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Polymorphism of *t*-complex genes in European wild mice

BY JAN KLEIN, PETER SIPOS AND FELIPE FIGUEROA

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

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SUMMARY

Thirty-two *t* haplotypes were extracted from wild mice captured in Central Europe, Spain, the Soviet Union, Israel, Egypt, the Orkneys and South and North America, and tested for lethality in the homozygous state. Twenty-two proved to be homozygous lethals, 8 semilethals and 2 viables. The lethal *t* haplotypes were then tested by the genetic complementation test for identity with representatives of known complementation groups and with each other. Five of the 22 haplotypes proved to carry previously identified lethality factors (t^{w5}, t^{w73} and $t^{Lub\text{-}1}$), while the rest carried new factors. The 17 haplotypes fell into 8 new complementation groups. Two of the new groups are partially overlapping in that they seem to share some lethality factors and differ in others. These tests raise the total number of known complementation groups to 16. The distribution of the individual *t* haplotypes among wild mice populations seems to reflect their differentiation from a common ancestor haplotype.

1. INTRODUCTION

Some 20–40% of wild mice, *Mus domesticus*, carry chromosome 17 with an abnormal region, the *t* complex, proximal to the centromere (Klein, 1975; Bennett, 1975; Lyon, 1981). A marker for this abnormal chromosome is a mutation that, when combined with the laboratory mutation *short tail* or *T*, leads to a complete absence of the tail. Some of the abnormal chromosomes carry, in addition, mutations that, in a homozygous state, cause all (lethal *t* haplotypes) or some (semilethal *t* haplotypes) embryos to die; others lack these 'lethality factors' (viable *t* haplotypes) (Klein, 1975; Bennett, 1975; Lyon, 1981). When lethal haplotypes derived from different wild mice are combined in a hybrid and all the embryos die, the two haplotypes are said to belong to the same complementation group. When at least some of the hybrids survive, the haplotypes are considered members of different complementation groups. Until 1973 only five complementation groups had been known, which are represented by the haplotypes t^{12}, t^{0}, t^{9}, t^{w5} and t^{w1}, derived from North American wild mice. Since then three other complementation groups have been discovered, t^{w73}, $t^{Lub\text{-}1}$ and $t^{wPa\text{-}1}$ (Dunn, Bennett & Cookingham, 1973; Winking, 1978; Guenet *et al.* 1980), all in European wild mice. Here we describe eight new complementation groups, provide two examples of partially overlapping groups, and report on a geographical differentiation of European mouse populations in regard to *t* haplotypes.

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2. MATERIALS AND METHODS

Wild mice of the species *Mus domesticus* or *M. musculus* L. were trapped at the localities indicated in Table 1. Mice trapped outside Germany were supplied to us by the following people: mice from the Orkney island of Eday by Professors 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 Clinico y Provincial, Barcelona, Spain; from Moscow, U.S.S.R. by Dr I. Egorov, The Jackson Laboratory, Bar Harbor, Maine; from Israel by Professor E. Nevo, University of Haifa; from Nahya, Egypt, by Dr Harry Hoogstraal, U.S. Naval Medical Research Unit No. 3, Cairo; from the area around Tübingen, F.R.G., by Drs 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.

3. RESULTS AND DISCUSSION

In 1978–9 we collected a sample of wild mice, *Mus domesticus* and *M. musculus* (Sage, 1981), from different parts of the world, and mated the males with $T/+$ females. We took the appearance of tailless progeny as an indication that the wild parent was a $t/+$ heterozygote, and presumed that the tailless mice were T/t heterozygotes. The frequency of t haplotypes in the entire sample tested was about 25 %. The frequency in individual localities varied from 0 to 50 %. However, these estimates may not reflect precisely the actual frequencies because the samples from some localities were small and because some of the mated males failed to produce progeny. We then mated taillness males with tailless females from the same cross and established t-bearing lines. In some of these lines the matings produced only tailless animals, presumably because the t haplotype was a homozygous lethal. Others produced both tailless and normal-tail animals because the t haplotypes were either semilethal or viable in the homozygous state. Altogether we produced 32 t-lines of which 22 turned out to carry lethal haplotypes, 8 semilethal and 2 viable haplotypes (Table 1).

Lines designated as viable were those in which $t/t \times t/t$ matings produced only normal-tail progeny. The two viable haplotypes are the first of this kind isolated from wild mouse populations. Full viability of the lines was also indicated by the normal litter size (the average was 8·3 for one line and 9·1 for the other, which is comparable to lines without t haplotypes), and by the fact that the t/t males were fertile. We designated the lines t^{Tuw1} to t^{Tuw32}, where *Tu* stands for Tübingen. We then *H-2*-typed the lines and mated *inter se* those that appeared to have identical *H-2* haplotypes and came from the same locality. If only tailless mice appeared in the progeny, we maintained further only one representative line of the group because we presumed that all the lines from that locality carried the same t haplotype. We now maintain 19 of the original 32 lines.

To determine the complementation groups of the t haplotypes in the 19 lines, we set up matings in a chequerboard fashion so that each line was mated with all

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Table 1. *Lines with* t *haplotypes*

t haplotype	Strain	Origin	Group	H-2 haplotype	Status
Tuw1	BNK265	Wendelsheim, F.R.G.	t^{Tuw2}	*w36*	D
Tuw2	BNK266	Wendelsheim, F.R.G.	t^{Tuw2}	*w36*	M
Tuw3	BNK280	Wendelsheim, F.R.G.	t^{Tuw2}	*w36*	D
Tuw4	BNK756	Wendelsheim, F.R.G.	t^{Tuw2}	*w36*	D
Tuw5	BRU337	Brno, Czechoslovakia	t^{w73}	*w58*	D
Tuw6	BRU382	Brno, Czechoslovakia	t^{w73}	*w58*	M
Tuw7	CRO435	Nahya, Giza Governorate, Egypt	SL	*w37*	M
Tuw8	CRO437	Nahya, Giza Governorate, Egypt	SL	*w57*	M
Tuw9	CRO447	Nahya, Giza Governorate, Egypt	SL	*w37*	D
Tuw10	EDY589	Eday, Orkney Islands	SL	*w2*	M
Tuw11	GPC882	Buin, Chile	t^{Tuw11}	*w30*	M
Tuw12	LRA410	La Roca, Spain	t^{Tuw12}	*w30*	M
Tuw13	LRA414	La Roca, Spain	t^{Tuw12}	*w30*	D
Tuw14	MOY331	Moya, Spain	t^{Tuw12}	*w30*	D
Tuw15	MOY336	Moya, Spain	t^{Tuw12}	*w30*	M
Tuw16	ISL18	Haifa, Israel	SL	*w2*	D
Tuw17	ISL20	Haifa, Israel	SL	*w2*	D
Tuw18	ISL37	Haifa, Israel	SL	*w2*	M
Tuw19	MSW250	Ryazan, Astrakhan, U.S.S.R	t^{Tuw20}	*w38*	D
Tuw20	MSW251	Ryazan, Astrakhan, U.S.S.R	t^{Tuw20}	*w38*	M
Tuw21	BNK761	Wendelsheim, F.R.G.	t^{Tuw2}	*w36*	M
Tuw22	GPC881	Buin, Chile	t^{Tuw11}	*w30*	D
Tuw23	GPC183	Temuco, Chile	t^{w5}	*w31*	M
Tuw24	LGN925	Langenargen, F.R.G.	t^{Tuw24}	*w36*	M
Tuw25	OBL984	Oberer Lindenhof, F.R.G.	t^{Tuw25}	*w36*	M
Tuw26	PLD826	Bialowieza, Poland	t^{w73}	*w59*	M
Tuw27	ROD1455	Dudelhof, F.R.G.	t^{Tuw27}	*w36*	M
Tuw28	ERP1465	Erpenhausen, F.R.G.	t^{Tuw28}	*w60*	M
Tuw29	BRW942	Aulendorf, F.R.G.	t^{Lub1}	*w61*	M
Tuw30	B10.KPB68	Ann Arbor, Michigan, U.S.A.	SL	*w2*	M
Tuw31	ISL26	Haifa, Israel	V	*w56*	D
Tuw32	ISL33	Haifa, Israel	V	*w56*	M

D, discarded; M, maintained in the colony; SL, semilethal; V, viable.

the other new *t* lines and also with representatives of the previously established groups. (The only haplotype not represented was $t^{wPa\text{-}1}$, which we could not obtain.) Absence of normal-tail progeny in any of the crosses was taken to mean that the *t* haplotypes in the parental lines involved belonged to the same complementation group; appearance of normal-tail progeny signified that the *t* haplotypes belonged to different groups (Table 2). In this manner, we could divide the *t*-lethal lines into 11 complementation groups, of which only 3 were described previously (Table 3). The 8 new complementation groups double the number of groups described in the last 50 years or so.

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Table 2. *Complementation test with available* t *strains*

	BNK	BRU	BRW 942	GPC 882	LGN 925	LRA 410	OBL 984	MOY	MSW	PLD 826	ROD 1455	ERP 1465	GPC 183
BNK	14/42												
BRU	13/24	1/3											
BRW942	3/5	6/11	5/5										
GPC882	10/7	12/11	5/11	4/2									
LGN925	2/7	2/5	6/8	5/7	2/6								
LRA410	**0/58**	8/4	6/6	10/18	8/8	8/7							
OBL984	10/14	5/5	7/5	6/1	7/8	**0/35**	9/9						
MOY	6/5	**0/68**	3/2	6/2	10/8	3/2	5/5	16/9					
MSW	3/9	**0/26**	1/3	3/3	2/2	5/4	4/3	1/1	**0/22**				
PLD826	**0108**	10/10	4/12	3/3	4/12	6/3	7/49	3/6	2/2	3/3			
ROD1455	6/4	5/9	5/5	10/13	2/1	5/8	2/3	1/1	1/6	7/3	2/4		
ERP1465	2/4	14/8	2/1	9/9	5/7	12/6	3/5	6/3	4/0	2/0	2/3	3/12	
GPC183			1/4	2/4	3/2	19/25	2/9	12/14	9/35	4/3	2/5	**0/6**	3/5
t^{w1}, t^{w12}	3/6	5/9	7/7	7/11	9/11	8/6	5/4	7/4	10/6	5/1	5/15	9/8	**0/44**
t^{w5}, t^{w94}	10/19	5/14	**0/30**	8/10	1/2	2/2	6/9	4/13	2/2	4/3	4/5	4/6	2/3
t^{Lub-1}	3/13	8/9	6/4	2/4	4/9		2/1	2/1	**0/78**	4/4	4/9	3/6	2/4
t^{9}, t^{6}	1/3	14/19	7/5	6/9	1/1	3/8	3/6	34/28	1/5	1/5	2/3	1/0	6/9
t^{12}, t^{w32}	3/4	**0/80**	1/3	6/6	2/8	2/10	6/5	2/3	**0/78**	**0/29**	3/4	2/5	1/2
t^{w73}	1/1	1/3	1/6	7/5	1/7	4/4	2/4	1/4	2/4	1/5	3/8	1/5	2/3
t^{w18}													

Tailless mice of the strain indicated in the vertical column were mated with mice in the horizontal column. The two numbers indicate the number of normal tail/tailless progeny. Where there is no number in the line designation, it means that more than one line (with the same *t* haplotype) was used for matings. Bold face figures indicate lack of complementation.

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Special relationships exist among some of the groups as depicted, together with a suggested interpretation, in Table 4. The unusual feature of these relationships is that a given *t* haplotype appears to belong to the same complementation group as two other haplotypes, but these other haplotypes clearly belong to different groups. One example of such a situation was reported previously: Artzt and her

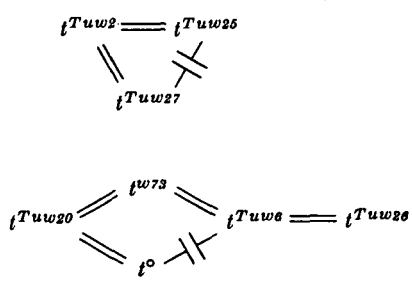
Table 3. *Groups of* t *haplotypes carried by the new lines*

Complementation group	Strain*	
$t^{Lub\text{-}1}$	BRW942	
t^{w5}	GPC183	
t^{w73}	BRU382, BRU337, PLD826	
t^{Tuw20}	MSW251, MSW250 (contains t^0 l.f.)	share t^{w73} l.f.
t^{Tuw2}	BNK266, BNK761, BNK265, BNK280, BNK756	
t^{Tuw25}	OBL984	share OBL l.f.
t^{Tuw27}	ROD1455	share BNK l.f.
t^{Tuw24}	LGN925	
t^{Tuw28}	ERP1465	
t^{Tuw12}	LRA410, LRA414, MOY336, MOY331	
t^{Tuw11}	GPC882, GPC881	
Semilethal haplotypes		
t^{Tuw7}	CRO435	
t^{Tuw8}	CRO437	
t^{Tuw10}	EDY589	
t^{Tuw18}	ISL37	
t^{Tuw30}	B10.KPB68	
Viable haplotypes		
t^{Tuw31}	ISL26	
t^{Tuw32}	ISL33	

* l.f., lethal factor

Table 4. *Partially overlapping complementation groups*

Relationship between haplotypes



Interpretation

t^{Tuw2}:	t^{Tuw2}	t^{Tuw25}
t^{Tuw25}:	+	t^{Tuw25}
t^{Tuw27}	t^{Tuw2}	+
t^{Tuw20}:	t^0	t^{w73}
t^{w73}:	+	t^{w73}
t^{Tuw6}:	+	t^{w73}
t^0:	t^0	+

= indicates absence of complementation; ⊣ ⊢ indicates complementation between the connected *t* haplotypes. Under 'Interpretation' are represented the chromosomal segments with the presumed lethality factors for each *t* haplotype.

co-workers described the t^{w75} haplotype that overlaps two complementation groups, t^{w5} and t^{w1} (Artzt, Babiarz & Bennett, 1979). We interpret the overlapping groups by postulating that some of the haplotypes carry at least two lethality factors, one of which they share with one haplotype and the other with another haplotype (Table 4). Conditions favouring the association or separation of lethality

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factors occur when two or more *t* haplotypes are present in the same population, as Silver & Artzt have demonstrated that in heterozygotes bearing two different *t* haplotypes, crossing over occurs with relatively high frequency (in comparison to the *T*/*t* heterozygotes) in the *t* region (Silver & Artzt, 1981).

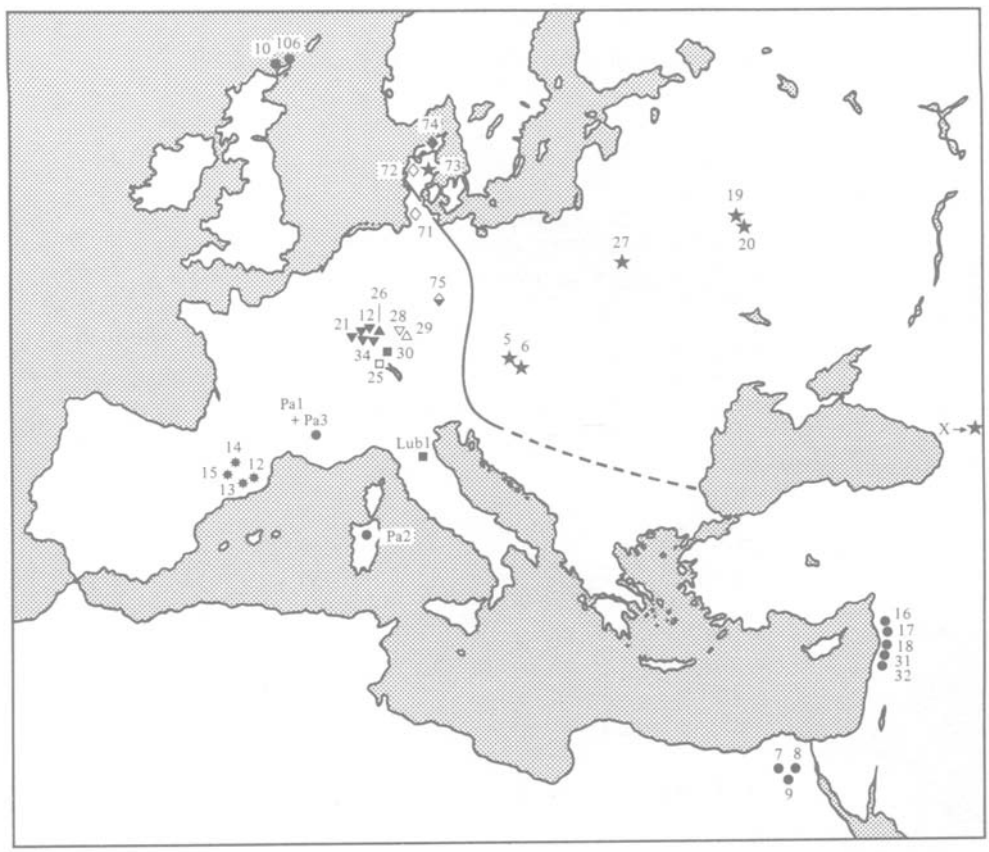

Fig. 1. Distribution of *t* haplotypes in Europe and neighbouring countries. Each symbol indicates one kind of complementation group. The numbers indicate individual *t* haplotypes. They are: 71, t^{w71}; 72, t^{w72}, 73, t^{w73}; and 74, t^{w74} (Dunn, Bennett & Cookingham, 1973); 75, t^{w75} (Artzt, Babiarz & Bennett, 1979); 106, t^{w106} (Dooher *et al.* 1981). Pa-1 to Pa-3, $t^{wPa\text{-}1}$ to $t^{wPa\text{-}3}$ (Guenet *et al.* 1980); Lub1, $t^{Lub\text{-}1}$ (Winking, 1978); X, undesignated *t* haplotype (Demin, Krjukov & Orlov, 1979); all other numbers refer to t^{Tuw} haplotypes described in this communication. The line drawn across from Denmark southwards indicates the border between the distribution of the two species, *Mus domesticus* (western form) and *M. musculus* (eastern form). The dotted lines are an extrapolation for the region for which data on the distribution of these species are available.

Until recently, information concerning *t* polymorphism was obtained mostly from studies of wild mice in North America (Klein, 1975; Bennett, 1975; Klein & Hammerberg, 1977). These studies revealed the occurrence, in appreciable frequencies, of only two lethal *t* haplotypes, t^{w1} and t^{w5}, in addition to semilethal haplotypes. Members of other complementation groups were found either only in

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laboratory stocks or only once in wild populations. In this regard the situation among European wild mice is clearly different. The three haplotypes found in North America (t^{w1}, t^{w5}, and semilethal) occur also in Europe, although possibly only in the westernmost parts of the continent, but in addition to these one finds at least 8 other lethal *t* haplotypes, and also viable haplotypes, all in appreciable frequencies. The *t* complex is, therefore, much more polymorphic among European than among North American wild mice.

When plotting the geographical distribution of the *t* haplotypes (Fig. 1), one can discern the emergence of certain patterns, possibly reflecting the evolutionary history of European wild mouse populations. The most striking observation is that in the regions occupied by the eastern form of the house mouse, *M. musculus* (Sage, 1981), all the *t* haplotypes thus far found carry that t^{w73} lethality factor. (The t^{Tuw20} haplotype may carry, in addition the t^0 lethality factor.) Should this observation be upheld by more extensive sampling, it would have an important bearing on the origin of the *t* complex, particularly since the t^{w73} lethality factor has not been found in the western form of the house mouse, *M. domesticus*.

It is in the *domesticus* species or subspecies that most of the *t* polymorphism occurs. However, even here there is at least a hint of geographical differentiation. Thus the viable and semilethal *t* haplotypes have thus far been found mostly in the regions around the Mediterranean Sea. One exception is the region of the Orkney Islands, where two semilethal *t* haplotypes have been found: t^{Tuw10} and t^{w106} (this report and Dooher *et al.* 1981). The one found by us is associated with the same *H-2* haplotype as the semilethal haplotypes found in Michigan and in Israel, suggesting that there might be an evolutionary connection among the three. Although the identity of semilethal *t* haplotypes found in different areas cannot be established, the fact that they are all associated with only one or two *H-2* haplotypes suggests that they might be all related to one another.

The $t^{Lub\text{-}1}$ haplotype occurs on both sides of the Alps in mouse populations that are apparently related because they share Robertsonian translocations (Adolph & Klein, 1983; Capanna *et al.* 1976). Although the two regions are separated by a formidable physical barrier, it should be noted that they have been connected since prehistoric times by old trading routes (Müller-Beck, 1983).

Several *t* haplotypes occur in southern Germany but they all seem to be related to one another. The t^{Tuw2}, t^{Tuw25} and t^{Tuw27} haplotypes are related by the sharing of at least one lethality factor (Table 4), and by their association with the same *H-2* haplotype (Table 1, Sturm, Figueroa & Klein, 1982, and Nižetič, Figueroa & Klein, 1984). The same *H-2* haplotype is also associated with t^{Tuw24}, and a very similar haplotype is associated with t^{Tuw28}. Thus there appear to exist, in this region, two families of *t* haplotypes, one related to $t^{Lub\text{-}1}$ and the other representing variants of the t^{Tuw2} haplotype.

The region around Barcelona, Spain, is characterized by the occurrence of the t^{Tuw12} haplotype. Interestingly, the *H-2* haplotype present in this *t* chromosome is indistinguishable from that associated with t^{Tuw11}, found in Chile. Thus, again, there is a hint that the *t* haplotypes in the coastal regions of western Europe are the same as those found on the American continent, and that the colonization of the Americas by man and mice went hand in hand.

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Archaeologists believe that agriculture has spread into central and western Europe in two courses originating in the Near East (McNeill, 1963). One course travelled through the Mediterranean and via Spain and Italy into central Europe; and the other took the northern route through Turkey and Russia. They both met approximately where today the borders between *M. domesticus* and *M. musculus* lie. As it is widely believed that mice were introduced into Europe with agriculture, the borders between the two species or subspecies might be some 5000–7000 years old (McNeill, 1963). The geographical distribution of the *t* haplotypes, too, suggests that certain regions might have been occupied by the same populations since prehistoric times.

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