Genet. Res. Camb. (1985), **45**, *pp.* 251–264
Printed in Great Britain

Recombination between the t^6 complex and linked loci in the house mouse

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(*Received 31 December 1984*)

SUMMARY

Recombination between the t^6 complex, three allozyme-encoding loci, two antigen-encoding loci, and four molecular markers was studied. The allozyme-encoding loci were complement component-3 (*C-3*), kidney catalase (*Ce-2*), and glyoxalase-1 (*Glo-1*); the antigen-encoding loci were the *H-2* Class I genes *H-2K* and *H-2D*; the four molecular markers were *Tu66*, an α-globin pseudogene (*Hba-4ps*), an unidentified *H-2* Class I gene, and the *H-2* Class II gene *I-Aβ*. The latter six loci were used as markers for the *t* complex. Recombination was detected between *Glo-1*, *Ce-2* and *C-3*, but not between the markers for the *t* complex *Tu66*, *Hba-4ps*, and the *H-2* loci. These data indicate that *Ce-2* and *C-3* are located outside the t^6 complex, while the latter are located within. These data also indicate that the telomeric boundary of the t^6 complex is located between *H-2* and *Ce-2*. Recently published studies have shown that complete gametic disequilibrium exists between the *t* complex and loci located centromeric to the *H-2* – *Ce-2* interval, while disequilibrium was not detected between loci located telomeric to this interval. Loci included within the region of recombination suppression are also those in disequilibrium with the *t* complex. As a result, recombination suppression probably resulting from chromosomal rearrangements associated with the *t* complex appears to be a sufficient explanation for the gametic disequilibrium observed between certain loci and the *t* complex.

1. INTRODUCTION

The *t* complex is a cluster of genes on Chromosome 17 of the house mouse with effects on recombination, meiotic transmission, embryonic viability, male fertility and gametic equilibrium (for reviews of the genetics and developmental biology of the *t* complex, see Bennett, 1975; Klein & Hammerberg, 1977; and Lyon, 1981). While many loci affect viability and fertility, the *t* complex is the only well-characterized example of transmission ratio distortion and one of the few examples of recombination suppression and gametic disequilibrium in house mice. However, since neither the genes nor the proteins involved have been identified, the mechanisms by which the *t* complex causes these diverse effects on genetics and development remain poorly understood.

Recently, however, progress has been made towards understanding the basis for recombination suppression between chromosomes bearing a *t* complex and

chromosomes bearing its wild-type homologue. Experiments by Artzt, Bennett, Shin, Silver and others (Silver & Artzt, 1981; Artzt, McCormick & Bennett, 1982*a*; Artzt, Shin & Bennett, 1982*b*); Shin *et al.* 1983) suggest that chromosome rearrangements are responsible for suppressing recombination. Because of the limited number of genetic markers available, however, it has not been possible to determine the kind, extent or number of rearrangements involved. Although a number of loci located on Chromosome 17 are potentially useful markers for characterizing these rearrangements, many have not been mapped relative to the *t* complex. To identify loci included within the *t* complex, recombination was studied in an outcross involving mice heterozygous for the t^6 complex and for three biochemical, two serological, and four molecular markers.

2. MATERIALS AND METHODS

(i) *Mice.* Mice of the following inbred or segregating inbred strains were used: AKR/J, C57L/J, MA/MyJ, SJL/J, TT6/Le-*T tf*/t^6 +. These were obtained from the research and production colonies of the Jackson Laboratory.

(ii) *Experimental design.* Three allozyme-encoding loci, two antigen-encoding loci, and four loci detectable with molecular probes were used as markers to study recombination. The allozyme-encoding loci were complement component-3 (*C-3*), kidney catalase (*Ce-2*), and glyoxalase-1 (*Glo-1*). The antigen-encoding loci were *H-2K* and *H-2D* Class I loci. The loci detected with molecular probes were *Tu66* (Röhme *et al.* 1984), an α-globin pseudogene (*Hba-4ps*) (Fox, Silver & Martin 1984; d'Eustachio *et al.* 1984), an unidentified *H-2* Class I gene (Evans *et al.* 1982; Margulies *et al.* 1982), and an *H-2* Class II gene (*I-Aβ*) (Choi *et al.* 1983; Robinson *et al.* 1983). The chromosomal location of these and other relevant loci on wild-type chromosomes is given in Fig. 1. Two approaches could be used for studying recombination between these loci: (*a*) a series of crosses each involving some but not all markers, or (*b*) a single nine-point cross. The second alternative was used. The advantage of the latter alternative is that multiple recombination events can be detected; the disadvantage is that a chromosome having alternative alleles for each locus was needed.

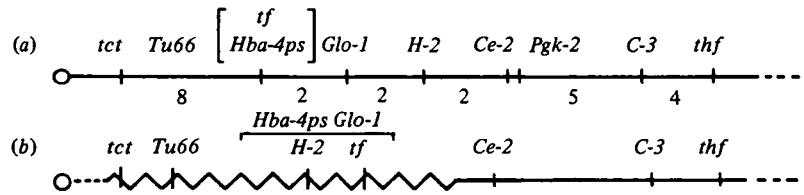

Fig. 1. Chromosomal location of relevant loci in wild-type chromosomes (a) and in chromosomes with a t^6-complex (b). *H-2* denotes the location of both the Class I and the Class II loci studied. (*a*) Brackets indicate that the gene order of *tf* and *Hba-4ps* has not been determined. Recombination frequencies in centiMorgans are also given (cf. Davisson & Roderick, 1981). (*b*) The waved line at the centromeric end of the chromosome denotes the region in which recombination is suppressed between the t^6 complex and its wild-type homologue. The bracket indicates that the locations of *Hba-4ps* and *Glo-1* relative to *H-2* and *tf* have not been determined. Preliminary studies suggest but do not firmly establish that *Pgk-2* is located outside the t^6 complex.

(a) Crosses to produce the tester chromosome:

MA/MyJ female × SJL/J male

$$\frac{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a}{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a} \times \frac{Glo\text{-}1^a\ H\text{-}2^s\ I\text{-}A\beta^s\ Ce\text{-}2^a\ C\text{-}3^b}{Glo\text{-}1^a\ H\text{-}2^s\ I\text{-}A\beta^s\ Ce\text{-}2^a\ C\text{-}3^b}$$

MA/MyJ female × F_1 male

$$\frac{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a}{Glo\text{-}1^a\ H\text{-}2^s\ I\text{-}A\beta^s\ Ce\text{-}2^a\ C\text{-}3^b}$$

Select recombinant between *Ce-2* and *C-3* and intercross

$$\frac{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^b}{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a} \times \frac{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^b}{Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a}$$

(b) Experimental crosses

Tester chromosome heterozygote

$$\frac{+^t\ Tu66^a\ Hba\text{-}4ps^b\ Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^b}{+^t\ Tu66^a\ Hba\text{-}4ps^b\ Glo\text{-}1^b\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a}$$

$$\times\ TT6/Le\text{-}(Tlf/t^6+)$$

$$\frac{T \quad \cdot \quad \cdot \quad Glo\text{-}1^a \quad \cdot \quad \cdot \quad Ce\text{-}2^a\ C\text{-}3^a}{tct\ Tu66^t\ Hba\text{-}4ps^t\ Glo\text{-}1^c\ H\text{-}2^{w30}\ I\text{-}A\beta^{w30}\ Ce\text{-}2^a\ C\text{-}3^a}$$

Select appropriate heterozygotes

$$\frac{tct\ Tu66^t\ Hba\text{-}4ps^t\ Glo\text{-}1^c\ H\text{-}2^{w30}\ I\text{-}A\beta^{w30}\ Ce\text{-}2^a\ C\text{-}3^a}{+^t\ Tu66^a\ Hba\text{-}4ps^b\ Glo\text{-}1^b\ H\text{-}2^k \quad I\text{-}A\beta^k \quad Ce\text{-}2^b\ C\text{-}3^b}$$

× C57L/J

$$\frac{+^t\ Tu66^a\ Hba\text{-}4ps^b\ Glo\text{-}1^a\ H\text{-}2^b\ I\text{-}A\beta^b\ Ce\text{-}2^a\ C\text{-}3^a}{+^t\ Tu66^a\ Hba\text{-}4ps^b\ Glo\text{-}1^a\ H\text{-}2^b\ I\text{-}A\beta^b\ Ce\text{-}2^a\ C\text{-}3^a}$$

× 129/Sv

$$\frac{+^t\ Tu66^a\ Hba\text{-}4ps^a\ Glo\text{-}1^a\ H\text{-}2^b\ I\text{-}A\beta^b\ Ce\text{-}2^a\ C\text{-}3^a}{+^t\ Tu66^a\ Hba\text{-}4ps^a\ Glo\text{-}1^a\ H\text{-}2^b\ I\text{-}A\beta^b\ Ce\text{-}2^a\ C\text{-}3^a}$$

× AKR/J

$$\frac{+^t\ Tu66^a\ Hba\text{-}4ps^a\ Glo\text{-}1^a\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b C\text{-}3^a}{+^t\ Tu66^a\ Hba\text{-}4ps^a\ Glo\text{-}1^a\ H\text{-}2^k\ I\text{-}A\beta^k\ Ce\text{-}2^b\ C\text{-}3^a}$$

Test progeny for recombination

(c) Control crosses (see above for genotypes)

Tester homozygote × C57L/J

F_1 × C57L/J, 129/Sv, AKR/J

Test progeny for recombination

Fig. 2. Genetic crosses used (*a*) to produce the tester chromosome (see text) and (*b*) to study recombination. Loci have been listed in order from the centromere (left) to the telomere (right) for a wild-type chromosome. Dot (·) indicates not typed.

Alleles of the nine markers in chromosomes bearing the t^6 complex are *Tu66*^{t}, *Hba-4ps*^{t}, *Glo-1*^{c}, *H-2*^{w30} (three Class I markers), *I-A*β^{w30}, *Ce-2*^{a}, and *Ce-3*^{a} (cf. Fox *et al.* 1984; Nadeau 1983; J. H. Nadeau, in preparation). Because none of the inbred strains at the Jackson Laboratory had alternative alleles for each of these nine markers, the appropriate chromosome had to be constructed. The breeding scheme used is outlined in Fig. 2*a*. MA/MyJ females, which have the alleles *Glo-1*^{b}, *Ce-2*^{b}

https://doi.org/10.1017/S0016672300022242 Published online by Cambridge University Press

and *C-3*^{a}, were crossed to SJL/J males, which have the alleles *Glo-1*^{a}, *Ce-2*^{a} and *C-3*^{b}. F_1 males of this cross were backcrossed to MA/MyJ females. Backcross progeny in which a crossover occurred between *Glo-1* and *C-3* were selected and typed for *Ce-2* by using partial nephrectomy to obtain a kidney sample for allozyme analysis. Those mice in which a crossover occurred between *Ce-2* and *C-3* but not between *Glo-1* and *Ce-2* were selected. This chromosome is hereafter designated tester chromosome (Fig. 2).

Females heterozygous for the tester chromosome and the standard nonrecombinant homologous chromosome from MA/MyJ were crossed to TT6/Le-*T tf*/*t*^{6}+ males (Fig. 2B). Eight male and eleven female progeny with normal tails, i.e. heterozygous for the *t*^{6}-bearing chromosome and for the tester chromosome, were selected for use as heterozygotes in which to study recombination. Both male and female heterozygotes were sequentially crossed to C57L/J, 129/Sv, and AKR/J and several litters were obtained from each. *C-3* and *Glo-1* were typed in progeny of all three crosses and *Ce-2* only in crosses involving AKR/J. (See the electrophoresis section below for the reasons for these restrictions.) (Outcrosses to C57L/J and 129/Sv were originally made in order to type *Pgk-2*. Apparent irregularities were encountered in the typing, however, and the results have not been presented here.)

To assess the contribution of the tester chromosome to the results obtained, control crosses were made. In these crosses, Chromosome 17 bearing the *t*^{6} complex from TT6/Le-*T tf*/*t*^{6}+ was replaced by Chromosome 17 from C57L/J (Fig. 2). These heterozygotes were outcrossed to C57L/J, 129/Sv and AKR/J. *Glo-1*, *Ce-2*, and *C-3* were typed in progeny of these outcrosses (Fig. 2C).

(iii) *Electrophoresis*. Electrophoretic methods provided by E. M. Eicher (personal communication) were used to type *Ce-2* and *Glo-1* and methods described by Alper (1973) and Alper & Propp (1968) were used with modifications (J. Kömpf, personal communication) to type *C-3*. *Ce-2* was typed only in progeny of outcrosses to AKR/J because *Ce-2*^{b}, which is present in AKR/J, is a recessive allele.

The allozyme encoded by the *Glo-1* locus within the *t* complex has unusually low activity. This allele, which is designated *Glo-1*^{c}, will be described in detail elsewhere (J. H. Nadeau, in preparation).

(iv) *Molecular analysis*. Isolation of DNA. To prepare plasmid DNAs, equilibration centrifugation in cesium chloride-ethidium bromide gradients or alkaline lysis was used (Maniatis, Fritsch & Sambrook, 1982). To prepare mouse genomic DNA, one freshly dissected mouse spleen was gently homogenized in 3 ml 20 mM Tris pH 7·4, 10 mM-NaCl, 3 mM-$MgCl_2$ at 4 °C by using a Dounce homogenizer fitted with a 'B' pestle. The suspension of spleen cells was lysed by adding 1 ml 4X TNLB (5 % sucrose (w/w), 4 % NP-40 (w/w) in 20 mM Tris pH 7·4, 10 mM-NaCl, 3 mM-$MgCl_2$). After gently mixing, nuclei were recovered by centrifugation at 3500 ***g*** for 5 min. The nuclear pellet was redissolved by vortexing in 5 ml 50 mM sodium acetate, 10 mM-Na_2EDTA, pH 5·1. Nuclei were lysed by adding 0·5 ml 10 % SDS. The lysate was then phenol-extracted twice and chloroform-extracted. Two volumes of ethanol were added to precipitate the DNA. The DNA was redissolved in 2 ml H_2O and stored at −20 °C. This procedure routinely yielded 0·5–2·0 mg DNA per spleen.

Genomic DNA (4 μg/lane) was digested with excess restriction enzyme (20 units) for 3–6 h in buffer recommended by the enzyme supplier (Bethesda Research Labs, Gaithersburg, MD).

(v) *Electrophoresis and transfer.* Digested DNAs were electrophoresed in horizontal 1% agarose gels using 0·04 M Tris, 0·02 M sodium acetate, 0·001 M-Na_2EDTA, pH 7·2 as buffer. Gels were electrophoresed for 16–20 h at 30 V and then photographed after staining with ethidium bromide (0·5 μg/ml). Gels were prepared for transfer by soaking for 1 h in 0·2 M-NaOH, 0·6 M-NaCl, 0·15% thymol blue followed by neutralization for 1 h in 1 M-Tris pH 7·4, 0·6 M-NaCl. Transfers were set up according to the methods of Southern (1975) except that 10 × SSC was used as the transfer buffer and nylon Zeta-bind (AMF-Cuno, Stamford, Conn.) was used as the recipient filter. Filters were baked *in vacuo* at 80 °C until dry (20 min to 2 h).

(vi) *Preparation of probes and hybridization.* Plasmid DNAs were nick-translated using ^{32}P (Maniatis, Jeffrey & Kleid, 1975) and labelled DNA was separated from unincorporated nucleotides by means of a 5 ml G-100 column (Pharmacia, Piscataway, NJ). After baking, the nylon filter was washed with three changes of 0·1 × SSC, 0·5% SDS at 65 °C for 1 h, and then presoaked in 5% Denhardt's, 5 × SSCP (20 × SSCP = 0·3 M sodium citrate, 2·3 M-NaCl, 0·3 M-Na_2HPO_4, pH 7·4) and 50% formamide (MCB FXO/420, EM Science, Gibbestown, NJ) at 42 °C for 2–5 h. Filters were hybridized using $1–3 \times 10^6$ cpm/ml of nick-translated probe in 6 × SSC, 1% Denhardt's, 50% formamide, 0·5% SDS, and 1 × S256 (10 × S256 = 8 μg/ml poly A, 8 μg/ml poly C, 200 μg/ml yeast tRNA, 10 μg/ml E. coli DNA, and 50 μg/ml salmon sperm DNA in 30 mM-Tris, pH 8·1) at 42 °C for 16–20 h. Unbound probe was removed by washing in 2 × SSC, 0·1% SDS at room temperature for 30 min and then at 52 °C through several changes of 0·1 × SSC, 0·1% SDS. Filters were air-dried and exposed to Kodak XAR film with Dupont Lightening-plus intensifying screens at −70 °C for 2–4 days.

(vii) *Serological typing of* H-2. *H-2* phenotypes were determined by using a microcytotoxicity assay with monoclonal and conventional *H-2* specific antibodies. A one-step complement mediated dye-exclusion test was carried out in 60-well microtiter plates (Falcon Plastics, Los Angeles, CA). Mice were bled from the retro-orbital sinus and peripheral blood lymphocytes were isolated from blood samples by using lymphocyte separation medium (Litton Bionetics, Charleston, SC). The cells were washed and suspended in Hank's balanced salt solution supplemented with 5% foetal calf serum and 25 mM-HEPES buffer at a concentration of 3×16^6 cells ml^{-1}. In each well of a microtiter plate, 1 μl of an appropriate dilution of the antibody and cell suspension was mixed and 2 μl of a diluted preselected rabbit complement was added. The plates were incubated for 1 h at 37 °C. After incubation, 4 μl of a 0·2% solution of Trypan Blue was added, the cells were fixed with 5% formaldehyde and the results evaluated by estimating the percentage of dead (stained) cells in each well. Background levels of staining were always kept under 5%. The reaction was considered to be weakly positive if 20–33% of the cells were stained, positive if 34–67% of the cells were stained, and strongly positive if more than 67% of the cells were stained.

Hybridoma cell lines 11–4·1 (reacts with antigenic determinant m93 on K^k

molecule) (Oi *et al.* 1978), 16–3–22 (m93, K^k), 14–4–4 (m7, $E\alpha^k$), 17–3–3 (m26, $E\beta^k$:$E\alpha^k$), 15–5–5 (m91, D^k), 20–8–4 (m57, K^b), 28–8–6 (m61, K^b), 25–9–3 (m20, A^b), and 28–14–8 (m64, D^b) (Ozato, Mayer & Sachs, 1980) were gifts from Drs David H. Sachs and Keiko Ozato. Ascites fluids were produced in mice by injecting them with hybridoma cells and subsequently used in serological tests. Conventional antisera specific for H-2^{w30} antigens and for H-2^s antigens were produced in our laboratory by repeated injections of donor spleen cells into appropriate recipient mice.

3. RESULTS

A total of 503 progeny in the experimental crosses and 254 progeny in the control crosses were used to study recombination. Since the data consist of groups of animals tested for some markers but not others, the results were presented separately for each of these groups.

(i) *Recombination involving* Glo-1 *and* C-3. In experimental crosses, a total of 503 mice consisting of 234 progeny of heterozygous males and 269 progeny of heterozygous females were typed for both *Glo-1* and *C-3* (Table 1). Chi-square contingency tests were used for identifying data that can be justifiably pooled for calculating recombination frequencies. Among progeny of heterozygous males in experimental crosses, significant heterogeneity in the frequency of recombination between *Glo-1* and *C-3* ($\chi^2_2 = 0{\cdot}28$, $P > 0{\cdot}005$) was not detected (Table 2). The absence of significant heterogeneity in recombination frequencies justified pooling data from the three outcrosses for estimating the recombination frequency between *Glo-1* and *C-3*; this estimate was $17{\cdot}1 \pm 2{\cdot}5\,\%$.

Among progeny of heterozygous females in experimental crosses, significant heterogeneity of recombination frequencies between the three outcrosses was observed ($\chi^2_2 = 6{\cdot}38$, $P < 0{\cdot}025$; Table 2). This heterogeneity resulted primarily from an increased recombination frequency between *Glo-1* and *C-3* among progeny of outcrosses to C57L/J ($\chi^2_1 = 4{\cdot}66$, $P < 0{\cdot}05$; Table 2). As a result, the recombination frequency between *Glo-1* and *C-3* in heterozygous females was calculated for C57L/J alone and for 129/Sv and AKR/J together. These estimates were $34{\cdot}9 \pm 7{\cdot}3\,\%$ and $19{\cdot}9 \pm 2{\cdot}7\,\%$, respectively.

In control crosses 254 mice consisting of 99 progeny of heterozygous males and 155 progeny of heterozygous females were typed for *Glo-1* and *C-3* (Table 1). Significant heterogeneity of recombination frequencies was not detected among progeny of heterozygous males, among progeny of heterozygous females, or between sexes (Table 2; results of the statistical analysis are not shown). For the pooled data the estimated recombination frequency between *Glo-1* and *C-3* was $13{\cdot}8 \pm 2{\cdot}2\,\%$ and was not significantly different from the estimates obtained in the experimental crosses (males: $\chi^2_1 = 1{\cdot}22$, $P > 0{\cdot}05$; females: (129/Sv and AKR/J only) $\chi^2_1 = 3{\cdot}20$, $P > 0{\cdot}05$). Thus, presence of the t^6 complex in experimental crosses did not significantly reduce the recombination frequency between *Glo-1* and *C-3*.

Production of the tester chromosome yeilded an independent estimate of the recombination frequency between *Glo-1* and *C3* for backcrosses of (MA/MyJ × SJL/J) F_1 to MA/MyJ. This estimate was $6{\cdot}1 \pm 1{\cdot}4\,\%$ ($N = 313$) and was signifi-

Table 1. *Recombination between* Glo-1 *and* C-3: *Allelic combination inherited from heterozygous males and females outcrossed to one of three inbred strains**

Allelic combination	Heterozygous males outcrossed to:			Heterozygous females outcrossed to:		
	C57L/J	129/Sv	AKR/J	C57L/J	129/Sv	AKR/J
	Experimental cross: results for *Glo-1* and *C-3*					
Glo-1^c C-3^a	107	29	43	18	49	60
Glo-1^b C-3^b	2	5	8	10	31	41
Glo-1^c × C-3^b	23	6	7	8	7	10
Glo-1^b × C-3^a	1	1	2	7	18	10
Experimental cross: results for *Glo-1*, *C-3*, and the four loci detected with molecular probes†						
Mt Glo-1^c C-3^a	12	7	5	1	16	12
M^w Glo-1^b C-3^b	1	2	5	2	12	25
M^t × Glo-1^b C-3^b	0	0	0	0	1	1
M^w × Glo-1^c C-3^a	0	0	0	0	0	1
M^t Glo-1^c × C-3^b	12	3	7	2	6	8
M^w Glo-1^b × C-3^a	1	1	1	1	12	10
	Control cross: results for *Glo-1* and *C-3*					
Glo-1^a C-3^a	22	11	17	—‡	14	54
Glo-1^b C-3^b	14	8	16	—	15	48
Glo-1^a × C-3^b	3	0	2	—	2	7
Glo-1^b × C-3^a	2	3	1	—	2	13

* × indicates the interval in which recombination has occurred.

† Since recombination was not detected between *Tu66*, *Hba-4ps*, *I-Aβ* and *H-2*, these loci were treated as a single marker designated *M^t* or *M^w*, where *t* and *w* designated the combination of alleles originally associated with the *t^6* complex or its wild-type homologue, respectively.

‡ Dash (—) indicates not done.

cantly less than the estimate obtained both for the experimental cross ($\chi_1^2 = 24{\cdot}60$, $P < 0{\cdot}001$; data for experimental males and females were pooled) and for the control cross ($\chi_1^2 = 9{\cdot}65$, $P < 0{\cdot}001$). The reason for the difference in recombination frequency between these backcrosses and the experimental and control crosses is known.

(ii) *Recombination involving* Ce-2. Data for recombination involving *Ce-2* were obtained only from outcrosses to AKR/J. For experimental crosses 135 animals including 42 derived from heterozygous males and 93 from heterozygous females were typed for *Glo-1*, *Ce-2* and *C-3* (Table 3). Differences in recombination frequencies between males and females were not significantly different (*Glo-1 – Ce-2*: $\chi_1^2 = 3{\cdot}38$, $P > 0{\cdot}05$; *Ce-2 – C-3*: $\chi_1^2 = 0{\cdot}01$, $P > 0{\cdot}05$). The pooled typing results were consistent with the gene order and recombination frequencies *Glo-1* – $5{\cdot}2 \pm 1{\cdot}9$ – *Ce-2* – $14{\cdot}8 \pm 3{\cdot}1$ – *C-3*. For control crosses (heterozygous females only), the gene order and recombination frequencies were *Glo-1* – $6{\cdot}9 \pm 2{\cdot}5\,\%$ – *Ce-2* – $12{\cdot}7 \pm 3{\cdot}3\,\%$ – *C-3*, a result that was not significantly different from the result obtained for progeny of heterozygous females in experimental crosses (*Glo-1 – Ce-2*: $\chi_1^2 = 0{\cdot}02$, $P > 0{\cdot}05$; *Ce-2 – C-3*: $\chi_1^2 = 0{\cdot}21$, $P > 0{\cdot}05$).

(iii) *Recombination between markers for the* t *complex* Tu66, Hba-4ps, H-2 *and*

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Table 2. *Transmission ratios for* Glo-1 *and* C-3 *and recombination frequencies between* Glo-1 *and* C-3 *among progeny of heterozygous mice outcrossed to C57L/J, 129/Sv and AKR/J**

	Cross†	*C57L/J*	*129/Sv*	*AKR/J*
		Recombination frequency (×100)		
Heterozygous males	E	18·0±3·3%	17·1±5·9%	15·0±4·6%
	C	12·2±5·1%	13·6±7·3%	8·3±4·6%
Heterozygous females	E	34·9±7·3%	23·8±4·2%	16·5±3·4%
	C	—	12·1±5·7%	16·4±3·4%
		Transmission ratio‡		
Heterozygous males				
Glo-1^c	E	0·98±0·01	0·85±0·06	0·83±0·05
Glo-1^a	C	0·61±0·08	0·50±0·11	0·53±0·08
C-3^a	E	0·81±0·03	0·73±0·07	0·75±0·06
C-3^a	C	0·59±0·08	0·64±0·10	0·50±0·08
Heterozygous females				
Glo-1^c	E	0·60±0·07	0·53±0·05	0·58±0·04
Glo-1^a	C	—	0·48±0·09	0·50±0·05
C-3^a	E	0·58±0·08	0·64±0·05	0·58±0·04
C-3^a	C	—	0·48±0·09	0·55±0·05

* Sample sizes are given in Table 1.

† Experimental (E) or control (C) cross.

‡ Transmission ratios are expressed as the proportion of progeny having the *Glo-1^c* (or *Glo-1^a*) or the *C-3^a* allele.

I-Aβ. Among the 167 animals tested from the experimental cross (Table 1), 57 were derived from heterozygous males and of these 46 were *Tu66^t Hba-4ps^t H-2^{w30} I-Aβ^{w30}* and 11 were *Tu66^a Hba-4ps^b H-2^k I-Aβ^k*. The remaining 110 chromosomes were derived from heterozygous females and of these 63 were *Tu66^t Hba-4ps^t H-2^{w30} I-Aβ^{w30}* and 47 were *Tu66^a Hba-4ps^b H-2^k I-Aβ^k*. Each of these represented the parental combination of alleles; recombinants were not detected. The absence of recombination between these four markers in the 167 animals tested was significantly less ($\chi^2_1 = 6{\cdot}98$, $P < 0{\cdot}01$) than the 4% frequency observed between *Hba-4ps* and *H-2* in wild-type chromosomes (d'Eustachio *et al.* 1984). These results support other evidence that these loci are located within the t^6 complex and formally demonstrate that recombination was suppressed.

Of the 167 studied with molecular probes, 103 had the parental combination of *Glo-1* and *C-3* alleles, while 64 had a recombinant combination. This result clearly demonstrates that *C-3* is located outside the t^6 complex. However, since recombinant chromosomes were preferentially selected for analysis with molecular probes, these data can not be used for calculating recombination frequencies.

Only three crossovers between *Glo-1* and loci detectable with molecular probes were detected (Table 1). These data suggest *Glo-1* may be separable from the t^6 complex. However, since animals with these apparently recombinant chromosomes could not be progeny tested, little significance can be attributed to them at least until further experiments verify these results.

Sixty-four of the animals from experimental crosses that were typed with

Table 3. *Recombination involving* Ce-2*: Allele combination inherited from heterozygous males or females outcrossed to AKR/J**

Allelic combination	Heterozygous males	Heterozygous females
Experimental cross: results for *Glo-1*, *Ce-2* and *C-3*		
Glo-1^c Ce-2^a C-3^a	30	54
Glo-1^b Ce-2^b C-3^b	6	21
Glo-1^c × *Ce-2^b C-3^b*	0	1
Glo-1^b × *Ce-2^a C-3^a*	0	3
Glo-1^c Ce-2^a × *C-3^b*	5	7
Glo-1^b Ce-2^b × *C-3^a*	1	4
Glo-1^c × *Ce-2^b* × *C-3^a*	0	1
Glo-1^b × *Ce-2^a* × *C-3^b*	0	2
Experimental cross: results for *Glo-1*, *Ce-2 C-3*, and the four loci detected with molecular probes†		
M^t Glo-1^c Ce-2^a C-3^a	5	11
M^w Glo-1^b Ce-2^b C-3^b	4	21
M^t Glo-1^c × *Ce-2^b C-3^b*	0	1
M^w Glo-1^b × *Ce-2^a C-3^a*	0	3
M^t Glo-1^c Ce-2^a × *C-3^b*	6	5
M^w Glo-1^b Ce-2^b × *C-3^a*	1	4
M^w Glo-1^b × *Ce-2^a* × *C-3^b*	0	2
M^w × *Glo-1^c* × *Ce-2^b* × *C-3^a*	0	1
Control cross: results for *Glo-1*, *Ce-2* and *C-3*‡		
Glo-1^a Ce-2^a C-3^a	—	44
Glo-1^b Ce-2^b C-3^b	—	39
Glo-1^a × *Ce-2^b C-3^b*	—	1
Glo-1^b × *Ce-2^a C-3^a*	—	5
Glo-1^a Ce-2^a × *C-3^b*	—	5
Glo-1^b Ce-2^b × *C-3^a*	—	7
Glo-1^a × *Ce-2^b* × *C-3^a*	—	0
Glo-1^b × *Ce-2^a* × *C-3^b*	—	1

* × indicates the interval in which recombination occurred.

† *M* designates the four loci *Tu66*, *Hba-4ps*, *I-Aβ* and *H-2* and superscripts *t* or *w* designate the combination of alleles originally associated with the *t*^{6} complex or its wild-type homologue, respectively.

‡ Too few progeny of heterozygous males were tested for meaningful results to be obtained.

molecular probes were also typed for *Ce-2*. Sixteen were derived from heterozygous males and 48 from heterozygous females (Table 3). Nineteen of the 23 crossovers detected occurred between *Ce-2* and *C-3* (including two double and one triple crossover); only seven crossovers were detected between *Glo-1* and *Ce-2*. Again since the animal with the triply recombinant chromosome could not be progeny tested, little significance can be attached to this observation at least until it is verified.

(iv) *Correlation between serological and molecular markers for the* t^6 *complex.* Twenty-one animals were selected for serological typing of *H-2* (Table 4). Recombination between the two serological markers for *H-2*, the *t* complex and *Glo-1*

https://doi.org/10.1017/S0016672300022242 Published online by Cambridge University Press

was not detected. The results are consistent with the hypothesis that the serological and molecular markers are located within the t^6 complex.

(v) *Transmission ratios.* As expected, the average transmission ratio of *Glo-1*^{c} ($0{\cdot}92 \pm 0{\cdot}02$), which is a marker for transmission of the t^6 complex in experimental crosses was significantly higher than the ratio for the corresponding *Glo-1* allele *Glo-1*^{a} in control crosses ($0{\cdot}56 \pm 0{\cdot}05$; $\chi^2_1 = 60{\cdot}02$, $P < 0{\cdot}001$). While higher than the characteristic value of 0·6–0·7 for the t^6 complex, it is known that a variety of genetic factors influence transmission ratio of the *t* complex (Demin & Safronova, 1979; Olds-Clarke & McCabe, 1981; Bennett, Alton & Artzt, 1983; Lyon, 1984).

Table 4. *Serological typing of* H-2 *in a sample of progeny*

	Allelic combination				
Serological marker	*M*	*Glo-1*	*Ce-2*	*C-3*	Number sampled
t†	*t*	*t*	*t*	*t*	2
w	*w*	*w*	*w*	*w*	2
t	*t*	*t*	—*	*w*	10
w	*w*	*w*	—	*t*	6
w	*w*	*w*	*t*	*t*	1

* Dash (—) indicates not tested (see Materials and Methods).

† *t* and *w* designate alleles of both *H-2K* and *H-2D* originally associated either with the t^6 complex or its wild-type homologue, respectively.

Considerable heterogeneity was observed between the frequencies of *Glo-1* alleles transmitted to offspring of the three strains to which each heterozygous male was crossed in experimental crosses ($\chi^2_6 = 15{\cdot}05$, $P < 0{\cdot}025$; Table 2). Partitioning this chi-square value showed that most of the heterogeneity resulted from differences in the transmission ratio of *Glo-1* between strains ($\chi^2_2 = 14{\cdot}32$, $P < 0{\cdot}001$); the transmission ratio of the allele *Glo-1*^{c} in outcrosses to C57L/J was significantly higher than the ratio in outcrosses to 129/Sv and AKR/J together ($\chi^2_1 = 14{\cdot}21$, $P < 0{\cdot}001$; Table 2).

By contrast, heterogeneity of transmission ratios was not detected between the three strains to which heterozygous females were outcrossed ($\chi^2_6 = 9{\cdot}62$, $P > 0{\cdot}05$; Table 2). In the pooled data for heterozygous females, however, transmission ratios of both *Glo-1*^{a} ($0{\cdot}57 \pm 0{\cdot}03$) and *C-3*^{a} ($0{\cdot}60 \pm 0{\cdot}03$) were unexpectedly both higher than the expected value of 0·50, *Glo-1*: $\chi^2_1 = 4{\cdot}55$ $P < 0{\cdot}05$; *C-3*: $\chi^2_1 = 11{\cdot}25$, $P < 0{\cdot}005$).

In control crosses, significant heterogeneity of transmission ratios was not detected among progeny of heterozygous females (Table 2; results of the statistical analysis are not shown).

4. DISCUSSION

Recent evidence suggests that not only are *H-2* Class I loci located within the *t* complex (Green & Snell, 1969; Pizarro & Dunn, 1970; Hammerberg & Klein, 1975*a*) but also that some of these Class I loci are rearranged compared to their order within the *H-2* complex of wild-type chromosomes (Artzt *et al.* 1982*b*; Shin

et al. 1983). *H-2* Class II and Class III loci are also thought to be located with the *t* complex. The absence of recombination between markers for the *t* complex and the *H-2* Class II locus *I-Aβ* suggests that *I-Aβ* is located within the t^6 complex (cf Shin *et al.* 1983; Shin, Bennett & Artzt, 1984); it remains to be determined whether Class III are located within the *t* complex and whether these loci are in their expected arrangements.

Hammerberg & Klein (1975*a*) showed that the locus thin fur (*thf*), which is located near *C-3* (Fig. 1), is located outside the *t* complex and that the telomeric boundary of the t-complex is located between *H-2* and *thf*. Results of the present study indicate that the telomeric boundary is located between *H-2* and *Ce-2*, an interval of about 2 cM in wild-type chromosomes. If chromosomal rearrangements are responsible for the suppression of recombination associated with the *t* complex, then these rearrangements do not include *Ce-2* or loci located more telomerically. This more precise definition is important because it demonstrates that loci located telomerically to the *H-2* – *Ce-2* interval are probably not responsible for any of the properties of the *t* complex. For example, it has been proposed that *Pgk-2*, a locus which is expressed only in the testes of sexually mature males, causes transmission ratio distortion (Rudolph & Vanderberg, 1981; Olds-Clarke & McCabe, 1981). This hypothesis seems unlikely since *Pgk-2* is probably located outside the *t* complex and since *t* complexes are associated with at least three different *Pgk-2* alleles (Nadeau, 1983).

By contrast to *Ce-2* and *C-3*, *Glo-1* is located centromeric to the *H-2* complex in wild-type chromosomes and is therefore expected to be located within the region of recombination suppression. *Glo-1* was, however, apparently separable from the t^6 complex (*Tu66*, *Hba-4ps*, *H-2*, *I-Aβ*) at a low frequency (Table 1). This frequency cannot be estimated, however, since animals studied with molecular probes were a nonrandom sample of the animals available. Because animals with these recombinant chromosomes could not be progeny tested, we can not be certain that these are legitimate recombinants. Indeed, preliminary results ($N = 270$) from crosses designed specifically to detect recombination between the t^6 complex and *Glo-1* have failed to reveal any evidence for recombination between these markers. As a result, *Glo-1* can probably be regarded as a reliable marker for the *t* complex.

Differences in the frequency of recombination between *Glo-1* and *C-3* in the three outcrosses were detected in heterozygous females. These differences suggest that gametes or zygotes with certain recombinant chromosomes differ in their viability in the three outcrosses. Differences in recombination frequency are occasionally observed between crosses (for review see Davisson & Roderick, 1981). Because crosses in these other studies usually differed in the strains involved for both the heterozygous and the backcross parent, it was not possible to determine whether differences in recombination frequency result from differences in the physical distance between loci on chromosomes from different inbred strains or from differences in the viability of gametes or zygotes with recombinant chromosomes. In the present study, the same heterozygote was used in each of three outcrosses. As a result, differences in physical distance can be ruled out as an explanation. There is, however, a third confounding explanation that must be considered. Each heterozygote was mated first to C57L/J for several litters, then to 129/J for several

litters, and finally to AKR/J for several more litters. As a result, a potential age effect was introduced. Thus we cannot be certain whether the differences in recombination detected result from differences in the viability of gametes or zygotes with recombinant chromosomes, or from differences in the frequency of recombination with increasing age of the heterozygous female parent. Although previous studies failed to detect a consistent age effect on recombination (Fisher, 1949; Wallace, 1957; Bodmer, 1961; Reid & Parsons, 1963; Wallace, MacSwiney & Edwards, 1976), it cannot be ruled out in the present study.

Differences in the transmission ratio of *Glo-1* between outcrosses of heterozygous males to C57L/J and to 129/Sv and AKR/J were detected. Previous studies have shown that transmission ratio depends on a variety of factors including loci within the *t* complex (Lyon, 1984), loci within the wild-type homologue of the *t* complex (Bennett *et al.* 1983) and genotype of the female to which heterozygous males are crossed (Demin & Safronova, 1979). Since in the present study the same heterozygote was used in each of the outcrosses, variation involving loci within the t^6 complex or between the t^6 complex and its wild-type homologue can be ruled out as explanations for the differences in transmission ratio observed. Instead, results presented here suggest either that factors determined by the genotype of the female accentuate the degree of distortion or, less likely, that the degree of distortion depends on the age of the heterozygous male.

Identification of the distal boundary of the t^6 complex allows interpretation of data for gametic disequilibrium between the *t* complex and linked loci if it is assumed that the boundary defined for the t^6 complex is located in the same position in other *t* complexes. Disequilibrium has been detected between the *t* complex and loci located centromeric to *Ce-2* (Hammerberg & Klein, 1975*b*; Hauptfeld *et al.* 1976; Levinson & McDevitt, 1976; Silver, White & Artzt, 1980), but not between the *t* complex, *Ce-2* and loci located telomerically (Nadeau, 1983). It is noteworthy that loci in disequilibrium are those located within the region of recombination suppression, while loci not in disequilibrium are located outside. Thus recombination suppression appears to be a sufficient explanation for the disequilibrium detected between the *t* complex and linked loci (cf. Hammerberg & Klein, 1975*a*, *b*), while functional constraints (Snell, 1968; Rudolph & Vanderberg, 1981) do not seem to be important (cf. Ishi & Charlesworth, 1979).

We thank Karen Artzt, Mary Lyon, and Lee Silver for discussing this work with us, Hans Lehrach, Jon Seidman, and Lee Silver for providing molecular probes, Eva Eicher for the *Ce-2* and *Glo-1* electrophoretic methods, Jost Kömpf for the *C-3* electrophoretic methods, and Don Bailey and Margaret Green for critically reading a previous draft of this manuscript. This work was supported by NIH grant GM 28017 to I.K.E. and by NSF grant PCM 8215004 to J.H.N., and by ACS grant IN 155.

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