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The relationship between *t* and *H-2* complexes in wild mice

I. The *H-2* haplotypes of 20 *t*-bearing strains

BY SABINE STURM, FELIPE FIGUEROA AND JAN KLEIN

Max-Planck-Institut für Biologie, Abteilung Immungenetik, Corrensstr. 42, D-7400 Tübingen, F.R.G.

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SUMMARY

Twenty *t* haplotypes were extracted from wild mice captured at several locations in Europe, Israel, North Africa, and South America. The haplotypes were designated t^{Tuw1} through t^{Tuw20}. The *H-2* haplotypes of the lines were defined using antisera and monoclonal antibodies specific for private antigenic determinants controlled by known *H-2* alleles and by antisera produced using the new *t* lines as donors. The t^{Tuw} haplotypes fall into four groups according to the *H-2* haplotype associated with them. Haplotypes t^{Tuw10} through t^{Tuw18} are associated with $H\text{-}2^{w30}$, previously found to be linked with haplotypes of the t^{w1} group. Haplotypes t^{Tuw1} through t^{Tuw6} are associated with a new *H-2* haplotype, $H\text{-}2^{w36}$, characterized by the determinant H-2.107. Haplotypes t^{Tuw7} through t^{Tuw9} are associated with another new *H-2* haplotype, $H\text{-}2^{w37}$, characterized by determinants H-2.108 and H-2.111. And finally, haplotypes t^{Tuw19} and t^{Tuw20} are associated with yet another new *H-2* haplotype, $H\text{-}2^{w38}$, characterized by determinants H-2.33 and H-2.109. These findings suggest that the *t* polymorphism might be more extensive and more intricate than it was previously thought to be and that at least some of the *t*-associated *H-2* haplotypes, and probably also the *t* haplotypes themselves, are related to one another in their origin.

1. INTRODUCTION

The *t*-complex is a family of loci affecting embryonic differentiation, sperm differentiation, segregation of chromosomes in spermatozoa, and frequency of genetic recombination (reviewed by Bennett, 1975; Klein & Hammerberg, 1977; Lyon, 1981). The *H-2* complex is another family of loci, the products of which restrict the specificity of thymus-derived lymphocytes (T cells): a given T cell recognizes not only the foreign antigen via its receptors but also the *H-2* molecules of the antigen-presenting cell (for a review, see Klein *et al.* 1981). Both complexes are located on the same chromosome (No. 17) and both are polymorphic, occurring among wild mice as a series of genetically different haplotypes.

Individual *t* haplotypes are discerned on the basis of, first, the survival of *t*/*t* homozygotes and, second, a genetic complementation test. By the first criterion

one distinguishes lethal, semilethal and viable *t* haplotypes; by the second one then divides the lethal haplotypes into complementation groups: the survival of t^x/t^y heterozygotes, where t^x and t^y are two independently derived *t* haplotypes, is interpreted as indicating that the t^x and t^y haplotypes are distinct; failure of the t^x/t^y embryos to survive is taken to mean that the t^x and t^y haplotypes belong to the same complementation group.

The assessment of the *H-2* polymorphism is based on serological analysis of the *H-2* molecules: these molecules themselves act as antigens when injected into a mouse carrying a different set of *H-2* molecules from that of the donor and stimulate the immune system of such a mouse to produce H-2-specific antibodies (reviewed by Klein & Figueroa, 1981). The antigenic determinants defined by the H-2 antibodies are referred to by numbers (i.e. H-2.1, H-2.2, H-2.3, etc.). Most of these determinants are shared by molecules controlled by different *H-2* alleles (we shall refer to these molecules as allomorphs) but some are restricted to a given allomorph or to a group of closely related allomorphs. The shared determinants are referred to as 'public' and the restricted ones as 'private'. By typing wild mice with reagents containing antibodies specific for the individual H-2 allomorphs one can study the frequencies and distribution of *H-2* alleles and haplotypes (the latter being particular combinations of alleles at individual *H-2* loci on a single chromosome). Such a study has revealed that, among wild mice, more than a hundred different alleles may exist at some of the *H*-2 loci (Klein & Figueroa, 1981).

The H-2 typing of laboratory stocks carrying *t* haplotypes has demonstrated strong correlation between the *t* and *H-2* polymorphism: with few exceptions, a given *t* haplotype was found to be associated with a particular *H-2* haplotype irrespective of the origin of the wild mouse from which the *t* haplotype was extracted (Hammerberg & Klein, 1975). This finding suggested the existence of strong linkage disequilibrium between the two complexes in wild mouse populations – most likely as a result of suppression of crossing over by *t* genes in the centromeric region of chromosome 17. To study this postulated disequilibrium, we extracted *t* haplotypes from some 36 wild mice captured in different parts of the world. In this communication, we describe the *H-2* analysis of 20 of these new *t* lines; the results of the genetic analysis of the *t* haplotypes will be described in a later communication.

2. MATERIALS AND METHODS

(i) *Mice*

Wild mice of the species *Mus musculus L.* were trapped at the localities indicated in Table 1. Mice trapped outside of Germany were supplied to us by the following people: mice from the Orkney Island of Eday by Profs. R. J. Berry and M. Newton, Department of Genetics and Biometry, University College London, England; from La Roca and Moya in the vicinity of Barcelona, Spain, by Dr J. Vives, Hospital Clinica y Provincial, Barcelona, Spain; from Moscow, U.S.S.R., by Dr I. Egorov, Duke University Medical Center, Durham, North Carolina; from Israel by Dr E.

Nevo, University of Haifa, Haifa, Israel; from Nahya, Egypt, by Dr Harry Hoogstraal, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; from the area around Tübingen, FRG, by Dr J. H. Nadeau and S. Adolph: and from the area around Brno, Czechoslovakia, by Dr H. Winking, Klinikum der Medizinischen Hochschule Lübeck, Abteilung für Pathologie, Lübeck. All inbred lines used in this study came from our animal colony at the Max-Planck-Institute for Biology.

Table 1. *List of* t-*bearing lines*

Line	*t* Haplotype	Origin of wild *t*-mouse
BNK265	*Tuw1*	Wendelsheim (near Tübingen), Germany
BNK266	*Tuw2*	
BNK280	*Tuw3*	
BNK756	*Tuw4*	
BNK761	-*	
BRU337	*Tuw5*	Brno, Czechoslovakia
BRU382	*Tuw6*	
CRO435	*Tuw7*	Cairo, Egypt
CRO437	*Tuw8*	
CRO447	*Tuw9*	
EDY589	*Tuw10*	Eday, Orkney Islands
GPC881	—	Buin, Chile
GPC882	*Tuw11*	
GRL12	—	Greece
ISL15	—	Haifa, Israel
ISL16	—	
ISL18	*Tuw16*	
ISL20	*Tuw17*	
ISL26	—	
ISL33	—	
ISL37	*Tuw18*	
LRA410	*Tuw12*	La Roca, Spain
LRA414	*Tuw13*	
MOY331	*Tuw14*	Moya, Spain
MOY336	*Tuw15*	
MSW250	*Tuw19*	Moscow, Soviet Union
MSW251	*Tuw20*	

* not assigned.

(ii) *Antisera*

The strain combinations used for the production of antisera and the antigenic determinants defined by these antisera are given in Table 2. The K and T (E) series of antisera were produced at the University of Texas Southwestern Medical School, Dallas, Texas, and the Max-Planck-Institute for Biology, Tübingen, respectively. The antisera were produced by giving four weekly injections of spleen, thymus and lymph-node cells, with 1 donor per 25 recipients. The cell suspensions were made

Table 2. *H-2 alloantisera detecting private determinants used for the typing of wild mice*

Code designation	Recipient	Donor	*H-2*-Haplotype combination: (recipient) donor	Absorbed by cells of *H-2* haplotype	Antigenic determinant detected
T117PI	(A.TL × DBA/2)F_1	A.TFR1	(*t1/d*)an*1*	*k*	9
T26	[A.CA × B10.A(2R)]F_1	B10.WB	(*f/h2*)*j*	*d, p, q, w13, w15*	15
K13PPI	(B10.A × A.SW)F_1	B10.P	(*a/s*)*p*	*d, f, j, q, r, w1, w13, w14*	16
T30PI	(A.AL × DBA/2)F_1	A.TL	(*a1/d*)*t1*	*v, w26*	19
T21	(A × B10)F_1	B10.NZW	(*a/b*)*z*	*w13, w14, w24*	20
T76	(DBA/2 × A.TL)F_1	A.AL	(*d/t1*)*a1*	*p, q, r, u, w1, w3, w23*	23
T37	(DBA/2 × B10.A)F_1	B10.M(11R)	(*d/a*)*ap1*	*p, w13*	26
K355	(B10.A × LP.RIII)F_1	B10.AKM	(*a/r*)*m*	*d, j, w1*	30
K548PIII	(A × B10.A)F_1	B10.D2	(*a*)*d*	*b, w15*	31
T28PI	(DBA/2 × B10.A)F_1	B10.OH	(*d/a*)*o2*	*w13*	32
K333PI	(B10.D2 × A)F_1	B10.A(5R)	(*d/a*)*i5*	*f, j, p, q, r, u, v, w7*	33
T29	(A × B10.CAS2)F_1	B10.SAA48	(*a/w17*)*w3*	*d, w18, w24, w27*	103
K561	(B10 × A)F_1	T/t^{12}	(*b/a*)*w28*	*f, s, w22, w29*	106
K560	(B10 × A)F_1	T/t^{w2}	(*b/a*)*w29*	*d, j, v, w13*	107
K559	(B10 × A)F_1	T/t^{w1}	(*b/a*)*w30*	*f, v, q, w27*	108, 126
T184	(A × B10)F_1	(T/t^{w5} × Rb7d)F_1	(*a/b*)*w31/d*	—	109
E6	(A × B10)F_1	T/t^{w5}	(*a/b*)*w31*	*q, r, w1, w4, w9, w16, w19*	109
T159	(B10.A × B10.PL)F_1	B10.NZW	(*a/u*)*z*	—	114
T48	(A × B10.CHR51)F_1	B10.STC90	(*a/w18*)*w15*	*u, w7, w16*	115
T167	(B10.A × B10.SAA48)F_1	B10.BUA16	(*a/w3*)*w22*	*k, w7, w24*	116
T174	(B10.S × A)F_1	B10.GAA37	(*s/a*)*w21*	—	117, 130, 139
T154	(B10 × A)F_1	(B10.A × $T/t^{Lüb1}$)F_1	(*b/a*)*a/w33*	*f, p, r, s, w16, w15, w27*	126?, 117
T152PI	(A × B10.BUA16)F_1	B10.BUA1	(*a/w22*)*w16*	*f, q, w27*	130
S1	C3H.OH	ISL37	(*o2*)*w30*	*b, f, k, p, q, r, s, u, v, w27*	117?
S2	DBR-7	LRA414	(*g*)*w30*	*d, f, k, p, q, r, s, u, v, w27*	117?
S7	C3H.OH	MOY336	(*o2*)*w30*	*b, d, f, k, p, q, r, s, u, v, w3, w15, w16, w21, w27*	117?
T147	(B10.M × B10.P)F_1	B10.CAA2	(*f/p*)*w11*	*d, w15*	131
T166	(B10.A × B10.BR)F_1	B10.WR7	(*a/r*)*wr7*	*s*	132
T131	(B10.G × A.TL)F_1	B10.CHA2	(*q/t1*)*w26*	*w8*	137
T149	(A × B10)F_1	B10.T7WF	(*a/b*)*w6*	*d, q, r, s, u*	143
T14	(A × B10)F_1	B10.CAS1	(*a/b*)*w23*	*f, r, s, u*	144
T169	(BALB/c × B10.P)F_1	B10.CHR51	(*d/p*)*w18*	*w14, w26*	147
S8	A.AL	CRO435	(*a1*)*w37*	*f, p, r, s, u, w17, w26*	?

in phosphate-buffered saline (PBS). One week after the last injection all recipients were bled from the retroorbital sinus, and thereafter immunizations were alternated with bleedings at weekly intervals. The serum from each bleeding was stored at −70 °C until the completion of the immunization when it was tested in the cytotoxicity test, and bleedings with similar titers were pooled, aliquoted and frozen again. All antisera were tested against a panel of cells carrying inbred *H-2* haplotypes *b*, *d*, *f*, *j*, *k*, *p*, *q*, *r*, *s*, *u*, *v* and *z* and wild-derived *H-2* haplotypes *w1* through *w27*. Antisera containing more than one antibody were made operationally monospecific by absorption (see Table 2).

Table 3. *Monoclonal antibodies used for the* H-2 *typing of* t-*bearing strains*

Antibody against determinant*	Hybridoma	Immunization
H-2.m2 (H-2.2)	B22-249.R1	BALB/c anti-C57BL/6
H-2.m17 (H-2.17)	F5.21.37	(A.BY × B10.A)F_1 anti-B10.Q
H-2.m18 (H-2.18)	T3.6	C3H/HeJ anti-RIII/2J
H-2.m21 (H-2.21)	T2.100.1	A.SW anti-B10.WOA105
H-2.m24 (H-2.4)	T1.51.C	CBA/J anti-B10.A
H-2.m36 (H-2.111)	T63.18P	(DBA/2 × B10.A)F_1 anti-B10.CAS2
H-2.m117 (H-2.117)	F25.15.2	(B10 × A)F_1 anti(B10.A × T/t^{Lub1})F_1

* Equivalent determinant defined by polyclonal antibodies in parentheses.

(iii) *Monoclonal antibodies*

Seven monoclonal antibodies specific for H-2 determinants were used (Table 3). The hybridomas secreting these antibodies were produced in our laboratory (Figueroa, Davies & Klein, 1981), with the exception of B22-249.R1 which was kindly given to us by Prof. Günther Hämmerling, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, Heidelberg, FRG.

(iv) *Absorptions*

To restrict their specificity, some of the antisera were absorbed in vitro by mixing them with washed spleen and lymph-node cells at a ratio of 1 vol. of packed cells to 1 vol. of antiserum. The mixture was incubated for 1 h at room temperature with occasional shaking and the antiserum was then retrieved by centrifugation.

(v) *Cytotoxicity test*

To determine the presence or absence of individual H-2 determinants cells were tested in the two-stage microcytotoxicity assay on Terasaki plates (Greiner, Nürtingen, FRG). The assay, originally described by Amos, Bashir & Boyle (1969), was modified as follows (see Zaleska-Rutczynska & Klein, 1977). Lymphocytes from spleen or lymph-node fragments were obtained by pressing the tissue through

a cell sieve (Cellector, mesh 50, Bellco Glass, Inc., Vineland, New Jersey) into Hanks' balanced salt solution (HBSS) supplemented with 10% foetal calf serum (FCS). To remove erythrocytes and dead cells, the suspension was fractionated on a Ficoll Paque column (Pharmacia, Uppsala, Sweden). The cells were then washed twice with HBSS-FCS and their concentration adjusted to 3×10^6/ml. Using the Hamilton syringe (Hamilton Deutschland GmbH, Darmstadt) the cell suspension was then distributed into the individual wells of the Terasaki plate each of which already contained 2 μl of the antiserum or monoclonal antibody. After incubation of the plates for 20 min and washing, 2 μl of complement (a mixture of normal rabbit serum, normal guinea pig serum and HBSS-FCS at a ratio of 1:1:8) were added to each well and the plates were incubated for an additional 30 min. The percentage of dead cells was estimated using an inverted phase-contrast microscope.

3. RESULTS

(i) *Extraction of t haplotypes from wild mice*

Wild males were crossed with females of a stock carrying the *T* mutation and, if the mating produced animals lacking a tail (presumably of the *T*/*t* genotype), these were intercrossed and a balanced lethal line was produced (if the *t* haplotype contained a lethality gene, the mating $T/t \times T/t$ produced only tailless animals since *t*/*t* and *T*/*T* homozygotes die *in utero*). Of 160 males tested in this manner, 36 proved to carry a *t* haplotype. The *H-2* haplotypes of the first 20 of these *t* haplotypes, designated here t^{Tuw1} through t^{Tuw20} (*Tu* for Tübingen, *w* for wild), are described in this publication. Sixteen of the 20 *t* haplotypes were found to carry a lethality gene while four (t^{Tuw7} through t^{Tuw9} and t^{Tuw18}) carry a gene for semilethality (among the latter four the $T/t \times T/t$ matings produced some normal-tailed in addition to the majority of tailless animals, the normal-tailed animals presumably being the *t*/*t* homozygotes).

(ii) *Typing of the t lines with antibodies defining private* H-2 *determinants*

As a first step in determining the *H-2* haplotypes of the *t* lines, the cells of the *T*/*t* animals were tested in the cytotoxicity assay on a battery of serological reagents (antisera and monoclonal antibodies) specific for private determinants of the known *H-2K* and *H-2D* alleles. All 20 lines typed as negative for the following H-2 determinants: H-2.2, 4, 9, 15, 16, 17, 18, 19, 20, 21, 23, 26, 30, 31, 32, 103, 106, 110, 112, 113, 114, 116, 118, 137, 138, 143, 144 and 147. To be precise, some of the *t* lines did react with some of these reagents but in every case the reactivity could be attributed either to determinants controlled by the *H-2* haplotype of the T chromosome or to extra antibodies not specific for the private H-2 determinant in question. To save space, we have not shown these results. Cells from at least one line reacted with reagents defining the following H-2 determinants: H-2.33, 107, 108, 109, 111, 126 and 117 (Table 4).

Table 4. *Reactivity of H-2 antisera and monoclonal antibodies with* t*-bearing lines*

		H-2 antisera or monoclonal antibody													
Line	*t* Haplotype	T37 (26)*	T26 (15)	K355 (30)	K333 (33)	T63.18P (111)	T174 (117, 130, 139)	T166 (132)	T29 (103)	T48 (115)	T154 (126? 117)	K559 (108, 126)	K560 (107)	E6 (109)	F25.15.2 (117)
BNK265	*Tuw1*	—	—	—	—	—	.	—	—	—	—	—	16	.	.
BNK266	*Tuw2*	—	—	—	—	—	—	—	—	—	—	—	16	—	—
BNK280	*Tuw3*	—	—	—	—	—	.	—	—	—	—	—	8	.	.
BNK756	*Tuw4*	—	—	—	—	—	.	—	—	—	—	—	16	.	.
BRU377	*Tuw5*	—	—	—	—	—	.	—	—	—	—	—	32	—	.
BRU382	*Tuw6*	—	—	—	—	—	—	—	—	—	—	—	32	—	—
CRO435	*Tuw7*	—	—	—	—	⩾32	—	—	—	—	—	⩾32	—	.	.
CRO437	*Tuw8*	.	.	.	.	⩾32	.	.	.	.	—	.	.	.	.
CRO447	*Tuw9*	.	.	.	.	⩾32	.	.	.	.	—	⩾32	—	—	.
EDY589	*Tuw10*	—	—	—	—	—	⩾32	—	—	—	8	⩾32	—	.	⩾32
GPC882	*Tuw11*	—	—	—	—	—	⩾32	—	—	—	8	⩾32	—	.	⩾32
ISL18	*Tuw16*	10	8	—	—	—	.	—	16	—	16	⩾16	—	.	.
ISL20	*Tuw17*	10	—	—	—	—	.	—	8	4	8	⩾16	—	.	.
ISL37	*Tuw18*	10	8	—	—	—	⩾32	—	4	4	4	⩾16	—	.	⩾32
LRA410	*Tuw12*	—	—	—	—	—	.	—	—	—	16	⩾64	—	.	.
LRA414	*Tuw13*	4	—	—	—	—	⩾32	—	—	—	8	⩾32	—	—	16
MOY331	*Tuw14*	—	—	—	—	—	.	—	—	—	8	⩾64	—	—	.
MOY336	*Tuw15*	—	—	—	—	—	⩾32	—	—	—	8	⩾32	—	—	16
MSW250	*Tuw19*	—	—	—	⩾32	—	8	—	—	—	16	—	—	⩾32	32
MSW251	*Tuw20*	—	—	—	128	—	—	—	—	—	—	—	—	⩾32	—

—: No reaction at antiserum dilution 1:2 or more; numbers indicate the reciprocals of the titre.
. : Not tested.
*: Antigenic determinant detected.

H-2.33, a private determinant controlled by the K^b allele, was found to be present in the two MSW lines (MSW250 and MSW251 carrying haplotypes t^{Tuw19} and t^{Tuw20}, respectively). The presence of this determinant in the MSW251 line was confirmed by absorption: cells from this line completely removed the anti-K^b activity of the K333 antiserum. Since none of the $T/+$ animals used for establishing the balanced lethal lines carries the K^b allele, we assume that H-2.33 of the MSW lines is controlled by the t chromosome.

Table 5. *Absorption analysis of antiserum K560: (A × B10) anti-*T/t^{w2}

	Reciprocal of titre against cells			
Absorbed by	T/t^{w2}	BRU382	BNK266	ISL33
T/t^{w2}	—	—	—	—
BRU382	—	—	—	—
BNK266	—	—	—	—
ISL33	⩾32	8	8	—

H-2.107 is a determinant thus far found only in association with t haplotypes. Previous typing (Hammerberg & Klein, 1975; Hammerberg *et al.* 1976) revealed the presence of this determinant in haplotypes *H-2*^{w28}, associated with t haplotypes t^{12} and t^{w32}, *H-2*^{w29} associated with t haplotypes t^0, t^1, t^{w2}, and t^{w8}, and *H-2*^{w33}, associated with t haplotypes $t^{Lüb1}$, t^{w101} and t^{w102}. In the present tests, H-2.107 was found in all the BNK and BRU lines (t haplotypes *Tuw1* through *Tuw6*). Not all were checked by absorption for the presence of H-2.107, but those that were revealed the identity of this determinant with that carried by previously typed strains. An example of the absorption analysis is given in Table 5, in which lines BNK266 and BRU382 are shown to absorb out the activity of the K560 antiserum against t^{w2}-bearing cells completely. The H-2.107-negative ISL33 cells, on the other hand, did not absorb the activity of the antiserum against BNK266, BRU382 or t^{w2}.

The H-2.108 determinant was previously demonstrated (Hammerberg & Klein, 1975; Hammerberg *et al.* 1976) to be controlled by the *H-2*^{w30} haplotype which is associated with t haplotypes of the t^{w1} complementation group and t^6. Here we demonstrate that the K559 antiserum reacts also with the $t^{Lüb1}$, t^{w73}, T^{Tuw10} through t^{Tuw18}, t^{Tuw7} and t^{Tuw9} haplotypes. Absorption analysis of the K559 antiserum gave the following results (Table 6). Absorption of the antisera by $t^{Lüb1}$ or by t^{w73} cells (data not shown) removed all activity against these two strains but left weak activity against t^{w1}, t^{Tuw7}, t^{Tuw10}, t^{Tuw11}, t^{Tuw12}, t^{Tuw15} and t^{Tuw18} cells. Absorption by t^{w1}, t^{Tuw10} through t^{Tuw12}, t^{Tuw15} and t^{Tuw18} cells removed activity against all the tested cells. Absorption by t^{Tuw7} cells removed the weak activity with these cells but did not remove the activity against any other positive cells, including $t^{Lüb1}$. We interpret these results to mean that the K559 antiserum contains at least two antibodies, one detecting the original determinant 108 and the other defining a new determinant 126. The *H-2*^{w30} haplotype codes for both

determinants, the *H-2* haplotype carried by the $t^{Lüb1}$ line codes for the 126 determinant while the *H-2* haplotypes of the t^{Tuw7} through t^{Tuw9} lines possibly code for the 108 determinant and the t^{w73} line codes only for the 126 determinant.

The H-2.109 determinant, previously found to be controlled by the $H\text{-}2^{w31}$ haplotype of the lines carrying the t^{w5}-like haplotypes, was also detected in our lines carrying the t^{Tuw19} and t^{Tuw20} haplotypes. The identity of the determinants carried by these different lines was established by absorption analysis (Table 7).

Table 6. *Absorption analysis of antiserum K559: (B10 × A) anti-*T/t^{w1}

Absorbed by	Reciprocal of titre against cells									
	T/t^{w1}	$T/t^{Lüb1}$	B10.A	CRO435	EDY589	GPC882	ISL37	LRA410	MOY336	MSW250
T/t^{w1}	—	—	—	—	—	—	—	—	—	—
$T/t^{Lüb1}$	8	—	—	16	w	8	16	8	4	—
B10.A	16	8	—	8	32	16	16	8	16	—
CRO435	4	8	—	—	4	8	8	8	8	—
EDY589	—	—	—	—	—	—	—	—	—	—
GPC882	—	—	—	—	—	—	—	—	—	—
ISL37	—	—	—	—	—	—	—	—	—	—
LRA410	—	—	—	—	—	—	—	—	—	—
MOY336	—	—	—	—	—	—	—	—	—	—
MSW250	16	8	—	w	4	8	16	8	16	—

Table 7. *Absorption analysis of antiserum T184 :(A × B10) anti-*$(T/t^{w5} \times Rb7d)F_1$

Absorbed by	Reciprocal of titre against cells			
	T/t^{w94}	A	MSW251	MSW250
T/t^{w94}*	—	—	—	—
A	4	—	2	⩾32
MSW251	w	—	—	—

* t^{w94} belongs to the same complementation group as t^{w5}.

However, the two groups of *H-2* haplotypes coding for the H-2.109 determinants are clearly different, since in the t^{w5}-like lines H-2.109 is associated with H-2.11-, 25- and 23-like determinants, whereas in the t^{Tuw19} and t^{Tuw20} lines the H-2.109 determinant is associated with H-2.33. We designate the *H-2* haplotype of the t^{Tuw19} and t^{Tuw20} lines *w38*.

The H-2.111 determinant was previously found only in the B10.CAS2 ($H\text{-}2^{w17}$) strain (Zaleska-Rutczynska & Klein, 1977). In the present study, we found the determinant in lines carrying the t^{Tuw7} through t^{Tuw9} haplotypes. In B10.CAS2 the H-2.111 determinant is associated with H-2.11, 25, 118, 119, 120 and 124 determinants. In the t^{Tuw} strains, on the other hand, the H-2.111 determinant possibly occurs together with H-2.108 which is absent in B10.CAS2. The combination of determinants H-2.111 and H-2.108 defines a new *H-2* haplotype which we designate *w37*.

The H-2.130 determinant was previously found to be present in the B10.GAA37

Table 8. *Absorption analysis of antiserum T174 : (B10.S × A/WySn)F$_1$ anti-B10.GA A37*

Reciprocal of titre against cells

Absorbed by	STA62	A	T/t^{w1}	T/t^{w73}	T/t^{Lib1}	BNK266	LRA410	MOY336	MSW250	MSW251
STA62	—	—	—	—	—	—	—	—	—	—
A	⩾32	—	⩾32	16	16	—	16	⩾32	8	—
T/t^{w1}	8	—	—	—	—	—	—	—	—	—
T/t^{w73}	16	—	—	—	4	—	—	—	—	—
T/t^{Lib1}	4	—	—	—	—	—	—	—	—	—
BNK266	16	—	⩾32	⩾32	⩾32	—	⩾32	⩾32	—	—
LRA410	4	—	—	—	2	—	—	—	—	—
MOY336	8	—	—	—	4	—	—	—	—	—
MSW250	4	—	w	8	4	—	2	—	—	—
MSW251	16	—	⩾32	⩾32	⩾32	—	16	⩾32	8	—

and B10.BUA1 strains carrying the $H\text{-}2^{w16}$ and $H\text{-}2^{w21}$ haplotype, respectively (Duncan, Wakeland & Klein, 1979; Klein & Figueroa, 1981). The antiserum T174 contains antibodies against the H-2.117, 130 and 139 determinants. It reacted with strains carrying t^{w1} and related haplotypes t^{6}, t^{w73}, t^{Lub1} and t^{Tuw10} through t^{Tuw18}. The absorption analysis of the antiserum is shown in Table 8. Strain B10.STA62 (H-2.130 negative, H-2.117 and H-2.139 positive) removed reactivity for all positive strains tested indicating that these strains carry a determinant different from H-2.130 and similar to H-2.117. These results were also confirmed by the reactivity of the monoclonal antibody F25.15.2 which defines the H-2.117 determinant when tested against the B10.W lines (F. Figueroa & J. Klein, unpublished data). The analysis also reveals the presence of a crossreactive determinant in the t^{Tuw19} and t^{Tuw20} strains that absorbs the antibody weakly and often irreproducibly. The association of H-2.117 with H-2.107 defines a new *H-2* haplotype, designated as *w33* and carried by the t^{Lub1} strain.

Table 9. *Reactivity of some new anti-*t *sera with* t-*bearing wild mice*

	Reciprocal of titre of antiserum				
Line	S1	S2	S7	S8	T154
BNK265	—	—	—	—	—
BNK266	—	—	—	—	—
BNK280	—	—	—	—	—
BNK756	—	—	—	—	—
BRU377	4	—	—	—	—
BRU382	4	—	—	—	—
CRO435	8	—	—	2	—
CRO437	8	—	—	2	—
CRO447	8	—	—	4	—
EDY589	16	4	4	4	8
GPC882	8	4	4	4	8
LRA410	8	8	4	2	16
LRA414	8	8	4	2	8
MOY331	8	4	4	4	8
MOY336	8	4	4	4	8
ISL18	4	4	4	4	16
ISL20	4	4	4	4	8
ISL37	4	4	4	4	8
MSW250	8	2	4	—	16
MSW251	2	—	—	—	—

(iii) *Production of antibodies against determinants of t-associated* H-2 *haplotypes*

In an attempt to define new H-2 determinants in some of the 20 new *t* strains, we made a series of immunizations using these strains as donors. Since the *t* strains are not inbred, one could expect the immunization to produce a mixture of antibodies against H-2 and non-H-2 determinants. This expectation was confirmed by the results. We succeeded in producing antisera against several *t* lines (Table 9) and all of these antisera proved to be quite complex. However, by appropriate

absorptions we could remove the non-H-2 antibodies and detect H-2-specific antibodies in several of the antisera. All these antibodies seem to be specific for the H-2.117 determinant (Table 9). (The immunizations were initiated before we knew the *H-2* haplotypes of the *t* lines and by chance we selected for the immunization lines with identical haplotypes.) These results confirm the assignment of *H-2* haplotypes to these *t* lines made on the basis of typing with known antibodies.

4. DISCUSSION

The distribution of the individual H-2 determinants among the 20 *t* lines tested in this study appears in Table 10. The *H-2* chart of all the lines tested thus far is given in Table 11. In both tables only determinants occurring in at least one of the lines are listed; determinants absent in all the *t* lines are not listed. Although most of the listed H-2 determinants have not been mapped as to whether they are controlled by the *K* or the *D* loci, a few have been (e.g. H-2.11, 25, 31, 33 and 117) and, using these as a starting point, one can predict the assignment to *K* or *D* of the other determinants (Table 12). The analysis described in this paper and summarized in Tables 10–12 adds four new haplotypes of *t* lines ($H\text{-}2^{w33}$, $H\text{-}2^{w36}$, $H\text{-}2^{w37}$ and $H\text{-}2^{w38}$) to the five already known ($H\text{-}2^{w28}$ through $H\text{-}2^{w32}$). Each of these haplotypes is defined by a new combination of antigenic determinants.

The first thing apparent from the summaries in Tables 10–12 is that lines derived from mice trapped at the same locality carry the same *H-2* haplotypes. Thus all lines derived from mice trapped in Germany (the BNK and BRU lines) carry the $H\text{-}2^{w36}$ haplotype; the lines derived from the Egyptian mice carry the $H\text{-}2^{w37}$ haplotype, and so on. This similarity of *H-2* haplotypes probably also extends to the *t* haplotypes. Spot checks of the lines by complementation testing demonstrated that lines originating from the same locality failed to complement each other – a finding normally interpreted as indicating similarity of the *t* haplotypes involved (unpublished data). Hence we assume that, for instance, haplotypes t^{Tuw1} through t^{Tuw6} are all similar; that t^{Tuw7} through t^{Tuw9} form another group of similar *t* haplotypes, and so on.

Of the *H-2* haplotypes identified previously, only one was found in the new collection of lines: $H\text{-}2^{w30}$ previously associated with *t* haplotypes of the *w1* group was found in mice from several geographically distant localities (*t* haplotypes t^{Tuw10} through t^{Tuw18}). The *H-2* haplotypes previously found in association with the t^{12}, t^{0}, t^{w5} and t^{w73} groups of haplotype were not found in the present sample of mice.

The next step in the analysis of the new *t* lines will be the characterization of the *t* haplotypes themselves, in particular the determination of the complementation groups to which these haplotypes belong. Such characterization is already in progress and the results will soon tell us to what degree the previously observed linkage disequilibrium between the *H-2* and *t* complexes (Hammerberg & Klein, 1975) also holds for our sample of wild mice. However, preliminary data already suggest that the situation will be more complex than previously anticipated. These

Table 10. *Distribution of H-2 antigenic determinants among lines carrying haplotypes* t^{Tuw1} *through* t^{Tuw20}

Line	*t* Haplotype	*H-2* Haplotype	H-2 Antigenic determinants						
			33	107	108	109	111	126	117
BNK265	*Tuw1*	*w36*	—	107	—	—	—	—	—
BNK266	*Tuw2*	*w36*	—	107	—	—	—	—	—
BNK280	*Tuw3*	*w36*	—	107	—	—	—	—	—
BNK756	*Tuw4*	*w36*	—	107	—	—	—	—	—
BRU377	*Tuw5*	*w36*	—	107	—	—	—	—	—
BRU382	*Tuw6*	*w36*	—	107	—	—	—	—	—
CRO435	*Tuw7*	*w37*	—	—	108	—	111	—	—
CRO437	*Tuw8*	*w37*	—	—	108	—	111	—	—
CRO447	*Tuw9*	*w37*	—	—	108	—	111	—	—
EDY589	*Tuw10*	*w30*	—	—	108	—	—	126	117
GPC882	*Tuw11*	*w30*	—	—	108	—	—	126	117
LRA410	*Tuw12*	*w30*	—	—	108	—	—	126	117
LRA414	*Tuw13*	*w30*	—	—	108	—	—	126	117
MOY331	*Tuw14*	*w30*	—	—	108	—	—	126	117
MOY336	*Tuw15*	*w30*	—	—	108	—	—	126	117
ISL18	*Tuw16*	*w30*	—	—	108	—	—	126	117
ISL20	*Tuw17*	*w30*	—	—	108	—	—	126	117
ISL37	*Tuw18*	*w30*	—	—	108	—	—	126	117
MSW250	*Tuw19*	*w38*	33	—	—	109	—	—	CR
MSW251	*Tuw20*	*w38*	33	—	—	109	—	—	CR

CR = cross reactive determinant.

Table 11. H-2 *haplotypes of all the tested* t-*bearing lines*

H-2 Haplotype	Group of *t* haplotypes	H-2 Antigenic determinants												
		11	23	25	31	33	105	106	107	108	109	111	126	117
w28	t^{12}, t^{w32}	—	—	—	—	—	—	106	107	—	—	—	—	—
w29	t^{0}, t^{1}, t^{w2}, t^{w8}	—	—	—	31	—	105	—	107	—	—	—	—	—
w30	t^{w1}, t^{w12}, $t^{w12}tf$, t^{w71}, t^{6} t^{Tuw10} through t^{Tuw18}	—	—	—	—	—	—	—	—	108	—	—	126	117
w31	t^{w5}, t^{w75}, t^{w93}, t^{w94}, t^{w97}, t^{w105}	11	CR	25	—	—	—	—	—	—	109	—	—	—
w32	t^{w73}	—	—	—	—	—	—	—	—	—	109	—	126	—
w33	$t^{Lüb1}$, t^{w101}, t^{w102}	—	—	—	—	—	.	—	107	—	—	—	126	117
w36	t^{Tuw1} through t^{Tuw6}	—	—	—	—	—	.	—	107	—	—	—	—	—
w37	t^{Tuw7} through t^{Tuw9}	—	—	—	—	—	.	—	—	108	—	111	—	—
w38	t^{Tuw19}, t^{Tuw20}	—	—	—	—	33	.	—	—	—	109	—	—	CR

CR = cross reactive determinant.

data indicate, for example, that at least some of the t^{Tuw} haplotypes carrying the *H-2*^{w30} complex of genes complement the t^{w1} haplotype, which apparently carries the same *H-2* complex. It appears, therefore, that the same *H-2* haplotype can be associated with seemingly different *t* haplotypes – at least as these haplotypes are defined by the complementation test. The extent of this discordance between *H-2* and *t* typing will have to be established and any conclusions about the relationship between the *H-2* and *t* complexes must await the completion of the genetic analysis of the new *t* lines. It may turn out, for example, that the complementation analysis is not a good test for establishing the relationship between *t* haplotypes extracted

Table 12. *Predicted distribution of H-2 determinants between K and D molecules of* t-*bearing strains*

H-2 Haplotype	H-2 Determinants carried by K	D
w28	107	106
w29	31, 107	105
w30	.	108, 126, 117
w31	11, 23^{Cr}, 25	109
w32	.	109, 126
w33	107	117, 126
w36	107	.
w37	111	108
w38	33	109, 117^{Cr}

from different wild mice. In fact, there are already indications that not all members of a complementation group are identical: see Bennett, 1975. The present study as well as those of Winking (1978) and Guenet *et al.* (1980), suggest that the neat division into sharply separated *t* complementation groups might be an illusion. This division may hold for mice on the North American continent which appear genetically more homogeneous than the European wild mice, but in Europe and Asia, with their abundance of species and subspecies of *Mus*, the division may break down. If this happens, new ways of classifying *t* haplotypes will have to be found and in this new classification, *H-2* typing of the individual *t* chromosomes may provide one of the most crucial markers.

A very striking result of the *H-2* typing done thus far is the indication that most of the *t*-associated *H-2* haplotypes present in European wild mice appear to be related to one another (see Table 11). Determinants H-2.107, 108, 109 and 126 are either completely absent or appear in very low frequencies in non-*t* wild mice thus far tested (F. Figueroa & J. Klein, unpublished data), yet in the *t* haplotypes they are shared by different *H-2* haplotypes (e.g., H-2.107 is shared by haplotypes *H-2*^{w29}, *H-2*^{w33} and *H-2*^{w36}). This observation suggests that at least some of the *t* associated *H-2* haplotypes are of common origin and they have been derived from a small number of original haplotypes by recombination and/or mutation. If so, then one can postulate similar relatedness and a similar mode or origin for the *t*

haplotypes themselves. It may eventually turn out that the European *t* haplotypes form a single complex consisting of geographically differentiated groups with individual groups being distinct yet clearly related to one another. In such a complex it might be difficult to define *t* haplotypes on the basis of a single trait such as embryonic lethality, for in different groups each trait might result from the interaction of several genes. One can envision, for example, a *t* haplotype behaving in one situation as a lethal and in another situation as a semilethal haplotype, depending on the constellation of linked genes or even genes not linked to the *t* complex. Whatever the correct interpretation might be, it is clear that the study of the population genetics of the *t* complex is only now beginning.

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