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Polymorphisms distinguishing different mouse species and *t* haplotypes

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Summary

Three anonymous chromosome 17 DNA markers, *D17Tu36*, *D17Tu43*, and *D17Le66B*, differentiate between house mouse species and/or between *t* chromosomes. The *D17Tu36* probe, which maps near the *Fu* locus and to the *In(17)4* on *t* chromosomes, identifies at least 15 haplotypes, each haplotype characterized by a particular combination of DNA fragments obtained after digestion with the *Taq* I restriction endonuclease. Ten of these haplotypes occur in *Mus domesticus*, while the remaining five occur in *M. musculus*. In each of these two species, one haplotype is borne by *t* chromosomes while the other haplotypes are present on non-*t* chromosomes. The *D17Tu43* probe, which maps near the *D17Leh122* locus and to the *In(17)3* on *t* chromosomes, also identifies at least 15 haplotypes in *Taq* I DNA digests, of which nine occur in *M. domesticus* and six in *M. musculus*. One of the nine *M. domesticus* haplotypes is borne by *t* chromosomes, the other haplotypes are borne by non-*t* chromosomes; two of the six *M. musculus* haplotypes are borne by *t* chromosomes and the remaining four by non-*t* chromosomes. Some of the *D17Tu43* haplotypes are widely distributed in a given species, while others appear to be population-specific. Exceptions to species-specificity are found only in a few mice captured near the *M. domesticus–M. musculus* hybrid zone or in *t* chromosomes that appear to be of hybrid origin. The *D17Leh66B* probe, which maps to the *In(17)2*, distinguishes three haplotypes of *M. domesticus*-derived *t* chromosomes and one haplotype of *M. musculus*-derived *t* chromosomes. Because of these characteristics, the three markers are well suited for the study of mouse population genetics in general and of *t* chromosome population genetics in particular. A preliminary survey of wild *M. domesticus* and *M. musculus* populations has not uncovered any evidence of widespread introgression of genes from one species to the other; possible minor introgressions were found only in the vicinity of the hybrid zone. Typing of inbred strains has revealed the contribution of only *M. domesticus* DNA to the chromosome 17 of the laboratory mouse.

1. Introduction

Eight thousand to ten thousand years ago, the most westerly outpost of the house mouse (the *Mus musculus* complex) in Eurasia was the Fertile Crescent – a region of the Middle East curving across the northern section of the Syrian desert, watered by the Tigris and Euphrates rivers, and including parts of Jordan, Iraq, Israel, Syria, and Lebanon. Here, human tribes began to cultivate grasses which were to become the forerunners of modern cereals, legumes, and other plants, as well as to domesticate wild ancestors of the sheep, goat, pig, cattle, and other animals. They thus started the process of neolithic transition, marked by the development of agriculture (Rindos, 1984; Ammerman & Cavalli-Sforza, 1984). Archaeological evidence indicates that wild mice spreading from the steppes of present-day Pakistan associated with the early farmers in the Fertile Crescent, and then spread into Europe with the expansion of agriculture (Tchernov, 1968; Auffray, Vanlergerghe & Britton-Davidian, 1990). To explain the presence of two major house mouse (sub)species in Europe, *M. musculus* and *M. domesticus*, which are believed to have diverged 1–2 million years (myr) ago (Yonekawa *et al.* 1981; Ferris *et al.* 1983*a*; Sage, 1981), we have speculated

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Table 1. *The* D17Tu36 *haplotypes found in inbred, congenic, and* t *strains*

Haplotype	Strain
a	A/J, A.BY, ABP/Le, ACA/J, BALB/cJ, CE/J, DA/HuSn, DC/Le, DW/J, DBA/1, DBA/2, FS/E, NMRI, LG/J, LP/J, RIIIS/J, RF/J, SEA/GnJ, SEC/1ReJ, THF, YBR/E, 129/SvJ, B10.STA62, B10.STA12, B10.STC77, B10.WOA105, B10.KPA132, B10.LIB18, B10.SAA48, B10.CHR51, C3H.WOA1
b	AKR, AU/SsJ, BDP/J, BUB/BnJ, C3H/HeJ, CBA/J, C57BL/6, C57BL/10Sn, FL/1Re, MA/MyJ, NZW/LacJ, NZB/B1NJ, B10.GAA20, B10.GAA37, B10.KEA2, B10.KEA5, B10.KPA42, B10.KPA44, B10.LIB55, B10.MOL1, B10.SNA57, B10.SNA70, B10.STA10, B10.STA39, B10.STC90, B10.BUA1, B10.BUA16, B10.CAA2, B10.CAS1, B10.CHA2.
c	I/LnJ, WB/ReJ-W, WC/ReJ
t^d	$t^0, t^6, t^{12}, t^{w1}, t^{w2}, t^{w5}, t^{w12}, t^{w32}, t^{w71}, t^{lub1}$
t^m	$t^{w73}, t^{wN8}, t^{wN11}, t^{wN15}, t^{wN19}, t^{wN20}, t^{wN25}, t^{wN27}, t^{wN29}, t^{wN36}, t^{wnN37}, t^{wN38}, t^{wN39}, t^{wN40}, t^{wN47}$

Table 2. *Frequencies of* D17Tu36 *haplotypes among wild mice*

Population	N	Frequency of haplotype															
		a	b	c	d	e	f	g	h	i	o	p	q	r	t^d	t^m	u
Germany	32	0·15	0·36	0·08	0·06	0·00	0·22	0·00	0·02	0·00	0·00	0·00	0·00	0·00	0·09	0·03	0·00
England	39	0·40	0·33	0·13	0·00	0·00	0·00	0·00	0·08	0·00	0·00	0·00	0·00	0·00	0·06	0·00	0·00
France	19	0·14	0·24	0·27	0·00	0·03	0·14	0·05	0·08	0·00	0·00	0·00	0·00	0·00	0·05	0·00	0·03
Spain	13	0·08	0·27	0·12	0·04	0·08	0·08	0·27	0·00	0·00	0·00	0·00	0·00	0·00	0·04	0·00	0·02
Italy	8	0·13	0·47	0·00	0·13	0·07	0·00	0·00	0·13	0·00	0·00	0·00	0·00	0·00	0·07	0·00	0·06
Greece	5	0·10	0·20	0·30	0·00	0·10	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·10	0·00	0·20
Syria	5	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·50	0·00	0·00	0·00	0·00	0·00	0·00	0·50
Turkey	2	0·00	0·50	0·25	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·25
USA	11	0·18	0·50	0·05	0·00	0·05	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·14	0·00	0·09
Brazil	4	0·13	0·63	0·25	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Madagascar	6	0·00	0·00	0·00	0·00	0·75	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·08	0·00	0·17
Australia	2	0·25	0·50	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·25	0·00	0·00
Soviet Union	31	0·02	0·00	0·00	0·00	0·08	0·05	0·00	0·00	0·00	0·27	0·15	0·05	0·13	0·02	0·15	0·10
Czechoslovakia	20	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·75	0·08	0·00	0·03	0·00	0·15	0·00
Yugoslavia	11	0·23	0·05	0·18	0·00	0·05	0·05	0·09	0·00	0·00	0·18	0·09	0·00	0·09	0·00	0·00	0·00
Total	208	0·16	0·23	0·09	0·02	0·06	0·06	0·03	0·03	0·01	0·12	0·04	0·01	0·03	0·06	0·04	0·05

that a similar association with early farmers occurred in the second center of plant cultivation in North China (Klein, Tichy & Figueroa, 1987). We have suggested that the *M. domesticus* form occupying western Europe is derived from Near East ancestors, whereas *M. musculus*, which colonized eastern Europe, spread from North China. Where the two forms met, they established a narrow hybrid zone running from the north to the south of Europe (Zimmerman, 1950; Reichstein, 1978; Sage, Whitney & Wilson, 1986). A number of minor forms (subspecies), such as *M. castaneus* of southern Asia and *M. molossinus* of Korea and Japan, apparently diverged from these two main species in different regions of Eurasia (Sage, 1981; Yonekawa *et al.* 1986, 1988).

The different forms comprising the *M. musculus* complex were originally defined by morphological characters, primarily coat colour and tail length relative to body length (Schwarz & Schwarz, 1943;

Sage, 1981; Marshall, 1981). Subsequently, however, the repertoire of defining characters was extended to include protein variants detected by electrophoretical techniques (Selander, Hunt & Yang, 1969; Bonhomme *et al.* 1984; Sage, 1981; Miyashita *et al.* 1985; Watanabe *et al.* 1987), antigens detected by serological methods (Figueroa *et al.* 1986; Kurihara *et al.* 1985, 1988; Robinson *et al.* 1984), DNA markers detected by methods of molecular biology (Ferris *et al.* 1983*a*; Yonekawa *et al.* 1981, 1986, 1988; Tucker, Lee & Eicher, 1989; Nobuhara *et al.* 1989; Suzuki *et al.* 1986; Riblet & Tutter, 1989; Redi *et al.* 1990), and chromosomal C-banding (Moriwaki, Miyashita & Yonekawa, 1985). Most of the differences between mouse species were found, however, in frequencies of the various markers, which were adequate for classifying populations but not suitable for identifying individual mice. The only species-specific markers reported thus far are the Thy-1 antigens (the Thy-1.1

Table 3. *Frequencies of* D17Tu43 *haplotypes among wild mice*

Population	N	Frequency of haplotype															
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*o*	*p*	*q*	*r*	*td*	*tm1*	*tm2*	*u*
England	40	0·75	0·17	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·08	0·00	0·00	0·00
USA	11	0·72	0·14	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·14	0·00	0·00	0·00
France	12	0·79	0·08	0·00	0·00	0·00	0·00	0·00	0·05	0·00	0·00	0·00	0·00	0·08	0·00	0·00	0·00
Italy	7	0·36	0·14	0·43	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·07	0·00	0·00	0·00
Spain	13	0·73	0·08	0·11	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·08	0·00	0·00	0·00
Greece	6	0·92	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·08	0·00	0·00	0·00
Syria	5	0·80	0·10	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·10	0·00	0·00	0·00
Turkey	2	0·75	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·25	0·00	0·00
Brazil	7	0·71	0·00	0·15	0·00	0·00	0·14	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Madagascar	7	0·00	0·00	0·00	0·79	0·21	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Australia	4	0·75	0·00	0·00	0·00	0·00	0·00	0·25	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Germany	31	0·81	0·08	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·08	0·03	0·00	0·00
Soviet Union	32	0·08	0·00	0·00	0·00	0·00	0·00	0·00	0·03	0·36	0·09	0·16	0·09	0·02	0·08	0·06	0·03
Czechoslovakia	15	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·07	0·23	0·07	0·46	0·00	0·17	0·00	0·00
Yugoslavia	13	0·73	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·15	0·08	0·00	0·00	0·00	0·06
Total	205	0·55	0·07	0·02	0·03	0·01	0·01	0·01	0·01	0·05	0·07	0·03	0·04	0·06	0·03	0·01	0·01

and Thy-1.2 antigens being *M. musculus*-specific and *M. domesticus*-specific, respectively, although there are exceptions to this specificity; see Kurihara *et al.* 1985; Figueroa *et al.* 1986). Obviously, more than one species-specific marker is needed to identify mouse species unambiguously.

The two major house mouse species also differ in the presence of particular *t* haplotypes, but these differences could not, up until now, be used for easy species differentiation. The *t* haplotypes, which occur at frequencies as high as 40% in some populations (Figueroa *et al.* 1988), are characterized by four nonoverlapping inversions in the proximal part of chromosome 17 (Artzt, Shin & Bennett, 1982; Hermann *et al.* 1986; Sarvetnick *et al.* 1986; Hammer, Schimenti & Silver, 1989). The inversions suppress recombination in a region that in non-*t* chromosomes is approximately 20 cM long. All complete *t* haplotypes (i.e. those with all four inversions) carry *t*-complex lethal (*tcl*) or semi-lethal genes which in the homozygous state arrest embryonic development at a specific stage (for review, see Silver, 1985; Klein, 1986). Individual *tcl* genes can be distinguished by the genetic complementation test in which some embryos heterozygous for different *tcl* genes survive, whereas all embryos homozygous for non-complementing *tcl* genes die. The two main mouse species carry haplotypes with different *tcl* genes. The *t* haplotypes of *M. domesticus* can carry one of at least 12 different *tcl* genes, whereas *t* haplotypes of *M. musculus* carry the *tcl^{w73}* gene which has not been found in *M. domesticus* (Klein, Sipos & Figueroa, 1984). However, the identification of the *tcl-w73* gene requires a laborious breeding test which is not practical for a large scale population study. Interestingly, the *tcl* genes have, until recently, been the only genes that differentiate the complete *t* haplotypes. Although a number of

DNA markers have been described that differentiate *t* and non-*t* chromosomes (Röhme *et al.* 1984; Silver *et al.* 1987; Neufeld *et al.* 1991), all but one of these markers have shown no polymorphism among the *t* haplotypes (Silver *et al.* 1987; Neufeld *et al.* 1991; Uehara *et al.* 1990). An exception is the *t*-specific element (TSE) probe which hybridizes to tandem repeats present on *t* chromosomes but absent on the wild-type homologues (Uehara *et al.* 1990). The probe apparently distinguishes most independent *t* haplotypes. The inability to differentiate *t* haplotypes easily has been a major hindrance in analysing the *t* haplotypes in wild mouse populations. Here we describe DNA markers which appear to be species-specific and which differentiate *t* haplotypes of *M. musculus* and *M. domesticus*, thus opening up the possibility for large-scale population studies.

2. Materials and methods

(i) *Mice*

The A/J, A.BY, A.CA, AKR, BALB/c, C57BL/6, C57BL/10, CBA, C3H, DBA/1, DBA/2, NMRI, THF, 129/Sv strains, as well as the B10.W congenic strains were from our animal colony at the Max-Planck-Institut für Biologie, Tübingen. All other inbred strains (see Table 1) were purchased from The Jackson Laboratory, Bar Harbor, Maine. The origin of the wild *M. domesticus* and *M. musculus* mice is indicated in Tables 2 and 3. All other rodent species came from our collection.

(ii) *Probes*

The isolation of the D17Tu36 and D17Tu43 probes as well as their map position on the mouse chromosome 17 was described elsewhere (Vincek *et al.* 1989; Sertic

et al. 1991). The D17Tu36 probe is a 1·8 kilobase (kb) long *Pst* I fragment and the D17Tu43 probe is a 1·0 kb *Pst* I/*Kpn* I fragment; both were isolated from a λ phage genomic library (Vincek *et al.* 1989). Both probes were cloned in Bluescript vectors (Stratagene, Heidelberg, Germany). The isolation of the D17Leh66B probe was described previously by Schimenti *et al.* (1987). The probe is a *Bam*H I/*Eco*R I 800 base pair (bp) fragment derived from a cosmid clone mapping to the *B* subregion of the *D17Leh66* family of elements.

(iii) *Southern blotting and hybridization*

Genomic DNA was isolated as previously described (Figueroa *et al.* 1985). For digestions, 11 μg of DNA were incubated with the restriction enzyme *Taq* I under conditions recommended by the supplier (Pharmacia LKB, Freiburg, Germany), separated by electrophoresis in 0·8 % agarose, and blotted on nylon filters (Amersham, Frankfurt, Germany) using the vacuum blotting system (Pharmacia LKB). Random primer labelling of the probes as well as hybridizations were performed as described previously (Vincek *et al.* 1989).

3. Results

As part of an effort to find species-specific DNA markers, we digested the DNA of individual mice with the *Taq* I restriction endonuclease, blotted the digest on filters, and hybridized the filters successively with three labelled probes: D17Tu36, D17Tu43, and D17Leh66B. The position of these probes on the map of mouse chromosome 17 is given in Figure 1. The DNA was isolated from three types of mice – inbred (congenic) strains, noninbred mice bearing *t* haplotypes and maintained in the laboratory, as well as mice caught in the wild. The hybridizations produced different restriction enzyme patterns consisting of 1–12 bands (DNA fragments) of different sizes. An attempt was then made to determine which fragments were derived from single chromosomes and hence represented haplotypes. The determination was based on the following criteria: First, patterns produced by inbred strains were assumed to be controlled by single haplotypes. Second, haplotypes controlled by *t* chromosomes were deduced by typing of either *t/t* homozygotes (if such existed) or compound heterozygotes of the t^x/t^y types, where t^x and t^y were two complementing haplotypes. Third, haplotypes borne by wild mice were deduced by subtraction: If a mouse could be shown to carry a *t* haplotype (by typing with other *t*-specific probes such as D17Leh122), any fragments in addition to those controlled by the *t* haplotype were assumed to be controlled by the second chromosome (haplotype); in non-*t* mice, patterns that could be explained by one or a combination of two previously identified haplotypes were assumed to be controlled by such haplotypes.
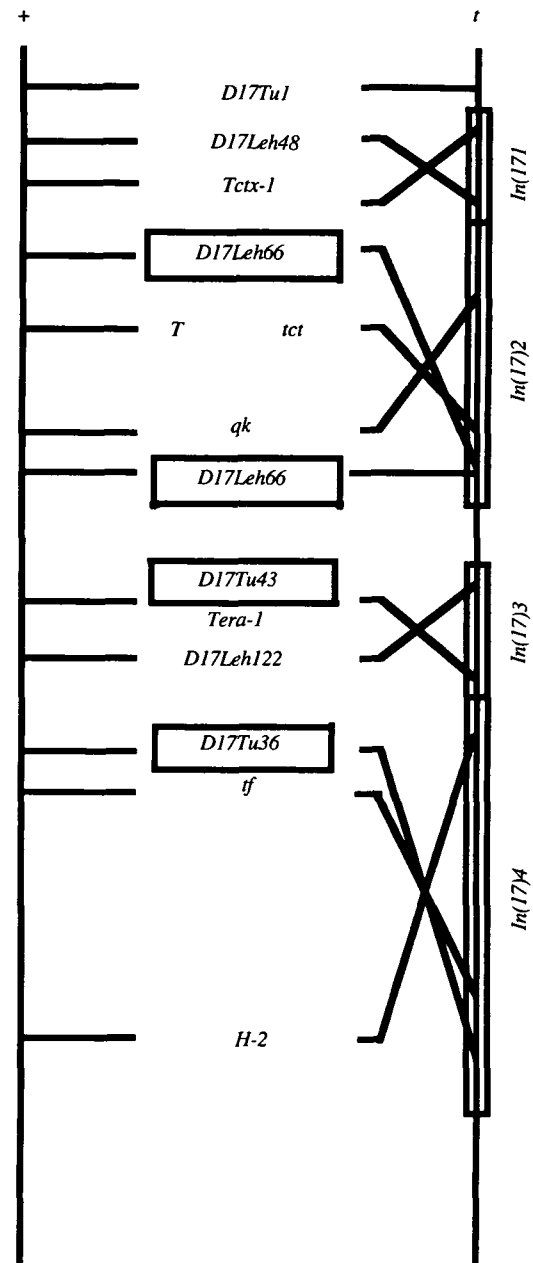

Fig. 1. Simplified map of the proximal part of the mouse chromosome 17 indicating the positions of the *D17Tu36*, *D17Tu43*, and *D17Leh66B* markers (in rectangles) in relation to other (anchor) markers. Both the *t* and non-*t* (+) versions of the chromosome are shown. The rectangles on the *t* chromosome indicate the extent of the four inversions, *In(17)1* through *In(17)4*.

The deduced haplotypes were designated by small letters, separately for each of the three tested markers. Frequencies of the individual haplotypes were then calculated based on these deductions.

(i) *The* D17Tu36 *haplotypes*

Typing of inbred and congenic strains revealed the presence of three *D17Tu36* haplotypes, two common ones (*a* and *b*), and a third (*c*) limited to the I, WB, and WC strains (Fig. 2, Table 1). The *c* haplotype seems to be a variant of the *b* haplotype from which it may have been derived by a slight shift in the size of
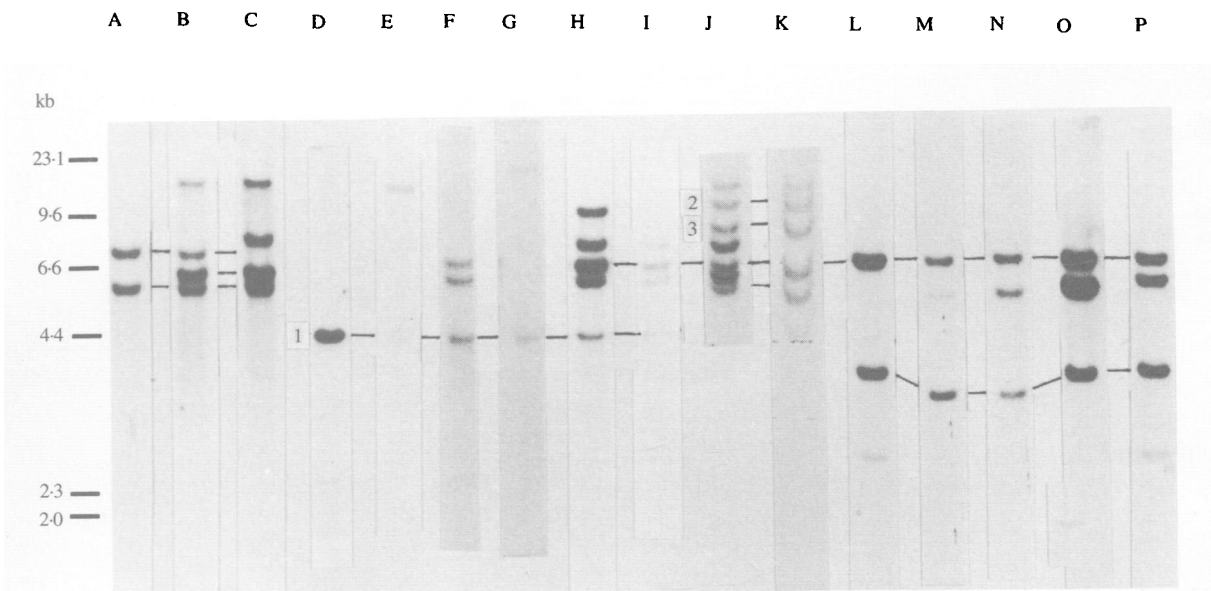
Fig. 2. Southern blots of *Taq* I-digested DNA hybridized with the D17Tu36 probe. Patterns representing the following haplotypes are shown: lane A, *a/a*; B, *b/b*; C, *c/c*; D, *td/td*; E, *d/td*; F, *e/td*; G, *f/td*; H, *g/td*; I, *h/td*; J, *a/tm*; K, *td/tm*; L, *o/o*; M, *o/p*; N, *p/p*; O, *q/q*; and P, *r/r*. *t*-specific bands are shown in lanes D–K. Bands 1 and 2/3 are *M. domesticus*- and *M. musculus*-specific, respectively.

the 7·5 kb fragment. The *t* chromosomes of mice maintained in the laboratory were associated with two *D17Tu36* haplotypes, *td* and *tm* (Fig. 2, Table 1). All *t* chromosomes extracted from *M. domesticus* wild mice carry the *td* haplotype, characterized by a single *t*-specific 4·4 kb fragment; all *t* chromosomes extracted from *M. musculus* wild mice carry the *tm* haplotype characterized by five fragments, two of which (10 and 8·5 kb) are *t*-specific. All the *tm*-bearing chromosomes carry the *tcl-w73* gene, but some of them also carry a second *tcl* gene. Similar results were also obtained by typing wild mice from different populations (Fig. 2, Table 2). All *M. domesticus* and *M. musculus* mice, in which the presence of *t* chromosomes was indicated by the D17Leh122 probe, could be shown to carry the *td* and *tm D17Tu36* haplotypes, respectively. The only exceptions to this rule were mice captured near *M. domesticus* and *M. musculus* hybrid zones in Germany (one mouse from the vicinity of Munich) and the Soviet Union (one mouse from the vicinity of the Black Sea). The German mouse was from the *M. domesticus* side of the zone but had the *tm* haplotype, whereas the mouse from the Soviet Union was from the *M. musculus* side of the zone but had the *td* haplotype. All populations from which adequate numbers of mice were tested carried *t* chromosomes; the overall *t*-chromosome frequency was 0·1. Wild mice not carrying *t* chromosomes and the non-*t* chromosomes from *t*-heterozygous wild mice typed differently depending on the species. The *M. domesticus* mice carried the *a*, *b*, and *c D17Tu36* haplotypes of the inbred strains, as well as six other additional haplotypes (*d–i*; Fig. 2, Table 2). The *a*, *b*, *c*, and *td* haplotypes were relatively common, comprising approximately 70% of all the haplotypes; the *d–i*

haplotypes were less common and some of them might be population-specific (e.g. haplotype *i* in Syrian mice). The *M. musculus* mice carried *D17Tu36* haplotypes *o*, *p*, *q*, and *r*, which were absent in *M. domesticus* populations. A few mice from the Soviet Union captured in areas near the *M. domesticus*–*M. musculus* hybrid zone carried the *D17Tu36 a*, *e*, or *f* haplotypes. Ten of the 208 tested mice produced complex patterns that appeared to be individual-specific. They were designated collectively as carrying the *u* haplotypes in Table 2. Of other *Mus* species and other members of the family Muridae, only *M. castaneus* and *M. molossinus* provided DNA that hybridized with the D17Tu36 probe. The hybridization patterns, however, were different from those found in *M. domesticus* and *M. musculus* (data not shown). No hybridization could be detected with DNA from the following rodent species: *M. spretus*, *M. matthey*, *M. pahari*, *Acomys* sp., *Apodemus flavicolis*, *Micromys minutus*, *Arvicanthis niloticus*, *Lemniscomys barbarus*, *Praomys* sp., *Rattus norvegicus*, *Clethriomys rutilus*, *Microtus arvalis*, *Mesocricetus auratus*, *Sigmodon* sp., *Gerbillus gerbillus*, *Tatera* sp. and *Pachyuromys duprasi*. We conclude therefore that the *D17Tu36* region displays a species-specific polymorphism in both *t* and non-*t* chromosomes of wild house mice and that it was either lost or diverged beyond recognition in other rodents.

(ii) *The* D17Tu43 *haplotypes*

All the inbred and congenic strains typed (i.e. those listed in Table 1) had the same *D17Tu43* haplotype designated as *a* (Fig. 3). The *t* chromosomes extracted from wild mice and maintained in the laboratory were
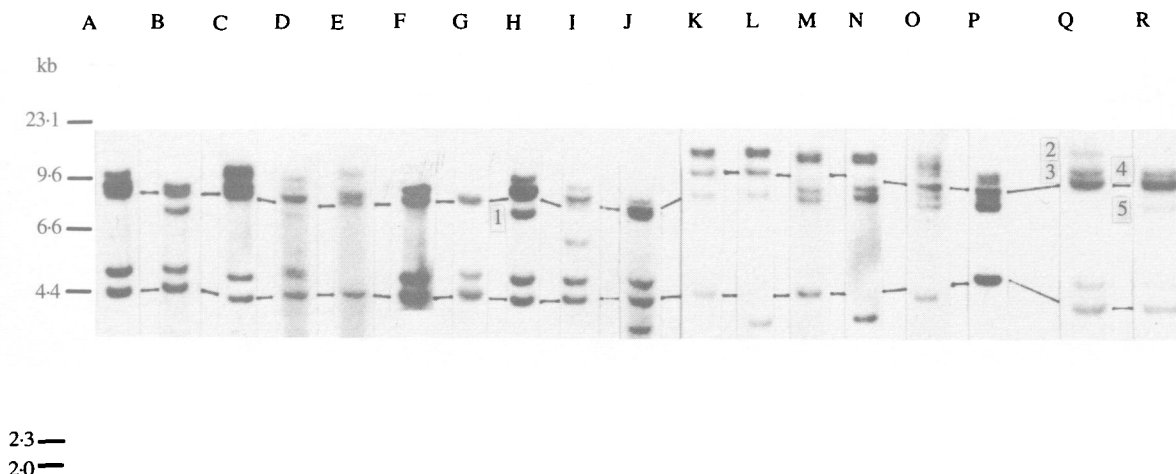
Fig. 3. Southern blots of *Taq* I-digested DNA hybridized with the D17Tu43 probe. Patterns representing the following haplotypes are shown: lane A, *a/a*; B, *b/b*; C, *c/c*; D, *d/d*; E, *e/e*; F, *f/f*; G, *g/g*; H, *td/td*; I, *h/h*; J, *u/a*; K, *o/o*; L, *p/p*; M, *q/q*; N, *r/r*; O, *tm1/q*; P, *tm2/q*; Q, *tm1/a*; R, *tm2/a*. *t*-specific bands are shown in lanes H, Q, and R. Band 1 is *M. domesticus t*-specific; bands 2, 3, 4, and 5 and *M. musculus*-specific.

associated with *D17Tu43* haplotypes *td*, *tm1*, and *tm2*. With one exception, all *t* chromosomes extracted from *M. domesticus* wild mice had the *td* haplotype, characterized by five restriction fragments, one of which (8·0 kb in size) seemed to be *t*-specific (Fig. 3). The exception was the t^{Tuw25} chromosome of the OBL984 strain, which was associated with the *tm1* haplotype. The *t* chromosomes extracted from *M. musculus* wild mice were associated either with *tm1* or *tm2* haplotypes. The *tm1* haplotype was characterized by four restriction fragments, one of which was *tm1*-specific (15 kb in size) and the other three (9·5, 9·4, and 4·4 kb in size) were shared with the *tm2* haplotype. The *tm2* haplotype was associated with the t^{wN} chromosomes *11*, *15*, *20*, *25*, *27*, *29*, *36*, *37*, *38*, *39*, and *40*; the *tm1* haplotype was associated with all the remaining *M. musculus*-derived *t* chromosomes.

The same wild mice typed with the D17Tu36 probe were also typed with the D17Tu43 probe. The *t*-bearing *M. domesticus* mice carried the *D17Tu43 td* haplotype; only one mouse from Germany (the same one that typed as *tm* with the D17Tu36 probe) typed as *tm1*. The non-*t* chromosomes of the *M. domesticus* wild mice were associated with eight *D17Tu43* haplotypes (Fig. 3; Table 2), of which the *a* haplotype was most common, followed by the *b* and *c* haplotypes. The remaining haplotypes seemed to be population-specific with *d* and *e* found in the Madagascar population, *f* in Brazilian mice, *g* in the Australian sample, and *h* in the French sample. The *a* and *h* haplotypes were found also in a few mice from the *M. musculus* side of the hybrid zone; all other haplotypes were detected only in regions occupied by *M. domesticus*.

The *t*-bearing *M. musculus* mice had either the *tm2* haplotype (mice from certain regions of the Soviet Union) or the *tm1* haplotype (all other mice). The *tm1* haplotype was also found in a few mice from Turkey and from Germany near the *M. domesticus–M. musculus* hybrid zone (Table 3). The non-*t* chromo-

somes of pure *M. musculus* mice had none of the *D17Tu43* haplotypes found in the *M. domesticus* mice; instead, they were associated with four new haplotypes *o*, *p*, *q*, and *r* (Fig. 3, Table 3). Of these, the *o* and *r* haplotypes were common in mice from the Soviet Union and Czechoslovakia, respectively. We conclude therefore that like the *D17Tu36* marker, the *D17Tu43* marker differentiates *M. domesticus* and *M. musculus t* as well as non-*t* chromosomes. Hybridization with the D17Tu43 probe was also obtained with *M. castaneus*, *M. molossinus*, *M. spretus*, *M. hortulanus*, and *M. abbotti*, but as with the D17Tu36 probe, the hybridization patterns were distinct from those found in *M. domesticus* or *M. musculus* (data not shown). None of the other rodent species tested hybridized with the probe.

(iii) *The* D17Leh66B *haplotypes*

Six *D17Leh66B* haplotypes were described by Vincek and associates (1990) among inbred and congenic strains. We found four additional haplotypes associated with the *t* chromosomes maintained in the laboratory (Fig. 4). Three of the four haplotypes (*g*, *i*, and *j*) were associated with *t* haplotypes extracted from *M. domesticus* mice; the fourth haplotype (*k*) was associated with *t* haplotypes extracted from *M. musculus* mice. The *g* haplotype was characterized by four restriction fragments (7·0, 4·4, 4·0, and 2·5 kb) of which one (the 7·0 kb fragment) seemed to be *t*-specific. It was associated with the t^6, t^{12}, t^{w32}, and t^{Tuw25} haplotypes. The *j* haplotype was identical with the *g* haplotype except that the 7·0 kb restriction fragment was replaced by a 6·9 kb fragment; it was associated with the t^{w2} and t^{Tuw10} haplotypes. The distinctiveness of the 7·0 and 6·9 kb fragments was confirmed by typing *g/j* heterozygotes in which these two hybridizing fragments could be demonstrated. The *i* haplotype differed from the *g* haplotype by the presence of one extra fragment 1·7 kb in size; it was
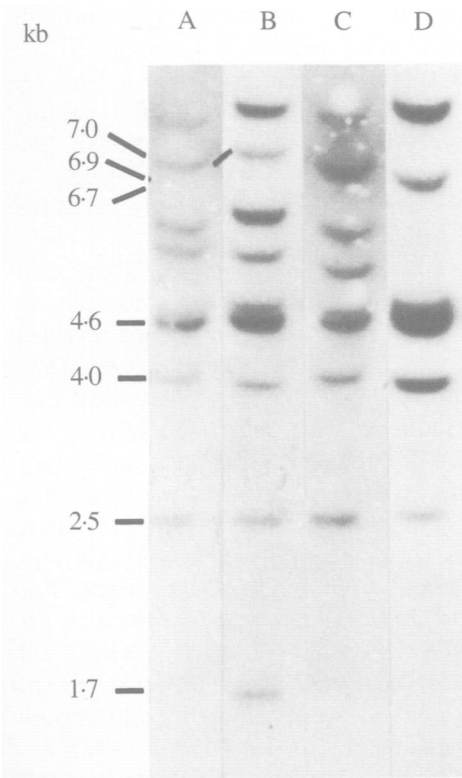
Fig. 4. Southern blots of *Taq* I-digested DNA hybridized with the D17Leh66B probe. Patterns representing the following haplotypes are shown: Lane A, *g/a*; B, *i/a*; C, *j/a*; D, *k/c*.

associated with the remaining *M. domesticus t* haplotypes. The *k* haplotype differed from the *g* haplotype in that it had a 6·7 kb instead of the 7·0 kb fragment (the distinctiveness of the two fragments was again demonstrated by typing of heterozygotes); it was associated with *M. musculus*-derived chromosomes t^{w73}, t^{Tuw6}, t^{Tuw20}, and t^{Tuw22}. Typing of a small panel of wild mice revealed the presence of the *i* and *k* haplotypes in *M. domesticus* (20 mice) and *M. musculus* (20 mice), respectively. The D17Leh66B probe thus differentiates between the *t* chromosomes of the two species and furthermore detects polymorphism among the *M. domesticus t* chromosomes.

4. Discussion

We used three markers, *D17Tu36*, *D17Tu43*, and *D17Leh66B* to identify at the DNA level chromosome 17 in different mouse populations. The D17Leh66B probe is known to detect three sets of basic elements in the different chromosomes. By using these three markers, it is now possible, first, to distinguish DNA of *M. domesticus* and *M. musculus* mice; second, to identify mice carrying *t* chromosomes; third, to differentiate between *M. domesticus* and *M. musculus t* chromosomes; fourth, to begin studying *t* chromosome polymorphism at the DNA level; and fifth, to differentiate populations of wild mice occupying different regions in the territories of *M. domesticus* and *M. musculus*. The markers will therefore be suitable

for determining the degree of introgression of one mouse species across the hybrid zone into the territories occupied predominantly by another species. This is particularly important in the Scandinavian countries, where there seems to be a great deal of confusion with regard to the genetic composition of their mouse populations (Vanlerberghe *et al.* 1988; Ferris *et al.* 1983*b*; Gyllensten & Wilson, 1987). The markers will further be useful for studying the geography and the width of the hybrid zone, migrations of mice in conjunction with human migrations, the origin of *t* chromosomes, and of other, related problems in mouse distribution and history. The data generated in the present study pertain to at least some of these problems. Thus with one possible exception, we have found no evidence of introgression of *M. musculus* markers deep into *M. domesticus* populations, or vice versa. This result is at odds with the claims of other investigators who believe they have uncovered signs of frequent gene exchange between *M. domesticus* and *M. musculus* populations. Thus, Ferris *et al.* (1983*b*) found that mice from Denmark and Sweden were of the *musculus* type on the basis of nuclear markers but of the *domesticus* type on the basis of mitochondrial (mt)DNA. The intragression of mtDNA seemed to extend into Sweden at least 750 km beyond the Danish hybrid zone (Gyllensten & Wilson, 1987). However, the presence of *domesticus* mtDNA into *musculus* appears to be the consequence of a founding event rather than a persistent and frequent mtDNA flow (Vanlerberghe *et al.* 1988).

The one exception to the general lack of introgression in the populations we studied is the t^{Tuw25} chromosome of the OBL984 strain. The chromosome was extracted in 1980 from a wild mouse captured at Oberer Lindenhof, a village near Tübingen in Germany, in an area occupied by *M. domesticus*. Several other *t* chromosomes (t^{Tuw2}, t^{Tuw21}, t^{Tuw24}, t^{Tuw27}, t^{Tuw28}) were extracted from the same area. While all these other *t*s behaved as typical *M. domesticus* chromosomes, the t^{Tuw25} chromosome was associated with the *M. domesticus D17Tu36* haplotype, but with the *M. musculus D17Tu43* haplotype. Interestingly, the t^{Tuw2} chromosome was shown to carry two *tcl* genes (tcl^{Tuw2} and tcl^{Tuw25}), a situation only encountered in a few other *t* chromosomes (Artzt, Babiarz & Bennett, 1979; Klein, Sipos & Figueroa, 1984; Ruvinsky *et al.* 1991), and generally assumed to be the result of recombination between two complementing *t* chromosomes. The t^{Tuw25} chromosome carries the *tcl*^{Tuw2} gene only. It is therefore possible that the presence of *M. domesticus D17Tu36* and *M. musculus D17Tu43* haplotypes on the t^{Tuw25} chromosome is the result of recombination between chromosomes derived from the two species, although it is not immediately obvious how and where such recombination occurred. Our breeding records show that the OLB984 strain was never crossed with any *M. musculus* mice.

Recombination might also have been involved in the derivation of the *D17Tu43 tm2* haplotype. As mentioned earlier, this haplotype lacks one fragment (15 kb) which is specific for *t* chromosomes derived from *M. musculus* (the *tm1* haplotype) but has another fragment (8·0 kb) which is otherwise present in *t* chromosomes derived from *M. domesticus* (the *td* haplotype). Other fragments are shared by *M. domesticus* and *M. musculus t* and non-*t* chromosomes. The *tm2* haplotype was found in t^{wN8} and t^{wNx} chromosomes extracted from wild mice captured in the Altai region (Siberia) of the Soviet Union; in the t^{wN19} chromosome extracted from a wild mouse captured in the Caucasus (Ruvinsky *et al.* 1991); in the t^{Tuw20} chromosome derived from an Astrachan wild mouse (Klein, Sipos & Figueroa, 1984); and in three wild mice from Siberia. Some of the t^{wN} chromosomes were shown previously to carry two *tcl* genes, one of them being the tcl^{w73}, which is present only in *M. musculus* mice, and the other a *tcl* gene otherwise characteristic of *M. domesticus*-derived *t* chromosomes (Ruvinsky *et al.* 1991). Recombination between two complementing *t* chromosomes, one derived from *M. domesticus* and the other from *M. musculus*, might have produced chromosomes with two *tcl* genes and the *D17Tu43 tm2* haplotype. *M. domesticus* admixture in the wild mice from Siberia is also indicated by serological typing for antigens controlled by the *H-2* complex, the major histocompatibility complex of the mouse (Ruvinsky *et al.* 1991). The admixture might be associated with the transsection of this region by the Trans-Siberian Magistral. Frequent recombinations between complementing *t* chromosomes have been documented in the laboratory (Silver & Artzt, 1981).

As far as non-*t* chromosomes of wild mice are concerned, distinct polymorphisms were detected in the different species at two of the three tested chromosome 17 regions, at *D17Tu36* and *D17Tu43*. At each of these two regions there were sets of haplotypes found only in *M. domesticus* and other sets found only in *M. musculus*. The only exceptions to this rule were mice captured near the hybrid zone, which in Europe transects the Jutland peninsula and then runs southward, passing near the cities of Dessau, Nürnberg, Regensburg, München, and Salzburg. From Austria it continues into Yugoslavia (where the coastal regions are colonized by *M. domesticus* and the inland by *M. musculus*) and then bends eastward toward the Black Sea. In the European part of the Soviet Union it runs across the Republics of Georgia and Azerbaijan (Zimmermann, 1950; Sage *et al.* 1986; Bonhomme, 1986; E. Kotenkova, personal communication). The exceptional mice were from the following regions: One mouse from Gessertshausen near Munich; one from Turkey (these mice had *M. musculus D17Tu36* and *D17Tu43* haplotypes although they were from the *M. domesticus* regions; the presence of *M. musculus* haplotypes in Turkey might be an indication that the hybrid zone actually runs through that country); and nine mice from Caucasus in the vicinity of the Black Sea (all these mice had *M. domesticus D17Tu36* and *D17Tu43* haplotypes although they were from *M. musculus* regions). The exceptional mice could be either *M. domesticus* × *M. musculus* hybrids or pure species that have entered the territory of the other species.

The tested inbred strains had *D17Tu36* and *D17Tu43* polymorphisms otherwise found only in *M. domesticus*; testing for these two regions revealed no admixture of *M. musculus*. The three *D17Tu36* haplotypes found in the inbred strains were those that are also most frequent in *M. domesticus* wild mouse populations. The distribution of the three haplotypes among the inbred strains indicates that their founding populations were apparently heterozygous for these three haplotypes since the haplotypes appear in strains which are presumably of independent origin. For example, the *D17Tu36 a* haplotype is present in the YBR/J, LP/J, A/J, RF/J, CE/J, SEA/GnJ, and DA/He strains, which belong to different inbred strain families derived from different wild mouse stocks (Potter & Liebermann, 1967; Klein, 1975).

Approximately one-half of the B10.W strains (*H-2* congenic strains derived from wild mice and the C57BL/10Sn inbred strain; see Klein, 1972; Zaleska-Rutczynska & Klein, 1977) had the *D17Tu36* haplotype of the C57BL/10Sn strain, while the other half had haplotypes of the wild mice donors. In the latter, the ten or more generations of backcrossing to the C57BL/10Sn strain were obviously not sufficient to replace long *H-2*-flanking, wild-derived segments by segments derived from the inbred strain. This conclusion is also supported by the results of typing for other chromosome 17 markers (Vincek *et al.* 1990).

The D17Leh66B probe is known to detect a family of genetic elements on chromosome 17 (Schimenti *et al.* 1987; Rosen, Bullard & Schimenti, 1990) and the haplotype variation described in the present study may reflect differences in the number of these elements. The organization of the regions revealed by the D17Tu36 and D17Tu43 probes is not yet known, but here, too, different haplotypes may arise from variation in the number of basic elements in the different chromosomes.

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