Proceedings of the National Academy of Sciences
Vol. 67, No. 2, pp. 961–967, October 1970

Two Radiation-Induced Chromosomal Inversions in Mice (*Mus musculus*)

Thomas H. Roderick and Norman L. Hawes

THE JACKSON LABORATORY, BAR HARBOR, MAINE 04609

Communicated by George D. Snell, June 8, 1970

Abstract. Whole-body x-irradiation of male mice has produced presumptive paracentric inversions in 15 animals, as evidenced by high frequencies of first meiotic anaphase bridges. Two of the highest frequencies observed have been propagated through several generations and found to behave as dominant genes. Acentric fragments were observed associated with about 10% of the bridges. The first inversion, in linkage group XIII, has been designated *In(13)1Rk*, and the second, in linkage group XVII, *In(17)2Rk*. For *In(13)1Rk*, recombination was reduced between loci inside and outside the inverted segment.

Identified chromosomal inversions in laboratory mammals would be valuable tools for studies in diverse fields of genetics. There is evidence for the existence of mammalian inversions, but none has heretofore been genetically defined and maintained. We have attempted to produce inversions in mice by irradiation of sperm and spermatids, and to detect induced inversions by searching for high frequencies of first-meiotic anaphase bridges in males of the next generation. This report describes the first two inversions produced by this method. They are called *In(13)1Rk* and *In(17)2Rk*, respectively.

Materials and Methods. Males of inbred strain DBA/2J were subjected to 700–900 R of whole body x-irradiation and each male was then mated immediately with two females of inbred strain C57BL/6J. Matings were terminated after three weeks. At three months of age all male offspring were weighed and anesthetized. One testis was removed, fixed in Tellyesniczky-Fekete fixative[1] for two days, and then transferred to 70% alcohol. Each was cut into 8-μm sections, stained with gallocyanin, and searched for meiotic anaphase bridges.

When comparisons between cellular manifestations of the inversions were desired, we coded slides from all animals including controls (see below), and observed them without knowledge of their identities. This procedure was particularly important in our study of the incidence of fragments associated with bridges of presumed inversion-heterozygotes and controls. For this study, we had already screened all slides, recorded locations of anaphase bridges for each slide, and determined whether males had high or normal frequencies of anaphase bridges. For reasons explained later, animals with a mean bridge frequency per slide of less than 10% were classified as normal, and those with higher bridge frequencies were classified as "high frequency." By random sampling, we chose up to four bridges per slide to observe for bridge characteristics and associated fragments. The control animals for this study were normal siblings of high-frequency males as well as normal B6D2F_1 males.

Preparations of primary spermatocytes were made by the air-drying method of Evans *et al.*[2] Cells from the bone marrow were prepared by Ford and Hamerton's method.[3] All values for genetic recombination were derived from males.

Results. We noticed a small but consistent frequency of first anaphase bridges in males with no ancestral irradiation. Of 915 anaphases observed in 30 animals of inbred strains C57BL/6J, A/J, and DBA/2J, 31 (3.39%) showed bridges. This we considered the "control" or normal percentage from which the higher percentages caused by inversions must be distinguished. In only one case did the percentage of bridges exceed 10% in a sample of 30 anaphases per animal. In further counts to a total of 72 anaphases in this animal, the frequency of bridges dropped to 8.3%.

Approximately 30 first meiotic anaphases were also examined in each male with paternal irradiation. If three or more anaphases had bridges, additional anaphases were examined up to a total of about 130 for each male. Of the 541 animals screened, 42 showed frequencies of bridges of about 10% in the first count, but only 15 of these 42 remained above 11% at the final count. These 15 we consider to be presumptive inversions. The three highest were males 471 with 38%, male 618 with 34%, and male 816 with 21%. Male 471 was sterile. The other two were mated and followed through more than 2 generations. In their male offspring, there were approximately equal numbers with high and normal percentages of bridges. The high frequencies of these male offspring were also characteristic of their sires. Of the 12 remaining males who were presumptive inversion heterozygotes, 6 were mated to produce male offspring for observation. Five were fertile, and four of the five had some sons with bridge percentages between 11 and 20%. Since such high percentages were not found among control animals, these data suggest that small inversions had been induced and transmitted. No attempts were made to propagate these lines further.

Hereafter we will refer to animals with the normal percentage of bridges as "normals" and those with a percentage clearly higher as "highs."

Mouse 618 and his descendants have been studied most extensively so far. In this study all descendants of male 618 were mated to unrelated animals with no ancestral irradiation. Between 20 and 30 anaphases were observed for each animal to determine its percentage of anaphase bridges. The average sample size was 24 anaphases. Daughter carriers were identified by the appearance of high-frequency sons among their offspring. About half of the daughters of male 618 were carriers. The distribution of grandsons was not affected by whether a male's percentage was at the upper or lower end of the high half. Normal sons produced nothing but normal grandsons. In all subsequent crosses from high males, the distribution of frequencies in sons was bimodal and nonoverlapping.

Fig. 1 shows the combined distributions of offspring of male 618, his high sons, and carrier daughters. There was no evidence for any differences between the three distributions of progeny; the means and variation were about the same. The number of high and normal offspring appear in equal frequencies in the offspring of high animals. High frequency therefore behaves as a dominant gene as would be expected in this backcross situation. The mean percentage of bridges of the high descendants of male 618 (34.0%) is almost exactly that of male 618 who had 61 bridges out of 181 anaphases observed, or 33.7%. The

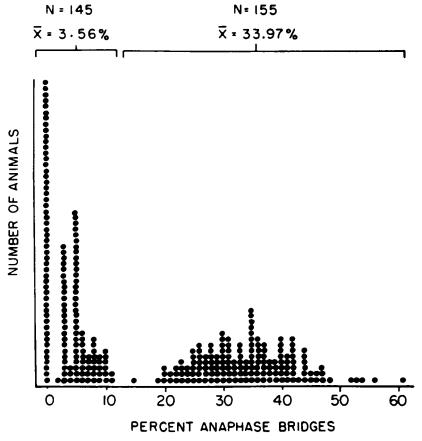

FIG. 1. Per cent anaphase bridges of sons of male 618 and his grandsons through his high sons (sons with high frequency of anaphase bridges) and carrier daughters. N = number of animals.

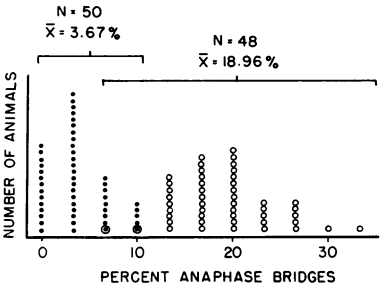

FIG. 2. Per cent anaphase bridges of sons of male 816 and his grandsons through his high sons and carrier daughters. N = number of animals.

mean percentage of all the normal sons of high males or carrier females (3.56%) is about the same as the mean of normals (3.39%) found in the inbred controls. The variation around these modes is therefore presumed to be due to sampling variation and not to any biological differences among high or normal males.

We assume that the genetic phenomena responsible for high frequencies are inversions, and hereafter will call them such.

Fig. 2 shows the equivalent distribution for descendants of male 816. The transmission of the high frequency in this case also behaved as a dominant gene and the mean percentage of the high descendants (19.0%) was similar to that of male 816 himself who showed 29 bridges out of 137 anaphases observed (21.2%). In this case all percentages were based on a sample size of the first 30 anaphases observed. There are quite evidently two distributions, even though there is some overlap. The final identification of high or normal percentage for those in the overlapping area was based on further counts.

Because acentric fragments are a manifestation of crossing over in a paracentric inversion heterozygote, we looked for the occurrence of fragments together with the bridges. Fragments are not always readily apparent, and it was disconcerting to discover that finding them depended in part on how much effort was spent looking for them. All the data given on fragments, therefore, were derived from coded slides. Furthermore, only those chromatin bodies which were clearly separate from the bridge, and yet within reasonable proximity to it were characterized as fragments. Many bridges had one or more clumps along their lengths which may have been in part due to closely associated fragments which had not fully separated. Thus, many fragments may have been present but not recorded as such. Occasionally we observed separated chromatin bodies which we felt were too large to be acentric fragments.

Fig. 3 shows anaphase bridges and typical associated fragments found in

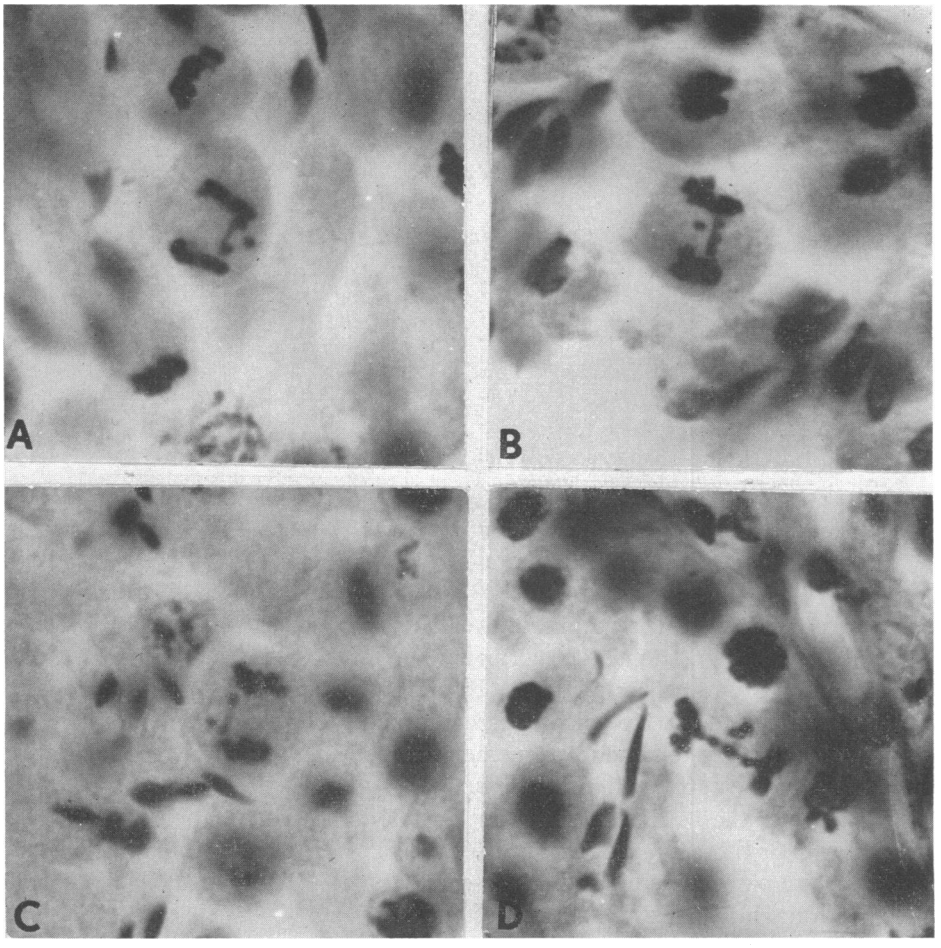

FIG. 3. Anaphase bridges with associated fragments. The chromatin body in Fig. 3*D* seems too large to be classified as an acentric fragment.

descendants of male 618. Table 1 gives the percentages of bridges with fragments for bridges from high and normal males descending from both male 618 and male 816. The differences between the highs and normals are nearly statistically significant at the 0.05 level.

Genetic tests demonstrated that the inversion found in male 618 is probably

TABLE 1. *Anaphase bridges with and without fragments in high males (descendants of males 618 and 816) and normal males.*

	Number of bridges with fragments	Number of bridges without fragments	Per cent bridges with fragments (% ± S.E.)*
High males from 618	14	148	8.6 ± 2.2
High males from 816	18	144	11.1 ± 2.5
Normals	2	62	3.1 ± 2.2

* Standard error of percentage = $(pq/N)^{1/2}$, where p = percentage and q = 100–p.

completely linked to some markers on linkage group XIII and closely linked to other markers of the same group. Markers from linkage groups I–IX, XI, XII, XVII, and XVIII were also tested for linkage with the inversion, but no linkage was found. This linkage is therefore specific to linkage group XIII and does not represent some general inhibition of recombination. In a backcross to a stock with the leaden phenotype (*ln/ln*), the inversion did not recombine with *ln* in 71 animals observed, which means it probably overlaps the *ln* locus. The splotch locus (*Sp*) is approximately 4.5 map units from *ln*[4], and *Sp* is about 11.5 map units from a locus controlling the structure of isocitrate dehydrogenase (*Id-1*)[5] (Fig. 4). We performed a three-point backcross in animals heterozygous for the inversion and at the *Sp* and *Id-1* loci. In 48 sons observed, there was no recombination among the three. The inversion therefore probably extends beyond the *Sp* locus as far as *Id-1*.

In an inversion heterozygote, all markers within the inverted segment should show reduced recombination with genetic markers outside the segment. Thus, the extent of the inversion relative to outside markers can be ascertained. Crosses were made involving the inversion and fuzzy (*fz*), which is about 37 map units from *ln* on the *Sp* side.[4] The first cross was between a high male and a female homozygous for leaden and fuzzy (*ln fz/ln fz*). High and normal littermate males from this cross were backcrossed to the same leaden-fuzzy stock. Reduced recombination in inversion heterozygotes is expected for regions in or near the inversion. Table 2 summarizes the results. The recombination frequency in normal males agrees with the values reported elsewhere. The differ-

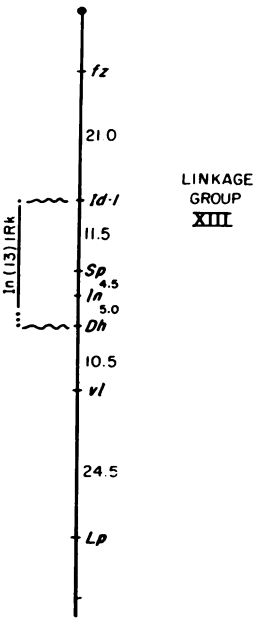

FIG. 4. Linkage group XIII showing approximate location of the first inversion. Recombination values are for males.

TABLE 2. *Recombination in linkage group XIII in high and normal littermate males descended from male 618.*

Cross* ♀	♂	Phenotypes of Offspring				Recombination
		ln fz	*ln* +	+ *fz*	+ +	% ± S.E.
$\frac{+\ ln\ fz}{+\ ln\ fz}$	$\times\ \frac{+\ +\ +}{+\ ln\ fz}$ (normal ♂)	28	26	16	27	43.3 ± 5.0
$\frac{+\ ln\ fz}{+\ ln\ fz}$	$\times\ \frac{In\text{-}1\ +\ +}{+\ \ ln\ fz}$ (high ♂)	96	29	32	101	23.6 ± 2.6
		Sp +	*Sp fz*	+ +	+ *fz*	
$\frac{+\ +\ fz}{+\ +\ fz}$	$\times\ \frac{In\text{-}1\ +\ \ fz}{+\ \ Sp\ \ +}$ (high ♂)	13	4	4	13	23.5 ± 7.1

* *In-1* is *In(13)1Rk*.

ences in recombination percentages between high and normal males is 19.7% which, with this number of animals observed, is statistically highly significant.

The next cross was made between a high, fuzzy male (*In-1 fz/ + fz*) and a splotch female (*Sp/+*). A high splotch son (*In-1 + fz/ + Sp +*) was then crossed to a stock homozygous for fuzzy (*+ + fz/+ + fz*). The results of this mating are shown in Table 2. In our laboratory we have not checked the distance between *Sp* and *fz* without the inversion, but data from other studies[4] showed it to be about 32.5 map units in males. It would therefore appear that our value is less and is also a manifestation of reduced recombination in the presence of an inversion. Moreover, if both of the loci *ln* and *Sp* are within the inverted segment they should both, barring double crossing over within the segment, exhibit the same percentage of recombination with loci outside the inverted segment in an inversion heterozygote. The similar values of the recombinations of *ln-fz* (23.6%) and *Sp-fz* (23.5%) in the inversion heterozygote manifest this characteristic of inversion. The normal distance from *ln* to *fz* is about 37 map units from published data[4] and about 43 map units from our data. The reduction of this distance by 20 map units in the presence of the inversion indicates that the inversion extends about 20 map units from *ln* to a distance of about 17 to 23 map units from *fz*. The inversion must therefore terminate very close to locus *Id-1*.

Data from a test cross of a double heterozygote with the inversion and dominant hemimelia (*Dh*) in repulsion showed no recombination in 15 males observed. Because *Dh* is only 5 map units from *ln* in the other direction,[4] these data are not sufficient to tell if the inversion overlaps *Dh*.

An estimate of the minimum length of the inversion is half the frequency of anaphase bridges, since a first meiotic anaphase bridge represents two crossover and two noncrossover strands in a tetrad. This inversion is therefore at least 17 map units long and probably longer depending on how near the ends of the inversion crossing over can occur in a heterozygote, and to what extent the frequency of crossing over is reduced throughout the inverted region. Locus *Dh* may be near the end of the inversion opposite *Id-1*, since the distance from *Id-1* to *Dh* is about 21 map units. Fig. 4 shows the probable range of the inversion, which we call *In(13)1Rk*, a nomenclature consistent with that in use for translocations.

Limited data on the comparison of reproductive performances of 16 high males (heterozygous for *In(13)1Rk*) and their normal brothers showed a possible reduction in litter size of male inversion heterozygotes of about 1 mouse per litter. Thirteen inversion heterozygotes and 28 normal brothers showed no differences in 90-day body weights. No phenotypic differences between them were observable. There is therefore little if any reduction in general fitness in these inversion heterozygotes.

The other inversion which first occurred in male 816 was found to be on linkage group XVII. Table 3 shows that the inversion is very close to the buff (*bf*) locus. Preliminary data also show that this inversion is linked with retinal degeneration (*rd*) and phosphoglucomutase-1 (*Pgm-1*) loci that are also of linkage group XVII.[4,5] This inversion we called *In(17)2Rk*.

TABLE 3. *Evidence for the location of inversion In(17)2Rk in linkage group XVII.*

Cross*		Phenotypes of Offspring			
♀	♂	I +	I *bf*	+ +	+ *bf*
$\frac{+\ bf}{+\ bf} \times$	$\frac{In\text{-}2\ +}{+\ bf}$	7	1	0	10

Recombination (±S.E.) = 5.6 ± 5.4%

* *In-2* is *In(17)2Rk*.

Primary spermatocytes and cells from bone marrow of four inversion heterozygotes of each kind were examined. In all cells where chromosomes could be counted, there was the normal complement (2N = 40), and no evidence for centromeric shifts or altered sizes of chromosomes. These inversions therefore apparently do not have other concomitant chromosomal aberrations such as translocations. Pachytene preparations in inversion heterozygotes were intensively examined, but we saw no loops which clearly represented inversion loops. Loops resulting from these inversions would be expected to be small. Successful production of stocks homozygous for *In(13)1Rk* is in the initial stages. Homozygous males show the low percentage of bridge frequency but when outcrossed, pass the high frequency to all sons. Phenotypes of these inversion-homozygotes are normal.

Discussion. The previous evidence for the existence of *pericentric* inversions in mammals comes from observations of feral populations of various species. The presumption of an inversion is based on observations of karyograms that appear normal except for an apparent shift in the position of a centromere in one chromosome.

Our evidence for inversions comes from other characteristic manifestations of inversions: (1) high frequencies of anaphase bridges were found only in descendants of irradiated males; (2) the frequency acts as a dominant gene when heterozygous but recessive and indistinguishable from normal homozygotes when it is homozygous; (3) homozygotes when outcrossed pass the high frequency to all their offspring; (4) the high frequency in a given line is stable within the line; (5) high frequency is "allelic" with three or more loci covering a map length comparable to the length estimated from the frequency of anaphase bridges; and (6) recombination is reduced between loci inferred to be within the inverted segment and those outside it.

This work was supported by Contracts AT(30-1)-3671 and AT(30-1)-1979 with the U.S. Atomic Energy Commission, N.I.H. research grant GM-07249 from the Division of General Medical Sciences, and in part by the Robert Sterling Clark Foundation. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. We are grateful for the advice and assistance of Drs. W. J. Welshons, J. B. Storer, Margaret C. Green, and C. W. Edington and Miss Carol Waldman. This paper is dedicated with affection and respect to Professor E. R. Dempster.

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