MASTER

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OBJECTIVE

The purpose of this project is to produce chromosomal inversion by chemical treatment or irradiation of sperm in mice and to detect inversions by observing high frequencies of first meiotic anaphase bridges of their sons or by using chromosomal banding techniques to detect inverted segments cytologically. For each new inversion, which is either of considerable length or which has particularly useful experimental properties, we will (1) determine its linkage group, (2) mark it genetically, if possible, or place it with a genetically marked homologous chromosome, and (3) study its cytological, physiological, and anatomical effects. The inversions are being used (1) to construct recessive lethal testing systems for estimating mutational loads in populations exposed to radiation or either proved or potential chemical mutagens, (2) to mark and maintain induced lethals for analysis of their potential dominant effects on fitness, and (3) to study other basic problems in mammalian genetics.

MAIN RESEARCH ACCOMPLISHMENTS

Detection of inversions. Our usual procedure is to treat strain DBA/2J male mice with radiation 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 5 per cent) in the first anaphases of almost every male (Roderick, 1971; Roderick and Hawes, 1974).

The nature of these bridges is not known, but it is necessary to distinguish them quantitatively from a higher frequency of bridges that indicates 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.

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 look for the linkage of the new inversion with 12 isozyme loci and two coat color loci simultaneously. Assuming we can detect the presence of linkage at least 30 cM from each marker in the backcross, and with knowledge of the location of the markers with respect to each other and the relative position within the chromosome, we estimate that in this single backcross we cover 632 cM of the autosomal genome of the mouse which is approximately 1500 cM in total. Thus, in the first backcross we can expect to find linkage of a new inversion 42 per cent of the time (Table 1). For mammalian genetics, this is an important and powerful tool. If we are unsuccessful in the initial cross we use other appropriate linkage testing stocks. At the same time we use peripheral lymphocyte culture and chromosomal banding techniques to cytologically detect the inverted segment.

TABLE 1. THE 14 LOCI CHARACTERIZED IN A BACKCROSS USING STRAINS C57BL/6J AND DBA/2J.

Gene	Name	Chromo- some	Alleles		cM screened in backcross
			C57BL/6J	DBA/2J	
<u>Dip-1</u>	Dipeptidase-1	1	a	b	87
<u>Id-1</u>	Isocitrate dehydrogenase-1	1	a	b	
<u>Car-2</u>	Carbonic anhydrase-2	3	a	b	30
<u>b</u>	Brown	4	+	b	92
<u>Gpd-1</u>	Hexosephosphate dehydrogenase-1	4	a	b	
<u>Pgm-1</u>	Phosphoglucomutase-1	5	a	b	60
<u>Gpi-1</u>	Glucosephosphate dehydrogenase-1	7	b	a	75
<u>Hbb</u>	Hemoglobin beta chain	7	s	d	
<u>Es-1</u>	Esterase-1	8	a	b	60
<u>d</u>	Dilute	9	+	d	68
<u>Mod-1</u>	Malate dehydrogenase-1	9	b	a	
<u>Es-3</u>	Esterase-3	11	a	c	40
<u>Es-10</u>	Esterase-10	14	a	b	60
<u>Lvp-1</u>	Liver protein-1	?	a	b	60
				Sum	632 cM

Biochemical markers. The above experimental tools have in a large part been developed by our laboratory in collaboration with other, particularly Drs. F. H. Ruddle, J. J. Hutton, and J. E. Womack. This has been a continual search for electrophoretic markers which are polymorphic with alleles that are neutral in selective value. The markers so far discovered have been useful in a variety of experimental programs. In the inversion projects, we use them as described above chiefly to map newly induced inversions. More importantly, they can be used in themselves to mark inversions genetically when inversions happen to include these markers. The most useful inversions for our purposes in studying mutagenesis are those which completely "inhibit" recombination. In these cases, there is no possibility of marking the inversions with useful phenotypic markers after the inversions have been produced; but the polymorphic nature of the common electrophoretic markers makes it possible to mark some inversions simply by choosing stocks to use against them which have different alleles at the loci of interest. In these cases we can take advantage of all the isozyme markers known and not just those that differ between our two parental strains. At least 40 such loci are now available in the animals available at the Jackson Laboratory. Of the 17 inversions which we have mapped nearly all overlap or are closely linked with some of these markers.

Inversions produced. A male is characterized as carrying a presumptive inversion if, upon continued counting of his first meiotic anaphases, 15 per cent or more show clear bridges. Ordinarily a presumptive inversion is given a symbol only after the animal produces offspring half of which show the same high bridge frequency. The exceptions are those few cases in which the animal carrying the presumed inversion did not reproduce, but cytological banding techniques clearly revealed an inverted segment on a particular chromosome and no other apparent chromosomal (karyotypic) aberration.

We continue to use intraperitoneally injected triethylene melamine (TEM) exclusively to produce inversions. We use a dose of 0.3 mg/kg of body weight, which is about as high as we can go without producing a significant incidence of sterility in the treated males.

Table 2 shows all the inversion and presumptive inversions produced in our laboratory to date. The last two were produced within this past year. The table shows the different lengths of the inversions, the various chromosomes affected, and the variety of possible physiological decrements associated with inversions. In27Rk and In28Rk were both induced in mice carrying Robertsonian chromosomes but they do not involve the Robertsonian chromosomes. We have also recently added In(X)1H to our inversion colony. This is the only mouse inversion discovered outside our laboratory. It's very important because it is the only inversion known on the X-chromosome. With it, sex linked lethals may be readily detected.

Maintaining inversions. The inversions, where the original carrier was viable and fertile, have usually been found to be homozygous viable and fertile. The only exceptions are inversions In(2)5Rk, which appears to be homozygous lethal in males (occasionally homozygous viable in females), and In(10)17Rk, in which inversion homozygotes are smaller and sterile. Nevertheless, the general character of homozygous viability of inversions means they can be easily maintained in inbred lines with no need for complicated breeding schemes. We maintain In(2)5Rk balanced against the gene pa and In(10)17Rk balanced against the gene sl.

TABLE 2. INVERSIONS INDUCED IN THE MOUSE.

Male number	Inversion symbol	Anaphase bridge frequency	Induced* by	Induced** on	Comments
471		.379 ± .033	800 R	<u>Ra, Sd</u>	Functionally sterile
587		.176 ± .034	"	DBA/2J	Died before mating
618	In(1)1Rk	.340 ± .008	900 R	"	
639		.151 ± .026	"	"	Fertile, discarded
816	In(5)2Rk	.192 ± .010	850 R	"	
2414		.340 ± .048	900 R	"	Small, died
2425		.158 ± .033	"	"	Discarded
2457	In3Rk	.143 ± .016	"	"	Discarded
2576		.164 ± .050	"	"	Discarded
2624		.175 ± .050	"	"	Discarded
2913	In4Rk	.188 ± .013	"	"	Lost
2916	In(2)5Rk	.345 ± .014	"	"	
3563	In(10)6Rk	.262 ± .018	"	"	
4587	In7Rk	.150 ± .024	800 R	"	Discarded
4609	In8Rk	.179 ± .019	"	"	Lost
5204	In(5)9Rk	.737 ± .020	TEM .2	C3D2F ₁	
6567	In(1)10Rk	.741 ± .033	EMS	"	Functionally sterile
6845	In(12)11Rk	.294 ± .055	TEM .2	C57BL/6J	
6857	In(1)12Rk	.228 ± .047	"	"	
7877	In(7)13Rk	.447 ± .040	"	C3D2F ₁	
8677	In14Rk	.347 ± .043	"	DBA/2J	
10434	In(3)15Rk	.689 ± .038	"	B6D2F ₁	Functionally sterile
11318	In16Rk	.458 ± .059	TEM .3	DBA/2J	T(1;In8)1Rk, sterile
11323	In(10)17Rk	.648 ± .040	"	"	Small homozygotes
11449	In(15)18Rk	.366 ± .024	"	"	
11675	In(2)19Rk	.609 ± .035	"	"	
11684	In(11)20Rk	.473 ± .037	"	"	Litter size reduced
11865	In(18)21Rk	.469 ± .032	TEM .4	"	Possibly semi-sterile
11951	In(14)22Rk	.663 ± .036	"	"	
12164	In(1)23Rk	.364 ± .040	"	"	Probably In(1)1Rk
12174	In(1)24Rk	.728 ± .046	"	"	
12452	In25Rk	.459 ± .048	"	"	
12953	In(9)26Rk	.237 ± .028	"	"	Identified cytologically
16567	In27Rk	.500 ± .067	TEM .3	POS (M)	Died before mating
16647	In28Rk	.559 ± .085	"	POS (AxD)	

*Numbers by TEM indicate dosage in mg/kg body weight.

**Symbols represent strains, stocks, or hybrids. POS are stocks carrying Robertsonian chromosomes.

Inversion symbol: In refers to inversion.

Number in parenthesis is the chromosome in which the inversion occurs.
Number following parenthesis or alone is the number of the inversion found in our laboratory.

Rk is the symbol of our laboratory.

Phenotypic effects of inversions. Paracentric inversions still have not been found between inbred strains, or in wild populations that we have examined. This is perplexing because there is little evidence of either reduction of viability or reproductive performance in homozygotes or heterozygous inversion carriers. One might expect selective disadvantages for new gene arrangements or at least for possible complications as inversions pass through meiosis. Possible phenotypic effects of some inversions are mentioned in Table 1. As yet no phenotypic effects can be attributed definitely to an inversion. We now know that In(2)5Rk has a small deletion as we suspected. This has been shown by Dr. Montrose Moses, Duke Medical School, using a method developed by him for visualizing meiotic chromosomal pairing using the synaptonemal complex.

Linkage of inversions. We have three systems for determining linkage of inversions. The first is to establish whether a presumptive inversion is "allelic" with known genetic markers, principally electrophoretic markers. Most of the inversions produced do not permit two-strand double crossing over within the inversion and, thus, none of the loci included in the inverted region and immediately adjacent to it recombine with the inversion. Therefore, these loci appear allelic with the inversion. Inversion In(7)13Rk is the longest inversion (45 per cent of first meiotic anaphases have bridges) to "inhibit" recombination along its entire length. In(11)20Rk, which has an anaphase bridge count of 47 per cent does appear to permit some two-strand double recombination. Even when two-strand double crossing over is possible, as for example with In(5)9Rk, loci included in the inverted region recombine with the inversion at a much lesser rate than would be expected from independent segregation of nonlinked genes.

The second method we use is to identify the inverted segment cytologically by chromosomal banding techniques. With our method for preparing mouse leukocytes for banding and karyotypic analysis (Triman, Davisson, and Roderick, 1975), this is now an easy procedure and permits the full breeding function of the characterized males and females. Seven chromosomal locations have been identified solely or first by cytological banding techniques.

The third method for identifying chromosomal locations of inversions is possible because of the meiotic effects inversion display when they are combined in a single animal. Each inversion exhibits a constant frequency of bridges at first meiotic anaphase which is proportional to its length. A male heterozygous for two inversions on nonhomologous chromosomes shows a frequency of double anaphase bridges which is approximately equal to the product of the expected frequencies of the independent inversions. Double anaphase bridges in normal individuals or single inversion heterozygotes are very rare. In individuals who are doubly heterozygous for different inversions on homologous chromosomes, such double bridges appear as a single, sometimes thicker bridge, and generally are indistinguishable from a single chromatid bridge. Furthermore, in two cases where this has been possible in mice (combining In(1)1Rk with In(1)12Rk, and combining In(5)2Rk with In(5)9Rk), doubly heterozygous males were also sterile as noted by reduced frequency of meiotic divisions. Five more such paired inversions are presently being tested to determine the generality of this effect.

Thus, it appears that once an inversion stock is produced and the inversion is located, the stock can be used to test that entire chromosome for linkage with any newly induced inversion. An advantage of this type of system is that it takes only one generation to produce evidence for linkage association rather than the two necessary for evidence using genetic markers. We now have inversions on 11 of the 20 chromosomes of the mouse.

Since the last report we have determined the chromosomal location of six additional inversions. In18Rk is located on the proximal end of Chr. 15. There have been no recombinants in 55 mice with uw (underwhite), which is near the centromere, and no recombinants in 53 mice with Gpt-1 (glutamate pyruvate transaminase), which is 33 cM distal to uw. The inversion does recombine with Ca (Caracul) which is probably located near the end of Chr. 15. Crosses are now in progress with an intermediate marker, Gdc-1 (NAD-alpha-glycerol phosphate dehydrogenase). This inversion may represent a third example of cross-over enhancement just outside the inverted region (others are In(1)1Rk and In(5)2Rk). There have been 12/27 recombinants (44 per cent) with Ca and no recombinants with Gpt-1 and on the current linkage map the distance between these two loci is 27 cM.

In two different linkage tests there has been recombinant in 115 animals between the agouti locus on Chr. 2 and In19Rk. Linkage tests with several other markers on Chr. 2 are now in progress to define the genetic limits of In(2)19Rk. In(14)22Rk has shown one recombinant among 22 mice with hr (hairless).

Two additional inversions have been located on Chr. 1. In(1)23Rk is so far cytologically and genetically indistinguishable from In(1)1Rk. It is possibly either a second induction of In(1)1Rk or more likely a mix-up with the In1Rk stock. We have produced double inversion heterozygotes and their bridge counts are low as would be expected in an animal homozygous for one inversion. We will karyotype these same mice to do measurements of the inverted segments and will analyze their meiotic pairing using the synaptonemal complex method of Moses (1978) to determine if the inversions are identical. The mixing of two stocks is not impossible in a colony where numerous mice of different genetic constitution are housed in close proximity. We have characterized each of our homozygous inversion stocks as well as our other stocks and strains for their alleles at isozyme loci. These allelic patterns are for the most part unique for any particular strain we maintain. Thus we are in a good position to determine genetic contamination when it occurs, and pin-point rather precisely which two strains have been mixed. We are also considering other measures to help in controlling such occurrences.

In(1)24Rk was induced simultaneously with T(1;13)2Rk. The inversion and translocation have separated so that mice carrying only the inversion are being used to maintain the line. Linkage data indicate the inversion encompasses most of Chr. 1 and permits two-strand double cross-overs. In the order of their distance from the centromere, there have been 0/48 recombinants with fz (fuzzy), the most proximal marker known on Chr. 1, 9/48 recombinants with ln (leaden), 3/29 with Id-1 (isocitrate dehydrogenase-1) and 2/38 recombinants with Dip-1 (dipeptidase-1). Crosses are in progress with two more distal markers, vl (vacuolated lens) and Lp (Loop tail).

In(9)26Rk has not yet recombined with Mod-1 (malic enzyme) ($n = 33$) or d (dilute) ($n = 7$). There have been 4/10 recombinants with cw (curly whiskers) which is the most proximal known marker on Chr. 9. We have crosses going with Mpi-1 (mannose phosphate isomerase) which is between cw and Mod-1.

Of the two inversions produced in mice with Robertsonian chromosomes In27Rk died before mating and In28Rk died after producing 29 offspring. Three of the sons were bridged but only one has produced a litter (of two). Several daughters have had litters but their sons are not yet old enough to do bridge counts. From preliminary karyotypic analysis and limited segregation data it appears the inversion does not involve one of the Robertsonian chromosomes.

All but one of the above chromosomal locations determined by linkage have been confirmed by cytological data (see next section). For In(14)22Rk the original male was not karyotyped and we are now in the process of karyotyping his offspring.

Cytology of inversions. We now have characterized seventeen of the induced inversions using the trypsin-Giemsa method for banding chromosomes. Table 3 gives the breakpoints of all the inversions identified. The identification of In(15)18Rk as the proximal two thirds of Chr. 15 has been confirmed by the linkage data with uw, Gpt-1 and Ca. In(2)19Rk involves over 60 per cent of Chr. 2, and, thus, should show linkage with the agouti locus as it does. In(11)20Rk occupies almost 70 per cent of Chr. 11 which is consistent with the high bridge count and the linkage data collected so far. In(9)26Rk includes the distal half of Chr. 9 which is consistent with the linkage data with cw, Mod-1 and d.

Cytological analysis was used to show the close similarity between In(1)1Rk and In(1)23Rk and detailed analysis of the karyotypes of doubly heterozygous mice will be used as one means of determining whether the two inversions are identical.

Chromosomal banding revealed that the original In24Rk male had both an inversion and a translocation involving Chr. 1. The other chromosome involved in the translocation is Chr. 13. The inversion and translocation have separated from each other and comparison of the Chr. 1 banding patterns in mice with the inversion only and mice with both suggests a more complex rearrangement than we originally thought. Karyotypic analysis is in progress and we plan to send mice with both abnormalities to Dr. Moses for meiotic analysis.

Inversion In21Rk appears to involve most or all of Chr. 18 with the proximal break in the centric heterochromatin. We have used a C-banding technique to demonstrate that about one half the pericentric heterochromatin is shifted to the distal part of the chromosome. We believe this to be the first mammalian paracentric chromosomal inversion to involve a shift of pericentric heterochromatin to within the chromosome. Although testicular sections resemble those of partially sterile males, there is no appreciable reduction in fertility. There is no meiotic or mitotic evidence for a translocation in these animals. Preliminary linkage tests with Chr. 18 markers show 6/13 recombinants with sy^{1p} (fused phalanges) and 5/7 recombinants with Tw (Twirler). Although two- or three-strand double crossovers within a large inversion are possible, our experience with In(5)9Rk, another large inversion, has shown reduced recombination with markers inside the inverted region. These preliminary data create some doubt about the assignment of this linkage group on Chr. 18. We are continuing the linkage tests and analyzing karyotypes from descendants of the original male to try to find the answer to the discrepancy. It is possible that one of the markers is in the middle of the inversion and shows high two-strand double crossing over and the other is outside the inversion distally and shows enhanced recombination as we have observed in In(1)1Rk and, probably, In(15)18Rk.

In(1)1Rk and In(1)12Rk have already been shown to have a common overlapping region equal to about 20 per cent of the physical chromosome. Dip-1, which is very near the distal end of In-1 has now recombined once with In-12. The recombinant male is being tested by breeding to determine if he is a real recombinant. If Dip-1 is really just outside the proximal end of In-12, this tells us three things:

- 1) Dip-1 must be within In1Rk and not just outside,
- 2) inhibition of recombination just outside the inverted segment is much less than we had previously thought, and
- 3) the Dip-1 locus must be located at the distal side of negative chromosomal band 1D.

It also strongly implies that the large negative band 1E contains very little genetic material which can be measured by recombinational frequencies.

TABLE 3. CHROMOSOMAL BREAKPOINTS OF CYTOLOGICALLY IDENTIFIED INVERSIONS. THE BREAKPOINTS ARE IDENTIFIED USING THE NOMENCLATURE OF BANDS DEvised BY NESBITT AND FRANCHE (1973).

Inversions	Breakpoints		Approx. per cent of chr. involved
	Proximal	Distal	
In(1)1Rk	1B	1F	50
In(1)10Rk	1A4	1H5	84
In(1)12Rk	1D	1H5	45
In(1)24Rk	1A4 or 1B	1H1	61
In(2)5Rk	2B	2F3	50
In(2)19Rk	2B	2H1	62
In(3)15Rk	3A2	3H3	77
In(3)27Rk	3A2	3D	30
In(5)2Rk	5D	5G2	42
In(5)9Rk	5A2	5G2	81
In(7)13Rk	(Prob. 7B1)	(Prob. 7F1)	(If so 54)
T(1;In8)1Rk	8A2, 1H5*	8E1	73
In(9)26Rk	9B	9F1	42
In(10)17Rk	10A4	10D2	75
In(11)20Rk	11A2	11D	68
In(15)18Rk	15A2	15E	61
In(18)21Rk	18A1	18E3	80

*Translocation breakpoint in Chromosome 1.

Use of inversions. The major purpose of these studies is to produce inversions that can be used in balanced lethal systems like those that 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 that has good genetic markers, and (3) it is viable in homozygotes. Id-1 (isocitrate dehydrogenase-1), Sp (splotch), ln (leaden), and Dip-1 (dipeptidase-1) are the most useful loci in the inverted segment. Splotch is a viable dominant spotting marker that is lethal when homozygous. There also appears to be a slight reduction in viability of Sp/+ heterozygotes. The mutant allele is fully penetrant but the expression is 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 a few 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 known effect 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.

We are using this inversion and suitable markers on the homologous normal chromosome in a breeding system for detecting recessive lethal and recessive deleterious mutations. This balanced lethal test system is similar to those used in Drosophila and necessitates three generations of breeding beyond the generation exposed to the mutagen. We have used high doses of radiation to sperm and spermatids to test the ability of this system to uncover recessives. Calculations based on (1) the relative length of the inversion to the total genome length and (2) knowledge of the approximate induced lethality per R per genome in the mouse provided an estimate of induction of recessive lethals of about 2 per cent in test animals. We now use 0.3 mg/kg TEM as a mutagen as well. At present 1088 animals have been tested completely and 277 are in the process of being tested.

Using the balanced lethal system four lethals have been induced. Lethal 1 is closely linked but probably outside the inverted segment because it recombines with the inversion. The lethal acts after implantation, as is evidenced by deciduomas (moles) of high frequency. In some litters there are occasionally abnormal embryos alive at 15 days gestation. These embryos are all characterized by at least one eye missing, low set ears, and a bugle-like snout. These may also be lethal-bearing mice which somehow survived the postimplantation crisis which kills the major portion of lethal bearers.

Two other lethals (lethals No. 2 and No. 4) have been studied in some detail this year. Both show characteristic early lethality as evidenced by moles and thus are presumed to have died early in development, between four and nine days after implantation. Inversion In(2)5Rk, also homozygous lethal, shows the same characteristics. Lethals No. 3 and 4 both show the leaden phenotype when crossed with a homozygous leaden (ln/ln) animal. This finding indicates that both lethals overlap the leaden locus and probably are small deletions.

We are trying to use two or more inversions simultaneously to improve the efficiency of mutation testing. Combination of inversions on nonhomologous chromosomes have been possible in every pair put together but combinations of different inversions on the same chromosome, as noted earlier, have so far been impossible. We began to combine In(1)lRk and In(7)l3Rk. We believe these two inversions, used together in a balanced lethal testing system, will cover more than 10 per cent of the genome. The combination is perfectly viable and inversion homozygotes are now being produced in an incipient inbred strain. These double inversion homozygotes

have been crossed to mice homozygous for In(5)9Rk and we have animals heterozygous for three different inversions. Preliminary breeding tests with these mice indicate females heterozygous for three inversions have reduced fertility.

Robertsonian metacentric translocations. The tobacco mouse, Mus m. poschiavinus, has 7 metacentric and 6 acrocentric chromosomes instead of the usual 20 acrocentric haploid chromosomes of laboratory mice. Fortunately, tobacco mice are interfertile with laboratory mice. We have established three lines homozygous for a single Robertsonian chromosome and several lines homozygous for two or three. These stocks are being used regularly for linkage testing since each Robertsonian chromosome is a marker for the centromeres of two different acrocentrics. In recombining the seven Robertsonian chromosomes we now have mice homozygous for three and heterozygous for three more.

Within the past week a presumptive pericentric inversion of Rb(11.13)Bnr has been detected. We are in the process of doing karyotypes of the mouse carrying it. If it is indeed a pericentric inversion it will be the first in the mouse.

During the past month we have found two new Robertsonian chromosomes. One arose in an inversion stock homozygous for In(1)12Rk. It appears to include Chromosomes 4 and 15. A second arose in one of the lines already carrying Poschiavinus Robertsonian chromosomes 3 and probably 15. Both animals have reproduced and we are now karyotyping the offspring for positive identification of the chromosomes involved. Chr. 15 may be unusually susceptible to centric fusion.

We are treating with TEM mice from three lines each homozygous for three Robertsonian chromosomes. All seven are represented among the three lines. All sons of the treated males are screened for paracentric inversions in the usual manner using counts of anaphase bridges and for pericentric inversions using leukocyte culture and banding methods. All daughters are screened for pericentric inversions and X inversions using leukocyte culture. Since we are examining banded chromosomes we can also detect other chromosomal rearrangements. So far we have identified at least three new translocations in the pericentric screening project in addition to 5 others which were induced simultaneously with paracentric inversions. A list of all the translocations to date is given in Table 4. Two of the new translocations involve Robertsonian chromosomes (T6Rk and T8Rk). Two additional presumptive translocations have also been detected but not positively identified yet. The three males carrying translocations or presumptive translocations involving Robertsonian chromosomes appear to be sterile. The two females have only recently been mated. We believe these translocations to be the first involving Robertsonian chromosomes in the mouse.

This system also should produce translocation dicentrics and ring chromosomes, both so far unavailable in the germ line of experimental mammals.

One of our objectives is to combine the use of metacentric translocations and paracentric inversions. If we can get good inversions in both arms of the metacentrics, we will have a better system for examining more of the genome simultaneously in balanced lethal systems. The problem is, therefore, to place suitable inversion in the Robertsonian metacentrics. We are in the process of combining In(1)1Rk with Rb(1.3)1Bnr and In(5)9Rk and In(15)18Rk with Rb(5.15)3Bnr. In both cases we are now backcrossing F_1 's to parental strains.

TABLE 4. TRANSLOCATIONS INDUCED IN SEARCHING FOR INVERSIONS.

Male number	Translocation symbol	Induced [*] by	Induced on	Induced with	Comments
2913	--	900 R	DBA/2J	In4Rk	Lost
4609	--	800 R	"	In8Rk	Lost
11318	T(1;In8)1Rk	TEM .3	"	In16Rk ^{**}	Sterile
12174	T(1;13)2Rk	TEM .4	"	In(1)24Rk	
12164	T(5;8)3Rk	" "	"	In(1)23Rk	
"	T(12;17)4Rk	" "	"	"	
"	T(3;4)5Rk	" "	"	"	
"	T(1.3;17?)6Rk	" .3		Screen ^{***}	
"	T(4;15)7Rk	" "		"	
"	T(9.4;10?)8Rk	" "		"	

* Numbers by TEM indicate dosage in mg/kg body weight.

** An inverted insertion; translocation terminology takes precedence.

*** A cytological screen of Robertsonian stocks for pericentric inversions.

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-round programs of research participation wherein each student carries out a research project under a Staff member's sponsorship. Visiting Investigators join in collaborative research of mutual interest. During the period covered by this report the following students and Visiting Investigators have been sponsored by Drs. Davisson and Roderick and have participated in projects directly relating to research of this contract:

GROUP

Name and address

Title of project

SUMMER PROGRAM FOR PRECOLLEGE STUDENTS

Summer, 1976

Melinda Randall
Needham High School
Needham, MA

Detection of sister chromatid
exchange with murine lymphocytes

Summer, 1977

Leslie A. Denson
Trinity School
New York, NY

Analysis of three induced recessive
lethal mutations in the mouse

Michael A. Werner
Harvard School
N. Hollywood, CA

Effects of paracentric chromosomal
inversions on spermatogenesis

SUMMER PROGRAM FOR COLLEGE, GRADUATE, AND MEDICAL STUDENTS

Summer, 1977

Michael G. Absatz
The Johns Hopkins University

The use of sister chromatid exchange
analysis in vivo to find mouse
models of human chromosomal breakage
syndromes

VISITING INVESTIGATOR PROGRAM

Summers, 1976 and 1977

Richard Doherty, M.D.
The University of Rochester
School of Medicine and Dentistry

1976 - Search for new biochemical
markers in the mouse
1977 - Linkage analysis of a new
esterase variant

Heinrich Malling, Ph.D.
The National Institutes of
Environmental Health Sciences

Developing histochemical stains for
enzymes of mouse sperm

POSTDOCTORAL FELLOWSHIP PROGRAM

1976-1978

Loren Skow
Texas A & M University

Mammalian biochemical genetics

PRESENT STATUS OF RESEARCH IN THIS FIELD

At this time, the specific locus mutation test is the most reliable system measuring heritable genic mutations in mammals. This test has given us considerable information on mutational events in mammals, and it is the one we look to for providing standards and baselines upon which further mutagen testing in mammals can be compared. The test has limitations in that it is expensive; no more than two laboratories have had the facilities and support to use it until now. Furthermore, the information that can be derived from it about the total spectrum of possible single gene mutational events is limited.

Simpler and more efficient methodologies are greatly needed. We need to know the kind and extent of genetic loads induced in specific regions of chromosomes where map distances can be estimated precisely. We need a method which does not have possible bias because of the specific loci studied and which does not have the uncertainties involved in estimating lethals from an increase in uterine deaths alone, the essence of the dominant and recessive lethal tests. Finally, we need a method which will permit identification and easy maintenance of specific mammalian recessive lethals and detrimental mutations so that they can be propagated and analyzed. Systems using chromosomal inversions in combination with each other and in combination with Robertsonian translocations in mice provide the necessary attributes for achieving this improved methodology.

The particular system we developed for detecting recessive lethals and detrimental mutations using In(1)1Rk covers only about 5 per cent of the genome, if we include a portion slightly outside the inverted segment. We have combined this inversion with another to improve the efficiency of the test. It is our opinion, therefore, that the inversion system as yet is not sufficiently satisfactory for screening mutagens on a large scale, even though the system in its present form is about equal to the specific locus test method in efficiency per locus studied. There is no reason why all inversions on nonhomologous chromosomes cannot be placed together in a single stock so that a larger portion of the genome can be tested. The major problem with combining two or more inversions in a single test system is that more animals need to be raised in each generation to get the gene combinations necessary to continue the test. We see this as a surmountable problem and believe we can combine at least two large inversions and possibly both within a Robertsonian metacentric chromosome which could tie up as much as 15 to 20 per cent of the genome in a single test.

There are recessive lethals in mice which have dominant phenotypic effects. These phenotypic effects are often coat color or pattern effects, and the fertility or viability of the heterozygote is usually reduced. Ehling also found recessive lethals with a dominant heterozygous effect on the skeleton. Russell's lethals at specific loci had deleterious heterozygous effects in some cases. It is possible that in those cases the seriousness of the heterozygous effect was correlated with the size of the deletion. We need now to study recessive lethals and detrimentals which have no outward phenotypic expression in the heterozygous state and which occur at loci where there are no viable allelic alternatives. The vast majority of recessive changes may be of this kind. Lüning and Sheridan found little if any heterozygous effect of this kind of lethal, but it was difficult for them to be certain about identification of lethal carriers in all cases. Inversions make it possible to detect such lethals and propagate them easily for study.

It is also possible now, with the variety of inversions available, to study many fundamental problems in mammalian genetics such as the effects of inversions on recombination elsewhere in the genome, the nature of the break points of inversions and possible position effects in mammals, and the extent of somatic recombination in vivo in mammals. Inversions also can be used to study the frequency of naturally occurring lethals in feral populations of mice.

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