PRELIMINARY DATA ON CROSSING OVER BETWEEN H-2 AND *Fu, Ki* AND *T* IN THE MOUSE *

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1. INTRODUCTION

PREVIOUS work (Gorer, Lyman and Snell, 1948 ; Snell and Higgins, 1951 ; Snell, 1951) has demonstrated the existence on chromosome 9 of the mouse of a locus, histocompatibility-2 ($H-2$), important in determining susceptibility and resistance to tumour transplants. Five alleles have been identified. These are $H-2$, characteristic of strain A ; $H-2^d$, characteristic of strains DBA/2 and BALB/c ; $H-2^b$, characteristic of strains C57BL/6 and C57BL/10 ; $H-2^p$, characteristic of strain P ; and $H-2^k$, characteristic of a non-inbred kinky line called K8 and probably also of strain CBA.

The locus, histocompatibility-2, is closely linked with fused tail (Fu), kinky tail (Ki) and brachy or short tail (T). Because classification of animals as genetically susceptible or resistant on the basis of tumour inoculation is subject to occasional error, results so far published have failed to demonstrate conclusively the occurrence of crossing over between $H-2$ and the other three loci. This paper is concerned with the demonstration of such crossing over.

The data come from several different types of crosses. None of these was designed primarily to provide linkage information but all produced such information as a useful byproduct.

2. LINKAGE WITH *T*

The gene T was introduced into strain A by 4 or 5 successive back-cross matings. It brought with it into the cross a histocompatibility-2 allele distinct from $H-2$, and probably identical with $H-2^b$ (unpublished data). The resulting stock was crossed again to mice of strain A. This cross was therefore

$$H-2^bT/H-2+ \times H-2+/H-2+$$

Brachy offspring could be either $H-2^bT/H-2+$ (non-crossovers) or $H-2T/H-2+$ (crossovers).

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Non-brachy mice were discarded, and the brachy mice were mated to strain C57BL/6.   This mating was

$$H-2^bT/H-2+ \times H-2^b+/H-2^b+ \quad \text{for non-crossovers,}$$
$$H-2T/H-2+ \times H-2^b+/H-2^b+ \quad \text{for crossovers.}$$

Non-brachy mice were discarded and brachy mice inoculated subcutaneously with strain A tumour 15091a. Non-crossovers should throw, except for new crossovers, all T resistant $(-)$ offspring ; crossovers should throw all T susceptible $(+)$ offspring. The inoculation of 5 young was set as a satisfactory test, but owing to poor breeding by some of the brachy mice, particularly by brachy females, this number was not always attained.

The results are summarised in table 1. Results of tests where only 1, 2 or 3 T young were inoculated are included in this table, but are not used in the totals or in any subsequent tables. For animals tested by 4 or 5 or more inoculated offspring, the crossover values were 4·1 per cent. for heterozygous males (total of 73 mice) and 12·5 per cent. for heterozygous females (total of 8 mice). The combined figure is 4·9 per cent.

TABLE 1

Number of brachy (T) *mice and number of presumed brachy crossovers from the mating* $H-2+/H-2^bT \times H-2+/H-2+$, *grouped according to the number of brachy* (T) *offspring raised and inoculated to test each animal. Only mice tested by 4 or more inoculated young are used in calculating crossover per cent.*

	Number of T offspring inoculated						Crossover per cent.
	1	2	3	4	5 or more	Total (last 2 columns)	
Mice by heterozygous males—							
Number of mice	2	6	10	15	58	73	
Number with all positive offspring	0	1	1	1	2	3	*4·1*
Mice by heterozygous females—							
Number of mice	0	1	3	2	6	8	
Number with all positive offspring	0	1	1	0	1	1	*12·5*

Combined per cent. with all positive offspring (crossover per cent.) = 4·9±2·4

The one crossover in the group tested by 4 inoculated T offspring was additionally tested by the fact that he had been used prior to testing, in matings to A females, to sire some mice who were themselves tested. All of six such offspring proved to be genetically $H-2T/H-2+$. He was therefore unquestionably a crossover. It remains to consider the adequacy of the test applied to the mice from whom 5 or more T young were raised and inoculated.

Table 1 shows that there were 3 probable crossovers tested by the inoculation of 5 or more offspring. Actually 1 of these was tested

by the inoculation of 12 young, all of which were positive, 1 by the inoculation of 9 young, all of which were positive, and 1 by the inoculation of 5 young, all of which were positive. There would seem to be no question that the first two were crossovers. Data pertinent to the question as to whether the third may have been diagnosed falsely as a crossover are given in tables 2 and 3.

From table 2 it will be seen that T males diagnosed as non-crossovers gave 32 offspring that grew the tumour and 256 that failed to grow it, while for T non-crossover females the figures were 20 and

TABLE 2

Outcome of tumour inoculation of brachy (T) offspring by brachy non-crossover males and females from cross $H-2+/H-2^bT \times H-2^b+/H-2^b+$

Sex of parent	Positive	Negative	Per cent. positive
Male . . .	32	256	*11·1*
Female . . .	20	99	*16·8*
Total . .	52	355	*14·6*

99. This is 11·1 per cent. and 16·8 per cent. positive respectively, with a combined value of 14·6 per cent. Some of the positive mice could have been, and presumably were, crossovers. However, the combined value is significantly higher (P = ·04) than the combined crossover per cent. shown in table 1. Presumably, then, some of the positive mice in table 2 must be otherwise explained.

In view of the well-known fact that some tumours will grow in a percentage of animals of certain foreign and hence presumably resistant strains, the obvious explanation is that some of the genetically resistant mice succumbed. Our past experience would have led us to expect such false positives to be rare with tumour 15091a when the genotype of the inoculated animals was $H-2^b/H-2^b$. However, some of the stocks used in this cross were obviously in rather poor physical condition, due to unknown causes, and this may have decreased resistance to the tumour. In any case we regard the figure 4·9 per cent. from table 1 as the more reliable measure of crossing over between $H-2$ and T.

Table 3 shows the frequency distribution of brachy males and females from which 5 or more young were inoculated, tabulated according to the per cent. positive offspring. It will be seen that 2 out of 61 non-crossovers gave 50-60 per cent. positive offspring, but that none gave more than 60 per cent. It seems unlikely therefore that any non-crossovers would give 5 out of 5 or 100 per cent. positive offspring. The one mouse previously mentioned, which was diagnosed as a crossover on the basis of 5 positive offspring, is not likely to have been incorrectly classified.

Error from false negatives is ruled out by the fact that all inoculated mice were genetically F_1 hybrids between strains A and C57BL/6

TABLE 3

Frequency distribution of brachy males and females from which 5 or more young were inoculated, tabulated according to per cent. positive offspring

	Non-crossovers					Crossovers
Per cent. positive .	0	12-20	27-40	50-60	61-99	100
Sex of parent—						
Male . .	28	10	6	1	0	3
Female . .	7	2	6	1	0	0

except for the chromosome segment under test. The crossover value of $4\cdot9\pm2\cdot4$ per cent. should therefore be reliable.

3. LINKAGE WITH *Fu*

The data came from two different sources.

A chromosome segment tagged by the gene *Fu* was introduced into strain A by repeated backcrosses. This segment was derived from the CA strain, genetically *CaCaFufuWw*, and carried with it a histocompatibility-2 allele distinct from *H*—2 (Snell and Higgins, 1951) but not otherwise identified. The mice were thus *H*—2^x*Fu*/ *H*—2+. A number of them, from several different backcross generations, were used in crosses set up to identify the *H*—2 allele present in certain inbred strains.

One mouse, male ACA80, in the third backcross generation, mated to strains C57BL·10, RIII and ST, gave 25 normal tailed young and 24 fused tail young which all succumbed to strain A tumour 15091*a*. Since strains C57BL/10, RIII and ST all lack allele *H*—2 (Snell and Higgins, 1951 and unpublished data), male ACA80 must have had the genotype *H*—2*Fu*/*H*—2+. This genotype could have been derived by mutation from *H*—2^x to *H*—2. Since there are at least 5 (and probably more) alleles at the histocompatibility-2 locus, there is a degree of improbability in the assumption that mutation would give the particular allele born by the homologous chromosome. The acquisition of *H*—2 by crossing over seems more probable.

The total number of tested mice from the backcross of *Fu* to strain A was 18. Only male ACA80 proved to be a crossover. The indicated crossover per cent. is 5·6 (table 4), but the probable error is of course high.

Additional information comes from another group of crosses. These had the form

$$(M \times F^h) \times N \text{ or } (M \times F^H) \times N$$

where M and N are any two inbred strains, F^h is a strain carrying

the gene *Fu* linked with an unidentified histocompatibility-2 allele which however was not $H-2$, and F^H is a strain carrying the gene

TABLE 4

Number of mice and number of genetically tested crossovers from mating
$$H-2+/H-2^zFu \times H-2+/H-2+$$

Number of mice	Number of crossovers	Per cent. crossovers
18	1	5·6

Fu linked with $H-2$.   Offspring of the double cross were inoculated with a tumour native to the strain in the M position.
The results are summarised in table 5.

TABLE 5

Crossover data from crosses involving fused (Fu). Animals were classified as positive (+) only if they succumbed to the tumour

Cross	Tumour	Results			
		++	+−	*Fu*+	*Fu*−
(A × F^h) × C57BL/6, C57BL/10, C57BR/cd, C57L, MA, RIII *	*15091a*	31	11 (3 §)	1 ‡	34
(BALB/c × F^h) × A, AK, C3H, C57BL/10, C57BR/cd, C57L, LINE 11, P, RIII, ST	*S621*	75 (1 †)	13	4 (3 ‡)	80
(C57BL/6 × F^H) × A, AK, BALB/c, DBA/2, P, RIII	*C1498*	55	15 (1 §)	2 (1 ‡)	58
Total	...	161	39 (4 §)	7 (5 ‡)	172
(C57BL/6 × F^H) × ST . .	*C1498*	7	4	5 (1 ‡)	15

* In this group, data from some crosses were excluded on the ground that the inbred strain used in the final cross shows partial susceptibility to 15091a. For example, about 30 per cent. of DBA/2 mice succumb to 15091a, probably because of the virulence of the tumour plus a relationship between the alleles *H-2* and *H-2^d* (Snell, 1951). Since all *Fu* mice from this cross would receive the allele *H-2^d* from the DBA/2 parent, some of the *Fu* animals might be expected to succumb even though possessing a genotype ordinarily classified as resistant.

† Because S621 and C1498 are slightly less virulent tumours than 15091a, and because good data for choosing between the more and less satisfactory crosses were lacking, all crosses were included in these two cases. However, data for the cross involving C57BL/6 and ST appear aberrant and are given separately.

‡ These mice succumbed to the tumour, but survived longer than other susceptible mice. They probably should be classified as genetically resistant. For detailed data on length of survival of comparable cases see table 7 of Gorer, Lyman and Snell (1948). The numbers outside the parentheses are the totals, including the presumably resistant mice.

§ These mice had normal tails but were proved by genetic tests to carry the fused gene.

It will be seen that in general the non-fused mice succumbed to the tumour while the fused mice survived. The exceptions ($+-$ and $Fu+$ mice) may be crossovers, but we need first to exclude other possibilities. The gene Fu is known to be subject to the rather frequent occurrence of normal overlaps (Reed, 1937). There is every reason to suppose that many of the normal tailed resistant ($+-$) mice belong in this category. Some were proved to belong here by genetic tests. These are indicated in the $+-$ column of table 5 by the numbers in parentheses. The occurrence of these overlaps renders the normal tailed ($++$ and $+-$) mice unsuitable for use in estimating crossover per cent. We shall therefore confine our consideration to the fused mice.

Some of the fused survivors may possibly have been genetically susceptible and hence crossovers. However, we have good evidence from several sources that 15091a usually kills any mice with the allele $H-2$. (See for example, the results of inoculating offspring of male ACA80 referred to above.) The evidence is not so clear in the case of the other tumours, but in any case the frequency with which " susceptible " mice survive is low and the resulting error small.

More important is the occurrence of false positives. We have already pointed out that these occur in discussing the linkage of $H-2$ and T. A clue as to which animals fall in this category may be obtained from the records as to the length of survival of, and progress of tumour growth in, the $Fu+$ mice. Normal tailed susceptible mice inoculated with 15091a usually die in 3 to 5 weeks and very rarely live to 8 weeks. The one mouse included in table 5, first cross, which was Fu and which succumbed to tumour 15091a, lived 10 weeks and showed partial tumour regression at 4 to 6 weeks before the final spurt of tumour growth which killed it. Presumably this mouse was genetically resistant. Other such mice are indicated in parentheses in the $Fu+$ column.

The $Fu+$ mice which succumbed after the normal interval are probably crossovers. Possible exceptions are the 4 $Fu+$ mice from the cross (C57BL/6 $\times$ F^H) $\times$ ST which succumbed promptly to tumour C1498. These are separated from the total because the high number of positives in this one cross suggests some unusual condition. Omitting both the long survivors and the mice from this one cross, there are 2 probable crossovers out of 174 mice. The indicated crossover value is 1·2 per cent. However, the sources of error are obviously such that this value should be regarded as possibly subject to future emendation. Its principal utility lies in the indication that the accurate but limited data in table 4 may give too high a figure.

4. LINKAGE WITH Ki

Data pertinent to the linkage of $H-2$ and Ki were derived from crosses of the type

$$(A \times K) \times N$$

where A is strain A, N is any other inbred strain (all strains used lacked $H-2$) and where K is a strain carrying Ki associated with a histocompatibility-2 allele other than $H-2$. Mice from the double cross were inoculated with strain A tumour 15091a. Results are summarised in table 6. The indicated crossover value, including

TABLE 6

Crossover data from crosses involving kink (Ki). All mice were inoculated with A strain tumour 15091a. Mice from crosses to strains DBA/2 and ST are excluded because these strains show partial susceptibility to this tumour. Mice were classified as positive (+) only if they succumbed to the tumour.

Cross	$++$	$+-$	$Ki+$	$Ki-$
$(A \times K) \times AK$, CBA, C57BL/6, C57BL/10, P	47	4 *	1	48

* Mice tested genetically, not normal overlaps.

all mice, is 5 per cent., and including only Ki mice, 2 per cent. Since none of the presumed crossovers were proved susceptible or resistant by a breeding test, some question must remain as to the correctness of the classification. The occurrence of crossing over between $H-2$ and Ki should be confirmed by further tests before the separability of the $H-2$ and Ki genes is regarded as proved.

5. DISCUSSION

Dunn and Caspari (1945) have shown that Fu, Ki and T lie in a chromosome segment not exceeding 8 units in length. The occurrence of overlaps in fused heterozygotes and certain other difficulties inherent in the nature of the material leave some uncertainty as to the details of the arrangement. The best available estimates of crossover per cent. are :—

Fu and T, 4·3 per cent. in heterozygous males.
Ki and T, 4·3 per cent. average for both males and females, with the rate being higher in females than in males. Another cross gave 4·8 per cent. in males.
Fu and Ki, 2 per cent. The poor viability of $FuKi$ mice makes this cross particularly difficult.

The order of the four identified loci on chromosome 9 is uncertain, but there is indication that $H-2$, Fu and Ki are bunched at one end of the linkage group in an interval of perhaps 2 units, while T is separated from the nearest of the other three by perhaps 4 units.

6. SUMMARY

1. The locus histocompatibility-2 $(H-2)$, which is important in determining susceptibility and resistance to tumour transplants, lies on chromosome 9 in close association with the genes Fu, Ki and T.

2. Crossing over between $H-2$ and T was $4\cdot9\pm 2\cdot4$ per cent.

3. Crossing over between $H-2$ and Fu was $5\cdot6$ per cent. in one cross and an estimated $1\cdot2$ per cent. in another. The first figure is based on small numbers and the second is derived from a cross where there is a considerable element of uncertainty in the detection of crossovers. Further data are needed.

4. There is evidence indicating but not finally proving that crossing over occurs between $H-2$ and Ki.

5. The locus of $H-2$ is distinct from the loci of T, Fu and probably Ki, but its order relative to these loci is not yet determined.

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