

A *t*-haplotype (t^{w75}) overlapping two complementation groups

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(Received 12 March 1979)

SUMMARY

The recessive lethal T/*t*-complex haplotype t^{w75} was isolated from Jena, East Germany, prior to 1973. It was at first thought to be a standard member of the t^{w5} complementation group because matings of $T/t^{w75} \times T/t^{w5}$ produced tailless offspring only (Dunn, Bennett & Cookingham, 1973). Cross tests with all other complementation groups and embryological studies have recently been completed, with results demonstrating that t^{w75} does not complement the members of either the t^{w5} or the t^{w1} complementation groups. Histological studies show that compound embryos t^{w75}/t^{w5} and t^{w75}/t^{w1} die with symptoms indistinguishable from t^{w5}/t^{w5} and t^{w1}/t^{w1} homozygotes respectively. Thus t^{w75} represents the first confirmed isolation of a *t*-haplotype that overlaps two complementation groups.

1. INTRODUCTION

Recessive lethal *t*-haplotypes in the T/*t*-complex have traditionally been defined by genetic complementation tests. These tests take advantage of the fact that crosses between tailless (T/t) mice carrying the same lethal recessive chromosome behave as balanced lethal systems, and produce viable offspring of the T/t genotype only. Recessive haplotypes are identified as different from one another when crosses between tailless animals of different origin ($T/t^x \times T/t^y$) produce an additional class of viable normal-tailed t^x/t^y progeny. Six different complementation groups have so far been defined by these criteria, each containing from one to more than a dozen independently isolated members. As a general rule, with one apparent exception noted below (Dunn & Bennett, 1971), complementation has been found to be non-overlapping; that is, members of any one group fail to complement all members of that group, and only the members of that group. Likewise, the embryological effects in homozygotes were found to be unique in each complementation group, and shared by all members (Bennett, 1975 for review). We report here new studies on the t^{w75} haplotype, which falls by genetic test into two different complementation groups (t^{w5} and t^{w1}) and produces lethal homozygous embryos of the t^{w5} type; in crosses to t^{w5} and to t^{w1} the lethal phenotype resembles respectively t^{w5} and t^{w1} . The implication of these findings for the structure and origin of lethal *t*-haplotypes is discussed.

2. MATERIALS AND METHODS

(i) *Mice*

All lethal *t*-haplotypes are maintained in balanced lethal stocks ($T/t^x \times T/t^x$) such that the only viable offspring expected in the absence of exceptional recombination are also tailless. Cross tests for complementation analysis are made by mating tailless mice derived from two different balanced lethal stocks ($T/t^x \times T/t^y$). If only tailless mice are produced, t^x/t^y is lethal and t^x and t^y are said to be members of the same complementation group. If t^x/t^y survives, normal-tailed offspring are born and t^x and t^y can be assigned to two different complementation groups.

(ii) *Embryos*

The presence of a vaginal plug was considered day 0 of gestation. $T/t^{w75} \times T/t^{w75}$ and $T/t^{w75} \times T/t^{w5}$ conceptuses were dissected on day 7 and $T/t^{w75} \times T/t^{w1}$ on day 10. The younger embryos were restaged at dissection according to Sobotta's (1911) criteria, fixed in Bouin's and embedded in paraffin. Serial 6 μm thick sections were cut and stained with haematoxylin and eosin.

3. RESULTS

The two lethal haplotypes t^{w5} and t^{w1} are those found most often in wild populations, with t^{w5} actually by far the more frequent. When t^{w75} was first isolated from a population in Jena, East Germany, and tested for complementation group (Dunn *et al.* 1973), cross tests of $T/t^{w75} \times T/t^{w5}$ produced 170 tailless offspring and no complementing t^{w5}/t^{w75} animals. The conclusion was drawn correctly for the specific instance (but misleading in the long run) that t^{w75} was yet another typical member of the common t^{w5} group, and further cross tests with other haplotypes were not done. We recently crossed T/t^{w75} by T/t^{w1} for other reasons and, as Table 1 shows, found that t^{w75} also fails to complement t^{w1} . Further tests made it unequivocally clear that t^{w75} behaves by genetic test as a member of both the t^{w1} and t^{w5} complementation groups but does complement all others (Table 1). This raised the interesting question of whether the t^{w75} haplotype contains two separate lesions identifiable as the specific lethal factors of t^{w5} and t^{w1} , or whether it represents another more general change.

Since the phenotypes of embryos homozygous for either t^{w5} or t^{w1} are strikingly different, both in terms of time of death and histological abnormalities, examination of embryos segregating from inter-crosses of T/t^{w75} animals and from crosses of T/t^{w75} mice by either T/t^{w5} or T/t^{w1} mates provided a way of answering this question.

Embryos homozygous for t^{w5} are grossly recognizable as abnormal or dead by 8 days of gestation (Bennett & Dunn, 1958), whereas t^{w1} homozygotes do not begin to die until about 10 days (Bennett, Badenhausen & Dunn, 1959). Table 2 shows that litters examined at 8 days from inter-crosses of T/t^{w75} and from the cross of $T/t^{w75} \times T/t^{w5}$ both have a high proportion of abnormal embryos, and

Table 1. Summary of complementation tests of t^{w75} with other groups, presented as normal tail/tailless offspring

	t^{w1}	t^{w5}	t^0	t^9	t^{12}	t^{w73}	t^{wPA}^*
t^{w75}	0/184	0/188†	2/14	11/27	7/21	32/65	5/7
t^{w1}		422/715					

* 170 of the 188 were previously reported in Dunn *et al.* 1973.

† t^{wPA} is a new complementation group (Jean-Louis Guenet, personal communication).

Table 2. Histological observations on litters segregating for *t*-haplotypes

Crosses*	No. of litters	Total no. of embryos	Typical abnormalities	Observed (%)	Expected† (%)
$T/t^{w75} \times T/t^{w75}$	10	105	45	43	42
$T/t^{w75} \times T/t^{w5}$	7	78	33	42	43
$T/t^{w75} \times T/t^{w1}$	5	41	16	39	38

* Crosses between different *t*-haplotypes were made using males of both haplotypes.

† Percentage expected calculated from the known distorted transmission ratios for *t*-bearing males, which varied from 75–95%.

furthermore that litters obtained from $T/t^{w75} \times T/t^{w1}$ parents and examined at 11 days also have a high proportion of abnormal embryos at that stage. The percentage of abnormal embryos found in each cross corresponds well with that expected because of the transmission ratio distortion in the male parent. The conclusion can be made that the time of embryonic lethality in the first two crosses is consistent with that expected for t^{w5} homozygotes, and likewise that t^{w75}/t^{w1} embryos are dying at the time expected for t^{w1} homozygotes.

These results were confirmed by histological observations. At 7 days gestation, embryos homozygous for t^{w5} show extensive pycnosis and degeneration of embryonic ectoderm (epiblast) cells (Bennett & Dunn, 1958). Figure 1 compares at 7 days of gestation a t^{w5} homozygote, a t^{w75} homozygote, and an embryo obtained from the $T/t^{w75} \times T/t^{w5}$ cross; the three are clearly indistinguishable. There is little question that embryos with this degree of abnormality would be dead and virtually resorbed by 10 days of gestation. Homozygotes for t^{w1} show abnormalities at a much later stage; by about 10 days cells in the entire ventral half of the neural tube, brain and otic vesicles are pycnotic and dying (Bennett *et al.* 1959). In Fig. 2, two embryos respectively t^{w1}/t^{w1} and t^{w1}/t^{w75} are seen to have apparently identical specific defects.

Thus, the t^{w75} haplotype appears to contain the specific genetic defect typical of both t^{w1} and t^{w5} . The reason that t^{w75} homozygotes die at the stage typical of t^{w5} homozygotes is presumably because the t^{w5} lesion is the earliest acting one, and therefore there is no opportunity for their t^{w1}/t^{w1} defect to be expressed since no neural tube develops.

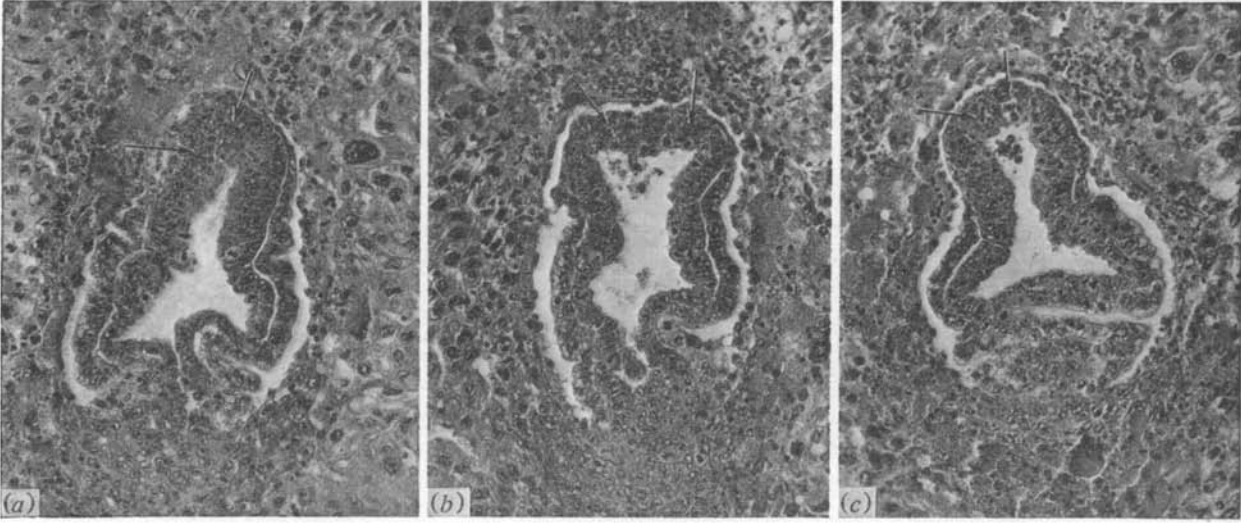


Fig. 1. Light micrographs of day 7 mutant embryos, sectioned longitudinally and stained with haematoxylin and eosin. All three embryos show a characteristic degeneration and pycnosis of the cells of the embryonic ectoderm (arrows). (a) t^{w5}/t^{w5} embryo; (b) t^{w75}/t^{w75} embryo; (c) t^{w75}/t^{w5} embryo. $\times 800$.

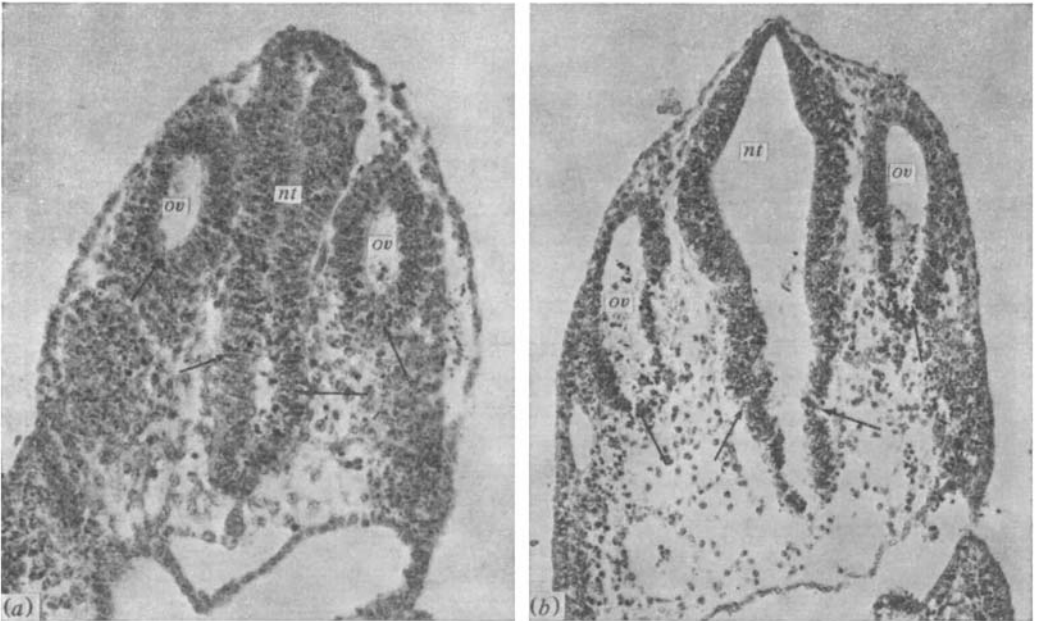


Fig. 2. Light micrographs of day 10 mutant embryos cut in cross-section and stained with haematoxylin and eosin. A degeneration of the cells (arrows) of the ventral portions of the neural tube (nt) and otic vesicles (ov) is obvious in both. (a) t^{w1}/t^{w1} embryo, $\times 800$; (b) t^{w1}/t^{w75} embryo, $\times 500$.

4. DISCUSSION

The t^{w75} haplotype does not show genetic complementation with two other lethal haplotypes with very different embryological effects, and thus must overlap them both. This is the first completely analysed case in which a lethal recessive *t*-haplotype has fallen sharply into two 'complementation groups' by both genetic and embryological criteria. An apparent precedent was reported by Dunn & Bennett (1971). In this case the t^{w74} haplotype isolated from a wild population in Denmark failed to complement either of two groups (t^{w73} and t^{w5}), but it was lost before more genetic tests and embryological studies could be completed. It is noteworthy that European populations of *Mus* may present a somewhat different sample of *t*-haplotype than North American ones. Prior to 1971 virtually all known wild haplotypes had been isolated from North American populations and included only t^{w5} , t^{w1} and $t^{semilethals}$ (Bennett, 1975). While these also exist in Europe, sampling there since 1971 has revealed t^{w73} , t^{wPA} (a new complementation group) (Guenet, personal communication) and the overlapping haplotypes t^{w74} and t^{w75} .

It is not clear whether t^{w75} represents a deletion that covers the lethal factors of both t^{w1} and t^{w5} , or whether it contains separate genetic lesions that are allelic to those in t^{w1} and t^{w5} . In the absence of any real knowledge of the structure of lethal *t*-haplotypes speculation is difficult. However, the very specific embryological interaction of t^{w75} with both t^{w5} and t^{w1} , the fact that t^{w75} complements all other *t*-lethals normally, and our observations that t^{w75} heterozygotes show none of the effects often associated with deletion-heterozygosity (Russell, 1962) argues against a deletion.

On the other hand, Lyon & Bechtol (1977) have described a lethal *t*-haplotype (t^{h20}) with characteristics in some ways similar to t^{w75} which they do interpret as a deletion. t^{h20} arose as a mutant on a chromosome carrying the t^6 haplotype, and was noted when mice having that chromosome heterozygous with one that carried the marker *tf* (a hair-loss mutation that maps about 7 units from *T*) were phenotypically tufted. This suggested either that the t^6 chromosome had undergone a mutation to *tf*, or that a deletion covering the region of *tf* had occurred. The deletion hypothesis was supported when it was shown that the t^6 (t^{h20}) chromosome produced embryonic lethality when opposite a chromosome carrying the mutation Knobbly; that mutation is closely linked to *tf* and is a homozygous lethal. With the idea that t^{h20} indeed must represent a deletion, since the elicitation of pseudodominance of two closely linked markers is generally accepted as a valid criterion (Russell, 1962), Lyon, Jarvis and Sayers (1979) went on to investigate the complementation interactions of t^{h20} with other *t*-haplotypes. They had already found (Lyon & Bechtol, 1977) that t^{h20} completely failed to complement its parent haplotype t^6 , so it appeared that t^{h20} represented an additional lesion on the original t^6 chromosome. Complementation tests with the haplotypes t^{w1} , t^{w5} , and t^{w32} revealed that t^{h20} was capable of some complementation with all of them, although it was much weaker than in the case of t^6 , being respectively about 50 %,

16 % and 5 % of that found for t^6 in their experiments. These results led Lyon and colleagues to suggest that the deletion in t^{h20} included the lethal factors present in t^{w1} , t^{w5} and t^{w32} . However, this situation would seem to be very unlikely since there is no precedent for assuming that complementation can occur between a recessive lethal gene and a deletion. Thus it seems that there is little rationale for assuming that t^{h20} is actually a deletion covering the lethal loci of t^{w1} , t^{w5} and t^{w32} , although to be sure the reason for weak complementation is also not clear. Lyon *et al.* (1979) do not present any embryological data that define the phenotype of lethal homozygotes of t^{h20} or of double heterozygotes of t^{h20} , t^{w1} , t^{w5} , and t^{w32} embryos, so it is not known why or when these poorly complementing genotypes die.

We also have found specific cases of very poor complementation between various different t -haplotypes. In the one instance that has been thoroughly investigated, Silagi (1962) showed that embryos carrying both t^0 and t^{12} , which had only about 10 % of normal viability at birth, died at a variety of stages from early to mid gestation. The dying embryos did not have any one genotype-specific abnormality such as has been found for homozygotes for recessive lethal t -haplotypes, although many of them showed resemblance to t^9/t^9 embryos.

The notion of a *cis-trans* test is implicit in this kind of reasoning but actually it is difficult to apply those criteria to t -haplotypes to decide whether they are lesions in the same gene or not. For example, the lethal factors t^0 and t^{12} mentioned above were classified as 'complementing' in the *trans* configuration and therefore considered as being mutations in separate genetic units by Dunn (1954) on the basis that t^0/t^{12} embryos were viable, even if only to the extent of 10 % of normal. In retrospect it would seem equally valid to classify them as different mutations in the same gene since 90 % of the embryos have a 'mutant' phenotype. The partial complementation that they do show must then represent intragenic complementation, the only precedent for which is in genes that control subunits of multimeric proteins.

Thus, the complexity of T/ t locus genetics prohibits a strict classification of the t^{w75} defect at this time. It is clear, however, that the t^{w75} mutation shares the genetic characteristics and embryological defects of two distinct 'complementation groups', t^{w5} and t^{w1} . This mutation represents a potentially informative haplotype for the elucidation of the genetic structure of lethal t -factors.

This work was supported by Grants CA-08748, CA-21651 from NIH; PCM 77-17835 from NSF and EE-77-S-02-4159 from DOE. Dr Bruce Babiarz was supported by NIH Fellowship 1 F32 HD-05647.

REFERENCES

- BENNETT, D. (1975). The T-locus of the mouse: a review. *Cell* **6**, 441–454.
- BENNETT, D., BADENHAUSEN, S. & DUNN, L. C. (1959). The embryological effects of 4 late lethal *t*-alleles in the mouse which affect the neural tube and skeleton. *Journal of Morphology* **105**, 105–144.
- BENNETT, D. & DUNN, L. C. (1958). Effects on embryonic development of a group of genetically similar lethal alleles derived from different populations of wild house mice. *Journal of Morphology* **103**, 135–158.
- DUNN, L. C. (1954). The study of complex loci. *Proc. Ninth International Congress of Genetics. Caryologia Suppl.* 155–166.
- DUNN, L. C. & BENNETT, D. (1971). Lethal alleles near Locus T in the house mouse populations on the Jutland Peninsular, Denmark. *Evolution* **25**, 451–453.
- DUNN, L. C., BENNETT, D. & COOKINGHAM, J. (1973). Polymorphisms for *t*-lethals in European populations of *Mus musculus*. *Journal of Mammalogy* **54**, 822–830.
- LYON, M. F. & BECHTOL, K. B. (1977). Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion. *Genetical Research* **30**, 63–76.
- LYON, M. F., JARVIS, S. E. & SAYERS, I. (1979) Complementation reactions of a lethal mouse *t*-haplotype believed to include a deletion. *General Research* **33**, 153–161.
- RUSSELL, L. B. (1962). Chromosome aberrations in experimental mammals. *Progress in Medical Genetics* **2**, 230–294.
- SILAGI, S. (1962). A genetic and embryological study of partial complementation between lethal alleles at the T-locus of the house mouse. *Developmental Biology* **5**, 35–67.
- SOBOTTA, J. (1911). Die Entwicklung des Eies der Maus vom ersten Auftreten des Mesoderms an bis zur Ausbildung der Embryonalanlage und dem Auftreten der Allantois. *Archiv für Mikroskopische Anatomie* **78**, 271–352.