Research Notes

Asahina, Kazuo Sex ratio of D. melanogaster. Negative results have been obtained in tests of some conditions which seem to have an influence on the sex determination of D. melanogaster. But on its sex-ratio can recognize certain influences of several ions (As, CN, etc.), and the age of spermatozoa at the time of fertilization. Moreover studying on which status of the genetic process they operate actively, it should be noted, has very important significance in the theory of so-called "environmental heredity." H-ion, ultraviolet rays, X-rays, and sexual hormones have no definite influences on it.

Auerbach, C. Tests of chemical substances for mutagenic ability. (1) Pyrogallol (in co-operation with Dr. M. Bird). This substance was tested because it was the most effective of the phenols which in the experiments of Levan and Hin Tjio produced chromosome fragmentation. Meanwhile Therman-Suomaleinen had shown that this effect is more or less restricted to Allium chromosomes; but this work was not known to us at the time. Treatment was given by injection into young imaginal females. Three concentrations were used: .0013%, .002%, and .005%. None of these was harmful to the flies; but, taking account of the dilution by abdominal fluid, they are in the range of the concentrations that were most effective on Allium chromosomes. The treated females of theconstitution +/sc^{S1} InS w^a sc^8; +/Cy were mated to sc^{S1} InS w^a sc^8; Cy/L males, and six days later they were transferred with their mates to fresh vials. The two broods were scored separately in order to detect a possible dependance of sensitivity to pyrogallol on the age of the germ cells. In all 6 series (3 treatments, 2 broods) both sex-linked and autosomal lethals on the wild-type chromosomes were scored. The result was entirely negative. Only 1 autosomal and 2 sex-linked lethals occurred in over 1,000 cultures for each test. Four second-chromosome lethals had been present at the start of the experiment. One occurred in 6 females, one in 3, and 2 singly. Thus 11 of 115 P_1 females, including controls, were heterozygous for a second-chromosome lethal. The one autosomal lethal that arose de novo was identical with one of those present in the stock. This, taken together with Mather's observation (John Innes Report for 1948) that the same second-chromosome lethal arose spontaneously twice in the same experiment, suggests that the second chromosome may possess some loci that mutate easily; it may have a bearing on Hadorn's finding of identical autosomal lethals after phenol treatment.

(2) Chloropicrin. This substance was tested because it is a strong sulfhydril poison. In the first tests, in which adult males were exposed to vapor of undiluted chloropicrin, the highest dose that could be tolerated by a minority was 3 minutes. Only 1 sex-linked lethal was found in 1318 chromosomes from males that had been exposed for 2-3 minutes. In later tests, chloropicrin was diluted with liquid paraffin; this raised the threshold of tolerance to 7 minutes or more, a time amply sufficient for the induction of mutations by mustard gas or nitrogen mustard. Males that had been exposed for 7 or more minutes were mated to a succession of fresh females, so as to obtain progeny from germ cells that at the time of treatment had been immature and presumably more sensitive toenzyme poisoning than mature sperm. Mutation rates in three such experiments did not exceed 0.1%. This, together with the negative results obtained previously with lewisite, another strong sulfhydril poison, indicates that attack on SH-groups is not responsible for the production of mutations by mustard gas and X-rays. It also makes it seem unlikely that the increased mutation rate in later broods, which has been observed after mustard gas treatment, is due to inhibition of phosphokinases in immature germ cells.

(3) Sodium desoxycholate. This substance has given positive results on bacteria (Witkin) and Drosophila (Demerec). It was injected into the abdomen of young imaginal males. The highest concentration tolerated was 0.3%. Only 1 sex-linked lethal was found in 1000 progeny of males that had been injected with concentrations varying between 0.2 and 0.3%.

(4) Nitrogen mistard as injection. A watery solution of bis-(2-chloroethyl)amine hydrochloride, buffered with $NaHCO_3$, was injected into young imaginal males. 0.1% was the highest dose tolerated. In 60 chromosomes from males treated with this dose, there were 2 sex-linked lethals and 1 almost-lethal; with lower doses the genetical effects became negligible. The injection method is obviously not suitable for work with such highly toxic substances.

(5) Formalin vapor. Adult males and females and larvae of various ages were exposed to formalin vapor for periods from 30 minutes to 2 hours. Their progeny--in the case of the adults, four successive broods separated from one another by 5 days--were examined for sex-linked lethals. All results were negative. This indicates that formalin by itself is not mutagenic, and that the mutagenic ability of formalin-treated food is due to some reaction product formed with a component of the food. Experiments to identify this component are under way.

Auerbach, C., and Moser, H. Analysis of the genetical action of formalin-treated food. (1) There exists a sensitive period of the larval germ cells to the mutagenic action of formalin food. It is not identical with the sensitive period of the larvae to the toxic effect of the food. Eggs from Ore-K females were collected twice daily. They and the larvae emerging from them were kept on normal food at 25° C. for a varying number of days before transfer to formalin food (0.2% HCHO, on the basis of the water used in the preparation of the food). In several series the larvae were put back onto normal food after one or two days on the treated food. The results showed that sensitivity of the larvae to the toxic action of the food is highest during the first day after emergence--that is, during the first instar--but that the great majority of mutations are produced during the following 48 hours--that is, during the second and early third instar. Larvae 48 hours old (counted from the laying of the eggs) were put on formalin food, and some of them were removed after 24 hours; mutation frequency in their progeny was sensibly the same as in the progeny of the remainder of the larvae, which had completed development on the treated food. Larvae more than 72 hours old no longer react genetically to formalin food, and larvae before the sensitive period--i.e., during the first day of life--react very little. In two experiments in which larvae 24 hours and 48 hours old were put on formalin food and left to complete development, the former developed fewer mutations than the latter. When males that have spent the sensitive period on formalin food are mated twice in succession, with an interval of 5 days between matings, mutation frequency is significantly lower in the second brood. This indicates that only or preferentially the oldest germ cells react during the sensitive period, an observation which may have a connection with the finding by Lamy and by Muller that spontaneous mutations occur mainly in the first sperm used.

(2) There exists a negative correlation between the amount of damage done to the larvae and the frequency of induced mutations. In various experiments it was found that, for a given concentration of formalin in the food, conditions that allow the highest rate of survival are most favorable

for the production of mutations. One example—the relatively low mutation rate in larvae that have been transferred to formalin food immediately after emergence—has been quoted under (1). Another came from an experiment in which the yeast in the food had been pretreated with different concentrations of formalin. Although the mutagenic effect in this experiment was small, probably because treatment of the yeast had been too short, it allowed a comparison between the two extreme treatments. For the highest concentration, survival rate was half and mutation frequency double that of the lowest. Finally, an experiment was made using the same concentration of formalin (.18% HCHO) and the same amount of yeast in three different media: A, with additional maizemeal and treacle; B, with additional dextrose; C, with no additional nutrients. Survival was good and development normal in A and B, possibly somewhat better in A; in C, development was prolonged, survival rate low, and the emerging flies small and weak. Mutation rates, determined for 3-400 X chromosomes in each series, were 9.4% in A, 5.7% in B, and 0 in C. Various possible explanations of these results are now under test. In any case, these data make it appear unlikely that formalin food produces mutation through starving the larvae or depriving them of some food constituent.

(3) Formalin produces the same types of mutation as X-rays and mustard gas. Lethals, visibles, small deficiencies, large deletions, inversions, and one probable translocation (under test) have been obtained. There is a suggestion that visibles are slightly less frequent and large deletions slightly more frequent than after mustard gas. Translocations are infrequent, as after mustard gas. Only one translocation between chromosomes 2 and 3 was found in over 200 spermatozoa from flies that had yielded 5.7% sex-linked lethals, and none in over 100 from flies that had yielded 9.4% lethals. Mosaics and gonadic mosaics are as frequent as after mustard gas, and are found even in the F_1 of flies that have been treated only during the second and third days of larval life. Gynandromorphs seem to be fairly frequent: 12 were found among 14,500 daughters of treated males and untreated y w sn females.

Barigozzi, C. Investigations reported previously (DIS-22) on the genetics of the Y chromosome have been continued. Three characters controlled by the Y chromosome are being systematically studied in melanogaster: size of cornea, frequency of hairs on the wing surface, and facet irregularities in heterozygotes with Me. The investigation is being made on stocks in which Y's from different wild strains are brought together, with the remaining elements of the genome identical in the different stocks. This is done using markers of the first, second, and third chromosomes. Analogous investigations are being started with D. pseudoobscura.

Barigozzi, C., and Semenza, L. A new Notch has been described, showing the typical wing aspect, without lethality in the male. In double doses (XX) it is less viable than in single (XY), and therefore the sex ratio is in favor of the males (♂♂ 871:♀♀ 676).

Bird, M. J. Three compounds in the class of nitrogen mustards were tested for their mutagenic effects. They were: R.44, NN-Di-(2-chloroethyl)-p-toluidine; R.45, NN-Di-(2-chloroethyl)-p-anisidine; R.48, beta-Naphthyldi-(2-chloroethyl)amine.

Except for R.48 0.1%, they were all dissolved in arachis oil and then added to a yeast agar medium. R.48 0.1% was used in an alcoholic solution. A counted number of newly hatched larvae from a wild-type stock of Drosophila melanogaster (Oregon-K) were reared on these media. Some of the males that emerged were tested for sex-linked recessive lethals, using the Muller-5 stock.

For two of the dosage levels of R.44 and one of R.48, these males were also mated to y v f dp:e females and the F_1 scored for large deletions and visibles. R.44 was the most toxic, and at 0.01% dosage level only 1.8% of the larvae completed their development. Of the adults obtained, 95% had abnormal abdomens, the sclerites were grossly deformed and the pigmentation disturbed. Table 1 shows the P_1 survival rates calculated as a percentage of the control flies that emerged in the same experiments. The percentage of flies with abnormal abdomens is also given.

Table 1 - P_1

Substance	Dosage level %	P_1 % survival	% with abnormal abdomens
R.44	0.003	89	.8
R.44	0.007	65	81
R.45	0.07	78	4
R.45	0.09	45	3
R.48	0.07	92	0
R.48	0.1	45	0

In R.44 0.007%, only males with abnormal abdomens were tested, but in the 0.003% dosage level all the males tested were normal. Table 2 shows the F_2 results of the tests for sex-linked recessive lethals. The test males were mated to several females and when 5-7 days old were remated, thus giving Brood I and Brood II. In all tests the mutation rate was higher in Brood I. Several males gave more than one lethal, and these lethals are being tested to see whether they form a cluster. The figures for the controls in Table 2 are the combined data of several experiments.

Table 2 - F_2

	Brood I		Brood II		Total		I	II	Total
Substance and dosage level	Chr.	l	Chr.	l	Chr.	l	% l	% l	% l
R.44 0.003%	745	14	235	2	980	16	1.9	0.9	1.6
R.44 0.007%	681	23	286	4	967	27	3.4	1.4	2.8
R.45 0.07%	317	9	1327	11	1644	20	2.9	0.8	1.2
R.45 0.09%	192	7	273	2	465	9	3.6	0.7	1.9
R.48 0.07%	591	5	277	2	868	7	0.8	0.7	0.8
R.48 0.1	558	5	398	0	956	5	0.9	0	0.5
Controls	1527	5	2466	3	3993	6	0.3	0.1	0.2

Chr. = chromosomes tested; l = lethals

In the test for large deletions and visibles, only one deleted fragment was found in R.44 0.003%. Most of the visibles found were Minutes; the numbers are given in Table 3. Of the 40 Minutes found, 14 were sterile, 10 produced a small second generation with no Minutes, and 16 bred as normal autosomal Minutes (these are shown in brackets in the table). A small number of sex-linked recessive visible mutations and autosomal dominants were found, as well as a large number of phenotypic effects such as blistered wings, which appeared to be nongenetic. Three fractional Minutes found are

not included in the data.

Table 3 - F_1

Substance and dosage level	Normal diploid F_1 females	Females hyperploid for a deleted-X	Wild-type F_1 males	Minutes	% Minutes
R.44 0.003%	1296	1	1456	19 (8)	1.3
R.44 0.007%	1276	0	1299	12 (5)	0.9
R.48 0.07%	916	0	1004	9 (3)	0.9
Controls	647	0	711	1 (1)	0.1

R.48 has been found to be a carcinogen (unpublished data), but R.44 and R.45 have not yet been tested. Chemically R.48 is the least reactive, and, from the results shown here, of the three it is also the weakest mutagen. The data show that all these substances are capable of producing lethals and visible mutations. Only R.44 was adequately tested for producing chromosome breaks, and gave negative results.

Bonnier, G. and Lüning, K. G. Influence of time and age after X-ray irradiation on chromosomal behavior.

Wild males were irradiated (about 2900 r) and mated to y w sn females. Four different series were made: (1) freshly hatched males were irradiated and mated immediately; (2) freshly hatched males were irradiated and thereafter kept without females for one week, with subsequent mating; (3) males were kept without females for one week and then irradiated and mated; (4) males were kept without females for one week, then irradiated and kept without females for another week and then mated. The female (and gynandromorph) progeny in each of these series numbered about 40000. The proportions of gynandromorphs and translocations (w sn and sn males) were very similar in the last three series, but differed considerably from the first series. These proportions (per 10,000) were in the first series 11.8 and 5.3, and in the pooled last three series 7.2 and 8.4. The differences are statistically significant.

As the first series was easier to keep and gave larger progenies, studies were undertaken of the hatchability of the eggs after different treatments of the males. Round 150,000 eggs have so far been counted in several repetitions of the experiments. Though a few series deviate somewhat too much from the bulk of the experiments, the results give a fairly consistent picture. Controls gave a hatchability of between 80 and 90 per cent. When freshly hatched wild males were irradiated and immediately mated, the hatchability was 50% or somewhat less. However, when the newly hatched males were irradiated and then kept for a week before mating, and also when both irradiation and mating were postponed for a week, the hatchability of the eggs decreased to 30% or less. Several variants of these experiments were performed. The keeping of the males before mating with or without females seems to be without influence.

In the four series mentioned in the first paragraph it was not possible to compare the frequencies of gynandromorphs due to maternal and paternal eliminations, as there were no marker genes in the irradiated males. To make such a comparison, and also to study in more detail Patterson's finding that the paternal X's also are eliminated after irradiation of females, we have irradiated w sn females (2200 r) and mated them to y f car males. Three series have

been carried out: (1) freshly hatched females were irradiated and mated immediately; (2) freshly hatched females were irradiated and mated after a lapse of one week; (3) 1-week-old females were irradiated and then mated. To these series was added a control series with mating of freshly hatched females. So far the total female (and gynandromorph) progeny (round figures) and the number of gynandromorphs due to maternal and paternal eliminations are (in that order): series (1), 60,000, 8, 7; series (2), 50,000, 10, 21; series (3), 60,000, 6, 14. In the controls the corresponding figures are 55,000, 6,7. Patterson's findings are thus confirmed by the present study. The proportion between the two kinds of gynandromorphs conforms well to a 1:1 ratio in series (1) and in the controls, but decidedly does not in series (2) and (3).

Brncic, D. and Briones, H. Tumors and nutrition (summary). The effects of variations in nutrition on the spontaneous incidence of melanic tumors were studied in one wild and two tumoral strains of Drosophila melanogaster (st, sr (tu), and bw (tu)). These variations were brought about by means of modifications of the concentration of Torulopsis utilis in Birsch's synthetic medium. During the course of five different experiments, in which 5126 larvae at the end-stage of their development were examined, it was observed that culture media that were rich in yeast lowered the tumoral incidence, whereas media poor in this fungus increased it, regardless of the decrease in viability. These results were observed in all three strains, but were statistically significant only in the two tumoral strains. The low tumoral incidence observed in culture media with high yeast concentration did not seem to be related to a decrease of viability or a shortening of the larval period, at least in the strains st and sr(tu). In the wild Oregon-R strain, the type of nutrition modified the duration of the developmental stage of the larvae, but in this case the antitumoral effect was extremely low and statistically not significant. Nothing is known yet about which element or group of elements of Torulopsis utilis is responsible for the protecting effect. The possibility of participation of the vitamin B complex and of proteins, which are abundantly present in this type of yeast, is discussed in the published work. It is not possible in this case, as it would be in mammals, to relate it to the antitumoral action of caloric restrictions, as we have observed in Drosophila a lowering of the tumoral incidence precisely in overfed and overweight larvae. This peculiar property discovered in T. utilis might be another argument for its use in human nutrition, besides its high nutritional value and easy and cheap manufacture.

Burdette, Walter J. Separation of sterility factor from sn. A stock of w^e with a sterility factor but without sn was obtained from a w^e sn stock in the following manner. Oregon-R virgins were mated to w^e sn males in order to obtain w^e flies that were not carried in the laboratory cultures. One w^e male was found among the progeny of the F_1 heterozygous females, and a pure stock was obtained. Although the w^e males were fertile, it was soon found that the w^e females were sterile. The ClB chromosome was utilized to provide a balanced stock. Eggs were laid by each of 30 w^e females mated to Oregon-R males, but none hatched. In another experiment, 16 of these females were observed to copulate; eggs were laid by all, but no larvae appeared. Sperm were very active in sperm receptacles and spermathecae shortly after copulation in 3 females dissected, and motile but sluggish 48 hours later in 3 others. Only 11 out of 40 heterozygous females (w^e/w^e sn) laid eggs when mated to Oregon-R males, and no larvae developed from them. Apparently the sterility factor in the w^e stock described and the one associated with sn are allelomorphic. Perhaps the simplest explanation is that a crossover occurred

between the factor and sn. However, other possibilities, such as mutation of sn to + and the presence of two sterility factors on the w^e sn chromosome, have not been eliminated.

Burla, Hans Key for species of Drosophila occurring in Switzerland.

1. Mesonotum yellow 2
- Mesonotum gray, every bristle arising from a dark spot. Repleta group 7
- Mesonotum brown or black 8
2. Yellow, small, slender. Three distinct brown or black longitudinal stripes on the mesonotum. The median stripe splits posteriorly. Black spots on the tergites of the abdomen .. busckii Coqu.
- Yellow, small, robust. Mesonotum without black stripes. Abdominal tergites with dark marginal bands not interrupted in the middle. Melanogaster group 3
- Small, yellow or brownish. Two presutural acrostichals on the mesonotum ..... testacea v. Ros.
- Not entirely as above 4
3. Male genital arch with a small hook-like preocess ..... melanogaster Meigen
- Male genital arch with a large clam-shell-like process ..... simulans Sturtevant
4. A row of short peg-like bristles inside on the front femur ..... immigrans Sturtevant
- No such bristles present 5
5. Abdominal tergites with blackish marginal bands which are interrupted in the middle; forming large triangular lateral spots .. histrio Meigen
- Abdominal marks different. Quinaria group 6
6. Yellow. Abdomen with dark marginal bands which are interrupted in the middle. Crossveins clouded.
Acrostichal hairs in 8 rows. Dark abdominal bands with straight anterior margins ..... Kuntzei Duda
- Indistinct marginal bands widely interrupted in the middle and laterally narrowed. The first two oral bristles of about the same size ..... limbata v. Ros.
- Marginal bands on the abdomen broken in four separate spots. Two prominent oral bristles ..... transversa Fall.
- Marginal bands incomplete, broken into spots. Only one prominent oral bristle ..... phalerata Meig.
7. 1st costal section apically blackened. Front coxae darkened on the apex. Costal index 3,0. Cheeks narrow .... repleta Wollast.
- Appex of first costal section blackened. Costal index 2.6. Broad cheeks ..... buzzatti Patterson-Wheeler.
- Costal section not blackened. Costal index 3.4. Fore coxae light, males with long recurved hairs on medial side of fore tarsi .... hydei Sturtevant.
8. Large brown species. The marginal bands of the first two tergites in the middle indistinctly interrupted.
Arista with about 11 branches. The distal section of the 5th longitudinal vein of the wings shorter than the distal crossvein ..... funebris Fabr.
Brown small species. Carina prominent only on the upper half of the face.
Sex-combs on first and second tarsal segments of male forelegs, each

consisting of only 2-4 bristles..... helvetica Burla.
- Prescutellars present. Lowest part of the carina thickened. Victoria group 9
- Mesonotum brown with darker longitudinal stripes. Large species. Abdominal sternites relatively large and brownish. Crossveins clouded. Virilis group 10
- Blackish species. Two sex-combs on forelegs on males, consisting of 6-15 bristles. Obscura group 11

9. Brown, dull. Post-verticals crossed. Second orbital inserted outside of the first ..... guyénoti Burla.
- Blackish, shining. Post-verticals convergent but not crossing. 2nd orbital inserted behind but not outside of the 1st orbital ..... nitens Buzzati

10. Mesonotum yellowish brown, dull, with greyish-brown stripes .... unimaculata Strobl.
- Mesonotum darker, more polished ..... littoralis Meig.

11. Mesonotum greyish brown. Pleurae and shoulders yellow. Sex-combs long, extended almost over the whole length of the first two tarsal joints ..... alpina Burla.
- Not entirely as above 12

12. One terminal and one more proximal, equally long bristles on the palpi. Anterior apical part of the wings darkened in males ... . tristis Meig.
- 3rd costal section of the wings with heavy bristles on its basal half. Mesonotum uniformly dark, dull ..... subobscura Collin.
- 3rd costal section with heavy bristles on its basal two-fifths .. 14

14. Mesonotum uniformly dark. The two first tarsal joints on males equally long ..... ambigua Pomini.
- Mesonotum with two darker shining longitudinal stripes. Lateral yellow spots on the anterior margins of the 5th and the 6th tergites only in females ..... obscuroides Pomini.
- Females without yellow spots on the tergites ..bilineata Pomini.

This key is preliminary. A more detailed one with descriptions and drawings will be published elsewhere later.

Buzzati-Traverso, A. Preference of D. subobscura for wild yeasts. Flies have been set free in an empty room having on the floor a number of traps inseminated with yeasts isolated from free-living subobscura (DIS-22: 69) and with the usual baker's yeast. Experiments repeated several times have shown a definite preference of the flies for wild strains of yeasts. By making use of these strains, improvement in the breeding of this species has been obtained.

Buzzati-Traverso, A. (2) What is Drosophila obscura? At least five European species correspond to Fallen's description (1823) of D. obscura. Material collected at Esperöd (Sweden) through the kind offices of Professor K. Anders (Lund) show the presence in the terra typica of D. subobscura and of D. obscuroides. Since D. subobscura is numerically prevalent at Esperöd, and has the wider geographical distribution in continental and insular Europe among species of the obscura group, it seems convenient to consider D. subobscura Collin 1936 as being D. obscura

Fallén 1823. Before making a formal statement on some publication according to international rules of zoological nomenclature, comments and criticisms on this proposition would be appreciated.

Castiglioni, M. C. - An investigation has been begun on the histological structure of the eye in some white alleles and their compounds. Particular attention was paid to the distribution of the pigment granula. One preliminary result concerns the difference between the compounds of w^e, w^{bf}, w^{bl}, w^a with w, and those with w^i. The latter appear lighter than the former.

Gardner, Eldon J., and Stott, Gerald Genes producing a maternal effect on tumorous head found in "wild" populations of D. melanogaster. A maternal effect has been identified with an inherited abnormality characterized by abnormal growths in the head region of D. melanogaster. A sex-linked recessive gene controls the maternal effect, but acts only in the presence of a third-chromosome, semidominant, homozygous viable gene. A gene presumed to be an allele of the sex-linked gene controlling the maternal effect in the inbred tumorous-head stock was found in the Oregon-R stock. Another gene producing a more pronounced maternal effect than either the one in the inbred tumorous-head stock or that in the Oregon-R stock has been found in the Stephenville stock, recently obtained from the University of Texas. Nine other "wild" stocks and twenty-one laboratory mutant stocks have been tested for the presence of factors controlling the maternal effect. All have given negative results.

Gloor, H. Silver-reducing organ in Drosophila larvae. On the ventral side of Drosophila larvae, near the posterior end, there is a definite skin area that in a dilute silver nitrate solution stains selectively with silver. The structure in somewhat different shape is present in different species of Drosophila, but not in Calliphora larvae. It behaves like certain papillae of aquatic Dipteran larvae, which have been shown to be organs for osmotic regulation (salt uptake). Experiments are under way to check on this possibility for Drosophila.

Gowen, John W. Sex ratio genes. Continued inbreeding and homozygous matings have led to isolation of several important sex genes. The action of one of these genes leads to sex ratios comparable with those so often observed in economic animal breeding. The sex ratios are variable from family to family. The sex ratio is affected by selection. Some matings give surprising results; others give ordinary sex ratios. These differences can appear even in families carrying the gene in homozygous condition, as shown in the following table (sex ratios for individual pair matings).

Pedigree No.	No. of Males	No. of Females	Pedigree No.	No. of Males	No. of Females
446 a.....	65	0	442 e.....	50	6
h.....	55	6	j.....	106	39
j.....	57	9	o.....	72	0
r.....	66	1	q.....	74	3
A.....	35	17	u.....	58	4
D.....	27	24	y.....	94	2

The sex ratios range from near equality in the sexes (No. 446D) to all males and no females (442-o). Male progeny are greatly in excess of female progeny. This is shown in the more extensive data. In twelve general crosses involving some hundreds of individual matings, the general result was 26,768 males to 5,110 females, a ratio of males to females of 5.2 to 1.0.

Reciprocal crosses showed that when the male carries the gene and the female does not, the sex ratio is practically one male to one female, or the sex inheritance of the male does not affect progeny sex ratio (321 males to 347 females). When the female carries the gene for the male ratio, the average ratio is 1.6 males to 1.0 females, a significant deviation from the normal 1:1. This deviation (1.6) is much less pronounced than when both parents carry the sex gene (5.2). This makes it appear as though both sexes had an effect on the sex ratio, even though the female alone shows it in the reciprocal matings.

When both parents are heterozygous, the progeny have the sex ratio 1139 males to 1004 females. When both sexes are heterozygous, the sex ratio is slightly toward an excess of male progeny, 113 males to 100 females. This ratio may be compared with 93 males to 100 females where the female parent is homozygous normal, and 162 males to 100 females where the mother is homozygous for the male sex gene. These results seem to indicate a gradation in the effects of the inheritance. The scale is +/+, 93; +/ms, 113; ms/ms, 162; or the heterozygote is about one-third up from the normal sex ratio. The puzzling fact is the high sex ratio of 520 males to 100 females when both sexes are ms/ms x ms/ms.

Tracing the inheritance through the different chromosomes shows that the gene, or genes, is located in the second chromosome, but as yet the position within this chromosome is unknown.

Hoch, Milton O. Displacement of f B^i B by wild-type in D. melanogaster. A rapid displacement of flies bearing f B^i B factors on the X chromosome was observed when a wild-type male (WR+/+) was introduced into the f B^i B population. Ten f B^i B populations in 1/2-pint jars at 27°-30° C. were examined and transferred at 14-day intervals. The starting population consisted of 10 pairs of f B^i B and 1 WR+/+ male in each bottle. The initial frequency for f B^i B was .968, which reduced to .017 eighty-four days later, yielding a smooth curve of displacement meanwhile (data from 10,000 flies). f B^i B homozygotes were rare by the third census, so that the displacement that occurred was largely through the heterozygotes and f B^i B males. The males were more severely affected than the heterozygotes, as judged by their frequencies at each census. It is believed that the gene, forked, played little or no part in the displacement, and served merely as a marker to distinguish heterozygotes and homozygotes.

Other people here have observed displacement by wild-type in f B and f B^i populations. Their conditions were somewhat different, which makes comparison difficult, but it appears that displacement is faster in the f B^i B populations. Work is continuing, using larger populations at lower temperatures. Work is also being done to elucidate the causes of displacement.

Kikkawa, H. Tryptophane synthesis in insects. According to recent work on Neurospora, it is stated that tryptophane is synthesized by

the following processes: anthranilic acid ⟶ indole + serine ⟶ tryptophane. In order to find out whether or not insects are able to utilize anthranilic acid or indole as a precursor of tryptophane, I have injected anthranilic acid, indole with serine or without it into pupae of silkworms of normal or white-1 mutant type. If anthranilic acid on indole is utilized as a precursor of tryptophane in insects, the amount of + chromogen (hydroxykynurenine) or that of kynurenine in the pupae should increase as compared with the control (H_2O injection). But the result was negative. On one hand, indole-lactic acid is utilized by the silkworm. Similar results have been obtained in Drosophila. Assuming that the genes control enzyme functions or chemical reactions, the results seem to indicate that the gene-complex in insects is not the same as that of molds with respect to tryptophane synthesis. Presumably the genes for converting anthranilic acid or indole into tryptophane have become inactive or lost in the course of evolution.

Lamy, R. Production of 2-X sperm in males

A stock of yellow attached-X females mated to white males was observed to produce occasional white females with two free X chromosomes and a Y. The frequency of the occurrence and its relation to the age of the white males was measured b mating 24 males individually to attached-X females and changing the pairs to fresh food two or three times a week throughout the life of the males. 32 white females were obtained in a total of 14,313 offspring. The regular F1 males numbered 7587. Thus the over-all frequency with which the white chromosome showed equational doubling was .42%. The correlation of doubling with the age of the males was as follows: 1-14 days, .06%; 14-21 days, .34; 21-28 days, .52; 28-35 days, .83; and 35-38 days, 1.6.

All but four of the patroclinus females were mated to Muller-5 males and shown to contain two free X's and a Y. They gave about 1.5% of secondary nondisjunction. F_2 nondisjunctional females heterozygous for the white chromosome and M-5 gave, as expected, a much higher rate of secondary nondisjunction (33-50%). The only other known case of equational doubling of the X chromosome in males seems to be that reported by Schultz (Carnegie Inst. Wash. Year Book 33: 280, 1934) and attributed to the action of a particular gene. But Dr. Helen Slizynska reports finding patroclinus females in a stock of attached-X females mated to ring-chromosome males. This case has not been examined further.

Lefevre, George, Jr. Mutation in germinal and somatic tissue.

Wild-type Canton-S males were X-rayed in the larval stage with 1300 R, and 4700 adults were inspected for white eye-color mosaics. Examination of the adults immersed in paraffin oil permits a good separation of colorless mosaics from those possessing some color. Larvae were irradiated at 26, 48, and 70 hours after egg laying. From the incidence and average size of the white mosaics produced, a somatic mutation constant was calculated. A value of about 14×10^{-5} was found in both the one- and two-day series, but a much lower value was computed from the data for the three-day series. In that case the size distribution was such that many undetectable mosaics less than one facet in size must have occurred. Data on germinal mutation of w^+ to w from various sources yield a value of 12.5×10^{-5} for the germinal mutation constant.

Attempts were made to induce reverse mutations of w in both germinal and somatic tissue. Adult y w sn^3 males were irradiated with 5000 r; and no evidence of reverse mutation was found in the 50,000 progeny examined. Irradiation of white larvae yielded no evidence of reverse somatic mutation of w in tests equivalent to the exposure of 600,000 loci to 5000 r. White alleles of

three diverse origins were tested: spontaneous, X-ray-induced, and sulfur-mustard induced.

A comparison was made of the incidence of mosaics observed after the irradiation of larval w/+ females and +/sc^8 delta-49 w^a females. With a dose of 1300 r, given at the rate of 59 r per minute, 48 hours after egg laying, a mosaic incidence of 65% per eye was observed in normal w/+ females, 6% per eye in the inversion heterozygotes, and about 2% per eye in + males. These results indicate that of the various mechanisms that might produce mosaics in heterozygous females, mutation, delection, chromosome loss, chromatid translocation, and abnormal segregation can be responsible for a relatively small proportion of the mosaics observed in normal females. Somatic crossing over or nonhomologous segmental interchange produce by far the majority of mosaics, but no clear evaluation can be made of the relative effectiveness of these latter two mechanisms.

Lewis, E. B. A "new" balancer for the third chromosome. A derivative of the complex rearrangement T(2;3)Mé has been obtained from a T(2;3)Mé/ri stock. The complex of inversions in chromosome 3 is retained, but the insertion of the tip of 2R in heterochromatin of 3 is removed, probably as the result of double crossing over. This derivative-symbol, In(3LR)TM- carries Mé, ri, and as in T(2;3)Me, although not previously described, an inseparable sbd mutation (or position effect) such that TM/Sb is lethal and TM/sbd resembles sbd/sbd. It is at present used as a balancer for su^2-Hw (see this issue of DIS).

Lüers, H., and Borchert, R. Experiments on the induction of mutations by visible light. Experiments have been undertaken with D. melanogaster to induce mutations by visible light after photosensitizing males with a photodynamic substance (Acridinorange). Use of Acridinorange has the advantage that this substance is relatively nontoxic for the flies and also penetrates the chromosomes. Males of a y w stock are fed on porcelain filters saturated with dilutions of Acridinorange and sugar for several days. After having taken up a demonstrable quantity, they are irradiated, at a controlled temperature, with wave length 4356 A, the sphere of highest absorption of the substance within the visible spectrum. With increasing doses (8.8×10^6 erg/cm^2 to 8.8×10^7 erg/cm^2), a rising percentage of males is killed (several hours to several days after irradiation) in contrast to controls that have only been fed with the photodynamic substance. First experiments have given a greater number of visible mutations in irradiated flies than in the controls.

Lüning, K. G. Gynandromorph production after mustard gas treatment. In order to check the experiments by Bonnier and Lüning (see this section), a series of experiments was performed in which the flies were treated with sulfur mustard gas. In one of these, freshly hatched y w females and f males were treated together. The female progeny contained about 42,000 animals and gave 18 maternally and 20 paternally eliminated gynandromorphs. In a second experiment, in which freshly hatched w sn females were treated and crossed to freshly hatched y f car males, about 22,000 female progeny were counted. These included 18 gynandromorphs, of which 15 were due to maternal and only 3 to paternal elimination. Finally, one experiment in which freshly hatched wild males were treated and then mated to y w sn females has given a female progeny of about 30,000. As there are no marker genes in the males, only paternal eliminations can be checked. So far they amount to 31.

Luria, Z.; Valencia, J. I., and Muller, H.J. Simultaneous induction of chromatid and chromosome rearrangements of the same chromosome.

Among the F_1 of a cross of X-rayed sc.Y^l/sc w.B.Y^s males by y In 49 v f; bw females, a mosaic female was found by Z. Luria which was narrow B in one eye and + in the other, with forked bristles present unilaterally on the same side as the + eye. The female proved fertile, giving offspring which showed that the mosaicism had included her gonads. The progeny were of the type expected if two different females had been used, one of composition sc w BB. Y^s/y v f . Y^s bw and one of composition sc w (f B)$^-$.Y^s/ y v f .Y^s bw. In other words, the irradiated X of one part of the mosaic was nonlethal, nonsterilizing, and phenotypically like double Bar, whereas that of the other part behaved as if it had a small deletion of the region containing forked and Bar, being lethal in the male but viable in the heterozygous female.

Salivary study of both types of X's by Valencia, showed that the (f B)$^-$ chromosome has a deficiency extending from 16A3 or -4 to 16F4 or -5, and that the piece missing here is present as a duplication in the chromosome, giving the double Bar phenotype. (Thus the latter has the Bar region quadruply represented.) At the same time, an inversion has taken place, and is present in both the deficient and duplicated chromosome alike, This inversion extends from about 1-B5, a point well to the left of the deficiency or duplication and lying between the loci of fw and wy, to about 20A2, a point in the chromocentral heterochromatic region, to the left of the centromere. Thus it is larger in extent, and more extreme in effect on crossing over, than the B^{M1} and B^{M2} inversions, and it includes, without touching them, the BB duplication and the deficiency.

The apparent identity of position of all the breaks in the two original chromatids, along with the fact that the union of fragments was in part the same and in part different in the two (as has also been found by Valencia in studying the progeny of other mosaics produced by irradiation of spermatozoa) appears to indicate that breakage occurred at a stage when the chromosome was in effect single, but the union, of some of the pieces at any rate, at a stage when it consisted of two separate chromatids. It is noteworthy that chromosome rearrangements occurred on both sides of the chromatid rearrangements.

The sc w BB^L, Inh. Y^s chromosome, as we designate it, should be a useful one in several ways. For one thing, since it overlaps In49, chromosomes differing in both of these inversions should give even less crossing over than the In49 B^{M1} or In49 B^{M2} heterozygous combinations. For another thing, the Y^s arm should be less readily detachable than usual by crossing over with a Y or between X chromosomes of identical type, because of the fact that there is a shorter stretch of X heterochromatin homologous with that of a Y^s or Y^l present just to the left of the centromere. This chromosome should therefore provide more stable stocks when in combination with chromosomes of the Y^l and sc . Y^l types. We now have it in males in a stock with sc . Y^l, the females having y f = attached X's. Females homozygous for the BB^L were found able to breed, but rather small, weak, and infertile, as is usual with high multiples of Bar.

We designate the deficient chromosome sc w (f B)-L, Inh.Y^s, and have this balanced over sc^{sl} f In49. v.

Makino, Sajiro The geographic variation of some species of Drosophila in Hokkaido.

A survey of the geographic distribution of Drosophila in Hokkaido has been made in our Laboratory with the assistance of Mr. T. Mizuno, a student. Wild stocks of D. melanogaster, virilis, immigrans, and nigromaculata collected this summer in several localities of Hokkaido have been bred for the purpose of investigating the geographic variation of the chromosome structure. The survey of the geographic distribution will be extended over various areas of the island of Hokkaido in the future.

Mickey, George H. Association of species in an ecological niche.

Several adult flies were seen to emerge from a small hole in a tomato which was still attached to a vine in my garden but which was beginning to ferment. Larvae collected from this one tomato were reared in the laboratory, and yielded four species of Drosophila as follows: D. subquinaria, D. simulans, D. buskii, and D. tripunctata. Stocks of these species are being maintained, except for the first, which was lost.

Moree, Ray. Call for genes with incomplete dominance.

I should like to obtain stocks (either sex-linked or autosomal) either incompletely dominant or incompletely recessive to wild-type D. melanogaster, to be used in experimental population work, provided of course that holders of such stocks do not already plan to use them for the same purpose.

Nakamura, K. and Imaizumi, T.

Electric charge at the surface of developing embryos (D. virilis) was measured in liquid paraffin. It was found that embryos bear a positive charge in liquid paraffin, and that the charge changes as developmental stages proceed. Through stages in which polar cells appear at the posterior end of the embryo and the blastoderm is formed, the electric charge at the surface increases gradually, and then decreases to the former level at later stages. When killed with heat, embryos at each stage do not show such differences in surface charge.

Newby, W. W. Morphological studies on the tumorous-head stock.

Morphological studies on the development of the head through the larval and pupal stages have been carried out in the tumorous-head stock. Abnormalities of the head primordia may easily be recognized in the late larval stages, and probably even earlier.

Novitski, E. An inversion of the entire X chromosome.

In the F_1 of females presumed to be of the constitution X^c, y/sc cv v f, no single crossovers were found, as is expected of ring-X heterozygotes; but the doubles involving the "ring" were as frequent as those involving the rod X, and not about a third as frequent, as is expected with such heterozygotes. It was suspected that the ring-X had opened out, as had been previously observed by L.V. Morgan, but that in this case the opening had occurred in reverse order, to produce a long inversion with yellow at the base. Tests of the composition

$(X^c, y)?/In(1)sc^8$, sc^8 cv v f ♀♀ x y sc cv v f B ♂♂ showed that this was the case, since in such heterozygotes single crossing over occurred with normal frequency and one of the crossover classes was inviable in the male but viable as a yellow deficiency in the female.

It is possible that the new inversion arose by crossing over with the Y, which supplied two ends to the ring-X. Males carrying the opened ring and either Y^L or Y^S separately are sterile, so that neither an entire Y^S nor Y^L is attached to the X. However, when the single crossover product from $(X^c, y)?/In(1)sc^8$, sc^8 cv v f carrying the base of sc^8 and the tip of $(X^c, y)?$ is combined with bb^1, a bb phenotype results. This can be explained by either (1) the possession of a bb allele in the original ring stock, which was suppressed by a normal allele in the duplicate heterochromatic region carried by the ring and which was shifted to the longer free end when the ring opened out, or (2) the attachment of the end of Y^L, including that section which carries a bb allele, to the new free end of the X chromosome.

In neuroblast metaphases the chromosome appears to be a normal X plus a short arm about one-fourth the length of the longer arm of the X.

Since the entire X chromosome appears to be inverted, the inversion has been given the symbol In(1)EN.

Oftedal, Per. Analysis of a fore-and-aft gynandromorph.

This summer a gynandromorph of the fore-and-aft type was found in a sn^2 B & yy stock, at the Genetics Institute, the University of Oslo, Norway. Description: Head entirely male, with singed bristles and hairs. Both eyes Bar--the right of the normal homozygous Bar type, the left also Bar, but with a rather large area (B) of Sturtevant (Sturtevant, 1927), containing ommatidia, and a smaller area along the upper anterior margin of area (B) also composed of ommatidia. Thorax: All hairs and bristles singed, except those contained in a wedge including the right half posterior margin of the thorax, and extending anteriorly two-thirds of the length of the thorax--namely, both right scutellars, right posterior dorsocentral, both right postalars, and the posterior two-thirds of the dextral median and submedian rows of acrosticals. Appendages: Both fore legs have sex-combs of normal size. Both wings and all legs are singed. The right wing is slightly warped. Abdomen: Yellow; all hairs non-singed; external genitalia entirely female and normal.

The gynandromorph was confined with sn^2 B males. The males repeatedly attempted to mate with the gynandromorph. Usually the gynandromorph did not permit mounting, and under observation always decamped before copulation was completed. At least one attempt must have been successful, however, since one of the several eggs laid hatched. This larva did not pupate, and so no progeny was obtained. The gynandromorph was probably due to a binucleate egg, fertilized by two different sperms. The nucleus giving rise to the anterior, male part must have contained a Y chromosome, and must have been fertilized by a sperm carrying a sn^2 B X chromosome. The nucleus giving rise to the posterior, female part must have contained yy, fertilized by a Y-carrying sperm. Fertilization by an X-carrying sperm would have made the abdomen non-yellow.

Oshima, C. Hybrids between D. novamexicana and D. virilis.

Patterson, Stone, and Griffen (1942) reported that the hybrid female from D. virilis ♀ x D. novamexicana ♂ was fertile, but the hybrid male sterile. In the course of my experiment on D. novamexicana ♀ x D. virilis ♂, one hybrid male was obtained that was fertile when crossed with virilis females. The first back-cross females were crossed with virilis males, and this was repeated three times. When the mutant genes, yellow (2.9) and apricot (130.6), were given to the X chromosome of virilis, the males that had X^v (virilis's X) were distinguished from the males that had X^n (novamexicana's X), as well as from the males that had X^{v-n} (novamexicana and virilis compound); the case of the females was the same. The compound X came out of the crossing over in the distal part of X chromosome but not in the overlapping inversions which were the same with that of D. americana.

X^nX^v ♀ (y-ap/+) x virilis ♂ (y-ap)

	F_1 Female				Male				Female $\frac{(y)+(+)}{(y-ap)+(ap)}$	Male $\frac{(y)+(+)}{(y-ap)+(ap)}$
	(y-ap)	(ap)	(y)	(+)	(y-ap)	(ap)	(y)	(+)		
Backcross										
2nd	160	77	64	176	144	62	45	93	1.01	0.67
3rd	311	167	166	365	329	161	99	192	1.11	0.59
4th	203	95	102	242	194	98	57	92	1.15	0.51

The number of virilis autosomes would increase as the backcross was repeated, although it was statistically proved by the chi-square method that genic complementary relation between the two kinds of sex chromosomes was not effected by the autosomes. (D.F.= 6. ♀:chi-square = 3.7404; $0.80 > P > 0.70$. ♂:chi-square = 5.2894; $0.70 > P > 0.50$.) From the results shown in the table, it was found that the homozygous males (X^vY^v) were superior in vitality and fertility to the heterozygous males (X^nY^v). When (V(NV))V females were crossed with novamexicana males, 40 males (X^vY^n) and 54 males (X^nY^n) (Y^n: novamexicana's Y) were obtained, and the latter were superior in fertility to the former. In short, the backcrossed males that had the sex chromosomes of the same species were always superior in vitality and fertility to those that had the sex chromosomes of the different species. It seems that the X and Y chromosomes have the complementary genes that make the virilis-novamexicana hybrid males fertile as the virilis-americana hybrid males. This genic complementary relation, and the Y-autosomes relation which Patterson, Stone, and Griffen discovered, will control the fertility and vitality of the hybrid males from the virilis group's members.

The D. novamexicana strain (1714.4) was received from Professor J. T. Patterson, of the University of Texas, by the hand of Dr. M. Kodani in A.B.C.C. in Hiroshima. I should like to express here my appreciation.

Piternick, Leonie K. Reverse mutations in D. melanogaster.

D. melanogaster males were irradiated (2400-6000 r X-rays) in an attempt to produce reverse mutations. The following stocks were tested:

Stock	Number of Flies Examined
y^2 cv v_2 f	1411
ec ct g^2	818
w	12260
w sn	19971
In(1)y^{px} bl, In(1)w	1973
In(1)y^{px} bl, In(1)w, f	24448

All the "reversions" found occurred in the stock In(1)y^{px} bl, In(1)w,f, and were observed for f only: 23 partial reversions (i.e., phenotypic expression of f less extreme than in the parent flies); 8 "reversions" (bristles straight, but either slightly bb, or one or two bristles missing, or both characters combined). All flies with partial and complete reversions were tested. In their offspring, f appeared in a number of types of expression, but in no case could a line with normal bristles be isolated.

Power, Maxwell E. A report from a study of the correlation between flightless behavior and the central nervous system of D. melanogaster.

A neurological study is being made of nine mutant strains of D. melanogaster in order to determine the relationship between an aberrant behavior pattern and the central nervous system. The following stocks, which will not fly, were arbitrarily chosen for study: bi ct^6 g^2; al b c sp^2; Df(2) vg^B/Cy, L^4 sp^2; Ly/D^3; ru h th st cu sr e^s ca; ss^a; bx^{34e}/Payne, Dfd ca; tx; and Gl Sb/LVM-3L Payne. It is generally assumed that these stocks do not exhibit the normal flight reflex because of their respective possession of the following genes: cut, curved, Curly, Lyra, curled, aristapedia, bithorax, taxi, and Stubble; but this has not been clearly demonstrated in all cases. The material is being studied in silver- and gold-impregnated sections. The investigation has so far failed to reveal any detectable changes in the morphology of the central nervous system that can be correlated with the absence of flight. The known cortical neurons are present, the fiber pathways and glomeruli appear normally developed in the neuropile, and the pair of giant fibers seems not to be modified in any of the mutant lines. Because flight is a thoracic activity, volumetric measurements were made on the thoracico-abdominal nervous system; but these quantitative studies likewise failed to demonstrate any significant differences between the controls and the experimentals in respect to the volume of the entire thoracico-abdominal nerve mass, that of the cortex, or that of the neuropile. It would thus appear that, unlike alterations in peripheral sensory fields, these genetic deviations in motor behavioral expression are not associated with identifiable aberrations in the central nervous system. No attention has been given to physiology, musculature, skeleton, or the peripheral nerves.

Rosin, S. Chemically induced disturbance of bristle pattern.

Injection of nitrogen mustard into old larvae of D. melanogaster suppresses the development of the bristles in varying degrees. Different regions of head and thorax react in a specific manner. Eyes, wings, legs, and abdominal differentiation are often also abnormal. The analysis of the pattern of disturbance is still in progress.

Semenza, L.

A series of new mutants of D. melanogaster acting on wing are under investigation in order to determine the interaction during phenogenesis.

Sobels, F. H. Normal egg production of lethal ovaries after transplantation into normal hosts.

Ovaries of larvae homozygous for the gene lethal-translucida (ltr, 3-21+) were transplanted into female fes cn bw/fes cn bw larvae. In those cases where attachment of the implanted ovaries to the oviduct of the host took place, the sterile females, after mating with cn bw/cn bw males, gave normal offspring, which could be recognized genetically as originating from the lethal-translucida ovaries.

Spurway, H. A lethal allele of ct producing a high frequency of back-mutation in nonlethal alleles at the same locus.

Dr. R. Milani, while at University College, London, discovered in D. subobscura what behaved genetically as a deficiency for the cut locus i.e., a sex-linked lethal which when heterozygous with a ct allele produced females with a phenotype intermediate between the homozygous females and the hemizygous males. (There is great sexual dimorphism in most ct alleles in subobscura; only in one is the female fully penetrant, and in many the phenotype is entirely confined to the males.) He was unable to find any cytological abnormality.

Females heterozygous for this lethal and at least three other ct allelomorphs produce, as expected, twice as many daughters as sons; but those sons, instead of being entirely ct, contain among themselves 1.7% wild-type flies. These exceptional males and their descendants breed consistently as though they carried a ct^+ allele. Experiments with bnt, 4.5 units from ct, seem to show that it is the nonlethal allele which mutates to wild-type; but this is not critical, as the absence of markers closely straddling the ct locus makes it impossible to observe whether some unequal crossing over has occurred in this region.

The ct locus seems to be the most mutable in this species, in both directions, and there are several anomalies in both this mutation rate and the actual penetrance of the alleles, which seem to be connected with different chromosome orders. On the evidence of the complex pleiotropisms of the phenotypes of the many alleles, the homology of this locus with its namesake in melanogaster seems better established than most.

Sturtevant, A. H. Sequence of loci near the centromere of chromosome 2.

A series of multiple-mutant stocks has been made up (e.g., stw^{48} blt tuf, pk tuf, tuf ltd). The techniques used in making these gave information on the sequences involved, and they have also been used to make further tests on other loci. In addition a series of deficiencies has been tested against the mutants of this region. One of these is the crossover between bw^{VD32} and In(2R)Cy; this and the duplication from T(2;3)108 both give evidence (consistent in all cases) on the position of the left break point of the Cy(R) inversion in the genetic map--and this break point is already known to lie between salivary bands 42A2 and 42A3. The sequence of loci is as follows: Bl, lt, centromere, rl, stw, blt, In(2R)Cy left break point, pk, tuf, ltd. The total crossing over for this region is not far from 1%.

Suley, A. C. E. Transplantation experiments with the mutant grandchildless (Chr. 3) in D. subobscura.

gs/gs females produce sterile offspring, with reduced ovaries or testes, regardless of the males to which they are mated (Spurway, DIS-20). Transplants of gonad rudiments between larvae from fertile and sterile families, at third instar, show that both types of gonad are capable of

autonomous development from this stage (Suley and Milani, DIS-22) and appear unaffected by the host. Sterile individuals with ovaries transplanted from fertile ones are capable of bearing foster children. (The true relationship of these was checked by the use of genetical markers.) About one-half of the "sterile" foster mothers were known to be gs/gs themselves, as well as being the daughters of gs/gs mothers. But they did not appear to exercise the sterilizing effect on their foster children that a gs/gs female exerts on her own children. (Sixteen foster mothers produced nt offspring with no genital abnormality. Sixteen nt/nt mothers, as controls, also produced normal broods with the exception of one sterile male. Fifteen foster mothers produced ma int offspring, 1% of which were sterile; but ma int controls showed that a comparable proportion was present in the ma int stock.)

Valencia, R. H., Muller, H. J., and Valencia, J.I. Formation of attached X's by reverse crossing over in the heterochromatic region.

In the course of an experiment in which the oogonia of females of our "plond" stock (bl23 in DIS-22) were irradiated, it was noted that attached X's were formed with an unusually high frequency. The daughters containing them were always dark yellow, and frequently were also car, f car, or narrow B. The frequency of these attached-X daughters was about 1 in 250 following 4200 r.

The "plond" mothers regularly contain sc^{sl} B In49 lz^{s} in one X chromosome, and a series of recessive mutations called "ploc" (y ac sc pn w rb cm ct^{6} sn^{3} oc ras^{2} v dy g f car) in the other. In addition they contain a Y chromosome of the sc.Y^{l} type, marked with a dark yellow, and called $y^{3}.Y^{l}$. The males of the "plond" stock, which were used as fathers in this experiment, also have a $y^{3}.Y^{l}$, and their X is of composition y oc $lz^{3}.y^{s}$.

Analysis of the situation led to the following conclusions. The heterochromatic section lying near the distal end of the sc^{sl} chromosome occasionally crosses over, in reverse arrangement (as it does with a Y when chromosomes of sc.Y^{l} type arise), with the heterochromatin just to the left of the centromere in the "ploc" X chromosome. (See figure 1.) When this occurs, the result is attached X's, with the y^{+} ac^{+} sc^{sl}-containing region missing from the sc^{sl} chromosome. However, the $y^{3}.Y^{l}$ which the daughters receive from their father makes them diploid for the loci in this region. Such females appear dark yellow. It is to be noted that, unlike ordinary attached X's, these have a nearly terminal centromere but that, except for In49, their genes are in mirror-image order to one another, as they are in ordinary attached X's. Therefore, by folding of the heterochromatic bridge, alignment of all parts except the In49 region can occur.

After this attachment has taken place, then, the homologous regions at either side of the point at which the chromosomes have become attached to one another find and pair with one another in the oocyte stage, as shown in figure 2. Double crossing over can then occur fairly readily in this region, especially after irradiation, and when both crossings over involve the same two strands, strands unconnected with one another across their centromere, the result is attached X's homozygous for car, f car, or B. Although such double crossing over can also occur between the detached X's of ordinary females of the "plond" stock, and would then also produce car, f car, or BB exceptions, which however would be non-yellow, such non-yellow exceptions appear with very much greater rarity, even after irradiation, than the corresponding dark-yellow exceptions resulting from double crossing over between the attached X's. This shows that the fact of their attachment to one another

leads to much more complete synapsis of the regions in question (roughly extending from the loci of g to bb) than usually occurs between the same regions of these chromosomes when they are detached. This must be largely because the synapsis of the detached chromosomes, even in these regions, is much hindered by the heterozygous Inversion 49, which introduces competitive pairing of other parts. It must be concluded that the attachment, allowing the g-bb region of both chromosomes to be adjacent to one another from the start, and thus initiating a zipper action, succeeds in largely overcoming the interference which In49 would otherwise exert.

In view of the above analysis, it seems probable that the so-called double attached X chromosome described in DIS-17, pp. 61-62, and DIS-18, p. 57, which has given rise to our "y f:=" stocks, arose by the above method, rather than by deletion and double attachment as described, although the structure arrived at would be the same in either case and was correctly given. This chromosome also was originally heterozygous for f and B (in "repulsion" arrangement) and the homozygosity in regard to f arose through a later double crossing over, of the type described above.

Several attached-X stocks produced in this way later began to produce occasional disjunctional progeny again. This can be accounted for on the interpretation that here another heterochromatic crossing over had followed the first, the later one involving an exchange between the heterochromatic bridge that unites the X chromosomes, on the one hand, and the y^3 .Y^l chromosome, on the other hand, as shown in figure.3. Thus the attached X's become separated again and the region previously lost from the sc^{sl} chromosome resupplied, though in this case with a dark-yellow marker. At the same time, the "ploc" chromosome would receive a centromere and a Y^l "tail" to the right of the centromere. It is to be noted, however, that our "y f:=" chromosome, unlike some of the recently induced double X's described above, has been observed to undergo such detachment only with extreme rarity, and far less often than the ordinary attached X's with median centromere, at least when an ordinary Y is present.

(For figures see next two pages).

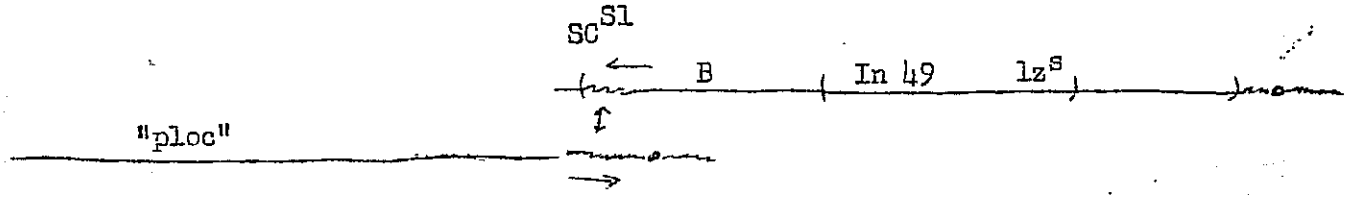


Figure 1

Formation of double X by reversed crossing over. Wavy lines represent heterochromatic regions; single arrows indicate the orientation which a given heterochromatic region would have towards the centromere if it were located in a chromosome of normal structure. The large dot represents the centromere as here placed; a double arrow marks the point of crossing over. It will be seen that only when crossing over involves reversed arrangement of parts at attachment point are monocentric (but double sized) chromosomes formed.

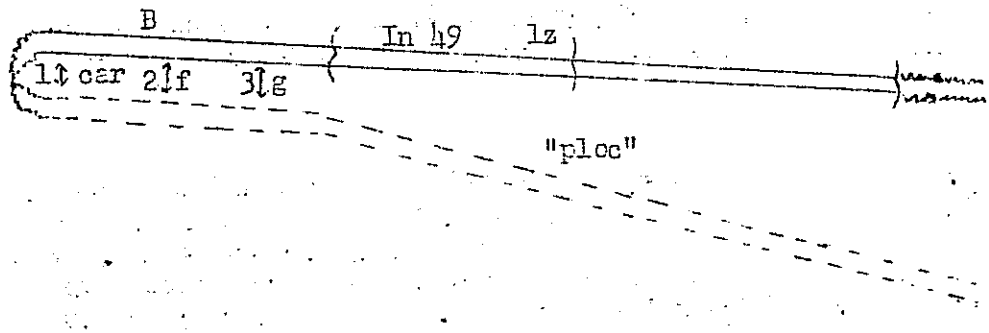


Figure 2

Double crossing over in attached X, giving rise to homozygous car, f car, or B. Points of crossing over are indicated by arrows. Note that the two strands involved must be unconnected. Crossing over at 1 and 3 would produce f car, and crossing over at 1 and 2 would produce car. The corresponding class in both cases would be B/B. Since no homozygous forkeds occurred, 2 and 3 must be too close together for double crossing over.

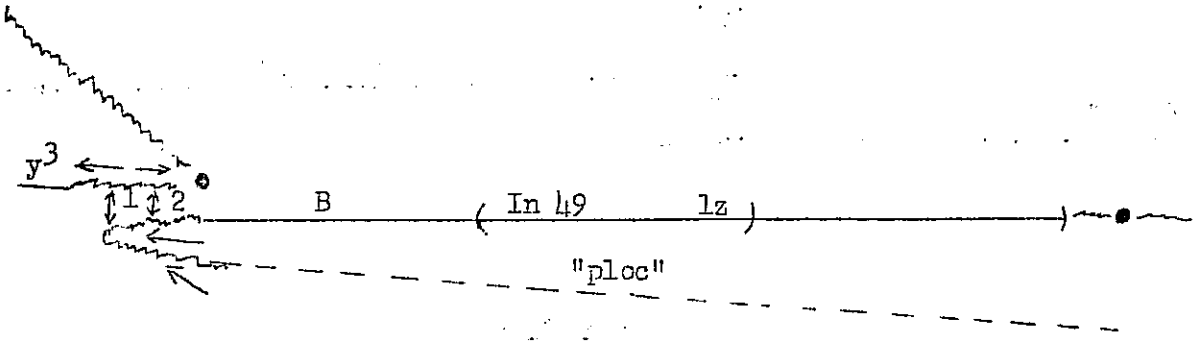


Figure 3

Detachment of double X by crossing over with y^3 .Y^{l}. Symbols same as in figure 1. The region of the y^3 .Y^{l} chromosome at 2 is derived from an original Y, and so has normal orientation, whereas the region of y^3 .Y^{l} at 1 is derived from heterochromatin of a sc^{Sl} X and is in inverted position with regard to centromere. Therefore if crossing over is at 2, it must be "reverse" in order so as to give monocentric chromosomes, but if it is at 1 it is of orthodox (nonreverse) type. On the other hand, the Y could as well cross over with the part of the heterochromatin shown below (that derived from the "ploc" chromosome), and in this case crossing over at 2 must be orthodox and that at 1 reverse, to give monocentrics.

Wallace, Bruce. Effects of radiations on populations

Recently we have started a program to determine the genetic effects of radiations on populations. The analyses will include a study of frequencies of dominant and recessive lethal gene mutations, chromosomal aberrations, and sterility genes in populations treated chronically or acutely with gamma or X-rays. So far, data are avilable only for the elimination of dominant lethals from populations; obtaining data for recessive lethals is much more time-consuming.

Three populations of D. melanogaster are being maintained. (For description of population cages, see Technical Notes.) Each of these populations was started with 16 strains (isolated from a mass culture of Ore-R) isogenic for their second chromosomes and free of lethal or semilethal genes on that chromosome. The flies used to start population #1 (3933 flies) were exposed to the following amounts of X-ray; males, 7125 r units; females, 1012.5 r units. Both males and females (total of 4219 flies) used to start population #2 were exposed to 1012.5 r units. The flies of population #3 (total of 2114) were untreated.

Egg samples (1000 or more eggs per sample per cage) have been taken from these populations every two weeks, starting the day after the original flies were first placed in their cages. The sampling procedure for dominant lehtals consists of inserting a food cup into a cage for approximately 6 hours. Eggs deposited on the food in this period are transferred to

food on a glass slide; usually 50 eggs are placed on each slide. These slides are then placed in shell vials, the vials are covered with moist toweling, and trays of vials are placed in a constant-temperature room (25° ± 1°). The unhatched eggs are counted after 24 hours and are transferred to fresh slides; a second and final count is made at 48 hours. The data that have been obtained are presented below (percentage of eggs unhatched after 48 hours):

	Population			Probability that data from 3 cages are homogeneous
	#1	#2	#3	
Sample 1 (parents)	96.6	58.3	17.3	0
2	52.3	39.4	28.1	0
3	13.5	7.8	8.8	0+
4	13.1	10.1	8.0	.001
5	7.6	7.5	5.6	.14
6	8.2	11.8	8.6	.01
7	9.5	16.8	8.2	.0+

Obviously, the bulk of the dominant lethals are eliminated at once, as expected. The high frequency of unhatched eggs of sample 2 merely indicates that the survivors of the original parents comprise most of the population, despite the emergence of the new generation. However, genetic conditions--presumably translocations and pericentric inversions--capable of giving rise to dominant-lethal combinations persist through the third and fourth samples (second and third generations). The heterogeneity of the sixth and seventh samples is, as yet, unexplained.

The frequencies of unhatched eggs in the control population (#3) demonstrate the effect of heterosis. The original strains of flies, isogenic for their second chromosomes, were chosen for the absence of genes deleterious to viability. Apparently, however, these flies were homozygous for fertility modifiers, and produced eggs of poor hatching quality. Sample 2 of cage #3 shows the effects of age on these same flies, but subsequent samples show a marked decrease in the frequency of unhatched eggs. This decrease probably results from random mating between the isogenic strains and the production of flies heterozygous for their second chromosomes. The increase in frequency of egg hatching is greater than is apparent, for the original flies were raised under reasonably good conditions in culture bottles, whereas the subsequent generations developed under the stringent conditions existing in population-cage food cups--conditions that should lower the percentage of hatching.

Wilson, Louise Palmer. The use of N-phenyl nile blue chloride as a vital stain for fat in Drosophila. N-phenyl nile blue chloride obtained from Calco Chemical Company was added to a sterile culture medium to which sterile eggs of D. melanogaster were added. Under these conditions cells of the fat body show bright-red droplets of neutral fat as the larvae develope. Parts of the gut show varying shades of blue and purple. Tumor cells in the fat body are not stained, or are faintly tinted, and it is thus possible to identify early premelanotic stages of tumors in the living larvae. The larvae grow and develop normally in a concentration of 0.002% of the dye. The use of this dye should facilitate study of the development of tumors as well as be useful in the study of general anatomical problems.

Yu, Sien-chiue. Studies of reverse mutations of several recessives in D. melanogaster.

X-ray-induced reverse mutations were looked for among the progeny of y sc w^e spl females mated to treated sc^7 w^a males (4500 r units). One apparent reversion of sc^7 was sterile, and no reversals of w^a were found among 7106 gametes tested. From a mating of y ac v; bw females and treated v; bw males (4500 r units), no v reversals were found among 6050 gametes tested. From cn px bw sp females mated to treated cn bw males (4500 r units), no cn reversals occurred among 14,432 gametes tested. From bw; th st cp females mated to treated px bw sp; st males (4500), no st reversals occurred among 29,195 gametes tested. No reversals of bw were detected in these experiments among a total of 55,723 gametes tested.

TECHNICAL NOTES

Begg, Michael, and Robertson, Forbes W. Aseptic culture of Drosophila.

Eggs are collected and transferred to a test tube containing sterile water, which is rotated about its long axis for several minutes, after which the eggs are allowed to settle. The water is then replaced by 50% ethyl alcohol. The tube is again rotated slowly for 3-4 minutes, and the eggs are allowed to collect at the bottom of the tube, then removed with a sterile pipette and transferred to a tube containing 5% antiformin in 10% formaldehyde solution (Glaser, 1943). After 10 minutes they are removed to another tube of 50% alcohol, and after settling are transferred in batches of about 20 tosmall round-bottomed tubes (3/8" x 2"), containing 50% alcohol. Two further changes are made into similar tubes, so that the total immersion time at this stage is about 25 minutes. The eggs are then transferred, with the usual precautions, to the sterile culture media. This is best carried out by using pipettes made by drawing out glass tube, squaring the ends off very carefully, and plugging with cotton wool. With the aid of a length of rubber tubing attached to the top of the pipette, the operator can suck the eggs into the end of the pipette and gently blow out excess alcohol, while holding the pipette against the glass bottom to prevent the eggs from escaping. The entire operation can be carried out by one person after practice, but at first it is safer for two persons to work together.

Latteraly a modification was used, involving transference of eggs, after immersion in antiformin and formaldehyde, to a small sterile glass tube close at one end by a platinum grid (100 mesh per square inch); the eggs can then be moved from one solution to another in this container. After 10 minutes in a mercuric chloride solution of the following composition:

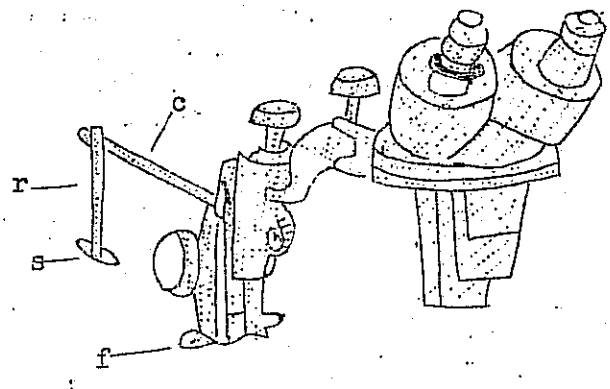
$HgCl_2$	0.50 g.
HCl	1.25 ml.
NaCl	6.5 g.
Ethyl alcohol	250 ml.
Water to	1 litre,

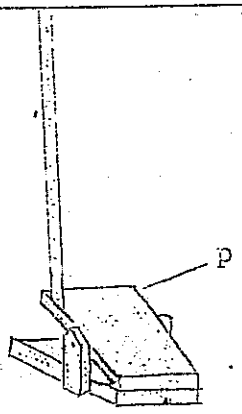
the eggs are given four 5-minute washes in alcohol. Finally the eggs are removed in batches to the small tubes, and thence into the cultures without further transfers.

Mickey, George H. Cellucotton in cultures. Routine use of cellucotton in culture vials and bottles (as indicated in 1941, J. N.Y. Ent. Soc. 49: 77-84) has consistently yielded larger numbers of flies than the use of paper toweling or other materials. We have found that the best method of applying the cellucotton to vials is to cool the food on a slant in such a way that one edge of the surface is near the bottom of the vial. Then a small bit of sterile cellucotton is inserted at the shallow margin. Fermentation gases can escape without "blowing up" the food.

Mickey, George H. (2) Water table for controlling temperature. A fairly uniform temperature can be maintained for stock cultures and for class experiments by placing cultures in metal containers and putting the latter in a water table. The rate of flow of tap water will regulate the temperature within a narrow range. Aluminum baking or loaf pans, 9 5/8 x 5 1/2 x 2 3/4 inches, weighted with a rectangle of sheet lead cut to fit the bottom, have been found to be suitable containers.

Mickey, George H. (3) Foot-focusing device for binocular. The need is acute for a means of focusing the binocular dissecting microscope for work where both hands are needed for dissection under high manification. The design described herein is much simpler than that reported by Stern (DIS-6, 1936), and can be constructed from materials available in any laboratory. The Bausch and Lomb model RKT-5 and the American Optical Company's Spencer model 28 LC are suitable types of instruments. The binocular is placed at about a 30-degree angle from the edge of the table and is screwed to the table top by means of a metal flange (f); a laboratory extention clamp (c) is attached to the focusing knob. A hole bored through the opposite end of the clamp allows fastening the clamp to a rod (r) by means of a small bolt. The rod passes through a slit (s) in the table top to join the pedal (p) below. The clamp can be attached to or removed from the focusing adjustment quickly, to allow for hand operation or for adjustment to a greater range of focus. (See accompanying sketch on following page).





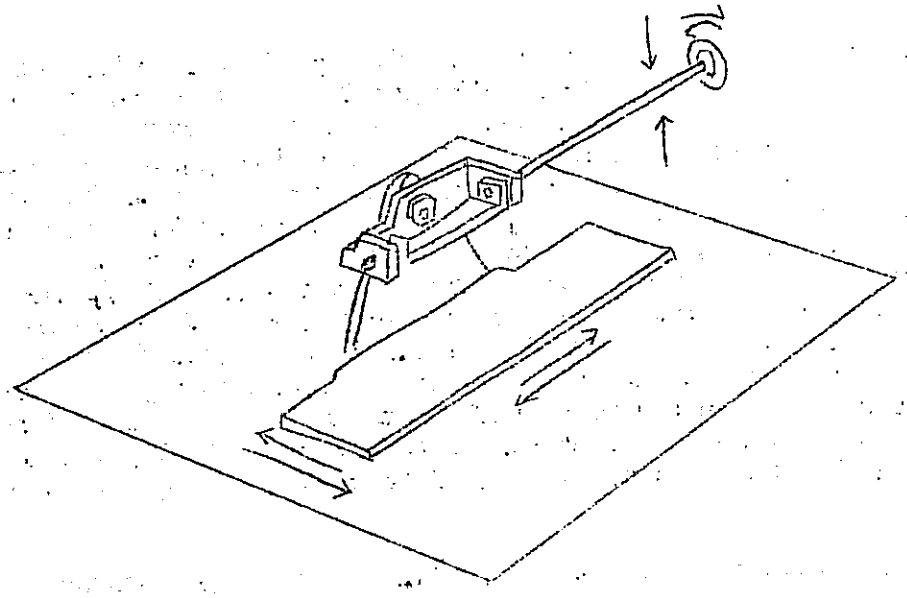
Newby, W. W. Sectioning of Drosophila.

When sectioning Drosophila, it has been found that clearing with several changes of chloroform instead of xylene gives consistently better results. Serial sections may be cut at 6 micra by the use of a safety razor blade, in such a way that about one-half millimeter of the razor blade's edge projects above the edge of the microtome blade. This method of sectioning, originally observed in the Texas laboratory, has proved invaluable in sectioning larvae, pupae, and imagoes.

Reeve, Erick, and Robertson, Forbes W. Measurement of living Drosophila.

In carrying out selection for body size in D. melanogaster, we had to devise a method of measuring the body size of live flies rapidly and accurately. We have used a small movable platform (see figure), which is attached to the moving stage of an ordinary microscope, and can be rotated about either of two horizontal axes at right angles to each other by twisting or moving vertically an attached lever. The platform is covered with thin cloth, to which the etherized fly readily adheres. The fly is placed on the platform in approximately the correct position, and the dimension required can be brought into the exact position and alignment for measuring with a calibrated ocular micrometer, by use of the moving stage and platform adjustments, and by rotating the axis of the micrometer. By

this method it is possible for a skilled operator to measure 150-200 flies in a day before fatigue ruins the accuracy of measurement. Provided that the two ends of the dimension to be measured are brought into focus simultaneously, accuracy is limited in practice only by the degree of definition of the two end-points.



Smith, Guinevere C. A substitute for Moldex.

Since "Moldex" is no longer available, a substitute, "Tegosept M," has been used successfully at the Carnegie Institution of Washington Department of Genetics for about two years. This product is available at the Goldschmidt Chemical Corp., 153 Waverly Place, New York City, and is used exactly the same as "Moldex" (10% in alcohol).

Spencer, W. P. Collecting wild Drosophila.

For making collections of wild Drosophila species, banana mash to which live Baker's yeast has been added provides an excellent bait. Quart-size freezer pail packs (of cardboard heavily impregnated with paraffin) are hung by heavy string to bushes or low branches of trees. These containers are filled one-third to one-half full of banana mash. From a pasteboard cylinder 8 inches to 12 inches in length and about 5 inches in diameter a "collector" may be constructed by covering one end with fine-mesh cheesecloth. A large cotton plug covered with cheesecloth fits snugly into the other end. For collecting flies the cotton plug is removed and the open end of the cylinder suddenly pushed down over the fly-trap, which is then shaken. Drosophila, which are positively phototropic, fly up to the cheesecloth end and remain there. After flies have been collected from several traps, the cotton

plug is inserted. Flies may be removed from the "collector" by pushing the plug up near the cheesecloth, placing a piece of cleansing tissue moistened with ether over the cheesecloth, and covering the end of the collector with cardboard or glass plate. Etherization may be done in the field or flies may be carried back to the laboratory in the "collector." Largest collections are made if the traps are exposed for two or three days. Freezer pail packs are light and, as they nest together, are easily carried in quantity. They are large enough so that bait does not dry out for several days. Overripe bananas for bait can generally be secured in any large town at a wholesale fruit house at little or no cost.

Wallace, Bruce. A new population cage.

The fundamental problem of maintaining Drosophila populations was solved by constructing a cage in a way that permits small amount of food to be given the flies periodically, rather than attempting to supply the flies with a large amount of food at once. In the cages developed and described by Dobzhansky, however, there remained irksome difficulties connected with keeping the food from drying out before the flies emerged (solved by periodic addition of fresh yeast suspension), and with fastening the food container securely to the cork (solved(?) by placing three or four small nails at the edge of the cork). In addition, we were faced with the problem of constructing a cage that would expose a population (larvae and pupae as well as adults) to a uniform amount of gamma radiation from a radium scource. (See Research Notes.) Since it would be impossible to handle the cage during its exposure, we wished to reduce all care of the cage to a minimum.

The cages we have built seem to fit our needs. They are plexiglass structures, semicircular in shape, outside diameter 23 inches, inside diameter 18 inches, and depth 4 inches. Consequently, the adult fly population occupies a curved volume, which looks something like half an angel-food cake, 2 1/2 inches from front to back and 4 inches from top to bottom. The 16 food cups are placed in a single row along the curved bottom, so that all are the same distance from the radium source. The curved surface facing the radium consists of fine aluminum screening. Tops are removable to facilitate cleaning. Each cage stands on three legs 6 inches high. By virtue of the shape of these cages, two populations can be placed about a radium source and receive exactly the same amount of radiation.

The food cups are also plastic (more transparent to gamma rays than glass) and are 1 3/4 inches deep by 1 3/8 inches in diameter (inside dimensions). The cups are filled to the top with food enriched with Brewer's Yeast; because of the combination of greater depth and extra yeast, it has proved unnecessary to add fresh yeast suspension to support the developing larvae and to keep the food from drying.

No corks are used in these cages. The floor of the cage is built of two layers of plastic separated by a sheet of rubber. The holes for the cups are cut slightly smaller in the rubber than in the plastic, so that a rim of rubber protrudes around the edge of the hole. This rim fits firmly about the sides of the cup and holds it in place. An extra precaution against flies' escaping is a thick rubber band around each cup; when the cup is in place, this band slips between the cup and the bottom plastic layer of the floor and presses firmly against the rubber layer.

Three of these cages have been in use for seven generations (D. melanogaster) and have proved satisfactory. We estimate that each cage supports

about 10,000 adult flies, although this has not been confirmed by actual count. Our cages do not have openings for easy removal of adult flies, but these can be added with little difficulty.

Wittinghill, M. A mosaic mother and a mosaic son. In classifying the testcross progeny of a gamma-ray-treated ru h th st + sr e^s ca/cu melanogaster female, of the 520 offspring was found to be mosaic in respect to the antennae. She had one thread antenna and one wild-type, and she showed the recessive mutants st sr e^s ca symmetrically. Although nonvirgin, she was isolated to see whether she represented crossing over in region 2 or region 3. The majority of her 220 offspring indicated that she was a crossover in the h-th region, but 5 offspring were of the phenotypes of region-3 crossovers. This might indicate a mosaic gonad. Furthermore, complementary classes of region-3 exchange were found: 3 were th^+ st like one side of the mother, but 2 were th st^+, and each type was verified by further breeding. These and other classes of offspring of the first mosaic could be explained if the mosaic female was triploid, and assuming a variable dominance at the thread locus, since recombinations in this region near the spindle attachment are greatly increased in triploids. Inspection in the living state and after preservation in balsam has not revealed any signs of a triploid phenotype externally. Yet there was no other source for th + chromosomes in the laboratory at that time. If she was neither triploid nor mosaic in still other ways in the ovaries, she must have mated to eight or more different phenotypes of brothers to explain the wide variety of her offspring. The difficulty is not solved by assuming that she was carried over from any other culture classified that day.

A second and different mosaic was found among the 220 offspring already mentioned. This son was a bilateral mosaic showing curled wing on the left side but not on the right. The right side showed thread antenna, scarlet claret eyes, stripe, and e^s. Somatically, then, this male was a mosaic of two phenotypes common among its sibs. An attempt to backcross this male to its mother failed, but when it was testcrossed to rucuca females cu and rucuca offspring resulted. This indicated a different mosaic for the gonads as compared with the somatic tissues. Subsequent generations revealed no more mosaics in well over 2500 flies. The lines are maintained in stock as "cu (gamma-rayed, mosaic)" and as "rucuca 1949" cultures.

Muller, H.J. The use of rearranged X's and Y's in facilitating class work with Drosophila.

The greatest handicap to the prosecution of laboratory work on Drosophila by undergraduates is the need for obtaining virgin offspring for the making of second-generation crosses. The virgin P_1 can be obtained for the students fairly readily, so that the first-generation cross does not present much of a problem. But for the second-generation cross the backcross, or "testcross" as Bridges called it, is usually desirable, especially for beginners, since it gives a simpler picture of the gamete frequencies than is obtainable from crosses of F_1 by F_1; and for the testcrossing of F_1 females it is necessary (except in the case of crosses of sex-linked genes in which the P_1 female had all the recessive traits) for these F_1 females to be virgins. In most courses, it is too much to expect undergraduate students to come in both in the evening and the morning (sometimes several times) to secure these virgins. But if the instructor secures the virgins for the student, the latter's role virtually becomes reduced to that of a watcher, and only a part-time watcher at that, at a demonstration. He then has to take too much on faith, much of his interest as the active agent in the work is gone, and it is doubtful if such laboratory work is worth the time and effort. Thus the difficulty of getting virgin F_1 has much reduced the extent of laboratory teaching in genetics below what it otherwise might have been.

The difficulty can be, and in our classes of the past year has been, avoided by the method, devised by Stern, of making crosses between stocks differing in the arrangement of their Y-chromosome arms in such a way as automatically to produce, in these crosses, sterile sons and therefore virgin daughters. For application of this method to elementary class use, we have made up a number of otherwise standard stocks, all having males with an $X.Y^s$ (X chromosome of normal structure except that it has a short arm of the Y attached at its right end) and a $\underline{Y^{lc}}$ (ring-shaped Y chromosome having a full set of long-arm but not short-arm genes for sperm motility); that is, the arrangement of $\underline{Y^{lc}}/X.Y^s$. These stocks, termed "sterlizers" (sz) for short, have the following designation ("signal") and composition, being listed in category h of our stock list.

"sz+": $\underline{Y^{lc}}/X.Y^s$♂ & $X.Y^s$ ♀
"sz w": $\underline{Y^{lc}}/w.Y^s$♂ & $w.Y^s$ ♀
"sz y w": $\underline{Y^{lc}}/yw.Y^s$♂ & $\underline{Y^{lc}}/y$ ct f. = ♀
"sz bw": $\underline{Y^{lc}}/X.Y^s$; bw ♂ & $X.Y^s$; bw ♀
"sz c": $\underline{Y^{lc}}/X.Y^s$; c ♂ & $\underline{Y^{lc}}/y$ v f. = ; c ♀
"sz e": $\underline{Y^{lc}}/X.Y$; e ♂ & $\underline{Y^{lc}}/y$ v f. = ; e ♀

The attached-X composition of the female is of no significance here, and is present only because the stocks were more easily constructed in this way. They can readily be replaced by separate X's through crossing with the "sz+" stock. However, the stocks are usable in their present form because only the males of the "sz" stocks are ordinarily used for crossing.

These stocks, when taken in connection with our non-sz stocks, suffice for most of the standard crosses illustrating segregation, random assortment, and crossing over involving two or three pairs of genes. Thus, for autosomal linkage of "repulsion" type, sz c males are crossed to virgin females of our ordinary (not sz) bw stock. The automatically virgin F_1 females are than obtained by the student and backcrossed to males of our ordinary c bw stock. At the same time, from a parallel cross of ordinary c males by bw females, the F_1 males, being fertile, are backcrossed to virgin

females of the c bw stock. (As before noted, the concession is made of supplying students with virgins whenever these are to be obtained, en masse, from stock cultures.) For the "coupling" arrangement, P_1 virgin females of the sz^+ stock are crossed to c bw males, and the automatically virgin F_1 females backcrossed to c bw males, while F_1 males from a parallel P_1 cross of ordinary + by c bw are backcrossed to c bw virgins. The same operations can be performed, for chromosome 3, with e and ss, using the cross of sz e males by ordinary ss females, and that of sz^+ males by females of our ordinary ss e stock; but these crosses are less to be recommended because of the low fertility of ss and ss e. For random assortment, corresponding crosses involving sz^+ ♂ by c e ♀ or bw e ♀ and sz cd or sz bw ♂ by c ♀ are made. For three or more point crosses in which all the mutant genes are recessive and enter from one side, the multiple-mutant females are crossed to sz males and the automatically virgin F_1 females are backcrossed to multiple-mutant males.

It is to be conceded that this method suffers from the disadvantage that the elementary student is not yet prepared to understand why the F_1 males are sterile. We have found it best to postpone the explanation until later, when it adds interest to the matter without detracting from the understanding of the primary principles that were to be illustrated. Naturally, there are a number of precautions to be taken, such as insuring that the P_1 females were virgin, that the P_1 were completely removed before the F_1 hatched, and that no flies of the wrong sex were put in as P_1 or P_2, but these precautions are always necessary anyway. Very rarely, an extra Y is present to prevent sterility, and for this reason two individual cultures of each cross are to be preferred to one mass culture. The fact that the Y chromosome of the sz stocks is ring-shaped prevents it from becoming transformed into an ordinary Y by crossing over with the $X.Y^s$, as happen in other $Y^l/X.Y^s$ stocks (and also, in the stocks whose females have attached X's, prevents the detachment of the latter). However, as Kenneth Cooper found through cytological examination of our Y^{lc} stocks, an occasional $Y^s.Y^s$ is formed (no doubt by crossing over between the two $X.Y^s$ chromosomes), and this if frequent would disturb the scheme. But in practice this method has been found to be a great help in our elementary teaching, and we believe it should overcome the greatest difficulty in the way of more widespread laboratory teaching of genetics to elementary classes.

An additional practical aid, if temperature regulated to between 24° C. and 27° C. is avilable, is the provision of two widely spaced laboratory periods per week, as on Mondays and Fridays, since it is then possible for the students to carry the flies through a generation every 10 or 11 days,--i.e., two generations in 3 weeks.