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1. OBJECTIVE

The purpose of this project is to produce chromosomal inversions by irradiation or chemical treatment of sperm in mice and to detect inversions by observing the frequency of first meiotic anaphase bridges of their sons. For each new inversion of considerable length, we will (1) determine its linkage group, (2) mark it genetically, if possible, or place it with a genetically marked homologous chromosome, (3) study its cytological, physiological, and anatomical effects, and (4) use it, possibly in combination with other inversions, to produce test systems for precise estimates of mutational loads in irradiated populations.

2. MAIN RESEARCH ACCOMPLISHMENTS

1. Detection of inversions. Our usual procedure is to treat strain DBA/2J males with irradiation or a chemical mutagen, mate them immediately to strain C57BL/6J females, and save offspring conceived in the first two weeks after treatment. When the male offspring are 60 days of age, one testis is removed, sectioned, and stained. High frequencies of bridges in the first meiotic anaphase are indicators of inversions. Repeatedly we have found a low percentage of bridges (about 3.5%) in the first anaphases of almost every male (Roderick, 1971). The reasons for these bridges are not known. But they do necessitate our distinguishing them quantitatively from a higher frequency of bridges that indicate an inversion. Other indicators of inversions that we do not usually see in normal animals are broken anaphase bridges and telophase bridges, neither of which are included in our count for anaphase bridges. Although anaphase bridges are the main indicator, we use these other characteristics as well in searching for new inversions. Numerous associated broken bridges suggest that the inversion is relatively near its centromere, whereas numerous telophase bridges suggest that the position of the inversion is much farther from its centromere. The frequency of anaphase bridges is an indication of the length of the inversion itself.

Since the beginning of the project we have screened 1750 males for new inversions. We have also examined the sectioned testes of an additional 4100 males in the handling and studying of the inversions we have produced. There are approximately 20 sections on a slide, and the time to count a slide depends on the frequency of anaphases present. The frequency of first anaphases on a slide ranges from none to about 40. Sometimes additional slides from the same animal must be observed to bring the count high enough to be meaningful. Our experience is that the frequency of anaphases is correlated with the animal's subsequent fertility. Therefore, it is not always worthwhile to count more than two slides in searching for anaphases, since the likelihood of the animal's usefulness diminishes with the more slides needed.

The smaller the inversion, the fewer the anaphase bridges, and the more anaphases we must score to be able to distinguish the bridge frequency due to the inversion from that of normal animals. In the past we have saved any animal for breeding with a bridge frequency consistently about 11%. Now we study animals with 15% or above but will usually keep only those with at least 20%. This change in procedure was made because of the difficulty of maintaining inversions with low bridge frequencies. Each male descendant requires much more effort to determine whether he is an inversion carrier. If the linkage of the particular small inversion is found quickly using our multiple linkage testing system (Roderick, 1971), we are

likely to keep the inversion. Also, if it can be made homozygous quickly and with certainty, we would be likely to keep it. Otherwise it would be discarded, since there are now enough longer inversions available.

Occasionally a male will show several anaphase bridges on a single portion of a slide confined to a portion of a tubule, but not in other areas of the slide nor in other recuts. These frequencies seem higher than one would expect from chance distribution of bridges of the background frequency. In the offspring of these animals, we have not found the usual bimodal distribution of bridge frequencies characteristic of the offspring of inversion heterozygotes (Roderick and Hawes, 1970). A possible explanation for these data is gonial mosaicism for an inversion. Continual propagation of such animals might produce a useful inversion carrying male.

As we breed a prospective new inversion heterozygote, we simultaneously backcross him to one of the parental strains, usually DBA/2J. This particular backcross enables us to check the linkage of the new inversion with nine isozyme loci and two coat color loci simultaneously (Roderick, 1971). We estimate with the location of these various loci and the number of backcross animals observed that we are testing about 1/4 to 1/3 of the genome of the mouse in this single cross. If unsuccessful in the initial cross, we use other appropriate linkage testing stocks. So far, in the 10 inversions we have produced, the linkage of four has been determined, two of which were linked to isozyme loci.

New staining techniques, developed recently, permit differentiation of mammalian chromosomes because of the unique banding patterns of each. The two major techniques are the quinacrine fluorescence technique (e.g., Evans, Buckton, and Sumner, 1971) and the Giemsa stain technique (e.g., Drets and Shaw, 1971). Both of these have been applied widely to human chromosome studies. They have also been usefully applied to studies of the mouse (Miller et al., 1972 and earlier; Buckland, Evans, and Sumner, 1971). We think that the banding of some of the chromosomes is sufficiently distinct so that some of our inversions may be seen cytologically. Specifically, since almost all the mouse chromosomes are now associated with linkage groups (Committee, 1972), we hope to identify linkages and relative positions of inversions more quickly using these techniques.

During the last year we have adapted our Zeiss photomicroscope for fluorescent microscopy. Figure 1 shows a fluorescent karyotype of a male heterozygous for In(1)IRk. The inversion in Chromosome 1 is not recognizable in this preparation.

We have also begun to examine the fluorescent banding patterns of first meiotic anaphase bridges in sections 5 microns in thickness. A long bridge should appear symmetrical at either end. This symmetrical segment would represent the part of the chromosome between the centromere and the inversion break-point. The center of the bridge should not be symmetrical and should represent the inverted segment. All bridges of a specific inversion should be similar in banding patterns. It is possible that, since the bridges are sometimes stretched extensively, this approach may lead to identification of chromosomes of new inversions. Also, because of the stretched chromatin more details of banding may appear. We have determined already that banding patterns do appear on fluorescent-stained chromatin bridges.

In the past year we have begun also to make preparations of meiotic pachytene stage in animals heterozygous for the different inversions. These loops not only would be worthwhile to demonstrate for mammalian inversions, but would be useful in estimating the relative length of the inverted segment compared to the total

length of the chromosome. To make these preparations from males we have modified the air-drying technique of Evans, Breckon, and Ford (1964). Essentially this method involves washing meiotic cells out of the testis tubules, treating the resulting cell suspension with hypotonic saline, fixing the preparation in methanol to acetic acid three to one, and making air-dried preparations on slides. We have increased the pipetting time in washing to collect more pachytene cells and have increased the hypotonic treatment to produce more spreading in pachytene figures. The tubules are saved and softened to obtain any pachytene cells not washed out by the pipetting.

For females the procedure is more complicated since the pachytene stage is completed in all oocytes during embryogenesis (Ohno, 1964). We made a series of timed matings using C57BL/6J females and determined that for this strain 70 to 80% of the meiotic cells are in pachytene just prior to birth. We have in progress timed matings between C57BL/6J females and males homozygous for In(1)lRk. Squash preparations will be made of ovaries from 17- to 18-day old embryos. This technique has been used successfully to study pachytene configurations in Cattanaach's fd translocation (Sliznyski, 1967).

2. Inversions produced. Table 1 summarizes the results of screening for new inversions. Roderick (1971) calculated the rate of induction of inversions of a length to produce at least 11% anaphase bridges. This estimate for sperm and spermatids, based on a total of 541 animals with an average radiation dose of 814 R was

$$15/541/814 = 3.4 \times 10^{-5} \text{ per R per gamete}$$

or 2.8% of animals screened.

Now because our data concern only longer inversions, we estimate the induction rate of inversions producing at least 15% anaphase bridges to be

$$16/1557/859 = 1.2 \times 10^{-5} \text{ per R per gamete}$$

or 1.0% of animals screened.

The rate is smaller and indicates, as the array of inversions also does (Table 2), that longer inversions are less frequently induced than smaller ones.

Our first experience with TEM (triethylene melamine) suggests it is a better inducer of inversions (6.2% of animals screened) than irradiation. The inversion produced by TEM, In1ORk, is also extraordinarily long compared to the others. In these 16 animals 11 translocations were found; 2 appeared simultaneously in four animals.

Flupromazine and triflupromazine were chemical mutagens that Dr. Kyle W. Petersen of the University of Maryland and the Food and Drug Administration used here last summer in studies of induction of dominant lethals. We took advantage of this opportunity to screen these males from his study.

Not all inversions can be maintained for one reason or another. For instance, one was found dead, and another was sterile.

Proof that we are dealing with inversions comes from several sources of evidence: The high frequency of anaphase bridges is passed on to half of the sons, and through half of the daughters to their sons. The high frequency acts

as a dominant gene when heterozygous, but recessive and indistinguishable from normal homozygotes when homozygous. Homozygotes when outcrossed pass the frequency to all their offspring. The high frequency in a given line is stable within that line, i.e., it is characteristic of a single inversion. The high frequency is "allelic" with more than one locus covering a segment of a chromosome. For In(1)1Rk we have much data showing that there has been no recombination of four loci within the inversion in inversion heterozygotes.

We have 10 inversions with which we are actively working. They are given symbols only after we can show that their characteristic high frequency is passed to half of the sons. Table 2 summarizes these data for the inversions and shows as well the high correlation between the original male's percentage of anaphase bridges and that of his inversion carrying descendants. (See also Fig. 2). The percentage of inversion carriers is about 50% as expected, and the other 50% show the percentage of anaphase bridges characteristic of normal males. For four inversions, linkages have been found. The chromosome numbers of the inversions are shown in the parentheses of their symbol.

The cytological characteristics of the inversions are variable. The histological slides of the original inversion males were coded and read in detail. They were characterized for the number of anaphases, bridges, fragments, general bridge length, number of broken bridges, telophase bridges, and double bridges. Furthermore, the number of sections for the entire slide was noted so that the frequency of anaphases per section could be determined. Finally each bridge was characterized as to its thickness, shape, and associated lumps. We suspect that short bridges with an associated lack of telophase bridges indicates that inversions are relatively close to their centromeres. This remains to be proved. We also suspect that morphological characteristics of the bridge will be clues either to specific chromosomes or perhaps to specific sizes of chromosomes involved. This also must be proved. In the meantime, we have recorded these idiosyncrasies with the objective of finding meaningful associations.

For example, there is a wide variation among the inversions in the frequency of associated acentric fragments. In10Rk shows one fragment in about every two anaphase bridges, whereas fragments are associated with bridges in In(5)2Rk about 2% of the time. In(1)1Rk, In(2)5Rk, and In10Rk show a number of associated telophase bridges, whereas most of the other inversions produce very few telophase bridges. The bridges of In(1)1Rk are consistently lumpy generally showing a lump at either end and sometimes a lump in the center. The center lump may be an attached acentric fragment, and the lumps at either end may be characteristic coilings of that particular chromosome. The bridges of In(2)5Rk are also consistently lumpy, but most of those of other inversions are thin and smooth. The bridges of In10Rk are uniformly solid in appearance much unlike the other inversion bridges. Bridges of In4Rk and In8Rk are often very irregular which may be the result of the associated translocation in each of those cases.

We derive an initial estimate of the length of an inversion by the percentage of anaphase bridges. If crossing over within the inversion is precisely correlated with its length, then the distance in centi-Morgans would be $1/2$ the frequency of anaphase bridges. There are two reasons why we feel that the length of smaller inversions will be more severely underestimated by bridge frequencies. First, we feel we cannot count broken bridges as part of the count for anaphase bridges, since sometimes artifacts may appear as broken bridges. Therefore any anaphase exhibiting such broken bridges are noted, but are not included in either the numerator or denominator of the fraction of bridges to anaphases. Maximum length of a bridge before it breaks is equal to $2A + B$, where A is the distance from the centromere to

the inversion and B is the length of the inversion. Even though B is only a fraction of this length, a smaller B will lead to a greater likelihood of a broken bridge. This greater frequency of broken bridges reduces the true anaphase bridge frequency as we estimate it.

The second reason for underestimating the length of smaller inversions is the fact that homologous pairing between an inversion segment and its normal homologous segment is likely to be more difficult with smaller segments. If this is true, crossing over would be reduced in the segment and anaphase bridges would be reduced. Since linkage data indicates that even larger inversions like In(1)lRk are underestimated by our bridge counting (described later). The final ascertainment of an inversion's effective length is best estimated by the loci along the chromosome with which it does not recombine.

3. Characteristics of each inversion in the mouse. Following is a summary of the information we have obtained for each useful inversion we have induced.

a. In(1)lRk. The first inversion has been the most extensively propagated and studied, and most of the knowledge we have on the behavior of inversions comes from these particular studies. This inversion has provided the best opportunity for establishing a good mammalian balanced lethal test system because (1) it is a long inversion on the longest chromosome (Roderick, 1971; Committee, 1972), (2) it is homozygous viable, and (3) good genetic markers are available in this chromosome to provide genotypic identification in the balanced lethal test system (Figure 8).

We performed a four-point cross involving four loci in and around the inversion which provided us with the best genetic information for this region of Chromosome 1. The data are summarized in Table 3 and show the gene order and distances are:

fz (fuzzy)

21.3 ± 2.4 cM

Id-1 (Isocitrate dehydrogenase - 1)

12.9 ± 2.0 cM

ln (leaden)

7.1 ± 1.4 cM

Dip-1 (Dipeptidase - 1)

Most important about this region of the chromosome is that it contains two isozyme loci, both of which are in the inversion. Hutton and Roderick (1970) identified the linkage group and position of Id-1. Chapman, Ruddle, and Roderick (1971) found the linkage group of Dip-1 because of its linkage with Id-1. Roderick (1971) suggested Dip-1 must be on the ln side of Id-1, since Dip-1 was found to be within the inversion In(1)lRk. The data above confirm the position of Dip-1.

Figure 3 shows the position of In(1)lRk on Chromosome 1. The criterion for determining the extent of the inversion is its lack of recombination with each of the loci within that segment. In all crosses the inversion has not recombined with loci tb, Id-1, Sp, ln, Dh, and Dip-1. The inversion does show recombination

with fz (fuzzy) and with vl (vacuolated lens) and thus these two loci represent the maximum effective limits of the inversion.

Preliminary data suggest that recombinations of the inversion with fz and vl are much more frequent than would be expected from our knowledge of their respective map distances to tb (tumbling) and Dip-1 (dipeptidase - 1), respectively. It is possible that our sampling of recombination sites is biased since all recombination occurring within the inversion are lost. We therefore may have an increased frequency of recombination of loci outside the inversion approximately equal to the ratio of the map length of the entire chromosome to that of the entire chromosome minus the inverted segment.

One can estimate the minimum length of an inversion as 1/2 the frequency of anaphase bridges, since only half of the chromatids in a tetrad with a bridge would be recombinational. This would be a minimal estimate because (1) there would be a possibility of asynapsis over the entire segment which would permit no crossovers, and (2) crossing over between an inversion paired with its normal homolog could not occur near the ends of the inversion. Table 2 gives anaphase bridge data which indicate a minimal length of 17 cM for this inversion, but the greatest distance between the loci with which it does not recombine is 34 cM (Figure 3). This second estimate is the effective length of the inversion, but it would be an overestimate of the actual length, since the inversion would not be expected to recombine with loci immediately adjacent to it.

b. In(5)2Rk. As was the case with the first inversion, In(5)2Rk was found closely linked with an isozyme locus, in this case Pgm-1 (phosphoglucumutase - 1) on Chromosome 5. The precise location is not known, because of some conflicting linkage data. We are presently establishing a stock which will enable us to make a four-point backcross which will include the inversion and loci bf (buff), rd (retinal degeneration), and Pgm-1. The linkage of Pgm-1 and W (dominant spotting) was found by Hutton and Roderick (1970), and, although its direction from W is not certain, preliminary evidence suggests it is in the direction of bf. The data at this time indicate the inversion is beyond bf toward the end of the chromosome (Figure 3). Because this particular inversion forms rather short anaphase bridges, we believe the centromere must be located at the bf end of the chromosome.

This inversion is one of the smaller ones, but it may have usefulness at a later time since it is viable in homozygous state. The inversion is also being placed on the standard C57BL/6J background by repeated backcrossing to that strain.

c. In3Rk. From knowledge of the anaphase bridge frequency observed in inversion-heterozygotes (15%), this is the smallest inversion we have saved to date. Ascertaining whether a male is an inversion heterozygote is more difficult since at least 40 anaphases must be counted and sometimes more. In addition, this particular inversion does not have associated telophase bridges characteristic of some of the other inversions. Ascertainment, then, is on bridge frequency alone. Of the normal sons of this original inversion-carrying male there were the following numbers of bridges in the first count of 40 anaphases: One male had zero, four had one, two had two, and one had three. Of the inversion-carrying sons, one had three, six had four, one had five, one had six, and one had seven. Half of the animals were characterized by two to four bridges in forty anaphases, and for these an additional 20 to 40 anaphases had to be counted.

In collaboration with Drs. George D. Snell and Marianna Cherry we performed a fourteen-point backcross hoping to uncover the linkage of this inversion as well

as genes for two erythrocytic antigens. We reasoned that if a linkage could be found, the propagation of the inversion would be vastly simplified and therefore worthwhile. Table 4 gives the data regarding this cross and shows no striking association between the inversion and any of the other loci. (One possible linkage was noted however between Ea-4 and Es-3. The possible close linkage between Ea-2 and H-2 should also be further studied.)

With the difficulty of ascertaining carriers for this inversion and with the lack of success in finding its linkage, we have decided not to continue to maintain this inversion. This study of a small inversion was useful in that it elucidated criteria for decision to keep or discard a small inversion. Because we are uncovering new inversions reasonably rapidly, we shall in the future not keep those with anaphase bridge frequencies below 20%.

d. In4Rk. Females and males carrying this inversion showed a typical reduction in litter size characteristic of most translocations. Cytological preparations of the first meiotic metaphase indeed show a quadrivalent in all inversion-carrying males (Figure 4A). The translocation and inversion are still inseparable after several generations of propagation. The simplest explanation is that this represents a reciprocal translocation where one of the translocated elements is inverted with respect to its new centromere.

One interesting cytological observation of this inversion is that the bridges are generally very long.

This inversion-translocation has been tested for linkage against several loci and two possible linkages have been uncovered in two separate backcrosses: (1) 6/22 or 27% recombination with mi (microphthalmia) on Chromosome 6, and (2) 6/25 or 24% recombination with hr (hairless) on Chromosome 14.

e. In(2)5Rk. This is a long inversion probably with an effective length of at least 30 cM. Its location is fairly well established in Chromosome 2 (Figure 3). Males heterozygous for this inversion have an unusually high frequency of first meiotic telophase bridges. This inversion has the potential of providing another balanced lethal testing system, but unfortunately our evidence so far suggests it is homozygous lethal itself.

f. In(10)6Rk. This inversion is at least 15 cM long and is located on Chromosome 10 (Figure 3). It is the third inversion we have been able to maintain in homozygous condition. Anaphases are characterized by many broken bridges which we cannot count in our total estimate of bridge frequency. The inversion may therefore be close to its centromere and probably considerably longer than our estimate from bridge frequency alone.

g. In7Rk. This inversion is at least 10 cM long. No linkage for it is known as yet.

h. In8Rk. This inversion, like In4Rk, is probably a reciprocal translocation where one of the translocated segments is inverted with respect to its new centromere. Associated with it is reduced litter size as well as a large quadrivalent in all Metaphase I cells. We have seen a variety of different quadrivalent configurations, including rings, which suggests that the translocation is reciprocal. Chains (Figure 4B) are about 59% of the cases, X-shaped configurations (Figure 4C) are about 24%, and rings (Figure 4D) are about 24%.

Anaphase bridges are often of the normal thickness, but in addition are sometimes bizarre and very thick as shown in Figure 5. Their unusual thickness may involve at least three chromosomes of the quadrivalent and may happen when in addition to an exchange to form a bridge, there is also another exchange preventing homologous centromeres from moving quickly to opposite poles. This same type of very thick bridge also occurs as expected in In4Rk, but to a lesser extent.

i. In9Rk. This is a new inversion at least 10 cM long. No first meiotic telophase bridges have been seen associated with it, suggesting it is relatively close to its centromere or it is on one of the smaller chromosomes. No linkage data are available as yet.

j. In10Rk. This inversion was induced by triethylene melamine (TEM) in a hybrid male offspring of DBA/2J and C3H/HeJ. Its extremely high anaphase bridge frequency of 70% indicates it is by far the longest we have induced to date. Meiotic and mitotic preparations show this is not a ring chromosome and that there is no associated translocation. A test for linkage of this inversion is just now underway.

k. Naturally occurring inversions. Two years ago we imported a stock of mice classified as Mus musculus molossinus which had been recently derived from a feral population on Kyushu Island in Japan. Crosses of this stock with standard inbred strains of Mus m. musculus showed a consistently high frequency of first meiotic anaphase bridges as well as double bridges. We have continued to backcross the descendants from this cross to inbred strain C57BL/6J and have saved only males with a high bridge frequency. Initial testing with certain isozymes has not been successful in locating a linkage group. The high frequency of double anaphase bridges suggests at least two inversion differences between these subspecies.

4. Lethality resulting from crossing over within the inverted segment. Our initial observations on each of the simple inversions showed that there was no reduction in the litter sizes from inversion heterozygotes. Crossing over between the inverted segment and its normal homologous segment produces chromatids with duplications and deficiencies, and one might expect to find these expressed as embryonic lethals. An additional factor in these particular inversions, because they are all paracentric, is that crossing over produces an anaphase bridge which may be involved in the selection against crossover products.

In the last year we did a study to assure that there was no reduction in litter size and to search for the stages at which selection against crossover products occurs.

There were essentially three groups of animals used in the study:

- (1) Males doubly heterozygous for inversions In(1)1Rk, and In(2)5Rk
- (2) Sibling males to the above that carried neither inversion, and
- (3) Males heterozygous for the inversion-translocation In4Rk.

We chose males heterozygous for two inversions to increase the frequency of anaphase bridges and thereby exaggerate the effects of inversion heterozygosity. The anaphase

bridge frequency of the double heterozygotes is approximately what is theoretically expected from our information on the individual frequencies of each inversion. In addition there is a fraction of double bridges which is approximately the product of the individual fractions. Figure 5 shows a section of the testis of one of these males with arrows pointing to (1) a normal anaphase, (2) a double anaphase bridge resulting from simultaneous bridges of both inversions, and (3) a telophase bridge of one of the inversions.

All males were mated with C57BL/6J females with no history of ancestral irradiation. The matings were handled in two ways: In the majority the cages were inspected daily for females in late pregnancy. Pregnant females were removed, killed, and examined. In the second group, females were all killed 20 days after introduction of the males into the cages. No differences were found between the groups, so the data from them were combined.

The uteri of pregnant females were examined by the method described by Green, Roderick, and Schlager (1964). Implants were scored as live embryos, resorptions, and late embryonic deaths.

The results in Table 5 show no differences in reproductive performance between the double inversion heterozygotes and their normal male siblings. Animals heterozygous for the inversion-translocation show, as expected, some reduction in live fetuses and greater numbers of dead fetuses per litter. This difference, however, is not as great as expected from translocations. Further work is necessary to determine why it is not as great.

From these data, we looked to earlier stages for the elimination of the products of recombination. Relative number of sperm produced by the three different genotypes were determined as follows: Sperm were collected from the vas deferens by removing the entire duct from the caudal epididymis to the bladder and stripping it into a measured volume of 0.85% saline solution. The assumption was made that the sperm count is constant for any unit volume collected in this location. The sperm were transferred to a test tube, agitated in the solution and allowed to stand. Four separate samples of each solution were transferred to hemacytometers where counts were made. Any variation in sperm production by these males might be shown in a variation of total number of sperm present in the vas. All animals used in this phase of the study were individually caged for at least five days prior to sacrifice.

We also estimated the percentage of sperm viability by a modification of the method of Lasley, Easley, and McKenzie (1942).

Sperm counts did not differ between genotypic groups, nor was there any detectable difference in sperm viability. In the course of this study no grossly abnormal sperm were seen. These preliminary studies suggest that selection against the products of crossing over takes place prior to spermiogenesis.

We studied the seminiferous epithelium of males using both paraffin sections and fresh material. Sectioned testes embedded in paraffin and stained with gallo-cyanin permitted us to follow the division of cells through the second meiotic anaphase. Figure 7 shows a section of a tubule roughly at the time of the second meiotic anaphase. To the left of the numeral 1 is a normal second anaphase. Immediately to the right of the numeral 2 is a late second anaphase with an apparent fragment left in the spindle. Further to the right is a second meiotic anaphase bridge. Although second anaphase bridges and fragments can occur together as a result of three strand double crossing over both within and outside the inverted

segment, we think the presence of an acentric fragment alone at second anaphase probably represents a first anaphase fragment which was carried to a daughter nucleus in the first division. If this is true, then lethality from crossing over in the first division does not occur until after the second anaphase division.

These studies indicate that the selection against crossing over products in inversion heterozygotes occurs earlier than fertilization and probably between the second anaphase division and the formation of sperm.

5. Balanced lethal test system. The major purpose of these studies is to produce inversions which can be used in a balanced lethal system of a kind which have had such practical advantages in the study of genetics in Drosophila. In(1)lRk is the most suitable inversion so far produced for this purpose, because (1) it is long, (2) it covers a chromosomal segment which has good genetic markers, and (3) it is homozygous viable. Id-1 (isocitrate dehydrogenase - 1), Sp (splotch), ln (leaden) and Dip-1 (dipeptidase - 1) are the most useful loci in the inverted segment (Figure 3). Splotch is a viable dominant spotting marker which is lethal in homozygous form. There also appears to be a slight reduction in viability of Sp/+ heterozygotes. The mutant allele is fully penetrant but quite variable. Some animals exhibit a large clear white belly spot, whereas in a few only the tips of the feet are white. In only one or two cases of the several hundred we have observed have we had trouble distinguishing a Sp/+ animal from the wild-type +/+. Leaden is a fully penetrant recessive coat color marker with no effect in the laboratory on fertility or viability. Loci Id-1 and Dip-1 are polymorphic in feral populations and are fully penetrant codominant loci. Both are determined by starch gel electrophoresis. Neither has any detectable effect on fertility or viability.

Figure 8 shows the mating scheme of this system which makes use of the two external phenotypic markers. Animals homozygous for the inversion are treated with the mutagen and mated to animals of the splotch-leaden stock. All offspring are potential bearers of recessive visibles or lethals. Propagation from the splotch (Sp/+) animals is shown in Figure 8 and from the wild-type (+/+) alternate animals in Figure 9. In the case of the splotch animals, each is mated to another animal from the splotch-leaden stock of the same genotype as its parent from that stock. Each test animal at this point (Generation IIa) becomes the progenitor of two more generations of descendants ending in the test Generation IV. In Generation III the leaden marker distinguishes the normal splotch from a splotch-inversion double heterozygote. The latter should be identical to the test parent with respect to the inverted and normal segments. All four genotypes of Generation III can be distinguished either through coat color or pattern, or because they die. The test animal can be mated to his offspring of identical genotype, or offspring of that genotype can be mated inter se. In the test generation one should expect a ratio of 2:1 of splotch animals to wild-type animals. Any appreciable and consistent reduction in wild-type animals suggests a lethal has been induced at a locus near the inversion. A complete absence of wild-type animals suggests a lethal has been induced within the inverted segment. Also the wild-type class may exhibit recessive visibles or subvitals. In using the alternate animals of Generation IIa, the mating system is altered only slightly and is different in genotypes only in Generation III. All four genotypic classes can be distinguished, and only the non-leaden splotch animals are used for mating to produce Generation IV. In this case matings can only be made between animals of this genotype in Generation III, since the test animal of Generation IIa cannot be used.

The induction rate for recessive lethals per genome in the mouse has been estimated by Luning and Searle (1971) for spermatogonia to be about 10^{-4} per roentgen per genome. For sperm and spermatids, which we are irradiating, it may be over twice that rate. Since the genome of the mouse is approximately 1600 cM in length, our inversion (about 34 cM) is approximately 2.1% of the genome. We can give about 1000 R to sperm and spermatids to induce lethals and detrimental. Our expectation would be:

$$10^{-4} \text{ (per R per genome)} \times 2 \text{ (increased factor for sperm and spermatids)} \\ \times 1000 \text{ R} \times 0.021 \text{ (fraction of the genome)} = 0.004.$$

If we also include about 20 cM on either side of the inversion which the system would scan, and if we also include the probability of inducing some recessive visibles and detrimental in this region of the chromosome, our probability of finding something may be in the neighborhood of 1/50. With the system we can also screen simultaneously for dominant mutations as well as mutations at the two isozyme loci included within the inversion.

Initially we have used high doses of radiation to sperm and spermatids to test the ability of the system to uncover recessives. Table 6 shows the number of animals we have tested from Generation IIa and those which are still in progress. Twelve have been completed and 60 are in progress. For each test animal of Generation IIa, we raise at least 30 offspring in Generation IV and examine the ratio of Sp/+ to +/+ in them. Also at least one Sp/+ and +/+ animal are examined for their alleles at loci Id-1 and Dip-1. Table 7 gives the numbers of animals tested for all genotypes in Generation IV. Only the two parental genotypes occurred in 80 animals. This combined with previous data (Roderick, 1971) brings to 108 the number of animals where at least 3 or more markers have been tested and found not to recombine in the presence of the inversion. Loci ln, Sp, and Id-1 are centrally located in the inversion and would be most likely involved in a double recombination when it occurs. We are searching for the products of double recombination, since that would provide us with allelic variation within the inverted segment. Figure 10 shows the starch gel electrophoretic patterns at Id-1 and Dip-1 for 17 males from those given in Table 6. Animals represented in slots 1 to 5, 9, and 11 were inversion homozygotes with genotypes (from Table 6) b + + b/b + + b. Animals of slots 6 to 8, 10, and 12 to 17 were heterozygous for the inversion and have genotypes b + + b/a Sp ln a. Slots 1 through 17 are patterns derived from kidneys, and slots 18 and 19 are testicular tissue from a homozygote and heterozygote respectively. That we could detect both of these isozymes in testicular tissue was a great advantage for using this inversion, since animals do not have to be killed to ascertain their alleles. One testis can be removed under general anesthesia, and the animal can be used for mating after starch gel analysis.

Another important feature about the isozyme loci is their codominance. We therefore have the opportunity of finding new allelic mutations which could be seen as a band in a new location in both heterozygotes and homozygotes. Codominance also permits us to see the absence of a band in the heterozygotes if a deletion of either locus is induced. We would expect all mutations at either of these loci to occur in the B band because the inversion homozygote exposed to the mutagen is homozygous for b at both loci.

In all of the matings between the homozygous stock and the splotch-leadon stock (Generation I, Figure 8), there were produced 49 +/+ animals and 39 Sp/+ animals, with about equal numbers in each sex. This should be a 1:1 ratio. Also

in the combined data of the Generation IV there have appeared 96 $+/+$ animals and 153 $Sp/+$ animals. This should be a 1:2 ratio. Both sets of data are consistent in suggesting about a 20% reduction in viability of $Sp/+$ genotype at least sometime before weaning when the classification is made. As mentioned earlier, even though $Sp/+$ produces a variable phenotype, there is almost no problem in misclassifying them with wild-type.

In the balanced lethal study the Generation IV descendants of one test animal (female 8010-1) showed a gross departure from a 1:2 ratio. These data are not included in the totals above but are given separately in Table 8. The 3 wild-type animals are perfectly viable and healthy in appearance, and thus this does not appear to be a recessive detrimental within the inverted segment. Most likely it is a lethal outside the inverted segment but closely linked. The lethal could be in the center of the inverted segment, but our data indicate that double crossovers within the segment could not be so frequent to produce 3 $+/+$ animals in this sized sample. We can assume that we should have produced about $1/2$ as many $+/+$ animals as $Sp/+$, or 27.5. Only one crossover between the inversion and the lethal is necessary to produce a viable $+/+$ animal, and therefore there were, among the gametes which produced this hypothetical 27.5 animals, 55 opportunities for recombination. The crossover value then is $3/55$ or 5.5%.

If a crossover between the inversion and the lethal occurs in an animal used to produce further animals of Generation IV, one would expect the ratio of that mating to go immediately back to 1:2 of $+/+$ to $Sp/+$. The data in Table 7 do not yet suggest that this has occurred.

No aberrations were seen in the allelic patterns at either Id-1 or Dip-1 in heterozygotes of Generation IV. Tests are underway to determine if a possible deletion has been induced in loci outside the inversion, but closely linked to it, such as fz and vl (Figure 3).

The important fact of having this lethal is in the verification of the system to detect lethals within a reasonable number of animals tested (i.e., one of 12). It is also important to have a recessive lethal which can be propagated and studied in itself.

6. Importation of animals. In addition to Mus m. molossinus we imported another subspecies called the Tobacco mouse, Mus m. poschiavinus. This interesting subspecies is fully fertile with laboratory mice, but most importantly has a haploid characteristic of metacentric and 6 acrocentric chromosomes instead of the 20 acrocentric chromosomes of laboratory mice. These metacentric chromosomes probably arose by Robertsonian translocation (Gropp, Tettenborn, and von Lehmann, 1970) since the chromosome arms are individually identifiable with specific acrocentric chromosomes of laboratory mice (Zech et al., 1971). The metacentric chromosomes are combinations of the following acrocentric chromosomes respectively: 3-1; 6-4; 15-5; 11-9; 12-8; 13-14; and 16-17. We have separated the metacentrics in several different stocks, and plan eventually to establish a stock homozygous for each one. We also plan to put inversion In(1)IRk into the largest metacentric which includes Chromosome 1. This stock then could be further treated with mutagen to produce a more complex inversion which might effectively reduce recombination for a major part of Chromosomes 1 and 3. Inducing inversions in the metacentrics alone may also achieve more efficient balanced lethal systems.

We have also recently imported Mus m. castaneus, a subspecies from Thailand, and another stock descended from a feral population from Ohio. These stocks together contain four new isozyme variants which are not closely linked to those we use in our standard linkage procedures (Verne M. Chapman, personal communication). Incorporating these new variants into our test system will substantially increase the efficiency of the search for linkages of new inversions.

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TABLE 1. SCREENING FOR INVERSIONS WITH VARIOUS MUTAGENIC AGENTS. ALL MALES WERE CONCEIVED WITHIN TWO WEEKS AFTER TREATMENT OF THEIR SIRE.

Treatment of sire	Number of male offspring observed	Number of inversions found*
None	29	0
Saline, I.P.	15	0
700 R	158	0
750 R	29	0
800 R	293	4
850 R	28	1
900 R	1024	11
1000 R	25	0
Ethylmethane-sulfonate		
(EMS) 400 mg/kg, I.P.	4	0
Triethylene melamine		
(TEM) 0.2 mg/kg, I.P.	16	1
Flupromazine		
(FP) 40 mg/kg, I.P.	36	0
Triflupromazine		
(TFP) 75 mg/kg, I.P.	44	0

*Males are classified as inversion carriers only if they show frequencies of first meiotic anaphase bridges consistently above 15%.

TABLE 2. COMPARISON OF PERCENT OF ANAPHASE BRIDGES BETWEEN ORIGINAL INVERSION-HETEROZYGOTES AND THEIR DESCENDANTS WITH HIGH FREQUENCIES.

	Number of	Percentage of	High descendants		Normal descendants		p*
	original ♂	original ♂	N	Percentage	N	Percentage	
In(1)1Rk	618	61/181 = 33.70	155	1264/3721 = 33.97	145	124/3480 = 3.56	0.94
In(5)2Rk	816	29/137 = 21.17	48	273/1440 = 18.96	50	55/1500 = 3.67	0.54
In3Rk	2457	20/135 = 14.81	10	45/320 = 14.06	8	11/320 = 3.44	0.97
In4Rk	2913	23/100 = 23.00	38	139/760 = 18.29	24	19/480 = 3.96	0.26
In(2)5Rk	2916	59/154 = 38.31	51	346/1020 = 33.92	69	76/1380 = 5.51	0.29
In(10)6Rk	3563	29/100 = 29.00	24	123/480 = 25.62	16	12/320 = 3.75	0.49
In7Rk	4587	7/40 = 17.50	9	26/180 = 14.44	9	11/180 = 6.11	0.64
In8Rk	4609	39/180 = 21.67	7	17/140 = 12.14	7	6/140 = 4.29	0.03
In9Rk	5161	20/97 = 20.62	2	6/40 = 15.00	6	3/120 = 2.50	0.46
In10Rk	5204	68/97 = 70.10	2	16/20 = 80.00	2	0/20 = 0.00	0.39

*p is the probability, based on a χ^2 test, that the difference between the percentages of the original male and his inversion-carrying descendants is due to chance.

TABLE 3. PROGENY OF A FOUR-POINT* BACKCROSS ON CHROMOSOME 1.

The cross: $\frac{fz\ b\ ln\ a}{fz\ b\ ln\ a} \text{♀} \times \frac{fz\ b\ ln\ a}{+ \ a \ + \ b} \text{♂}$

Parental		Double crossovers	
$\frac{fz\ b\ ln\ a}{fz\ b\ ln\ a}$	80	$\frac{fz\ b\ ln\ a}{fz\ b\ +\ a}$	0
$\frac{fz\ b\ ln\ a}{+ \ a \ + \ b}$	92	$\frac{fz\ b\ ln\ a}{+ \ a\ ln\ b}$	0
Single crossovers		$\frac{fz\ b\ ln\ a}{fz\ a\ ln\ a}$	2
$\frac{fz\ b\ ln\ a}{fz\ b\ ln\ b}$	10	$\frac{fz\ b\ ln\ a}{+ \ b \ + \ b}$	0
$\frac{fz\ b\ ln\ a}{+ \ a \ + \ a}$	8	$\frac{fz\ b\ ln\ a}{+ \ b\ ln\ b}$	0
$\frac{fz\ b\ ln\ a}{fz\ b\ + \ b}$	17	$\frac{fz\ b\ ln\ a}{fz\ a \ + \ a}$	1
$\frac{fz\ b\ ln\ a}{+ \ a\ ln\ a}$	18	Triple crossovers	
$\frac{fz\ b\ ln\ a}{fz\ a \ + \ b}$	27	$\frac{fz\ b\ ln\ a}{fz\ a\ ln\ b}$	0
$\frac{fz\ b\ ln\ a}{+ \ b\ ln\ a}$	31	$\frac{fz\ b\ ln\ a}{+ \ b \ + \ a}$	0

*The loci for each genotype are arranged in the order fz, Id-1, ln, Dip-1.

TABLE 4. ATTEMPT TO DETERMINE LINKAGE OF In3Rk*

Linkage Group	?	XIII	XIII	VIII	XVII	I	I	XVIII	II	IX	XII	VII	?	?
Chromosome	?	1	1	4	5	7	7	8	9	17	19	?	?	?
Locus	In	Id-1	Dip-1	Gpd-1	Pgm-1	Gpi-1	Hbb	Es-1	Mod-1	H-2	Ly-1	Es-3	Ea-2	Ea-4
Animal No.														
1	I/+	ab	ab	ab	ab	ab	s	ab	ab	b	ab	ac	b	ab
2	I/+	ab	ab	ab	ab	ab	sd	ab	b	b	b	ac	bd	ab
3	+	a	ab	a	a	b	s	ab	ab	b	b	a	b	ab
4	I/+	a	a	ab	a	b	s	a	b	bd	b	a	b	ab
5	I/+	ab	ab	a	ab	b	s	a	b	bd	ab	a	b	ab
6	I/+	ab	ab	a	ab	ab	sd	ab	b	bd	ab	a	bd	b
7	I/+	a	a	a	ab	ab	sd	ab	ab	bd	ab	a	bd	b
8	+	a	a	a	a	ab	sd	a	ab	bd	ab	ac	bd	ab
9	+	ab	ab	ab	a	b	sd	a	b	b	b	a	bd	b
10	I/+	ab	ab	a	a	b	sd	ab	ab	b	b	a	bd	b
11	I/+	ab	a	a	ab	b	s	ab	b	b	b	a	b	b
12	I/+	a	a	ab	a	ab	sd	a	ab	b	b	a	bd	b
13	+	ab	ab	ab	ab	ab	sd	a	b	bd	ab	ac	bd	b
14	I/+	a	ab	a	a	ab	sd	ab	b	bd	ab	ac	bd	ab
15	+	ab	ab	ab	ab	ab	sd	ab	b	b	b	a	bd	b
16	+	a	a	a	a	ab	sd	a	ab	b	ab	ac	b	ab
17	+	ab	ab	a	ab	ab	sd	a	ab	bd	ab	ac	b	ab
18	+	ab	ab	a	a	ab	sd	ab	b	b	ab	a	b	b

* For ease of comparison, only one allele of homozygotes is given.

TABLE 5. ZYGOTIC LETHALITY IN OFFSPRING OF INVERSION HETEROZYGOTES.

	Genotypes*		
	In1/+, In5/+	+/, +/+	In4/+
	N** = 32	N = 32	N = 21
	$\bar{X} \pm \text{S.E.}$	$\bar{X} \pm \text{S.E.}$	$\bar{X} \pm \text{S.E.}$
Live fetuses	6.8 ± 0.3	6.7 ± 0.4	6.0 ± 0.6
Dead fetuses	1.8 ± 0.2	1.6 ± 0.3	2.3 ± 0.5

* In1, In4, and In5 represent the first, fourth, and fifth inversions respectively.

** N is the number of matings on which the data are based.

TABLE 6. ANIMALS TESTED FOR RECESSIVE MUTATIONS IN THE BALANCED-LETHAL TEST SYSTEM.

Mutagen	Sp/+		+/+	
	♀♀	♂♂	♀♀	♂♂
<u>Test completed</u>				
900 R	4	5	0	0
850 R	2	1	0	0
800 R	0	0	0	0
				Total- - - 12
<u>Test in progress</u>				
900 R	2	6	7	5
850 R	0	0	0	0
800 R	8	7	13	12
				Total- - - 60

TABLE 7. GENOTYPES OF MALES OF THE TEST-GENERATION WHERE ALL LOCI AND THE INVERSION WERE SCORED*.

THE CROSS**:

	$\frac{\text{In}(b + + b)}{+ (a \text{ Sp } \ln a)}$	×	$\frac{\text{In}(b + + b)}{+ (a \text{ Sp } \ln a)}$
	$\frac{\text{In}(b + + b)}{\text{In}(b + + b)}$		$\frac{\text{In}(b + + b)}{+ (a \text{ Sp } \ln a)}$
♀♀	13		20
♂♂	13		34

* Females were not scored with respect to anaphase bridges. At the ln locus only +/? and ln/ln could be distinguished.

** Loci within the inversion are arranged in the order Id-1, Sp, ln, Dip-1.

TABLE 8. RESULT OF GENERATION IV FROM TEST FEMALE 8010-1.

Type of Mating	Dam	Sire	Offspring	
			+/+	Sp/+
			♀♀:♂♂	♀♀:♂♂
Daughter × son	8174-3,	8174-4	0:0	14:14
Granddaughter × grandson	8491-1,	8491-6	1:0	3:2
	8491-2,	8491-5	1:1	6:3
	8491-3,	8491-4	0:0	1:5
	8540-1,	8540-3	0:0	4:1
	8605-1,	8605-3	0:0	0:1
	8605-2,	8617-1	0:0	0:1
	Total*		2:1	28:27

* Departure from ratio 1:2 of +/+ to Sp/+: $\chi^2 = 20.7$; 1 df; $P < 0.001$.

Figure 1. Karyotype, produced by quinacrine fluorescence, of female

heterozygous for In(1)IRk.

Figure 2. The correlation of percent anaphase bridges between original inversion heterozygotes and their respective inversion-carrying descendants.

Figure 3. Inversion map of the mouse

Figure 4. Representative quadrivalents appearing in first meiotic metaphase
of inversion-translocations.

4A: In4Rk

4B,C,D: In8Rk

Figure 5. The unusually thick anaphase bridges often seen in heterozygotes
for In8Rk.

Figure 6. First meiotic anaphase of a male heterozygous for two inversions,

In(1)lRk, and In(2)5Rk.

(1) a normal anaphase

(2) double bridge at anaphase

(3) telophase bridge

Figure 7. Second meiotic anaphase of male heterozygous for inversions

In(1)lRk and In(2)5Rk. Note second anaphase bridge and a

second anaphase acentric fragment without associated bridge.

Figure 8. Balanced lethal test system using inversion-In(1)lRk.

Figure 9. Balanced lethal test system used when propagating from alternate wild type animals of generation II in Figure 8.

Figure 10. Starch gel electrophoretic patterns of loci Isocitrate dehydrogenase - 1 and Dipeptidase - 1 of 17 males in Generation IV of the balanced lethal test system. Slots 1-17 were prepared from kidney tissue, slots 18 and 19 from testicular tissue. Males of slots 1-4, 9, 11, and 18 were homozygous at four loci tested within the inverted segment as well as for the inversion. Animals of the other slots were heterozygous at all loci and the inversion.

Id-1

Dip-1

1 2 3 4 5 6 7 8 9

10 11 12 13 14 15 16 17 18 19

3. CONTINUATION PLANS

1. Inversion In(1)1Rk. We will increase using the balanced-lethal test system to induce recessive lethals and detrimentals by irradiation of sperm and spermatids. Further linkage tests will be made to determine more precisely the limits of this inversion. Linkage tests will be started to determine the location of the induced recessive lethal near the inversion. The inversion will be incorporated into the largest metacentric from the tobacco mouse population.

2. Other inversions. Eight of the other nine inversions will be studied further to determine their precise linkage positions and to develop each in a homozygous line. Wherever feasible, balanced lethal systems will be constructed with each inversion, and attempts made to combine two or more such systems.

3. Cytogenetic analysis. By quinacrine and Giemsa staining we will attempt to show visually the inverted segments of the various inversions. We shall also examine pachytene stages for inversion loops.

4. Further screening. We shall continue to induce and detect inversions using the chemical mutagen TEM.

5. Linkage test system. We shall incorporate four new isozyme loci into our linkage test system.

4. STUDENTS AND VISITING INVESTIGATORS

The Jackson Laboratory is primarily a research institution, but its Staff members are directly concerned with training students for careers in biomedical research. This training is accomplished through summer and year-around programs of research participation wherein each student carries out a research project under a Staff member's sponsorship. Visiting Investigators join in collaborative research efforts of mutual interest. During this past year the following students and Visiting Investigators have participated in projects directly relating to research of this contract. Their work was done in collaboration with the Principal Investigator in his laboratory.

GROUP

Name and address

Title of project

SUMMER PROGRAM FOR PRECOLLEGE STUDENTS

Philip W. Kantoff
Forest Hills High School
Forest Hills, N. Y.

Characterization of isozyme alleles and their use in locating linkages of chromosomal inversions

SUMMER PROGRAM FOR COLLEGE, GRADUATE, AND MEDICAL STUDENTS

Frederick R. Bieber
Oswego State College
Oswego, N. Y.

A study of the poschiavinus metacentric chromosome in incipient inbred lines derived from a cross of Mus m. musculus and Mus m. poschiavinus

PREDOCTORAL TRAINING PROGRAM

Keith T. McMahon
University of Maine
Orono, Maine

Effect of heterozygous chromosomal inversions on meiotic, gametic and zygotic viability in Mus musculus.

POSTDOCTORAL TRAINING PROGRAM

Eugene R. Soares, Ph.D.
The Jackson Laboratory

Chemical mutagenesis and new techniques for assessing dominant lethals

VISITING INVESTIGATOR PROGRAM

Kyle W. Petersen, Ph.D.
University of Maryland
School of Medicine and
Food and Drug Administration

The effect of chemical mutagens in producing dominant lethals and chromosomal inversions

Frank H. Ruddle, Ph.D.
Yale University
New Haven, Conn.

Chromosomal variations in mice

5. BIBLIOGRAPHY

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6. PRESENT STATUS OF RESEARCH IN THIS FIELD

Studies in mammalian mutagenesis are still hampered by the relatively inefficient systems available to assess and maintain recessive lethals. Furthermore, there is essentially no information available for mammals on the dominant effects of recessive lethals. The two major techniques for inducing recessive lethals have been the specific locus technique of W. L. Russell and the entire genome scan for recessive lethals of K. G. Lüning. The former technique is relatively inefficient in estimating rates of induction of lethals since only 7 loci can be tested simultaneously. But

the system is efficient for maintaining lethals once they have been induced. The latter system is more efficient in screening for lethals, but is less efficient in maintaining them.

Our approach is the first study to induce, genetically define, and use inversions in mice. With a balanced lethal system now available we have been able to screen about 5% of the genome for recessive visibles, detrimental, and lethals. Recessives induced with the system are easily propagated for study. Of 12 test animals where the father has received at least 800 R to the sperm or spermatids, one recessive lethal closely linked to the inverted segment has been revealed.