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Three new *t*-haplotypes of *Mus musculus* reveal structural similarities to *t*-haplotypes of *Mus domesticus*

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Summary

Three new *t*-haplotypes, t^{p4}, t^{p12} and t^{p14}, were isolated from *M. musculus* male mice captured in Central and East Bohemia, Czechoslovakia, about 400 km from the zone of hybridization between *M. musculus* and *M. domesticus* species. Complementation tests have shown that all three new *t*-haplotypes belong to t^{w73} group. When compared with 5 *t*-haplotypes from *M. domesticus* they displayed the same pattern of *Bam*HI restriction fragments with *H-2* class I genes, and they also shared the *t*-specific 5·2 kb *Taq*I fragment of the alpha globin pseudogene. However, they differed from *M. domesticus t*-haplotypes at the D17Leh443 locus since they all displayed a 10·5 kb *Msp*I fragment, labelled by the Tu443 probe, not found in wild type-chromosomes or in *M. domesticus t*-haplotypes. A hypothesis is proposed that *t*-haplotypes in *M. domesticus* originated by a single successful introgression from a parental species during speciation.

1. Introduction

Sixty years ago Dobrovolskaia-Zavadskaia & Kobozieff (1927) discovered the first *t*-complex mutation in the mouse, dominant *T* (*Brachyury*) which caused shortening of the tail in $T/+$ heterozygotes. *Brachyury* enabled subsequent detection of recessive *t*-alleles through the tailless phenotype of T/t compounds. The *t*-alleles, now designated as *t*-haplotypes, have been shown to occupy about 10 cM or 5×10^4 kilobases (kb) of the proximal portion of mouse chromosome 17 (Fig. 1). The whole chromosomal segment with a *t*-haplotype is transmitted to progeny in 99·8% of cases as a single genetic factor due to two non-overlapping inversions harbouring the *t*-chromatin and suppressing locally the crossing-over (Artzt & Bennett, 1982; Pla & Condamine, 1984; Herrmann *et al.* 1986). The majority of *t*-haplotypes observed in wild mouse populations affect specifically spermatogenesis by distorting their own transmission ratio from $t/+$ male parents to progeny and by causing male sterility in certain t^x/t^y compounds. Most of the *t*-haplotypes also carry recessive lethal factors affecting embryogenesis at defined stages and allowing classification of *t*-haplotypes into complementation groups. The complex properties of *t*-haplotypes in the mouse have been repeatedly reviewed, most recently by Silver (1985) and by Frischauf (1985). Detailed analysis of various *t*-haplotypes extracted from present-day populations of wild mice indicated that they could be descendants of a common ancestor (Silver, 1982; Nizetič, Figueroa & Klein, 1984; Klein, Sipos & Figueroa, 1984; Figueroa *et al.* 1985). However, most of the experimental evidence supporting the hypothesis came from comparisons of various *t*-haplotypes extracted from the *Mus domesticus* species.

Here we present the first analysis of three new *t*-haplotypes recently isolated from *Mus musculus* wild mice of Central and East Bohemia, Czechoslovakia. Our results lend support to the hypothesis suggesting that *t*-haplotypes from both mouse species, *M. musculus* and *M. domesticus*, share a single ancestor.

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2. Materials and methods

(i) *Mice*

All mouse strains used in this study were bred in the Institute of Molecular Genetics, Prague. The breeding nuclei of 129-*t* congenic strains were kindly provided by Dr J.-L. Guénet, Pasteur Institute, Paris in 1979. The wild-derived, non-*t* inbred strains of *M. musculus* PWK and PWD were established in our laboratory (Pavljuková & Forejt, 1981; Forejt, 1985). Three new *t*-haplotypes were extracted from wild mice of *M. musculus* species captured in Central and East Bohemia, Czechoslovakia, in 1979–1981. Two of them were found in mice trapped and initially bred by our former student Ms L. Michalová. The wild-

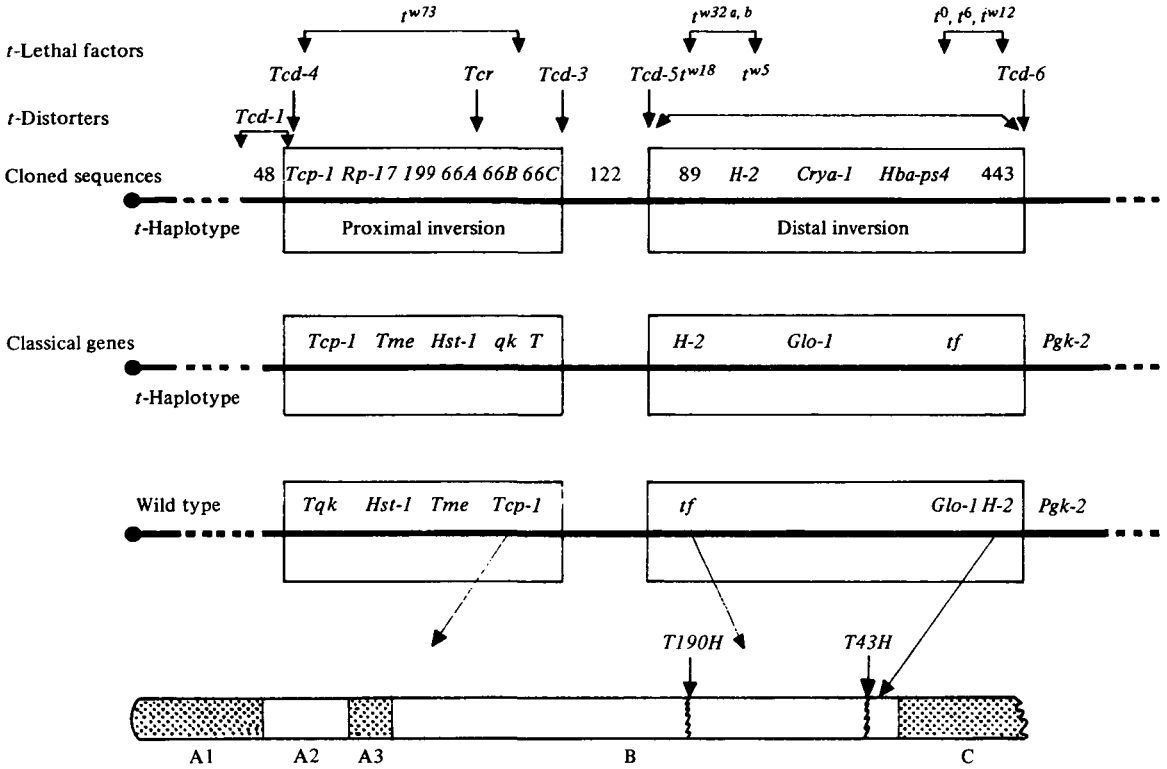

Fig. 1. Summary of the genetic and cytogenetic structure of *t*-complex region on chromosome 17. Mapping positions are not to scale *t*-Lethal factors are according to Artzt (1984), *t*-distorter factors are according to Lyon (1984), Fox *et al.* (1985) and Silver & Remis (1987). Not all DNA markers are shown. Those indicated by numbers are from the Tu-series described in Roehme *et al.* (1984), Fox *et al.* (1984), Bučan *et al.* (1987), The origin of other cloned sequences and gene markers is indicated in the text or can be found in *Mouse News Letter* (1987). Two inversions were observed in *t*-haplotypes when compared with wild-type chromosome 17. However, molecular analyses have shown that the proximal inversion occurred most probably in the wild-type chromosome and was fixed during evolution of *Mus*, while *t*-haplotypes represent an older, not rearranged form of the chromosome 17 (Schimenti *et al.* 1987; Herrman, Barlow & Lehrach, 1987). Positions of *t*-complex markers on G-banded chromosome 17 are according to Forejt, Čapková & Gregorová, 1981, Lader *et al.* (1985) and Lyon *et al.* (1986).

derived mice were transferred to the experimental mouse facilities of the Institute of Molecular Genetics after their delivery by Caesarean section, nursing by foster mothers and examination for selected microbial and viral pathogens. All three *t*-haplotypes have been made congenic on 129/Sv genetic background, Congenic resistant strain B10.W67 was prepared by Dr M. Loudová-Micková in our Institute. It carries the *H-2* haplotype from the non-*t* chromosome 17 of a wild mouse *M. musculus* (Micková & Iványi, 1976).

(ii) *Two-dimensional polyacrylamide gel electrophoresis of solubilized testicular cell proteins*

The suspension of testicular cells was prepared by short trypsin digestion (0·01%, Difco 1:250) and by mechanical shearing. The cells were cultured overnight to restore their membrane proteins (Silver, 1982) in Dulbecco's MEM with 10% foetal bovine serum, antibiotics and 8% CO_2 in the atmosphere. For radiolabelling 3·7 MBq of L-[^{35}S]methionine (Radiochemical Centre, Amersham, U.K.) was added to 1×10^7 cells in 1 ml of methionine-free culture medium. The cell pellet was repeatedly washed and lysed in 0·5 ml of complete lysis buffer (O'Farrell, 1975) consisting of 9·5 M urea, 2% (w/v) NP 40, 5% (v/v) beta-mercaptoethanol and 2% Ampholines (LKB), pH range 3–10 and 5–8, ratio 1:4. Isoelectric focusing was done with the same pH range and ratio of Ampholines (LKB) as used in the lysis buffer. The proteins were resolved in the second dimension by sodium dodecyl sulphate (SDS) electrophoresis in 10% polyacrylamide gels. The radioactive proteins were visualized by fluorography, and proteins from non-labelled samples were ascertained by the silver-staining technique of Oakley, Kirsch & Morris (1980).

(iii) *Isolation of high-molecular-weight DNA*

Mouse DNA was prepared by standard procedures. Briefly, one kidney was homogenized in liquid nitrogen and the frozen tissue powder was transferred to 5 ml of 200 mM-NaCl, 20 mM-EDTA, 40 mM-Tris-HCl pH 8·0 and 0·5% SDS at 60 °C for 20 min. The mixture was cooled to 37 °C and 5 ml of 0·5% SDS was added. Then self-digested Pronase (Serva) was added to a final concentration of 1 mg/ml and the mixture was

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left overnight at 37 °C. After repeated extractions with phenol, phenol:chloroform (1:1) and chloroform:isoamyl alcohol (49:1), on volume of ethanol was added and the precipitated high-molecular-weight DNA was spooled. The precipitate was washed in 70% ethanol in 10 mM-Tris-HCl pH 8·0, 1 mM-EDTA, briefly dried and dissolved in 10 mM-Tris-HCl, 1 mM-EDTA. Only traces of RNA were present in most samples, and they were not removed.

(iv) *Restriction enzyme digestions and Southern blot hybridizations*

Mouse DNA was digested to completion with a specified restriction endonulease (*Bam*HI, *Eco*RI, *Bg*1II – Sevac, Prague; *Msp*I – Pharmacia, *Taq*I – Boehringer, Mannheim), and 10 μg samples were resolved by electrophoresis in 0·7% agarose gels. *Eco*RI fragments of LP 52 phage DNA (Forstová *et al.* 1982) or *Hind*III fragments of lambda phage DNA were used as markers of known length. The denatured DNA was transferred from the gel on to a Schleicher & Schuell nitrocellulose membrane (BA85ND) or on to a Bio-Rad Zeta-Probe nylon blotting membrane. The subcloned *Pst*I 1·8 kb fragment of the *H-2K*^{d} gene (including the leader sequence and two additional exons), kindly provided by Dr G. Gachelin, Pasteur Institute, Paris, was used as a probe for class I *H-2* genes. Hybridization probe for *Hba-ps4* was an *Eco*RI fragment (2.4 kb) of the alpha globin pseudogene inserted into pBR322 (Leder *et al.* 1981) and for *D17Leh443* it was a 1·7 kb *Eco*RI fragment from the genomic microclone (Roehme *et al.* 1984) inserted in pUC19 (Bučan *et al.* 1987). The probes were labelled with [^{32}P]dCTP (Amersham) by nick translation (Rigby *et al.* 1977) to 0·9–1·6 × 10^{8} dpm/μg specific activity, or by using the Klenow fragment of DNA polymerase I and random hexamer priming (Feinberg & Vogelstein, 1983) to 2 × 10^{9} dpm/μg specific activity. The pre-hybridization and hydridization mix was 50% deionized formamide (BRL), 5 × SSC (1 × SSC is 0·15 M-NaCl plus 0·015 M sodium citrate), 50 mM-$NaPO_4$ pH 6.5, 1·25 × Denhardt's solution, 1% glycine, 2·5 mM-EDTA and 100 μg/ml of denatured salmon sperm DNA (Serva). For hybridization 10^{6} dpm of denatured probe was added per 1 ml of hybridization mix and the filter was incubated at 42 °C for 24 h. Washes were performed in 2 × SSC/0·1% SDS twice for 30 min at room temperature and in 0·1 × SSC/0·1% SDS twice for 30 min at 50 °C. Medix-Rapid film (Foma) was exposed to a filter with two intensifying screens for periods of 48 h to 6 days at −70 °C.

3. Results

(i) *Complementation tests and transmission ratios*

The haplotypes t^{p4} and t^{p14} were isolated from male mice (*M. musculus*) trapped in two different Prague localities, Lhotka and Suchdol, in 1979 and 1981. The haplotype t^{p12} was extracted from a wild male mouse (*M. musculus*) captured in the locality of Trpišov, East Bohemia, in 1981. Both localities are situated 300–400 km to the east of the zone of hybridization between the two mouse species, *M. musculus* (Eastern Europe) and *M. domesticus* (Western Europe) (Sage, 1981; Sage, Whitney III & Wilson, 1986). All three *t*-haplotypes behaved as recessive lethals since in crosses $T/t \times T/t$ only tailless offspring (T/t) were born, thus creating balanced lethal stocks. In complementation tests the *t*-haplotypes proved to belong to the t^{w73} complementation group (Table 1). The haplotype t^{p4} was tested for the presence of seven additional different *t*-lethal factors but none of them was found (Table 1). Testing of t^{p12} and t^{p14} was incomplete because of the low fertility of *t*-carrying animals. Nevertheless, t^{p12} (but not t^{p14}) apparently carried another lethal factor belonging to t^{0} complementation group. The transmission from T/t males was high in all three *t*-haplotypes: 92·3% in t^{p4} (121/131 – normal tailed progeny/total), 95·9% (143/148) in t^{p12}, and 94% (136/144) in t^{p14}.

(ii) t-*complex polypeptide-1*

Tcp-1 gene is located between the *T* and *tf* markers in the proximal portion of chromosome 17. It codes for a non-glycosylated peripheral membrane protein present most abundantly in haploid testicular cells. It was first detected by Silver, Artzt & Bennett (1979) on two-dimensional gels of electrophoretically separated testicular cell proteins. All complete *t*-haplotypes were shown to carry the *Tcp-1*^{a} allele coding for a more acidic variant of TCP-1 protein, while the *Tcp-1*^{b}

Table 1. *Complementation tests of three new* t-*haplotypes*

New *t*-haplotypes	Normal-tailed/tailless progeny[a]								
	t^{p12}	t^{w37}	t^{0}	t^{w5}	t^{w12}	t^{w18}	t^{Pa1}	t^{w1}	t^{w32}
t^{p4}	0/37	0/26	8/18	23/29	9/18	9/10	5/8	6/12	2/4
t^{p12}	—	—	0/18	3/6	2/4	—	—	—	—
t^{p14}	0/17	—	5/11	—	—	—	—	—	—

[a] Viable progeny of the cross: $T/t^{x} \times T/t^{y}$. The genotype of normal-tailed progeny is t^{x}/t^{y}, tailless animals are T/t.

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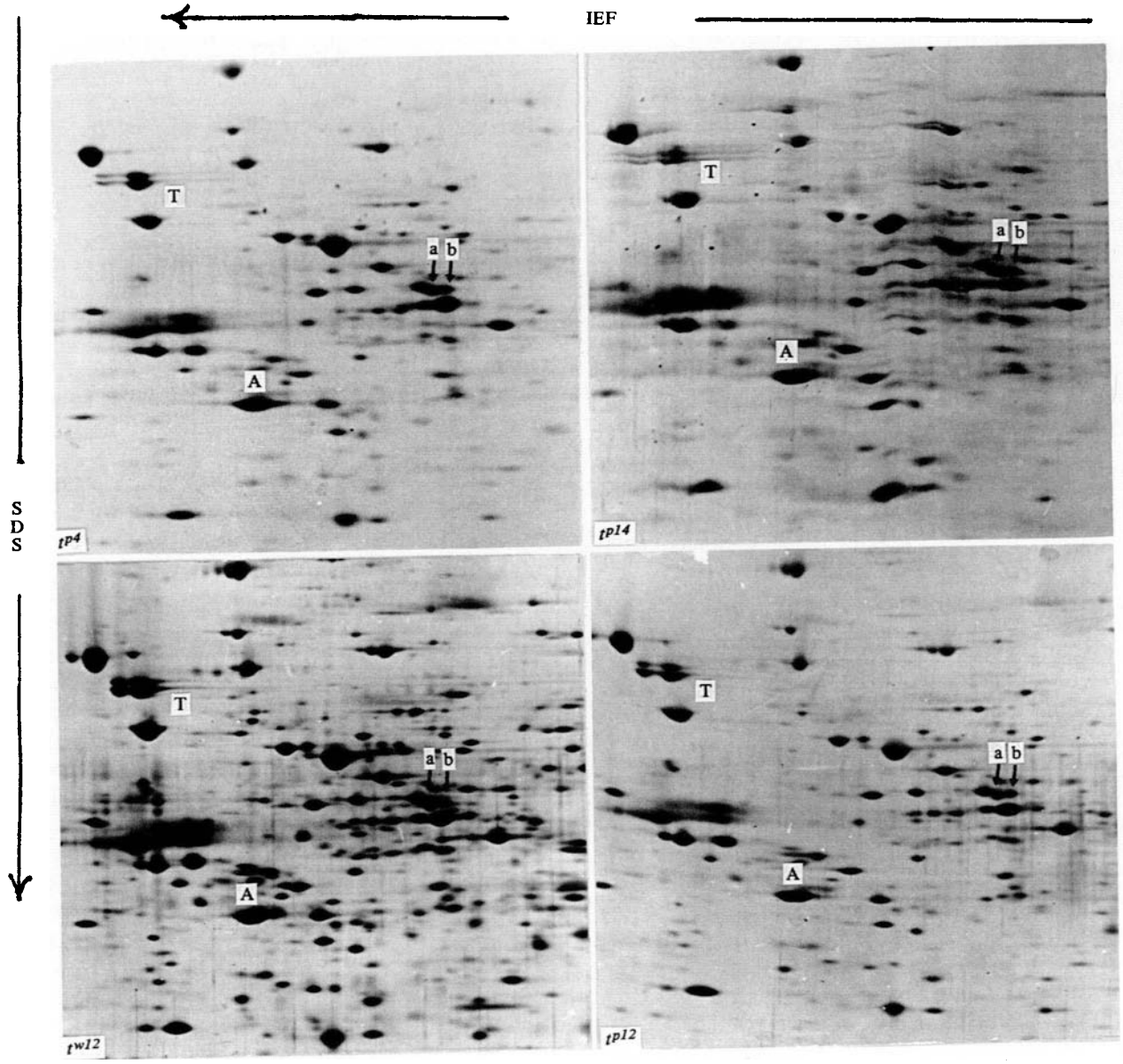

Fig. 2. Two-dimensional polyacrylamide gel electrophoresis of NP-40 solubilized proteins from testicular cells of 129-*t* mice. Three *t*-haplotypes extracted from *M. musculus* show the same mobility of TCP-1A polypeptide as does the t^{w12} haplotype originated from *M. domesticus*. A, actin; T, tubulin; a, TCP-1^a; b, TCP-1^b forms of *t*-complex polypeptide 1.

allele is characteristic of all wild-type, non-*t* chromosomes 17 examined so far. Here we examined *M. musculus*-derived *t*-haplotypes t^{p4}, t^{p12} and t^{p14} for their *Tcp-1* allelic form by carrying out two-dimensional gel electrophoresis of testicular cell proteins from males of appropriate genotypes (Fig. 2). All three *t*-haplotypes examined displayed *Tcp-*1^a allele as judged from the presence on gels of the TCP-1A protein, which is indistinguishable by its SDS–polyacrylamide-gel electrophoresis mobility or its isoelectric point from the gene product coded by *Tcp-*1^a genes in *t*-haplotypes of *M. domesticus* origin.

(iii) *Restriction fragment length polymorphism of class I* H-2 *genes*

Class I *H-2* genes constitute a family of approximately 30 closely related genes located within the *H-2* complex on chromosome 17. Inside a complete *t*-haplotype they are all trapped in the distal inversion. The probe used here to visualize the genomic restriction fragments carrying the class I genes was similar, but not identical to pH-2IIa used by Silver (1982) or to pH-2III used by Rogers, Lyon & Willison (1985). It was a *Pst*I fragment of the 5′ end of the *H-2K*^{d} gene spanning its first three exons (Fig. 3). It recognizes most of the *H-2* class I genes belonging to *K, D, L, Qa* and *T1a* regions of the *H-2* complex.

In previous studies (Steinmetz *et al.* 1981; Margulies

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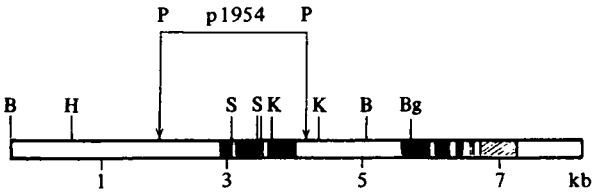

Fig. 3. Restriction map of the genomic clone of gene *H-2K*^{d} (Kvist, Roberts & Dobberstein, 1983). A subclone of 1·8-kb *Pst*I fragment in pBR322 was used as a probe for *H-2* class I genes. Solid boxes denote exons, hatched box indicates the 3′ noncoding region. Restriction enzymes: B, *Bam*HI; H, *Hin*dIII; S, *Sma*I; K, *Kpn*I, Bg, *Bgl*II.

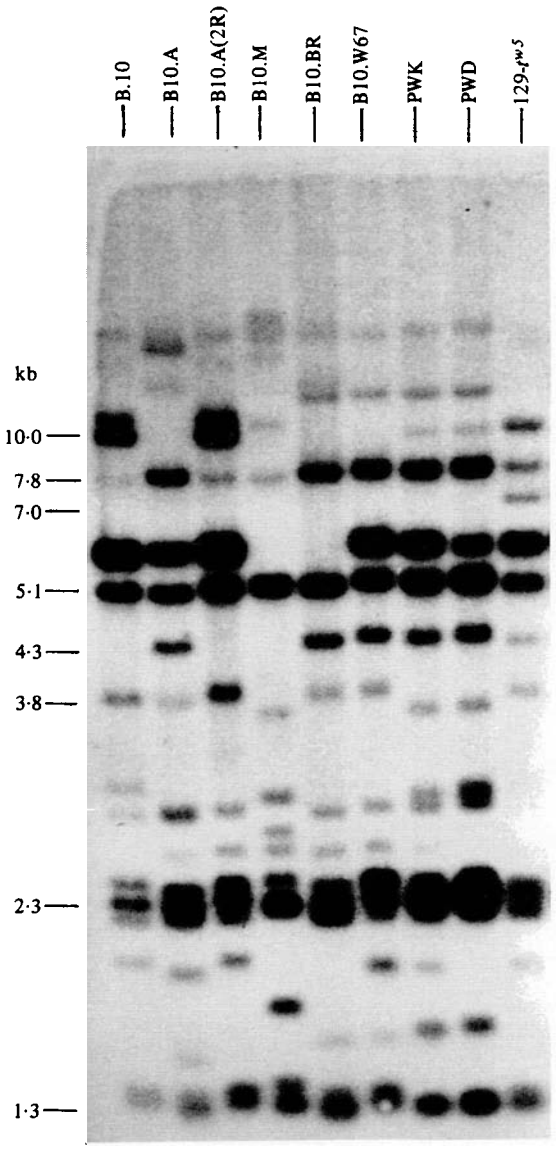

Fig. 4. Hybridization of the *H-2* class I probe to *Bam*HI-digested DNA from mice with different *H-2* haplotypes from wild-type chromosome 17. 129-t^{w5}/*T* is included for comparison. PWD, PWK and B10 . W67 are strains carrying chromosome 17 from wild mice of *M. musculus* origin. Other B10 congenic strains carry different *H-2* haplotypes from laboratory mice. The 7·0 kb fragment appears *t*-specific in our set of chromosomes 17. Each *H-2* haplotype displays a unique pattern of *Bam*HI restriction fragments.

et al. 1982) the *Bam*HI restriction sites appeared more conserved around and inside the class I genes than restriction sites for other enzymes. Therefore we first analysed Southern blots of *Bam*HI restricted genomic DNA of 10 different *H-2* haplotypes of non-*t* chromosomes. They included *H-2* haplotypes *d*, *k*, *b*, *a*, *f*, *i5*, *q* and *H-2* haplotypes from *M. musculus* fixed in strains B10 . W67, PWK and PWD. Part of this analysis is shown in Fig. 4. Altogether 33 bands of different size were distinguished, but only three of them were present in all the haplotypes tested. The highly conserved restriction sites defined a 5·1 kb fragment that was previously identified by Rogers, Lyon & Willison (1985) as an *H-2K* gene-carrying band. A second conserved band was observed in a cluster around 2·3 kb where *H-2D* and *H-2L* genes are situated, and a third such band, of 1·3 kb, might have been identical with the gene 8 of cluster I in Rogers' nomenclature (Rogers, 1985). Individual *H-2* haplotypes displayed 13–15 distinct bands. Of them, 10–12 were responsible for variations that caused the unique patterns of bands in each *H-2* haplotype analysed. On the other hand, by using the same restrictase and probe, the analysis of *H-2* class I genes in eight 129-*t* congenic strains, including 5 strains with *t*-haplotypes of *M. domesticus* origin and 3 strains with new *t*-haplotypes of *M. musculus* origin, revealed 15 bands in each genotype without a single difference in position of any band (Fig. 5). Within the limits of the examined panel of 10 *H-2* haplotypes from non-*t* haplotypes and 9 *H-2* haplotypes from chromosomes with *t*-haplotypes, the 7·0 kb *Bam*HI fragment was specific for *t*-haplotypes (Fig. 5). However, when we used the same probe to visualize the class I-bearing fragments created by *Eco*RI or *Bgl*II enzymes, certain heterogeneity of the restriction sites was apparent both within and between the *M. musculus* and *M. domesticus* *t*-haplotypes (data not shown).

(iv) *Haemoglobin alpha-4 pseudogene*

One of the globin pseudogenes, referred to as $\alpha\psi 4$, has been mapped to chromosome 17 (Leder *et al.* 1981), to *Hba-ps4* locus near the *tf* mutation (Fox, Silver & Martin, 1984; D'Eustachio *et al.* 1984). Following the observation of Fox *et al.* (1984) that the *Taq*I restrictase discloses a unique 5·2 kb *t*-specific DNA fragment on genomic Southern blots, we compared t^{p4}, t^{p12} and t^{p14} with t^{w12} and non-*t* chromosomes 17 using the same enzyme and $\alpha\psi 4$ probe (Fig. 6). Our *M. musculus*-derived *t*-haplotypes displayed the same 5·2 kb *Taq*I fragment characteristic of *t*-haplotypes of *M. domesticus* origin. Moreover, a new allele of the *Hba-ps4* locus was observed in the PWK inbred strain (derived from *M. musculus*). It is characterized by the occurrence of two *Taq*I fragments 1·4 kb and 1·9 kb and we designated it *Hba-ps4*^{p}.

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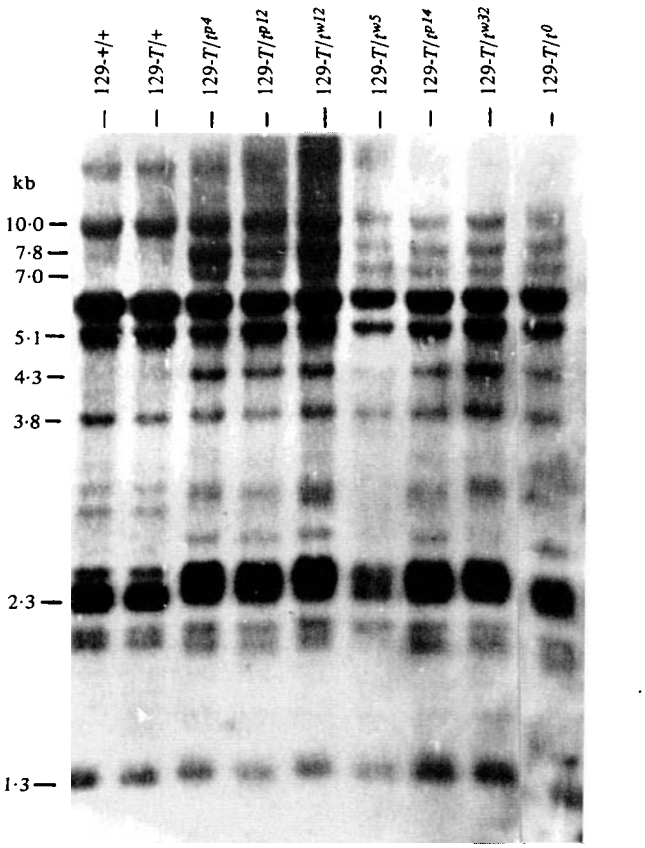

Fig. 5. Hybridization of the *H-2* class I probe to *Bam*HI-digested DNA from 129-*t* congenic mice. Mice with *Mus musculus* and *Mus domesticus* *t*-haplotypes display the same restriction fragment pattern.

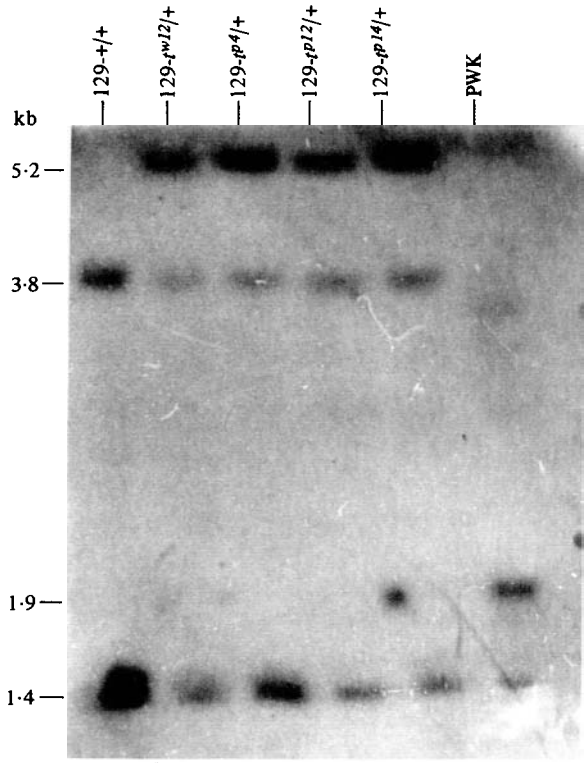

Fig. 6. Genomic blot analysis of Hba-ps4 DNA alleles by hybridization of *Taq*I-restricted DNA with $\alpha\psi 4$ genomic clone as a probe. All *t*-haplotypes display a 5·2 kb *t*-specific *Taq*I fragment. PWK inbred strain shows a new allele with 1·9 kb and 1·4 kb *Taq*I restriction fragments.

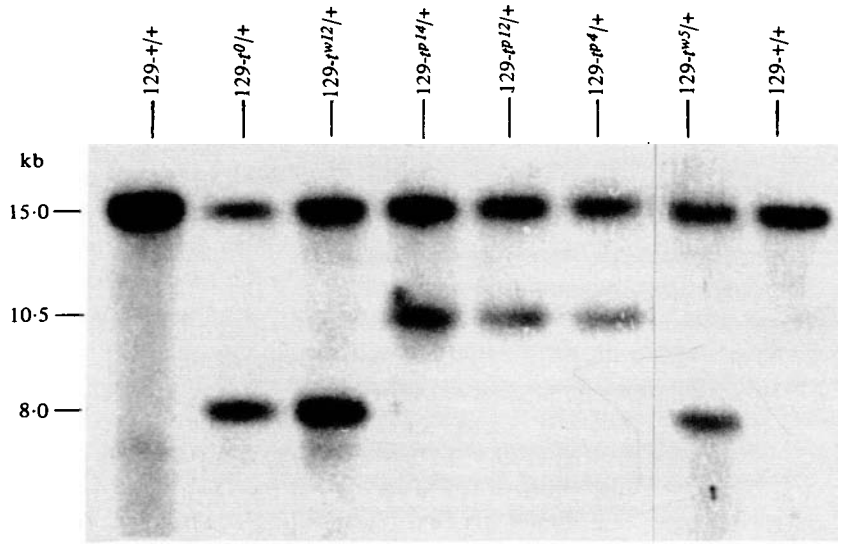

Fig. 7. Hybridization pattern of *Msp*I restriction fragments of genomic DNA using Tu443 probe. *t*-haplotypes t^{w5} t^{0} and t^{w12} display an 8·0-kb *M. domesticus*-*t*-specific fragment while t^{p4}, t^{p12} and t^{p14} haplotypes show a 10·5-kb *M. musculus*-*t*-specific *Msp*I fragment.

(v) *D17Leh443 locus*

Tu443 is one of 25 cloned DNA sequences obtained by microdissection of chromosome 17 by Lehrach and his coworkers (Roehme *et al.* 1984; Bučan *et al.* 1984). The *D17Leh443* locus identified by this probe has been mapped proximal to *tf* and also proximal to *Hba-ps4*. In complete *t*-haplotypes this locus apparently represents the most distal marker inside the distal inversion (Bučan *et al.* 1987). The Tu443 probe hydridizes to a single 15 kb *Msp*I fragment of DNA from 129+/+ and to an 8 kb *Msp*I fragment of

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DNA from t^{w5}/t^{w5} cells. We could confirm this polymorphism for t^{w5}, t^{0} and t^{w12} haplotypes. However, *M. musculus*-derived haplotypes t^{p4}, t^{p12} and t^{p14} displayed a new *Msp*I fragment of 10·5 kb (Fig. 7) and lacked the *M. domesticus*-*t*-specific 8·0 kb fragment.

4. Discussion

Two major species of the house mouse in Central Europe, *M. musculus* and *M. domesticus*, have been separated by incomplete reproductive isolation (Sage, Whitney & Wilson, 1986) for approximately one million years. The occurrence of *t*-haplotypes in both species might indicate either their introgression from one species to another through the zone of hybridization, or independent occurrence of the same type of chromosomal anomaly in both species, and finally transmission, during speciation, of *t*-haplotypes from an ancestral species to the newly established *M. domesticus* and *M. musculus* species. In order to evaluate these alternatives, we have performed a comparative analysis of several loci within *t*-haplotypes from *M. musculus* and *M. domesticus*.

(i) *Lethal factors*

The original t^{w73} haplotype was discovered by Dunn & Bennett (1971) in wild mice from Jutland Peninsula in Denmark at the zone of hybridization between both mouse species. The lethal factor of t^{w73} is located in the proximal inversion, while all other lethal factors from *t*-haplotypes of *M. domesticus* were found in the distal inversion around the *H-2* complex or even more distally (Artzt, 1984). Assignment of all three *t*-haplotypes described in this report to t^{w73} complementation group supports the prediction raised by Klein, Sipos & Figueroa (1984) on the uniformity of the lethal factors in *t*-haplotypes of *M. musculus* populations. In their experiments all 5 *t*-haplotypes from 3 European localities of *M. musculus* belonged to the t^{w73} complementation group and two of them harboured also t^{0} lethal factor. Taken together with our data, 9 *t*-haplotypes have been reported from 7 different localities of *M. musculus* species, all of them belonging to t^{w73} complementation group. This contrasts with the situation in *M. domesticus*, where 10 complementation groups of lethal *t*-haplotypes have been recognized after a survey of 27 *t*-bearing chromosomes (Klein, Sipos & Figueroa, 1984).

(ii) *Class I genes of* H-2 *complex*

Serological study of class I genes was the first instance when products of a particular gene from various *t*-haplotypes were compared. Results of the comparison were remarkable; with only one exception, various *t*-haplotypes that shared a particular lethal factor also displayed the same *H-2* haplotype (Hammerberg & Klein, 1975; Klein *et al.* 1981). However, the rule holds only for North American wild mouse populations, since a similar analysis in wild mice from Europe resulted in a much more complex picture (Nizetič, Figueroa & Klein, 1984). Silver (1982) was the first to perform a similar comparison of *H-2* class I genes on genomic Southern blots. He observed considerable homology in patterns of restriction fragments of class I genes in all of the 5 examined *t*-haplotypes, and concluded that *t*-haplotypes originated from a small number of related ancestors. Later, more detailed analyses were reported from different laboratories using gene-specific probes for class I, II and III genes (Rogers & Willison, 1983; Golubič *et al.* 1984; Delarbre *et al.* 1985; Rogers, Lyon & Willison, 1985; Figueroa *et al.* 1985). Quite unexpectedly, the class I and class II genes were much more variable than single-copy genes in other parts of *t*-haplotypes. A possible explanation for this fact could be sought in specific evolution of genes belonging to multigene families, where gene conversion-like events and unequal homologous recombination contribute to their diversification (Lalanne *et al.* 1982; Weiss *et al.* 1983; Geliebter *et al.* 1986; Geliebter & Nathenson, 1987). In the present study, the *Eco*RI and *Bgl*II restriction sites of class I genes showed differences even between *M. musculus* haplotypes t^{p12} versus t^{p14}. However, for answering the question of the origin of *t*-haplotypes in *M. musculus* and *M. domesticus*, the pattern of restriction fragments created by *Bam*HI was more informative. The polymorphism of *Bam*HI sites among class I genes of ten different *H-2* haplotypes of wild-type (non-*t*) chromosomes on the one hand, and their uniform, identical pattern in eight 129-*t* congenic strains on the other hand, strongly support the hypothesis of a common ancestor of *t*-haplotypes presently found in *M. musculus* and *M. domesticus* species.

(iii) *Exclusive allelic forms of* Tcp-1, Hba-ps4 *and* D17Leh443 *loci*

In contrast to *H-2* class I and class II genes, single-copy or low-copy genes or microcloned random sequences showed little or no polymorphism in complete *t*-haplotypes examined so far (Fox *et al.* 1985; Herrmann *et al.* 1986). Moreover, these markers repeatedly displayed unique allelic forms not found outside the *t*-haplotypes. Thus Willison, Dudley & Potter (1986), after cloning and sequencing the *t-complex polypeptide-1* gene, confirmed and extended the previous finding of Silver, Artzt & Bennett (1982), namely that the *Tcp-1*^{a} allele is exclusively associated with *t*-haplotypes. We have detected here, in agreement with these observations, the same allelic form of the *Tcp-1* gene in *t*-haplotypes newly derived from *M. musculus* species.

A similar conclusion could be drawn from an

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analysis of the *Haemoglobin alpha-4 pseudogene* in 28 independent chromosomes 17 (Fox, Silver & Martin, 1984; D'Eustachio *et al.* 1984; this paper). The *Taq*I endonuclease could distinguish four different patterns of restriction fragments defining four different alleles. One of them, characterized by a 5·2 kb *Taq*I restriction fragment, was found in all of the *t*-haplotypes examined and only in them. Again, this observation points to a single ancestor of *t*-haplotypes in both species, *M. musculus* and *M. domesticus*.

D17Leh443 locus defined by the microcloned random sequence Tu443 displayed a single 8-kb *Msp*I restriction fragment in six different *t*-haplotypes of *M. domesticus* origin. The whole locus was deleted in three tested *t*-haplotypes from t^9 complementation group and displayed only one allele (15 kb *Msp*I fragment) in eight tested inbred strain +/+ at the *t*-complex (Bučan *et al.* 1987; this paper). However, our observation of a new allele with a 10·5-kb *Msp*I fragment in three *t*-haplotypes from *M. musculus*, together with the occurrence of the t^{w73} lethal factor in all *t*-haplotypes from *M. musculus* so far tested point towards their recent origin from a single ancestor. Thus, if *t*-haplotypes spread in *M. musculus* after introgressing from *M. domesticus* as suggested by Willison, Dudley & Potter (1986), then such introgression might have happened successfully only once. Continuing this line of reasoning, an introgression might also explain the single-ancestor origin of *M. domesticus* *t*-haplotypes. In this case a *t*-haplotype would enter *M. domesticus* from a now extinct parental ancestor species, bringing allelic forms that had not been transmitted to the new species in the course of speciation and that would therefore behave in the new environment as *t*-haplotype-specific.

Note added in proof.

Additional testing of the t^{p12} haplotype in the cross $T/t^{p12} \times T/t^0$ yielded 5 normal-tailed and 61 tailless offspring in 25 litters. Thus, by definition, the t^{p12} haplotype does not carry t^0 lethal factor and the phenomenon could be formally attributed to the incomplete complementation between t^0 and t^{p12} haplotypes.

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