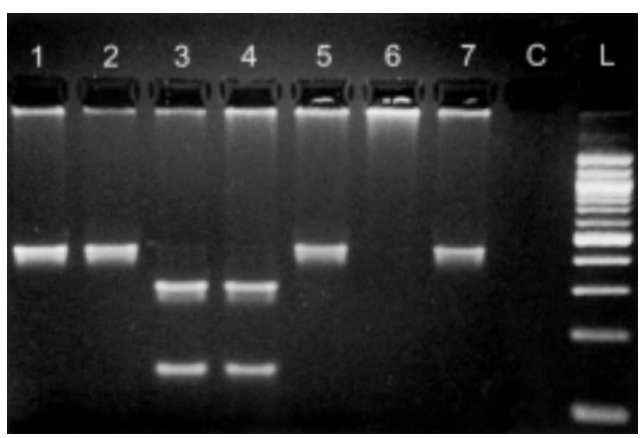

Fig. 3. Mitochondrial DNA of house mice from Czech Republic. An amplified 430-bp mtDNA fragment was digested with *Bam*H I. The fragment contains the *Bam*H I restriction site in *M. domestic*us whereas it is absent in *M. musculus*. Thus a single 430-bp fragment indicates absence of the restriction site (localities Hlavno, lanes 1, 2, 5; and Boučí, lane 7) while two fragments, 295-bp and 135-bp, indicate its presence (locality Poustka, lanes 3 and 4). Lane C contains PCR mixture without template DNA. Lane 6 shows an unsuccessful amplification attempt of a template DNA. Lane L contains a 100-bp DNA Ladder 323-1S (New England BioLabs® Inc.). The five smallest fragments are (in base pairs): 100, 200, 300, 400 and 500. Electrophoresis was run on a 2% agarose gel.

et al. 1981). The PCR conditions included 1.5 mM $MgCl_2$, 200 µM concentration of dNTPs, 0.5 u Taq polymerase (Promega) and 0.5 µM concentration of each primer. The 10× Buffer diluted 1:10 had a final composition of 10mM Tris-HCl, 50 mM KCl and 0,1% Triton® X-100. Reactions were performed using an MJ thermal cycler under the following conditions: 95 °C for 3 min and 35 cycles at 95 °C, 55 °C, and 72 °C, each step for 30 s. PCR products were then digested with the restriction endonuclease *Bam*H I and subsequently analysed on 2% agarose gels. The amplified fragment contained a *Bam*H I restriction site (position 3565 in the complete mtDNA; Bibb et al. 1981) in *M. domestic*us but is absent in *M. musculus* (B o u r s o t et al. 1996). Thus, a single 430-bp fragment indicates absence of the restriction site, while two fragments (295-bp and 135-bp) indicate its presence (Fig. 3).

t h a p l o t y p e s

Primers Hb.1 and Hb.2 (S c h i m e n t i & H a m m e r 1990) were used to genotype mice for the presence of *t* haplotypes. The primers flank a 16-bp insertion in the *Hba-4ps* locus, which is supposed to be present in most of the *t* haplotypes. PCR and electrophoretic conditions followed protocols given in S c h i m e n t i & H a m m e r (1990).

Results

As shown in Table 1, most of Czechia and Slovakia are occupied by *M. musculus*. This is apparent especially in the sex chromosomes markers (*Btk*, *Zfy2*) and mtDNA (*mt-Nd1*). Conversely, *M. domesticus* was found only in Germany (S Thuringia, NE Bavaria) and at sites located in the westernmost part of Czechia (Table 1).

A slightly different pattern was seen in allozymes (first two columns in Table 1), where *domesticus*-like alleles were found at low frequencies at sites even hundreds of kilometres east of the zone (Fig. 1). However, comparison of hybrid indices based on the full set of six loci (HI6) and those without *Es-2* (HI5) clearly shows the extensive introgression to be due to the esterase locus (this is shown more explicitly in Table 2, where hybrid indices are listed for each allozyme locus separately). If we only consider areas east of the hybrid zone (site nos. 23–49), *domesticus* alleles either do not appear at all (*Sod-1*, *Idh-1*) or they are found at two (*Np*, *Mpi*) or three (*Gpd-1*) sites, respectively. In contrast, *Es-2* was found to segregate in as many as nine of 27 *M. musculus* localities. Regardless of this large-scale introgression by some alleles, the transition of allozyme markers from the *musculus*-side to the *domesticus*-side is rather abrupt (Fig. 2), and even more so for the sex chromosomes and mtDNA markers (cf. Table 1).

Except for the westernmost localities (sites 1–16) all the mice studied were light-bellied, with the relative tail length (tail: body ratio) seldom exceeding 100%. Conversely, mice from within the *M. domesticus* range (except locality No. 1, Waldau) were dark-bellied, with tails longer than the body. Hybrids formed a mixture of dark-bellied, light-bellied and intermediate animals with various tail lengths. Values for tail: body ratios for each locality are listed in the Appendix.

The presence of *t* haplotypes was found in almost half the localities studied (23 of 49). The mean frequency of *t*/+ heterozygotes was 0.13 (range = 0.00 - 1.00). Even if samples comprising only a single specimen are excluded, the maximum frequency reaches 0.833 (Hohenberg). Owing to the low number of *M. domesticus* samples, we could not test if frequencies of *t* haplotypes were significantly different from those in *M. musculus* and/or hybrids. We did not find any *t*/*t* homozygotes.

Table 1. List of collecting sites with frequencies of *domesticus*-type markers for allozymes, *Btk*, *Zfy2*, and *mt-Nd1*. The last column shows frequencies of *t*/+ heterozygotes, which were mapped with a 16-bp insertion at the *Hba-4ps* locus. Allozyme data are summarised as hybrid indices computed as frequencies of *domesticus* alleles averaged across all loci within individual populations (HI6 = hybrid index base on the whole set of six loci; HI5 = hybrid index based on five loci, excluding *Es-2*). In parentheses, numbers of animals scored; in markers other than HI6, numbers are only indicated when different from allozymes.

No.	Locality	HI5	HI6	*Btk*	*Zfy2*	*mt-Nd1*	*Hba-4ps*
1	Waldau	1 (1)	0.917	1		1	0
2	Röslau	1 (2)	1	1	1 (1)	1	0
3	Thierstein	0.848 (14)	0.856	1 (13)	0.625 (8)	1 (12)	0.143
4	Neuenreuth	0.900 (11)	0.900	1	0.286 (7)	1	0.636
5	Hohenberg	0.948 (6)	0.900	0.666	0	1	0.833
6	Trojmezí	0.800 (3)	0.778	1		1	0.667
7	Hazlov	0.878 (9)	0.861	1	0 (1)	0.778	0
8	Poustka	0.712 (38)	0.731	0.918 (36)	0.063 (16)	0.472 (36)	0.194 (36)
9	Lužná	0.817 (12)	0.750	1	0 (4)	1	0
10	Dolní Pelhřimov	0.500 (6)	0.545	0.875	0 (4)	1	0
11	Plesná	0.840 (123)	0.842	0.978 (46)	0.846 (26)	0.886 (44)	0.174 (46)
12	Křižovatka	0.845 (9)	0.735	1 (8)	0.333 (6)	0.875 (8)	0.250 (8)
13	Střížov	0.779 (7)	0.744	0.500 (5)	0 (2)	0.400 (5)	0.167 (6)
14	Horní Ves	0.786 (20)	0.780	0.917 (18)	0.333 (12)	0.167 (18)	0.167 (18)
15	Dolnice	0.757 (30)	0.715	0.923	0.059 (19)	0.107 (28)	0
16	Kopanina	0.904 (13)	0.893	1	0 (6)	0	0
17	Nebanice	0.200 (8)	0.208	0.143	0 (2)	0.250	0
18	Kaceřov	0.130 (14)	0.133	0	0 (8)	0.429	0
19	Dolina	0.208 (21)	0.314	0.067	0 (10)	0	0.048
20	Boučí	0.030 (10)	0.085	0	0 (7)	0	0
21	Hlavno	0.200 (6)	0.236	0	0 (3)	0	0
22	Kostelní Bříza	0.028 (24)	0.024	0	0 (8)	0	0
23	Osvinov	0.033 (3)	0.028	0		0	0.667
24	Stráž nad Ohří	0 (4)	0	0	0 (2)	0	0
25	Horní Jiřetín	0.015 (7)	0.075	0	0 (1)	0	0.143
26	Filipov	0 (2)	0	0	0	0	0
27	Nový Dům	0 (1)	0	0	0	0	0
28	Na Kokrdech	0 (7)	0.060	0 (4)	0 (1)	0 (4)	0 (4)
29	Doupno	0 (2)	0	0 (2)	0 (1)	0 (2)	0.500 (2)
30	Božtěšice	0 (5)	0	0 (4)	0 (3)	0 (4)	0 (4)
31	Mladý Smolivec	0.077 (3)	0.063	0	0 (1)	0 (2)	0.333
32	Jaroškov	0 (1)	0	0		0	1
33	Benešova Hora	0 (23)	0.040	0 (2)	0 (2)	0 (1)	0 (2)
34	Masákova Lhota	0 (4)	0	0	0 (3)	0	0.500
35	Zdíkovec	0 (33)	0.011	0 (20)	0 (18)	0 (20)	0 (20)
36	Vimperk	0 (9)	0.020	0	0 (4)	0 (8)	0
37	Dvory nad Lužnicí	0.100 (2)	0.083	0	0 (1)	0	0
38	Telč	0 (1)	0	0		0	0
39	Havraníky	0 (1)	0	0	0	0	0
40	Šatov	0 (3)	0.056	0		0	0.667
41	Brno	0.067 (3)	0.059	0	0	0	0.667
42	Těchov	0 (3)	0	0	0 (1)	0	0
43	Rudické propadání	0 (1)	0	0	0	0	1
44	Vilémovice	0 (2)	0.083	0 (1)	0 (1)	0 (1)	0 (1)
45	Lipovec	0 (1)	0	0		0	1
46	Kulířov	0.025 (13)	0.034	0 (11)	0 (4)	0 (11)	0.500 (10)
47	Zemianska Oľča	0 (8)	0	0	0 (4)	0	0
48	Necpaly	0 (8)	0.042	0	0 (1)	0	0.250
49	Hraň	0.069 (7)	0.057	0	0 (3)	0	0.286

Table 2. Frequencies of *domesticus* alleles for six diagnostic allozyme loci scored at each locality under study.

No	Locality	*Es-2*	*Gpd-1*	*Idh-1*	*Mpi*	*Np*	*Sod-1*
1	Waldau	0.500 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
2	Röslau	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)
3	Thierstein	0.909 (11)	0.679 (14)	0.607 (14)	1 (14)	1 (14)	0.962 (13)
4	Neuenreuth	0.900 (10)	1 (11)	0.682 (11)	0.955 (11)	0.909 (11)	0.955 (11)
5	Hohenberg	0.667 (6)	1 (5)	0.833 (6)	1 (6)	0.917 (6)	1 (6)
6	Trojmezí	0.667 (3)	1 (3)	1 (3)	0.667 (3)	0.333 (3)	1 (3)
7	Hazlov	0.778 (9)	1 (9)	0.722 (9)	0.833 (9)	0.833 (9)	1 (9)
8	Poustka	0.816 (38)	0.845 (31)	0.656 (32)	0.500 (34)	0.684 (38)	0.865 (37)
9	Lužná	0.417 (12)	1 (12)	0.708 (12)	0.792 (12)	0.542 (12)	1 (12)
10	Dolní Pelhřimov	0.750 (6)	0.667 (3)	0.250 (6)	0.583 (6)	0.250 (6)	0.833 (6)
11	Plesná	0.857 (77)	0.129 (70)	0.955 (122)	0.914 (122)	0.915 (123)	0.984 (122)
12	Křižovatka	0.222 (9)	0.813 (8)	0.667 (9)	1 (8)	0.833 (9)	0.938 (8)
13	Střížov	0.571 (7)	0.929 (7)	0.750 (6)	0.571 (7)	0.857 (7)	0.786 (7)
14	Horní Ves	0.750 (20)	0.868 (19)	0.800 (20)	0.559 (17)	0.700 (20)	0.975 (20)
15	Dolnice	0.517 (30)	0.840 (25)	0.483 (29)	0.833 (30)	0.733 (30)	0.857 (28)
16	Kopanina	0.846 (13)	0.611 (9)	1 (13)	0.944 (9)	0.885 (13)	1 (13)
17	Nebanice	0.250 (8)	0.125 (8)	0.125 (8)	0.500 (8)	0.125 (8)	0.125 (8)
18	Kaceřov	0.142 (14)	0.077 (13)	0 (14)	0.429 (14)	0.071 (14)	0.071 (14)
19	Dolina	0.850 (20)	0.025 (20)	0.025 (20)	0 (21)	0.325 (20)	0.675 (20)
20	Boučí	0.389 (9)	0 (10)	0 (10)	0.050 (10)	0.050 (10)	0.050 (10)
21	Hlavno	0.417 (6)	0.333 (6)	0 (6)	0.583 (6)	0 (6)	0.083 (6)
22	Kostelní Bříza	0 (19)	0 (15)	0 (24)	0.125 (24)	0 (24)	0 (20)
23	Osvinov	0 (3)	0 (3)	0 (3)	0.167 (3)	0 (3)	0 (3)
24	Stráž nad Ohří	0 (4)	0 (3)	0 (4)	0 (4)	0 (4)	0 (4)
25	Horní Jiřetín	0.357 (7)	0 (6)	0 (7)	0.071 (7)	0 (6)	0 (7)
26	Filipov	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
27	Nový Dům	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
28	Na Kokrdech	0.357 (7)	0 (7)	0 (7)	0 (7)	0 (7)	0 (7)
29	Doupno	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
30	Božtěšice	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)	0 (5)
31	Mladý Smolivec	0 (3)	0.333 (3)	0 (1)	0 (3)	0 (3)	0 (3)
32	Jaroškov	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
33	Benešova Hora	0.262 (14)	0 (23)	0 (23)	0 (23)	0 (23)	0 (23)
34	Masákova Lhota	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
35	Zdíkovec	0.095 (21)	0 (33)	0 (21)	0 (33)	0 (33)	0 (33)
36	Vimperk	0.111 (9)	0 (9)	0 (4)	0 (9)	0 (9)	0 (9)
37	Dvory nad Lužnicí	0 (2)	0 (2)	0 (2)	0 (2)	0.500 (2)	0 (2)
38	Telč	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
39	Havraníky	--	0 (1)	--	0 (1)	0 (1)	0 (1)
40	Šatov	0.333 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
41	Brno	0 (2)	0.333 (3)	0 (3)	0 (3)	0 (3)	0 (3)
42	Těchov	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)
43	Rudické propadání	0 (1)	0 (1)	--	0 (1)	0 (1)	0 (1)
44	Vilémovice	0.500 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
45	Lipovec	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
46	Kulířov	0.077 (13)	0 (13)	0 (9)	0 (13)	0.115 (13)	0 (13)
47	Zemianska Oľča	0 (8)	0 (8)	0 (8)	0 (8)	0 (8)	0 (8)
48	Necpaly	0.250 (8)	0 (8)	0 (8)	0 (8)	0 (8)	0 (8)
49	Hraň	0 (6)	0.286 (7)	0 (4)	0 (6)	0 (6)	0 (6)

Discussion

Our genetic investigations have revealed that almost the entire territory of the Czech and Slovak Republics is populated by *M. musculus*. However, the westernmost part of Czechia harbours populations of *M. musculus*/*M. domesticus* hybrids and/or pure *M. domesticus* – as shown by M a c h o l á n & Z i m a (1994) and L a z a r o v á (1999). Although a detailed

Appendix. Values of the mean relative tail length for each sample for which relevant data were available. TL/HBL*100, the tail length to head-and-body length ratio (expressed as a percentage); *SE*, standard error of the mean; in parentheses, numbers of animals measured. Site numbers correspond to those in Table 1.

No.	Locality	TL/HBL*100±*SE*	No.	Locality	TL/HBL*100±*SE*
1	Waldau	93.07 (1)	25	Horní Jiřetín	97.72 ± 2.33 (7)
2	Röslau	107.00 ± 3.81 (2)	26	Filipov	94.03 ± 14.03 (2)
3	Thierstein	110.59 ± 1.70 (14)	27	Nový Dům	93.42 (1)
4	Neuenreuth	113.42 ± 2.60 (8)	28	Na Kokrdech	110.72 ± 4.23 (4)
5	Hohenberg	106.90 ± 2.52 (6)	29	Doupno	95.50 ± 1.95 (2)
6	Trojmezí	105.57 ± 3.34 (3)	30	Božtěšice	96.96 ± 0.92 (4)
7	Hazlov	107.50 ± 1.76 (9)	31	Mladý Smolivec	94.52 ± 0.35 (3)
8	Poustka	104.64 ± 1.19 (35)	32	Jaroškov	94.59 (1)
9	Lužná	102.54 ± 1.66 (15)	33	Benešova Hora	87.56 ± 2.30 (8)
10	Dolní Pelhřimov	104.49 ± 3.21 (6)	34	Masákova Lhota	99.15 ± 5.59 (4)
11	Plesná	105.19 ± 0.74 (109)	35	Zdíkovec	98.34 ± 1.75 (19)
12	Křižovatka	114.05 ± 4.18 (8)	36	Vimperk	97.84 ± 2.41 (8)
13	Střížov	106.69 ± 3.96 (14)	37	Dvory nad Lužnicí	105.74 ± 8.56 (2)
14	Horní Ves	108.60 ± 1.92 (18)	39	Havraníky	88.24 (1)
15	Dolnice	103.75 ± 1.61 (29)	40	Šatov	100.84 ± 3.41 (3)
16	Kopanina	112.43 ± 1.98 (13)	41	Brno	93.90 ± 2.44 (2)
17	Nebanice	99.37 ± 1.53 (15)	42	Těchov	95.23 ± 4.77 (4)
18	Kaceřov	99.71 ± 1.79 (13)	43	Rudické propadání	110.29 (1)
19	Dolina	94.96 ± 3.11 (21)	45	Lipovec	87.01 (1)
20	Boučí	109.90 ± 3.11 (11)	46	Kulířov	97.08 ± 2.34 (8)
21	Hlavno	93.65 ± 0.74 (6)	47	Zemianska Oľča	86.04 ± 1.86 (8)
22	Kostelní Bříza	96.67 ± 1.37 (23)	48	Necpaly	85.52 ± 2.61 (8)
23	Osvinov	98.62 ± 0.79 (3)	49	Hraň	90.00 ± 2.41 (7)
24	Stráž nad Ohří	96.31 ± 1.79 (4)			

study of the hybrid zone was not the focus of the present paper, it appears that the zone is quite narrow, and that the transition of allozyme markers between the two species takes place within less than 20 km. The position of the transition zone fits with the results of Kraft's (1984) morphological studies from the entirety of Bavaria, as well as with the results of genetic and parasitological studies along a transect across the hybrid zone in southern Bavaria and north-western Austria (Sage et al. 1986a,b, Tucker et al. 1992). In contrast, we contend that the precise position of the *M. domesticus/M. musculus* hybrid zone north of the area studied by us should still be treated as undetermined.

As far as external features are concerned, the animals under study corresponded with the data in published reports (e.g. Marshall & Sage 1981, Macholán 1996 and references therein). However, as already pointed out by Macholán (1996), morphological criteria such as coloration and relative tail length should only be considered as a rough guide. Mice from as many as five sites (nos. 20, 28, 37, 40, 43) from well within the *M. musculus* range included mice with average tail: body ratios exceeding 100% but, on the other hand, a mouse from Waldau (Thuringia) had a relatively short tail (93.07%, see Appendix). Obviously, the level of commensalism of a population, and perhaps other factors as well, play a role so that strictly commensal mice are generally darker and longer-tailed, whereas mice living outdoors tend to be lighter and shorter-tailed (Macholán 1996).

Extensive introgression of some allozyme alleles has been observed in Denmark (Hunt & Selander 1973, Vanlerberghe et al. 1986, 1988b, Dod et al. 1993), Bulgaria (Boursot et al. 1984, Vanlerberghe et al. 1986, 1988a) and southern Germany (Tucker et al. 1992). In the present study, we found *domesticus* alleles as far east of the zone as southern Moravia (SE Czechia) and eastern Slovakia, i.e. hundreds of kilometres

apart. However, occurrence of *M. domesticus* allozyme markers is rather incidental and their frequencies are usually extremely low (except in very small samples, cf. Table 1). Hence, it is not clear if this phenomenon has resulted from human-mediated long-distance dispersal, or from gene flow across the zone due to a weak barrier against supposedly neutral markers, or from incomplete fixation of diagnostic alleles due to incomplete lineage sorting for autosomal markers. [Under an assumption of neutrality, the expected time for lineage sorting is four times longer for nuclear markers than for mitochondrial ones due to a four times larger effective population size; see Avise (2000).] The last possibility seems to correspond well with our finding no such "introgression" in the mtDNA marker (Table 1). Finally, we cannot rule out the possibility that seemingly introgressed markers are, in fact, newly arisen alleles, unidentified by our methods. On the other hand, the introgression in western Czechia appears to be systematic at the locus. It is apparent from Table 1 that *Es-2* tends to decrease values of the hybrid index in the westernmost sites and, conversely, increases it at sites east of the supposed centre of the hybrid zone; this results in a broader cline.

The highest introgression of *domesticus* alleles at the *Es-2* locus was also found in Denmark (Hunt & Selander 1973) and Bulgaria (Boursot et al. 1984, Vanlerberghe et al. 1988a). However, it should be noted that large penetration of *Mpi* was also reported in Bulgaria (Boursot et al. 1984) and this was expressed more explicitly by Vanlerberghe et al. (1986), who found the *Es-2* introgression rather intermediate (being lower than that of *Mpi*, *Np* and *Pgm-1*). Similar results were reported from Denmark, where the *Es-2* introgression was lower than in *Mpi* and *Pgm-1* (Vanlerberghe et al. 1986). *Mus domesticus* alleles at *Mpi* were also found at least 50 km eastwards of the East Holstein Peninsula (Prager et al. 1993). In addition, Tucker et al. (1992) found *domesticus*-like alleles at *Np-1* up to 100 km east of the centre of the hybrid zone along a transect in southern Germany and western Austria. In all these cases, the introgression was asymmetrical – i.e. higher for *M. domesticus* alleles penetrating into the range of *M. musculus*. Unfortunately, these results can not be assessed with our data owing to the lack of appropriate sampling sites on the *M. domesticus* side.

Limited intogression of the X and Y chromosome markers is in accord with strongly impeded movement of sex chromosome loci found in other sections of the hybrid zone (Vanlerberghe et al. 1986, Tucker et al. 1992, Dod et al. 1993, Prager et al. 1997). A general lack of populations with both variants of the X chromosome marker (*Btk*) in the study area supports the view that X-linked markers are strongly selected against in the hybrid genome (Dod et al. 1993). Conversely, our analysis of the Y chromosome marker revealed an unexpectedly gradual transition between *domesticus*-type and *musculus*-type *Zfy2* alleles. Moreover, none but one of the localities studied was found to carry the fixed *M. domesticus* Y chromosome (the only exception was Röslau, where only a single male was studied). This contradicts the results of studies carried out in Denmark (Dod et al. 1993), East Holstein (Prager et al. 1997), southern Germany (Tucker et al. 1992) and Bulgaria (Vanlerberghe et al. 1986) which report steep clines for the Y chromosome. Our results should therefore be verified by comparison with material from southern Germany.

Since mtDNA is almost exclusively matrilineal and inherited as a single unit due to a lack of recombination (but see Gyllensten et al. 1991, Wallis 1999, Ladoukakis & Zouros 2001) one simple marker can be used to differentiate between the *M. domesticus* or *M. musculus* origin of the mtDNA in hybrid animals. In accord with previous studies on mtDNA variation in Europe (Sage et al. 1990, Prager et al. 1996) we have demonstrated the prevalence of *musculus*-type mtDNA throughout the

study area, *domesticus*-type mtDNA being confined to the westernmost parts. Similarly to the *Btk* gene, the transition from the *musculus* side to the *domesticus* side was rather steep; this is in agreement with Bulgarian data (Boursot et al. 1984, Vanlerberghe et al. 1988a). Limited evidence presented by Prager et al. (1996) suggests a rather different pattern, including the presence of both *domesticus* and *musculus* mtDNA about 100 km eastwards of the centre of the hybrid zone (locality 1 in the Bavarian transect, Branau). A remarkably different situation occurs in Scandinavia, where populations of *M. musculus* have been found to carry exclusively *M. domesticus* mtDNA (Ferris et al. 1983a,b, Gyllensten & Wilson 1987, Vanlerberghe et al. 1988b, Prager et al. 1993). This pattern was suggested as having resulted from a supposed colonisation event by *M. domesticus* females from East Holstein, where a high proportion of the mice appeared to carry the types of mtDNA found in Scandinavian *M. musculus*, as do most mice from the *musculus* side of the Holstein hybrid zone (Gyllensten & Wilson 1987, Prager et al. 1993). Thus the presence of *M. domesticus* mtDNA in northern Denmark, Sweden and Finland is not a result of permanent gene flow across the zone, but rather a consequence of a founder effect before the establishment of the Danish hybrid zone.

The frequency of *t*/+ heterozygotes ascertained in this study falls into the range based on previously studied populations of the house mouse (reviewed in Ardlie 1998). However, it is worth noting that populations (both of *M. musculus* and those with a high proportion of *M. domesticus* alleles) with more than 50% of *t*/+ animals were not exceptional. Since all known *M. musculus t* haplotypes belong to the same t^{w73} group (Klein et al. 1984, Forejt et al. 1988, Ruvinsky et al. 1991, Mazin 1994) all *t/t* carrying individuals in this species probably die early in gestation. Therefore, the absence of *t/t* homozygotes in *M. musculus* populations is not surprising. *t* haplotypes belonging to other complementation groups can only be expected in *M. domesticus* and in hybrid populations. Unfortunately, the method used did not allow us to ascertain any details about the haplotypes or complementation groups of the studied *t* complexes. Laboratory crosses with mice carrying known *t* haplotypes or microsatellite typing of *t*/+ animals (as suggested in Ardlie & Silver 1996) are needed to obtain more information about the *t* haplotypes found in the present study.

We conclude that the molecular markers and allozymes used in this study are capable of mapping the position of the hybrid zone in central Europe, and of revealing some details of the genetic features of wild mice populations, e.g. the frequency of *t* haplotypes. Since PCR-based markers are practicable for even low quality templates such as DNA from museum specimens (cf. Prager et al. 1998) they probably represent the best option for future studies. These will extend our knowledge of the exact position of the hybrid zone, which is still not satisfactorily resolved in some areas.

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