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t^{wPa-1}, t^{wPa-2}, t^{wPa-3}: three new *t*-haplotypes in the mouse

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SUMMARY

t^{wPa-1} is a new lethal *t*-haplotype isolated in the South of France. Homozygous embryos die at the time of implantation or shortly thereafter following general disorganization. t^{wPa-1} complements all previously known *t*-haplotypes and has thus to be considered as the first representant of a new complementation group. t^{wPa-2} and t^{wPa-3} are viable, male sterile haplotypes, but t^{wPa-2} is low transmitter.

The *t* region of the house mouse is a segment of the 17th chromosome located between the centromere and the H-2 histocompatibility complex. Several types of genetical variations have been reported in this system which fall in two, very different, categories (Gluecksohn-Waelsch & Erickson, 1970; Bennett, 1975; Sherman & Wudl, 1977).

(*a*) The *T* alleles, which have been detected exclusively in laboratory animal colonies; they are dominant and produce a shortening of the tail with a wide variation in expressivity. They are invariably homozygous lethals.

(*b*) The *t*-haplotypes which constitute a large series of different recessive variants. They have been found segregating in wild populations or in laboratory stocks because (and only because) of their interaction with *T*, the phenotype of *T*/*t* double heterozygotes resulting in taillessness.

The recessive *t* haplotypes have been classified according to the viability of the *t*/*t* homozygotes. Some are lethals *in utero*, others are more or less viable (the semi-lethals), finally others are viable and behave normally in all respects, except for the interaction with *T*.

On the basis of complementation tests (intercross matings $T/t^x \times T/t^y$), the lethal haplotypes have been sorted out in six different groups (t^0, t^{w1}, t^{w5}, t^9, t^{12} and t^{w73}). When homozygotes, the members of a given group die with a particular and unique sequence of events in opposition with the double heterozygous conceptuses which are viable although male-sterile. In contrast with the lethal ones, most (but not all) of the viable *t*-haplotypes have been found as a result of a genetical change (cross-over or mutation) in stocks carrying a *t*-haplotype. Being viable these haplotypes belong to the same complementation group. They are usually fertile.

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We report here on three new *t*-haplotypes* (t^{wPa-1}, t^{wPa-2}, t^{wPa-3}) which have been found in France in wild mouse populations and exhibit original properties.

(i) t^{wPa-1}, *a new lethal haplotype*

The t^{wPa-1} haplotype was discovered in a wild male trapped in Villeneuve-sur-Lot (France). The taxonomical identification (kindly performed by Dr Louis Thaler) showed the animal to be a member of the *Mus musculus brevirostris* group (Mus I) (Bonhomme *et al.* 1978). The animal was light bellied agouti (A^w/A^w).

When mated with $T/+$ females of a moderately inbred laboratory stock (BTBR/Nev F) obtained from Dr Karen Artzt (New York), this male sired three types of progenies: 106 normal-tailed, 101 tailless and one single short-tailed newborn, out of a total of 208 offspring classified.

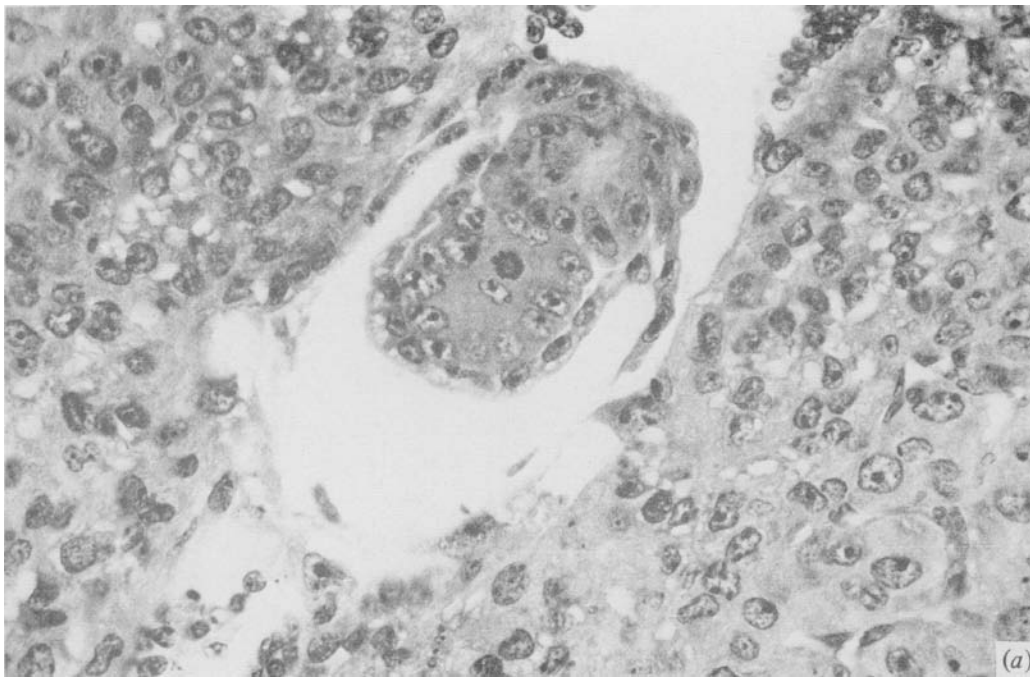
When intercrossed, the tailless F1 bred true as do the T/t double heterozygotes which carry a lethal *t*-haplotype. More than six hundred tailless and no normal-tailed young have been obtained so far in such intercrosses. This result indicates that crossing over occurs at a very low rate (if at all) between T and the lethal factor of t^{wPa-1}. This could be due either to close linkage between these two loci or to strong cross-over suppression in this region (a situation known to occur in most of the lethal *t*-haplotypes).

In order to define the lethal phenotype of t^{wPa-1}/t^{wPa-1} homozygous embryos, a stock of random bred $+/t^{wPa-1}$ mice was prepared. $+/t^{wPa-1}$ females which had been mated with $+/t^{wPa-1}$ males were sampled for dissection at various stages of pregnancy. Preliminary observations carried out at day 15 of embryogenesis indicated that, in addition to normal uterine implantations, moles were also present, with no recognizable embryonic structure in them. On the 7th day of pregnancy, 73 implantations with normal decidual components were found in a total of 10 $+/t^{wPa-1}$ female mice. In 45 decidua, microscopic dissection revealed a normal late cylinder stage (occasionally early primitive streak stage). The remaining 38 decidua, however, only yielded disorganized trophoblastic remnants, with no other embryonic structure recognizable under the binocular. These results point, therefore, to a segregation of normal and sterile implantations close to 1:1 in litters from $+/t^{wPa-1}$ females mated with $+/t^{wPa-1}$ males. This fits well with the expected segregation of $+/t^{wPa-1}$ and t^{wPa-1}/t^{wPa-1} embryos in such crosses, considering the transmission ratio of t^{wPa-1} versus + haplotypes in $t^{wPa-1}/+$ males (see below).

To study the phenotype of the lethal t^{wPa-1}:t^{wPa-1} embryos, serial histological sections of the uterine horns of pregnant $+/t^{wPa-1}$ mothers have been prepared.

A typical litter examined on day 6 consisted of 6 embryos. While no anomaly was evident on 3 of these in sections, the remaining three were quite runted or even (in one case) disorganized (Plates 1 and 2). Runted embryos consisted of 3 superimposed ectoblastic masses, with ectoplacental cone, extraembryonic and embryonic ectoderm still recognizable. Many dead cells were present in all areas

* In the nomenclature *wPa*, *w* stands for wild, *Pa* for Pasteur Institute.



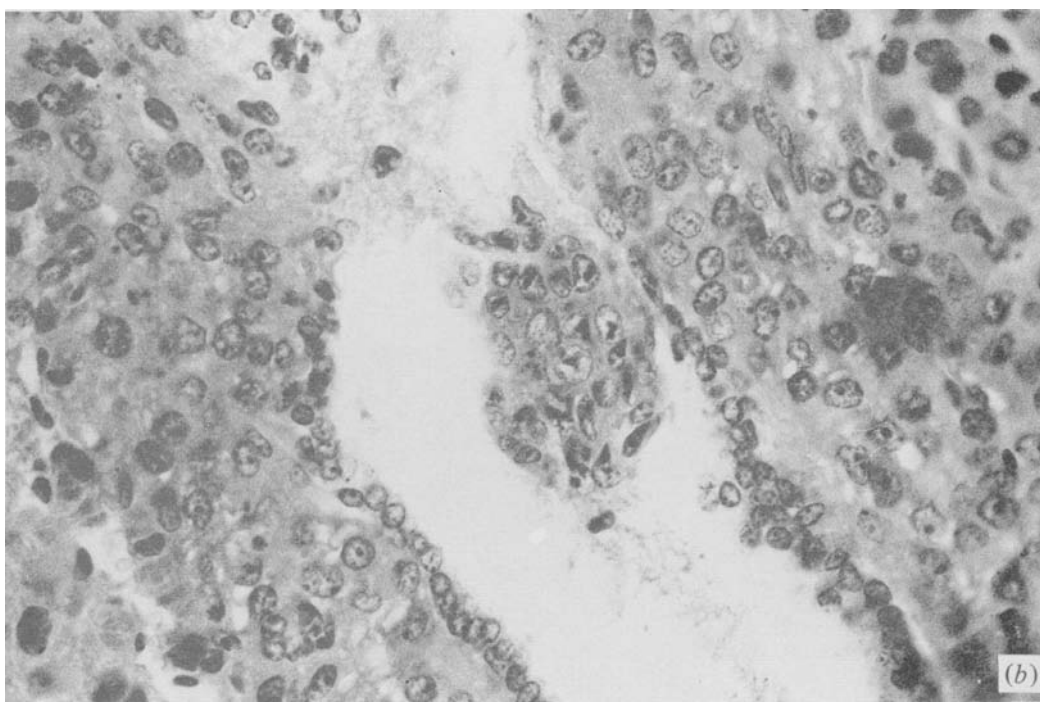


PLATE 1

Two 5½ day embryos from a $t^{wPa-1}/+$ female mated with a $t^{wPa-1}/+$ male. (1*a*) Embryo with normal appearance. (1*b*) Maximal section through an abnormal, presumably homozygous, t^{wPa-1}/t^{wPa-1} embryo.

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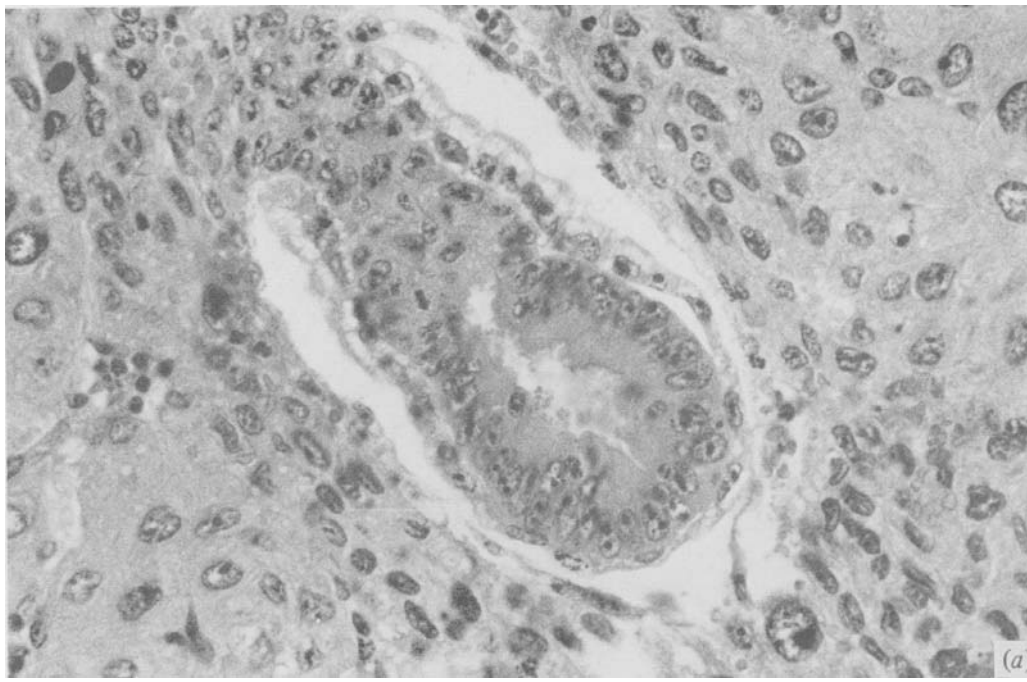

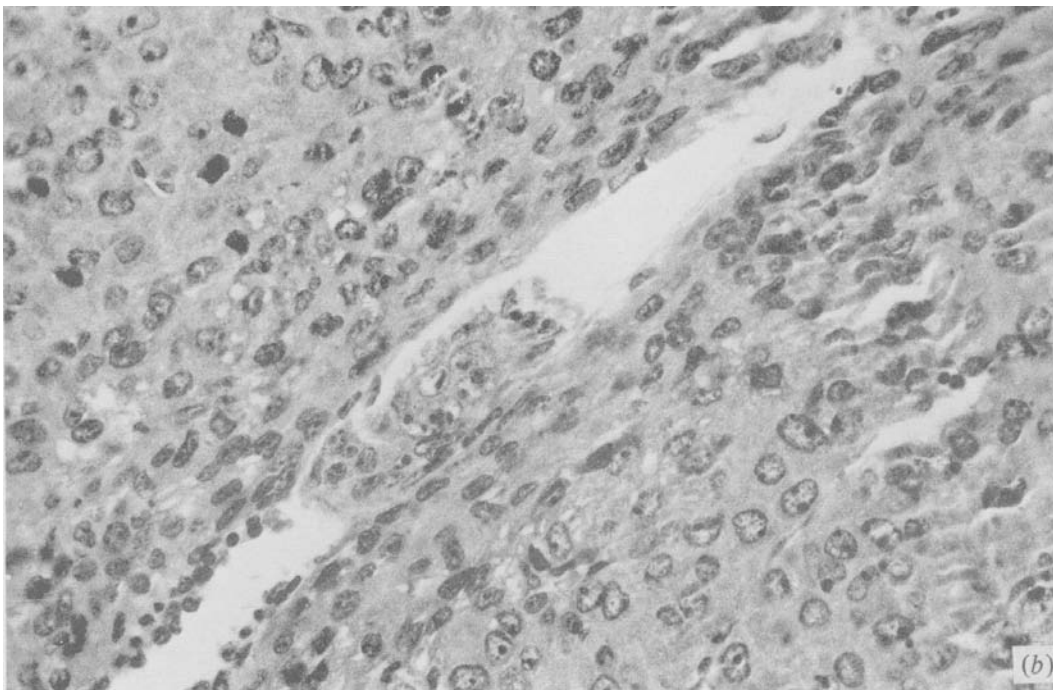

PLATE 2

Two $6\frac{1}{2}$ day embryos from a $t^{wPa-1}/+$ female mated with a $t^{wPa}/+$ male. (2*a*) Embryo with normal appearance. (2*b*) Maximal section through an abnormal, presumably homozygous, t^{wPa-1}/t^{wPa-1} embryo. Although ectoplacental cone, embryonic ectoblast and endoderm are still recognizable, their arrangement is desultory.

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of the embryo. The yolk sac cavity was clearly identifiable, with distal endoderm, however, difficult to recognize. In contrast, proximal endoderm was conspicuous, with possibly hypertrophic cells. The Reichert's membrane was seen not fully differentiated. A few primary trophoblast giant cells were present in contact with the decidua, but separated from the embryo by blood suffusion. Clearly, the development of these embryos was about to be (or already) stopped. Similar anomalies have been observed in an even more pronounced state on day 7. Complete resorption seems to take place around day 8.

Table 1. *Complementation tests with* t^{wPa-1}

Genotype of the female	Genotype of the male	Normal tailed offspring	Tailless offspring
$Ttf/t^{wPa-1}+$	$T+/t^{12}+$	3	11
$Ttf/t^{wPa-1}+$	$Ttf/t^{w32}+$	12	33
$Ttf/t^{w73}+$	$Ttf/t^{wPa-1}+$	11*	25*
$Ttf/t^{wPa-1}+$	$Ttf/t^{w73}+$ ‡	7†	17†
		2	6
$Ttf/t^{0}+$	$Ttf/t^{wPa-1}+$	2	5
$Ttf/t^{wPa-1}+$	$Ttf/t^{0}+$	3†	11†
		2	6
$Ttf/t^{w5}+$	$Ttf/t^{wPa-1}+$	14	29
$Ttf/t^{w18}+$	$Ttf/t^{wPa-1}+$	8	5
$Ttf/t^{wPa-1}+$	$Ttf/t^{w18}+$ ‡	6	28
$Ttf/t^{w1}+$	$Ttf/t^{wPa-1}+$	8	13
$Ttf/$ ^{w12} tf	$Ttf/t^{wPa-1}+$	1	1
$Ttf/t^{wPa-1}+$	Ttf/t^{Lub-1}§	2	3
$Ttf/t^{wPa-1}+$	$Ttf/t^{wPa-1}+$	0	217

* Tests done by Dr K. Artzt in New York City.

† In utero examination at 18 days of gestation.

‡ $Ttf/t^{w18}+$ and $Ttf/t^{w73}+$ males are normal (or low) transmitters at the Institut Pasteur.

§ t^{Lub-1} is a lethal *t*-haplotype discovered by H. Winking (1978) in a multimetacentric mouse population.

t^{wPa-1} and the t^{w75} haplotype show complementation (Arzt, Babiarz & Bennett, 1979).

In conclusion, it appears that the t^{wPa-1} haplotype does not prevent the homozygous embryos from being implanted. Disorganization and death, however, follow shortly, within about 24 h. Furthermore, from histological examination, no particular tissue can be identified in the embryos as an evident target for the lethal factor of t^{wPa-1}.

Complementation tests have been carried out as usual by mating tailless $Ttf/t^{wPa-1}+$ progenitors with a series of tailless animals belonging to the already known complementation groups. The results of these tests are summarized in table 1. In all instances, normal tailed offspring (t^{x}/t^{wPa-1}) have been recorded, none of which appeared to be runted in contrast to what is found with some other combinations (t^{0}/t^{12} or t^{0}/t^{w73} for example).

All of the t^{x}/t^{wPa-1} compound males studied (at least one for each of the *t*-haplotypes) have invariably been found to be sterile and thus conform to the general rule.

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Heterozygous males T/t^{wPa-1} exhibit a high distortion in the transmission ratio of the t and + haplotypes (Table 2). The t^{wPa-1} haplotype can thus be classified as a high distorter ($0{\cdot}90 \pm 0{\cdot}04$). From this point of view, however, the original male was apparently still higher in distortion since it sired only one $T/+$ * animal among more than 200 offspring.

(ii) t^{wPa-2} *and* t^{wPa-3}*: two viable alleles*

The t^{wPa-2} haplotype was discovered in a wild male trapped in Galeria (Corsica island). This animal was A^w/A^w and classified as *Mus musculus brevirostris*

Table 2. *Testcross progenies of* t^{wPa-1}, t^{wPa-2} *and* t^{wPa-3} *heterozygous males*

Genotype of the male	Genotype of the females B6CBA F1	Phenotype of the offspring Classified at birth	
		Short tailed	Normal tailed
T/t^{wPa-1}	+/+	14	132
T/t^{wPa-2}	+/+	80	29
T/t^{wPa-3}	+/+	6	107

Table 3. *Progenies of intercross matings with* t^{wPa-3} *and* t^{wPa-2}

Type of matings		Phenotype of progeny	
Male	Female	Tailless	Normal tailed
T/t^{wPa-2}	T/t^{wPa-2}	19 (17)	4 (6)
T/t^{wPa-3}	T/t^{wPa-3}	14 (16)	17 (15)

Note: The bracketed values correspond to the theoretical expectations on the basis of full viability of both haplotypes.

(Mus 1). When mated in the usual way with $T/+$ females, he sired occasional tailless progenies among a majority of short-tailed and normal-tailed animals. Out of a total of 37 young animals, 19 have been found normal-tailed, 14 short-tailed and 4 tailless. When intercrossed, the tailless F1 animals produced only tailless and normal-tailed offspring but no short-tailed, as expected from a viable t-haplotype (Table 3).

The normal tailed male offspring (t^{wPa-2}/t^{wPa-2}) are sterile. None has bred so far in spite of repeated matings with known prolific females. In contrast, the females breed normally.

The transmission ratio of the t^{wPa-2} haplotype has been calculated by examination of the testcross progenies of T/t^{wPa-2} tailless males and has been found to be $0{\cdot}26 \pm 0{\cdot}03$. The $t^{wPa-2}/+$ males are thus low transmitters of the t-haplotype (see Table 3).

The t^{wPa-3} haplotype has been found in a wild male trapped in Issus close to Toulouse in France. The male was A/A and classified as belonging to the *Mus*

* This animal was kept for the establishment of a stock homozygous for the original wild 17th chromosome carrying the + haplotype.

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musculus domesticus (group Mus 1). Mated with a short-tailed female, this male remained sterile for several months; then it produced two progenies and died. The first progeny was composed of three young (one tailless female, and two normal-tailed: one of each sex); the second progeny was composed of four young (one short-tailed female and three normal-tailed animals, two males, one female).

The tailless female and a normal-tailed male of the second progeny were then intercrossed and produced several additional tailless offspring. When intercrossed, these tailless animals behave as if they were heterozygous for a further viable *t*-haplotype. The normal-tailed male offspring again are sterile while the females are fully fertile. Both sexes are normal and healthy and have even better growth performances than their tailless littermates.

The transmission ratio of the t^{wPa-3} haplotype has been calculated by examination of the testcross progenies of T/t^{wPa-3} tailless males and has been found to be $0{\cdot}94 \pm 0{\cdot}03$ (see Table 2).

1. DISCUSSION

The discovery of new *t*-haplotypes has several implications for the understanding of the t-complex.

(1) The wild mouse populations sampled and studied by L. C. Dunn and successive coworkers have originated (with some exceptions) from North America. From these populations, a limited number of haplotypes have been isolated (t^{w5}, t^{w1}, and a variety of semi-lethal or viable haplotypes) (Klein & Hammerberg, 1977). As the Mus genus has been imported to the new world from the Euro-asiatic continent (Chaline, 1977), one can consider that a systematic sampling of the European populations (and particularly those from the Mediterranean border) could contribute to a better knowledge of the population genetics of the *t*-locus. This report, that of Winking and coworkers on an Italian mouse population (1978) and the initial report by Dunn & Bennett (1971) on North European populations have revealed several new *t*-haplotypes which indicate that such studies might be fruitful.

(2) According to Dunn, Bennett & Cookingham (1973) and Bennett, Dunn & Artzt (1976), t^{w5} might represent the original or most ancient *t*-haplotype which may have arisen before the divergence of the several subspecies of the Mus genus. If this assumption, which is of cardinal interest for the evolutionists, is true, one should find t^{w5} itself and a series of its derivatives generated by recombination in non-American populations of mice.

(3) The *t* mutations have been considered by developmental biologists (Bennett, 1975) to specifically affect the transitions by which ectodermal derivatives undergo progressive differentiation. When the number of complementation groups was limited to six, a non-overlapping pattern of pathological differentiation was presented for each of these. This, however, could result from chance only, and if the number of *t* mutations increases the model might have to be reconsidered.

(4) According to Lewontin (1962, 1968), computer simulated models indicate

that no degree of transmission ratio distortion is sufficient to maintain lethal or semi-lethal alleles in equilibrium in wild mouse populations. D. Bennett (1975) thus suggested that the overall persistence of *t* alleles in wild populations must be attributed either to a process of continual extinction of small populations with subsequent replacement by migration from others or to some additional unknown selective advantage.

In the case of t^{wPa-2} (viable, male sterile, with low transmission ratio), the disadvantage seems to be at its maximum and the presence of such a *t* haplotype in the wild is paradoxical at first sight. Several possibilities could account for it.

(*a*) In view of results collected in Bennett's (1978) and author's (unpublished observations) laboratories, it is conceivable that the transmission ratio fluctuates among wild males according to unknown genetic determinants. In that case, high transmitter males alone would be responsible for maintenance and propagation of *t* haplotypes.

(*b*) Lethal, high transmitting *t* haplotypes are known to give rise, in the laboratory, to viable low transmitting types (Bennett *et al.* 1976). One would expect such a phenomenon to occur in the wild also. t^{wPa-2} would be the result of such a rare event and its presence in the wild purely transitory.

(*c*) Alternatively the presence of *t* haplotypes in wild population might be accounted for by a continuous production via abnormal recombination in the centromeric region of chromosome 17. Similar hypotheses have already been proposed for different reasons (Jacob, 1977; Lyon *et al.* 1979).

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