

RESEARCH NOTES

Barigozzi, C. Mitoses in differentiated cells of D. melanogaster.

this, mitoses have been observed, showing chromosomes two to four times bigger than in neuroblast mitoses. The different pairs are easily classifiable, although synapsis is very strong.

Migrant hemocytes are cells which have already undergone histological differentiation. This is proved by the low polyteny of their nuclei. In spite of

Barigozzi, C., and Di Pasquale, A. Localization of genes controlling pseudotumor production in Drosophila.

on all pairs. The present report deals with an attempt to localize tu genes, in one stock, with the second chromosome, which is practically the only site of tu genes in some stocks. Using as markers cn c px, crossing over proved the existence of more than one tu locus, probably located on both sides of px. Thus, tu genes are a system of genes, acting as polygenes, restricted to a short portion of one single chromosome. The presence of modifiers can be proved by means of selection. Starting with stocks that are practically homozygous for tu genes, it is possible to select both for increasing and for decreasing tumor frequency. Using this technique, it is possible in some cases to raise the frequency up to nearly 100% or to lower it down to 3% or less. This is done merely by changing the modifier assortment. Thus, crosses between + and - selected lines give a high pseudotumor frequency because the recessive tu's are still present in both lines. In the stocks investigated so far we thus find two systems of polygenes, one restricted to a short chromosome portion, and the other (the modifiers) spread throughout the whole genome. The degree of homeostasis in different stocks, detected through interruption of selection, proves that carrying pseudotumors can be semilethal or deleterious in some stocks, but harmless and even advantageous in some others. No generalization can be made.

Previous investigations have proved that two different parts of the genome are at work in producing pseudotumors in some stocks of D. melanogaster: tu genes on the second chromosome, and many modifiers

Becker, H. J. On X-ray-induced somatic crossing over.

imagines were checked for single (w/w or w^{co}/w^{co}) and twin mosaic spots. The factor sn was not immediately significant in this connection; se was used in order to increase the color contrasts, and h for easier recognition of the se chromosome. In this experiment (inspection of the imagines first in air and afterwards under paraffin oil), 336 twin spots, and 115 white and 32 dark single spots were found. In a second experiment (only larvae of the first and second instar treated; inspection of the imagines in air; complete formula, see below), 1225 twin spots, and 203 white and 156 dark single spots were obtained. The different frequencies of white and dark single spots are probably due to the relative difficulty of distinguishing the dark spots, especially in older imagines. The discrepancy between the percentages of twin spots in the two experiments is probably due to the fact that in the first experiment younger stages were treated. Twin spots induced during these stages are more often situated, by reason of their size, with only one partner in the eye, and are thus counted as a single spot.

Embryos and larvae of constitution w sn⁺/w^{co} sn; se h/se h were X-rayed with a dose of 1200 r, and the eyes of the

Other data contribute to the problem of the primary X-ray effect resulting in somatic crossing over. At the end of the first larval instar the presumptive eye area in the head anlage consists of about 20 cells (see the next note). After treatment of larvae at this stage, about 30 spots resulting from somatic crossing over were found among 100 eyes, that is, 30 spots per 2000 treated cells, or 1.5 spots per 100 cells. However, a mosaic spot arises as a consequence of somatic crossing over only when the two crossover chromatids join different cells, not when they join the same cell. Both these types of segregation occur with equal probability. Thus, not taking into account special double and multiple exchanges that may also result in mosaic spots, crossover events in the X chromosome take place in about 3 out of 100 cells. Fano and Demerec, after treating *Drosophila* sperm with 400 r, found 1% dominant lethals due to single breaks in the X chromosome--that is, 3% with 1200 r; thus two breaks, one in each chromatid, are to be expected in 0.09% of the cells and at homologous points with a disproportionately lower frequency. Since in somatic cells values of a similar order are to be expected, a two-hit event cannot be the cause of somatic crossing over. Since Friesen (1937) and Shapiro (1941) found a deviation from a linear dose-frequency relation for X-ray-induced mosaic spots, it does not appear that a single-hit event, either, is the cause of somatic crossing over. Lefevre's (1947) finding that the spot frequency depends upon the dose rate points in the same direction.

Since somatic crossing over thus hardly seems to arise through chromosomal breaks as a consequence of ionization, an attempt was made to get further information about its origin. The larvae of the second experiment were made heterozygous for the factor ru^{δ} (3-0.0); thus the complete formula was $w^{co} sn/w sn^{+}; ru^{+} se h/ru^{\delta} se h$. Five twin spots were found in which the white partner showed the ru^{δ} phenotype; the same phenotype was shown by the dark partner of 8 other twin spots, and by 4 white and 6 dark single spots. In addition the following 8 spots were found: (1) both twin-spot partners ru^{δ} ; (2 and 3) only a part of the white partner ru^{δ} ; (4) only a part of the dark partner ru^{δ} ; (5) inside a large ru^{δ} region, a smaller twin spot; (6) inside a large ru^{δ} region, a smaller white single spot; (7 and 8) inside a large ru^{δ} region, a smaller dark single spot. The single color spots in (6)-(8) lie on the margin of the eye.

Provided that at least some of these types of spots are due to induced somatic crossing over in both the marked chromosomes, then these cases indicate that the X chromosome (in the case of the smaller color spots) and the 3rd chromosome (in the case of the smaller ru^{δ} spots) did not undergo crossing over immediately at the time of treatment, but only later on. This would mean that not breakage events caused by ionization, but rather a physiological change in the cells, is responsible for the origin of somatic crossing over. This change may provide the conditions for crossing over, and may sometimes be maintained for one or more cell divisions after the treatment.

Becker, H. J. On the development of the *Drosophila* eye.

After X-ray treatment of $w sn^{+}/w^{co} sn; se h/se h$ embryos and larvae, twin mosaic spots are to be found in the

eyes of the imagines. These spots are caused by somatic crossing over and consist each of a white w/w and a dark w^{co}/w^{co} partner (see the preceding note). The partners of spots induced before the end of the first larval instar in the lower half of the eye (this portion shows the clearest

conditions) are situated in a row, with the approximate direction, frontal-dorsal to caudal-ventral; in later-induced spots they are situated in a direction vertical to the former. The relative position of the two partners of the spots shows the direction of cell division of their common stem-cells. Thus at the end of the first larval instar a change takes place in the direction of the divisions.

This stage is further characterized by another phenomenon. Whereas spots induced before this time, that is, larger ones, have a random position within the eye, spots induced during this time occupy certain distinct sectors of the eye. It can be concluded, therefore, that the cells before this stage are not predestined to develop into distinct parts of the eye.

A third characteristic of the end of the first larval instar is that twin spots, having a total size of one of the above-mentioned sectors, are constituted of partners, the smaller of which is situated in the posterior middle of the eye. This situation is not found in the large, earlier-induced spots. The regular size differences in twin-spot partners show that a pattern of division intensity is built up.

It seems plausible that all three changes in condition are due to a uniform determination process, and that the designation of a number of cells of the head anlage as an eye anlage is connected with a change of orientation of the cell divisions and the creation of a pattern of division intensity. The total number of cells of the presumptive eye area is 18 to 20 at the time of determination, as calculated from the size of mosaic spots induced at the end of the first larval instar.

These findings were amplified by the analysis of a new mutant strain, in which the eyes of 35% of flies are normal at 18° C. In the other 65%, parts of the lower half of the eye do not form any ommatidia, but normal head cuticle instead. These parts are of the same shape as the X-ray-induced mosaic spots. They can be as large as a spot induced at the end of the first larval instar, or larger; the largest defect is the absence of the lower half of the eye. In spite of the different sizes of the defects, the peculiarities of the frequency distribution of the defective areas are best interpreted by supposing that the mutational effect is due to an interference with a process which gives certain cells of the head anlage the capacity for ommatidia formation. According to the size of the smallest defects, this process takes place at the stage when the presumptive eye anlage consists of about 18-20 cells. Obviously, this process is concurrent with the above-inferred determination of eye formation. These processes can be denoted as the creation of an ommatidia field.

In X-ray experiments with +/- sn heterozygotes, spots with singed bristles occurred close to white ommatidia on the margin of the eye. The stem-cells of these spots were found in considerable numbers up to the end of the second larval instar. This shows that within the field determination the outline of the imaginal eye is not definitely fixed.

After treatment of larvae of the third instar, irregularities of the facet pattern of the eye were observed. The position of the irregularities is dependent on the time of irradiation. The sensitive zone migrates over the anlage during the third instar in a posterior-anterior direction. Possibly this phenomenon is connected with the definitive establishment of the outline of the eye, which takes place during the third larval instar according to investigations made by several authors with the mutant Bar.

Belitz, H. J. The distribution of mutations induced by 2:5-bis-ethylene-imino-benzochinone-1:4 on the genetical map of the X chromosome of D. melanogaster.

In experiments with the compound 2:5-bis-ethylene-imino-benzochinone-1:4 (Chinon I, Bayer G 4073), which yielded a mutation rate of 5.5% lethals (H. Lüers, 1956), all lethals and visibles were localized by means of a strain marked by *sc ec ct v g f*. Fourteen lethals (10.4%) were con-

nected with gross structural changes of the sex chromosome. The distribution of the other 120 mutations on the genetical map of the X chromosome was compared with the distribution of 90 point mutations, 34 of which were obtained from different controls, the rest from negative mutation experiments. All mutations had arisen in the stock "Berlin wild" and were detected by the M-5 technique. As shown in the table, the relatively low mutation rate in the region *sc-ec* in the experiment with the chinone derivative is significant.

Region of chrom.		<i>sc-ec</i>	<i>ec-ct</i>	<i>ct-v</i>	<i>v-g</i>	<i>g-f</i>	<i>f-sp-f</i>	Total
Spontaneous	n	30	14	9	14	14	9	90
	%	33.3	15.5	10.0	15.5	15.5	10.0	
Chinon I	n	17	20	21	12	25	25	120
	%	14.2	16.7	17.5	10.0	20.8	20.8	
	χ^2	10.82	0.04	2.36	1.35	0.94	4.50	20.01

Bell, A. E. On the significance of fourth-chromosome polygenes in quantitative genetic studies with D. melanogaster.

The assumption of random distribution of polygenes among the various chromosomes is usually made in quantitative genetic studies. If one accepts this assumption, the need for genetic markers for the

fourth chromosome of D. melanogaster must be considered in the utilization of marked inversion stocks. While transferring the recessive fourth-chromosome gene *pol*, into a large-body-size stock as well as a small-body-size stock, some evidence was found which suggests that fourth-chromosome genes did not contribute to the genetic variation observed among these lines.

These large-and small-body-size lines were initiated from a common wild population and had been selected over a period of twenty generations for large and small body weight, respectively. For a related experiment, it was decided to insert the recessive marker, *pol*, into each of these lines. Males from a stock homozygous *pol/pol* and possessing dominant markers on the three major pairs of chromosomes were mated to virgin females of each body-size line. The F_1 male progeny were then backcrossed to virgin females from the parental size line. Among the segregating progeny those classified as wild type possessed chromosomes 1, 2, and 3 exclusively from the original body-size line and undisturbed by recombination. As for the fourth chromosome, these wild-type segregants would consist of $1/2 \text{ pol}/+$: $1/2 +/+$, where the + chromosomes would be in toto from the original body-size line. From matings among these wild-type segregants, selection for body size was reinstituted in the same direction as previously practiced. Over a period of three generations the following two phenotypic classes were classified and weighed within each body-size line: (1) polished (*pol/pol*), containing no fourth-chromosome genes from the original body-size line except for the possibility of limited

recombination in females; and (2) wild type, consisting of $pol/+$ and $+/+$ and possessing either one or two fourth chromosomes from the original body-size line. Thus, within each body-size line, a comparison is provided between the pol chromosome (unselected for body size) and the $+$ fourth chromosome (selected for body size for 20 generations) when expressed against a common genetic background on chromosomes 1, 2, and 3. The results were consistent for all three generations and are summarized in the accompanying table.

Body weights in selected lines of D. melanogaster where classes differed only in their fourth chromosome

Body-size line	Average body weights (micrograms)			
	Males		Females	
	polished	+	polished	+
Large	(650)* 1,038.0	(333) 1,023.3	(633) 1,426.9	(328) 1,397.1
Small	(804) 618.3	(363) 612.0	(855) 844.6	(370) 848.6

* Number weighed per class.

Comparing polished versus $+$ within sex and within line, the obvious conclusion is that fourth chromosomes exposed to twenty generations of selection for large or small body size did not differ from the unselected polished chromosome in their influence on body weight. Since comparisons were made with both large and small lines, one cannot attribute the results to the possibility that the polished chromosome possesses unusually large or small body-size genes.

If further studies show the above-described situation to be general for other populations and other quantitative traits, efforts to genetically mark the fourth chromosome in D. melanogaster hardly seem justified in quantitative genetic studies, unless one wished to check the validity of assuming random distribution of polygenes.

Braver, G. Crossing over in the distal euchromatin of homozygous $In(1)rst^3$.

$In(1)rst^3$ (Gruneberg), with one break near rst (1.7) and the other in the proximal heterochromatin of the X chromosome, has a small distal noninverted euchromatic region. Using $In(1)rst^3$, w, produced by Novitski by irradiating $In(1)rst^3$, and kindly supplied by Lefevre, tests were made of exchange in the y-w region of rst^3/rst^3 females (25° C). This region includes most of the distal euchromatin. Three crosses, made during the past year, gave crossover percentages of 5.5 (N=4624), 6.6 (N=2165) and 4.4 (N=6283). In the first two tests, crossovers in the w-car region were detectable in the females. Values obtained were 14.6 (N=2425) and 10.8 (N=1093). An additional cross, in which the w-car and car-f regions were tested, gave values of 10.8 and 10.1 respectively (N=5296). Salivary-gland studies of the homozygous rst^3 females showed no chromosomal aberrations of X chromosomes or autosomes (other than the homozygous inversion), and crossover values for the y-car and car-f regions were comparable to those reported by Gruneberg (1935) and Mather (1938) for this chromosome. Crosses are now in progress in order to insert br (0.6) in one case, and pn (0.3) in another, on the rst^3 chromosome. These markers will permit a more detailed study of the increased exchange in this distal euchromatic segment.

Brousseau, G. E., Jr. The recovery of detachments from a reversed acrocentric compound X chromosome.

In an experiment set up for other purposes, an unusually high frequency of detachments was recovered from Muller's double X, a reversed acrocentric.

Because of the reputed high stability of this compound and the unusual circumstances surrounding the recovery of the detachments, these data are particularly interesting. Virgin y f: females were mass-mated to X^{c2}/Y males that had previously received 3300 r of X-rays. Among the progeny, 5528 attached-X chromosomes and chromosome products were recovered. Among these chromosome products were five y f males and one + female. The y f males must represent recovered detachments, but the female could just as well have been a superfemale or a triploid. Three of the five males were tested for fertility and found to be sterile. No actual control crosses were carried out, but no detachments were found among an estimated 2700 y f: females from the stock cultures that provided the material for the X-ray experiment. The frequency of recovery in this experiment is about one detachment per 1000 compound X chromosomes. This must represent a minimum estimate of the detachment frequency, because one of the possible detachment products carries a lethal deficiency. The fact that most, if not all, of the detachments were recovered as males and the fact that these males were sterile suggest involvement of the paternal Y chromosome. Reversed acrocentrics give rise to second-anaphase bridges, which presumably break, yielding open ends that would most likely result in dominant lethality. The introduction of X-ray-fragmented chromosomes from the male might provide chromosome ends to cap these open ends and give rise to viable detachments. This problem is under further investigation with a different reversed acrocentric that is free of the complications in Muller's double X.

Brown, William P. A study of the mutant daughterless in a large laboratory population.

Since the discovery and description of the mutant daughterless, da (Genetics 39: 958-959), several interesting aspects of the mutant have been considered. One of

these is the selective advantage which da may or may not exhibit in a large population. The experiment presented here was an attempt to determine the activity of the mutant in a large random-mating population. A population cage was used to perform the study. The cage was kept in a room that had a temperature range of $76^{\circ} F \pm 4^{\circ}$. Fresh food was added twice weekly to the cage. The population was initiated by crossing wild-type virgin females obtained from a laboratory stock and homozygous da males. From the resulting progeny, 50 flies of each sex were etherized and introduced into the cage. By this procedure the initial gene frequency of da was .50. The presence of daughterless can be determined only by the progeny-testing of females. Those females which are homozygous for da, although wild type in appearance, produce normal sons but no daughters, regardless of the genotype of their mate. Eggs were collected from the population cage at weekly intervals, and a sample of 120 nonvirgin females hatched from these eggs were placed individually in food containers to produce progeny. The progeny in the successful cultures were observed and classified as to the presence or absence of female offspring, and the observation was recorded. In only one culture bottle, during the entire period, was the number of progeny so small that no definite classification could be made of the genotype of the female parent. The frequency of da for females in the population was calculated directly by application of the Hardy-Weinberg Law. The weekly gene-frequency values for the mutant are presented below. This 14-week period is equivalent to approximately 10 generations under conditions at this laboratory.

Weeks in cage	Successful cultures	No. da/da females	Per cent da/da	qda for females
0	-	-	-	.50
1	103	20	19.4	.44
2	111	11	9.9	.32
3	113	6	5.3	.23
4	105	1	1.0	.10
5	111	9	8.1	.28
6	111	6	5.4	.23
7	110	9	8.2	.29
8	111	9	8.1	.28
9	106	11	10.4	.32
10	115	5	4.3	.21
11	110	5	4.5	.21
12	112	7	6.2	.25
13	115	6	5.2	.23
14	113	8	7.1	.27

Tentatively, the mutant appears to exhibit some degree of selective advantage in a large random-mating population. It will be necessary to gather additional evidence over subsequent generations before reaching a conclusion as to the equilibrium frequency of this mutant.

Burdette, Walter J. Isolation of ecdysone from *Drosophila*.

Hormonal control of metamorphosis is easily demonstrated by ligation during the larval stage or by genic alteration

of the ring gland. Crude hormone capable of inducing pupation was isolated from the Oregon-R stock raised in large quantity at 25° C on yeast-enriched medium. Collections of pupae, along with a small number of larvae in the late third instar, were made at the onset of pupation, after larvae had migrated to the walls of the containers in which they were cultured. This material was preserved in 100% methanol, and the extraction described by Butenandt and Karlson was employed for separation of the active hormone. Parallel extractions of silkworm chrysalises were also carried out. The yield from *Drosophila* (.018%) was greater per gram dry weight than that from dried silkworm chrysalises (.002%). However, the activity of hormone (in *Calliphora* units) from fresh material was less for *Drosophila* than for *Bombyx*. The total amount of crude hormone obtained from *Drosophila* was 111.2 mg from 6015.4 cc volume of fresh material, representing the total collection in the laboratory for one year.

Yield of hormone

Extraction	Drosophila			Bombyx		
	Wt. of crude hormone (g)	Dry wt. of pupae (g)	% yield	Wt. of crude hormone (g)	Wt. of dried chrysalises	% yield
1	.0246	87.3	.028	.0670	2520.0	.003
2	.0211	125.8	.017	.0287	1719.2	.002
3	.0175	134.6	.013	.0142	1112.2	.001
4	.0045	112.2	.004	--	--	--
5	.0436	171.1	.025	--	--	--
Total	.1113	631.0	.018	.1099	5351.4	.002

Burdette, Walter J. Lethal mutation rate after injection of water-soluble carcinogenic hydrocarbons.

Extensive testing with methylcholanthrene failed to give any indication that it increased the mutation rate of lethals on the X chromosome in D. melanogaster, even though tumor incidence was increased con-

comitantly in certain experiments. In a smaller number of tests, 1,2,5,6-dibenzanthracene also was not found to be mutagenic. Additional studies were carried out to determine whether these hydrocarbons yielded negative results because they are not soluble in aqueous solution. Mutation rate in the st sr e^s ro ca; tu^{36a} strain was tested by the Muller-5 method after males were injected at three days of age with 0.1% aqueous solution of sodium 1,2,5,6-dibenzanthracene-9,10-endo- α,β -succinate. This experiment was then repeated, using sodium 3-methylcholanthrene-6,12b-endo- α,β -succinate. Lethal mutation rates on the X chromosome were 0.21% with the former and 0% with the latter. Since the control rate of mutation was 0.08% in this strain, no evidence was obtained that these water-soluble compounds are mutagenic in Drosophila.

Carcinogen	No. of lethals	Chromosomes tested	Percentage lethals	P
Sodium 1,2,5,6-di- benzanthracene- 9,10-endo- α,β - succinate	2	965	0.21	0.34
Sodium 3-methyl- cholanthrene-6,12b- endo- α,β -succinate	0	787	0.00	0.40
Control	1	1188	0.08	

Burdick, A. B., and Mukai, T. Viability of heterozygotes for 1(2)55i.

Lethal 1(2)55i occurs in the W-1(Erie) wild stock with $q = 0.17\pm$, and appears to have maintained this level for over a year. It is located at about 51 on

the second chromosome and is not associated with any crossing-over inhibition. When extracted with isogenic first, third, and fourth chromosomes and made heterozygous with a single (homozygous viable) second chromosome from W-1, 1(2)55i remains in the population with $q = .25$ after 16 random-mating generations. Our anticipated q for a recessive lethal in generation n being $1/(n + 1)$, leads us to expect a frequency of .06 after 16 generations, the difference (.25 - .06) apparently being due to the extraordinary viability of the lethal heterozygote. In addition, when 1(2)55i, with its associated W-1 genome is made heterozygous with a single, unrelated genome from W-11, it may be found in frequency .29 after five generations when it is expected to be about .17. We determine the lethal frequency in each generation of these two populations and expect to continue to do so until a steady frequency is reached, at which time we expect to re-extract the populations.

Carlson, Elof A. Relocalization of the mutant rotund in the third chromosome.

The mutant rn (rotund) was reported by Glass (DIS-2: 8, 1934) to be of probable X-ray origin and within 1.6 units of Bl (Bristle). Bridges, using hk (hook) and

Bl, thought it to lie between these loci (DIS-9: 85, 1938), but Muller found rn to be to the right of Bl, between Bl and tk (thick). More recently Muller (DIS-27: 106-107, 1953) reported that an irradiated stock of rn, selected for crossover suppression in the second chromosome, also contained "a large scale translocation with chromosome 3," which he designated as rn, T23. This rn, T23 was associated with a high degree of autosomal nondisjunction. According to Muller the original rn stocks were likely to have contained this translocation. In the same year Oksala (Proc. 9th Intern. Congr. Genet. II: 789) also noted the peculiar pairing relations of rn, which he localized between lt (light) and rl (rolled), concluding that rn coincides with the centromere, which he referred to as a "rotund-centromere."

In a stock of sc^{L6} , a scute, rotund male was observed. Since this rn^2 male (see New Mutants, this issue) was sterile, several of his sc^{L6} brothers were mated to $fes\ pr\ rn, T23/al^2\ Cy\ cn^2\ L^4\ sp^2$ females. From a line showing rn offspring, several males, some carrying the presumed $rn^2/al^2\ Cy\ cn^2\ L^4\ sp^2$ genotype, were mated to $S\ Sp\ rn, T23/dp^{txl}\ Cy\ pr\ Bl\ cn^2\ L^4\ sp^2$ females. The resulting $rn^2/dp^{txl}\ Cy\ pr\ Bl\ cn^2\ L^4\ sp^2$ offspring were used to establish a stock. However, several $Cy\ Bl\ L\ rn$ flies resulted from the cross, indicating independent assortment of the rn^2 from the second chromosome.

An attempt was then made to balance rn^2 with the third-chromosome balancer $Me, Ins\ ri\ Sb^1$, and a stock was successfully established. Crossover tests using R (Roughened) and Ly (Lyra) showed rn^2 to be located in the right arm of chromosome 3, in the neighborhood of Sb. From data obtained using W (Wrinkled) and Sb, rn^2 was found to lie about one-fifth of the distance between these markers, or about 47.7 ± 1.1 using the standard distance of 46.0 for W. This was based on a total of 51 crossovers in this region, 10 of which were between W and rn^2 .

It is thus evident that rn, T23 (or the original rotund) is a reciprocal translocation with breaks of both major autosomes very near their centromeres. Probably both breaks are slightly to the right of the centromere; if so, the left arm of II is joined to the right arm of III and vice versa.

(This work was supported by a predoctoral Public Health Service Research Fellowship of the National Institutes of Health for the year 1956-1957.)

Carson, H. L. A female-producing strain of D. borealis Patterson.

Fourteen wild virilis-group females caught at Selkirk, Manitoba, in July 1952 were separated from males at collection and

were allowed to exhaust the sperm which they had received in nature. Pair matings were then made with wild males captured at the same time, and strains were developed from these. All were proved to be D. borealis by spermatheca examination and by crossing F_1 individuals to a known strain of D. borealis (Univ. Texas Lab. 2077.4, Itasca State Park, Minnesota). The strain designated as Sel-12 produced an F_1 from the wild pair consisting of 34 females and no males. The small number of offspring counted is due to the fact that only one tube of larvae was saved from this cross. F_1 Sel-12 females were crossed to D. borealis males from three other strains--Sel-5, Sel-7, and the unrelated 2077.4. From each of these crosses only females were produced: these

numbered 99, 191, and 286, respectively. The female-producing strain was maintained thereafter for 8 generations by crossing 10-20 females en masse with 10-20 from Sel-5. As each generation emerged, at least 50 females were counted before the backcross to Sel-5 was made. Over these 8 generations and up to the time that the strain was accidentally lost in June 1953, 1327 females and no males were observed by actual count. The females were vigorous and produced very large quantities of offspring throughout the experiment. Brain smears of these females revealed no chromosome abnormalities; they showed the typical diploid montana-complex configuration of four pairs of rods and one pair of J's; salivary-gland-chromosome smears showed three inversions, two in chromosome 3 and one in chromosome 5. Individual females were found which were homozygous both for and against each of these inversions.

Certain genetic explanations of this female production may be eliminated. The source of the male has no effect on sex ratio, and as the strains from which males were taken were wholly normal in this respect, "sex ratio" of the male-influenced type, as in D. pseudoobscura, is thus precluded. The females will not reproduce unless mated, so that the possibility of thelytokous parthenogenesis cannot explain the data. By using the naturally occurring inversions as markers, it is possible to prove that, at least for chromosomes 5 and 3, the inheritance of the females in all-female sibships is biparental. This eliminates the possibility of diploid parthenogenesis set in motion by sperm entrance without syngamy (gynogenesis). All existing details of this case suggest similarity to the case described by Magni for D. bifasciata (Proc. 9th Intern. Congr. Genet., II: 1213, 1954), in which an extra-chromosomal cytoplasmic factor, transmitted only by females, is lethal to male zygotes.

Castiglioni, M. C. Phenogenetics of pseudotumor in D. melanogaster.

In some stocks under investigation the production of benign melanotic masses seems to be conditioned by a series of processes controlled by different and

independent genetical factors. The production of melanotic masses depends on: (1) The presence of a large number of a special kind of large migrant cells (hemocytes?), originating in the lymph gland, which have the tendency to clump and to produce melanin. (2) Disintegration of one portion of the lymph gland. Melanization occurs only when the big migrant cells are released in large quantity in the hemolymph, but their presence does not necessarily cause melanization. The number of these cells probably depends on their frequency in the lymph gland, although direct evidence is lacking. The total number of cells, and the frequency of big migrant cells in particular, has been determined for 11 stocks, in smears of body fluid (hemolymph) stained with May Grünwald-Giemsa. The histology of the lymph gland has been studied with microtome sections, as well as in toto.

Chung, Y. J., Paik, Y. K., Kim, D. U., and Kim, K. W. Further information regarding Drosophilid species in Korea.

Only a few species of Drosophila in Korea were reported by Kikkawa and Peng, and by Nakayma, in 1936 and 1940 respectively. Since then no one has reported Drosophila species in Korea.

We made a first report on Drosophila species found in Korea in DIS-29. As we had many other Drosophilid species in our field collections from May to October, 1956, at Kwangju, Mt. Chiri, and Quelpart Island, the following species can be added:

D. lutea, D. immigrans, D. histrio, D. bizonata, D. transversa-complex, D. nigromaculata, D. cheda, D. sternopleuralis, D. buskii, D. coracina, D. puncticeps, D. alboralis, D. histrioides, D. quadrivittata, D. (Hirtodrosophila) sp., Amiota alloguttata, Amiota (Phortica) sp., Scaptomyza disticha, Scaptomyza graminum, Leucophenga maculata, Leucophenga sp., Microdrosophila congester, Microdrosophila sp., Mycodrosophila sp-1, Mycodrosophila sp-2.

Edington, C. W. The production of dominant lethals by gamma rays, 1-Mev neutrons, and 14-Mev neutrons.

In comparisons of the relative biological effectiveness of radiations of different ion density it has been shown that the induced biological damage increases with increasing ion density. Since there is

also a great difference in the ion density produced in tissue by fast neutrons of different effective energies, a comparison was made of the effectiveness of fast neutrons of 1 Mev and 14 Mev and of Co^{60} gamma rays in the production of dominant lethals in *Drosophila*. Oregon-R males, 2-4 days old, were exposed to one of the three radiations at several doses and mated to virgin Oregon-R females for 24 hours, and the percentage hatch of the eggs was determined. The following results were observed:

Gamma rays			14-Mev neutrons			1-Mev neutrons		
Dose ($r \times 10^3$)	Eggs laid	% hatch	Dose ($\text{rep} \times 10^3$)	Eggs laid	% hatch	Dose (rep)	Eggs laid	% hatch
0	1294	98.3	0	2632	97.8	0	2933	96.9
1	1130	83.8	1	1780	64.7	463	2719	68.1
2	1017	70.4	2	2389	40.0	1083	3053	37.1
3	987	51.6	3	837	22.0	2154	3037	14.9
4	1093	37.1	4	1640	11.2	2922	3830	6.8
5	1120	27.8						
6	768	23.7						

It is obvious from these data that both types of neutrons are more effective than gamma rays in producing dominant lethals and that 1-Mev neutrons are more effective than 14-Mev neutrons. Furthermore, the shape of the hatchability curve for 1-Mev neutrons and 14-Mev neutrons is linear whereas the gamma-ray data depart significantly from linearity. These results indicate that the RBE of fast neutrons of different energies decreases with decreasing ion density and that the frequency-dose relation for neutrons of high energy is linear.

Fahmy, O. G., and Fahmy, Myrtle J. Differential response of specific gene loci to mutagens in *D. melanogaster*.

We have previously reported (DIS-29; Fahmy and Fahmy, 1956a) that selective mutagenicity of the alkylating compounds as compared to radiation is manifested in the differential yield of "visible"

mutations. Hundreds of these visibles have been induced on the X chromosome alone by the chemical agents; this does not seem to have been encountered in the radiation mutagenesis work. In order to determine the degree of response of these chemically mutated loci to radiation, we analyzed the mutability of 17 specific "new" visibles under the effect of X-rays. The selected loci were spread along the whole length of the X chromosome, and were most easily recognizable, and each had occurred more than once (mostly 3-5 times) in the chemical mutagenesis work. All mutants were fully viable and fertile in the homo-

zygous condition. It was further possible to combine these mutants in sets of 2-4 loci per stock without impairing either the fertility or the viability. For the sake of comparison we also tested the mutability of 7 X-chromosome loci which are known to be affected (to various degrees) by radiation: scute (sc), cut (ct), vermilion (v), wavy (wy), garnet (g), forked (f), and carnation (car).

Females homozygous for the "new" visibles were mated to irradiated males carrying the normal allelomorphs, and the F_1 daughters were scored for the genes tested. About 30,000 to 80,000 daughters receiving the marked and tested X chromosomes were scored per locus, and the X-ray dose ranged from 2,500 to 4,250 r. The F_1 daughters showing the character tested for were further analyzed to determine whether the treated chromosome carried (1) a visible allelomorph to the marker, (2) a visible together with a lethal somewhere else on the chromosome, or (3) a deficiency or a deletion (in itself a lethal) covering the locus of the marker.

Of the 17 chemically mutated visibles only one was affected intragenically by radiation (at a rate of 3.4×10^{-8} per r) and also eliminated within deletions (at a rate of 1.05×10^{-8} per r). Eight other visibles were eliminated within deletions--6 in the euchromatin at a rate of $1-2 \times 10^{-8}$ per locus per r, and 2 near the heterochromatin, at a much higher rate, $6-8 \times 10^{-8}$ per locus per r--but none of these 8 were mutated intragenically. The remaining 3 visibles were stable to radiation in the size samples utilized. That this size sample is reasonably adequate is shown by the fact that it was sufficient for the induction of intragenic mutations, as well as deletions for the tested known loci, and at roughly the same rate as ascertained by other radiation geneticists.

Details of this work will be soon published elsewhere, but the above brief note is sufficient to indicate that the results on mutability at specific loci add further support to our claim that the mutation process is not random but selective, and is dependent on the nature of the mutagen.

Fahmy, O. G., and Fahmy, Myrtle J. The mutagenic action of alkyl sulphonates.

In a recent paper (Fahmy and Fahmy, 1956b) it has been shown that a particular sulphonate (2-chloroethyl methane-sulphonate: $\text{ClCH}_2\text{CH}_2\text{OSO}_2\text{CH}_3$) exerts a unique mutagenic effect on the male germ line of *Drosophila*. This compound proved to be practically ineffective on mature sperm and late spermatids, but extremely active on the early germ mother cells, particularly the spermatogonia. Furthermore, in these latter cells, the sulphonate induced practically as many sex-linked recessive visibles as lethals.

An attempt was made to determine whether these mutagenic properties are characteristic of this compound only or are shared by related sulphonates. For this purpose we tested the mutagenic action of the unsubstituted sulphonate (ethyl methanesulphonate: $\text{CH}_3\text{CH}_2\text{OSO}_2\text{CH}_3$). This compound was administered by injection into adult males of the same age and average size as those used with the chloro derivative, and the progenies of these males were also fractionated in exactly the same manner, that is, in 3-day broods. The yield of sex-linked recessive lethals and visibles in the separate broods was determined by the Muller-5 technique. The concentration injected was 1.6×10^{-2} M and the volume received per male was 0.25 μl of solution, making the absolute dose 4.0×10^{-9} mole per male. The X-linked recessive mutations

induced by the above concentration in the successive broods are tabulated below.

Brood	Chromosomes tested	Visibles		Lethals	
		No.	%	No.	%
I	458	23	5.0	84	18.3
II	350	13	3.7	51	14.6
III	301	17	5.6	47	15.6
IV	383	9	2.3	46	12.0
V	316	-	-	1	0.3
VI	345	1	0.3	2	0.6
VII	328	-	-	3	0.9
Total	2481	63	2.5	234	9.4

Ratio visibles/lethals 0.27

It is clear that the mutagenic action of the ethyl methanesulphonate is completely different from that of the chloro derivative. The unsubstituted compound is most active on the mature sperm and spermatids and practically ineffective on the early germ cells. Furthermore the efficiency of ethyl methanesulphonate in mutating morphogenesis loci (visibles) is much lower than that of chloroethyl methanesulphonate, the over-all ratio of visibles to lethals for the two compounds being 0.27 and 0.47 respectively.

The drastic alteration, almost complete reversion, of the mode of mutagenic action of ethyl methanesulphonate by the substitution of a Cl atom for an H atom of the ethyl group is yet another example of the intricate biochemical nature of mutagenesis and its dependence on the agent.

Farnsworth, M. W. Localization of alkaline phosphatase in lethal embryos and larvae of Minute(4) and Minute(1)o.

The role of phosphatases in the transfer of various substances across cell membranes has been reported by many authors. In particular, the presence of these enzymes in vertebrate intestinal and

kidney epithelium, as well as in other tissues, is well known. Moog (Biol. Bull. 86: 51-80, 1944) and Yao (Quart. J. Micr. Sci. 91: 79-108, 1950) have associated alkaline phosphatase with the onset of histo-differentiation in chick and *Drosophila* embryos, respectively. Yao reported that this enzyme suddenly appears shortly after germ-band contraction (9 hours), and considered its point of origin to be the "differentiation center" of the *Drosophila* embryo. Studies of embryos homozygous for eight different Minutes have shown that the anomalies of all stocks originate around 10 to 12 hours of development, a period when alkaline phosphatase activity is presumably becoming widespread. Furthermore, all homozygous Minutes so far investigated are characterized by abnormalities of the midgut and slowness of yolk withdrawal (Farnsworth, Genetics, in press). In view of these findings, both an egg and a larval lethal were tested for the presence and localization of alkaline phosphatase.

Ten to sixteen-hour embryos of M(4) and M(1)o were used, as well as hatched homozygous first-instar M(1)o larvae and their control sibs. Initially, two fixatives were employed: chilled 80% ethanol and chilled 100% acetone. After sectioning at 6 to 10 μ , material fixed by both procedures was prepared by the method of Gomori (Microscopic Histochemistry, U. of Chicago Press, 1952) and by the method given by Yao (loc. cit.) and outlined

by Danielli (J. Exp. Biol. 22: 110-117, 1946). It was found that fixation in 80% alcohol followed by the technique of Danielli gave the best and most consistent results, and this procedure was then followed throughout the rest of the study. Sodium- β -glycerophosphate was used as substrate, and incubation at 37° C was varied from 2 to 20 hours, although 4 hours was used in most instances. Control slides were incubated without substrate. Additional controls used as a check on the incubating mixture consisted of frog skin and kidney, tissues previously tested and known to be highly active.

In general, no differences between controls and lethals were found with respect to the localization of this enzyme. In embryos, alkaline phosphatase appeared at the proper time, and its presence in salivary glands, Malpighian tubules, hypodermis, and to a much lesser extent the gut, was noted. The quantity of the enzyme, as compared with that in frog skin or kidney incubated for equal periods of time, was extremely low. Indeed, frog tissue required only a 15-minute incubation period for an intense and specific reaction, whereas *Drosophila* material, at the end of 4 hours, was only faintly positive. Long periods of incubation (12 or more hours) gave darker staining, but also resulted in diffusion artifacts, as judged from the presence of nonspecific nuclear adsorption.

Only first-instar larvae were studied, since M(1) homozygotes do not grow beyond this stage. As reported by Yao and found in the present work, only a very weak reaction can be obtained in larvae of this age. Again, no specific differences between controls and lethals were observed.

It is concluded that the presence and localization of alkaline phosphatase is not a significant factor in the causes of lethality in Minute homozygotes.

(Supported by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Farnsworth, M. W. Somatic mitosis in M(2)¹² homozygotes.

As part of an investigation dealing with the quantitative determination of DNA, RNA, and total protein in various states of larval growth in wild-type, Minute heterozygotes, and Minute homozygotes, studies of somatic mitoses in aceto-orcein brain smears have been carried out. Homozygous M(2)¹² larvae were used as material. Such larvae are approximately 1 mm in length and do not increase appreciably in size although they may live for several days. In smears prepared from approximately 50 of these individuals, the giant neuroblasts seemed fewer in number than in the wild type, although no cell counts were made. In addition, mitotic figures were exceedingly rare--only 6 were found in the material examined. Apparently, in Minute homozygotes, not only is increase in cell size greatly restricted or completely eliminated but, in addition, growth by increase in cell number is greatly reduced in tissues which normally undergo cell division in larval life.

(Supported by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Frydenberg, Ove. D.
pallidipennis from Peru.

D. pallidipennis centralis from Mexico is known to be genetically isolated from D. p. pallidipennis from southern Brazil by

F₁ male sterility. As to gene arrangement, the two subspecies differ only in a single inversion in chromosome D. Strains of D. pallidipennis from Peru have been testcrossed to South Brazilian strains of D. p. pallidipennis. There appeared to be no hybrid sterility whatsoever between the strains. The Peruvian strains are therefore regarded as belonging to the subspecies D. p. pallidipennis. However, the Peruvian strains have the same gene arrangement in chromosome D as has the subspecies D. p. centralis. Consequently there seems to be no direct connection between the differentiation of the gene arrangement and the development of isolating mechanisms in this case. A new inversion in chromosome A was discovered in the Peruvian strains. The results are to be published shortly.

Frydenberg, Ove. Two new species from Peru.

Two new *Drosophila* species, D. neoguaramunu of the Guarani group and D. procardinoides of the Cardini group have been

described. The description will appear in the *Revista Brasileira de Entomologia*.

Fung, S. T. C., and Gowen, J. W.
A major locus for sex determination in the third chromosome.

Different diploid and polyploid combinations of the genes +, Hr, and tra have been made for the study of their sex types.

Besides the familiar normal males and females, the triploid type of Bridges, the two Hr types of our stock, and the two male types of Sturtevant, there are now three other distinguishable sex phenotypes. The diploid genotype Hr/tra had male-like but retracted genitalia and no visible claspers, sex combs of 8-9 teeth, internal genitalia largely male. The Hr/tra/tra resembled the Hr/tra male, sex combs 8-9 teeth, internal genitalia predominately male, well developed duct and accessory organs, testes elongated slightly but much smaller than normal males. The external genitalia of Hr/tra/+ flies resembled diploid Hr/+, had rudimentary claspers and sex combs of 5-6 teeth. The internal genitalia were developed, with mixture of male and female sex organs. Besides the intersex genotype, 2X + 3A, which was little affected by these genes, there were ten distinguishable sex types which were produced by the action or interaction of these genes in the diploid and triploid flies. Dosage interactions in diploids and triploids proved that + of the wild type was like the Hr and tra, a sex gene. These genes were in the third chromosome. The results show that fundamental sex characters, like other characters, sometimes may be due to substitution of major sex genes occupying particular loci.

Gersh, Eileen Sutton.
Salivary analysis of Y:bw⁺.

The piece of chromosome 2 inserted in Y extends over a little more than one numbered section of Bridges' map. Its minimum

extent is from 59E1.2 to 60E3, and a few more bands may be included at each end. It is visible in salivary-gland nuclei as a loop situated at the chromocenter, and constitutes a useful salivary marker by means of which an extra Y chromosome (specifically Y:bw⁺) can be detected.

Glassman, Edward. Allelism between the tumor-producing loci of the *ell tu* and *bw tu* strains.

Genetic analysis indicates that the tumors of the *ell tu* stock are dependent upon a single gene located at about 88 on the second chromosome. It has been reported (Hartung, 1950, *J. Heredity* 41: 269) that

the tumor gene in the *bw tu* stock is on the same chromosome at about 84. A cross between the two stocks produced 334/435 (76%) tumorous offspring, and since both stocks show very low penetrance when heterozygous, these genes are probably allelic. The differences in linkage data are most likely a result of inaccuracies due to the incomplete penetrance of these genes.

Glassman, Edward. The occurrence of urea in *D. melanogaster*.

According to various authors, the end products of nitrogen metabolism in insects are the relatively insoluble uric acid

and allantoin. Since large amounts of uric acid and some allantoin were detected on paper chromatograms of *D. melanogaster* extracts, it was unexpected to find that a compound accumulated by a sable (body color) strain exhibited R_f values similar to urea in 5 solvents. The fact that the compound forms a yellow spot with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde, applied as a 2% solution in 5% HCl), and is destroyed by urease, indicates further that it is urea. Many stocks show traces of this compound, but it is most evident in the sable strain. Genetic analysis is in progress. The source of the urea is not known, but since large amounts of what appears to be allantoin are also present in these flies, as well as in their excreta, a relation may exist between them.

Henke, H., Hölne, G., and Kunkel, H. A. Recessive sex-linked lethals in successive broods of *D. melanogaster* after N oxide mustard treatment.

It has been evidenced by a number of investigations that during spermiogenesis in *Drosophila* the germ cells pass through some periods of different sensitivity to the effects of chemical mutagens.

Furthermore, different kinds of mutagenic action has been found among these agents. As indicated by Auerbach's results with *Drosophila*, mustard gas produces the highest mutagenic effect in a period of sperm development intermediate between early spermatogonia and mature spermatozoa. In our investigations a new derivate of the nitrogen-mustards, bis-(β -chloroethyl)-methylanineoxidehydrochloride, was tested for frequency of induction of mutations in successive stages of sperm development in *D. melanogaster*.

An aqueous solution (1.0%) of the nitrogen oxide mustard was injected intraabdominally one day before copulation. The treated males were given fresh females every three days, and the rates of recessive sex-linked lethals were determined by the Muller-5 technique in five successive broods. As is demonstrated in the table, no mutagenic effect was obtained in very early periods of sperm development. The rates of mutation found in the following stages of spermiogenesis and in mature sperm show particular differences. Thus the sensitivity to the mutagenic effect of nitrogen oxide mustard increases to a maximum in the third brood. The mutation rate here is twice as high as in the second or the fourth brood, and exceeds the frequency of mutations in mature sperm. A similar type of mutagenic action has been established by Auerbach's investigations with mustard gas. In relation to the correspondent stages of spermiogenesis determined by Auerbach, the maximum of mutation sensitivity to nitrogen oxide mustard shown in the table

occurs during or soon after meiosis. This kind of action differs somewhat from the mutagenic effect of X-rays.

Brood	Copulation intervals after treatment (days)	No. of X chromosomes tested	Induced lethals	
			No.	%
I	2-4	2678	71	2.65
II	5-7	2149	35	1.63
III	8-10	2178	74	3.40
IV	11-13	971	16	1.65
V	14-16	1537	4	0.26

Herskowitz, Irwin H. Studies on the nature of recessive lethal mutations induced in oocytes by X-rays.

It was reported (DIS-29: 125, 1955) that a concentrated treatment of oocytes with about 3264 r produced significantly more sex-linked recessive lethals than did this dose delivered in a protracted manner. A similar result was obtained, although the difference was not significant, in a subsequent experiment using 4000 r, in which $8.1 \pm 1.6\%$ lethals (25/308) were obtained with the concentrated treatment and $5.9 \pm 0.8\%$ lethals (53/905) with the protracted. Even though, in both experiments, the F_1 tested for lethals came from eggs oviposited within 4 days after irradiation, the results might have been due to an intensity effect on oviposition rate and not on mutation rate. For there is an intensity effect on oviposition rate, proved at about 2000 r with dehydrated females (Anat. Rec. 125: 639, 1956); and since the rate of induced recessive lethal mutations is known to decline in successive eggs laid, the smaller number of eggs laid after intense treatments might contain a significantly greater frequency of such mutations than the larger number of eggs laid in the same interval of time after diluter treatments.

Since no appreciable effect of intensity on oviposition rate has been found when normal (hydrated) females are given an X-ray dose of 2500 r or less, the present experiments were performed using a dose of 2300 r.

The females employed were free of recessive sex-linked lethals arising in a previous generation, and were of two types: "rod/rod," homozygous for a wild-type X chromosome; and "rod/ring," having one identical wild-type chromosome and one ring X (Xc2 y.B). The "rod/rod" females were given the X-ray dose either intensely (I) in 94 seconds, or protractedly (D) over a period of 5 hours 25 minutes, or were given no dose (C); and the "rod/ring" females were given either the intense treatment (I) or no irradiation (C).

Among eggs laid the first 4 days after treatment, sex-linked recessive lethal mutation percentages for rod/rod females were: for C, 0.125% (1/799); for I, $4.73 \pm 0.60\%$ (60/1268); and for D, $2.89 \pm 0.41\%$ (47/1625). In the rod/ring females the identical rod-X gave mutation rates of C = 0.0% (0/377) and I = $2.73 \pm 0.65\%$ (17/623), and the ring-X 0.0% (0/347) and $2.98 \pm 0.69\%$ (18/605) for C and I, respectively. These values show a significantly higher mutation rate for the rod chromosome irradiated intensely (I) than for the same rod chromosome irradiated protractedly (D) when it had a rod X as its homolog ($P = .015$), or irradiated intensely (I) when it had a ring X for its homolog ($P = .05$).

The average number of eggs laid per fertile female in the first 4 days after irradiation was for rod/rod females 46, 41, 42, for I, D, and C respectively, and for rod/ring females 55 and 50 for I and C, respectively. Thus the oviposition rate of irradiated flies was not less than in the unirradiated controls, and it is unlikely that the approximately 20% greater number of eggs laid after intense treatment by rod/ring females than by rod/rod could account for the significantly higher mutation rate of the rod X in the latter type of female as compared with the former.

The intensity effect on lethals demonstrates that a considerable proportion of such mutations induced in oöcytes are multi-hit events. Since it is known that broken ends produced by X-rays in oöcyte chromosomes can join soon after their production, it is suggested that the intensity-dependent lethals are connected in their origin with multi-break exchanges. Such exchanges could include small deficiencies and duplications acting as recessive lethals, produced by "pseudo crossing over"--intra-tetrad exchange between nearby but nonhomologous loci. Supporting this view is the lower lethal rate for a rod when its homolog is a ring rather than a rod, for pseudo crossing over in the former case would much more often form a dicentric, which would not be included in the haploid egg.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Herskowitz, Irwin H. The effect of dehydration upon the frequency of X-ray-induced crossover-like exchanges in oöcytes and oögonia.

A higher egg mortality, among eggs laid over approximately the first 8 days after irradiation, had been found when dehydrated females rather than normally hydrated ones were X-rayed. The present experiment, to determine

whether X-ray-induced exchanges of crossover-like nature likewise were sensitive to prior dehydration of the mothers, was performed in part to test whether the dehydration-increased egg mortality could have been the result of an effect on the genetic material.

Virgins ($y.Dp\ sc^{V1} y^+/y^2 v f car$) were dehydrated (D) or not (W) and irradiated (I) or not (C) with 2940 r delivered in 2 minutes 5 seconds (furnishing four series, DI, DC, WI, WC) and then mated en masse to equal numbers of $y^2 v f car/Y^+$ males in nylon-mesh-covered egg-laying cylinders. The cylinders were placed on nutrient-containing Petri dishes and the dishes replaced twice daily for 20 days. Egg counts were made for all 40 of these 1/2-day periods, and for a number of these periods adults were obtained from the eggs and scored for phenotype.

Some of the data are summarized in the table. For eggs oviposited within 4 1/2 days after treatment (periods 1-9), which were at the time of irradiation late oöcytes presumably past the stage in which spontaneous crossing over is thought to take place, there were in the car-centromere region significantly more exchanges induced by DI than by WI treatments. This was true also for the egg-laying periods 14-16, but not for periods 39-40. Since dehydration and a dose of 2940 r both inhibit egg laying, the data for different treatments should be compared also on the basis of approximately equal successive groups of eggs laid. Since in the first 8-day period of egg laying the percentages of induced exchanges for both DI and WI were rising, examination of the data in this way made the difference already noted

in this period even greater, whereas there was still no difference in the 39-40 periods. Thus dehydration increases the frequency of X-ray-induced cross-over-like exchanges as it does egg mortality, the dehydration effect extending over a similar period of egg laying in both cases.

1/2-Day ovipo- sition periods		DI	DC	% DI- % DC (A)	WI	WC	% WI- % WC (B)	A-B
1-9	No. F ₁	400	1969		2531	2116		
	% car-	8.25	2.69	5.56	5.13	2.69	2.44	3.12±1.53
	centromere exchanges	±1.34	±0.36	±1.42	±0.43	±0.35	±0.56	(P<.05)
14-16	No. F ₁	1846	631		2532	668		
	% car-	12.1	3.3	8.8	7.0	1.6	5.4	3.4 ±1.25
	centromere exchanges	±0.76	±0.51	±1.04	±0.51	±0.49	±0.69	(P<.01)
39-40	No. F ₁	2974	835		4629	569		
	% car-	6.4	2.9	3.5	6.0	3.0	3.0	0.5
	centromere exchanges	±0.45	±0.58	±0.73	±0.35	±0.72	±0.80	±1.1

These results permit one to propose that the dehydration effect on the egg mortality produced by radiation has a genetic basis. Since X-ray-induced exchanges of the type scored here were earlier shown to be multi-hit events, and some of these at least are multi-break events, it is suggested further that dehydration affects rearrangement frequency. A possible way of doing this would be by shrinking the nucleus and bringing independently produced broken ends closer to each other, proximity of such ends favoring interchange. The results establish that the exchanges in eggs laid 7-8 days after irradiation, considered to be X-ray-induced crossovers, are also dehydration dependent, as well as, as previously shown, intensity dependent.

(This work was supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195.)

Herskowitz, I. H., and Myers, Terry. Mortality induced by an X-ray dose given to sperm intensely and to oocytes intensely and protractedly.

After it was found that egg mortality after X-radiation of oocytes was in large measure intensity dependent (Abrahamson and Herskowitz), and that some genetic events (such as half-translocations and pseudo-crossovers) responsible for this

are much more frequent after irradiation of oocytes than of sperm (Herskowitz and Schalet; Muller and Herskowitz), it became desirable to compare the effectiveness, in producing mortality in the egg, larval, and pupal stages, of a concentrated X-ray dose administered to sperm with that of the same dose applied to oocytes in both a concentrated and a protracted manner.

Accordingly, virgin wild-type males and females, free of sex-linked recessive lethal or sublethal mutations arising in a previous generation, were collected and stored for 2-4 days, after which they were both divided at random into four groups: "C" in which neither sex was irradiated, "I" in

which only males were irradiated with 2300 r delivered intensely, "I♂" in which only females were given 2300 r delivered intensely, and "D♀" in which only females were treated with 2300 r delivered in a protracted manner. The concentrated dose was delivered at 940 r per minute in 2 minutes 28 seconds; the protracted at 33 r per minute in 7 irradiations, each 10 minutes long, with 30-minute nontreatment intervals between successive irradiations. The X-ray machine was run at a peak of 200 kv and 20 ma, and 1 mm Al was used to filter the rays. The single intense irradiation was given simultaneously to males and females midway in the course of the protracted irradiation treatment. All flies were motile and well aerated during the irradiation.

Beginning about one-half hour after the irradiations were completed, the flies were etherized and placed either in single pairs in the individual compartments of the egg-laying chambers (described in detail by Abrahamson and Herskowitz), to determine egg mortality; or large and equal numbers of males and females were placed in uncompartimented egg-laying chambers to provide additional larvae for the larval and pupal mortality studies. The egg-laying chambers were placed on nutrient-containing Petri dishes, which were replaced twice daily. The males were left with females for the entire 4 days after irradiation that eggs were collected. The results are summarized in the accompanying table.

Oviposited on days	Eggs				Larvae		Pupae	
	1/2-2		3-4		3-4		3-4	
	No.	% mortality	No.	% mortality	No.	% mortality	No.	% mortality
C	1111	2.5	1845	1.8	800	15.2	638	27
I♂	2095	46.0	4171	35.4	800	15.0	643	25
I♀	1143	37.3	3557	31.1	800	23.6	575	31
D♀	1217	32.4	3489	14.9	600	15.7	591	24

The already-mentioned intensity effect on egg mortality when oöcytes are treated is found here also. Although the egg mortality for I♂ is in general higher than for I♀, it varies according to period, that at 1/2-2 days for I♀ being higher than that at 3-4 days for I♂. The rate in the I♂ is lower in the later (3-4 day) period than the earlier (1/2-2 day) one, probably because by that time the highly mutable sperm delivered in the first copulation were diluted by less mutable sperm of a subsequent copulation. While there does not appear to be any increase in mortality in the larval and pupal stages over the control rate after the I♂ or D♀ treatments, this does seem to be the case after the I♀ treatment. This suggests that a concentrated dose of 2300 r to oöcytes produces multi-hit events (probably genetic), which kill in later developmental stages and which are less frequent when this dose is delivered to oöcytes protractedly or to sperm intensely. It is thought probable on the basis of other work (Herskowitz) that these events are pseudo-crossovers.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Hexter, W. M. Pseudo-allelism at the *g* locus.

Tests for crossing over between g^{53d} and g^2 -Caltech in attached-X chromosomes appropriately marked on both sides of garnet resulted in 7 wild-type females in

78,000 females tested. Four of the 7 wild-type females yielded progeny, which indicated that each wild-type female was associated with a recombination of the markers, strongly suggesting that they resulted from a crossover between pseudoallelic loci. The other 3 wild-type females gave no offspring. Three of the four crossovers were reciprocal and one was nonreciprocal. The presumed double garnet ($g^{53d} g^2$) chromosome has been detached, and tests to verify conclusively that both garnets are present are now in progress.

Hinton, Claude W., and Schmidt, Jean A. A variegated $sc^8.Y$ chromosome.

A compound $w^{vc}+/sc^8.Y$ female which received 1000 r X-rays produced among her $y w spl sn^3/sc^8.Y$ sons one which was strongly yellow variegated. Tests of the

$sc^8.Y$ chromosome (called $sc^{8V}.Y$) carried by this male indicate the variegation is a typical V-type position effect. The variegation is partially suppressed in Y^{SXYL} , sc^8 , $dl-49$, $y v f car/sc^{8V}.Y$ males as compared with $dl-49$, $y v f car/sc^{8V}.Y$ males; and males of the latter genotype reared at 18° C exhibit more variegation than those reared at 26° C. The variegation is manifested in all males carrying $sc^{8V}.Y$, and from 10 to 15 per cent of the bristles on the dorsal thorax of such males are yellow.

Hinton, Taylor, and Dagg, Martha Kushida. The lethals associated with twenty-two related chromosomal arrangements.

Phenotypically, $In(2LR)40d$ is identified by an abnormal eye, and is lethal in the homozygous condition. Further irradiation of the inversion was carried out, and twenty-four reversions of the eye phenotype were isolated and cytological

analyses were made (Hinton, 1950). It was disclosed that the reversions were associated with further chromosomal lateration. Of these stocks, the following were used in the present study: IIA, IIP, IIV, IIBI, IICQ, IICR, IIDC, IIDH, and IIDJ. These ten stocks will be referred to as the reversion series. They were balanced with Cy.

One case was found, $In(2LR)IIDD$, in which the cytological picture remained identical with that of $In(2LR)40d$, although the eye phenotype had reverted completely to normal. The combination $IIDD/In(2LR)40d$ was lethal.

Samples of $In(2LR)40d$ and $In(2LR)IIDD$ were further irradiated, and offspring which genetically manifested translocations involving the second and third chromosomes were selected. These were balanced with Cy; D.

The descriptions of most of these genetically selected translocations have appeared in previous issues of DIS. The ones used in the present study are: $T(2:3)Hin 102$, $T(2:3)Hin 105$, $T(2:3)Hin 106$, $T(2:3)Hin 111$, $T(2:3)Hin 114$, $T(2:3)Hin 119$, $T(2:3)Hin 120$, and $T(2:3)Hin 121$. These eight stocks will be referred to as the translocation series.

This varied collection of chromosomal arrangements in the two series, all derived from the same arrangement, has been tested in all possible combinations two by two in order to determine whether the lethality associated with the original one is allelic to the others (Dagg, 1955). Since Cy is used to

balance the second chromosome in all cases, the presence of non-Cy in the offspring of a cross would indicate that allelic lethals did not exist between the test stocks. All crosses were repeated at least twice.

Six of the stocks in the translocation series (T(2:3)Hin 102, 106, 111, 114, 119, and 120) proved to have a lethal that was allelic to the lethal in the original inversions, In(2IR)40d and In(2IR)IIDD, and in all of the stocks in the reversion series, except IIDC. The lethal shared in common by these seventeen stocks will arbitrarily be referred to as lethal #1.

One stock in the translocation series (T(2:3)Hin 105) had a lethal that was allelic only to a lethal in In(2IR)IIDD and not to those in any of the other stocks. This lethal has been designated #2. In(2IR)IIDD had previously been shown to contain lethal #1.

A lethal (#3) was found in T(2:3)Hin 121 not allelic to any of the other lethals in this study.

A lethal (#4) was found in one stock in the reversion series (IIDC) which was allelic only to T(2:3)Hin 119, which was also shown to contain lethal #1. The IIDC lethal had previously been found to be allelic with another stock in the reversion series, IICO (Hinton, 1950). The IICO stock was lost before the present study was begun and thus Hin 119/IICO combination could not be tested. Hinton (1950) also reported that IICO and Plum had a lethal in common (#5) but IIDC and Plum did not. Likewise Hin 119 and Plum do not have a lethal in common.

It is possible to conclude that the same region of the chromosomes is responsible for the lethality in all of the cases (rather than having to postulate five different lethals in the series) if the lethalities are the result of interrupting the sequential action of a region of the chromosome concerned with some vital function. Two lethals will act as alleles only if between them they fail to supply all the steps in the sequence.

Horikawa, M. Tryptophan metabolism in the eye discs of D. melanogaster in tissue culture.

The eye-antennal discs and cephalic complexes from mature third-instar larvae (95 hours after hatching at 25° C) of D. melanogaster were cultured in vitro in a synthetic medium (see Technical Notes, this issue), to investigate tryptophan metabolism in the eye discs. In comparison with culture of the eye-antennal discs alone, the culture of the eye-antennal discs together with the cephalic complexes showed more pronounced growth, differentiation, and pigmentation of the eye discs.

In the synthetic medium containing 5 mg/ml L-tryptophan, brown pigment was deposited in the eye discs of Oregon and bw after culturing for about 72 hours. In the medium containing 4 mg/ml DL-kynurenine, the pigment was deposited in the eye discs of Oregon, bw, and v after culturing for about 30 hours. The eye discs of v bw, however, deposited pigment after 55 hours. In the medium containing 2 mg/ml DL-3-hydroxykynurenine, the eye discs of Oregon, v, cn, and bw deposited pigment after culturing for 5 hours, whereas the eye discs of v bw and cn bw deposited pigment after about 15 hours. Amounts of pigments deposited in the eye discs of v bw and cn bw were less than those of Oregon, v, cn, and bw.

The fact that in the medium containing 2 mg/ml DL-3-hydroxykynurenine the eye discs of Oregon deposited brown pigment after culturing for 5 hours seems to show that all enzymes relating to the tryptophan metabolic system were present in the eye discs of the mature third-instar larvae of Oregon.

The smaller amount of pigment in the eye discs of the double recessive mutants, *v bw* and *cn bw*, seems to indicate that there may be some interaction between tryptophan metabolism and pteridine metabolism.

Hunter, Preston E. Observations on length of larval and pupal periods in *D. melanogaster*.

It is generally noted in *Drosophila* laboratories that the flies first emerging from a culture bottle are predominantly females, regardless of the final sex ratio.

In a study conducted at the *Drosophila* laboratory of the University of Kansas, in which separate lines of *melanogaster* were selected for long and short larval periods, respectively, particular attention was paid to a correct record of larval and pupal periods. The larvae were kept individually in small medium vials and checked for pupation and emergence at regular intervals throughout a 24-hour period. In both selected and control lines the female flies always had a shorter pupal period than male flies. Average differences in length of pupal period between females and males for a representative sample of 18 generations were: short larval period line, 5.8 ± 0.59 hours; control line, 5.4 ± 0.64 hours; long larval period line, 5.0 ± 0.72 hours. No difference in length of the larval period was found between male and female flies. In contrast to adult emergence, which occurs chiefly in the early morning hours, it was noted that in all lines studied pupation occurred uniformly throughout any 24-hour period.

Jacobs, M. E. Studies on melanism in *D. melanogaster*.

From a grocery garbage can at Beaufort, North Carolina were selected a light strain (wild type), a dark strain with

dark trident and scutellum and slight darkening of the sclerites in general (but less dark than the mutant black), and an ebony strain with light puparia. The dark and ebony genes are semidominant alleles of the laboratory mutant, ebony. The mean larval period is: light (shortest), dark (intermediate), and ebony (longest). Colorimetric determination of tyrosinase activity of mature larvae showed: light (least), dark (intermediate), and ebony (greatest). Amino acid determinations of mature larvae by means of two-dimensional paper chromatography showed ebony to have more tyrosine and less of an unknown than light larvae. Ebony larvae that were fed methionine showed more of the unknown and less tyrosine than ordinary ebony larvae. Colorimetric determinations of tyrosine confirmed the chromatographic findings.

Kato, Mikio, and Kato, Masaru. Lipids in some mutants of *D. melanogaster*.

The following stocks may be divided into three groups according to color of lipochromes: group A (+); B (*v*, *cn*, *se*, *bw*); and C (*w*, *cn-bw*, *v-bw*). In the B group

this color is pale yellow or yellowish orange, particularly dark in *cn*; in the A and C groups it is a watery whitish. The appearance of saponified lipochrome in "wild" is watery, and in *w* is whitish cream in color, but in both *cn-bw* and *v-bw* it is a faint tint of pale yellow.

On the other hand, lipochromes in these groups present marked differ-

ences from each other in refractive index, iodine value, saponification value, neutralization value, Reichert-Meissl value, Polenske value, intensity of absorption extinction (Du-Bekman spectrophotometer). Paper chromatographic examination reveals differences in fatty acids, especially in unsaturated fatty acids; lecithin and lysolecithin are found in the B group, cepharin and choline in the C group.

Khishin, Aziz F. Drosophila in Egypt.

The *Drosophila* fauna of Egypt was never investigated until April, 1956, when the writer started to collect species in an attempt to survey distribution and to study various problems related to suspected adaptation to environment, particularly temperature. The occurrence of a number of oases sufficiently far apart, and the possibility of their being inhabited by some *Drosophila* species, necessarily inbred and isolated, should present interesting material for population and other studies.

At the present time, the work has only begun, and collections--for the sake of convenience only--have been restricted to Cairo, and a suburb called Matareya. Collections were made during April, May, and June, a dry season with temperature seldom falling below 30° C during the daytime. Traps used were one-pint milk bottles into which over-ripe bananas were mashed. These were usually set up shortly before sunset and left for about two hours before being removed. The bottles were either tied to branches or put on the ground under trees.

So far, four species of *Drosophila* have been found: *D. melanogaster*, *D. simulans*, *D. busckii*, and species of the *repleta* group. By far the most predominant species is *D. melanogaster*, closely followed by *repleta* and *simulans*. *D. busckii* seems to be very rare, as only one female was caught over a period of three months. It may also be of interest to mention that *D. simulans*, *repleta*, and *busckii* were never found indoors, whereas *D. melanogaster* was found both in and out of doors.

Khishin, Aziz F. Pupation habits of Drosophila.

Stocks of *D. melanogaster*, *simulans*, and *repleta* caught in Cairo and suburbs were raised on laboratory food containing baker's yeast, molasses, flour, and agar. It was noticed that in all cultures, in vials or bottles, larvae about to pupate did not crawl up the walls as usual, but instead pupated on the surface of the food. This was observed again in the second generation. However, beginning with the third generation some of the larvae started to crawl up and pupate on the walls in the usual way. Now, all the introduced *Drosophila* behave in exactly the same way as laboratory ones.

Kikkawa, H. Metal analyses of mutants of the w series in *D. melanogaster*.

As shown in a previous issue (DIS-29, p. 130, 1955), mutants belonging to the w series are divided into three or four main groups or types from the view point of metal absorption.

- (1) Ni group w
- (2) Cu group w^e, w^{e2}, w^t, w^{bf2}, w^{co}
- (3) Fe group w^a, w^h, w^{sat}, w^{col}
- (4) Cu + Fe group ... w^{ch}, w^{ch2} (discovered by the author)

In my opinion, the first three groups (1-3), at least, may be looked upon as different genes, and furthermore mutants within one group having the same metal pattern may be looked upon as multiple alleles.

King, R. C., and Rudden, M. J.
Studies on hybrids between
"tumorous" strains of D. melano-
gaster.

Hybrids between the pseudotumor strains tu^W (Wilson et al., Growth 19) and tu^{531} (King, DIS-29) were studied. In the case of tu^W , pseudotumors result from encapsulation of the caudal fat masses by lamellocytes (Rizki, Anat. Rec. 125). Tumors in the tu^{531} stock are associated with an X-chromosomal mutant; whereas the factors responsible for pseudotumor expression in the case of tu^W reside in chromosome II. The most important results of the study are presented below.

		<u>Genotype</u>		
	<u>tu incidence</u>	<u>I</u>	<u>II</u>	<u>III</u>
1	100%	tu^W	tu^W	tu^W
2	50%	tu^W	tu^W	Sb/D
3	15%	tu^{531}	tu^W	Sb/D
4	5%	tu^{531}	Cy/Pm	tu^W
5	5%	tu^{531}	tu^{531}	tu^{531}
6	5%	tu^{531}	Cy/Pm	tu^{531}

Comparison of rows 1 and 2 shows that chromosome III of the tu^W strain enhances tu incidence in that strain. However, tu^W III does not enhance tu incidence when substituted in the tu^{531} genome (see rows 4, 5, and 6). Furthermore, the X chromosome of tu^{531} , which is responsible for tu incidence in this stock, reduces tu incidence when substituted in the tu^W genome (compare rows 2 and 3). Many pseudotumor strains exist, and the tendency has been to assume that information obtained for one strain applies to all strains. This work shows that such generalizations are hazardous. Blackened cell aggregations will probably be shown to be a generalized response to various stimuli. Factors which enhance this response in one strain may have no effect or suppress the response in other strains.

Komai, Taku, Yamada, Yukio,
Hiraizumi, Yuichiro, and
Kitagawa, Osamu. Effect of
selection after X-ray
irradiation.

Selection was conducted for larger and smaller numbers of chaetae on the fourth and fifth abdominal plates of D. melano-
gaster, starting with a cross of Oregon-R and Samarkand stocks. The flies of each successive generation were irradiated with

1500 r X-rays. The H (high chaeta number) and L (low chaeta number) lines were classified in four lots according to whether (1) both sexes, (2) only females, (3) only males, or (4) neither sex was treated. The selection intensity was 30% up to the fourth generation, and 20% in the fifth and sixth generations. Variance analyses revealed significant differences in chaeta number between H and L. Also, the lots among H lines in which only females had been irradiated showed significantly higher chaeta numbers than the lots in which only males had been irradiated. This seems to indicate that the apparent effect of X-rays on the induction of new mutations controlling chaeta number is at least partly (or perhaps mostly) due to the release of already existing genes by the X-rays through enhancement of recombination. This work will be continued by Kitagawa in the Genetics Laboratory of Tokyo Metropolitan University.

Kuroda, Y., and Tamura, S.
Effects of Cu^{++} on the
melanotic growth of tumors
in D. melanogaster in
tissue culture.

Melanotic tumors in the hindgut of mature
third-instar larvae (95 hours after
hatching at 25°C) of v tu and st tu
strains of D. melanogaster were cultured
to investigate the effects of Cu^{++} on
melanotic growth in a synthetic medium

(see Technical Notes, this issue) involving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in concentrations of
1.0 mM, 2.5 mM, and 5.0 mM.

Conc. of Cu^{++} added	No. of cultures	Melanotic growth of tumors					
		Excellent		Progressive		Slight	
		No.	%	No.	%	No.	%
1.0 mM	50	6	12	25	50	19	38
2.5 mM	50	13	26	35	70	2	4
5.0 mM	50	22	44	20	40	8	16
0	50	8	16	37	74	5	10

As shown in the table, melanotic growth was inhibited markedly when 1.0 mM Cu^{++} was added to the synthetic medium. The addition of 2.5 mM Cu^{++} to the synthetic medium resulted in no significant difference from culturing without Cu^{++} . The effect of 5.0 mM in the synthetic medium was to accelerate the melanotic growth pronouncedly. In the basis of these findings it is assumed that a substance inhibiting the phenol oxidase system is present, and this inhibitory substance seems to be enhanced by 1.0 mM Cu^{++} .

Kuroda, Y., and Tamura, S.
Effects of DDC (diethyldithio-
carbamate) on the melanotic
growth of tumors in D. melano-
gaster in tissue culture.

DDC is known to inhibit melanin formation
by chelating some metals. Melanotic
tumors in the hindgut of mature third-
instar larvae were cultured in vitro, by
the procedures described in the preceding
note, to investigate the effect of DDC

upon the melanotic growth of tumors. The results are shown in the table.

Conc. of DDC added	No. of cultures	Melanotic growth of tumors					
		Excellent		Progressive		Slight	
		No.	%	No.	%	No.	%
1.0 mM	50	15	30	18	36	17	34
2.5 mM	50	5	10	24	48	21	42
5.0 mM	50	0	0	2	4	48	96
0	50	8	16	37	74	5	10

It was observed that the higher the concentration of DDC in the synthetic medium was, the more markedly the melanotic growth of tumors was inhibited. The addition of 5.0 mM DDC to the synthetic medium inhibited the melanotic growth of tumors almost completely.

Kuroda, Y., and Tamura, S.
Effects of Fe^{+++} on the melano-
tic growth of tumors in D.
melanogaster in tissue culture.

Melanotic tumors in the hindgut of mature
third-instar larvae of v tu and st tu
strains of D. melanogaster were cultured
in synthetic medium involving

$\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in concentrations of 1.0 mM, 2.5 mM, and 5.0 mM. The results are shown in the table.

Conc. of Fe^{+++} added	No. of cultures	Melanotic growth of tumors					
		Excellent		Progressive		Slight	
		No.	%	No.	%	No.	%
1.0 mM	50	15	30	24	48	11	22
2.5 mM	50	17	34	19	38	14	28
5.0 mM	50	22	44	19	38	9	18
0	50	8	16	37	74	5	10

When 1.0 mM Fe^{+++} was added to the synthetic medium, it was observed that progressive melanotic growth of tumors was decreased, and excellent and slight growth were increased. The addition of 2.5 mM Fe^{+++} to the synthetic medium had similar effects on melanotic growth. Addition of 5.0 mM Fe^{+++} to the synthetic medium accelerated melanotic growth pronouncedly. In relation to the effects of Cu^{++} upon the melanotic growth of tumors, described in the preceding note, these results seem to indicate that transition metals play an important role in the formation of melanotic tumors.

Kuroda, Y., Tamura, S., Abe, K., and Doi, K. Relation between hereditary tumors and transition metals in D. melanogaster.

Amounts of metals contained in pupae of D. melanogaster, the first day after pupation, were determined in two melanotic tumorous strains, v tu and st tu, and a nonmelanotic tumorous strain, tu-h,

for comparison with those contained in the nontumorous strains v, st, and Oregon. The results are shown in the table.

Expt. no.	v tu		v	
	Fe	Cu	Fe	Cu
1	294	211	270	199
2	369	225	273	154
3	320	198	308	129
Mean	328	211	284	161

Expt. no.	st tu		st	
	Fe	Cu	Fe	Cu
1	282	346	140	356
2	209	386	131	340
3	201	340	163	342
Mean	231	357	145	346

(table continued on following page)

Expt. no.	tu-h		Oregon	
	Fe	Cu	Fe	Cu
1	381	242	167	159
2	391	265	152	154
3	404	301	140	185
Mean	392	269	153	166

Greater amounts of iron and copper were detected in the tumorous strains, v tu, st tu, and tu-h, as compared with the nontumorous strains, v, st, and Oregon, respectively. As a result of these facts, it is assumed that the formation of hereditary tumors in D. melanogaster is closely related with the transition metals.

Kurokawa, H. Sexual isolation among the three races of D. auraria.

The three races (A, B, C) of D. auraria are distributed sympatrically in Japan. In the laboratory, these races can be intercrossed regardless of the localities

of capture, though it is not so easy as intraracial crossing. The F_1 hybrids of the interracial crosses are fertile in both sexes, and are intermediate between parental races in characteristics. So far, however, no natural hybrids have ever been found among many samples from localities where two or three races occur together. Judging by this fact, gene transfer among the three seems to be precluded mainly by sexual isolation.

In experiments, using the multiple-choice technique, between intraracial strains (A-A; B-B; C-C), none showed significant sexual isolation, irrespective of the strain used. In interracial experiments, on the other hand, significant isolation was detected; the results are given in tables 1, 2, and 3. Letters in parentheses indicate the races. Stalker's "Isolation Index" (I) was used for analysis of the data; significance was tested by chi square.

Table 1. A-B

Crosses	I
$\frac{\text{♀}}{\text{G(A), S(B)}} \times \frac{\text{♂}}{\text{G(A)}}$	0.56
$\frac{\text{♀}}{\text{G(A), S(B)}} \times \frac{\text{♂}}{\text{S(B)}}$	0.77
$\frac{\text{♀}}{\text{G(A), K(B)}} \times \frac{\text{♂}}{\text{G(A)}}$	0.79
$\frac{\text{♀}}{\text{G(A), K(B)}} \times \frac{\text{♂}}{\text{K(B)}}$	1.00

Table 2. A-C

Crosses	I
$\frac{\text{♀}}{\text{H(A), U(C)}} \times \frac{\text{♂}}{\text{H(A)}}$	0.49
$\frac{\text{♀}}{\text{H(A), U(C)}} \times \frac{\text{♂}}{\text{U(C)}}$	0.99
$\frac{\text{♀}}{\text{H(A), S(C)}} \times \frac{\text{♂}}{\text{H(A)}}$	0.57
$\frac{\text{♀}}{\text{H(A), S(C)}} \times \frac{\text{♂}}{\text{S(C)}}$	0.98
$\frac{\text{♀}}{\text{H(A), M(C)}} \times \frac{\text{♂}}{\text{H(A)}}$	0.59
$\frac{\text{♀}}{\text{H(A), M(C)}} \times \frac{\text{♂}}{\text{M(C)}}$	0.89

Table 3. B-C

Crosses		I
♀	♂	
K(B), U(C) x K(B)		0.74
K(B), U(C) x U(C)		0.89
K(B), S(C) x K(B)		0.43
K(B), S(C) x S(C)		0.66
K(B), M(C) x K(B)		0.40
K(B), M(C) x M(C)		0.67

Kurokawa, T. Effect of starvation on the phenotypic expression of vg^{np} flies.

Flies of vg^{np} stock, which normally have strap- or antlered-like wings, were raised on peptone food (partial starvation) during certain periods of larval development. (3.3 g peptone, 1 g agar, 1.7 g glucose, in 100 ml water.) When larvae were transferred to peptone food two days after oviposition and kept there until pupation, the wing phenotype of all female flies was shifted toward notched type, whereas the male phenotype was scarcely affected. The frequency of notched wings varied with the duration of starvation and the larval age at which starvation began, although no definite effective period could be determined. In experiments in which larvae were kept in a vial containing only filter paper soaked in Ringer's solution (complete starvation), development was delayed considerably, but no marked effect on wing phenotype was seen except that a few flies had strongly notched wings with a V-shaped incision. According to Akita (1955), vg^{np} flies have vestigial wings at 30° C. However, the results of a number of experiments in which the effects of starvation and temperature were examined together showed that the starvation effect is covered almost completely by the temperature effect.

Lefevre, G., Jr., and Bartlett, Alan C. Mutant incidence after irradiation of females.

Three-day-old $v f^{3N}$ car (homozygous) D. melanogaster females were exposed to 4200-r doses of X-rays and were subsequently mated individually to $y^{s1} sc^8 f v dl-49 w^a$ males. Each female was transferred through 10 successive subcultures, extending over a period of 4 weeks. The incidence of the following visible mutants was determined for each subculture: yellow, white, Notch, and reversions of f^{3N} to f^+ . Also, sex-linked recessive lethals were studied, testing all daughters of each irradiated female insofar as possible. In this way, clusters of mutations could be detected, and their size and persistence in time could be analyzed. Very large numbers of F_1 offspring have been examined.

The trend of mutant incidence with time is best illustrated by the sex-linked-lethal studies. After an initial incidence equivalent to that resulting from irradiation of mature sperm, sex-linked lethals declined in frequency for the first week. The frequency was relatively constant during the second and third weeks, but in the fourth week it increased erratically, in some runs becoming as high as or higher than the initial rate. The visible mutants showed the same general trend, but with considerably less variation in incidence throughout the 4-week period.

The size of mutant clusters tended to be small. Most frequent were

clusters of 3-4 or 7-8 individuals. In only one case, a white mutant, were there more than 8; in that case 9 individuals were found. The method of culturing made it likely that most, if not all, of the mutant eggs were recovered. Only during the first week, before clusters occurred was the fertility of the irradiated females below normal. In the extreme, over 1000 progeny were produced by one female in the 4-week interval.

Further experiments of this sort will be delayed because of a disaster that wiped out the entire fly colony at Salt Lake City just at the end of the current series of tests. New stocks are being obtained as replacements.

Lewis, E. B. Additions and corrections to the cytology of rearrangements.

The following locations of breakage points in the salivary-gland chromosomes supplement those found in the work of Bridges-Brehme (1944) (see also DIS-25:

108-109):

<u>Rearrangement</u>	<u>Breakage Points</u>
C(3)x	Two inversions apparently identical to 3L and 3R Payne.
T(1;4)B ^s	The break in four is in 102F.
T(2;3)101	44B / 83E or F.
T(2;3)Hn	54A or B / 76 or 77. In addition there is a deficiency (associated with the Henna effect) in the region of 66A and B (exact limits not determined).
T(2;3)p ^{Gr}	57C / 81F.
T(2;4)d	55E or F / break in four not determined.
T(3;4)e	79E / 102F.
T(3;4)f	Insertion of at least seven bands of chromosome four (bands not identified) into 3L, probably just after 65D1-2.

Lewis, E. B. Addition and corrections to the list of mutants in the work of Bridges-Brehme (1944).

Tft: Tufted Since "tufted" (symbol: tuf) is already in use (see DIS-22: 56), it is proposed that the name associated with the symbol Tft be changed from Tufted to Tuft.

Hn^{r3}: Henna-recessive-3 New symbol and name proposed for sed, which proves to be allelic to Hn^{r1} (and Hn). More extreme, and therefore generally more useful, than Hn^{r1}.

sed: sepioid Name and symbol discarded. Symbol changed to Hn^{r3}.

ld: loboid Locus between ca and bv at 102± (instead of 100±).

Lindsley, D. L., and Novitski, E. Influence of the proximal regions of the fourth chromosomes on their meiotic behavior.

Females heterozygous for In(1)sc⁸, f v cv sc⁸ or In(1)sc⁸L, EN^R.Y^L, y⁺ f v cv y and X(Y^L.)4, y² su-w^a w^a (the order of the centromere and Y^L in the last case is unknown) were found to fall into two groups: those that gave haplo-4 progeny

and were presumed to carry one free fourth chromosome (4/0), and those that gave no haplo-4 progeny and were presumed to have two free fourth chromosomes (4/4). Consider what happens when the inverted chromosome separates from the $XY^{L.4}$ chromosome in females with one free 4. If the free 4 pairs with and separates from the fourth-chromosome portion of the $XY^{L.4}$, each meiotic product will receive a fourth chromosome. If, on the other hand, the free 4 passes to the same pole as the $XY^{L.4}$, the inverted chromosome must pass to a null-4 pole and give rise to a haplo-4 zygote. Perfect disjunction of the free 4 from the $XY^{L.4}$ produces no haplo-4 inversion zygotes, whereas random disjunction of the free 4 should render half of the inversion-bearing zygotes haplo-4 and consequently extremely inviable.

Since presumably every zygote produced by the 4/4 females will receive a fourth chromosome from the mother, data from such females provide a control. Furthermore, since $XY^{L.4}$ progeny will never be haplo-4, the ratio of inversion zygotes from both 4/4 and 4/0 females. If one assumes complete lethality of haplo-4 zygotes by ignoring all haplo-4 individuals recovered, any decrease in the above ratio in the 4/0 as opposed to 4/4 females indicates something less than perfect separation of a single free 4 from the $XY^{L.4}$. A ratio from 4/0 females that is half the ratio from 4/4 females indicates random assortment of the $XY^{L.4}$ and the free 4.

From females of constitution $y^2 su-w^a wa bb (Y^{L.})_4 \#179-8/In(1)sc^8, f v cv sc^8$ there were 258 sc^8 daughters and 484 $XY^{L.4}$ daughters for a ratio of 0.553 from 4/0 females, and 638 sc^8 daughters and 690 $XY^{L.4}$ daughters for a ratio of 0.914 from 4/4 females. The recovery of the inversion from 4/0 mothers was 0.583 that from 4/4 mothers. Similarly, $y^2 su-w^a wa bb (Y^{L.})_4 \#179-8/In(1)sc^{8L}, EN^R.Y^L, y^+ f v cv y; 4/0$ females yielded 87 $sc^8 EN$ and 157 $XY^{L.4}$ daughters (= 0.554), whereas $y^2 su-w^a wa bb (Y^{L.})_4 \#179-8/In(1)sc^{8L}, EN^R.Y^L, y^+ f v cv y; 4/4$ females yielded 968 $sc^8 EN$ and 841 $XY^{L.4}$ daughters (= 1.153). Here the recovery of the inversion from 4/0 mothers was 0.481 that from the 4/4 mothers.

The fact that a single free 4 fails to pair with and disjoin from the fourth-chromosome portion of an $XY^{L.4}$ chromosome suggests that the portion of the fourth chromosome which controls pairing and disjunction is absent from the $XY^{L.4}$ chromosome. Since the $XY^{L.4}$ chromosome is an induced detachment of an $XY^{L.4}$ chromosome the region of the four missing is almost certainly proximal, but its extent is unknown, since sv^+ is the only fourth-chromosome gene identified on the $XY^{L.4}$, and this is the most distal gene on chromosome 4 according to Sturtevant (1951).

Lindsley, D. L., and Sandler, L. The effect of a free heterochromatic X-chromosome duplication on the disjunction of normal fours.

certain of the duplications. It was further noticed that the high incidence

of haplo-4 offspring was correlated with high nondisjunction of the attached-X and the duplication, and that the haplo-4 individuals nearly always carried the duplication (39 of 43 cases recorded). One case in which careful counts were made on the Minute progeny was a cross of $y w/Dp(1;f)135$ females x $Y^{SX.Y^L}, In(1)EN, y B/0$ males. The progeny included 1684 $y^2 B$ males, 416 $y B$

In a series of crosses in which attached-X females carrying different free heterochromatic X-chromosome duplications marked with y^+ or y^2 were crossed with $Y^{SX.Y^L}, In(1)EN, y B/0$ males, a high incidence of haplo-4 progeny was noticed from females carrying

of haplo-4 offspring was correlated with high nondisjunction of the attached-X and the duplication, and that the haplo-4 individuals nearly always carried the duplication (39 of 43 cases recorded). One case in which careful counts were made on the Minute progeny was a cross of $y w/Dp(1;f)135$ females x $Y^{SX.Y^L}, In(1)EN, y B/0$ males. The progeny included 1684 $y^2 B$ males, 416 $y B$

males, 1978 y w females, 417 y² w females, 14 y² B M males, 2 y w M females, and 13 y² w M females.

The interpretation of these observations is that those duplications that do not have a particularly strong affinity for the attached-X tend, in a proportion of the cases, to pair with^{and} separate from one of the fourth chromosomes. When the remaining fourth chromosome passes to the same pole as the duplication, each of the products receives one four; but when it passes to the opposite pole, that pole gets two fours and the cell that receives the duplication lacks a fourth chromosome. Since a number of different duplications show this effect and since duplications that show the effect definitely carry proximal-X heterochromatin as shown by the presence of bb⁺, it seems likely that these observations provide additional evidence of pairing homology between the X and 4. These observations are similar to those of Gershenson (1940), who found that similar heterochromatic X duplications separate regularly from the third four in triplo-four flies.

Miers, T. Cyto-architectonic studies in the central nervous system of the adult *Drosophila*.

The structure of the cortex of the central nervous system has been studied in late pupae and young adults of *D. melanogaster* and *D. funebris*. The material, sectioned

in series in different directions, has been stained by Nissl's method (Kresylechtviolett). The different cell types, characterized by their size, form, and internal structure, build up typical bilaterally symmetrical patterns. In the cortex of the brain there could be demonstrated eleven main cyto-architectonic regions, some of which are separated from each other by sharp boundaries. An extremely sharp boundary is seen between the protocerebral and the optic lobe. In the cortex of the latter are found the smallest cells of the whole nervous system, forming a homogenous cell area. Nearly all the other areas are characterized by different combinations of the different cell types, especially by scattered clusters of giant cells. In the cortex of the thoracico-abdominal ganglion an adequate arrangement is given. The areas can be classified according to their position in relation to the neuromeres. In this ganglion the biggest cells of the whole system are found between the prothoracic and mesothoracic neuromeres. In respect to the different types of nerve cells the Nissl-method makes possible the best analysis of the internal structure. There are great differences with regard to the nucleus-cytoplasm relation among cells of different size, with a shift from the smallest to the largest in favor of the cytoplasm. This stains dark blue, with a very fine granulation in the giant cells. On the whole, a highly differentiated cellular organization can be established in the cortex of *Drosophila*.

Miers, H. Examination of the number of active primary germ cells in the late imago of *Drosophila*.

On the basis of results of radiation experiments, H. J. Muller has established that the number of proliferating primary germ cells diminishes in the aging male imago of *Drosophila*, usually to about two

in each testis. Mutagenicity experiments were made with 2:5-bis-ethylene-imino-benzochinone-1:4, administered in three days' feeding, to *D. melanogaster* males 1-2 days old. Each P₁ male was tested individually by the Muller-5 technique in ten successive broods, each of three days' duration. It was found that in experiment no. 7 male no. 3 produced a cluster of about 50% lethal mutations (33/80) in broods six to ten, and male no. 30 gave 100% lethal mutations (81/81) in the same broods. The percentages of lethals in these two

males in broods one to five had been 5.6% and 14.6%, respectively. Thus there is the possibility that in both males only one primary cell in each testis had been actively proliferating from brood six on (age of the males at this time, 19-23 days). These cells carried a lethal in the one and no lethal in the other testis in male no. 3, and one lethal in each testis in male no. 30. All lethals of the two clusters will be tested, by locating them through the use of an X-ple stock, in order to check on the expectation of identical lethals in male no. 3 and of two different sorts of lethals in male no. 30.

Ufers, T., and Küpf, H.
Morphological observations
on the neurosecretory cells
of adult D. funebris.

Neurosecretory cells have been demonstrated in many groups of insects. Although in Drosophila physiological investigations have been numerous, descriptions of the morphology and distribu-

tion of these cells are rare. M. Vogt (1942) observed 4 to 8 fuchsinophilic cells in the pars intercerebralis of the larval brain in D. melanogaster. We stained sections of late pupae and adults of D. funebris at different ages, by Gomori's method. In the brain it was possible to recognize a main group of at least 20 cells in the pars intercerebralis. Two lateral groups of about three cells each are situated at the transition zone between the protocerebral lobes and the optic lobes. A few isolated large neurosecretory cells are scattered in other parts of the cerebral ganglion, a cluster of two situated in the cortex of the antennal lobes. In the thoracic ganglion several groups of neurosecretory cells can be found, bilaterally placed, adjacent to the neuropiles. All these cells are relatively large. The large and light nuclei are more or less rounded, containing chromonemata and chromocenters and red-colored nucleoli. In respect to the cytoplasm, there can be found two different types of cells in the same clusters, one type dark blue in color and the other dark reddish. In comparison with neurosecretory cells of Calliphora and Musca stained by the same technique, Drosophila showed no distinct granules, but a diffuse coloring resembling colloids. In some cases a large vacuole can be observed in the neurosecretory cells of the thoracic ganglion. Up to now no transport of the secretory products along the axons has been found.

Makino, S., Momma, E., and Wakahama, K. Fluctuation of predominant species of Drosophila at Sapporo.

Trappings were made in the University Botanical Garden at Sapporo during every month except the snowfall season (November to April) for three years, 1954-1956. D. nigromaculata, D. auraria, and D. trans-

versa were found to be most common in each year; but their frequencies of occurrence showed variations. D. nigromaculata ranked first in 1954 and 1956, but showed a striking decrease in 1955 and 1956. D. auraria ranked first in 1955, when it showed the highest frequency of the three years. D. transversa ranked third in each year, with slight variations in frequency. The order of frequencies is given below.

(Table on following page.)

Year	Rank			Total flies collected
	I	II	III	
1954	D. nigromaculata (52.68%)	D. auraria (20.65%)	D. transversa (12.94%)	2295
1955	D. auraria (35.50%)	D. nigromaculata (24.25%)	D. transversa (15.00%)	800
1956	D. nigromaculata (23.12%)	D. auraria (21.16%)	D. transversa (14.02%)	3609

Matthews, P. A sex-limited semilethal in *D. melanogaster*

During the course of a series of in-breeding experiments a sex-limited semilethal was discovered in a wild-type stock, Hampton Hill. Matings between homozygous males and females gave progenies greatly varying in their sex ratios, generally deviating significantly from an expected 1:1 ratio. Lethality was limited to females, although a few did break through the lethality barrier. Hence the stock could be maintained in a homozygous condition. Progenies of single-pair matings from the lethal stock gave percentages of females varying from 14.8% to 51.4%. Data from a large number of such matings gave an over-all excess of males to females of 2 to 1. Reciprocal matings to normal stocks gave 1:1 sex ratios. Other reported cases of sex-limited lethals in *D. melanogaster* (Bonnier, 1923; Morgan, 1929; Gowan, 1949; and Bell, 1954) all gave reciprocal differences in sex ratios when the lethal stock was mated to wild-type stocks--excesses of males being observed when the lethal stock was used as female. In three of the above cases the action of the lethal occurred in the egg stage (Morgan, 1929; Gowan, 1949; and Bell, 1954), so that one might assume that the reciprocal difference is in part a reflection of the genetic structure of the female.

The time of action in the present lethal appears to be during the late pupal stages and in the pupal-imago transition period. The exact time of action varies in different cultures. Upwards of 50-to-1 of the dead flies recovered from lethal cultures have proved to be females. These females were characterized by a series of abnormalities affecting the chaetae of the dorsal surfaces of head, thorax, and abdomen; the structure of the dorsal surface of the thorax; and the normal inflation of the wings. The regular pattern of the minor chaetae of the dorsal thorax was disorganised: the posterior scutellars were upright; dorsocentrals either upright or missing; postverticals, verticals, and orbitals missing or arranged in an irregular fashion. The lethal was not without its effect on the males. The wings of the majority of males were slightly upcurved, in extreme cases resembling Curly. Occasionally the same characteristic was noticed in females, the curling being nowhere near as marked as in the males.

An extensive series of salivary-gland studies of the lethal stock has revealed no major inversions or alterations in chromosomes X, 2, or 3. Tests to locate the position of the lethal are at present under way, and a more detailed report will be presented elsewhere.

Meyer, Helen U. Failure of inseminated females to produce fertilized eggs unless additional copulation takes place.

When in tests for autosomal lethals the final inbreeding to obtain homozygosity of the treated chromosome is attempted, it is sometimes impossible to obtain heterozygous flies of both sexes having the desired composition (autosome to be tested/balancing chromosome). This happens mainly in poorly going, moldy vials, where some of the flies may have died before one attends to selecting the suitable parents for the next generation.

If in such a case only female, but no male, heterozygotes can be obtained --not even at a later date--it is our custom to culture such females for at least one week, assuming that they had been fertilized by brothers which since had died. After this waiting period the vial is checked for collapsed eggs or larvae; only when no sign of fertilization can be found do we remate the female to suitable males of another composition, in order to complete the test for lethals. (We have found that the frequency of lethals in the poorly going cultures is somewhat higher than in the rest of the group which they represent; ignoring such cultures would therefore bias the results.)

Judging from the offspring obtained from females that had laid unfertilized eggs only during the first waiting period, it was found that each must indeed have mated with a brother and contained stored sperm from such a first mating, but could produce fertilized eggs only after being given additional males.

We conclude from this observation that, oftentimes, females do not or cannot utilize stored sperm. The reason for this, we think, may be an insufficient amount of sperm or of glandular secretions--or probably both--delivered at the first copulation. Another possibility may be that the need for polyspermy cannot be adequately met without more sperm.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Meyer, Helen U. Frequency of detachments of attached-X chromosomes in the presence of sc.Y^L or Y^{Lc} spontaneously and after irradiation of polar caps.

Since it is known that most spontaneously occurring detachments of attached-X chromosomes are the result of crossing over with the Y chromosome (Kaufmann, 1933; Philip, 1934), it was expected that the type of Y chromosome present would greatly influence the rate of spontaneous detachments. This was confirmed by Parker (1954) for radiation-induced detachments. Comparing several types of females having different Y chromosomes or not having a Y at all, he found that the frequency of exchanges was greater when a two-armed Y chromosome was present in the attached-X females than when the Y was ring shaped or entirely absent.

Even though radiation-induced exchanges most certainly do not take place at the stage of premeiotic crossing over, Parker could confirm that exchanges with the Y chromosome accounted for by far the most numerous cases of detachments if a Y chromosome had been present, or if it was not a ring. In the latter two instances, almost all exchanges involved the fourth chromosome, which always ranks second as a supplier of a new chromosome end for the detached-X chromosome. This was also reported by Abrahamson, Herskowitz, and Muller (1954, 1956) for detachments obtained from irradiated females without a free Y chromosome.

In our experiments we compared the detachment frequency in attached-X females which had either a $sc.Y^L$ (Crew and Lamy, 1940) or a Y^{Lc} (Muller, 1948); both had also been studied by Parker in his comparisons. The $sc.Y^L$ is V shaped and contains a great amount of heterochromatin, whereas the Y^{Lc} is a ring and seems only about half the mitotic length or bulk of the $sc.Y^L$. The attached-X chromosomes were of "snoc" type ($sc\ ct^n\ oc\ ptg\ car$. In49sn^{x2} ct^1 , In y), and such females with either a $sc.Y^L$ or a Y^{Lc} were mated to males $oc\ ptg.Y^S/sc.Y^L$ or Y^{Lc} , respectively.

From such a cross, all daughters should be ct and all sons ct^+ , $oc\ ptg$, like the parents. However, if detachment of the attached-X's should have occurred, we would find some non-cut females and ct males, which could then be tested to determine whether they really were the products of detachments.

By far the greatest number of females in our experiment were untreated; a smaller number from both groups had been treated at an early embryonic stage (polar cap stage) with either ultraviolet (300 ergs/mm² of mainly 2537 Å), or X-rays (1500 and 2000 r, 200 kvp) applied to the region which then contained the pole cells. Only a few of the X-ray group survived. To check the effectiveness of the treatment, male embryos which had been irradiated with the females were tested for autosomal lethals. We obtained the following results:

Group	Treatment	No. of P females	Confirmed detachments in F ₁ females*		Lethals, chrom. 2**	
			No.	%	No. tests	%
$sc.Y^L$ group	untreated	131	4/8164	.049	1232	.32
	ultraviolet	53	1/3256	.031	1143	5.0
	X-rays	31	-/1512	0.0	754	2.6
Y^{Lc} group	untreated	115	-/6307	0.0	1315	.61
	ultraviolet	23	-/1011	0.0	178	8.4
	X-rays	10	-/ 458	0.0	129	0.0

* No evidence of detachments found in a similar number of F₁ males.

** Found in offspring from brothers of P females, see text.

We see then that our data for the spontaneous rate of detachments agree with the results which Parker obtained in his irradiation experiments; whereas .049% of the F₁ females of the $sc.Y^L$ group had detached-X chromosomes, none were found in the group having the ring Y. This was no doubt due to the shape of the latter, which allows only double crossovers to survive.

No exceptions at all were found among F₁ males. Parker, who also found many fewer male than female exceptions in irradiated material, attributed it only in part to induced lethals in the detached chromosome portion, but had reason to believe that some of the attached-X's had pre-existing lethals accumulated in regions near the centromere. In our case we expected only half as many male as female exceptions to begin with, since one arm of the "snoc" attached-X chromosome carries a known lethal (ct^1), and with the low number of female exceptions in our untreated material an explanation on statistical grounds might be sufficient. Since the frequency found from F₁

females alone is no doubt closer to the true detachment frequency than if based on the sum of the males and females, only the figures for females are listed in the table.

We further see from the data in the table above that, apparently, the frequency of detachments is not increased by irradiating the future germ cells at such an early stage. We do not believe that this is a true result, for the following reasons: (1) both X-rays and ultraviolet are known to increase non-meiotic crossing over, especially in heterochromatic regions; (2) we had evidence that the treatment must have affected the germ cells from the increase in autosomal lethals obtained from corresponding males, except for the X-ray-treated Y^{Lc} group; (3) we found in a more recent experiment, also using females, but designed to discover a different type of detachment (of translocated parts attached to free X chromosomes), that detachments can be obtained by either ultraviolet or X-ray treatment of early germ cells. Therefore we are inclined to think that there probably were cases of detachment caused by the treatments, which we were unable to detect. The reason might well be, as suggested by Muller, that in those pole cells in which detachment of one chromosome arm had occurred at this early stage of germ-cell development, a segregation of the arms followed, so that the cells became genetically male and failed to furnish properly functioning germ cells and nurse cells within a female gonad.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Milani, R. Emendation.

The last issue of DIS (DIS-29: 139) included a contribution by the present author with the title, "Effect of natural selection on the full expression of the gene countercoiled (cc)." This note did not mention the organism involved, which was Musca domestica. It seems desirable to clarify this point, because a similar mutant type has been detected in D. melanogaster.

Milani, R. Countercoiled genitalia in D. melanogaster.

Toward the end of 1951, in scoring the F₂ of a wild D. melanogaster female it was found that some males had the hypopigium rotated out of its normal position. These males, sexually active and otherwise perfectly normal, were sterile through a mechanical impediment to copulation. A line was started from single-pair matings of sibs of the abnormal males. Observations carried through a few generations provided evidence of monofactoriality for the abnormal rotation, which behaves as a recessive. Dissection of preserved specimens has recently shown that this abnormality involves a counterclockwise looping of the sperm duct around the rectum. A search among normal strains having a common origin with the one in which this abnormality was first observed revealed some counterclockwise males (homozygous?; about 2%). The morphological effect of this mutant type is closely similar to that of the mutant of Musca domestica which I called "countercoiled genitalia. However, it appears that in D. melanogaster the countercoiling of the hypopigium can very rarely be fully accomplished through 360°, whereas in some strains of the housefly the countercoiling is usually fully accomplished in nearly all males.

Milani, R. Housefly genetics. Evidence of linkage between two markers and of male crossing over in the housefly.

The markers divergent (div) and brown body (bwb) of the housefly can be found combined in a double-recessive class in the F_2 of crosses in which they have been introduced one from each parent.

However, the segregation ratios do not fit the expectation, owing to a great shortage in the double-recessive class; there are gross differences between lines. Backcross tests have shown a rather regular 40% crossover rate when the tested heterozygous flies were females; when the tested flies were males, close linkage has been found in most families, but in a few families crossover classes have been found. Comparison of the female crossover rate (about 40%) with the recombination values observed among intercrossed F_2 flies suggests that crossing over between bwb and div can reach 10% in the males. All the gynandromorphs found so far (7) showing mosaicism for these two markers had a topographic distribution of marked tissues in keeping with the nature of the original cross.

Miller, D. D. "Choice matings" involving D. athabasca from Wyoming, Michigan, and New York.

These "choice matings" were made with 7-8-day adult flies, and lasted 3 to 4 days. Males were combined with a mixture of females from two different localities,

in all the possible combinations of Wyoming (Jackson Hole), Michigan (University of Michigan Biological Station), and New York (Cold Spring Harbor). In all cases the frequencies of insemination of the two kinds of females were significantly different. Wyoming males inseminated 5/90 (6%) Michigan and 39/100 (39%) New York females; 3/87 (3%) Michigan and 67/90 (74%) Wyoming females; and 35/100 (35%) New York and 85/100 (85%) Wyoming females. Michigan males inseminated 79/102 (79%) Michigan and 1/101 (1%) New York females; 68/97 (70%) Michigan and 7/98 (7%) Wyoming females; and 0/106 (0%) New York and 5/101 (5%) Wyoming females. New York males inseminated 0/101 (0%) Michigan and 62/102 (61%) New York females; 0/87 (0%) Michigan and 15/87 (17%) Wyoming females; and 72/100 (72%) and 24/99 (24%) Wyoming females. It may be seen that interlocality insemination frequencies involving Michigan D. athabasca were consistently lower than those involving Wyoming and New York strains, with almost complete sexual isolation between Michigan and New York. However, frequencies of insemination between Michigan and Wyoming were appreciably higher than those observed the year before in "no-choice" combinations of D. athabasca from these localities (Miller, DIS-29). Additional "no-choice" matings of Wyoming and New York D. athabasca yielded abundant fertile offspring. These results help reconcile the findings of Novitski (1946), who did not report sexual isolation between western and eastern D. athabasca, with those of Miller (DIS-29), who reported a high degree of sexual isolation between western (Wyoming, North Dakota, and western Ontario) and eastern (Michigan) D. athabasca.

Miller, D. D. Geographical variation in copulation time in D. athabasca.

As has already been reported, copulation in D. athabasca is long in strains from Wyoming (Jackson Hole), North Dakota (Minnewaukan), and western Ontario (Cedar

Lake) (3'57"-15'29", Miller, DIS-29); short in strains from Michigan (Cheboygan) (1'6"-2'0", Miller, DIS-29), New York (Cold Spring Harbor), and New Jersey (Princeton) (1'12"-1'48", Miller, DIS-25). A few copulations have since been observed in recently derived (summer of 1956) D. athabasca strains, and the following durations have been determined: Iron River, Wisconsin,

4'56"; Iron Mountain, Michigan, 1'25" and 1'26" (these strains kindly provided by Dr. H. D. Stalker of Washington University); Algonquin Park, Ontario, 1'17", 1'30", 1'39", 2'16", and 2'42"; Gatineau Park, Quebec, 1'11", 1'24", 1'26", 1'56", 1'59", and 4'11"; Ste. Anne de Bellevue, Quebec, 1'35" and 1'59"; and Laurentides Park, Quebec, 5'56", 7'18", 7'20", 7'59", 10'7", and 20'56". (As before, all these have been first copulations of week-old flies.) The results show that the long-copulation-time characteristic extends into the east in the northern part of the known range of this species, with some localities (Algonquin and Gatineau Parks) having both short copulation times and copulation times longer than previously reported for eastern strains.

Three copulations have been observed between "short"- and "long"-copulation strains: New York female by Wyoming male, 4'18"; Wyoming female by New York male, 1'3" and 1'11". These results are at least consistent with male determination of copulation time, such as was reported by Merrell (1949) for inbred *D. melanogaster* strains. Also observed have been a few copulations of hybrids between "short" and "long" strains: F_1 (N.Y. female by Wyo. male), 1'13", 2'2", 2'16", 2'25", 2'26", 2'50", and 3'9"; F_1 (Wyo. female by N.Y. male), 5'19". These results show that hybrids may have copulation times intermediate between those characteristic of the parent strains. Work is in progress to augment the observations of copulation time in the new strains and between "long" and "short" strains and their hybrids.

Mather, W. B. Genetic relationships of four *Drosophila* species from Australia.

(The following is the summary of a paper to be published in a forthcoming University of Texas Publication.) By breeding tests it has been established that: (1)

D. serrata Malloch is a biological species distinct from *D. kikkawai* Burla. (2) The fly previously recorded as *D. takahashii* Sturtevant from Australia is in fact a new species, *D. pseudotakahashii*, morphologically distinguishable from *D. takahashii* by two features of the internal male genitalia, which are controlled multifactorially. (3) The synonymy of *D. levis* Mather with *D. bryani* Malloch, previously established on morphological grounds, is confirmed biologically. (4) *D. versicolor* Mather from Australia is synonymous with *D. buzzatii* Patterson and Wheeler, and contains an inversion previously recorded only from Lebanon.

Mather, W. B. Relationships between species groups of the *Pholadoris* subgenus.

(The following is the summary of a paper to be published in a forthcoming University of Texas Publication.) The failure of hybridization between species groups

of the *Pholadoris* subgenus, established on morphological grounds, supports the biological reality of these groups. However, sexual isolation is incomplete between the *levis* and *mirim* groups, between the *maculosa* and *victoria* groups, and between the *maculosa* and *coracina* groups, indicating the close biological relationships of the crossmating groups.

Mitchell, D. F. Persistence of a *sepia* allele in an inbred line.

In a strain derived by sib mating from the offspring of a single female from a wild collection, an allele of *se* has persisted for 20 generations of brother-sister

matings. The allele was presumably present in heterozygous condition in the wild female. The strain has been derived by making four single-pair sib

matings each generation, and selecting wild-type individuals for the next generation from the most productive, or one of the most productive, cultures. Thus, inbreeding and selection for viability under the culture conditions has been involved. Approximately one-half of the cultures contain surviving flies which are heterozygous for the *se* alleles. Homozygotes for *sepia* are not necessarily produced, or do not survive in every generation. It appears, therefore, that the allele has been maintained in the line through the superior fitness of the heterozygotes, and that, under the culture conditions, the homozygous wild type is significantly inferior.

Momma, E. Spermatogenesis in
D. lacertosa.

This is a new species described by Okada (1956), and is rather common in the forests of Hokkaido. Spermatogenesis in the newly emerged larva was investigated with both fixed and living materials. In material fixed with modified PFA 3 or Champy, the chromosomes make their clear appearance after Heidenhain's iron-hematoxylin staining. Material stained with Regaud's iron-hematoxylin shows the mitochondria with their characteristic features. The mitochondria differ slightly in shape from those observed in the living material, but Golgi bodies or dictyosomes are nearly identical with those studied in Champy material. Successive stages ranging from the maturation of germ cells to spermioteleosis were observed in living cells by phase-contrast microscopy. The cell body at metaphase I is very large in size (40-50 micra in diameter), showing the smaller spindle body (10-15 micra in diameter). Through the course of spermatogenesis the behavior of the mitochondria could be traced in the living state.

Morita, T. Tyrosinase activity
and free tyrosine content in
some strains of *D. virilis*.

Tyrosinase activities and free-tyrosine contents of larvae and pupae of a wild and two mutant strains were compared with one another. The ebony mutant has a darker color both in the puparium and in the imaginal integument than the wild type, whereas the yellow mutant has a lighter color in both respects. Tyrosinase activity was measured by the manometric technique. In mature third-instar larvae, tyrosinase activity of ebony was higher and that of yellow lower than that of the wild type. Tyrosinase activity of pupae after 24 hours of pupation was remarkably lower than that of larvae of any kind. The estimation of free-tyrosine content was carried out by the method reported in DIS-29. In mature larvae, free-tyrosine content of ebony was clearly higher than that of wild type or of yellow, but no difference could be detected between the latter two. Free-tyrosine content was reduced remarkably in pupae, and this reduction proceeded gradually during the pupal stage. However, the free-tyrosine content of yellow was maintained at a higher level than that of the others. Thus, the differences in degree of puparium pigmentation between wild, ebony, and yellow strains seem to be due to both tyrosinase activity and tyrosine content.

Muller, H. J. Another entire
inversion formed by opening of
a ring X.

The X chromosome in our stock b85 (b87 in DIS-29), which had originally been of the ring structure designated as X^{c2} (closed X of Beadle, 1934), has proved now to be an open X containing an inversion (*InEN2*) of the entire euchromatic region. Its crossover properties are like those described by Novitski (DIS-23: 94) for the entire inversion (*InEN*) obtained by him as a result of the opening of

X^c (closed X of L. V. Morgan). This information was not obtained until our present stock list had been submitted to DIS-30. Users of stocks designated as X^{c2} in this list are warned that any such stock may contain an open X instead of a closed one. Whether the new open X carries pieces of Y or of IV has not yet been determined. Like X^{c2} , but unlike Novitski's opened X, it contains the normal allele of yellow in its main euchromatic region.

Muller, H. J., and Herskowitz, I. H. Reciprocal and half-translocations with a rod-X chromosome produced by X-raying sperm and oocytes.

Irradiated (4000 r) $y\ sc^5.Dp\ sc^{V1}\ y^+/Y^+$ males crossed to females homozygous for y in separate X's gave in F_1 13 y^+ males and 15 y females among 10,956 offspring, a total of 0.26%, which were produced by intra-X rearrangements, point mutation, or the retention of a viable eucentric half-translocation. Unexceptional virgin F_1 females (whose maternal chromosome carried inversions in addition to y) were mated individually to y males, and the F_2 examined. Of 1949 F_1 females tested, 22, or 1.1%, carried a paternal X which had undergone a reciprocal translocation of such a kind that one or both half-translocations derived from it were separately viable. Eleven of the 22 were analyzed; of these, 7 were shown to be X-IV's, 1 was X-III, 1 was X-II, and 2 were X-II-III's.

Thus there were among the F_1 about 4 times as many reciprocal translocations as there were all types of gross rearrangement (and point mutation) which became viable aneuploids before advanced cleavage stages. This must mean that if centric unjoined fragments are frequent these usually cause death, and that such fragments once joined are relatively infrequently lost in early development, at least when the breaks are produced in sperm. Of the reciprocal translocations identified, 64% (7/11) were X-IV, a value not significantly lower than the 84% of the half-translocations identified which were shown to be X-IV by similar tests, after irradiation of attached-X's in the absence of a Y (Abrahamson, Herskowitz, and Muller, 1956). The preponderance of X-IV's in both studies is attributed largely to the relatively high viability of the aneuploid of IV, but proximity of the parts undergoing exchange may also play a role here.

Either 3600 r or 4250 r were delivered in a concentrated treatment to females homozygous for $y\ sc^5.Dp\ sc^{V1}\ y^+$, which were then crossed to $y\ sc^{S1}\ B\ In49\ v/Y^+$ males. From eggs oviposited within 4 days after irradiation, there were in F_1 , for the respective treatments, $0.58 \pm 0.13\%$ (21/3597) and $0.67 \pm 0.072\%$ (86/12,931) exceptional individuals, representing the frequency of y^+ -deficient half-translocations and/or deficiencies of $sc^{V1}\ y^+$ of the maternal X present in mature eggs. Of 82 exceptions tested, 39, or 48%, were proved X-IV half-translocations. This is in agreement with the 52% and 46% of all half-translocations tested which were proved to be X-IV after irradiation of sperm containing the same X chromosome and irradiation of attached-X's in the absence of a Y, respectively. Phenotypically unexceptional F_1 virgin females were mated individually to males like the P_1 fathers, and each F_2 culture examined for y^+ -deficient half-translocations. For the respective doses, the frequencies obtained were $< 0.079 \pm 0.08\%$ ($< 1/1263$) and $0.031 \pm 0.03\%$ (1/3253) for reciprocal translocations among F_1 females whose y^+ -deficient half-translocation (obtained in F_2) proved viable.

If all half-translocations in mature eggs were produced by the sorting into the polar bodies of the other part of a reciprocal translocation produced in the oocyte, then there could be, among the eggs producing the F_1 , no more

than three times as many half-translocations of a given type as reciprocal translocations producing the same type of half-translocation. However, the frequency of these reciprocal translocations is significantly less than one-third the frequency of half-translocations detected as such in F_1 , even without correcting for the fact that the experimental procedure favored the detection of a reciprocal translocation giving a y^+ -deficient half-translocation. This result must mean that half-translocations derived from irradiated oöcytes do not always (or usually) arise from reciprocal translocations, but frequently result from two-break events in which only one eucentric exchange union occurred, the other centric piece having been cast off into a polar body unjoined. Accordingly, the reverse situation probably happens equally frequently, in which the centric piece that has joined in a eucentric half-translocation is discarded in a polar body while the unjoined centric piece is retained in the mature egg. This would make a significant contribution to X-ray-induced egg mortality.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195.)

Nicoletti, B., and Solima, A.
Differential fitness in *D.*
melanogaster populations.

Fecundity, hatchability, fertility, and rate of development have been studied in Oregon-R, in Oregon inbred (370th brother-sister matings), in a population from the wild (Perugia), and in reciprocal crosses between Perugia and Oregon-R strains, as measurements of Darwinian fitness. It has been found that fitness is inversely related to the degree of inbreeding. The population from the wild shows the highest values of fitness, the Oregon inbred the lowest, and the Oregon-R strain intermediate values. The flies obtained from reciprocal crosses exhibit a high degree of heterosis, whose expression is related, however, with the strain used as female parent.

Nicoletti, B., and Solima, A.
Sexual selection on *D. melano-*
gaster.

Experiments on sexual selection in *D. melanogaster* have been continued, using Oregon-R and ss^a strains. When placed in competition with the wild type, the mutant males show lower sexual activity and the mutant females seem to be less sensitive to the male courtship. The experiments made suggest that in intraspecific crosses, when it is possible to obtain evidence of sexual selection, such selection depends not only on the choice made by females, but also on differences in sexual activity of males. Thus, in a given population, sexual selection depends in part upon which males are able to reach most promptly the necessary stimulus for mating, and thereby realize the copulation.

Nolte, D. J. Segregation
of modifiers.

The quantitative differences, reported previously, in the red and brown eye pigments of different South African strains of *D. melanogaster*, were studied further by outcrossing three strains, two from the same locality but collected during different years and the third from a widely separated area. The crosses were Inhaca-3 x Inhaca-1, Inhaca-3 x Inhaca-2, Inhaca-3 x Graaff-Reinet-2. From the F_2 of each cross, 15 fertilized females were taken at random to establish inbred lines over a period of ten generations. From these lines 10 were retained of each group

of 15, and these were tested quantitatively for eye pigments. The amount of red pigment differed by about 97% between the highest and lowest lines, and the amount of brown pigment by 58% between the highest and lowest lines for this pigment, the variation of pigment amount in the various lines being differential for the two pigments.

The two ranges of variation for the 30 inbred lines, due to segregation, are fairly continuous but show certain groupings. For the red pigment the number of groupings could be accounted for by the segregation of 10 pairs of modifying genes, and for the brown pigment the number of groupings could be accounted for by the segregation of 7 pairs of genes. There is no correlation between the amounts of red and brown pigment in this series of inbred lines, and it thus appears that the two pigments are influenced independently by two series of polygenes.

The conclusion is that South African wild-type strains are heterogeneous for various combinations of two series of modifiers which are segregating in various populations.

Novitski, E. The possibility of another cytoplasmically inherited factor in D. melanogaster.

In a set of 25 pair matings of males carrying a specially derived XY chromosome provided by D. L. Lindsley, to females carrying an attached-X, homozygous for y and w, and $sc^8.Y$, the progeny consisted of 847 females and 1 male. Subsequent tests established the following points. The aberrant sex ratio is obtained only when males from certain stocks are mated to these females, the off ratio being obtained also when X^{C2} males from the same stock from which the female was derived are used, and not when males from Canton-S; y v/w^a v, tra/Cx and y w^a cv v f stocks are used. Daughters of a female giving an aberrant ratio mated to a "sensitive" male may behave like their mother, may give a normal ratio, or an intermediate ratio, but in general they all behave similarly, suggesting some kind of maternal effect. Because of the presence in these females of an attached-X, the distinction between a cytoplasmic factor and the presence of a factor on that attached-X cannot be made with certainty and awaits the analysis of detachments occurring in lines giving the aberrant ratio. Ordinarily the possibility of cytoplasmic inheritance would be considered quite unlikely, but it is considered more so here because this kind of disturbance in sex ratio has been shown by Magni to be cytoplasmic in D. bifasciata.

Okada, T. The relation between wing indices and wing length.

It was found that among the individuals of a drosophilid species or among the various species of the genus *Drosophila* the costal index is roughly proportional to, and each of the other three indices is inversely proportional to, the wing length. In some species a female usually shows a costal index larger than that of a male of the same species, even when the male and female have the same wing length.

Oshima, C. Studies of DDT resistance in D. melanogaster from the viewpoint of population genetics.

The object of this study was to investigate changes in DDT resistance in populations of D. melanogaster by natural selection. Three wild strains and a mutant strain were used in the experiments: Hikone, which was

highly resistant to DDT; Kanmuri-jima, slightly resistant; and Canton-S, highly susceptible. A mutant strain which had two recessive marker genes, *sca* and *ss^a*, on the second and third chromosomes, respectively, was used as a tester.

Two kinds of analytic populations were prepared and cultured in population cages. One had heterozygous second chromosomes of one of the wild strains and the tester strain, homozygous third chromosomes having the *ss^a* gene, and the X chromosome of the tester. The other had homozygous second chromosomes carrying the *sca* gene, heterozygous third chromosomes of one of the wild strains and the tester strain, and the X chromosome of the tester.

After 150 days of culture, these analytic populations were mixed and synthetic populations were made. These populations were cultured in population bottles. Mortality of offspring of several pairs, removed from a population, was measured by exposing for 24 hours to a filter paper impregnated with a DDT concentration of 25 $\mu\text{g}/\text{cm}^2$. The change in mortality (300 flies tested) of each analytic and synthetic population was investigated during certain generations.

From a statistical analysis of the results it was concluded that the effect of the dominant factor on the second chromosome of the Hikone strain was highest and that of the Canton-S strain was lowest, and that there was a positive interaction between dominant factors on the two major autosomes of each wild strain.

A marked variance in mortality was recognized in the analytic population derived from the Kanmuri-jima strain. The level of DDT resistance of each synthetic population approached that of the original resistant strain. The effects of resistant factors on each autosome were detected, but the interaction between them was extremely limited. From these results, it is suggested that the dominant factors would gradually be eliminated and, on the other hand, the recessive factors would accumulate in a population by natural selection.

Oshima, C., and Hiroyoshi, T. The degree of DDT and nicotine sulfate resistance in various wild and mutant strains of *D. virilis* was determined. Using the Hikone strain, the most highly resistant to both insecticides, genetic analyses were performed. The dominant genes responsible for the DDT resistance were found to be located on the second and fifth chromosomes, respectively. The statistical analysis showed that the main effects of the two chromosomal factors were almost equivalent, and that their interaction was positive.

The dominant genes relating to nicotine sulfate resistance were found to be located on the second and fifth chromosomes, respectively; but the main effect exerted by the former was greater than that of the latter and their interaction was not significant. From these experiments it could not be discovered whether or not the genes for resistance to the two insecticides are the same, but it may be assumed that these genes (if there are more than one) might have some common physiological reactions to both insecticides.

It is of significance to an understanding of the processes of evolution

that DDT and nicotine sulfate dominant resistance genes have been located on the homologous chromosome elements of D. virilis and D. melanogaster.

Oster, I. I. A new crossing-over suppressor in chromosome 2 effective in the presence of heterologous inversions.

With a view toward finding a more effective crossing-over suppressor in the second chromosome than the combination of Curly (Cy) and its two large paracentric inversions, one in each arm of the second

chromosome, which occasionally undergoes single or double crossing over in the centromeric region and double crossing over in the right arm, we irradiated males containing $\Delta p^{txl} Cy, InL pr cn^2 InCyR$ with 4000 r. Following a genetic scheme suggested by Muller, the treated males were mated to virgin non-Curly females containing $InCyR$. The F_1 females were bred individually, and Curly flies from crosses which gave a reduction of recombination in the right arm of the second chromosome were saved for further examination. Such cases were subsequently tested for their effect on crossing over in combination with a normal second chromosome but in the presence of the inversions in the third associated with Moiré and/or $sc^{S1} In49 sc^8$. These tests indicated that one of our newly induced inversions, which probably contains a pericentric inversion in addition to $InCyL$ and $InCyR$, effectively reduces crossing over throughout the length of chromosome 2 even when heterologous inversions are present. This chromosome, containing the complex of inversions which we are designating $Cy, Ins05$, is viable when heterozygous, contains two dominants with lethal recessive effects, Δp^{txl} and Cy , thereby enabling one to use it in methods utilizing Muller's "criss-cross lethal" technique, and should prove useful in breeding schemes involving inversions in the other chromosomes.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195, and by a postdoctoral fellowship from the National Science Foundation.)

Oster, I. I. A stock ("Taxy") for detecting translocations of the autosomes and the X and Y chromosomes.

In order to be able to detect not only whole or partial losses of the X or Y, sex-linked lethal mutations, but also translocations involving the autosomes and the X or Y in the offspring of the

same treated individuals, a new breeding scheme was worked out. In practice, untreated or treated males of the composition $Y:bw^+ / y sc^4 B InS w^r sc^8$; $cn bw$ are crossed to females of Bloomington stock j100, composed of $sc^8.Y / y In49 sn^{x2} B^{M1} / y oc lz.Y^S$; $twl bw$; st^{541} females and $sc^8.Y / y sn oc$; $twl bw$; st^{541} males, designated "Taxy" (to denote a stock for detecting translocations involving the autosomes and the X or Y). Males containing either sc^{S1} or sc^4 can be mated, depending upon the main purpose for which one is using the scheme. That is, if one is chiefly interested in detecting lethals and translocations it is better to use males from the stock containing a $y sc^4 B InS w^r sc^8$ chromosome, since this is more viable than $y sc^{S1} B InS$; whereas if one is mainly interested in detecting losses and partial losses of the X or Y and translocations, it is better to use males containing a sc^{S1} chromosome since this is better than the $sc^4 sc^8$ chromosome, which being deficient for the proximal heterochromatic region, undergoes a high rate of spontaneous nondisjunction. The "Taxy" stock is only partially balanced and should be maintained by selection of $y^+ sn^+ oc^+$ females and $y^+ sn oc$ males. Regardless of which type of male is crossed to the "Taxy" females, the F_1

offspring can be scored for whole or partial losses of the X or Y, and Bar-eyed females which contain an X derived from their fathers can be tested for sex-linked lethal mutations by being crossed to non-Bar males and looking for the absence of Bar (B) males in the next generation. By virtue of its having been supplied with inversions in one X chromosome and a $sc^8.Y$, crosses of the "Taxy" females give rise to two classes of males in the F_1 , those receiving a treated Y from their fathers (y non-Bar) and those receiving a treated X from their fathers (y^+ Bar). Thus, individual matings of the former males to y ; $twl\ bw$; st^{541} virgins, which will detect translocations involving the Y, and of the latter males to $sc^8.Y / y\ f$; $twl\ bw$; st^{541} virgins, which will detect translocations involving the X, and looking for the absence of one or both classes of recombinants involving any of the three pairs of markers (y vs. y^+ , twl vs. twl^+ , and st vs. st^+), considered two pairs at a time, will indicate the presence of a translocation between the chromosomes with those markers or their alleles. The use of two stocks for testing for either X or Y translocations will enable one to carry out retests by repeating with the F_2 crosses like those of the F_1 of cases which yield insufficient flies to determine whether or not a translocation is present. In addition, this scheme should allow one to detect many more translocations of the Y, which are sometimes sterile if they include position effects on the fertility genes of the Y^S , because one-half of the males bearing such altered Y's are here rendered fertile since they are supplied with a Y^S from their mothers.

(This work was supported by a grant for work of Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195, and by a postdoctoral fellowship from the National Science Foundation.)

Pipkin, Sarah B. Balanced polymorphism in D. lebanonensis.

Populations of D. lebanonensis in four different areas in the Lebanon Mountains were found to be polymorphic for an autosomal dominant mutant determining a light scutellum and light area extending anteriorly and laterally as far as the level of the anterior dorsocentral bristles. About half of each of the natural populations showed the mutant "Spot," and about half was homozygous for its recessive allele, "non-Spot" or full color. The dominant mutant "Spot" maintains itself in about half of the populations of crowded culture bottles, according to counts made of the Beirut strain. This is then a new case of balanced polymorphism in Drosophila. In pair matings, D. lebanonensis individuals heterozygous for "Spot" derived from the Beirut strain, when crossed with the closely related D. victoria (Utah strain), which is homozygous for "non-Spot," gave 135 "Spot" and 144 "non-Spot" individuals. Preliminary tests with population cages indicate that a model similar to the one used by Da Cunha (1949) must be used in further studies of this species, since the larvae are extremely active and migratory.

Poulson, D. F. Differences with regard to copper toxicity in ebony strains.

An investigation of a series of ebony alleles with regard to their response to copper concentrations in the medium clarifies an apparent contradiction between my findings and those of Kikkawa, Ogita, and Fujito (DIS-28). The standard ebony strain (e), while showing lower viability than wild strains, will survive at all concentrations at which Ore-R can survive. On the other hand, the ebony-11 strain (e^{11}) is extremely sensitive to added copper and does not survive at the higher copper concentrations used. Sooty (e^S)

appears intermediate in this respect. Whether these are strain or allelic differences is being thoroughly investigated.

Poulson, D. F. Spontaneous reversions of ebony, vermilion, and apricot.

these a non-ebony v, bw, ey² strain was derived, which when crossed to the original v, bw, e, ey², gives typical wild-ebony segregation. The reversion is being subjected to intensive study with regard to its copper-accumulating properties.

The ebony reversion was found when a number of flies appearing to be heterozygous for ebony were observed in a single bottle of the v, bw, e, ey² strain. From

In the same v, bw, e, ey² stock a single female was found in which a symmetrical brown-pigmented area, including about one-quarter of the ommatidia, was present in one eye. This is the first time such a patch has even been observed in this stock, which has been rather closely examined over a considerable period of time. This clearly is a case of somatic reversion of vermilion. Another case interpretable only as a somatic back-mutation appeared in a single male of our K-5 stock. In this individual a symmetrical red-pigmented patch was present in one eye and presumably represents a change at the apricot locus. No such patches have been previously observed in any of our apricot-carrying stocks.

Raimondi, G. Lethality in a tumorous stock of D. melanogaster (tu-So^C).

an incidence of 33%. Counts of surviving larvae hatched from a fixed number of eggs (300 for each experiment) have shown without exception a death rate of 23% at a stage corresponding to a length of 2.5-3.5 mm. A certain amount of death has been detected also in the egg. Corresponding lethality fails to occur in the So^C stock without tumors. The relation between production of the melanotic mass and cause of death remains obscure.

In a stock marked by the character Sine oculis (So^C), melanotic masses have been found with an incidence of 5% in the adult stage, whereas larvae 2-2.5 mm long show

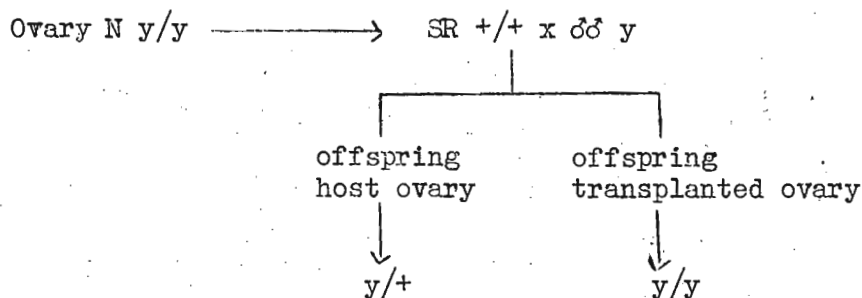
Rasmuson, B. Resistance to organo-phosphorous insecticides.

Four different strains of D. melanogaster have been found which show a high resistance toward insecticides of the organo-phosphorous type. Such resistance has not been reported previously. It was possible to increase the resistance by means of selection, which was performed during development by adding increasing amounts of para-oxon to the substrate. By now the resistant strains tolerate a concentration of 20-30 ppm, whereas two sensitive strains used as controls tolerate less than 1 ppm and have resisted all attempts to increase their tolerance. Insecticides of this type are known as cholinesterase inhibitors, and the cholinesterase activity of the flies has been determined to find out if the differences in resistance are due directly to differences in that enzyme system. This is not the case, as all the strains have identical enzyme activity. Radioactive parathion marked with S³⁵, has been used to study the uptake of the insecticide. This has been accomplished by means of the interrelations between radioactivity, enzyme activity, and LD-50. It was found that the LD-50 of the sensitive strains corresponds to a radioactivity of 30-35 c/min, whereas the resistant strains have a radioactivity of 125-300 c/min, when LD-50 is reached. All strains coincide in their enzyme activity,

which is inhibited to 25-30% by LD-50. As no other enzyme system is known which might cause different reactions to the organo-phosphorous insecticides, these are probably due to a metabolism of these compounds, which can be accomplished only by the resistant strains.

Rasmussen, Inge E. Ovary transplantation in "sex-ratio" stock of D. bifasciata.

In D. bifasciata an abnormal sex ratio showing cytoplasmic inheritance has been described (Magni, 1953). In order to test the possibility of transmitting the "sex-ratio" cytoplasmic particles from SR (sex-ratio) flies to N (normal) flies, ovaries marked with y from N larvae have been transplanted into SR wild larvae according to the scheme:



In 6 females the implanted ovary was functioning. The results are given in the table.

No.	Progeny			
	♀♀ +/-	♂♂ +	♀♀ y/y	♂♂ y
1	-	-	1	-
2	15	-	5	3
3	10	-	5	4
4	80	-	22	28
5	14	-	3	1
6	106	-	6	3

The offspring of the implanted N ovaries consisted of males and females, whereas the host ovaries produced only females. In order to control the sex-ratio condition of these flies, they were cultured singly. The females descending from the implanted ovary proved to be all N, the females from the host ovary all SR. As a reasonable conclusion, we exclude the possibility of infection of normal D. bifasciata with the sex-ratio cytoplasmic particle by means of ovary transplantation.

Röhrborn, G. Induction of pseudotumors in D. melanogaster by injection of acellular extracts.

On the basis of experiments by Harnly (1954), an attempt has been made to induce melanotic pseudotumors in 120-hour-old larvae of the strains "Berlin wild" and y w, by injecting the following

agents: (1) acellular extract of 120-hour-old larvae of the strain tu^g; (2) an analogous extract of the strain tu^{49h}; (3) an analogous extract of the pseudotumor-free strain ell; (4) an extract like (1) except that the larvae were reared on a dead-bakers'-yeast medium (designated as tu^{gx}); (5) Waddington's insect Ringer's solution. Since hereditary and induced pseudotumors

outlast pupal and adult stages as pigmented rest bodies, the adults were dissected, and the presence of observable pseudotumors was recorded. The search for pseudotumors was negative in the "Berlin wild" strain as host. On the contrary, as shown in the table, a considerable number of melanotic pseudotumors could be found in all experiments in which y w larvae were used as hosts. The results of experiments 1-3 are homogeneous ($P = 0.4$). The differences between the collective experiments 1-2-3-4 and 4-5 are significant ($P < 10^{-6}$ and $P = 0.0025$). In another control experiment it was attempted to induce pseudotumors by inserting the empty injection needle only. The outcome was negative. The induced pigmented growths correspond in their morphological characteristics to the hereditary melanotic pseudotumors in *Drosophila*, the so-called "Drosophila tumors." Thus the experiments show that pseudotumors in *Drosophila* can be caused by unspecific agents.

Expt.	Donor	No. adults dissected	% adults tumorous
1	tu ^g	72	77.8
2	tu ^{49h}	38	73.7
3	ell	38	65.8
4	tu ^{gx}	89	43.8
5	Ringer's solution	87	18.4

Sakai, Kan-Ichi, Hiraizumi, Y.,
Narise, T., and Iyama, S.
Experimental studies on migration in *D. melanogaster*.

By means of a set of four "population tubes" (see Technical Notes), migration of flies was studied with *D. melanogaster*. A certain number of flies of the Samarkand strain were kept for one day in a tube,

and then the tube was connected with three new tubes through three passages. The first series of experiments dealt with the relation between the number of flies that migrated and the length of time during which migration was allowed to occur. These experiments gave the following results:

Initial no. flies in original tube	No. of expts.	% flies migrating to new tubes after (hours)						
		6	24	48	72	96	120	144
100-149	5	1.84	0.95	2.25	3.32	5.52	6.51	7.21
150-199	4	14.66	11.02	13.31	14.51	20.95	22.47	25.01
200-249	4	14.92	14.47	15.41	18.47	23.50	24.59	24.00
250-300	5	19.59	21.35	24.39	27.55	29.90	32.46	32.91

It seems that a good deal of migration occurred within 6 hours, if the number of flies in the original tube was more than 150, although migration continued even after 6 hours.

The second series of experiments explored the problem of whether migration was dependent on the number of flies present in the original tube. In these experiments, number of migrating flies was counted two days after the new tubes were connected. The results of these experiments were as follows:

	Initial number flies in original tube					
	0-50	50-99	100-149	150-199	200-249	250-300
No. experiments	2	2	6	7	6	5
% migrating flies	5.88	0.65	1.89	23.47	26.49	24.93

The results indicate that migration occurred largely as the effect of pressure of population density, and that the critical population size was around 150.

Another experiment appeared to demonstrate that different species of *Drosophila* behaved differently with regard to the population density associated with migration.

Sandler, L. Additional evidence on the role of the centromere in determining disjunctional patterns.

From a cross of females carrying an attached-X chromosome, one arm of which was $y^+ \text{In}(1)sc^{S1} y \text{In}(1)EN$ and the other $y^+ \text{In}(1)sc^8$, and no homolog, by males carrying the YSX.YL, y B chromosome with

no homolog, a number of non-y, B males were recovered. The females had been X-irradiated with about 2000r. Although a stock was established from each of the recovered B males, in all but one case the normal allele of y segregated independently of the YSX.YL chromosome in males, suggesting that possibly one of the tips of the attached-X (including y^+) had capped an autosome; such stocks were discarded. In the one remaining line, on the other hand, the normal allele of y separated regularly from the YSX.YL chromosome in males, and from an attached-X chromosome in females, suggesting here that y^+ was present on a free centric chromosome fragment (designated FR-IV). The following data on the segregation of FR-IV have been collected: (1) the progeny from a cross of $y^2 su-w^a wa bb/FR-IV \times YSX.YL, y B/O$ included 317 $y^2 su-w^a wa bb$ females, 391 B males, 6 $su-w^a wa$ females, and 14 y B males; (2) from a cross of $y^2 su-w^a wa bb/O \times YSX.YL, y B/FR-IV$, 171 $su-w^a wa$ females, 224 y B males, and no exceptions were recovered. This regularity in the separation of FR-IV from the sex chromosomes parallels that of X-chromosome duplications generally. The following additional information about FR-IV has been obtained: (1) FR-IV carries bb^+ ; (2) it carries neither the *ci* nor the *ey* locus; and (3) translocation tests for linkage between y^+ and either chromosome II or chromosome III were negative.

From the nature of the cross from which FR-IV was recovered, and from the information about its composition, it appears very unlikely that the heterochromatic fragment carries a sex-chromosome centromere (for, indeed, no sex-chromosome centromeres were available unless a minimum of four breaks had been produced by the irradiation), and it seems exceedingly likely that the centromere involved comes from chromosome IV. Crossovers of essentially this type have been reported numerous times in the past. The origin of FR-IV can then simply be explained by supposing a heterochromatic eucentric exchange between chromosome IV and the sc^8 or sc^{S1} tip of the attached-X, yielding a chromosome of the composition: $y^+ sc^8$ (or sc^{S1}) bb^+ from the X chromosome, plus the basal region and centromere of chromosome IV. If this is so, then the regular separation of FR-IV from the sex chromosomes would support the idea that the centromere itself is not active in determining the patterns of segregation.

Sandler, I. Segregation in females heterozygous for T(1;4)B^S.

In order to determine the frequencies with which the various gamete types are formed in females carrying T(1;4)B^S and a normal X chromosome, females of the constitution

y w^a m f car/sc w^{e-2} cv/B^S were mated to males carrying T(1;4)B^S with the markers y sc cv m/B^S car/sc⁸.Y. Progeny resulting from this cross can be readily classified as to their zygotic constitution, and hence the constitution of the various female gametes that are formed can be determined. The evidence indicates that in parental females bearing the translocation and the normal X, gametes arise from alternate, adjacent I and adjacent II segregations. However, whereas in most translocations analyzed to date alternate segregation seems to occur with a frequency somewhat exceeding the sum of the frequencies with which adjacent I and adjacent II segregations occur, with adjacent II segregation occurring less frequently than adjacent I, estimations of the frequencies with which such segregations occur in a T(1;4)B^S heterozygote indicate that adjacent II segregation occurs with about the same frequency as does alternate segregation. Rough determinations of these frequencies are: alternate segregation, 43%; adjacent I, 18%; adjacent II, 38%. The complementary products resulting from each of the segregations are produced with the same frequency.

Shiomi, T. Lethal effect of C²¹ mutant of D. virilis.

Confluent-21 is an allele of C (2:45.0) found by Imaizumi among the progeny of X-rayed flies (Imaizumi, unpubl.). It

has been found that C²¹ has a recessive lethal effect at the egg stage. The normal embryonic development of homozygous C²¹ advances until about 12 hours after egg laying. Before germ-band contraction, lethal eggs show a delay in development. In lethal eggs the germ band never contracts, and gradually ceases to exist. This lethal effect is very interesting in connection with the mechanism of germ-band shortening in the development of *Drosophila* eggs.

Shiomi, T., and Kitazume, Y. Changes in glycogen content during early embryonic development in D. melanogaster.

The glycogen content in eggs of D. melanogaster, and its changes during early embryonic development, have been worked out. Five hundred dechorionated eggs of Oregon-R-S and of an attached-X strain

were used; glycogen determinations were made by the anthrone method. Results are given in the table. (U = unfertilized egg; C = stage of contraction after 30 minutes of egg laying; B = blastodermal stage; B N = normal blastema of attached-X strain; B L = Nullo-X embryos at the normal blastodermal stage; G = gastrulation stage.)

mg/500 eggs	Oregon-R-S				Attached-X			
	U	C	B	G	U	C	B	N L
Lyoglycogen								0.177
	0.268	0.187	0.167	0.152	0.250	0.185	0.185	
%	100.0	69.8	62.3	56.7	100.0	74.0	70.8	74.0
Desmoglycogen								0.019
	0.017	0.025	0.022	0.020	0.020	0.025	0.017	
%	6.4	9.3	8.1	7.5	8.0	10.0	7.6	6.8

As developmental stages proceed, there is a marked tendency toward decrease of lyoglycogen content, in contrast with desmoglycogen content. In the attached-X strain, Nullo-X embryos cannot develop to the blastodermal stage, and the glycogen content of these lethal embryos at the B stage of normal development remains unchanged from that at the C stage. It is considered that during the early embryonic stages lyoglycogen may be utilized for embryonic development.

*Steffensen, Dale, Fingerma,
Louis, and Anderson, Lulu F.

Failure to increase the recessive lethal frequency in *Drosophila* with ethylene diamine tetra acetic acid (EDTA).

The metal chelating agent, ethylene diamine tetra acetic acid, (EDTA) was added to food of *D. melanogaster*, presumably to bind calcium. The hypothesis was that mutational events might be induced, since calcium and magnesium deficiencies have been shown to produce

chromosomal aberrations in the plant *Tradescantia paludosa*. Wild-type males of an inbred Oregon-R stock were raised on EDTA food. Adult males were mated to Muller-6 females (y^2 In(1)sc⁸ dl-49 v w^a f). Female progeny were tested in creamers for recessive lethals. The table shows the results with five concentrations of EDTA in food (oatmeal, cornmeal, dried yeast, agar, Karo, and molasses) and as a control, food without EDTA. The pH of each food treatment was adjusted to 7.5 with KOH. A chi-square test showed no significant difference between the pooled EDTA treatments and controls.

Females should be grown on EDTA food to determine their frequency of lethals, because of the increased crossing-over response obtained with female larvae grown on EDTA food.

(Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

EDTA molarity in food	Number of lethals	Per cent lethals	No. of male chromosomes
.004	3	0.6	499
.005	0	--	1243
.006	0	--	1099
.007	0	--	646
.008	1	0.2	500
Pooled EDTA treatments	4	0.1	3987**
Control	6	0.3	2171

**Chi-square test between control and pooled EDTA treatments,
P = 0.3-0.2.

Strangio, V. A. Studies
on a wing mutant in *D.*
melanogaster.

In a stock derived from a single sepia-eyed female trapped in Queensland during 1951, a new character affecting wing morphology appeared during the following year. The new mutant resembles blistered (bs) and balloon (ba) in that the gross structure is deformed by the production of fluid-filled

bubbles during the process of wing expansion on emergence from the pupal case, but gene expression is more extreme than in either published account. Penetration is complete at the three experimental temperatures--20°, 25°, 30° C. Expressivity varies, ranging from a small blister at the tip of the fifth longitudinal vein to the extreme case in which the whole wing is converted to a fluid-filled sac. Wings with small blisters display a variable degree of plexus formation and extra venation, especially in the submarginal and second posterior cells. A marked temperature sensitivity is apparent: at the higher temperature (30° C) blistering is reduced; whereas at 20° C all wings have warped surfaces, heavy chitination of the intervein material, and chaotic venation. Semidominance, manifested at 25° C in the female heterozygote alone as small vein-flecks at the posterior crossvein and along the second longitudinal vein, is extended at 20° C to both male and female. A striking interaction of the heterozygote with the third-chromosome dominant Delta (Dl³) produces in the majority of cases a single large blister in the center of the wing, presumably due to a threshold effect. Cross-over data placed the mutant locus in the second chromosome, slightly to the left of blistered and balloon; and, pending the results of allelism tests, the term edematous wing (ew) has been adopted. Further tests of fertility, fecundity, relative viability and temperature-effective period in relation to gene action during development are in progress.

Taira, T., and Nawa, S.

Quantitative measurement of riboflavin, folic acid, and uric acid during metamorphosis of some mutants in D. melanogaster.

A working hypothesis that the red eye pigments of D. melanogaster are probably the derivatives of pteridine has been proposed by H. S. Forrest and H. K. Mitchell, by E. Hadorn et al., and also by us. The process of change of riboflavin, folic acid, or uric acid to pteridine derivatives has

been investigated.

The quantity of riboflavin and folic acid during the metamorphosis of v, bw, and se strains was measured by the method of bioassay. The results are shown in the first table below (r/mg; PR = prepupae, PS = postpupae).

	v		bw		se	
	PR	PS	PR	PS	PR	PS
Riboflavine	10.0	10.0	20.0	18.0	70.0	120.0
Folic Acid	.75	.60	.80	.65	.60	.70

As the table shows, no definite change in amount of folic acid during metamorphosis was observed. As regards riboflavin, no definite change in amount during metamorphosis was seen in the v and bw strains. The se strain contains much more riboflavin than the other strains, and its increase from the prepupal stage to the postpupal stage is quite significant.

We also measured spectrophotometrically, using uricase, the micro-amounts of uric acid during metamorphosis of strains Oregon-2, v, se, sed, bw, and w. These are shown in the next table (r/mg).

(See table on following page.)

	3rd-instar Larvae	Prepupae	Postpupae	Young adults
Oregon-2	.47	3.00	3.80	2.30
v	.35	2.30	3.00	1.90
se	.42	2.00	3.00	1.90
sed	.25	3.50	4.70	3.60
bw	.54	3.40	4.70	2.60
w	.27	2.30	3.90	5.30

These results seem to show that the formation of uric acid is independent of the increase in amount of pteridine during metamorphosis. Therefore we seem to be justified in concluding that the amount of none of these substances parallels the production of pteridine.

8. Flies of the bw stock, grown on a culture medium with an excess of N⁸-azaguanine, which inhibits the metabolism of purines, have a larger amount of isoxanthopterin than do flies cultured on normal medium.

These evidences suggest that the precursor of pteridine is some kind of purine. Further details concerning this problem will be published elsewhere.

Takada, H. Notes on the vertical distribution of Drosophilidae.

This survey was made on Mt. Rishiri (altitude, 1719 m.) on a small island located in the northern part of Hokkaido, in August 1956. Three traps baited with

fermenting banana and tomato were set up at each altitude. The scheme of the collection and the results are summarized in the table.

Species	Altitude (m.)							
	0-100	100-300	300-500	500-700	700-900	900-1100	1100-1300	1300-1719
Parascaptomyza disticha	2							
Hirtodrosophila sp.			1		4			
Drosophila coracina	1							
D. testacea	3	5	16	24	47	30	23	16
D. bifasciata	53	59	70	43	15	11	2	
D. nigromaculata	3	2	1		3			
D. transversa (III)	1	1						
D. sp. (quinaria gr.)	1	1						
D. histrio	2	1	2	2				
D. immigrans			1					
D. funebris	4							
D. busckii	2							
D. sp.				1				
D. sp. (obscura gr.)	3	4	2					
Total	75	73	93	70	69	41	25	16

0-1100 m., forest zone; 1100 m-1300 m, shrub zone; 1300 m-, alpine plant zone.

Tattersfield, F. Resistance to insecticides.

The John Innes stock has been used for some years in investigations at Rothamsted upon factors that influence resistance to insecticides. It has been

shown that (1) resistance to DDT is increased by the addition of yeast or of Casein to the food medium; (2) population pressure on food supplies affects resistance in the adults--high populations cause a decline--but the pattern of resistance differs between the two sexes; (3) high population density in the larval stage affects the weight of the adults, but resistance to DDT is not necessarily correlated with the size of the insect.

Thoday, J. M. A line responding to selection in one direction only.

One of our lines under selection for sternopleural chaeta number has given results showing that a population can be heterozygous for loci that, though readily

exploited by selection in one direction, cannot be exploited by selection in the opposite direction. The line is homozygous *dp* and is maintained as four single-pair cultures in each generation. The four cultures are kept as one population by a rotational mating system. The line began with 19.3 chaetae per fly and was selected for high chaeta number. The mean rose steadily to 24.3 chaetae at generation 21, and then rapidly to 28 at generation 24, then more slowly to a plateau of 30 chaeta at generation 30. It is now at generation 50.

The main interest lies in a subline taken out at generation 9 when the mean was 22.6 chaetae. At this point, of course, the main line was still heterozygous for the loci that ultimately raised it to 30 chaetae. The subline was selected for low chaeta number for 15 generations and showed no response to this back selection. It was then (generation 24) selected for high chaeta number, and its subsequent history was almost identical with that of the main line. It rose steadily to 24.3 chaetae at generation 35, rapidly to 28 at generation 42 and more slowly thereafter. It is now at 29 chaetae and may have reached a plateau.

It is clear that the subline, despite the progress made in the first 9 generations, was, during the period of back-selection, heterozygous for loci that could be exploited to raise chaeta number but could not be exploited to lower it. The observation is relevant to interpretations of genetic homeostasis.

Thompson, Peter E. A bilateral mosaic gynandromorph with second and third chromosomes marked.

From the cross of an *Ins(2)SMI, al Cy sp²/Ins(2)Pm, dp b Pm ds^{33k}; Ubx¹³⁰ e^s/C Sb* female with a *dp/dp* male, an offspring was obtained which was gynandromorphic and a bilateral mosaic for markers on each of

the second and third chromosomes contributed by the female parent. The left side of this individual was Curly, non-Plum, non-dumpy, Stubble, and non-Ultrabithorax, and lacked sex combs. The right side was Plum, dumpy, non-Curly, Ultrabithorax, and non-Stubble, and had sex combs. The left side of the abdomen was distended, but the genitalia appeared to be entirely male. Mating behavior was apparently male, although actual copulation with females was not observed. This individual is believed to have arisen by double fertilization of a binucleate egg.

Tinderholt, Victor E., and Hinton, Taylor. The ability of heterochromatin to produce position effects when removed to a new location in the chromosome.

In(2LR)40d had a region of heterochromatin from the base of 2R placed in the 27 region of 2L. This arrangement produced a position effect upon the eye. From this arrangement, In(2LR)ICQ was derived. It has the heterochromatin in question located between regions 23A and 23B and

lacks the eye effect. The question was asked, does this heterochromatin exert an effect on the wild-type alleles of any of the genes in region 23. To test this, as many recessives as possible, suspected of being in the area, were collected and crossed to In(2LR)ICQ. To date, positive results have been obtained in one case. The gene "rubroad" (rub), whose phenotypic expression is broadened and shortened wings and which is at 5.0+ on the linkage map, consistently shows a broadened and shortened wing when heterozygous with In(2LR)ICQ. The expression is less than that of the homozygous recessive mutant. In all cases, series of wings were permanently mounted on microscope slides and measurements made. The genotypes rub/+ and +/In(2LR)ICQ give a wild-type wing. This shows that heterochromatin removed to a new location in the chromosome can still act to produce V-type position effects. It also locates the gene rub on the salivary chromosome as being to the right of and near 23B.

Toyofuku, Y. Chromosomal polymorphisms found in natural populations in Hokkaido.

An investigation was made of polymorphisms of the salivary-gland chromosomes in natural populations of Hokkaido. Eleven different kinds of chromosomal variations

represented by heterozygous inversions were found in nine strains of the following six species: D. bifasciata, D. immigrans, D. melanogaster, D. nigromaculata, D. sordidula, and D. virilis. Six of these types were found in D. immigrans collected in the University Botanical Garden at Sapporo; in all the specimens the aberrations occurred in one or two chromosomes.

Ulrich, Hans. Effect of oxygen on the mutagenic action of X-rays on uncleaved Drosophila eggs.

Drosophila eggs of wild-type females mated to Muller-5 males were X-rayed at the age of 10-20 minutes, in air or in nitrogen, with the same dose of about 1500 r (50 kv, 10 ma; FD, 50 cm; time of exposure, 192

sec.). Each surviving F₁ female adult was mated to a single F₁ male derived from an untreated F₁ egg. Each F₂ offspring should consist of +/+ females, M5/+ females, + males, and M-5 males. Absence of + males or M-5 males indicates the induction of a sex-linked recessive lethal in the normal X or the Muller-5 X of the irradiated F₁ egg from which the F₁ mother in question developed. The results summarized below demonstrate that absence of oxygen during irradiation reduces the mutagenic action of X-rays.

Treatment	No. of eggs	Hatching larvae %	Surviving adults %	No. of F ₂ cultures with more than 20 adults	No. of F ₂ without (or with single) + males	No. of F ₂ without (or with single) M-5 males	Sex-linked recessive lethals %
X-rayed in air	8611	5.4	2.8	112	5	8	5.8
X-rayed in N ₂	3495	21.7	14.4	221	9	8	3.85
Control, untreated	1200	93.5	87.0	100	-	-	0.0

Van Alten, Pierson J. The induction of sex-linked recessive lethals by high-energy electrons.

The following data are of interest in connection with the induction of sex-linked lethals by high-energy electrons. The source of electrons was a General Electric million-volt resonant-transformer

type of electron-beam machine, with an energy range of 0-1 Mev., with a root mean square of 0.707 Mev. Adult (Oregon-R inbred) males, 4 days old, were irradiated and immediately mated to Muller-5 ("Basc") virgin females; and after 96 hours these parent flies were removed from the bottles. The results suggest that the rate of mutations to dose was not linear as with X-ray-induced mutations. At higher doses (3000, 4000, and 5000 rep.) the high-energy electrons seem to be less effective than X-rays.

Dose in rep.	No. treated males	No. X chromosomes tested	No. lethals	Per cent mutation
0	40	838	6	0.7
1000	30	608	26	4.3
2000	8	129	7	5.4
3000	31	341	26	7.6
4000	44	355	36	10.1
5000	20	48	8	16.6

(I wish to thank Dr. A. S. Fox for his suggestions and help with this study, and Mr. D. E. Wiant and Mr. R. Nicholas of the Department of Agricultural Engineering for operating the electron beam machine.)

Wakahama, K. A new type belonging to the genus *Amiota*.

Thirty flies belonging to this type were collected at Numanohata in Hokkaido, by means of banana traps, in June 1954. The

presence of milky white areas at the wing base and humerus is characteristic of the subgenus *Amiota*. External characteristics closely resemble *A. gigantia* and *A. leucostoma*; but there are clear differences in the shape of the clasper and the genital arch. A brief description of the male genitalia follows: Genitalia, very large. Genital arch, broad and roundish below. Anterior margin sinuated a little; posterior margin stair-like; heel, right angle. About 26 bristles on lower and middle portions; upper portion has about 9 bristles. Anterior margin and lower posterior margins chitinized. Anal plate separated from arch and very small, with dense bristles. Clasper, one; primary teeth, about 10 arranged in a straight row, with the first one or two shorter. There are about 5 thin bristles above the teeth, also a number of thinner hairs at the outer lower corner.

Welshons, W. J. Dosage experiments with split mutants in the presence of an enhancer of split.

Dr. M. M. Green was kind enough to send the author an enhancer of the sex-linked recessive split. The enhancer is associated with T(2;3)Xa. It was utilized in the performance of some dosage experiments,

which are reported here in a preliminary form. When the enhancer is present in females heterozygous for split, the flies have a phenotype quite similar to spl/spl. Hemizygous split males have a very extreme split phenotype when the enhancer is present; homozygous split females show the same extreme expression of split in the presence of the enhancer. These findings were conveyed to the author by M. M. Green in a personal communication. Since one split allele and

wild allele of split yielded a split phenotype in the presence of the enhancer, it was of interest to see if the effect could be eliminated by the addition of another wild-type allele. Therefore, homozygous $Dp(1;1)Co$ females were crossed with $y^2 su-w^a w^a spl; Xa-En-spl$ males. The F_1 females, $Dp(1;1)Co/y^2 su-w^a w^a spl; Xa-En-spl$, had one mutant allele and two wild alleles of split in the presence of the enhancer. They had a split phenotype, which was similar to that of homozygous split females.

The $Dp(1;1)Co/y^2 su-w^a w^a spl; Xa-En-spl$ females were then crossed to $y w^{def} rst^3; Dp(1;2R)w^{51b7}$ males (Lefevre, DIS-26). The $y^2 su-w^a w^a spl/y w^{def} rst^3; Xa-En-spl$ females with one wild and one mutant allele were compared with $y^2 su-w^a w^a spl/y w^{def} rst^3; Xa-En-spl/Dp(1;2R)w^{51b7}$ females which had two wild-type alleles and one mutant allele. It could be seen by this comparison that the females with two wild alleles were less extreme in phenotype than those with only one wild allele, although in both cases the expression of the mutant phenotype was within the range of expression found in spl/spl females. Because the addition of an extra wild allele does not greatly alter the expression of the phenotype when the enhancer is present, the effect was not noticed in the initial cross.

As might be expected from the above cross, $y^2 su-w^a w^a spl; Xa-En-spl/Dp(1;2R)w^{51b7}$ males with one wild and one mutant allele are less extreme in phenotype than $y^2 su-w^a w^a spl; Xa-En-spl$ males which have no extra wild allele present.

In previous experiments with different N/spl heterozygotes it had been observed by the author that the pseudodominant expression of spl is somewhat reduced as compared with that of spl/spl homozygotes. This is not the case with N/fa heterozygotes, in which the expression of facet is more extreme than in homozygous facet flies. In the one case tested so far, $N^{Co}/spl; Xa-En-spl$, as expected, was less extreme than $spl/spl; Xa-En-spl$.

These results can be understood if the mutant allele split is a mutant by virtue of the fact that it causes to be produced some substance which is different than that substance produced by the wild allele, and if the wild-type and split alleles are in competition for the same substrate. Then, if the production of a split substance is enhanced, the production of a + substance is indirectly inhibited. Conversely, an enhanced production of the + substance caused by the addition of wild alleles would inhibit the production of a split substance.

Widmer, Elmer Andreas. A rediscovery of the original rp mutation in D. melanogaster Oregon-R-C stock. (Master's thesis).

A study was made of the rotated genitalia in Oregon-R-C males. Investigations involved fertility, effects of temperature, mode of inheritance, location of the gene responsible for it in its proper linkage group, its locus on the chromosome, and its relationship to the original rp mutation described in 1929.

Matings between rotated-genitalia males and Oregon-R females proved that only males with a degree of rotation between 10° and 60° are fertile. The appearance of the mutant is correlated with temperature; it has a much reduced penetrance at low temperatures, and its penetrance increases progressively with an increase of temperature from $18^\circ C$ to $26^\circ C$. Flies with a 90° rotation were the most numerous in cultures kept at $26^\circ C$. The degree of

rotation was determined by means of an ocular protractor.

The gene for this condition is carried on the third chromosome at approximately locus 95.7. Because of reduced penetrance this position must be considered as only approximate. Tests with the original *rp* mutation (1929) suggest that the two mutant genes are the same. They agree in being recessive and on the third chromosome, but differ in their position on the chromosome.

TECHNICAL NOTES

Annan, Murvel E. A method for collecting eggs from individual *Drosophila* females.

Individual *Drosophila* females were placed with two males in 3/4-ounce creamers, which were closed with regular cardboard caps. Large paper straws (1/4 inch in diameter) were cut into lengths of approximately three inches. A length of straw was first dipped in water and then forced into a dish of standard cornmeal-agar-molasses food medium of sufficient depth so that when the straw was withdrawn it would be filled with food for at least an inch. The end of the straw containing the food was then sliced at an angle. Varying the angle of slice would vary the amount of food surface exposed. The knife or scalpel used must be sharp. It was found that the slice was most readily executed when the straw was held on end--food end down. The other end of the straw was flattened and folded to effect a closure. Identification data were written on the folded end of each straw and on the creamer cap with a copying pencil. The exposed food surface was lightly seeded with yeast and then inserted through a 1/4-inch hole punched in the cardboard creamer cap. A standard paper punch was of the proper size. The caps were secured by application of two or three spots of Duco cement. The brood chambers were stored in an upright position (for economy of space) in 6 x 10 inch baking pans. The pans were placed in an incubator at 25° C, in which a high humidity was maintained to prevent drying and shrinkage of the food. The straws could be changed as often as the investigator wished. This method was effective in minimizing the escape of flies while changing food straws.

Bennett, Jack. Inexpensive population cages.

In this laboratory (Department of Genetics, University of Wisconsin) we have been successfully using population cages made from 1 1/2-pint polyethylene refrigerator boxes (as Montgomery Ward, cat. no. 86C4578D, 12 for \$2.89). These boxes have 4-inch square tops, are 4 inches high with tapering sides, and feature a tight-fitting interlocking cover. Four holes are punched in each of two opposite sides, and one for ventilation in the top. The holes are a very tight fit for our 25 x 95 mm shell vials, which with food are used to fill all the side holes; the top hole is plugged with cotton for ventilation and can be used for a collecting vial to extract samples. Extra cotton-plugged ventilation holes may be necessary with some species, to keep down internal humidity. Food vials are changed one at a time

at five-day intervals for D. robusta, D. virilis, and D. melanogaster, and all three seem to do well. Obviously, more holes could be added or larger boxes used if a cage with greater capacity were needed.

Brosseau, G. E., Jr. Fast green as a useful counterstain for neuroblast and salivary smears.

Zeilinga (1956) has reported the use of fast green as a counterstain for aceto-orcein squash preparations in plant material. The green counterstain improves contrast and inhibits fading of the

orcein. His procedure was successful on *Drosophila* smears. In salivaries the nucleolus stains a clear, pale green; but inconsistent results were obtained with the nucleoli in neuroblast preparations. The green background and improved contrast do facilitate screening of brain preparations for division figures. The following procedure was successful. The dissected salivary glands or neuroblasts are fixed in 3:1 acetic acid-alcohol and then placed in 2% aceto-orcein in 70% HAC. After staining is complete, the excess stain is removed and the tissue gently blotted with Kleenex to remove any remaining stain. The tissue is then placed on a clean slide in a drop of 0.1% fast green in 45% HAC and allowed to remain for about 1 minute. Then the material is squashed in the fast green by the usual method. The preparations may be sealed with wax or made permanent by any of the common techniques.

Burton, L., and Friedman, F. A technique for the tissue culture of *Drosophila* tumors.

This communication describes the preparation of a nonsynthetic culture medium and its successful application in the culturing of *Drosophila* tumors.

All materials utilized in the preparation of the tissue cultures are autoclaved for 30 minutes. The tissue culture medium is prepared as follows. A sample (5 grams) of fresh 96-hour larvae (a genetically tumor-free strain) is ground in a mortar and suspended in 15 ml of Ringer's solution. This mixture is centrifuged at 35,000 x g for 30 minutes at 0° C in a Spinco refrigerated centrifuge. The resultant supernatant is then filtered through a Swinny filter.

All culture procedures are conducted in an enclosed hood, which has been washed with 70% alcohol and bathed in ultraviolet light (6 hours). The larvae containing pigmented tumors are washed in four consecutive baths of Ringer's salt solution, three baths of 70% alcohol, and a final bath of sterile Ringer's solution. The tumors are carefully removed without injury to the larval gut (a tear in the larval gut could liberate yeast cells and bacteria which might contaminate the culture). The liberated tumors are drawn into a constricted glass micropipette. The tumor is lodged at the constriction in the pipette, and is washed by drawing sterile Ringer's solution through the pipette. The tumor is placed, with a drop of sterile Ringer's solution, upon a cover slip. This fluid is withdrawn and a drop of the culture medium added carefully (to prevent the tumor from floating). The cover-slip is inverted over a depression slide, and the culture is sealed with liquid paraffin. The hanging-drop culture is incubated at 25° C.

After one week of incubation, the tissue culture can be opened, the old culture medium removed, and fresh medium added. Through the utilization of these techniques, *Drosophila* tumors in culture, with and without normal tissue, can grow.

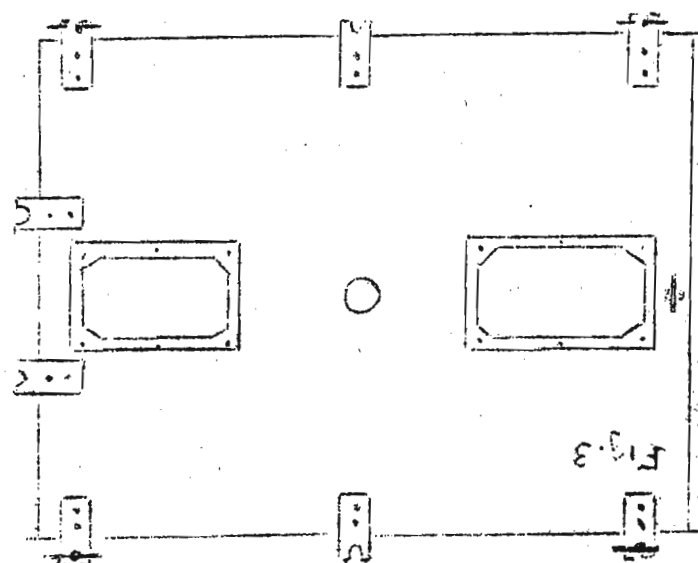


FIG. 3

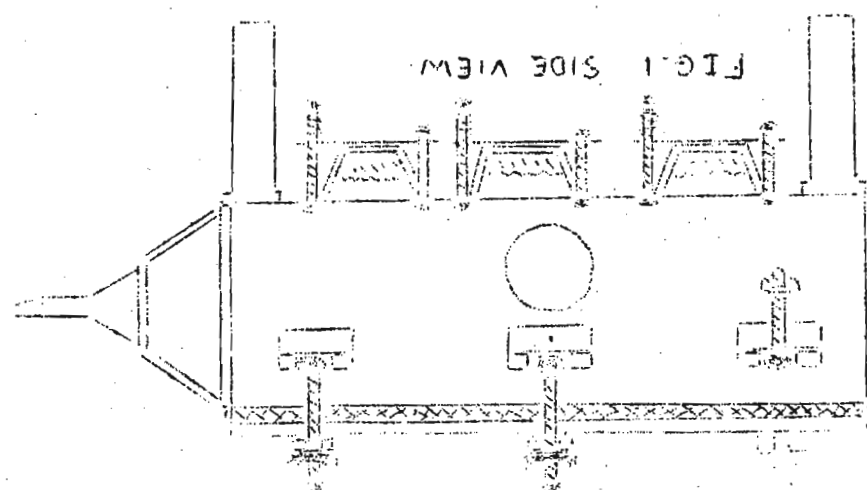


FIG. 1 SIDE VIEW

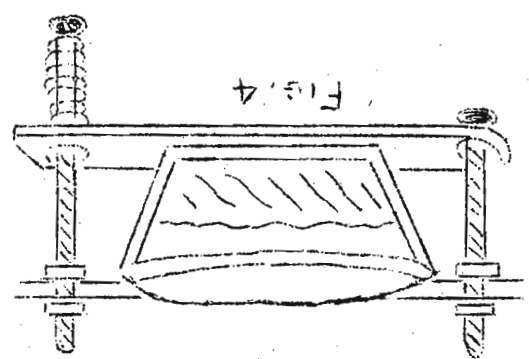


FIG. 4

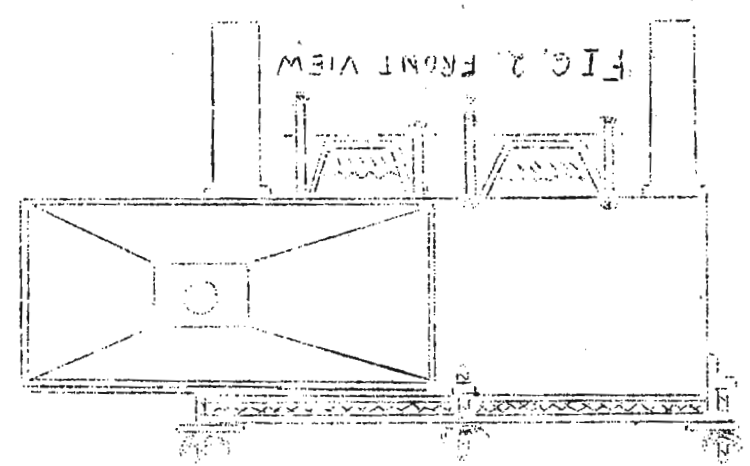


FIG. 2 FRONT VIEW

Friedman, Frank, Ostrander, Frank, Burton, Lawrence, and Solomon, Stanley. A technique and collection cage for the acquisition of large numbers of specific-age larvae.

The acquisition of large numbers of larvae of a specific age is vital to certain experimental procedures. To meet this need, a collection cage was built to provide a continual supply of specific-age larvae.

The method of collection is as follows. The cage is sealed, and stenders of media are fitted into place (figs. 1 & 4). A funnel is inserted into the hole in the top of the cage (fig. 3, top view of upper plate) and adult flies are passed through. After the flies have been placed in the cage, the funnel is removed and the hole stoppered. After a given egg-laying period, the stenders are removed and replaced. The egg-laden stenders are placed in a 25° C incubator for the desired developmental time. Flies may be removed from the cage, without etherization, and collected. This is accomplished by replacing the blank panel by a funnel panel (fig. 2) and forcing the flies through the funnel. The cage may then be dismantled, cleaned, and immediately readied for use.

The cage is constructed of 1/4-inch plexiglas. The L-shaped brass brackets are notched to accommodate a 10-32 Fillister head screw, with wing-top nuts. These brackets are fitted atop the upper plate (fig. 3). The cut-outs atop and in the sides of the cage are fitted with 60-mesh wire screen by means of aluminum panels (figs. 1 & 3). A gasket (rubber weather stripping, 5/16" x 3/8") is fitted to the upper edges of the cage, so that a tight seal is insured when the upper plate is fixed to the cage. Holes (2 1/2") are cut into the bottom plate and counterbored from the under side to accommodate stenders (62 mm diameter). These stenders are held in place, and rest upon an aluminum strip (fig. 4). The pressure of the compression spring upon the metal strip keeps the stender firmly seated (fig. 4). Stenders are removed by swinging the metal strip outward, and new stenders are seated into place by hooking the metal strip onto the supporting screw (fig. 4).

Dimensions and other details for the construction of this collection cage will be furnished upon request.

[The accompanying mimeographed illustrations were kindly supplied by the authors.]

Kuroda, Y. Synthetic medium for the tissue culture of *Drosophila*.

Although a brief description of this technique for tissue culture was given in DIS-28 (p. 127), some improvements have been made in the synthetic medium to

obtain better growth of tissues, and detailed directions for preparing the synthetic medium are given below. The ingredients were dissolved separately to make the following ten stock solutions, which were stored at 0° C.

1. Stock solution A: NaCl, 14.0 gm; KCl, 0.4 gm; CaCl₂·2H₂O, 0.04 gm; MgCl₂·6H₂O, 0.2 gm; NaHCO₃, 0.1 gm; NaH₂PO₄·2H₂O, 0.4 gm; glucose, 1.6 gm; water to 100 ml.

2. Stock solution B: Casein hydrolyzate, 10.0 gm; tryptophan, 0.2 gm; cystine, 0.2 gm. These substances were dissolved by gently heating in 100 ml of 0.075 N HCl.

3. Stock solution C: Cysteine, 52 mg; water to 10 ml.

4. Stock solution D: Thiamine, 1.0 mg; riboflavin, 1.0 mg; pyridoxine HCl, 2.5 mg; niacinamide, 2.5 mg; Ca-pantothenate, 1.0 mg; water to 1000 ml.
5. Stock solution E: Ascorbic acid, 50 mg; water to 10 ml.
6. Stock solution F: Choline HCl, 50 mg; inositol, 5 mg; p-aminobenzoic acid, 5 mg; water to 1000 ml.
7. Stock solution G: One-hundredth ampoule of vitamin A palmitate (30 mg in 1 ml water); water to 3000 ml.
8. Stock solution H: One ampoule of vitamin B₁₂ (10 µg in 1 ml water) was used as it stood.
9. Stock solution I: Sodium acetate, 10 mg; glutathione, 2 mg; glutamic acid, 20 mg; water to 10 ml.
10. Stock solution J: DPN, 2 mg; water to 100 ml.

The culture medium was prepared by mixing together equal proportions from the above stock solutions, just before using. After the mixture had adjusted to pH 7.2, 0.5 mg/ml of PNA (from yeast) was added. This culture medium was sterilized by filtration through a Seitz filter.

Lewontin, R. C. A simple technique for measuring egg laying.

The egg-laying surface is made from boiling cornstarch and water. Use the recipe for thick cornstarch which is printed on the back of the box. Enough carbon black is added to the hot mixture to make it quite black. The medium is spread on the surface of ice-cream sticks, which can be purchased in lots of 10,000 for about \$8.00. This is an extremely inexpensive, disposable carrier for the medium. Spreading the food on sticks is best done by a plastic mustard dispenser of the flexible polyester type. The aperture in the nozzle should be slit so that a slight pressure on the container will disperse a ribbon of cornstarch medium of the right width onto the stick. Several hundred sticks can be prepared in a short time, and if kept in a moist container in the refrigerator they will keep for several days. The blackened cornstarch provides a smooth flat surface, making visibility of the eggs good without refocusing of the microscope. The linear arrangement of the medium on the stick reduces errors in counting, as the sticks may be moved from left to right in a continuous motion without having to retrace steps.

Nawa, S., and Taira, T.
Simple microdetermination of uric acid by using uricase fermentation.

Fifty individuals of *D. melanogaster* at various stages are used as material. After the material is homogenized in a solution of 5 ml glycine buffer (pH 9.4), it is boiled in a water bath for 5 minutes at 100° C. After cooling, the solution is centrifuged at 4000 rpm for 2 minutes. Ether is added to the supernatant, and centrifuged. As a measurement solution, 4.5 ml of 4 mg crystalline uricase dissolved in 100 ml of glycine buffer is used. A sample of 0.5 ml of the test solution is measured spectrophotometrically at wave length 292 mµ, and incubated at 37° C. After 4 hours, the solution is again measured spectrophotometrically. Thus, the amount of uric acid is calculated from the value of standard fermentation of uric acid by using the same uricase solution.

Oftedal, Per. Handling
radioactive flies.

When measuring radioactivity in flies it is usual to put the fly or flies in a small gelatin capsule, which is then

placed under the counter. In some experiments it has been found desirable to make several measurements per day, and in this case the many etherizations necessary in order to place the flies in the capsules and remove them becomes the limiting factor to the study of normal physiology.

To avoid these etherizations, the following two little gadgets have been designed (figs. 1 & 2). Figure 1 shows the apparatus for putting flies into the capsule. It utilizes the same principle as was recently recommended by King and Wilson (J. Exptl. Zool. 130, 1955). The procedure is as follows. One half of the capsule is placed in position I. The vial is next inverted over the funnel, and the fly shaken down into the capsule. The knob of the stopcock (A) is turned, thus confining the fly to the lower compartment. The other half of the capsule is inserted (II), the stopcock is opened, and the upper half of the capsule is pushed home. After it is ascertained that the capsule is really closed, it is pushed out with a piece of blunt 1.5-mm wire and is ready for the counter.

To remove the fly from the capsule to the vial, the apparatus in figure 2 is used. The apparatus is put on top of the vial, the capsule is inserted in the hole across the top (B), and the entrance is stoppered (C). With two needles, one straight and one hooked, the two halves of the capsule are pulled apart through the slit in the top, and the fly is shaken down into the vial.

The two gadgets are best made from Perspex, which can be polished so that one can see the flies at all stages of operation. Special care should be taken while drilling the holes, to avoid blistering. The apparatus should be made as light as possible for ease in handling, but at the same time the walls should not be thinner than 5-7 mm, to give protection against soft beta emitters, and even a certain amount against hard beta.

The apparatus may be of help even in handling nonradioactive flies which should not be etherized. With slight modifications--e.g., one or more extra holes (F) in the stopcock--it should enable one to separate pairs of flies, etc., as well.

Thanks are due to our health physicist, Dr. Per Grande, for helpful discussion.

(See figures 1 and 2 on following page.)

Oftedal, Per. Measuring the volume of injection needles.

It is somewhat difficult to calculate the volume of the glass injection needles used in *Drosophila* work on the basis of measurements under the microscope, because the internal diameter of the needle is hard to determine. One can measure the diameter of drops of liquid expelled into another liquid, the two being immiscible. But here, again, one may find measurement difficult, since the drops are rather larger than is suitable for that method. This note has the purpose of bringing to the attention of *Drosophila* workers the possibility of using tracer methods for volume measurements. Even if the counting equipment is not available in most genetics institutes, the necessary apparatus will be found in almost any chemistry or physics laboratory. The procedure is simple and involves very little work.

The volume in question is measured out from a very weak solution of some radioactive isotope, preferably a fairly high-energy beta emitter, e.g., P^{32} . It is expelled into a drop of water on one of the usual plaquettes used in most scaler assemblies. Thereafter the solution is diluted 1:100 or 1:1000, and a known volume--e.g., 5 μ l--of this solution is pipetted onto a similar plaquette. After evaporation to dryness, the plaquettes are placed under the G.-M. tube for assay of radioactivity. A comparison of the two activities, corrected for the relevant dilution factor, gives the volume with a high degree of exactitude.

To minimize inaccuracies due to adsorption to the pipette walls, the solution used should contain inactive carrier isotope. If one tries to expel from the pipette onto the dry plaquette, one often finds that the drop creeps up along the outside of the pipette instead of settling on the plaquette, thus yielding inaccurate measurements.

Paik, Y. K. An improved technique, using the phase microscope, for studies of male genitalia in *Drosophila*.

Since Salles (1948) reported an effective technique for making preparations of male genitalia, it has been widely used among the workers in this field. However, as examination of some details is unsatisfactory and cumbersome in preparations made by Salles's technique, it was modified by Malogo (1952), who reported a coloration method for better examination. His preparations were made by Salles's technique and were stained with safranin of Johansen. This method was found to be effective in showing better contrast between the minor structures of the genitalia, but it was apt to bring about distortion or some damage to the genitalia. An improved technique which we have found is based on some modification of Salles's preparation technique and the use of the phase microscope. It is described below.

(1) Preparation technique: (a) Separate the terminalia in phenol. (b) Boil in an adequate amount of 10% sodium hydroxide on a slide glass. (c) Clarify in phenol. (d) Let the genitalia stand in creosote for more than one hour. (e) Mount in Diaphan. (f) Examine the preparations under the phase microscope.

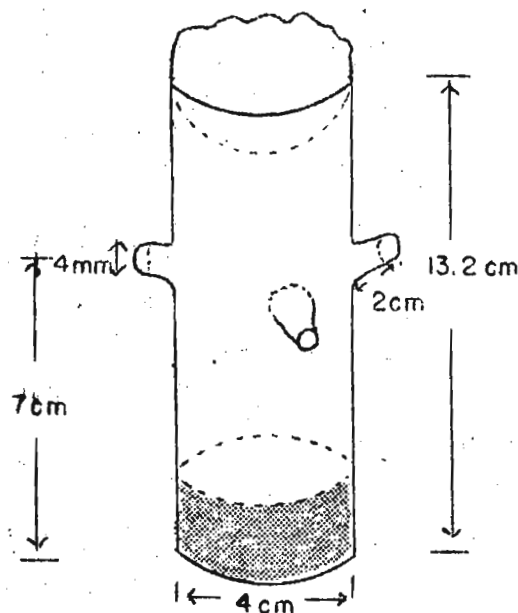
(2) Observation: For observation of the preparations, we used a phase-contrast microscope (Olympus Optical Co.). Among the objectives, we found that Dark (A-) contrast-Low (PL 10x in our microscope) gave the best results for examining and drawing the genital structures. For illumination we used a 6 v, 5 A transformer and a ground blue filter. Use of the phase microscope with the above-described preparation technique gave us excellent results.

This technique is much better than any other, with or without staining, in terms of showing clear-cut contrast between structures, and of avoiding distortion or damage to the genitalia. We found it completely satisfactory for purposes of examining or drawing the genital structures.

Sakai, Kan-Ichi. "Population-tubes": an apparatus for the study of population genetics in *Drosophila*.

This is an apparatus devised for the study of population genetics in *Drosophila* and allied insects. It consists of a variable number of unit tubes, each 4 cm in diameter and 13.2 cm in height.

The tube has a bottom, and contains an appropriate amount of food. The other end is stuffed with cotton. The characteristic feature of this tube is its three radial branches, 4 mm in diameter, protruding from the side.



Connecting these unit tubes with each other by short small tubes of vinyl resin, we can investigate changes in an insect population due to migration, competition, and so forth.

Travaglini, E. C. A method for collecting and counting large numbers of *Drosophila* eggs.

The following method, devised at the suggestion of Dr. Jack Schultz, makes it possible to obtain approximately 2000 eggs from each collection box at three-hour intervals during a two-week period.

Two hundred and fifty to three hundred females with approximately twice that number of males are placed in cotton-stoppered, quart Mason jars equipped with stainless steel trays, 5 x 2 x 3/8 inches in size. These trays contain portions of a molasses-agar medium made from 420 ml molasses, 35 ml Moldex, 2800 ml water, and 70 gm Bacto-agar, upon which two lumps of yeast approximately 1 cm in diameter are spaced. In this way, the females are fed adequately for as much as a two-week collection period, and continue to lay well for most of this time.

For collection, the bottom of the jar is gently tapped on a rubber pad so that the flies fall to the bottom; the tray upon which the eggs were laid is removed and a fresh one inserted in one operation. Since very little egg laying occurs the first half-hour after a new tray is placed in the jar, two hours is taken as a minimum period when large numbers of eggs are desired. The eggs are brushed off the molasses-agar with a soft camel's-hair brush into a Petri dish containing 70% alcohol. By gently swirling the dish, the eggs are concentrated at the center of the dish and the adherent medium washed away. This process is repeated with fresh 70% alcohol until all the contaminants are removed. Then the eggs are transferred to a 3:1 alcohol-ether solution contained in a vial, which is immersed in a dry ice-acetone bath. After the eggs are frozen, they are stored at -40°C until used.

For counting the eggs, the following method was devised with the help of Dr. Jerome J. Freed. Before being transferred to a vial for freezing, the eggs are dispersed in a single layer just covered by 70% alcohol over the bottom of the Petri dish. They are then photographed against a dark background at 1x magnification. From this negative superposed on a similar negative taken from a millimeter grid, 3x enlarged prints are made. Meanwhile, the eggs are swirled again in 70% alcohol, transferred to a 3:1 (v/v) alcohol-ether solution, and put into a calibrated Bauer-Schenck centrifuge tube whose tip from 0 to .05 ml is filled with paraffin to form a cushion for the eggs. The eggs are centrifuged ten minutes at 2000 rpm and their volume is measured. Finally, they are transferred to a vial and stored as described above.

From the photograph, the number of eggs can be counted. By using the counts and the volumes of eight different egg collections, it was found that one D. melanogaster (Oregon-R) egg has a volume of $28.5 \pm .4 \times 10^{-6}$ ml. Thus, for subsequent egg collections, the number of eggs collected could be determined by measuring only their volume. This entire procedure, from the time of removing the egg tray from the jar to the time of freezing the egg, takes twenty minutes or less.

TEACHING NOTES

Burdick, A. B. Duplicate sex-linked factors with about 28% recombination.

We have a stock homozygous for both *rb* and *g*(A-42). Since *rb* and *g* are almost indistinguishable and *rb g* has about the same phenotype (which we call "dull"),

these genes serve as duplicate factors for the "dull" phenotype. Genes *rb* and *g* are linked on the sex chromosome, with about 37 map units between them. I ask my advanced class to make the cross: dull(*rb g*) ♀ x + ♂, from which they obtain (one student's result) 382 + ♀♀ and 316 dull ♂♂. I ask them to mass-mate the F₁ to obtain: 304 dull ♀♀, 168 + ♀♀; 275 dull ♂♂, 156 + ♂♂, or about 2 dulls : 1 wild-type in F₂. In addition, I ask them to make the original cross reciprocally and, again, to mass-mate the F₁ to obtain: 0 dull ♀♀, 399 + ♀♀; 256 dull ♂♂, 127 + ♂♂. In interpreting these data they soon see that lethals and viability factors will not explain the high frequency of dull types in F₂ and the obvious sex-linkage. The only reasonable explanation that fits these data is duplicate factors, sex-linked with, in this case, about 28% recombination.

MATERIALS REQUESTED OR AVAILABLE

Haruo Kurokawa, of the Department of Biology, Tokyo Metropolitan University would like to receive reprints of papers on speciation in *Drosophila*.

Mutant dq. (See "Melanogaster - New Mutants") Byron R. Kadel, 77-A Fenway North, Baltimore 21, Maryland, will be happy to supply stock of cn vg dq to anyone interested.

I. H. Herskowitz (Jordon Hall, Indiana University, Bloomington, Ind.) is preparing Bibliography on the genetics of Drosophila. Part III. It is planned to include the literature from 1951 through 1956 and any titles not included in the two previous Parts, and to have a title index. He would appreciate receiving references to any abstracts, papers, or theses not included in Part I or II or in the current or previous issues of DIS..