

RESEARCH NOTES*

Abrahamson, S. Oxygen depletion and viability.

Drosophila oocytes (Abrahamson, 1956), it became important to learn how long females could be maintained in an environment devoid of oxygen. Females were placed in sealed chambers containing nitrogen (obtained from Linde "hi-purity" cylinders) for periods of 1, 3, and 18 hours. After the nitrogen exposure the flies were observed for four days. Better than 95% survival was obtained in the first two groups; the group confined for eighteen hours suffered 100% mortality.

In the course of X-ray experiments concerned with the effects of oxygen concentration on chromosome breakage in

Altenburg, Luolin S., and Edgar Altenburg. Absence of detectable mutagenic effect of sodium formate, ethyl acetate, or Frey's salt when administered to the polar-cap cells of *D. melanogaster*.

A number of compounds have been tested for mutagenicity in this laboratory by exposing the pole cells of developing *D. melanogaster* eggs to the chemicals and testing the survivors for recessive lethals in the second pair of autosomes by Muller's "sifter" technique. This report will be confined to certain of the chemicals, shown in the table below, which have so far yielded mutation rates as low as (or lower than) the control rates usually obtained with this material (between 0.3% and 0.7% in different experiments). Each dosage was sufficient to cause noticeable mortality of the eggs.

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<u>Agent</u>	<u>Chemical Formula</u>	<u>Conc.</u>	<u>No. ♂♂ Tested</u>	<u>No. Chroms. Tested</u>	<u>No. Rec. Lethals</u>	<u>Mutation Rate</u>
Sodium formate	HCOONa	1 M	40	1266	4	0.3±0.2%
Ethyl acetate	CH ₃ COOC ₂ H ₅	100% vapor for 20 min.	29	1006	2	0.2±0.1%
Frey's salt	*O-N(SO ₃ K) ₂	10 ⁻² M	52	933	1	0.1±0.1%

Sodium formate and ethyl acetate appear to be nonmutagenic, in contrast to ethyl formate vapor, which produced a mutation rate of 4.7±1.6% (Rec. Genet. Soc. 25: 632, 1956). Frey's salt is a strong oxidizing agent, which in water dissociates into moderately stable free radicals. The mutation rate of 0.1±0.1% produced by this compound is significantly below the control rate, but this apparent lowering of the rate below the spontaneous level must so far be accepted only tentatively, inasmuch as the rates being dealt with are so small. A fuller account of these experiments will appear elsewhere. (This work has been supported by a grant from the American Cancer Society for work of Edgar Altenburg and associates.)

Annan, Murvel E. Effects of degree of desiccation on X-ray-induced egg mortality in *Drosophila* females.

Virgin *D. melanogaster* (Oregon-R) females were exposed in a desiccation chamber for periods of 0, 6, 12, or 18 hours immediately before irradiation with 5000 r X-rays. The X-rays were provided by the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor. Immediately after irradiation, the females were placed

Virgin *D. melanogaster* (Oregon-R) females were exposed in a desiccation chamber for periods of 0, 6, 12, or 18 hours immediately before irradiation with 5000 r X-rays. The X-rays were

*An asterisk before the title of a note indicates that the author has given unrestricted consent for its citation in publications.

with males (3 males to 2 females) in egg-collecting chambers. The eggs were collected every 12 hours for 108 hours, were counted, and were cultured at 25° C. Failure to hatch was the criterion of egg mortality. The percentages of egg mortality for the nonirradiated females were subtracted from the corresponding percentages for the irradiated groups. The remainder was divided by the percentage of eggs hatched in that nonirradiated group, and the result was multiplied by 100 to give the percentage of X-ray-induced egg mortality (after Herskowitz, Genetics, 1957). These values are given in the table below.

Hours of desiccation	Hours after treatment									
	12	24	36	48	60	72	84	96	108	
0	72	-36	14	12	13	6	2	-4	-6	
6	100	73	75	80	76	62	73	83	81	
12	-80	-6	15	-20	-11	-12	-8	-10	-7	
18	83	86	25	60	64	58	58	50	28	

A minus figure indicates that eggs from the X-rayed females concerned had a lower mortality than did those from nonirradiated females in the same desiccation treatment group. The variability obtained for the first 36 hours may have been due, in part, to variability in fertilization. The periods from 48 hours on are believed to establish a reproducible pattern of X-ray-induced egg mortality characteristic for the degree of desiccation. The difference between induced egg mortality with 12 hours of desiccation and that with either 6 or 18 hours is the most significant aspect of the results. (This work was supported by a U.S.P.H.S. research grant.)

Baglioni, C. Studies of ormo-chrome biosynthesis in D. melanogaster.

The mutant lightoid was found to block ormo-chrome synthesis, like *v*, *cn*, and *st*. By crossing *ltd* with the mutant *brown*, which completely blocks the synthesis of the red pteridinic pigments, a white-eyed strain was obtained (*bw ltd*) which is almost identical in phenotype to the strains *v bw*, *cn bw* and *st bw*. An attempt was made to localize the metabolic block in *ltd* by means of feeding experiments. Larvae of the tested strains were grown on a medium containing kynurenine or 3-OH-kynurenine. As a control, *v* flies were cultured under the same conditions. At emergence *ltd* flies showed no brown pigment, whereas the *v* flies were normally pigmented. It seems that the *ltd* metabolic block involves a reaction affecting 3-OH-kynurenine or some 3-OH-kynurenine derivative. In feeding experiments *ltd* cannot be distinguished from *st* or *cd* flies, which also do not become normally pigmented when given kynurenine or 3-OH-kynurenine.

In order to reveal the accumulation of different unmetabolized compounds in these strains, a chromatographic analysis of tryptophan metabolites was made by the two-dimensional paper chromatographic method of Dalglish (Bioch. J. 64: 481, 1956). Preliminary results indicated that kynurenine and 3-OH-kynurenine are always present in the two-dimensional chromatograms of *ltd*, *st*, and *cd* strains in larger amounts than in the wild type. In the chromatograms of *cd* or *st* flies a pale blue fluorescent spot was found, which was not found in *v*, *cn*, or *ltd* chromatograms. This spot was present in the wild type, but at a lower concentration than in *cd* or *st* flies. We shall refer to the substance of this spot as the *ltd*⁺ substance.

Attempts aimed at identifying the ltd^+ substance, by making chromatograms of ltd fly extracts mixed with some known tryptophan metabolites, showed an overlapping of the ltd^+ spot with the spot given by a sample of synthetic xanthurenic acid. In other solvents, better suited for the separation of xanthurenic acid derivatives (like methanol, butanol, benzene, water, ammonia in the proportions 40:20:20:20:1, or butanol saturated with 0.2 M ammonia), it was possible to separate the ltd^+ substance from added xanthurenic acid. Further work will be necessary to get more precise details about the chemical structure of the ltd^+ substance.

Baglioni, C. Two new pteridins, found in D. melanogaster.

Research of Viscontini and his collaborators showed that the red pigment of *Drosophila* is made of at least three components: drosopterin, isodrosopterin, and neodrosopterin, which were isolated and chemically defined. Our aim was to find a simple paper chromatographic method which would allow separation of the pteridinic compounds without any previous chemical treatment of the flies. The best resolution of the pteridins was obtained by two-dimensional chromatography, using n-butanol-acetic acid-water (4:1:5) as solvent for the first run and KCl 20% for the second run. It was possible to separate quite well the compounds isolated by Viscontini and to demonstrate the presence of two undescribed pteridins, which have the same Rf values as drosopterin and isodrosopterin in the first run, but may be differentiated from them in the second run. Since these pteridins have the same fluorescence color and absorption maxima as drosopterin and isodrosopterin, we propose the names paradrosopterin and paraisodrosopterin (as suggested by Professor Hadorn). The amount of these five components of the red pigment was determined in 30 of the commonest eye-color mutants; the concentration of the pteridins was estimated fluorimetrically.

Previous results indicate that drosopterin, isodrosopterin, and neodrosopterin, even though reduced in many mutants, nevertheless maintain a constant relative ratio. Paradrosopterin and paraisodrosopterin seem to behave in an independent manner, not maintaining a constant ratio with the other pteridins. An extreme situation was observed in the mutant *sed*, which has no paradrosopterin or paraisodrosopterin. Although extractions were made with a large amount of flies, and a concentrated solution of the pigment was chromatographed, no trace of these two substances was found. Further studies of the metabolic significance of this finding are now in progress.

Bart, Carol. A retest of the frequency of spontaneous loss of the yellow⁺ region of the scute-8 chromosome.

Because of the far higher rate of spontaneous loss of the region (containing y^+) adjacent to the left end of the scute-8 chromosome in some of the experiments reported by Belgovsky (1938, 1939)

and in those by Sidorov (1941) and by Lindsley (1955) than in those by Frye (1957, 1958), and because of the importance of the control (spontaneous) rate in studies of radiation-dose dependence, further tests of the spontaneous frequency were carried out on the stock used by Frye. As before $sc^8 B$ males were mated to $y w In49 f$ virgins. The males were 0-24 hours old, the females 48-72 hours old, when first mated. After 48 hours with these females (lot a) in vials containing 5 flies of each sex, the males were separated out and mated to a second lot of females (b) for another 48 hours, again in groups of 5 of each sex. Both a and b females were put through 5 broods of 48 hours each. The offspring scored were as follows:

	Regular B ♀	yellow B ♀	Regular y w f ♂	Non-dis. y w f ♀	Non-dis. B ♂
From lot a	13,532	1 1/2	13,229	11	3
From lot b	20,203	1	19,550	29	18

The y B ♀ designated as 1/2 was a mosaic that did not transmit the mutant yellow phenotype of the sc⁸ B chromosome. There was in addition a forked-mosaic B ♀, which failed to transmit the new forked, and a non-Bar female that transmitted the phenotype without lethality. Counting only the transmitted yellows, the total is 2 among 33,740 disjunctionally produced daughters or about 1 in 17,000. This result is not significantly different from that of 7 yellows among 263,794, or about 1 in 38,000, found by Frye, although 4 of Frye's yellows proved to have attached X's. Both yellows found here had separate X's, and their y sc⁸ B X proved lethal to males. The present low control rate is also like that found for scute-S1 by Luning (1952) and for scute-8 in one of Belgovsky's experiments.

On the basis of Sidorov's analyses, confirmed by those of Lindsley, we assume that in all probability our two exceptional yellows resulted from exchange between Y^S and the left heterochromatic region of the scute-8 chromosome. It seems likely that differences in the amount and structure of the heterochromatin would occur not uncommonly both in scute-8 and in Y chromosomes and would affect the frequency of their interchange.

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

Barzilay, Roy. Tumor phenocopy produced by cold.

Larvae of several *Drosophila* strains were exposed for 24 hours to 5° C ± 1 in a refrigerator, at different stages

of their development. Many of the treated larvae developed dark masses, resembling the so-called "melanotic tumors." The work described here was carried out with strain D/118 of *D. melanogaster* (D balancing a lethal extracted from a local population) and *D. simulans* (see table). Positive results were also obtained with a number of other strains.

		Non-tumorous	Tumorous	S	Tumor Incidence
D/118	Treated	1474	647	2121	30.4%
	Control	494	22	516	4.3%
<i>D. simulans</i>	Treated	552	108	660	16.4%
	Control	1155	-	1155	-

In D/118, the highest tumor incidence was scored in individuals treated during the 60th to the 84th hour of larval life. This "sensitive period" includes the stage which according to Rizki (1957) is characterized by a peak in the frequency of lamellocytes in the hemolymph. The participation of the lamellocytes in the formation of tumorous aggregates was observed both by light and phase microscopy, thus confirming Rizki's conclusions.

Bateman, A. J. Mutations in irradiated spermatocytes.

References to the radiation responses of premeiotic male germ cells are usually concerned with spermatogonia,

though spermatocytes, which also belong to this category, are likely to have very different responses. In order to isolate the various stages of spermatogenesis, the classical brood pattern based on 3-day mating periods is too coarse, as 3 broods will include all the stages from spermatogonia onwards. A study of crossing over between *b pr* and *vg* in males irradiated with 1000 r and mated each day to 2-3 females has been started. This mating rate is adequate to prevent mixture of sperm maturing on successive days. Results so far are as follows (missing days were unsampled):

Days from irradiation	2	5	6	7	8	9	10	11
Crossovers	0	0	6	7	3	7	13	0
Noncrossovers	1651	1836	1400	1452	687	1540	2313	1990
% crossovers	0.00	0.00	0.43	0.48	0.44	0.45	0.56	0.00

Concurrent tests for egg-hatching and deleted X's confirmed earlier experiments (Bateman, 1956, 1957), indicating that the same day corresponded to the same germ-cell stage throughout. Day 8 consistently showed a drop in fertility (shortage of sperm), which is presumed to be due to the lethal action of X-rays on late spermatogonia. Then days 6 and 7, and possibly 8, will yield the products of irradiated spermatocytes. Some responses of spermatocytes compared with earlier (spermatogonia) and later (spermatids) stages:

Dominant lethals: gonias, nil; cytes, very high; tids high
 Deleted X's: gonias, nil; cytes, very high; tids low
 Induced c-o: gonias and cytes equal; tids nil

If a broad classification of germ cells according to their response to mutagens is to be made, it should be into spermatogonial and post-spermatogonial rather than into pre- and post-meiotic.

Baumiller, R. C. The pre-adult viability of spontaneous mutations in *D. melanogaster*.

In order to obtain an accurate estimate of mutation frequency when mutants can be scored only as adults, the pre-adult viability of mutants compared with

normals must be taken into account in an environment where the mutant is represented by one individual existing among a preponderance of individuals that are normal (i.e., nonmutant and otherwise identical), since this is the circumstance under which mutants occur. Accordingly, pre-adult viabilities were determined in both the "Maxy" stock and the same stock in which spontaneous mutations had recently occurred. The mutants *y^{A241}*, *f⁵⁸⁵*, *w⁵⁶⁸*, *w^{A659}*, and *w^{ch}* were each compared to the parental "Maxy" stock normal for these genes. The stocks were kindly supplied by Dr. A. Schalet.

Normal and mutant flies were placed each in their own dacron-net-enclosed cylinder, which was placed netting-down on individual petri dishes containing nutrient medium seeded with yeast. After a 24-hour egg-laying period parents were removed; the plates were incubated for another 24 hours, when larvae were picked and placed in vials containing yeast-seeded standard medium. One hundred normal larvae and 1 mutant larva were transferred into each vial. (Ratios of 300:1 and 10:1 were also tested. The ratio 300:1

showed a lower percentage of survival for both the normal and mutant flies than was found in the tests using the ratio 100:1, but the comparative rate of survival was approximately the same. Thus pre-adult viability differences were shown not to be an effect of crowding nor to be grossly affected by it. Therefore, the use of a lower ratio was indicated as more economical. The ratio 10:1 also gave about the same comparative rate of survival, but the wide variance in numbers surviving in individual vials made this ratio unsatisfactory. Thus the ratio 100:1 was used exclusively.)

The results are shown in the table.

Normal:mutant::100:1					
Mutant	No. vials	% normal emerging (%A)	% mutants emerging (%B)	<u>%B/%A</u>	<u>P</u>
1 w ^{A659}	19	60.8±1.14	79.0± 9.41	1.30	.05
2 y ^{A241}	20	70.0±1.04	80.0± 8.91	1.14	.27
3 w ^{ch}	20	72.3±1.03	85.0± 7.89	1.17	.12
4 w ⁵⁶⁸	20	73.1±0.98	95.0± 4.87	1.30	.0001
5 f ⁵⁸⁵	20	83.9±0.82	40.0±10.90	0.48	.0001

The sex ratios for emerging normal and mutant flies were similar, females outnumbering males as is expected in the "Maxy" stock. Tests are listed in the table in the order in which they were done. From the percentage of normal adults, it is evident that as the experiment proceeded the technique improved. This, however, does not affect the validity of the conclusions to be drawn, since the percentage of mutants emerging improved concomitantly. In the case of y^{A241} and w^{ch} the results suggest a higher relative pre-adult viability than in normal flies, but the differences are not significant. On the other hand, w^{A659} and w⁵⁶⁸ both show the same high rate of comparative larval viability (1.30), although the separate tests do not have the same level of significance. The difference between w⁵⁶⁸ and normal flies is highly significant, whereas the difference in the experiment using w^{A659} is barely significant because of the small number of adults obtained. The f⁵⁸⁵ stock showed a significantly lower relative pre-adult viability (0.48). In all cases where the rate of pre-adult viability favored the mutant, it was found that mutants were generally among the first group of flies to hatch. The forked mutant, however, in all but one test hatched out at least one day after the earliest group.

The data confirm that there are mutations which, though considered to be detrimental to the over-all productivity of the mutants, are actually supra-vital at least during the larval stage of their life cycle. It is also shown that in the "Maxy" stock the mutation frequency from w⁺ to w might be over-estimated by a factor of 0.3, and the frequency of mutation of f⁺ to f might be underestimated by a factor of about 2 if relative larval viability were not taken into account. The viability differences found between individual mutant and normal flies in either the positive or negative direction, depending on the mutant tested, demonstrate the necessity for making approximate viability allowances when calculating actual, as opposed to observed, spontaneous (or induced) mutation rates. The results do suggest, however, that adult-determined mutation rates are probably wrong by no more than a factor of 2 or so.

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Brehme-Warren, K. Valuation or rank of mutants. In preparing the second edition of "The Mutants of *Drosophila melanogaster*" by C. B. Bridges and K. S. Brehme, a new set of criteria for ranking the mutants has been devised. The older system, printed on page 8 of the 1944 edition, worked well while Bridges made most of the judgments. Since his death, however, it has proved to be so subjective as to give variable results in the hands of different investigators. Accordingly, at a seminar in the Genetics Department of Indiana University in February, 1958, a new and more objective system was worked out, with the assistance of Drs. H. J. Muller, I. I. Oster, E. A. Carlson, and H. U. Meyer. It reads as follows:

Valuation or Rank. Rank 1. A mutant allowing sharp classification; of good viability and fertility in both sexes in the condition (heterozygous, homozygous, or hemizygous) in which it is used for classification; and accurately located.

Rank 2. A mutant less good in any one of the above characteristics, but still of considerable use as a marker.

Rank 3. A mutant faulty in any two of the above characteristics but still retaining enough classifiability to be useful.

Rank 4. Poor markers.

The letter A is added to indicate association with a chromosome aberration. If a mutant has been insufficiently studied, it should not be given a rank.

At the request of the author and through the courtesy of Drs. O. G. and M. B. Fahmy, more than 75 new mutants from the Fahmy laboratory have been assigned rank by Dr. I. I. Oster, and the results, ranging from RK1 to RK4, have shown the new system to be easily applicable. The author is applying it to both new and old mutants.

Brousseau, G. E., Jr. Crossing over between Y chromosomes in male *Drosophila*.

Results of experiments with sterile Y chromosomes indicated that exchange between two Y's (or an X-Yarm and a Y) takes place with a higher frequency

than has been reported for other types of exchange in the male. To test this possibility, males carrying two marked Y chromosomes were tested for recombination. Males of the constitution $y v/Y^S/sc^8.Y:bw^+$; bw were crossed to $y v/y v$ females. Recombinant sons were recovered in the progeny of two males. One of these was a single $y v/Y:bw^+$ son; in the other case there were 4 $y v/Y:bw^+$ sons and a single $y v/Y^S/Y:bw^+$ son. In this latter case a recombinant Y and a nonrecombinant Y were recovered in the same gamete, therefore the exchange must have occurred at the four-strand stage. In other experiments, recombinants between a sterile Y chromosome (a $sc^8.Y$ chromosome with an induced deletion in the fertility region of the long arm) and Y^L of $Y^S X.Y^L$ have been recovered in comparable frequencies.

The frequency of Y/Y recombinants is about 1 in 2000-3000, whereas the frequency of exchange between the X and Y chromosomes in the male is about 10^{-4} and the frequency of recombination between autosomes is even less. Several clusters have been recovered as well as the simultaneous recovery of reciprocal recombination from the same male, indicating that the exchanges are gonial. The process of recombination between Y chromosomes is under further investigation.

One of the recombinant Y chromosomes recovered from males carrying the

two marked Y's mentioned above is a potentially useful Y. This Y has B^S at the end of the long arm and y^+ (sc^S tip) at the end of the short arm; thus both arms of the Y are terminally marked. It should be very useful in such studies as detachment of attached-X's and translocations involving the Y.

Brosseau, G. E., Jr., and D. L. Lindsley. A dominantly marked Y chromosome: YB^S .

The available marked Y chromosomes carry as duplications normal alleles of sex-linked or autosomal genes, and their utility relies on the use of crosses carrying the appropriate recessives. An exception to this is the Hw effect of $sc^S.Y$. The desire for a Y chromosome marked with a readily classifiable dominant gene has led to the construction of a Y with its long arm marked terminally with B^S .

From a cross of $T(1;4)B^S, B^S \text{ car}/XY^L.Y^S, y^2 \text{ su-w}^a \text{ w}^a \text{ (bb?) } Y^L.Y^S$ females by $XY, y \text{ cv } v/0$ males we recovered $B^S \text{ car}^+$ sons. These males could have been the product of exchange between w^a and the $T(1;4)B^S$ breakpoint followed by nondisjunction of X centromeres, and consequently $XY^L.Y^S/B^S/0$ in constitution. Such males are sterile owing to the B^S duplication. Alternatively they could have been the product of exchange between the $T(1;4)B^S$ breakpoint and car , and $X^D.B^S Y^L.Y^S/0$ in constitution, X^D and B^S representing the distal and proximal segments of $T(1;4)B^S$ respectively. Such males are fertile. All recovered B^S males were crossed to $y \text{ v } bb/0$ females, and several fertile cultures resulted; they yielded $y \text{ v } bb$ daughters, $y \text{ v } B^S$ daughters, and fertile B^S sons which were $X^D./B^S Y^L.Y^S$ in constitution. These males were irradiated in an attempt to delete enough of the euchromatin of $B^S Y^L.Y^S$ to allow males carrying the resultant derivative in addition to a normal X to be fertile. The irradiated males were crossed to free-X females; the two classes of sons produced normally were $X/0$ and $X/B^S Y^L.Y^S$, both sterile. The only fertile sons would be those carrying the desired derivative chromosome and the $X^D./B^S Y^L.Y^S$ sons resulting from maternal nondisjunction. Consequently, the progenies of the above mating were transferred to fresh medium and fertile cultures were recovered. The desired product was recovered from one such fertile culture. Since YB^S carries the tip of the B^S duplication it must also carry the distal part of chromosome 4 translocated to the X by $T(1;4)B^S$; it is consequently thought to be of the following constitution: $4^D B^S \text{ (bb?) } Y^L.bb^+ Y^S$. This YB^S chromosome is characterized by good viability and normal disjunction from an X chromosome in the male. The B^S is a strong Bar, and both males and females carrying it show a narrow Bar phenotype.

Burdette, Walter J. Stability of tumor incidence in *Drosophila* after injection of mammary tumor agent.

Numerous viruses are known to be carried by insect vectors. Recently the polyoma virus known to be associated with tumors in mammals has been found to cause tumors of multiple tissues in different

species, and the incidence of inherited tumors in *Drosophila* has been altered by the introduction of susceptibility genes into cytoplasm carrying the genoid for CO_2 sensitivity. In order to test whether the milk agent affects the appearance of tumors in *Drosophila*, a preparation of the virus from $C3H$ mice was injected into females of the $tu \text{ vg } bw, tu^{36a}$, and Oregon-R strains. Tumor incidence in the progeny was then compared to that in parallel control cultures. The results may be summarized as follows:

Strain	tu vg bw				tu ^{36a}				Oregon-R	
	Injected		Control		Injected		Control		Injected	
	No.	%	No.	%	No.	%	No.	%	No.	%
Males	$\frac{3242}{3245}$	99.9	$\frac{604}{608}$	99.3	$\frac{56}{1775}$	3.2	$\frac{15}{491}$	3.1	$\frac{2}{7572}$	0.03
Females	$\frac{3565}{3575}$	99.7	$\frac{546}{546}$	100.0	$\frac{77}{1810}$	4.3	$\frac{49}{566}$	8.7	$\frac{1}{7518}$	0.01
Total	$\frac{6807}{6820}$	99.8	$\frac{1150}{1154}$	99.7	$\frac{133}{3538}$	3.7	$\frac{64}{1059}$	6.0	$\frac{3}{15090}$	0.02

This evidence does not suggest that the injections increased the incidence of tumors. Whether the negative results are due to failure of the virus to propagate or to its being inert with reference to tumorigenesis in *Drosophila* has not been determined.

Carlson, Elof A. Variegated position effect at the dumpy locus in *D. melanogaster*.

Several induced mutations at the dumpy locus (dp--2, 13.0) were produced with high doses (4000 r) of X-rays in Oregon-R wild-type males which were mated to

echinoid dumpy clot (ed dp cl) females. A small number of these (about 10%) showed considerable asymmetry of wing and thorax effects. Therefore these variegated mutants have been designated dp^W, dumpy-warped. Most were homozygous lethal, although occasionally a few homozygotes were obtained with an extremely weak and deformed phenotype, showing dull variegation of the eyes, necrosis of the thorax, and club-like wings. There were three which only showed the variegation in the male offspring, the females always remaining homozygous ed dp cl when testcrossed with males of the composition dp^W/ed dp cl, indicating Y-2 translocations. All together, ten have been obtained. Two are lost; one is an X-2 translocation; three are 2-3 translocations; and the remaining exception has not yet been analyzed, although it too prevents crossing over in the ed-cl region. A sample of these were tested with an extra Y chromosome, using the stock X.Y y v; ed dp cl. The variegated position effect was completely suppressed in all tested cases. When individuals heterozygous for the rearrangement and the tester stock were inbred, the F₂ homozygotes, genotypically X.Y y v; R(dp^W), were almost completely normal. At a warmer temperature (28° C) an occasional slight vortex or mild oblique wing may be expressed in a few of these homozygotes. The variegated position effect does not extend to the outside markers ed (11.0) and cl (16.5). Since the dumpy region also controls a lethal factor, it is possible that the position effect is also affecting this character. However, with dp^T (olv^m) the compounds with dp^W are similar to the homozygotes but are somewhat more viable. Stocks of these variegated mutants may be kept without too much difficulty when dp^{txI} Cy is used as a balancer. The compound produces a peculiar effect on the Curly wings, which become opaque and deformed, probably as a consequence of the weak oblique effect exerted by dp^{txI} (lvⁱ). No effect is observed for the Curly when the balancer does not contain the dp^{txI}.

These typical variegated position-effect mutants are of interest primarily because the dumpy series is located in a complex locus with at least seven separable mutant sites which can express numerous pleiotropic effects.

Usually the wing and thorax expressions go together, although in at least one-third of the cases the variegation for the right and left sides may be expressed independently. No case has been found yet among the induced exceptions in which variegation affects exclusively the thorax or the wing. Only one of these exceptions is moderately expressed; the rest are indistinguishable in phenotype from one another. Several other induced mutants at the dp locus involve rearrangements, but no variegation is present. Some of these may represent intra-locus (perhaps inter-sublocus) breaks, and others euchromatic position effects of breaks outside the dp region. The total induced mutation rate is high (about 1/300) at this dose. Tests are being carried out to determine if the variegated types can, in some cases, represent intra-locus breaks.

(These mutants were obtained during a series of experiments at Indiana University which were partially supported by grants to Dr. H. J. Muller and associates from the U.S. Atomic Energy Commission, Contract AT(11-1)-195. Their analysis is part of a project sponsored by the National Research Council of Canada, Annual Grant No. A 776.)

Castiglioni, M. C. A new stock with lethal pseudotumors in D. melanogaster.

The stock tu-mwh (tumor-multiple wing hairs) shows pupal lethality. Nearly 88% of the dying pupae carry pseudotumors, and show, at the same time,

abnormalities in the organs originating from the imaginal discs. Examinations in toto and on microtome sections of larvae at different ages revealed irregularities in the disc folds and also alterations within the lymph gland, which clearly is transformed into a melanotic mass. In extreme cases there is also an excess of hemolymph fluid, so that swollen larvae are produced, which are also larger than the normal ones. Invasion of hemolymph cells has never been observed; therefore the cause of lethality is probably complete degeneration of the lymph gland.

Castiglioni, M. C. Cell melanization induced in larvae of D. melanogaster.

When larvae are treated in Bouin fixative solution at a temperature of 60° C for 60 seconds, dark points and spots appear in the body. The dark points

are nearly black and correspond to two types of blood cells, referred to in my papers as small and mid-sized cells. The spots are larger than the points but not so dark; they correspond to large cells. Response to the treatment is different in different stocks (wild stocks-Varese, S. Maria, Valdagno, Gaiano, Moltrasio; tumorous stocks-tu A₂, tu B₃, e 144 melanotic). The difference consists in the percentage of blackening larvae, and in the type and distribution of points or spots. The type depends on the composition of the hemolymph formula (frequency of the different cell types), so that with this technique it is possible to reveal the cytological composition of the lymph gland. Patterns of distribution show greater or lesser uniformity in different parts of the body. The melanization of the large cells parallels somewhat the effect of the tu genes of tumorous stocks.

Cooper, K. W. A probable heterochromatic deficiency in In(1)sc¹⁸, the approximate location of bobbed, and the size of block A.

In large neuroblasts, the heterochromatic region of the prophase X chromosome consists of the small right limb (XR) followed by four nearly equal-sized

heterochromatic segments (hA, hB, hC, and hD). Each of these heterochromatic segments is roughly the size of a fourth chromosome, and together they make up the whole of the heterochromatic region (Xh) of the large, left limb of X, with the nucleolus organizer lying between the second and third respectively. The structure of the heterochromatic half of X, then, from left to right, is: hD, hC, NO, hB, hA, sfa, XR.

In the case of $\text{In}(1)\text{sc}^{\text{L8}}$, the rightmost break of the inverted piece lies in hB, and a large series of prophase figures shows that only half or so of hB is inverted. The remaining, uninverted, proximal heterochromatic length, however, is reduced in size, and it is very likely that a segment approximately half the size of hB (or perhaps somewhat more, including a distal piece of hA) has been lost from the chromosome. $\text{In}(1)\text{sc}^{\text{L8}}$, therefore, very likely arose as a three-break event, and possesses a deficiency to the right of the nucleolus organizer which, at present, contains no known genes. The proximal break of this deficiency very likely lies close to, or at, the proximal break of the deficiency in $\text{In}(1)\text{bb}^-$.

Inasmuch as the rightmost break of the inverted piece of the bobbed-deficient $\text{In}(1)\text{bb}^-$ lies in hC, and as bobbed is inverted by $\text{In}(1)\text{sc}^{\text{L8}}$, it follows that bobbed is close to the nucleolus organizer and either in the proximal half of hC or the distal half of hB; that is, bobbed lies in the mid sector of Xh, and not close to the junction of the euchromatic and heterochromatic regions as generally believed. Furthermore, since block A is defined as a distal portion of the length of Xh that lies between the most proximal breaks of $\text{Ins}(1)\text{sc}^{\text{L8}}$ and sc^{B} , it follows that the region between the proximal breaks of the inverted segments of $\text{Ins}(1)\text{sc}^{\text{L8}}$ and sc^{B} possesses at least one breakable site (namely, that giving the deficiency- sc^{L8}), and that block A itself must be considerably smaller in size than the morphological element hA.

Crowell, Villa B. Experiment to determine percentage of larval-pupal death due to X-irradiation of P_1 males.

Despite some earlier reports that dominant lethal effects found in F_1 zygotes derived from irradiated spermatozoa are confined to the egg stage, some mortality in the larval-pupal

period is to be expected on the ground that the great majority of mutations are detrimental and have some dominance. This question was investigated by comparing the mortality, in this period, of zygotes derived from irradiated and control males that had been obtained from the same Oregon-R stock, divided randomly into the treated and control groups, and crossed to un-irradiated $y\ t^2\ v\ f$ virgins. The irradiated males were given 4000 r 3 to 3 1/2 days after eclosion, were at once mated for 22 1/2 hours to a first lot of females, whose offspring were discarded, and then remated to a second lot for 7 1/2 to 8 hours. The larvae that hatched from eggs laid during a 4-to-6-hour period by the females of the second lot were gently collected on needles, 24 to 32 hours after the beginning of the egg laying; and another collection, of the later-hatching larvae, was made some 40 to 42 hours after the beginning of the egg laying. The larvae were counted, scored as to color (yellow mouth parts here indicating males), and carefully placed in fresh, uncrowded culture vials. The same procedure was followed with the control series.

Because of complications arising from the possibility of sometimes mistaking the color of mouth parts, the results for both sexes will be reported collectively here. Somewhat more than 1700 control larvae and 2700 larvae from treated fathers were placed in vials. Survival to maturity

fluctuated between about 90% and 95% among the controls of the six different experimental series, and was usually distinctly lower in the corresponding treated lots. To avoid as far as possible the bias that might be caused by determinate cultural differences between series in which the ratios of control to treated larvae might also differ, use was made of the harmonic mean method of statistical analysis developed by Muller (1941, Amer. Nat. 75: 264-271). The results showed that approximately 6% (with a standard error of not more than 1%) of the just-hatched larvae were prevented from reaching maturity by dominant detrimental effects of the radiation given to the paternal spermatozoa. This result is in the range of our expectation based on previous work on dominance and on the frequency of lethal and detrimental mutations.

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

Di Pasquale, A., and Santibanez S. Koref Pseudo-tumors in D. melanogaster and D. simulans.

Flies were collected at Palermo, Sicily, in August and October, 1957. From the first collection, 16 D. melanogaster and 46 D. simulans were kept, and from the second collection, 21 melanogaster

and 75 simulans females. Each female was cultured singly, and lines were established by taking 4 pairs at random from each of the following generations to constitute the subsequent one. Pseudotumor incidence was recorded each generation for 12 generations.

In the first generation, over-all tumor incidence was as follows: D. melanogaster, 1st collection, 4.6%; D. melanogaster, 2nd collection, 1.9%; D. simulans, 1st collection, 2.7%; D. simulans, 2nd collection, 9.6%. In D. melanogaster in the 1st collection, 81.2% of the lines presented tumors, against 47.6% in the second collection. In D. simulans, 85.4% and 87.8% of the lines of the 1st and 2nd collections, respectively, showed the trait.

By the third generation, all but two of the lines of D. simulans from the August collection had presented the tumoral trait. Up to the twelfth generation, the character was not lost in any of the lines, and its incidence remained on the whole constant. An attempt to study the genetic factors responsible for the trait revealed them to be partially dominant in D. simulans. In D. melanogaster, however, as has been previously described, they were recessive.

These results seem to us to indicate the following: (1) Pseudotumors are relatively frequent in the natural populations of D. melanogaster and D. simulans studied. (2) There seem to be seasonal differences in the manifestation of the trait. (3) The genetic factors responsible for it are present in practically all the individuals captured. (4) There seems to be a difference in the physiological mechanisms by which the phenotypically similar trait is manifested in the two sibling species studied. (5) It is suggested that the factors responsible for the pseudotumors may confer certain adaptive advantages on their carriers, so that natural selection favors them. Seasonal differences may also be attributed to the plasticity of the genetic constitution, which may be responsible for differential responses in accordance with environmental pressures. Further studies are under way to clarify these hypotheses.

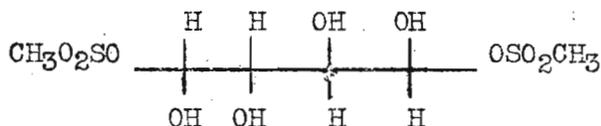
Doane, W. W. Meiosis in unfertilized eggs of D. melanogaster.

Young unfertilized eggs laid by virgin females of an Oregon-R strain of D. melanogaster were sectioned and analyzed. Cytological details studied in 93 such eggs indicated that in every case meiosis had gone to completion, contrary to views expressed in the literature (e.g., Huettner, J. Morph. 39, 1924; Sonnenblick, Biology of Drosophila, 1950). In twenty of these eggs, four interphase or prophase nuclei were found and identified as the products of maturation. The remaining eggs contained one, two, or three groups of chromosomes whose appearance resembled that of the polar-body chromosomal groups described by Rabinowitz (J. Morph. 69, 1941) in fertilized eggs. The sequence of events following maturation varies somewhat in different eggs, depending on which of the meiotic products fuse with one another. Details of the events following maturation will be described elsewhere.

Fahmy, O. G., and Myrtle J. Fahmy A nontoxic mutagenic sulphonate in D. melanogaster.

It has been shown (DIS-25; J. Genet. 54, 1956; Nature 180, 1957) that the alkyl-methanesulphonates, both mono- and difunctional as regards the methanesulphonoxy ($-\text{OSO}_2\text{CH}_3$) group, are mutagenic when injected intra-abdominally into adult male Drosophila. The over-all activity of difunctional compounds of this series on the postmeiotic germ cells was found to vary according to the size and molecular configuration of the alkyl moiety of the molecule, in between the active groups.

Recently a compound of the above series, in which the central moiety is the sugar mannitol, has been tested for mutagenicity.



1:6-dimethanesulphonoxy mannitol.

This compound is highly soluble in water and completely nontoxic to the injected flies, even at doses more than 10 times the highest tolerated dose of the least toxic of the other dimethanesulphonates. In spite of this low toxicity the mannitol derivative possesses decisive mutagenic activity. An injected concentration of 8×10^{-2} M (corresponding to a dose of 2.4×10^{-8} Mol. per male) induces an average of $5.6 \pm 0.5\%$ sex-linked recessive lethals among postmeiotic sperm. Like the rest of the alkyl-methanesulphonates, the mannitol derivative has very low activity on the premeiotic stages of the germ line.

Farnsworth, M. W. Quantitative studies of DNA in wild-type and Minute larvae.

One aspect of the over-all problem of delayed growth and development characteristic of Minute larvae is DNA production. In order to rule out the possibility that lag in DNA synthesis is a major factor in the delayed development of these mutants, estimates of DNA content and concentration in homogenates of wild-type and Minute larvae of D. melanogaster have been made.

Methods of isolation of nucleic acids were those of Schmidt, Thannhauser, and Schneider. Estimation of DNA in $\mu\text{g}/\text{ml}$ of final extract was carried out

according to Ceriotti's microchemical method, employing the indole reaction (Ceriotti, J.B.C. 198: 297, 1952). Control values were obtained from 15 separate determinations on homogenized Canton wild-type larvae. For each determination, from 200 to 800 72-hour larvae were used, the average number being around 450. Experimental stocks were M(2) $\underline{1}^2$ and M(3)w heterozygous larvae. Nine determinations were carried out with M(2) $\underline{1}^2$, and seven with M(3)w. The number of larvae used per determination was approximately the same as with control material.

All data were calculated as (1) μg DNA/ml of final extract (concentration), and (2) μg DNA/larva (content). On the basis of these calculations, the following data were obtained:

Canton wild-type

Concentration: $0.3 \pm .02 \mu\text{g/ml}$
Content: $0.7 \pm .04 \mu\text{g/larva}$

Minute (2) $\underline{1}^2$

Concentration: $0.3 \pm .03 \mu\text{g/ml}$
Content: $0.4 \pm .05 \mu\text{g/larva}$

Minute (3) w

Concentration: $0.4 \pm .08 \mu\text{g/ml}$
Content: $0.3 \pm .08 \mu\text{g/larva}$

From these data it is obvious that there is no significant difference in DNA concentration in the three stocks tested. The lower values for DNA content per larva in the Minutes as compared to wild type undoubtedly reflect the somewhat smaller body size of the Minute larvae. This size difference is probably due to the smaller cell size of the Minutes. In general, the data indicate that delays in growth and development cannot be ascribed specifically to defects in DNA metabolism.

Forbes, Clifford. Marked-Y technique for the detection of primary nondisjunction.

makes use of tester males from a stock with marked Y chromosomes. Females homozygous for yellow and vermilion are mated to $y^w.Y^S/sc.Y^L$ males. These males carry the wild-type allele of yellow on Y^L . Expected females and males are yellow and not-yellow vermilion, respectively. Exceptional females are not-yellow vermilion, and exceptional males are yellow white.

An improved method of detecting non-disjunction, which gives particularly reliable evidence concerning the origin of each exceptional female,

Frye, Sara. More X-ray-induced forkeds found not suppressible by su^{W-f} .

Tests of 12 forked mutants produced by X-raying spermatozoa in the male in experiments to ascertain the frequency of induction of yellow deficiencies in the scute-8 chromosome have shown that none of them is suppressed by Whittinghill's suppressor, su^{W-f} . This result is in agreement with findings reported by Green (1956), but it differs from the findings regarding the X-ray-produced mutant f^X (supposedly a sublocus deficiency), discussed by Muller, Oster, and Ehrlich in this and previous issues of DIS.

Tests of 12 forked mutants produced by X-raying spermatozoa in the male in experiments to ascertain the frequency of induction of yellow defi-

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

Glassman, E. Further studies of the maroon-like (ma-1) and rosy (ry) eye-color mutants.

did not occur. These two mutants, although on separate chromosomes, are related in that both lack xanthine dehydrogenase, and accumulate excess amounts of this enzyme's substrates (hypoxanthine and 2-amino-4-hydroxy-pteridine) because the conversions to uric acid and isoxanthopterin do not occur. The simplest explanation of why ma-1 is maternally affected is that these flies can utilize a maternal substance, whose synthesis they cannot carry out because of the genetic block. The reason ry is not maternally affected is that these flies are probably blocked in a biochemical reaction concerned with utilization of the maternal substance. This assumption is supported by the fact that females homozygous for ry can still exert a maternal effect on ma-1, indicating that ry flies can synthesize the necessary maternal substance for ma-1 progeny.

In DIS-31 (p. 121), it was reported that ma-1⁺/ma-1 females had a maternal effect on their ma-1 progeny, whereas a similar effect of ry⁺/ry on ry progeny

Biochemical studies have now shown that the maternally affected ma-1 progeny differ from typical ma-1 flies in that they have normal amounts of red eye pigments and exhibit traces of xanthine dehydrogenase, and its reaction produces uric acid and isoxanthopterin. Whether the enzyme or some necessary factor for activity is the maternal substance cannot be determined; but it is also possible that it is the enzyme-forming system which is involved, mainly because the maternal effect persists to the adult stage, and proteins or simple activators might not be expected to persist that long without new synthesis.

The genetic localization of ma-1 must now be reconsidered. It is obvious that the maternal effect (which diminishes as the bottle gets older) will interfere with crossover data unless progeny testing is carried out. Through the use of sn, m, f, and Bx in various combinations with ma-1, it was possible to relocate ma-1 to the right of Bx on the sex chromosome, and not near v as reported in Bridges and Brehme.

Grell, E. H. The autonomy of reciprocal eye transplants between pn and K-pn.

phenotype (Sturtevant, GENETICS 41: 118-123, 1956). Prune is an eye-color mutant which affects the pteridine pigments. It seems reasonable that K-pn might cause a further modification in the amounts of pteridines if the combination were not lethal.

Prune-killer is a third-chromosome dominant which in combination with prune causes second-instar larvae to die and has no other known mutant

In an attempt to circumvent the lethality and learn something about the interaction between K-pn and pn, eye discs were reciprocally transplanted between K-pn and pn larvae. The transplanted eyes were dissected out of the adult hosts, examined for color and chromatographed on filter paper in propanol-1:water:NH₄OH (60:30:1). No differences could be detected between pn eyes grown in K-pn or + hosts or between K-pn eyes grown in pn or + hosts. The pigmentation of the transplanted eyes was autonomous in all cases and the experiments did not give any indication of an effect of K-pn on pteridines.

Therefore the interaction between pn and K-pn is autonomous; and an effect on pteridines, if it exists, is expressed only when pn and K-pn are in the same cell.

Grell, Rhoda F. The effect of X-chromosome loss on variegation.

The cross of a v; bw male which carried an extra Y chromosome to a v; bw^{VDel}/SM1, al² Cy sp² female yielded one non-Curly gynandromorph. The left portion of the thorax of this fly was presumably female, since the prothoracic leg carried no sex comb and the wing on this side was considerably longer than the right wing. The right side of the thorax was male-like, for a normal sex comb was present on the first leg. The genital apparatus and pigmentation of the abdomen were also male.

The eye on the left side of the head was vermilion, and since the wings were not Cy it was assumed that the brown-variegation of this tissue was suppressed by the presence of an extra Y. The genotype of this tissue was interpreted as v/v/Y; bw^{VDel}/bw. The eye on the right or male side showed a white background with a few specks of color typical of unsuppressed brown-variegation in combination with vermilion. The genotype of this tissue was interpreted as v; Y; bw^{VDel}/bw.

The gynandromorph must have arisen through the loss of an X chromosome from an XXY female. That the unsuppressed condition did not result from the simultaneous loss of an X and Y chromosome was indicated by the fact that the fly was sufficiently fertile to produce four offspring. The X-chromosome loss also resulted in an unsuppressed brown-variegation. Thus it appears that an X chromosome is as effective as a Y chromosome in suppressing variegation.

Gruber, F. Frequency of heterozygous inversions in a natural population of D. immigrans from Israel.

The offspring of wild D. immigrans females, collected during two consecutive spring seasons at Qiryat "Anavim (near Jerusalem), were examined cytologically for the types and frequencies of inversions. So far, only the long median inversion in the II L chromosome (inversion "A") has been found, with an over-all frequency of 33.9±4.7%. The rate of this inversion in the Q.A. population is higher than that reported for Brazil (19.6±2.6%, Freire-Maia et al., 1953) and for Chile, where "A" exists side by side with 2 other inversions (Brncic, 1955). The data are summarized in the table.

The offspring of wild D. immigrans females, collected during two consecutive spring seasons at Qiryat "Anavim (near Jerusalem), were examined cytologically for the types and frequencies

<u>Year</u>	<u>No. of chromosomes studied</u>	<u>No. of heterozygotes for inversion "A"</u>
1957	45	17
1958	56	17
Total	101	34

Hillman, Ralph. Female sterility in singed^{55a}.

In a report of a new mutant in DIS-31 (1957), sn^{55a} was classified as a female-fertile allele at the singed locus. Recently, this mutant was used in a routine cross, where it was observed that homozygous sn^{55a} females did not lay eggs, but hemizygous males were fertile. The stock used at the time was y w^a cv sn^{55a} v/M5. Heterozygous

females from this stock were outcrossed to wild type, and the following homozygous chromosomes were recovered and tested.

<u>Chromosome tested</u>	<u>Number</u>	<u>Fertility</u>
y w ^a cv sn ^{55a} v	53	-
+ + + + +	14	+
y w ^a cv + +	35	+
y w ^a cv sn ^{55a} +	33	-
+ + + sn ^{55a} v	27	-
+ + + + v	26	+
y w ^a cv + v	7	+
+ + + sn ^{55a} +	4	-

Although the numbers involved were small and the map distances tested are 7 map units to the left and 12 map units to the right of singed, it appears that there has been the addition of a female-sterility factor associated with this singed allele. The stock has been sent to Dr. Harvey Bender at Northwestern University for further investigation.

Hoenigsberg, H. F., and Santibanez S. Koréf. Courtship and mating preferences in D. melanogaster.

their own type both in proximal and distant stimuli in inbred male choices.

By direct observation of the courtships of inbred and outbred lines of D. melanogaster from a common source, the authors were able to find the existence of discrimination and preference for

Hoenigsberg, H. F., and G. P. Sironi. Chromatographic studies within the obscura group of species.

the same studies were extended to D. melanogaster. Preliminary results indicate that the third-instar larvae do not serve to distinguish pseudobscura from persimilis. However, in 2-day-old male adults there is a marked quantitative difference as to intensity of fluorescence of the bluish and the yellow spots, Rf 0.353 and 0.411, respectively. In older flies (4 to 6 days) the differences between the two species are limited to the last blue-green fluorescent spot (kynurenin), which is considerably more intense in pseudobscura than in persimilis females. D. bifasciata is different from pseudobscura and persimilis. In third-instar larvae, D. bifasciata has greater fluorescence than the other two obscura species in the middle yellow spot, Rf 0.420. The second-day adult D. bifasciata lacks the uppermost pale spot of the series normally present in pseudobscura and persimilis. D. melanogaster differs from the obscura species in the intensity of fluorescence of all compounds.

One-dimensional paper chromatographic studies have been made with third-instar larvae and 48-144-hour adults of D. pseudoobscura, D. persimilis, and D. bifasciata. For comparative purposes,

The authors believe that sibling species and species whose morphological phenotypes do not differentiate them from each other may reveal their differences at the physiological level. Therefore, as routine procedure, the

systematist may find chromatographic analysis helpful in identifying members of a species about which he does not feel certain. Such analytical information may serve to confirm taxonomic classifications, especially if developmental as well as the adult stages are chromatographed. From our experience we point out that although larval chromatograms did not discriminate between pseudoobscura and persimilis, chromatography of adults revealed the difference. D. bifasciata, on the other hand, was distinguished from the other two species by chromatographic patterns of third-instar larvae.

Horikawa, M. Tissue culture analysis of delayed lethal irradiation effect in D. melanogaster.

It is well known that the effect of radiation on Drosophila larvae is not their immediate death but a delay of pupation and a decrease in pupation and imagination rates, within a wide

range of radiation doses. Furthermore, it was observed by the author that the body weight of irradiated larvae, whose pupation was delayed, not only increased but was considerably greater than that of control larvae. In the present investigation, the mechanism of this phenomenon was studied in some detail by the tissue culture method.

Wild strains (Oregon, Canton-S, Kochi, and Samarkand) and several eye-color mutants (bw, w, v, cn, v-bw, cn-bw, Bar, bar-3, and Dp/In(3L)P, In(3R)C, Sb e 1(3)e of D. melanogaster were used as material. Third-instar larvae (about 80 hours old after hatching at 25° C), grown under sterile conditions, were irradiated with various doses of X-rays (160 Kvp, 25 mA, Imm. Al filter, 370 r/min, at a distance of 30 cm). They were dissected, and organs and tissues were removed under a binocular microscope in a sterilized glass chamber. They were cultured in a synthetic medium, and sensitivity to radiation was determined by observing their growth and differentiation. Some of the results obtained with a wild strain (Oregon) are shown in the table.

G=Growth, D=Differentiation, CC=Cephalic complex (ten bodies), N=Normal, I=Irradiated.

Organs and discs cultured	G and D rate	Doses (Kr)									
		0	0.5	1	3	5	10	15	20	25	
N eye disc + ICC	G	+	+	+	+	+	+	+	+	+	-
	D	+	+	+	-	-	-	-	-	-	-
I eye disc + NCC	G	+	+	+	+	+	+	+	+	+	-
	D	+	+	+	+	+	+	+	+	+	-
I eye disc + ICC	G	+	+	+	+	+	+	+	+	-	-
	D	+	+	+	-	-	-	-	-	-	-
Culture medium	G	+	+				+		+	+	
	D	+	+				+		+	+	
Extracted metamorphic Hormone	G	+	+				+		+	+	
	D	+	+				+		+	+	

When irradiated (0-20-Kr) eye discs were cultured together with normal cephalic complexes (ten bodies), they showed pronounced growth and differentiation, as in normal eye discs. After high doses (25 Kr), however, neither growth nor differentiation was observed. When normal eye discs were cultured together with irradiated (3 Kr) cephalic complexes (ten bodies), the eye discs showed pronounced growth, but no differentiation. Since the cephalic complex

is known to exert hormonal control on growth and metamorphosis, it seems that the normal differentiation mechanism of organs and discs of the cephalic complex was virtually destroyed after low doses (3 Kr). Hence, one might suspect that the radiosensitivity of the bar-3 mutant, which showed the highest sensitivity in whole-body irradiation tests of the thirteen strains used, would be due to the higher sensitivity to radiation damage of the cephalic complex than the other strains. Furthermore, the metamorphic hormones already secreted from the cephalic complexes were not influenced even after high doses (25 Kr). The results of this experiment show that the delay in pupation and decrease in imago rate are due to radiation damage of the cephalic complex.

Imaizumi, T. Standardization of developmental stages in the *Drosophila* embryo.

In order to facilitate studies of embryonic development, standard developmental stages have been established. The characteristics of embryos at

different stages, as described below, can easily be recognized in dechorionated eggs through the transparent vitelline membrane. The standardization reported here was worked out with *D. melanogaster* and *D. virilis*. There is no essential difference between them as far as embryonic morphogenesis is concerned. Therefore the standards are probably applicable to other species of *Drosophila*.

Stage 1. Just after fertilization. Egg is filled with homogeneous protoplasm.

Stage 2. Egg contracted along the long axis, nuclei are multiplying interiorly.

Stage 3. Preblastodermic or blastema stage. A narrow zone of nucleated peripheral oöplasm differentiated from central yolk mass but not divided by cell walls. The stage is divided into two substages:

Substage 3a. Young blastema, in which pole cells are pushed out from the posterior surface.

Substage 3b. Late blastema, which is characterized by simultaneous multiplication of nuclei in the cortical zone.

Stage 4. Characterized by formation of the blastoderm, a unicellular layer on the surface of the embryo.

Stage 5. The stage during which both ventral and cephalic furrows are formed. It is divided into two substages in *melanogaster* and three in *virilis*:

Substage 5a. Early period. Secondary contraction of embryo is found only at the anterior part; in the meantime, the formation of ventral furrow takes place along the midventral line commencing anteriorly.

Substage 5b. Middle period, in which the ventral furrow extends onto the dorsal side beyond the posterior pole.

Substage 5c. Late period, in which the cephalic furrow is formed.

Substages 5a and 5b are distinct in *virilis*; in *melanogaster* the transformations at these stages occur almost simultaneously.

Stage 6. The stage in which a mass of pole cells moves toward the

center of the dorsal side; some transverse fissures (folds) appear at the same time.

Stage 7. Characterized by the formation of both proctodaeal and stomodaeal invaginations. Divided into two substages:

Substage 7a. Formation of proctodaeal invagination just completed.

Substage 7b. The stomodaeal invagination is formed; and at the same time the development of the germinal band formed by prolongation of the proctodaeal invagination advances into the interior of embryo.

Stage 8. The head and trunk are clearly distinguishable in the embryo.

Stage 9. The stage of preblastokinesis, preceding shortening of the embryo and dorsal closure. It may be distinguished from the next stage by the position of the yolk mass.

Stage 10. Involution of the head and dorsal closure take place. The posterior spiracles also are formed.

Stage 11. The stage at which segmentation of the body is evident.

Stage 12. This and the next three stages are identified especially by the shape of the midintestine. At this stage it is saclike and not coiled.

Stage 13. Coiling midintestine.

Stage 14. Ringed midintestine.

Stage 15. The stage before eruption of gas into the tracheae. The contents of the midintestine have become translucent and hardly visible through the body wall. There is also active movement of muscles.

Stage 16. The stage before hatching. The tracheae are filled with a sort of gas, which is not inhaled air but is produced inside the body.

Some characteristic photographs of the standard stages described above are now in press and will be published soon in Cytologia.

Imaizumi, T. The metabolic pattern of fluorescent substances in the development of *Drosophila*.

An attempt was made to demonstrate changes in patterns of fluorescent substances during the embryonic development of *D. melanogaster*, by means of two-dimensional paper chromatography.

(Regarding other substances such as amino acids, sugars, and so on, reports will be made in the future.) More than 1000 dechorionated eggs were smashed directly on filter paper (Toyo-Roshi No. 50). The paper was then irrigated with hot water (75°-80° C) in a dark place. After drying, it was irrigated for a second time in the dark with the following mixtures: n-butanol, glacial acetic acid, and water (4:1:5 v/v, upper phase); n-butanol, n-propanol, water (2:2:1 v/v); n-butanol, 5 N acetic acid (2:1 v/v); n-propanol, 1% ammonia (2:1 v/v); phenol, n-butanol, water (160 g:30 cc:100 cc v/v); water saturated with isoamylalcohol; 5% Na₂HPO₄ aqueous solution; and collidine saturated with water.

The following fluorescent substances were found in the *D. melanogaster* embryo: kynurenine, 3-hydroxykynurenine, flavin-adenine-dinucleotide (FAD), flavin-monomonucleotide (FMN), riboflavinyl glucoside (FG), two unknown flavin

compounds, probably new, and one unknown pteridine. Besides these components, a violet fluorescent spot was observed in place of kynurenine in the embryo of a vermilion strain. This may indicate the presence of an oxide of tryptophan. In brief, the fluorescent contents are rich in compounds derived from flavin throughout embryonic development, but very poor in pteridines. On the contrary, large amounts of pteridine compounds have been found, by the same method, in the embryo of *Bombyx* as well as in pupae of *Drosophila*. This is interesting and needs further investigation.

Changes in the fluorescent substances during embryonic development are tabulated below. The observations were made with 200 eggs per stage; the two-dimensional paper chromatography was performed by the irrigation with hot water the first time and with a mixture of butanol acetic acid and water the second time.

(1) Wild strain Oregon-R-S

Substances	Fl. color	Stages							
		1	2	4	7	9-10	13	14	16
Kynurenine	blue	++++	+++++	+++++	++++	+++	++	+	+(±)
3-OH kynurenine	green	-	-	-	-	+(±)	++	+++	++++
FAD	yellow	+	+	+	++	++	+++	+++	+++
FMN	yellow	+	+	+	+(±)	±	±	±	±
FG	yellowish	+	+	+	+	+(++)	++	++	++
Flavin compound I	yellowish	++	+	+	++	++	++	++	+++
Flavin compound II	yellowish	++	+++	+++	++	+	+(±)	-(±)	-
Pteridine	violet	+	+	+	+	+(±)	+(±)	+(±)	+(±)

(2) Eye-color mutants

Mutants	Substances	Stages						
		2	4	7	9-10	13	14	16
v	Kynurenine	-	-	-	-	-	-	-
	3-OH kyn.	-	-	-	-	-	-	-
	violet spot	++	++	++	+	±(-)	±(-)	±(-)
		Other components are similar to those in wild type.						
cn	Kynurenine	+++++	+++++	+++++	+++++	+++++	+++++	+++++
	3-OH kyn.	-	-	-	-	-	-	-
		Other components are similar to those in wild type.						
w	Kynurenine	+++++	++++	++++	+++	++	±	±(-)
	3-OH kyn.	-	-	-	±	+	++	+++
		Other components are similar to those in wild type.						

No remarkable difference could be found between the patterns of three mutants--st, se, and bw--and that of wild type. The tryptophan metabolism of the white mutant should be noted particularly; tryptophan is converted into kynurenine and further into 3-hydroxykynurenine in the embryonic stage

of white. This may indicate that no block exists, at least between tryptophan and 3-hydroxykynurenine, in this mutant.

The time of conversion of kynurenine into 3-hydroxykynurenine, or activation of kynurenine oxydase, may be learned by a comparison of tryptophan metabolism in the wild strain, vermilion, and cinnabar. The time of this conversion is at stages 9-10, the stage of involution of the head and dorsal closure, which corresponds to the stage of blastokinesis in the embryonic development of Bombyx. It seems that the conversion of tryptophan into kynurenine has already taken place in the ovary.

Details of this study will be published later.

*Jacobs, M. E. Influence of desiccation on tyrosine-oxidizing activity in D. melanogaster.

Previous studies (DIS-31) showed that desiccation of late larvae accelerated dopa-oxidizing activity. Further studies have shown that tyrosine-oxidizing activity was similarly increased after as little as one hour in a large air-tight chamber containing Dreirite, as compared with that of sibling larvae kept in a similar chamber containing moist gauze covered with wire cloth. Counts failed to show an increase in pupation rate accompanying the increase in tyrosinase activity, a fact that supports recent views of Dannel that melanization is not primarily related to puparium formation.

*Jacobs, M. E. Relation of age to sexual attraction in D. melanogaster.

Studies of ebony and non-ebony flies from a wild population in observation cells in light and diminished (red) light showed that non-ebony males more frequently mated with old (six-day) than with young (one-day) virgin non-ebony females; but with ebony females this difference was not noted. Males succeeded in mating with ebony females earlier than with non-ebony females; the latter showed avoidance behavior by kicking and bending down the abdomen. Males courted heads of one- and two-day-old females, in light or diminished light, more frequently than those of older or teneral females, and failed to court male heads only in diminished light.

*Jacobs, M. E., J. T. Bowman, and V. Walliser. Studies of a melanoma.

The stock of D. melanogaster used, herein named tu-55g, was discovered by the senior author in a wild population at Beaufort, North Carolina on July 28, 1955. Melanomas appear in the larvae, starting 55 hours after hatching. Every adult in a culture may show at least one melanoma, commonly on the abdomen, less commonly on the thorax, and rarely on the head. The largest larval melanoma is caudal, and this melanoma develops later than the others. The primary tumor chromosome is number two, as determined by use of dominant inversion marker stocks.

Kikkawa, H. Genetical analyses of resistance to parathion in a Swedish strain of D. melanogaster.

During a stay at the Biological Laboratory in Cold Spring Harbor during the summer of 1958, I obtained from Professor B. Rasmuson of Uppsala, Sweden a strain of D. melanogaster called "KSL" which was very resistant to parathion.

Genetical analyses have shown that the major gene for parathion resistance in this strain is also located at a locus near 65 on the second chromosome, as in Japanese strains like Hikone-R and WMB (see DIS-31, p. 125). Thus, resistance to parathion in D. melanogaster seems to be controlled by a single gene.

Kikkawa, H., and K. Abe.
Genetic control of amylase
in D. melanogaster.

Amylase activity in larval, pupal, and adult stages of D. melanogaster varies in different strains. The gene responsible for strong amylase activity

is semidominant, and probably located at 80+ on the second chromosome. This gene seems to be concerned with amylase function in both digestive organs and body fluids.

King, R. C. Further studies
of oögenesis in D. melanogaster.

Oregon-R wild-type females (0-1 hours old) were fed for various lengths of time on medium containing 5-aminouracil

(an analogue of both uracil and thymine). The medium contained 2% agar, 2% brewers' yeast, 0.5% sucrose, 0.5% propionic acid, and 0.1% of the analogue. Ovaries from flies fed 5 days on 5-aminouracil are small, and show a retardation in the replication of nurse-cell chromosomes somewhat similar to that produced in the mutants fs2.1 and sn^{36a}. Chambers are produced as a result, which contain a nonhomogeneous assemblage of nurse-cell nuclei (some resemble stage 3, others stage 4, and others stages 6-10). Numerous Feulgen-positive granules are present in the cytoplasm of oögonia and early nurse cells. Fusions of adjacent chambers occur commonly. Ovaries from flies fed for 10 days show chambers which contain reduced numbers of nurse cells; perhaps the result of nuclear fusions. In these chambers are found two or three giant nuclei, which contain banded chromosomes. A chamber from a fly fed 14 days on 5-aminouracil contained a single nucleus 70 micra in diameter (2.5 X the maximum volume normally observed). Most ovaries from the 14-day series, however, were completely pycnotic.

Further information necessitates modification of the staging of oögenesis of D. melanogaster as given in Growth 20: 121-157. Stage 10 is defined there as the stage at which the oöcyte makes up about half the total volume of the chamber. Stage 10 should be divided into stage 10A, which is common, and stage 10B, which is rare. The dimensions given for stage 10 (~140 x 360 μ) in the Growth article are for stage 10A. The dimensions for stage 10B are ~190 x 460 μ . Thus the stage-10B chamber is almost the size of a mature primary ovarian oöcyte. In both 10A and 10B the oöcyte and the nurse chamber have equal volumes. Because of shrinkage, the dimensions in Feulgen-stained whole mounts will be 70%-80% the values given above. Feulgen-stained stage-10B nurse nuclei are much paler than those in stage-10A chambers. Thus during stage 10B the 16-cell cyst reaches its maximum volume, and the concentration of DNA in the nurse nuclei falls below that of stage-10A oöcytes. During stage 11 the oöcyte grows at the expense of the nurse cells and eventually reaches its maximum size.

King, R. C., and J. H. Sang.
Modification of ovogenesis
in D. melanogaster.

The Ore-S wild strain studied has about 14 ovarioles per ovary. Freshly hatched flies contain no oöcytes more advanced than stage 7. By one day, all ovarioles

contain an oöcyte in active vitellogenesis (stages 8-11) and half the ovari-

oles contain a mature egg (stage 14). By the third day, all ovarioles contain a stage 14 and a stage 8-11; by seven days, all ovarioles have two stage 14's and a third of them have also an oöcyte in active vitellogenesis. If flies are fed on a protein-free diet, active vitellogenesis declines, and after a week no stages 8-11 are found. When such flies are then placed on live yeast, vitellogenesis restarts immediately and over half the ovarioles contain stages 8-11 within 24 hours of the yeast meal. By three days the females are laying normally (30 eggs/day), and they are still laying at this rate two weeks later when each ovariole has an oöcyte in active vitellogenesis. The first dozen or so eggs laid (which were formed during the period of protein deprivation) have a high mortality, but thereafter hatchability is 90% (provided fresh males are supplied).

When the females were placed on yeast their primary oöcytes averaged about 6 per ovariole, and during the subsequent two weeks each fly laid about 340 eggs; so that each ovariole must have produced at least 12 oöcytes, of which half must have originated from oögonia. It follows that keeping a fly on a protein-free diet for a week does not prevent oögonia from forming oöcytes, or oöcytes from synthesizing yolk once protein is again supplied. Protein deprivation for a fortnight does appear to lead to degeneration of germaria and early oöcytes.

When flies are fed on sugar-agar for a week, no stage 8-11 oöcytes remain in the ovary. Yeast feeding for a day then produces large numbers of oöcytes in active vitellogenesis (in line with the above). When such females are irradiated, at least half the ovarioles contain an oöcyte whose nucleus lies in yolkly oöplasm, which is increasing in volume. It would be of interest to contrast the mutation rate of such oöcytes with that of normal stage 7's (where the nucleus sits in a yolk-free cytoplasm) and of stage 14 oöcytes, in which the compact genetic material (which is not surrounded by a nuclear membrane) lies in the completely grown yolkly oöplasm.

Depriving adults of dietary Mg lowers the frequency of oöcytes in active vitellogenesis, but it does so more slowly than protein elimination, which suggests that the protein store runs out before the reserves of Mg salts. In this case, however, replication of Feulgen-positive material is retarded in the nurse cells. Subsequently, all chambers more advanced than stage 6 degenerate, the nurse cells going before the follicle cells. In spite of this, chambers continue to be produced by the germarium; so that an ovariole may eventually contain as many as eleven oöcytes, of which the posterior six will be degenerating stage 6 and 7 oöcytes and the anterior five will be normal stage 1-6 chambers. So Mg appears to be essential for yolk synthesis, nucleic acid replication, and the transformation of oöcytes from stage 6 to stage 7. Since chamber proliferation fails to occur in the protein-deficient ovary, it appears that protein is required for this process.

Oregon-S, Crianlarach-6, and hybrid females were compared with respect to their ability to synthesize yolk from larval reserves when reared on an inadequate adult diet. The flies were fed on an aseptic 15% starch--0.5% fructose diet during days 0-7. During this period all strains synthesized 8-11 eggs. However, the C-6 and hybrid females laid most of the eggs synthesized at once; whereas the Ore-S females stored the majority of synthesized eggs in their ovarioles. Ovaries from 83 Ore-S females were examined. This corresponds to an ovariole population of 2300. About one-third of the ovarioles contained stage 14 oöcytes. One contained an active first-instar larva. When adults from the three strains were fed on a Mg-free diet, the ovaries of the hybrid were the first to show abnormalities. Thus genotype

markedly influences the effect of dietary deficiencies upon oögenesis.

(This study is part of a collaborative program carried out during the tenure by R. C. King of a National Science Foundation Postdoctoral Fellowship while on leave from Northwestern University.)

King, R. C., and J. H. Sang.

Additional description of ap^4 .

Adult wild-type females have been fed dead yeast containing various pH indicators (bromophenol blue, bromocresol purple, bromothymol blue, lacmoid), and their digestive tracts have been examined to determine the resulting colors of the material in the lumens. Most of the ventriculus has a pH of about 6. The mid-ventriculus is generally about pH 5, although it can sometimes go as low as pH 3 or as high as pH 6. The pH of the crop varies between 5 and 6. Adult ap^4/ap^4 females live for only 3-4 days and are active only during the first day or so. During this first day the ventriculus of such females is generally pH 5 throughout its entire length. $ap^4/+$ females are normal in this respect. The fore-ventriculus of most ap^4/ap^4 females over a day old is swollen, since it is packed with a transparent material which appears to be disorganized peritrophic membrane. Their cardia appear normal in Feulgen-stained whole mounts. The situation here thus seems similar to that described by Rizki (J.E.Z. 131: 211) for 1(1)48j.

Normally, females accumulate sufficient reserves during larval feeding to allow the synthesis of more than half a dozen eggs, even in the absence of an adequate adult diet; but ap^4/ap^4 females fail to elaborate yolk under these conditions, even though they contain large amounts of adipose tissue. No oöcytes more advanced than stage 7 were found in 79 ovaries from 1-2-day-old females: one stage 10 and thirteen stage 14 oöcytes were found in a total of forty 3-day-old ovaries. All other oöcytes were in pre-yolk stages. No fully formed eggs are ever laid, since ap^4/ap^4 females are completely motionless and their only obvious movement is that of their pulsating hearts and ovaries. The testis of ap^4 males appears normal and contains mature sperm, although the adults are likewise inert. The failure of yolk formation and the blockage of the fore-ventriculus suggested that ap^4/ap^4 females might suffer from a lowered protein intake. Consequently, larvae from the ap^4/al^2 $Cy\ 1t^v$ sp^2 stock were reared aseptically on Medium C (Sang, J. Exp. Biol. 33: 45) and on the same medium with supplemented casein hydrolysate in place of casein, in the hope that they would use these diets more effectively than yeast. However, the diets produced no change in the morphology of wings, halteres, gut, or ovaries, nor did they affect the ability of adults to produce eggs when supplied to them.

(This study is part of a collaborative program carried out during the tenure by R. C. King of a National Science Foundation Postdoctoral Fellowship while on leave from Northwestern University.)

Kroman, R. A. The effect of Ag and Hg ions on melanotic tumor incidence.

In order to test the hypothesis that observed variation in incidence of melanotic pseudotumors is due to the proportion of tumors that become pigmented, and hence recognizable, and not due to the number actually formed by cell aggregation, the effects on tumor incidence of both inhibitors and enhancers of melanin are being studied. Four strains, tu^1 , tu^8 , tu^{49k} , and tu^{WPS} are being tested.

Ag, which inhibits melanin formation, was tested as AgNO_3 in concentrations of 0.05, 0.1, 0.15 and 0.2%. Ag ions have a similar effect on frequency in the tu^{49k} and tu^1 strains, with lower concentrations reducing, and higher concentrations increasing the incidence. Statistically, the minimum frequency, which was observed with concentrations of 0.1 and 0.15%, respectively, in the two lines, was highly significantly less than that of the controls, whereas with 0.2% concentration the incidence was significantly greater than in the controls. The tu^8 strain exhibited an increasing reduction in tumor incidence with increasing concentrations, which became significantly less than the control value at concentrations greater than 0.1%; the incidence in tu^{WPS} remained unchanged at all concentrations tested. It is interesting to note that no correlation was observed between the degree of integumental depigmentation and the presence or absence of a tumor in any individual.

Hg was tested as HgCl_2 , in concentrations of 0.001 and 0.005%, and in equimolar concentrations of the sulfhydryl inhibitor *p*-chloromercuribenzoic acid. The compounds had similar effects on tumor incidence, although the latter should increase the incidence if sulfhydryl groups inhibit tumor pigmentation. In the tu^1 strain there was a marked increase in both penetrance and expressivity, and the penetrance became nearly complete at the higher concentration. In the tu^{WPS} strain the incidence was significantly reduced at the 0.005% concentration. In the tu^8 and tu^{49k} strains, where the controls showed the greatest variation, the incidence was found to be correlated with the variation in incidence of the controls: when the control incidence was high, Hg ions decreased it significantly; when low, they increased it significantly. The last observation suggests that Hg influences the tumor frequency indirectly by its effect on other factors, perhaps the microflora of the culture.

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Kuroda, Y. Comparative study of the wing discs of vestigial series in *D. melanogaster* in tissue culture.

Wing discs from third-instar larvae of vg , vg^{no} , and vg^{np} strains of *D. melanogaster* were cultured in synthetic medium (see DIS-30, p. 161), to compare their development with that of wing

discs of a wild strain (Oregon).

Wing discs of the vg strain, when taken from mature third-instar larvae grown at 25° C, were distinguishable from those of the wild strain by the size of the wing pouch. When the vg wing discs were cultured in synthetic medium for 24 hours, evagination was observed, with extension of the wing pouch as a cone. When wing discs from the wild strain were cultured, the evagination was characterized by extension of the wing pouch as a cylinder. The difference in evagination between the two strains was observed more and more markedly in further cultivation. The wing discs of vg^{no} and vg^{np} showed characters intermediate between those of vg and those of the wild strain (though more resembling the wild strain) when they were taken from third-instar larvae grown at 25° C. Culture of the vg^{no} and vg^{np} wing discs resulted in evagination with extension of the wing pouch as a smaller cylinder, after 24 hours.

When wing discs from vg larvae grown at 31° C were cultured in synthetic medium at 31° C, evagination was characterized by extension of the wing pouch as a cylinder, as in the wild strain. Cultures of wing discs

of vg^{no} and of the wild strain in synthetic medium at $31^{\circ}C$ showed no marked differences from discs of these strains cultured at $25^{\circ}C$. When wing discs from vg^{np} larvae grown at $31^{\circ}C$ was cultured in synthetic medium at $31^{\circ}C$, they showed the evagination characteristic of vg wing discs, that is, extension of the wing pouch as a cone. These observations are consistent with the response of the characters of adult wings in these strains to high temperature.

Kuroda, Y., and S. Tamura.
Resistance to parathion of various organs in *D. melanogaster* in tissue culture.

Eye discs, wing discs, cephalic complexes, leg discs, haltere discs, and salivary glands of mature third-instar larvae (about 96 hours after hatching) of the insecticide-resistant strains

Hikone and WMB and the insecticide-susceptible strains Fukuoka and Canton-S of *D. melanogaster* were cultured in synthetic media containing 1.0, 5.0, 10.0, 25.0, and 50.0 ppm of parathion.

When 1.0, 5.0, or 10.0 ppm of parathion was added to the culture medium, no marked effect on any of the organs was observed, even after they were cultured for 48 hours. When 25.0 ppm parathion was added to the medium, it was observed after 24 hours' culture that differentiation and growth of eye discs, wing discs, cephalic complexes, and leg discs were more pronouncedly inhibited in Fukuoka and Canton-S cultures than in Hikone and WMB. Culture for 48 hours in this medium produced marked inhibition of differentiation and growth of these organs, from the resistant as well as from the susceptible strains. The haltere discs of all strains were markedly inhibited after culture for 24 hours in medium containing 25.0 ppm parathion. The growth of salivary glands of all the strains was less inhibited by 25.0 ppm parathion in the medium.

When 50.0 ppm parathion was added to the medium, all the organs mentioned above, from both resistant and susceptible strains, were pronouncedly inhibited after culture for 24 hours.

Lewis, H. W., and H. S. Lewis.
Interaction of the tyrosinase-activating systems of Canton-S and sable adults.

Comparison of the rates of activation of tyrosinase extracted from young Canton-S and sable flies revealed that the enzyme from Canton flies becomes activated at a faster rate than does

the enzyme from sable flies. This difference in rate of activation of tyrosinase in the wild-type and mutant extracts suggested the possibility of the presence of an inhibitor or inhibition of inhibitors in the extracts of one genotype and their absence in the other genotype. To test this possibility, mixing experiments were performed. The kinetics of the experiments showed that the difference in activation rate between the two genotypes is not due to any inhibition. Furthermore, the maximum activity of the mixed extracts was found to be greater than would be expected on an additive basis. This was explained by the fact that the Canton activator system enhances the activation of the sable proenzyme. This enhancement has been observed in mixtures of 1 part Canton to more than 50 parts sable, whereas the reciprocal mixtures have not shown the phenomenon. Mixtures of the activating system of yellow flies with the sable proenzyme do not show the enhancing effect. The major component of the activating system is a protein.

To test whether the in vitro enhancement could also be demonstrated in

a fly, the activation kinetics of Canton/sable heterozygotes was studied. These experiments revealed that the rate of activation of the heterozygote extract is like that of the Canton enzyme extract, whereas the maximum activity attained is like that of the sable enzyme extract.

Lindsley, D. L., and E. H. Grell.
The genetic extent of Dp(3;1)0-5.

Dp(3;1)0-5 is an insertion of a section of 3R extending from 88A-C to 92 into the X chromosome near 4F (Lewis, DIS-27).

This segment has been inserted dyscentrically, that is, with region 92 proximal to 88A-C. The ability of the duplication to cover the following third-chromosome markers has been checked: cu (50.0), ry (51.0), kar (52.0), red (55.5), jvl (56.7), cv-c (57.9), sbd (58.2), ss (58.5), bx (58.8), sr (62.0), gl (63.1), k (64.0), and e (70.1). Bridges and Brehme report that sr and e are covered by the duplication, but in the present tests the markers red (55.5) through gl (63.1) are covered and the rest are not. Consequently the genetic length of the inserted piece of 3R is between 7.6 and 12.0 units.

Makino, S., E. Momma, and H. Takada. Drosophila survey in Shiretoko Peninsula, Hokkaido.

A preliminary survey was made on Mt. Raus (altitude 1661 m) on Shiretoko Peninsula at the eastern extremity of Hokkaido, in August 1958. The flies

are listed in the table.

<u>Species</u>	<u>Female</u>	<u>Male</u>	<u>Total</u>
Leucophenga sp.	0	1	1
Drosophila coracina	0	3	3
D. bifasciata	50	150	200
D. helvetica	2	3	5
D. testacea	30	32	62
D. nigromaculata	31	8	39
D. brachynephros	3	1	4
D. funebris	4	3	7
D. moriwaki	22	24	46
D. laeertosa	6	15	21
D. sp. (robusta gr.)	3	5	8
D. ezoana	14	7	21
D. histrio	1	1	2
Totals	166	253	419

Meyer, Helen U. A case of double fertilization resulting in parasitism of a genetically lethal portion in a mosaic female.

In the course of breeding tests to detect lethals in ultraviolet-treated second chromosomes, an interesting mosaic female was found in one of the cultures, which (as was ascertained later) contained an induced allele of the dumpy series, a Truncate designated dp^{T57g} (this issue of DIS New Mutants section). The mutant is completely lethal in homozygous condition.

The culture giving the mosaic had contained a cross of a single male of composition dp^{T57g}.../S² CyInL lt³ cn bw sp by females of composition S Sp en.../dp^{txI} CyInL&R Bl lt³ cn² L⁴ sp². Offspring representing the class dp^{T57g}.../dp^{txI}...were absent, with the exception of one longitudinally

divided mosaic female that manifested the otherwise lethal compound on one side. That side had strong vortices, a blistered wing, and also the characters of Bl, L⁴, cn² eye color and sp wing. The other side of the mosaic had the constitution dp^{txl} Cy Bl lt⁵ cn² L⁴ sp²/S² Cy lt⁵ cn bw sp, as indicated by its phenotype. Homozygous Curly flies of the latter type occasionally survive at a low frequency in uncrowded cultures.

This mosaic female must have arisen by double fertilization of an egg containing the dp^{txl} Cy... chromosome, by the two different kinds of sperm. Though she produced no offspring, she lived for five days, the lethal half of the body obviously being supported by the nonlethal half. It is quite possible that, in a complementary way, the homozygous Curly half was helped to live by the half manifesting the dumpy compound, since the causes of death of the two genotypes would be different.

(This work was supported by a grant to H. J. Muller and associates from the U. S. Public Health Service, RG-5286 (Cl).)

Meyer, Helen U., and H. J. Muller. Genetic effects of high doses of X-rays in oögonia.

Even if given as many as 6 doses of 4000 r, adult females were found to undergo some recovery of fertility, provided that these doses were spaced at intervals of several days, 4 days

being the interval used in these studies. Some of the females thus accumulated as much as 24,000 r. During the intervals between irradiations the females were kept with males to promote egg production and, thereby, regeneration of oögonial tissue. F₁ females derived from eggs laid from 8 to 12 days after the final treatment were tested for lethals in their irradiated X chromosomes. To reduce the chance of testing more than one F₁ female carrying the same irradiated X chromosome, the P₁ females were bred in groups of 5, and not more than 4 females from any one such group were tested. In a case where two lethals were found in offspring from such a group, localization tests were carried out to throw light on whether they were of independent origin. In all cases they were found to be independent. The P₁ females themselves had obtained the X chromosomes to be tested from their fathers ("P₀"), in which generation it had therefore been nonlethal. Tests of crossing-over frequency, carried out on 9 of the sex-linked lethals, gave one case of marked reduction of crossing over, indicative of a gross structural change. The following results were obtained in experiments utilizing different numbers of treatments according to the above-described plan.

Accumulated X-ray dose	Distribution of doses (4-day intervals)	No. lethals in total no. tests	% lethals
12,000 r	4 x 3000 r	13/244	5.3
16,000	4 x 4000	15/175	8.6
24,000	6 x 4000	8/63	12.7
24,000	6 x 4000	11/62	17.7

Thus the rate of production of sex-linked lethals was approximately 1% per 2000 r (or $5 \times 10^{-6}/r$). The results suggest but do not prove that there is a small dose-dependent contingent of lethals that result in a somewhat

higher rate when individual fractions of 4000 r rather than 3000 r are used; on the other hand, the accumulation of more fractions that are so widely separated as these cannot have caused a real rise above linearity. However, that may be, the present induced rate is approximately one-sixth that ordinarily obtained from spermatozoa. Moreover, it is in excellent agreement with the rate of $5 \times 10^{-6}/r$ obtained by Oster for *Ogonia* irradiated in third-instar larvae, in experiments using doses of 600 r and 2400 r (Proc. X Intern. Congr. Genet.: 210-211, 1958). It also is in satisfactory agreement with the frequency of autosomal lethals obtained previously by Meyer after X-ray treatment of the embryonic pole cells (Genetics 42: 385, 1957), where 1500 r to 2000 r gave a rate of $2.3 \pm 1.0\%$ lethals in the second chromosome, since this would be equivalent to about 1% in the X chromosome.

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Meyer, Helen U., and H. J. Muller. Preliminary evidence of detrimental mutations originating at a comparatively high rate in untreated females.

Previous work on the frequency of detrimental mutations (invisible, not fully lethal, somewhat impairing viability and/or fertility) has been confined to those arising in spermatozoa as a result of irradiation, with

the exception of that reported in a recent paper by Bonnier, in which X chromosomes derived from irradiated females, although containing lethals at about the expected frequency, gave no evidence of having had detrimental mutations induced in them. In our present experiments a group of X chromosomes which at the start had been co-isogenic were passed down in separate lines of descent through 53 generations, during all of which they were kept in heterozygous condition in females by means of a genetic mechanism that made use of balanced sterility. At the end of this period one X chromosome of each line was tested for lethals, and if not lethal, for detrimental mutations. The tests indicated a lethal frequency of some 10%-15%. Unfortunately, many of the lines were lost, but 14 nonlethals of different lines were obtained for testing with regard to detrimental mutations. The tests were of a special kind (to be described in a later report, which involved multiplying the given chromosome and then getting it into males that were placed with attached-X females along with other males containing a similarly multiplied control X chromosome that had originally been co-isogenic with the one to be tested but had not had the latter's opportunity to accumulate mutations. Competition between the two groups of males for the same females was allowed to continue for about 12 generations, and the relative numbers of the two types were then determined by special means. This competition involved both the viability and the fertility of the males--in other words, their total productivity. Several sublimes of each of the X chromosomes to be tested were bred in parallel in the same way during this competition period, as checks on the repeatability of the effects found. Among 12 X chromosomes already partially tested in this way, two have so far been found to be definitely inferior to the control chromosomes, and two or three others (on which the tests have not yet been completed) probably inferior. Although the numbers are small, these preliminary results already indicate a frequency of spontaneous origination of demonstrable detrimental mutations in females which is higher than that of lethals.

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Miyoshi, Y. A new strain of D. melanogaster resistant to NaCl.

A strain resistant to a high concentration of NaCl in the culture medium has been found. It is a strain of bw which has been maintained in our laboratory for a long time but is of unknown origin. To test salt resistance, eggs were removed to a culture medium containing NaCl at a concentration of 1.0 M and left to grow to imagoes. This bw strain is far more resistant than the previously reported se strain (DIS-31); the survival rate of the former is about three times that of the latter (bw, 50.2%; se, 16.7%). By selecting from generation to generation on NaCl-containing culture medium, the survival rate was raised to a level of 90% after 10 generations, and remained almost constant in subsequent generations. In some progenies of the selected strain that were transferred to normal culture medium, the resistance to NaCl has remained at the level of the selected strain through many generations.

Morita, T. Purine contents and xanthine dehydrogenase in D. melanogaster.

It is a well-known fact that the rosy² (ry²) eye-color mutant of D. melanogaster does not contain isoxanthopterin, which occurs widely in Drosophila.

Another mutant, ry¹, also contains no trace of isoxanthopterin at any developmental stage. On the other hand, it accumulates larger amounts of hypoxanthine and xanthine at pupal and imaginal stages, but no uric acid, which is an end product of nitrogen metabolism in insects. Purine contents, detected by chromatographic methods, at different developmental stages of Oregon-R and ry are shown in the table. It is very interesting that hypoxanthine is accumulated in the head of the adult fly.

An enzyme, prepared from Drosophila pupae, can catalyze the oxidation of 2-amino-4-hydroxypteridine (AHP) to isoxanthopterin, as well as of hypoxanthine and xanthine to uric acid. This enzyme, therefore, has been called xanthine oxidase or pterin oxidase, but it is a true dehydrogenase, because it requires electron acceptors, DPN or methylene blue, for oxidation. As the homogenate can also catalyze the oxidation of DPNH to DPN, xanthine dehydrogenase seems to link the DPNH-oxidase system by the medium of DPN in vivo. The ry strain does not show any xanthine dehydrogenase activity, but it has DPNH-oxidation activity. The same phenomenon is found in such double-recessive mutants homozygous for ry as v; ry, bw; ry, cn; ry, se; ry, and stw³; ry.

	Oregon-R		ry		
	Hypoxanthine ug/mg	Uric acid ug/mg	Hypoxanthine ug/mg	Xanthine ug/mg	Uric acid ug/mg
3rd-instar larva	0.0	0.06	0.10	0.10	0.0
Early pupa	0.11	0.70	0.85	0.28	0.0
Mid pupa	0.06	0.80	1.55	0.48	0.0
Late pupa	0.0	1.10	1.88	0.52	0.0
Adult: male					
head	0.33	0.0	2.63	0.0	0.0
body	0.03	0.65	0.37	0.19	0.0
female					
head	0.09	0.0	1.38	0.0	0.0
body	0.0	0.30	0.20	0.20	0.0

Muller, H. J. An androgenetic homozygous male.

From a cross of a heterozygous male having one second chromosome containing *cn bw* and one third chromosome

containing *ri e* to a female having only normal alleles of these genes, one F_1 male arose that was of homozygous *cn bw ri e* constitution. There were approximately 100 sibs of expected type. The exception arose too early to be an F_2 and there was no chance for a contamination of the kind in question to have occurred. Since this exception afforded the opportunity of quickly obtaining a stock isogenic and nonlethal for all major chromosomes, it was subjected to crosses for this purpose. It bred satisfactorily but through inadvertence some of the desired descendants were lost and only the third chromosome, *ri e*, was obtained in homozygous condition. The exceptional male was evidently formed by the union of the nuclei of two paternal germ cells, either after fertilization or during some prefertilization mitotic or meiotic stage, and development must have proceeded with this chromosomal equipment even though the female pronucleus did not make its genetic contribution to the zygote.

Muller, H. J. Pseudo crossing over near centromeres of the third chromosomes induced in late oöcytes by X-rays.

Pursuant of the findings of Herskowitz, Muller, Abrahamson, and Schalet concerning induction of exchanges of diverse kinds between heterochromatic regions by X-rays applied to late

oöcytes, a study was made of the frequency of recombinants induced by these means between the central regions of the third chromosomes. The heterozygous mothers were provided with one third chromosome containing *ri* and p^D and the other third chromosome having inserted into it between *ri* and p^D close to the left of the centromere an insertion containing a portion of the X with the normal allele of *cut*. This insertion had been found by Hannah in 1947 as a result of irradiation of the ring X^{C2} and designated $Dp(sn R 13aH1)$. In order that this insertion might serve as a marker in the third chromosome, the females were provided with ordinary attached X's homozygous for *y ct* and *f*. They were testcrossed to *ri p^D* males. The daughters were diagnostic for all three markers in the third chromosomes, the sons only for the two outer ones. The mothers were treated with 2500 r at 125 KVP and changed daily to new broods.

The offspring from untreated mothers contained 6 recombinants between *ri* and p^D among a total of 1127, or 0.53%, among broods of the first six days. Of these recombinants only 1 occurred among the 433 daughters; this was in the left (*ri-Dp*) region. The treated females gave 604 offspring (not including here cases of detachment of the X's) from eggs laid in the first four days. These included 20 crossovers between *ri* and p^D , or 3.3%. This frequency is significantly higher than that in the controls, and when the control value is subtracted from it it indicates that a recombination frequency of 2.8% was induced in these chromosomes by the 2500 r. Among the 206 daughters of treated females here included there were 2 crossovers in the *ri-Dp* region and 8 in the $Dp-p^D$ region (that containing the centromere).

As a further test of the distribution of crossovers in these two regions in control material, untreated females of the same composition as before were crossed to *ct ri p^D* males, so that both sons and daughters could now be scored for the ct^+ duplication. Among the 1003 offspring, 5, or .5%, were crossovers in the *ri-Dp* region and 10, or 1%, in the $Dp-p^D$ region. Although the frequencies here are higher than in the previous controls they remain significantly lower in the right or centromere region than found in the

treated material (1% versus 4%, where they could be scored as such).

These results, taken in connection with others, support the conception that the exchanges induced at this stage are confined largely to the heterochromatic regions near the centromere. It is likely that they occur preferentially between homologous chromosomes, because of the latter's propinquity, but that they do not necessarily occur at precisely homologous positions in the two chromosomes.

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

Nawa, S., T. Taira, and
B. Sakaguchi. Pterine
oxidation in *D. melanogaster*.

Drosophila homogenate is capable of oxidizing both xanthine and 2-amino-4-hydroxypteridine (AHP). It is likely that the pterine dehydrogenase in

Drosophila is a dehydrogenase, not an oxidase, that diphosphopyridine nucleotide (DPN) is a more effective hydrogen acceptor than methylene blue (MB), and that the conversion of AHP to isoxanthopterin is carried out by pterine dehydrogenase in the presence of any DPN in vivo. An enzyme preparation was made from pupae of wild-type *D. melanogaster*. The freshly prepared supernatant produced a considerable amount of isoxanthopterin from AHP in the absence of any external (exogenous) hydrogen acceptor. When dialyzed enzyme or aged supernatant was used, however, no appreciable production of isoxanthopterin was observed without an external acceptor as shown in the table. Activity is expressed in micromoles of isoxanthopterin produced per gram of whole pupae (wet weight) per hour, pH 8.0.

Acceptor	Preparation		
	Fresh	Aged	Dialyzed
Water	0.55	0.08	0.02
MB	0.9	0.58	0.7
DPN	0.9	0.6	0.75
Cytochrome C	-	0.1	0.02

The activity of pterine dehydrogenase in the presence of DPN has been found to be about equal to or rather higher than that of MB. It seems that the DPN naturally present in *Drosophila* acts as a hydrogen acceptor. Under aerobic conditions, much more isoxanthopterin is produced than is accounted for by the amount of DPN added. This may be because pterine dehydrogenase is linked to the DPNH oxidase system. The pH optimum of DPNH oxidase in *Drosophila* is in the neighborhood of 6.5. The ratio of the reaction rate with DPN and with MB as an acceptor is very different at various pH levels. This may be due to the participation of DPNH oxidase in the reaction. The action of the enzyme for pterine oxidation in *Drosophila* is influenced by the concentration of DPN and the rate of DPNH reoxidation. For example, it has been known that the mutant *ry* lacks pterine dehydrogenase. The ratio of reaction rates with DPN and with MB as acceptors varies considerably in several different mutants. The variation may be due to differences in the rate of DPNH oxidation. Further experiments along this line are in progress.

Ogaki, M. Effect of genetical background on facet number in some eye mutants of D. melanogaster.

Small-eyed mutants such as B, bar-3, ey², and L² were subjected to a series of crosses designed to render them co-isogenic with a highly inbred strain of the wild Oregon stock. The shapes of the compound eyes of these co-isogenic strains were compared with those of the original mutant stocks. In B, bar-3, and ey² stocks the facet number was increased in the co-isogenic conditions, especially in the case of bar-3 and ey². On the contrary, the co-isogenic L² stock had a decreased facet number, and in almost all individuals eyes are completely lacking. The facet-increasing effect in the co-isogenic bar-3 stock was analyzed, and was found referable to a recessive gene (or genes) located on the second chromosome of the Oregon isogenic stock. This modifier gene is strong enough in homozygous condition to increase the facets to the extent of twice the number found in the original bar-3 eyes.

Ogita, Z. Genetical study concerning a new type of mixed insecticide for D. melanogaster.

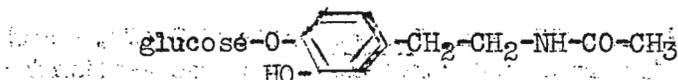
The author (1957) found that the cross-resistance pattern of DDT, BHC, parathion, and PU (phenylurea) is negatively correlated with PTU (phenylthiourea). Genetical analyses suggest that the dominant gene at II-64~66 which confers resistance to DDT, BHC, and parathion also confers resistance to PU and abnormal susceptibility to PTU, whereas the dominant gene at III-50+ which confers resistance to nicotine sulfate also confers resistance to PTU as well as PU.

Thus, resistance to PTU and PU is due to polygenic system, which simultaneously requires two main factors on the second and third chromosomes. Therefore, all strains of D. melanogaster may be killed by exposure to a mixture of the minimum amount of PTU that will kill DDT-resistant strains and the small amount of DDT that is enough to kill PTU-resistant strains.

These experiments suggest the possibility that a mixture of DDT, BHC, or parathion with a substance negatively correlated to these compounds, such as PTU, would have effective insecticidal action and would not bring about resistance even after continuous use. Further experiments are now in progress from the standpoint of biochemical genetics.

Okubo, S. N-acetylhydroxytyramine glucoside in D. melanogaster.

It was reported previously (DIS-31) that pupae of the mutant claret (ca) contain a specific phenol. This compound is hydrolyzed by the action of beta-glucosidase, yielding glucose and a hydroxyphenolic compound which exhibits an R_f value of 0.8 in paper chromatography with a solvent containing n-butanol, acetic acid, and water (4:1:1). The hydroxyphenolic compound dissolved in N hydrochloric acid was heated in a sealed glass tube for 12 hours at 100° C, and then the hydrolysate was examined by means of column or paper chromatography (Kirshner et al., J.B.C. 226: 207, 1957; Seki, J. Biochem., in press 1958). It was clear from these experiments that hydroxytyramine (dopamine) and acetic acid were liberated from the dihydroxyphenolic compound exhibiting R_f 0.8. Reaction with hydroxylamine, paper electrophoresis, and absorption spectrum were also used to determine the chemical structure. From the results of the above experiments, the specific phenol contained in the mutant claret seems to be N-acetylhydroxytyramine glucoside.



A detailed report will be published in a Japanese periodical.

Oster, I. I., and Astrid Cicak. Although a considerable amount of work has been carried out on the sensitivity of irradiated pre-imaginal stages of *Drosophila* to X-rays of the pre-imaginal somatic cells of *D. melanogaster*, the possible influence of sex has not been investigated. Although Mavor had already reported a somewhat higher mortality for male imagoes irradiated during the pupal stage in 1927 (J. Exp. Zool. 47: 63-83), and Patterson had detected a slightly higher sensitivity to X-rays for females than males treated during the early stages of development in 1929 (J. Exp. Zool. 53: 327-372), subsequent investigators (H. Fritz-Niggli, Fortschr. Röntgenstr. 76: 218-254, 1952) have failed to take the role of this factor into account in their work.

By the use of marked stocks, which facilitated the separation of the sexes, it was possible to segregate large numbers of male and female first- and third-instar larvae. F₁ larvae from a cross of two unrelated stocks were used to avoid treating individuals homozygous for deleterious genes. In order to rule out the presence of pre-existing lethals in the female, which would result in a higher mortality among the males, larvae for the controls as well as the irradiated series were collected from the same matings. These were irradiated with either 1280 r or 3500 r (135 kV; 20 ma; 1 mm. Al. filtration; 160 r/min.) when their outer surfaces were fairly dry. Unirradiated but similarly handled larvae served as controls. The following results were obtained:

<u>Treatment</u>	<u>Sex</u>	<u>No. of larvae</u>	<u>No. of dead pupae (late)</u>	<u>No. of imagoes hatching</u>	<u>Unaccounted for</u>	<u>% mortality</u>
Controls	♀	500	5	490	5	2.0
	♂	500	20	480	0	4.0
1280 r (1st-instar larvae)	♀	500	120	250	130	50.0
	♂	500	290	80	130	84.0
Controls	♀	500	12	485	3	3.0
	♂	500	10	482	8	3.6
3500 r (3rd-instar larvae)	♀	500	136	304	60	39.2
	♂	500	340	148	12	70.4

Death very rarely occurred during the larval stages, being very frequent among late pupae. The individuals unaccounted for in the table presumably died during the third larval instar, but because they underwent lytic degeneration we could not detect them when the culture vials were scored. All the individuals that reached adulthood after being irradiated as third-instar larvae showed extreme wing abnormalities, lack of many bristles, and marked weakness. It can be seen in the table that third-instar larvae are about three times more radiation resistant than first-instar larvae, and that female larvae of both stages are much more resistant than males.

Thus far, the only known biochemical difference between males and females which might explain this differential sensitivity, namely, that the

latter contain more free methionine (Kaplan, Science 127: 473-474, 1958) probably does not account for the observed variation, because methionine has been shown to be ineffective as a protective substance against X-rays in mice, although it does protect complex polymers from degradation by X-rays.

It will be of interest to determine whether the difference in radio-sensitivity between male and female larvae is of genetic origin. This would presumably be due to the fact that males, having only one X chromosome, would suffer the deleterious effects of its loss after breakage by X-rays more often than females, which have two chromosomes.

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Oster, I. I., Elizabeth Ehrlich,
and H. J. Muller. Further study
of the mutants f^X and f^{+ih} .

In order to throw further light on the peculiarities of the mutants f^X and f^{+ih} , previously described (Muller, DIS-20: 88, 1946, and DIS-21: 71, 1947; Muller and Oster, DIS-31: 141-144, 1957, the sublocus of f^X was investigated by testing this mutant for crossing over with the mutants f^3 and f^1 , the subloci of which normally lie in this order, as shown by Green. One crossover, of normal type, was found between f^3 and f^X among 23,712 inspected offspring, and the markers present here showed f^X to be to the right of f^3 . No crossovers between f^X and f^1 were found in a count of 44,934. Thus f^X belongs in the group of those forked mutants which Green found to be to the right of f^{3n} and f^3 and to be closely (perhaps completely) linked with f^1 .

To test the effect of increasing the dose of the region containing f^X , a chromosome of composition $f^X B$ was made up and irradiated, and five deleted X's giving the Bar phenotype were obtained therefrom. All these deleted X's were found, when present in females containing attached X chromosomes homozygous for f^1 , to have a distinctly normalizing effect on the expression of forked. Thus the region containing f^X acts as a hypomorph, not an amorph, even though f^X itself has been judged to be a deficiency. The resolution of this seeming paradox can be found in the interpretation that only a sublocus is deficient, so that the locus as a whole, in the chromosome having f^X , is in effect acting hypomorphically, in comparison with a normal chromosome. It may be concluded, for one thing, that the absence of induced back mutations of f^X in the above-cited work of Muller and Oster, where six would have been expected in the given total number in the case of f^1 , attests not only to the deficiency nature of f^X but also to the infrequency with which duplications arise when spermatozoa are irradiated. The latter circumstance had also been inferred from the sensibly equal frequency with which back mutations of f^1 had been obtained from irradiated rings and rods, respectively.

That f^X is a deficiency, even though of only one or some of the subloci of a complex locus, had been inferred not only from its failure to give back mutations, but (prior to that) from its origination in an irradiated chromosome that was found to carry at the same time a more or less complementary duplication, f^{+ih} , located in the proximal heterochromatin. Tests of f^{+ih} carried out by us have shown that unlike su^W-f it exerts considerable suppressing action (dominant) on all other forkeds tried, no matter which sublocus they occupy. Included in these tests, which involved obtaining males with the forked mutant in its usual position and the f^{+ih} near the

centromere of the same chromosome, were f^3 , f^{3n} , f^1 , f^x , f^5 , and f^{36a} , the last two being of an extreme type.

The paradox still remains that f^{+ih} , especially when its variegation is reduced by an extra Y, virtually normalizes the phenotype of a male when f^x (or some other forked) is present in its usual position, despite the distance between the two genetic components here involved, whereas f^x (or any other forked) gives a distinctly forked phenotype in females when in compound with any forked of either region, even though in any such trans combination there are two normal alleles present in much closer proximity to one another than in the former type of case. It is further noteworthy that in the combination of a forked of the left-hand sublocus with f^{+ih} the phenotype is normalized despite the fact that the duplicated element seems to have been derived from the right-hand sublocus, so that the normalizing effect seems only additive here rather than complementary, just as it does when a deleted X containing f^x is added to a male genotype containing f^x itself in its usual position.

The interpretation of these relations which seems at present to be most plausible is that f^{+ih} contains somewhat more of the forked⁺ region than is absent in f^x . For this to be true, the inserted piece would have had to be derived from a different chromatid, or from a different "singlet" of the Watson-Crick double nucleotide chain, than the piece lost in the f^x deletion, but it would have been inserted into the chromatid or singlet that underwent the latter deletion. Thus the originally left-hand break of the recovered insertion would probably have been further to the left than that of the recovered deletion. At the same time, the argument that f^x does consist of a deletion would remain valid. But the evidence would be weakened that the subgenes of forked represent duplications that occurred in past evolution, since it would not yet have been shown that the normal allele of the f^x sublocus, acting by itself, is able to produce an effect of the f^+ kind.

(This work was supported by a grant given under Contract AT(11-1)-195 of the U. S. Atomic Energy Commission.)

Paik, Y. K. Genetic analyses of lethal mutations in Korean populations of D. melanogaster.

Samples of natural populations of D. melanogaster were collected from three remote geographical regions: one each from Najoo, Daegoo, and Quilpart Island

in September, October, and September, 1957, respectively. The primary purpose in studying them was to secure more information about the dynamics of natural populations with respect to concentrations of lethals, distribution pattern of lethal genes on the chromosome, and selection of the lethal heterozygotes for two lethals against those for a single lethal. The present report includes only part of the results obtained in connection with the first item mentioned. In all, 472 wild second chromosomes from those regions were analyzed by means of $Cy/+ \times Cy/+$ test matings. The results are summarized in the first table. A glance at the table shows that the proportions of lethal and semilethal concentrations in the populations are extremely low, in comparison with results obtained so far by most other workers. Among the 472 chromosomes tested, the incidence of lethals is only $9.32 \pm 1.33\%$, that of semilethals $2.75 \pm 0.75\%$, and the combined incidence $12.07 \pm 1.50\%$. The table indicates, however, that the frequencies of lethals and semilethals are in general uniform among these three populations. Chi-squares for homogeneity were calculated for the three regions studied, as

follows (d.f. equals 2 for each chi-square):

	χ^2	Probability
Lethals	0.938	0.60
Semilethals	6.935	0.031
Lethals and semilethals	4.36	0.12

The frequencies of intra- and interpopulational allelism were determined for these lethals, as summarized in the second table.

Class viabilities (%)	QI-57i chromosomes		DG-57j chromosomes		NJ-57i chromosomes	
	No.	% (SE)	No.	% (SE)	No.	% (SE)
0 - 3.33	11	7.48 ± 2.16	15	9.74 ± 2.38	18	10.53 ± 2.34
3.34- 6.66	--	---	1	0.65 ± 0.65	--	---
6.67- 9.99	--	---	3	1.95 ± 1.11	1	0.58 ± 0.58
10.00-13.33	--	---	4	2.60 ± 1.28	1	0.58 ± 0.58
13.34-16.66	--	---	--	---	3	1.76 ± 1.00
Total (le. + semi-l.)	11	7.48 ± 2.16	23	14.94 ± 2.87	23	13.45 ± 2.61

Samples	No. lethal chromosomes	No. of crosses	Identical crosses	Allelism rate	No. lethal genes	Frequencies of appearance					
						1	2	3	4	5	6
NJ-57i	18	153	12	7.48 ± 2.17	10	5	3	1	1	-	-
DG-57j	15	105	16	15.24 ± 3.51	9	7	1	-	-	-	1
QI-57i	11	55	1	1.82 ± 1.80	10	9	1	-	-	-	-

Chi-square 8.67
d.f. 2
Probability 0.013

Crosses between populations	No. of cross tests	No. of identical crosses	Allelism rate (SE)
QI-57i x NJ-57i	198	0	-
QI-57i x DG-57j*	198	1	0.505 ± 0.504
DG-57j*x NJ-57i	324	0	-
Total	720	1	0.138 ± 0.138

*Three semilethals are included.

The results show rather significant differences in rate of lethal allelism among the populations. Further, they show relatively high incidence of allelism, although this does not seem to be the case in the QI-57i population; with that exception, about half the lethal chromosomes found in each population occurred repeatedly. On the other hand, all possible intercrosses between the lethals from the different populations showed extremely low frequencies of allelism. If all three interpopulational crosses are pooled as a group, the incidence of allelism is only about 0.14%.

These preliminary data suggest that natural populations of D. melanogaster in Korea are not continuously large but consist of smaller breeding units, with annual cycles of numerical size (Paik, 1957), in contrast to most of the other populations of D. melanogaster or related species that have been studied. However, it seems that there are some similarities, in breeding structure and numerical size of natural populations of this species, between Korea and Russia (Dubinin, 1945). The full results of the present work and the theoretical considerations for it will be published soon.

Plaine, Henry L., and Cheng-Mei Fradkin. The high mutating system in the Swedish-b erupt strain of D. melanogaster.

Following the appearance of erupt eyes, caused by a change in the second chromosome, possibly at the suppressor-of-erupt locus, the Swedish-b strain has been observed to yield a large number of spontaneous mutations. It appears

that this increase in the mutation rate is limited also to the second chromosome (DIS-29, DIS-31). During the past year 1,430,676 loci have been tested. Twelve recessive loci on the second chromosome have been tested in 120,552 flies: 68,917 males and 51,635 females. In the males only 5 of the 12 loci yielded mutations, and the average rate per locus was 3.3×10^{-5} (or 7.8×10^{-5} for the 5 loci alone). In the females, mutations occurred at 8 of the loci, with an average rate per locus of 8.96×10^{-5} (or 13.1×10^{-5} for the 8 loci alone). For the males it has not been possible, in the present study, to approximate the 4.2×10^{-4} rate previously reported (DIS-31); however, it is apparent that mutation rates vary in the different subcultures of the Swedish-b erupt strain. In the study reported here, males and females were from the same subculture. Therefore, it seems that the female rate is almost 3 times higher than that of the males. If this is true, it is of considerable interest, not so much because it differs from the findings of other workers, but because it adds import to the possible role of the suppressor-erupt gene, at least in this strain. The expression of erupt eyes is more extreme in the females and there is a significant difference between the low frequencies of affected males and the high frequencies of affected females. The suppressor locus, or at least the second chromosome on which it's located, is solely responsible for these differences in the expression of erupt between the sexes; and these differences are even greater when the Swedish-b second chromosome is derived from the female (DIS-31). The correlation, between the sex-differential suppressor of erupt and the female on one hand and the mutating system and the female on the other, is striking. It is still too early, however, to speculate that the suppressor locus is responsible for the increased mutation rate and may act, therefore, as a "mutator"!

From the tested males, four mosaic male progeny were obtained, but two were sterile and the other two produced no mutant offspring. Thus, the germ

line was probably not affected. However, the tested females produced one mosaic male and two mosaic females, and although the male produced no mutant offspring, each of the two females produced both mutant and normal offspring. Hence, the germ line was partially affected. This supports the concept, offered earlier (DSI-31), that the mutational process may be a delayed one. This is substantiated further by the fact that, in the tested males, the mutation rate steadily increased from 1.9×10^{-5} in the first backcross generation to 7.6×10^{-5} in the sixth backcross generation. Lastly, in at least four cases, normal females gave rise to clusters containing from two to seven mutant offspring. This, together with other data, suggests that the mutator is probably more effective in the heterozygous state.

(Supported by a grant from the National Science Foundation.)

Prout, Timothy. A possible difference in genetic variance between wild and laboratory populations.

The following experiment was designed to detect the operation of stabilizing selection in the wild. It was reasoned that if a wild population was being subjected to some form of stabilizing

selection (the favoring of heterozygous genotypes or the favoring of intermediate phenotypes) then when a sample of this population was brought into the laboratory and raised under optimal conditions an increase in genetic variance might ensue. The genetic variances of two groups of male D. melanogaster were assessed by means of a progeny test. One group of 50 males constituted a sample taken directly from a citrus grove population. The other group of 50 males constituted a sample from a laboratory population which had been reared in uncrowded culture bottles and which had been derived one generation previously from the same citrus grove.

Each male was mated to two females, and eggs from each female were collected and distributed in two culture bottles. The females used with both groups of males were from the laboratory population. Wing length was determined for ten females and five males emerging from each culture bottle. The analysis of variance of the resulting data produced a hierarchy of mean squares, and the appropriate "F" tests showed a highly significant culture effect within mothers, mother effect within fathers, and a father effect, but no significant differences between the means of the two populations, fathered by wild and laboratory males respectively. From the mean squares, components of variance were extracted. These components, together with certain other statistics, are set out in the accompanying table. Figures in parentheses are the degrees of freedom for the mean squares from which the components were extracted.

From the point of view of the primary objective of the experiment, the variance components found in the "among fathers" row are important. It will be seen that for both male and female progeny the variance component due to genetic differences among wild fathers is smaller than that due to differences among fathers derived from the same wild population but allowed to grow up under laboratory conditions. At the same time there are no differences in means. These results are consistent with the hypothesis stated above. However, it should be added that according to a statistical test suggested by Crump (Biometrics, 1947) this increase in variance component attributable to laboratory fathers in both cases (2.49 vs. 1.49 for female progeny and 2.33 vs. 1.77 for male progeny) is not significant. Nevertheless the data do suggest that there may have been an increase in genetic variance under the

relaxed conditions of laboratory culture. That such an increase should have shown itself at all indicates that further work along these lines may reveal that joint heterozygosity at a few major loci is strongly favored in the wild.

Variance Components

(1 unit variance = 10^{-4} sq mm)

	Female Progeny		Male Progeny	
	Laboratory Fathers	Wild Fathers	Laboratory Fathers	Wild Fathers
Among fathers	2.49 (48)	1.49 (48)	2.33 (48)	1.77 (48)
Among mothers				
within fathers	1.30 (43)	2.50 (43)	1.11 (43)	.99 (43)
Among cultures				
within mothers	1.47 (89)	1.45 (82)	1.09 (88)	1.72 (82)
Within cultures	12.62 (1559)	10.04 (1546)	8.18 (698)	9.65 (698)
Total variance	17.88 (1739)	15.48 (1719)	12.71 (877)	14.13 (871)
Mean wing length	1.536 mm	1.540 mm	1.338 mm	1.341 mm

Parent-Offspring Covariances

Intrafather covariance of mother and mean of her offspring	2.52 (34)	2.28 (30)	1.78 (35)	3.60 (30)
Intrafather covariance of father and mean of his offspring	2.85 (44)	-.93 (47)	2.79 (49)	-2.13 (47)

No serious effort was made to relate these components of variance to statistical models of genic action. However, values of h^2 for wing length ranging from 28% to 73% may be obtained depending upon what one wishes to include in h^2 . Using the parent-offspring covariances, h^2 ranges around 36%. This might be the best estimate of heritability "in the narrow sense," since it does not include dominance and particularly is not disturbed by linkage (Cockerham, Genetics, 1956). The appropriate combination of father and mother components (excluding components due to wild fathers) yields a value of h^2 around 48%, which includes various fractions of the nonadditive components plus an unknown disturbance due to linkage. (There is an interesting hint in the data that linkage disturbance may be more important in the fathers' contribution than in that of the mothers, which might be expected in *Drosophila*.)

Finally, it may be mentioned that the negative covariances between wild fathers and the means of their offspring is interpreted to mean that the wing length of a fly picked up in the field allows no prediction as to the wing length of his laboratory offspring. This fact might be borne in mind by those who have surveyed and are currently surveying quantitative characters in wild populations.

Ramel, C. Interchromosomal effects of inversions on the segregation of Y chromosomes in females.

The effect of autosomal inversions on segregation of the Y chromosome in females with attached and nonattached X's was studied. As autosomal inversions, $Ins(2L+2R)Cy$ and $In(3IR)Dcx^F$ were used. The following crosses were made: $y\ f. = /sc^8\ Y\ x\ y\ w\ sn/sc^8\ Y$, $y\ v\ f. = /sc^8\ Y\ x\ y\ w\ sn/sc^8\ Y$ and $y^{16}/y^{16}/Y\ x\ Canton-S$. In the series with attached X's, missegregation of the Y chromosome was indicated by yellow males in the offspring. In the series with nonattached X's, secondary nondisjunction was counted in both male and female offspring. The following results were obtained:

Autosomes	$y\ f. = /sc^8\ Y$		$y\ v\ f. = /sc^8\ Y$		$y^{16}/y^{16}/Y$	
	n	% exc.	n	% exc.	n	% exc.
+/+ +/+	5022	2.7	2713	0.2	4632	2.6
Cy/+ +/+	4075	5.4	4999	3.8	4589	1.1
+/+ D/+	1796	7.6	3623	1.7	1735	1.9
Cy/+ D/+	2055	3.0	2143	2.9	1103	0.5

In agreement with previous findings (Sturtevant, 1944, and others), the rate of secondary nondisjunction is decreased by the introduction of autosomal inversions. These inversions have an opposite effect on the number of exceptions in the attached-X series. These results could be explained either by assuming an increased pairing affinity between the X chromosomes caused by the autosomal inversions, which would tend to leave the Y chromosome unpaired, or by assuming an interchromosomal pairing of the Y chromosome with an autosome, as has been suggested by Oksala (DIS-31, 1957). If the latter is the case, the Y chromosome would presumably cause nondisjunction of autosomes, resulting in dominant lethals.

An attempt was made to find out whether such an increase in dominant lethals was caused by the Y chromosome. Egg mortality was studied, using $y\ v\ f. =$ females with and without a Y chromosome and having the same autosomal inversions as in the preceding experiments.

Autosomes	Egg mortality in per cent		
	With Y (n)	Without Y (n)	Difference
+/+ +/+	30.2 (1822)	32.6 (3250)	2.4
Cy/+ +/+	48.9 (2711)	57.2 (3228)	8.3
+/+ D/+	68.7 (3613)	71.7 (2051)	3.0
Cy/+ D/+	70.2 (4214)	73.2 (2406)	3.0

All the series show a higher mortality without a Y chromosome. Further experiments are planned to reveal the cause. At present, however, the data do not give any evidence of a mortality caused by the combined effect of autosomal inversions and a Y chromosome. The difference in mortality with versus without Y shows no correlation with the corresponding data on the segregation of the Y chromosome in the preceding studies.

Rasmison, B., and D. Björkman.
The fatty acid content in
D. melanogaster.

As part of an investigation concerning the lipid constituents of D. melanogaster, the different fatty acids in the neutral fat fraction was studied

by means of gas-liquid chromatography.

The fatty acid fraction from imagoes was methylated and the methyl esters chromatographed. Nine fatty acids were found, and four of these remained after bromination, showing that they were saturated. These were identified as lauric, myristic, palmitic, and stearic acid. Myristic and palmitic acid were present in about equal concentrations; the concentration of lauric acid was lower, and that of stearic acid still lower. The following unsaturated acids were found: oleic, linolic, a C₁₆ acid (possibly palmitoleic), a C₁₄ acid, and traces of a C₁₃ acid. Oleic acid predominated over linolic; the C₁₆ acid occurred in a concentration equal to those of palmitic and oleic acid. The amount of the C₁₄ acid was considerably smaller.

Repeated experiments with some different inbred strains of D. melanogaster gave completely identical results, both qualitatively and quantitatively. As corn meal is the major fat-containing constituent of the substrate, the fatty acid content of corn meal was also analyzed. Palmitic acid was the only saturated acid found, oleic and linolic the only unsaturated acids. The concentration of the palmitic acid is lower than that of oleic acid, and the ratio of linolic to oleic acid is reversed in comparison with that obtained from D. melanogaster. Determinations of the fatty acids in the complete substrate, including yeast and the products of microbial action, is under progress.

Rendel, J. M. Selection for scutellar bristle number.

Selection for high scutellar bristle number in sc flies results in an increase in average scutellar bristle

number. This number is far more variable in sc than in + genotypes. As the number increased in sc flies of a selection line segregating for sc and +, no change in the number on + sibs was found until the average number on sc flies was about 3; a few + flies with 5 scutellar bristles then started to appear. As the bristle number of sc flies increased to 4, the variability began to be reduced; and no sc flies with 5 bristles have yet been observed. As a few + flies with 5 bristles began to appear, the variability of the + segregants began to increase, and flies with 6 scutellar bristles soon followed. There is thus a region around 4 bristles in which genetic changes do not show at all readily. A change which will turn a + 5-bristle into a + 6 or a sc 1 into a sc 3 has little or no visible effect on a + 4-bristle of unselected type.

Rizki, M. T. M. Effect of ligation on tumor production in the tu^W strain.

In tu^W larvae the caudal fat cells become encapsulated by hemocytes (lamellocytes), and the masses thus formed are melanized in the late third

instar. In one series of experiments, tu^W larvae were ligated so that the brain and ring-gland hormone centers (BRH) were excluded from the posterior part of the body; in the control series for these experiments, tu^W larvae were ligated in such a way that these hormone centers were included in the posterior region of the body. Beginning 6 hours after the time of ligation and continuing at various timed intervals for 120 hours after ligation, larvae in both groups were examined for the development of melanotic masses. In addition to melanization of the caudal fat, which is the specific phenotype of the tu^W larvae, small melanotic masses were noted in various parts

of the body of the ligated larvae in both groups. Such masses are rarely encountered in unligated tu^W larvae, and they occurred with equal frequency in both BRH-excluded and BRH-included larvae. Other experimental evidence has shown that these atypical tumors are due to injury effects. Exclusion of BRH from the posterior part of the body decreases the frequency of the typical melanotic masses of caudal fat of tu^W larvae, and this decrease in frequency of typical tumors is correlated with the age of the tu^W larvae at the time of ligation. BRH-excluded larvae ligated at age 60 hours ($24^\circ C$) show a complete suppression of the typical melanotic pattern, whereas ligation after 85 hours will slow down the time of appearance of melanosis but will not prevent the formation of the typical melanotic masses. The BRH-included larvae developed typical masses with a frequency of 75% (over-all average based on larvae of all ages) as compared to a penetrance of 95% in the unoperated tu^W strain.

In another series of experiments tu^W larvae were ligated at a time when encapsulation of the fat cells presumably had already occurred but no melanosis was evident by external examination of the larvae. Control larvae were fixed at this time of ligation, and histological examination revealed that encapsulation of fat cells had occurred. The groups of BRH-included and BRH-excluded larvae were fixed for histological examination when 75% of the BRH-included larvae had developed melanotic masses. None of the BRH-excluded larvae had melanized masses. In the BRH-excluded larvae, where melanosis had been inhibited, phagocytosis of the encapsulated fat masses was evident. On the other hand, the melanized masses in the BRH-included series had the typical appearance found in tu^W larvae, and no sign of phagocytosis was observed. Details of these experiments will be published elsewhere.

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Rizki, M. T. M. Feulgen whole-mount preparations of imaginal discs.

Feulgen whole-mount preparations of imaginal discs of the larvae of D. melanogaster have been prepared to study various karyotypes. Larvae (60-

65 hours larval age, $24^\circ C$) were washed clean and opened in Carnoy solution (3 parts of 95% ethyl alcohol to 1 part of glacial acetic acid). The imaginal disc complex was removed while attached to the mouthparts and the brain, and placed in fresh Carnoy solution for a period of 12-24 hours. The Carnoy was replaced with 95% ethyl alcohol by changing the alcohol solution at least three times, and the material was then left in 95% alcohol for 12 hours. It was then hydrated by passing through a graded series of alcohols to distilled water, with approximately five minutes allowed for each step. The buds were transferred to cold 1 N HCl for 1 minute and then transferred to 1 N HCl at $60^\circ C$ for 13 minutes. They were placed in Feulgen stain prepared according to Stowell (1945, Stain Tech. 20: 45) for 1-2 hours, and then passed through three changes of freshly prepared bleaches (Stowell method), remaining 10 minutes in each bleach. They were washed in distilled water for 5 minutes, dehydrated through a graded series of alcohols, and cleared in xylol. The entire complex was then placed on a slide and covered with a drop of xylol. Gentle tapping of the mouth parts with a needle freed the imaginal discs and the brain. The mouthparts and the brain were removed from the slide, and the imaginal discs were mounted in Permount. Slides prepared this way must be stored flat to avoid movement of the imaginal discs to the edge of the coverslip. Examinations were made under oil immersion with a green filter on the

microscope lamp.

Somatic pairing can be seen nicely in these preparations. Further, the imaginal discs from five heterozygous inversion strains and X^{C2} show anaphase bridges, and these discs have numerous pycnotic nuclei. The anaphase bridges and pycnotic nuclei are not found in the normal Ore-R strain. Apparently, the high frequency of pycnotic nuclei is due to chromosomal aberrations resulting from somatic crossing over within the inversion heterozygotes. Despite the presence of many pycnotic nuclei in the imaginal discs of these strains with inversions, the imago is by no means morphologically asymmetric or aberrant. A considerable degree of autoregulation of imaginal tissue must, therefore, take place. Perhaps data about frequency of pycnotic nuclei and anaphase bridges in imaginal discs of these strains may be helpful in understanding the development of genetic mosaics resulting from somatic crossing over, by demonstrating the degree of autoregulation of the imaginal tissues that must occur in different karyotypes of *Drosophila*.

(Work supported by Grant G-3381 from the National Science Foundation and Grant RG-5285 from the Public Health Service.)

Rizki, M. T. M. Telobiosis of normal and tumorous larvae of *D. melanogaster*.

and cell-free extracts from genetically determined tumorous larvae. In view of these experiments we have attempted telobiosis of third-instar larvae of the Ore-R strain with tu^W larvae by means of a fine glass capillary. The telobiotic pairs were: $tu^W + Ore-R$; $tu^W + tu^W$; and $Ore-R + Ore-R$. Such telobiotic pairs were kept alive as long as four days. During this period the telescopic movement of the joined larvae was sufficient to produce enough pressure to result in exchange of hemolymph between the partners, and the blood cells could be observed moving through the glass capillary with the phase microscope. In all experiments, the tu^W larvae developed typical tumors characteristic of this strain, but the Ore-R larvae remained free of melanotic tumors even when joined with tu^W larvae. The Ore-R larvae generally pupated.

(Work supported by Grant G-3381 from the National Science Foundation and Grant RG-5285 from the Public Health Service.)

Sakai, K., T. Narise, T. Ito, and S. Iyama. Migrating activity in inbred lines derived from two wild populations of *D. melanogaster*.

Fifteen inbred lines have been derived from each of two wild populations of *D. melanogaster*, which differed markedly from each other with respect to migrating activity. Migrating activity was investigated at every generation of inbreeding. The method of investigation was to count the number of flies that had migrated from the original tube 48 hours after the establishment of an experimental set of population tubes. Eighty flies were tested at a time, and the test was repeated three times. The inbreeding was continued to the 20th generation. The following facts were found: (1) The migrating activity of the flies did not show any definite tendency to decrease with inbreeding, though there was a statistically significant variation from generation to generation. (2) The difference between the two populations

was not statistically significant, but intrapopulation variation was highly significant. The line means ranged from 25% to 60% among the lines. (3) Interaction between generations and lines was insignificant. These facts suggest that both populations involve genotypes responsible for either high or low migrating activity, and they were separated from each other by inbreeding. Despite our initial expectation, the inbreeding has not brought about any marked decrease in migrating activity.

Sandler, I., and L. Sandler. An additional case of apparent aberrant segregation of an attached-XY chromosome.

An attached-XY chromosome of the constitution y^+ , YSX.YL, y^+ (with the X chromosome in inverted sequence), constructed by Lindsley and Eddington, was tested in the following crosses:

(1) $y/y \times y^+$, YSX.YL, $y^+/0$; (2) $y/y \times y^+$, YSX.YL, $y^+/FR2$ (= YL, y^+); (3) $y/y \times y^+$, YSX.YL, y^+/Y^m . The y^+ , YSX.YL, y^+ chromosomes were all derived from a single male. The results were as follows:

	<u>Reduction</u>
Cross (1): 877 + ♀♀; 1948 y ♂♂; 2 + ♂♂	55%
Cross (2): 539 + ♀♀; 872 + ♂♂; 2 y ♂♂	58%
Cross (3): 416 + ♀♀; 727 y ♂♂; 1 + ♂♂	43%

In each case it can be seen that the attached-XY chromosome was recovered far less frequently than the expected 50 per cent. The amount of this reduction is shown in the righthand column. There is a great deal of culture-to-culture variability in the ratios, which means that the differences between the different crosses may not be meaningful. In fact, the excess reduction in cross (1) may be due simply to meiotic loss of the univalent attached-XY chromosome.

There are two matters of immediate interest. First, there is the possibility that this depression in the recovery of the y^+ , YSX.YL, y^+ chromosome is a consequence of a reduced viability of the X-Y-bearing class. As a test of this possibility, females carrying a normal chromosome marked with y and the y^+ , YSX.YL, y^+ chromosome were crossed to YSX.YL, y B/0 males. The progeny included 922 y B ♀♀, 670 B ♀♀, 1169 y ♂♂, and 1065 + ♂♂. Although this is perhaps not the most critical type of viability test, the equality of the y and + male classes suggests that no striking viability depression is associated with the y^+ , YSX.YL, y^+ chromosome. A second possibility is that the depression is a result of meiotic loss of the type known to occur with other X-Y chromosomes when such chromosomes are univalent. Cross (2) shows that this is not the case because (a) FR2 acts as a homologue for the X-Y chromosome (as evidenced by the lack of y males) but does not eliminate the reduced recovery of y^+ , YSX.YL, y^+ , and (b) simple loss of this chromosome should yield a large y male class, which has not been observed.

Although the question of zygote mortality (as measured by unhatched eggs) has not yet been explored, it seems reasonable to suppose that this is a case similar to those reported by Lindsley and Sandler (Genetics, in press), in which other attached-XY chromosomes were recovered much less frequently than the expected 50 per cent from males carrying various X-chromosome heterochromatic duplications. This, therefore, appears to be another case of meiotic drive.

Schlager, G. Fluctuations of pupation site in replicated experiments.

For several years the University of Kansas Drosophila Laboratory has been plagued by unexplainable fluctuations in various quantitative characters.

Replicates of an experiment would show significant differences between means, even though the medium was made under standard conditions, the eggs were from the same stock bottles, and the flies were reared in constant environment chambers. Statistical control of these fluctuations was only partially successful (Sokal and Hunter, Proc. Xth Congr. Ent., 1958). It was hoped that these fluctuations could be greatly reduced or eliminated by achieving better control over the environment. The remarks below are confined to pupation site of D. melanogaster (see Sokal and Hunter, Science 119, 1955). The unselected laboratory stock (COSU-2) was used in these experiments.

The quantity and/or quality of the microflora present in the cultures was thought to be the primary cause of the fluctuations. Eggs were washed to remove the yeasts, bacteria, and molds adhering to the exochorion in a manner similar to that of J. H. Sang (J. Exp. Biol. 33, 1956) but using 400 ml of .5% solution of filtered hypochlorite and 400 ml of 2% solution of the commercial germicide "Roccal." Washed eggs were transferred to the rearing medium in a sterile chamber previously washed with 95% alcohol and exposed to ultraviolet light for 24 hours. Smears from the surface of the vials were subcultured in a medium closely resembling the Drosophila rearing medium, to check for contamination.

In each experiment a lot of medium was divided into two parts: washed eggs were transferred to one part and unwashed eggs to the other. Even though possible sources of variation were controlled (i.e., pH of medium, water content of medium, temperature and pressure during autoclaving, temperature and humidity during rearing, microclimatic gradients in rearing chamber, differences between containers of ingredients used in preparation of the medium, differences in eggs collected), the fluctuations still persisted when the standard medium containing corn-meal, agar, yeast, Karo syrup, and Brer Rabbit molasses was used. These differences were maintained in both the "washed" and the "unwashed" vials.

When the eggs were transferred to a medium substituting sugar and mineral salts for the Karo syrup and molasses, the fluctuations were no longer significant in the "unwashed" method but were still significant in the "washed" method. Because of these findings a closer look was taken at the original medium, containing Karo and molasses. When eggs were transferred to original-formula medium containing twice the quantity of Karo but no molasses, the fluctuations disappeared. On the other hand, if the medium was made with twice the quantity of molasses and no Karo, the differences were significant ($P < .01$).

We can conclude (1) that the normal quality and quantity of microflora in the rearing cultures do not alter the expression of the character "pupation-site" enough to cause significant differences in replicated experiments; (2) that differences in pupation site between replicate batches appear to be due to chemical changes undergone by the molasses during medium preparation.

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Schnick, S. M. Viability of heterozygotes and homozygotes for l(2)55i.

Lethal l(2)55i was first found in the W-1(Erie) wild stock. It was maintained by random mating in this stock and in experimental populations at

higher-than-expected frequencies. (See Burdick and Mukai, DIS-30, p. 108). Further studies of this lethal have been made with the following results.

Frequency of l(2)55i: After 4 years of random mating $q = .1175 \pm$ in the original W-1 wild stock. Gene frequencies based on 5 generations each of four random-mating experimental populations range from $q = .0957 \pm$ to $q = .1647 \pm$. These four experimental populations range in age from 31 to 61 generations and are maintained by random mating.

Time of lethality: The gene was found to produce its homozygous lethal effect solely during the larval period. The pupae-larvae ratio of the cross $+/l \times +/l = .7094$, that of $+/+ \times +/+ = .9305$, giving a relative difference in pupation of .2377 which is quite near the expected difference of .25 for a lethal which produces its effects in only one stage. There were no significant differences in larva/egg or in adult/pupa ratios.

Factors influencing high heterozygote viability: The fecundity of females heterozygous for l(2)55i as compared to wild type was measured. Two estimates based on egg laying of 175 females were made. The eggs of each female were counted daily for 6 days, giving a total of 1038 egg counts. One estimate of average number of eggs/female/day gave the values $+/l = 77.97$ and $+/+ = 57.62$. This gives a relative fecundity value for the lethal heterozygote of 1.354, that of the wild type being 1. Another estimate of fecundity gives a relative value of 1.21 for the lethal heterozygote, that of the wild type being 1. No difference was found between the wild type and the lethal heterozygote, either in relative zygotic viability or in sperm competitive ability. Differences in mating ability between the wild-type and lethal heterozygous male have not yet been tested.

Seiger, Marvin Barr. Inbred stock of D. melanogaster.

Stocks inbred by single brother-sister pair matings (the number succeeding the name of each strain represents the

number of inbred generations as of 58k17):

Oregon-R 250 Single pair received from Ives 56a from the 116th generation.

Iv Oregon-R 270 Same as Ore-R 250 but received 57c at the 231st generation.

M Oregon-R 243 Received from Aloha Alava as a single pair of the 201st inbred generation, 57b. Originally received at Berkeley from Ives as a single pair of the 116th generation 53g. Not inbred from generation 163 to 170.

P₁ I Oregon-R 291 Received from Buzzati-Traverso at the 236th generation 56b.

2b Oregon-R-C 221 Obtained from Aloha Alava as a single pair in the 181st generation, 57b. Stock somewhat sterile and has been mass mated on two occasions for several generations.

f Oregon 180 Obtained from Buzzati-Traverso as a single pair 56b in the 123rd generation. Phenotype: forked.

y Oregon 179 Obtained from Buzzati-Traverso as a single pair 56b in the 121st generation. Phenotype: yellow.

Canton S 56 Received as a single pair in the 14th generation from Aloha Alava 57b. Stock made isogenic by Stern (ClB; Cy/Pm; H/Sb) about 15 years ago. Mass matings until 56h. Single pair matings since '56.

Oregon 100 From Amherst stock #2 (DIS-30) in the 100th generation. Mass mated since.

Oregon 200 From Amherst stock #2 (DIS-30) in the 200th generation. Mass mated since.

2b Oregon-R-C 200 From 2b Ore-R-C in the 200th generation. Mass mated since.

Seki, T., and S. Okubo.
Dihydroxyphenolic compounds
in D. melanogaster.

Since the work of Schmalfuss in 1927, dihydroxyphenolic compounds such as protocatechuic acid have been isolated from various insect cuticles. However, the occurrence of such phenols has not yet been reported in D. melanogaster.

Pupae of an Oregon-R stock were homogenized with 80% methanol, and the homogenate was centrifuged. The supernatant was concentrated under reduced pressure. The residue dissolved in water was acidified with hydrochloric acid and extracted with ethyl acetate. The extract was evaporated under reduced pressure and the residue was dissolved in a mixed solvent composed of acetone, methylethylketone, and 0.2 N hydrochloric acid (1:2:9 v/v). One ml of the resulting solution was placed on a column (0.9 x 110 cm) of Amberlite IRC-50 resin (H-form), which had been equilibrated with the same solvent. Elution was effected with the same solvent, and the effluent was collected with an automatic fraction collector (20 drops per fraction). After the addition of 0.2 ml of ethylenediamine to each fraction, they were heated at 50° C for 1 hour and measured fluorophotometrically. Six peaks with yellow fluorescence were found, four of which corresponded with the positions occupied by authentic samples--protocatechuic acid, homoprotocatechuic acid, catechol, and homogentisic acid. Judging from the chromatographic properties, other peaks seemed to represent more polar compounds such as dihydroxyphenyllactic acid and dihydroxymandelic acid.

Seto, Frank. Pupal
lethals in combination.

As part of a more comprehensive study of the developmental effects of a series of pupal lethals (mentioned in DIS-31, p. 160), different lethals were combined two at a time and the phenotypic expression examined. At the present time 29 combinations have been made and others are in the process of being synthesized. The results of preliminary investigations on the times of action of these "double lethal" combinations are summarized in the accompanying table. The numbers listed in the table are explained in the key and refer to the specific stages at which mortality appeared to have occurred. The stage(s) at which cessation of development occurred for the various lethals taken singly are given in the two below the table and those for the lethals in combination are in the body of the table.

The data show that in practically all cases the double-lethal homozygotes do not develop farther than the stage of the earlier-acting lethal,

and in some cases die earlier. Although the table does not indicate the quantitative effects of these lethals in combination, the numerical data (not presented here) further show that (1) in some cases the nonlethal (Cy/1) heterozygotes are reduced in number and the cultures have increased egg mortality, and (2) there is a general decrease in number reaching the pupal stage and an over-all increase in mortality at earlier stages. Studies are not in progress to determine the patterns of damage produced by the combined lethal effects and to compare them with the effects of single lethals.

	Key: 1-egg larval 2-larval 3-prepupal 4-early pupal 5-late pupal 6-adult												
N42	*												
X3	2	*											
N61	1,2	2,3	*										
N51	-	2,3	-	*									
N4	-	2,3	2,3	3,2	*								
N50	-	-	-	-	-	*							
N32	-	3	3	-	2,3	-	*						
Co7	-	2,3	-	-	2,3	-	-	*					
Co3A	-	3	1-3	3	3	-	-	-	*				
N1A	-	2	2,3	-	3,4	3	5	-	-	*			
N45	-	2	-	-	-	-	4	-	-	-	*		
N55	-	2,3	2,3	-	2,3	3	-	-	-	4,5	2-5	*	
	2,3	2,3	3	3	3-5	4	4,5	4,5	4,5	5	5,6	5,6	
	N42	X3	N61	N51	N4	N50	N32	Co7	Co3A	N1A	N45	N55	

Shima, T. *Drosophila* collection in the vicinity of Lake Tōya and the suburbs of Iwamizawa City, Hokkaido.

Flies were collected in the vicinity of Lake Tōya in the summer, and in the suburbs of Iwamizawa City, Hokkaido, during the period from May to October, 1958. The flies obtained are as

follows: *Aulacigaster leucopéza*, *D. histrioides*, *D. coracina*, *D. busckii*, *D. auraria A*, *D. auraria B*, *D. melanogaster*, *D. bifasciata*, *D. brachynephros*, *D. nigromaculata*, *D. lacertosa*, *D. sordidula*, *D. virilis*, *D. funebris*, *D. testacea*, *D. immigrans*, and *D. kuntzei*. *A. leucopéza* and *D. kuntzei* are found rather rarely in Hokkaido.

Shiomi, T. Changes of free ninhydrin-positive substances in the development of *D. melanogaster*.

In the wild Oregon-RS strain, eggs in three stages, larvae in four stages, pupae in two stages, and imagoes were employed as materials. Ninhydrin-positive substances extracted with

ethanol, the final concentration being 80%, were analyzed by means of two-dimensional chromatography. The spots identified were of alpha-alanine, beta-alanine, arginine, aspartic acid, glutamic acid, glutamine, glycine, histidine, leucine, lysine, proline, serine, threonine, tyrosine, and valine. Of these substances, alpha-alanine, aspartic acid, glutamine, glutamic acid, glycine, histidine, proline, and valine occurred constantly in each stage of development, whereas the others were not always recognized. Besides these, there were several spots of unidentified substances, of which some were supposed from their R_f values to be peptides, gamma-amino butyric acid, and taurine. The pattern of the ninhydrin-positive substances changes with the advance of the developmental process; each developmental stage shows its specific pattern. Quantitative analyses of these substances are still under way.

Sobels, F. H. Lack of mutagenic effectiveness of two organic peroxides in D. melanogaster.

Radiomimetic properties have been reported by Latarjet (1956) for cumene hydroperoxide and di-succinyl-monoperoxide (DSP) in microorganisms.

Samples of these peroxides, synthesized at the Institut du Radium in Paris were kindly placed at our disposal by Dr. R. Latarjet. Oregon-K males were injected with different concentrations of these compounds and their offspring were tested for incidence of sex-linked lethals by means of the Basc (Muller-5) technique.

Cumene hydroperoxide gave 2 lethals in 532 chromosomes and no lethals in 451 chromosomes at the two concentrations tested. No lethals were induced in two successive broods by a highly toxic concentration of 0.1% DSP. Injection of 0.08% DSP produced 9% mortality and 24% sterility. In four successive three-day broods, the following frequencies of sex-linked lethals were observed 3/685, 1/664, 0/545, and 0/531.

Since the spontaneous-mutation rate in this stock is 0.2-0.3%, it is clear that under the conditions tested no positive results have been obtained. Similar observations have been made for DSP in *Aspergillus* by G. A. van Arkel (1958) and by R. F. Kimball (cited by Latarjet, 1956) in *Paramecium*. Application of high concentrations of DSP to polar caps of *Drosophila* by L. S. Altenburg (1958) raised the frequency of second-chromosome lethals slightly over that of the controls.

(This investigation was carried out with support of the Health Research Council T.N.O.)

Sobels, F. H. The effect of pretreatment with cyanide on radiosensitivity in nitrogen and oxygen.

Pretreatment with cyanide enhances the radiosensitivity of spermatids after irradiation in air (Sobels, 1955). A possible interpretation of this effect is that cyanide, by depressing oxygen

utilization, makes more oxygen available in the irradiated cells. To test this idea, flies were pretreated with HCN in N₂ or O₂ and then irradiated in N₂ or O₂, respectively. Tests for sex-linked lethals were made by the Basc (Muller-5) method (three virgin females per male per brood). With this mating scheme, the most sensitive stages, corresponding to spermatids, are samples in the second brood. The data of two experiments with N₂ are shown in the upper part of the table; the data of two experiments with O₂ are pooled and presented in the bottom part. Considering the data of experiment 1, it is clear that, compared to radiosensitivity in air, the reduction of radiosensitivity after irradiation in N₂ is more pronounced in spermatids (brood b) than in mature sperm (brood a). In the second experiment, which was aimed at a more effective replacement of air present in the cells by nitrogen, the radiosensitivity of spermatids is leveled off entirely to that of mature sperm. The data suggest that the greater radiosensitivity of spermatids compared to mature sperm is due, at least in part, to a greater availability of oxygen in spermatids when irradiated under normal conditions in air. Our findings are in complete agreement with recent observations of Oster (1957, 1958), who sampled spermatids by irradiating 48-hour pupae.

As to the effect of cyanide in a nitrogen atmosphere, it is apparent that in experiment 1 such pretreatment resulted in a significant enhancement ($\chi^2 = 13.15$; $P < 0.001$) of radiosensitivity in spermatids. After more effective replacement of oxygen by nitrogen in experiment 2, the effect of cyanide

Treatment	Broods (days after treatment)					
	a (1-4)		b (4-7)		c (7-10)	
	no. chrom.	% leth.	no. chrom.	% leth.	no. chrom.	% leth.
<u>Experiment 1</u>						
CN in N ₂	462	0.4	474	0.2	478	0.0
1800 r in N ₂	597	4.0	712	5.1	712	3.1
CN + 1800 r in N ₂	600	3.3	709	10.2	460	2.8
1800 r in air	609	5.4	669	11.7	695	2.4
<u>Experiment 2</u>						
CN in N ₂	500	0.4	493	0.2	490	0.0
1800 r in N ₂	521	4.4	883	4.1	895	2.8
CN + 1800 r in N ₂	524	4.0	885	6.6	899	3.2
1800 r in air	517	5.2	861	10.9	881	3.3
<u>Experiments 3 & 4</u>						
CN in O ₂	1123	0.3	1209	0.1	1112	0.1
1000 r in O ₂	1139	4.4	1556	8.7	1218	5.3
CN + 1000 r in O ₂	1144	4.4	1550	9.7	1002	4.4
1000 r in air	1150	2.3	1217	6.2	1173	4.2

was less pronounced. The results indicate that a small amount of oxygen, which is used up by cellular respiration in the absence of cyanide, markedly increases radiosensitivity in the presence of a respiratory inhibitor. Our findings in *Drosophila* are comparable to those of Kihlman (1958) with *Vicia* root tips, where a respiratory inhibitor enhanced radiosensitivity in the presence of minute amounts of oxygen, but not in an atmosphere of pure nitrogen. It seems as if the conditions realized in experiment 2 approach those of pretreatment and irradiation in pure nitrogen in Kihlman's experiments.

A comparison of radiosensitivity in mature sperm and spermatids after irradiation in oxygen and air shows that the effect of oxygen is more pronounced in mature sperm than in spermatids. This observation confirms the idea that compared to the spermatids, mature sperm is relatively anoxic (cf. also Oster, cited above). Contrary to the observations on plant material by Lilly and Thoday (1956) and Kihlman (1957), cyanide if applied in an oxygen atmosphere has no radiomimetic effect by itself. Further, it is seen that pretreatment with cyanide in an oxygen atmosphere does not raise radiosensitivity above that observed after irradiation in oxygen only.

From the data presented above one is inclined to conclude that the enhancing effect of pretreatment with cyanide on radiosensitivity of spermatids in air is due to a greater availability of oxygen. Other findings, however, preclude such an interpretation as the only explanation of this phenomenon. There is no correlation between the effect of cyanide and that of oxygen in spermatids and in mature sperm. That is, compared to radiosensitivity in air, cyanide has a more pronounced effect on radiosensitivity in spermatids than oxygen, whereas the reverse is true for mature sperm. Mature sperm, however, is characterized by a lower availability of oxygen, so that if cyanide acted exclusively by raising the oxygen tension an enhancement of radiosensitivity should be expected in mature sperm and not in spermatids. Both this finding and the fact that posttreatment with cyanide enhances the

effect of high-intensity X-rays (Sobels, 1958) could be explained on the assumption that inhibition of catalase by cyanide favors the accumulation of mutagenic peroxides produced by the irradiation. Since oxygen would be essential for the formation of peroxides by irradiation, the fact that cyanide treatment (pre- or post-) only affects spermatids is in keeping with this hypothesis, because it has been shown that more oxygen is available within spermatids than within mature sperm.

(This investigation was carried out with support of the Health Research Council T.N.O.)

Takada, H. An unrecorded species of the robusta group in Hokkaido.

Eight specimens, 3 females and 5 males, of the robusta group were collected on Mt. Raus on Shiretoko Peninsula at the eastern end of Hokkaido by means of banana-yeast traps.

Their characteristics are as follows:

Male and female: Body large, dark brown, about 4 mm in length. Antenna dark brown. Arista with about 7 branches including a fork, 2 below it. Palpus with 2 long and several shorter bristles. Orb-2 about 1/3 orb-1, 1/2 orb-3. orb-2 about 1/2 size of vibrissa. Ocellar triangle large and black. Periorbits black. Carina yellowish brown and high. Cheeks dark brown, about 1/3 as broad as the greatest diameter of eye. Mesonotum dark brownish black, with black median longitudinal stripe. Scutellum brownish black. Sterno index about 0.75. Abdominal tergites brownish black, and with a broad blackish band on each tergite. Legs dark brown. Wings slightly fuscous, veins brown, crossveins clear. C index about 3.7; 4V index about 1.6; 4C index about 0.7; 5X index about 1.4. C3 bristles on basal 2/3. Phallic organs and egg guides unrecorded. Resembles D. lacer-tosa, Okada 1956.

Takada, H. On the ecological characteristics of D. ezoana (virilis group).

D. ezoana shows a definite ecological specialization. The habitat is restricted to cold and rather damp regions along mountain streams and lakes in eastern parts of Hokkaido. Flies were collected at a place about 100 m high at the foot of Mt. Raus (altitude 1661 m) in the summer of 1958. There were many butter-burs (Petasites japonicus) and smartweeds (Polygonum reynoutria) covering the area. The meteorological conditions were as follows: average humidity, 100%; range of temperature, 16°-21° C; illumination, 500 lux.

Tamura, S. Resistance to parathion during the developmental stages of D. melanogaster.

First-instar (just after hatching), second-instar (about 35 hours after hatching), and mature third-instar (about 96 hours after hatching) larvae of insecticide-resistant strains (Hikone and WMB) and insecticide-susceptible strains (Fukuoka and Canton-S) of D. melanogaster were raised on foods containing parathion in various concentrations, to investigate the effects of parathion on their pupation and emergence.

In first-instar larvae of Fukuoka and Canton-S, pupation rates and emergence rates were decreased to 4% and 1%, respectively, by 0.1 ppm parathion. In first-instar larvae of Hikone and WMB, pupation rates were decreased to 35% and 26%, respectively, by 3.0 ppm parathion, and emergence

rates to 34% and 26%, respectively. In second-instar larvae of Fukuoka and Canton-S, pupation and emergence were completely inhibited by 0.1 ppm parathion. In second-instar larvae of Hikone and WMB, pupation rates were decreased to 27% and 14%, respectively, by 3.0 ppm parathion, and emergence rates to 26% and 14%, respectively. In mature third-instar larvae of Fukuoka and Canton-S, pupation rates were decreased to 21% and 39%, respectively, by 50.0 ppm parathion, and emergence was completely inhibited. In mature third-instar larvae of Hikone and WMB, pupation rates were decreased to 21% and 19% respectively, by 50.0 ppm parathion, and emergence rate to 3% in both strains.

In the case of first- and second-instar larvae, a slight difference was found between the lengths of the larval periods in the resistant and the susceptible strains, but no significant difference was found between the lengths of the pupal periods.

Toyofuku, Y. Salivary-gland chromosomes of D. lacertosa.

three short, and one dot. The two long arms have been identified as the X chromosomes. No chromosomal polymorphism has been observed in the salivary-gland chromosomes in natural populations of D. lacertosa, so far as material collected in seven different localities of Hokkaido is concerned.

The salivary-gland chromosomes of D. lacertosa (♀) are characterized by nine arms: two long, three medium-sized,

Tsukamoto, M. DDT metabolism in D. melanogaster.

metabolite, DDE. In this laboratory there are several insecticide-resistant strains of Drosophila (see stock list), and all of them are highly resistant to DDT, although some of them were selected for resistance to other insecticides. Metabolism of DDT to DDE was tested in these resistant Drosophila strains, and the presence of DDE was suggested by the Schnechter-Haller test in ether extracts of larvae, pupae, and adults after rearing on DDT-containing media. However, results of paper chromatography of body extract showed that DDT was not dehydrochlorinated to DDE but converted into an unknown metabolite (or metabolites) having a different Rf value than DDT or DDE. This metabolite appears to be more polar than DDT or DDE. When resistant flies were reared on medium containing DDE, no metabolite was detected on the same chromatographic systems; and the evidence suggests that this unknown metabolite is not a derivative of DDE. Preliminary in vitro experiments for formation of DDE from DDT had negative results. The chemical nature of the unknown metabolite is now under investigation.

It is well known that in DDT-resistant houseflies, DDT is detoxified by dehydrochlorination to an ethylene-type

*Van Valen, Leigh. Interspecific competition between D. melanogaster and D. willistoni.

long enough to give information--one by each of 12 students, and two by myself. In all cases but one, D. willistoni was rapidly replaced, adult selection apparently being important.

This experiment was performed last year by the students in the beginning genetics lab at Columbia. Fourteen polyethylene cages were maintained

Except as specified below, each cage was started with 150 males and 150 females of each species. Egg samples of 120 to 1100 eggs were taken a week after the cage was started and at 10- to 20-day intervals thereafter; pupae or adults were counted. Eleven cages were kept at 25°; one at 19°, and two

at 15°. In two cages, strips of absorbent paper toweling were put into the food cups to help regulate cup moisture and to provide additional pupation space for D. melanogaster (D. willistoni usually pupates in the food). In ten cages D. melanogaster was weakened by the presence of either sc cv v f or bw; st. D. willistoni was favored in nine cages by initial proportions of 400:200 or 500:100, and in 8 cages by being placed in one day earlier than D. melanogaster. The generation length is the same within 12 hours in bottles at 25°.

The egg samples from the P₁ generation contained in all but one case a considerably higher proportion of D. melanogaster than the starting frequency, in two cases ostensibly 100% and in three others over 98%. The frequency continued to climb rapidly, although two cages reporting 100% D. melanogaster eggs contained 7% and 3% D. willistoni adults at the end of the experiment. There may have been some interference by D. melanogaster in the egg laying of D. willistoni; D. willistoni imagoes also had a greater propensity to become stuck in the food. Each variable other than temperature had a small effect in the direction expected. The single anomalous cage, at 19°, had the following frequencies of D. melanogaster eggs: 99.2% at 5 days, 99.1% at 21 days, and 52% at 36 days. At 48 days mites were found and the cage had to be discontinued. The adult population then consisted of 74% D. melanogaster. The reason for this situation is unknown; the keeper of another cage at 19° dropped the course before taking any samples.

Waddington, C. H. Genetic assimilation of adaptation to saline media.

Four strains (two derived from Oregon wild types, sp² bs² and al b c sp²) have been cultivated for about 20 generations on increasing concentrations of NaCl incorporated in normal cornmeal-molasses media. At the end of this period of intense natural selection, a survival rate of about 15%-20% was achieved by the adapted stocks on 7% NaCl. The length and width of the anal glands (reported to be an osmotic regulatory organs) have been measured in the pupae. Typical figures for the ratio anal length x breadth/pupal length are 0.068 for adapted Ore-K(L) grown in 7% salt, 0.054 for the same stock in normal medium, and 0.044 for the unselected Ore-K(L) in normal medium. Thus the (presumably adaptive) increase in size of the anal organ brought about by selection is partly retained when the adapted strain is returned to normal surroundings.

Wolfsberg, Marilyn F. A note on the egg-laying behavior of mated D. busckii.

In the course of a study involving oögenesis and egg-laying behavior in a standard wild type of D. pseudoobscura, some data were collected for some other species of Drosophila as well. The technique of King (Am. Nat. 89: 369, 1955) was employed to study the daily egg production of individual females. Records of 14 D. busckii females (obtained from Dr. P. S. Woods) were maintained for the first 10 days after eclosion. At 25° C, the developmental period from egg to adult in this species is 12 days; mated D. busckii females reach sexual maturity 2 days after eclosion, on the average. Unlike D. pseudoobscura, D. busckii females do not exhibit any egg-laying rhythm. Under our conditions, the average number of eggs per female per day ranged from 32 to 44 (mean = 38.3). Females occasionally laid large numbers of eggs within 24 hours, for example, 101, 105, 114, 159; but these instances were rare and such females reverted to more characteristic egg-laying be-

havior after the initial period of heavy laying. Kahle-fixed, Feulgen-stained whole mounts of the ovaries of some representative females are being studied. Both the number of ovarioles per ovary and the number of oocytes per ovariole are greater than those found in the more common species of *Drosophila* (cf. King and Wolfsberg, Growth 21: 281, 1957).

Wolfsberg, Marilyn F. The effect of ovarian growth on P^{32} distribution in adult *D. pseudoobscura*.

Newly emerged females were dissected after having fed for various lengths of time (3 hours to 14 days) on a standard cornmeal-molasses-agar medium to which had been added tracer.

amounts of $H_3P^{32}O_4$. The radioactivity of wings, legs, head, thorax, reproductive system, gut, abdominal residue or fluid residue (hemolymph), which was determined by means of an alpha, beta, gamma restricted atmosphere proportional counter, was expressed as percentage of the total incorporated P^{32} . A record was also kept of the stage of the most mature oocyte present in the ovary, in accordance with the terminology of King and his collaborators (Growth 20: 121, 1956).

Although there is much variation in the relative distribution of P^{32} in the organs of females during the first 24 to 48 hours, the relative distribution after this time is characterized by a steady state (no change in relative percentages), which lasts until the ovary begins to develop. Growth of the ovary from stage 5 at emergence to stage 14 at sexual maturity upsets the steady-state distribution. After the cessation of ovarian growth, however, the relative percentages of P^{32} in each organ once again become constant. Thus, the relative percentage of P^{32} within the ovary may be correlated with the amount of growth that has occurred in this organ, for example, 5%-8% in the immature (stage 7) ovary, 12%-15% in the intermediate (stage 10) ovary, and 20%-30% in the mature (stage 14) ovary.

Recent experiments have involved keeping females on labeled food until the relative percentages of P^{32} in the various organs reach the distribution characteristic of the female with immature gonads. The flies are then removed to unlabeled standard food and are dissected after various intervals. The relative percentage of P^{32} remaining in each organ is then determined. The results show that during the time the flies are fed on unlabeled food the ovary grows from the immature (stage 7) to the mature condition (stage 14). Despite the fact that the fly has been feeding on unlabeled food during this growth period, the ovary has continued to accumulate P^{32} . Moreover, it accumulates P^{32} until the relative percentage of P^{32} in the gonad has reached the same percentage as that found in the mature ovary of females feeding on labeled food. One cannot explain the data by assuming that the ovary loses P^{32} more slowly than any other organ. The tentative assumption is that the ovary accumulates endogenous P^{32} from other parts of the body during its growth. A metabolic pool of P^{32} mediated by means of the hemolymph has been suggested. Additional experiments are being planned to test these assumptions.

Yamada, Y., and O. Kitagawa. Polygenic mutation, induced by X-rays, in quantitative characters in *D. melanogaster*.

The CMI technique proposed by Burdick (1954) was used for this study, except that the fourth chromosome was neglected because our preliminary test showed that the fourth chromosome had little if any effect on hair counts. We treated each sample of five males,

taken at random from an isohomozygous line extracted by the CMI technique from a long-inbred line, with (A) 0 r, control, (B) 2000 r, and (C) 4000 r of X-rays, and obtained 36, 47, and 30 homozygous lines, respectively. In each line 40 females and 40 males were scored with respect to number of micro-hairs of abdominals and sternopleurals. The pooled scores of females and males were analyzed. The results so far available are given in the table.

The means for the three treatments show good agreement as regards both abdominals and sternopleurals. However, variances among lines for the same treatment were much higher in B and C than in control A. Variance in A could possibly be attributed to spontaneous mutation, to rarely occurring recombination during isogenization, or to chance sampling; but it still serves as a basis for comparison with B and C. Higher variance after those two treatments, therefore, should be ascribed only to polygenic mutation induced by X-rays. Increment in variance per unit dose was calculated by linear regression and amounts to 0.000167 for abdominals, but is negative for sternopleurals, although the variance in B is significantly larger than in A.

Taking the increment in variance due to spontaneous mutation to be 0.00475, which is merely an average of the values reported by Clayton and Robertson (1955) and Paxman (1956) cited by Mather (1956), the tentatively calculated doubling dose for abdominals is 28.39 or approximately 30 rad. To our surprise, the figure falls within the range of doubling doses estimated for major genes in various organisms. The experiment is still in progress.

Treatment	No. of genomes sampled	Abdominals		Sternopleurals	
		Means	Variances	Means	Variances
(A) 0 r	36	31.2903	0.271378	15.1881	0.439861
(B) 2000 r	47	31.1801	0.924582*	15.1165	0.903444**
(C) 4000 r	30	31.1736	0.940674*	14.9538	0.374808

*Significantly different from control at 1% level.

**Significantly different from control at 5% level.

TECHNICAL NOTES

Arnold, Lloyd L. Culturing flies
in disposable paper containers.

Drosophila may be bred and nur-
tured in plastic-lined paper
containers, dispensing entirely

with the odious and expensive labor of washing and sterilizing fly bottles. The containers can be autoclaved, but we have found it unnecessary. When in use, the containers are covered by a window of clear plastic film, which leaves the entire exposed surface of the medium and the chamber above it open to undistorted observation, either directly or with the aid of a dissection microscope, without removal of the cover.

The basic unit is an 8-ounce plastic-lined "Nestyle" container manufactured by the Sealright Company, Fulton, New York. The containers are 44 mm deep and have an inside diameter of 82 mm at the top and 76 mm at the bottom. When ordered in case lots (250) they cost less than 3 1/2 cents apiece. The feature which particularly recommends the "Nestyle" container for this purpose is the fact that the lip is rolled outwards, down and under, to form a resilient cuff about 3 mm thick and 9 mm wide around the top of the container. This cuff is sufficiently resilient to make a firm closure with the cover, even after considerable use.

The containers are supplied with a hood-type cover consisting of a paperboard ring or hoop about 20 mm high, which fits down snugly over the outside of the cuffed lip, and two paperboard discs, which are folded into the top edge of the ring and cover the mouth of the container. When cultures are set up the discs are pressed out of the ring and discarded. The ring is placed upside down and covered by a sheet of "Saran Wrap." The anesthetized flies are placed on the "Saran" and the container is pressed down into the ring. Small holes may be punched in the "Saran" to increase ventilation.

It is most convenient to anesthetize the flies before opening the containers. For this purpose a simple anesthesia machine can be easily made by placing two or three facial tissues saturated with ether in a polyethylene catsup bottle to the nozzle of which has been fitted a hypodermic needle. The only critical item in this apparatus is the needle; needles larger than #20 make holes large enough for flies to crawl through. The needle pierces the wall of an inverted container and ether-laden air is circulated back and forth between chamber and bellows until the flies are immobilized on the cover. When the cultures are crowded or when the weather is hot, it is better to use a piece of nylon stocking to cover the containers.

For our aging studies we use a cage fashioned from a 16-oz "Nestyle" which permits changing the medium without anesthetizing the flies. The bottom of the pint container is cut off so that it fits nicely into an 8-oz container above the medium, and covered with "Saran" or stocking net. The lower container is changed as indicated by inverting the cage and shaking the flies into the large chamber, while the old bottom is removed and a new container put in its place.

Basden, E. B. A *Drosophila* suction-collecting apparatus.

A sucking-tube or aspirator (as figured in Galtsoff et al., 1937, "Culture Methods for Invertebrate Animals,"

p. 46) connected to a small vacuum filter pump powered by a water tap makes a very satisfactory apparatus for collecting *Drosophila* from culture bottles or for transferring them to new bottles when there is no need to examine the flies. A suitably sized cork on the aspirator is fitted into the new vial or new bottle, and the flies can be sucked directly into it from the old culture and may be counted at the same time. This method eliminates the undue handling entailed in the three separate actions of banging into the etherizer, counting on a plate, and transferring. Since the flies are not etherized, there is no risk of their becoming stuck in the medium. If the numbers in the culture bottles are too large and the flies too active for collecting conveniently, they can be shaken into a large glass jar and kept down by a light directed towards the base. By picking the flies off the sides an accurate count is obtained.

Forbes, Clifford. Method of egg collection.

Petri half-plates with a 2-inch diameter have been used to collect eggs. The plate bottoms contain the agar medium,

which has been moistened with yeast suspension. The parent flies are placed in an empty half-pint milk bottle. Then the Petri dish is put on as a lid. The plates are held in place with masking tape, which is wrapped nearly all the way around, leaving space for air. The bottles are inverted when incubated. This technique allows the collection of as many as 800 eggs from 15 to 20 pairs of parents in a 20-hour period.

Frydenberg, Ove. The Bennett population cages.

Bennett (DIS-30, p. 159) described an inexpensive population cage that seems especially useful in experiments where

a large number of populations is required. During the last year Bennett cages have been used for different purposes in this laboratory (Institute of Genetics, Copenhagen). Our cages are slightly smaller than Bennett's, and we have found it convenient when working with *D. melanogaster* populations to change one of the eight food vials every second day, thus leaving any vial 16 days in the cage. Otherwise our set-up is identical with Bennett's.

In sampling the populations, we initially inserted an ordinary 100-mm food vial in the cage and left it for oviposition 24 hours. Comparisons of replicate egg samples obtained in this way from 10 cages showed that the variance between replicates was much higher than the binomial variance. The total homogeneity chi-square was 20.6, with 10 degrees of freedom. This was mainly due to the fact that egg-laying females tended to stay in the rather long vial once they had entered it. A new set of 15 replicate egg samples was then collected from 15 cages, using 50 mm vials with slanted medium; and this considerably reduced the variance between replicates. The total homogeneity chi-square decreased to an insignificant 12.0, with 15 degrees of freedom.

As to the effective population size, one might fear that this at times would be very low in cages that small. We have not yet been able to get any direct measurement of N , but experiments on 16 polymorphic systems in duplicate populations show a high degree of repeatability between duplicates, and thus indicate that random genetic drift is not serious in these cages--at

least not as long as the systems studied are subject to considerable selective drift.

The generation length, defined as the average length in days between two eggs in a line of descent, has been determined by introducing eggs of known age into a marked tester population and recovering the eggs laid by the introduced eggs. The average generation length turned out to be somewhere between 11 and 12 days at 25° C for both populations studied. The distribution of eggs laid shows that the average egg-laying period of the individual females is very short, certainly not more than 48 hours and probably considerably less. The fast turn-over indicated by these preliminary studies may make the Bennett cages favorable tools in selection studies.

Kirschbaum, W. F. A new container for handling vials.

Special boxes have been designed which greatly facilitate the handling of culture vials. The latter are placed

in the rack inside the box at the time they are washed and they need not be removed again until they are used, since they are dried, filled, and prepared in situ. The boxes are constructed entirely of 1-mm aluminum sheet, painted on the outside. The size of the boxes is such that they are easy to handle and fit two on each shelf of a standard 9-foot refrigerator. Each has a capacity of 80 tubes.

The washed vials are placed in the anodized aluminum rack (which is built into the box) and the box covered with a sliding perforated top. The box is then inverted and placed on the shelf of a drying rack, made especially to hold these boxes. Under each shelf is an inclined aluminum sheet to run off the water and prevent wetting of boxes on the shelf below. Once dry, the tubes may be stored by replacing the perforated top with an entire one. The vials may later be filled with medium without being removed from the rack. (A simple apparatus which fills a whole box of tubes at a time is being perfected and will probably be described in next year's DIS.) The box is then covered with a top made of bronze wire mesh (38 wires per cm) and placed (inclined side down) in a stand, which holds tubes at the proper angle for making the agar slant, for drying. When the agar has hardened, the boxes may be stored in the refrigerator after replacement of the mesh top with an entire one. To prepare the vials for use, cellucotton, yeast, and stoppers may be added without removing the tubes from the rack. On the front of each box is a holder for a card on which may be indicated the contents, date of preparation, etc. If the vials are not to be used immediately, the boxes may be covered again with the entire tops and stored in the refrigerator.

These containers have been in use in our laboratory for the past year and have proved advantageous in the following ways:

- (1) There is less manipulation of vials, and thus a saving of time and personnel.
- (2) Washed tubes remain thoroughly clean during storage.
- (3) The containers facilitate filling and accelerate preparation of vials.
- (4) They diminish the probability of contamination.
- (5) Facilitate storage in the refrigerator.
- (6) Aid cooling and drying of recently prepared tubes.
- (7) Aid the warming to room temperature of prepared vials that have been in the refrigerator, without formation of moisture on or inside the tubes.

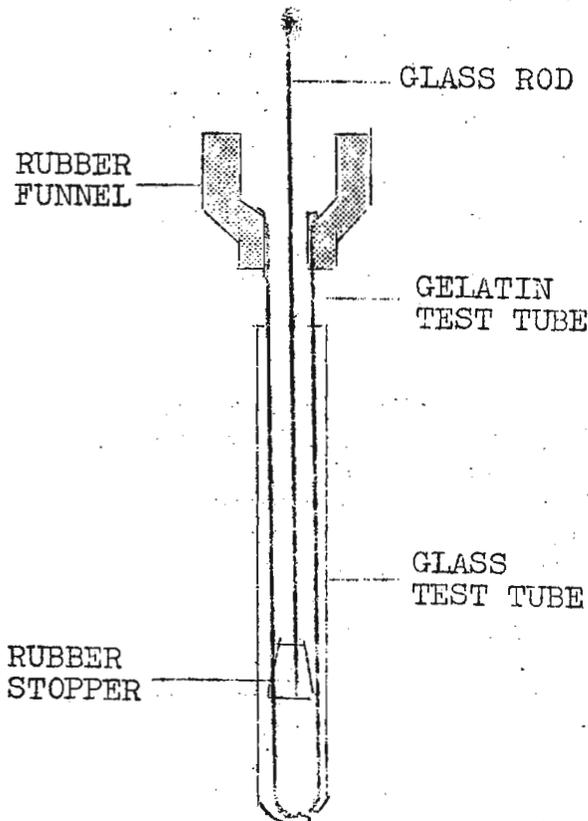
Marques, E. K., and C. M. Paiva Maciel. Improved soybean-banana-agar culture medium for *Drosophila*.

The high protein content of soybean is advantageous for the improvement of banana-agar media. Experiments have been performed with 5%, 10%, 20%, 30%, 40%, and 50% finely ground soybean flour added to banana-agar medium. The flies hatch earlier and in larger numbers than in the banana-agar medium. Ten pairs of *D. melanogaster* white mutants were distributed in each tube with approximately 4 cc of food. The tubes were overcrowded. A total of ten control tubes and ten tubes for each soybean-flour supplement were kept at 25° C. On the fifth day the parents were discarded. On the tenth day the flies were counted, and on the fifteenth another count was made. The total yield for 10 tubes on the tenth day was: control, 0; 5%, 0; 10%, 138; 20%, 25; 30%, 40; 40%, 143; 50%, 125. The totals on the fifteenth day were: control, 24; 5%, 11; 10%, 676; 20%, 339; 30%, 500; 40%, 709; 50%, 740.

Maud, G. D., and J. F. Ellis. An apparatus for handling flies without anesthetization.

In our work on modification of low-dose radiation effects by various anesthetics, it became necessary to pair-mate individual flies over a succession of days without the use of ether to immobilize them. An apparatus, pictured in the accompanying diagram, was constructed which permits the operator to eject single unanesthetized flies into mating vials quite rapidly and, with routine care, without loss. The apparatus consists of two nested test tubes, a rubber funnel such as that used with an etherizer, and a glass rod fitted into a small rubber stopper. The outer tube is of glass with a small hole, large enough to permit a single fly through, formed acentrically in the bottom. A corresponding and slightly larger hole is made in the inner gelatin test tube. A one-hole rubber stopper with a glass rod inserted forms a plunger which just fits inside the gelatin tube. The rubber funnel placed on the gelatin tube facilitates shaking the unanesthetized flies into the inner test tube. Twisting the outer tube to align the holes permits the controlled escape of single flies; the plunger helping to concentrate the flies near the openings speed up their escape. By taking advantage of the positive phototactic response of the flies, a light placed behind the recipient mating vials makes it possible to spread the flies even more rapidly and efficiently without anesthetization and without loss.

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Mitchell, D. F. A device for obtaining accurate measurements in large samples.

A device constructed by the Drummond Scientific Co. of Philadelphia has proved to be of great value in measuring physical dimensions in

large samples of flies. The device consists of a grooved plastic plate, mounted on a stage which moves on ball bearings. This stage is mounted on a second ball-bearing stage, to which is attached a plunger which activates a Federal Pressure Gauge. To obtain the measurements the flies are etherized and positioned in the grooves on the plastic plate; the upper stage is then moved by a screw dial to align one limit of the character with a hair line in the eye piece of a binocular microscope. A second dial then moves the lower stage until the other limit of the character is aligned with the hair piece. Movement of the lower stage activates the gauge, which is calibrated to .01 mm. The dimension is then recorded directly from the gauge, the gauge corrected to zero by turning the lower stage dial, and the next fly aligned with the hair piece by turning the upper stage dial. The device has been used successfully in measuring adult body length, wing dimensions, and puparium dimensions. The accuracy and rapidity of measurement of samples is limited only by the time required to position the flies. Further information concerning the device will be gladly provided upon request.

Pipkin, S. B. Containers for exposing *Drosophila* larvae to cosmic rays at extreme altitude.

To expose *Drosophila* larvae to cosmic rays at extreme altitude in balloon flights, light-weight celluloid centrifuge vials 1 1/2 x 6 inches were

used. The rounded bottoms of the vials were cut off and shallow stoppers fitted for the cut-off ends. The celluloid vials were sterilized with ultraviolet light; corks and cotton plugs were sterilized in an autoclave. Under an ultraviolet hood, autoclaved corn meal medium was poured into the vials, which were then tilted so as to cause the medium to slant and offer a large surface for egg-laying. After being plugged with sterile cotton, vials were sealed with a paraffin-beeswax mixture at both ends. After exposure, the culture medium containing larvae could easily be taken from a celluloid vial, by opening the cork end with a knife, and shaken into a half-pint milk bottle of sterile culture medium. There the exposed larvae could complete development without being crowded. Sealed and sterilized celluloid vials with culture medium retained moisture and developed no growth of microorganisms for two weeks before use.

Prout, Timothy. A rapid method for measuring wing length.

Recently many investigators are finding wing length a most useful quantitative character. For those experimental situations where large numbers

of individuals are required and where the measured flies may be discarded, the writer suggests the following method. This procedure essentially involves making the measurement after the flies are mounted on microscope slides in Canada balsam. First the flies are overetherized, which almost invariably leaves the wings in a vertical position. Then a microscope slide is streaked with Canada balsam that has been thinned with Xylol. The overetherized flies are then dropped on their sides onto the balsam and allowed to sink so that one wing will be on the surface of the slide itself. If the overetherized flies are first spread on a white card or some other surface they can be transferred to the balsam very rapidly with a moistened pencil or probe. Little or no time is required for positioning the flies after they

are in the balsam. A dozen or more flies can be mounted on each slide, and no cover slip is necessary.

After the balsam has dried (or before) an accurate wing measurement can be made through the back of the slide. The measurement may be made in the usual way with an ocular micrometer; the writer finds the use of a microprojector preferable. By adjusting the height of the microprojector (e.g., Bausch & Lomb Tri-Simplex Microprojector, Cat. No. 42-63-59), the image of the wing can be projected onto a horizontal surface so that with an appropriate ruler the measurement can be made in mm x 100 or some other simple multiple of absolute units. Since the base of the wing is often obscure, the best measurement seems to be between the point of intersection of the third longitudinal vein and the wing margin and the point of intersection of the third longitudinal vein and the anterior crossvein.

As to the permanence of the slides, the writer has taken a series of measurements of the same mounted flies over an interval of five months without detecting any change in this wing dimension. The value of the procedure described is that very large numbers of flies can be collected and mounted at one time and the measurements deferred until later. On one occasion the writer and two assistants were able to collect and mount more than 5000 newly hatched flies from 400 culture bottles in one day.

Shapard, P. B. An inexpensive device for dispensing *Drosophila* medium.

pared in a pressure cooker.

We have found that an inexpensive cream separator (Montgomery Ward 87FG 4245 MO) serves very well for dispensing *Drosophila* medium that has been pre-

Slizynski, B. M., and
H. Slizynski. Orcein.

salivary-gland preparation and do not have good results. The Orcein used in our laboratory here gives very good results; it is Orcein from G. T. Gurr, 136-138 New Kings Road, London, S.W.6.

During our recent visit in the United States we noticed that some laboratories use "Orcein--extract from lichen" for

Sokoloff, A. Types of bristles on the anterior margin of *Drosophila* wings conspicuous as a result of treatment.

Radius₁, the first longitudinal vein, actually possesses two rows of bristles. The dorsal row consists of acute bristles, as indicated by the detail drawing in figure 16, page 408, of

Biology of *Drosophila*, M. Demerec editor. The ventral row consists of blunt bristles shaped somewhat like elongated bullets. In dry-mounted wings the two types of bristles are difficult to discern, even with the highest magnification. They become evident, however, after the following treatment. The flies are killed in 95% alcohol and allowed to remain for two days. A number of flies are transferred to a slide, the alcohol blotted out, and a few drops of paraffin oil added to the mass of flies. The flies are then oriented in a supine position and the wings removed, so that the ventral surface of the wing lies uppermost on the slide. If examined with 40X magnification, a certain proportion of the wings will have an odd appearance. The first longitudinal vein will appear only half as wide as in the "normal" wing, and the anterior margin of the wing will seem darker. After examination with the compound microscope, it is clear that a folding back of the

blunt (ventral) bristles has taken place, leaving the acute dorsal bristles exposed throughout their length. In the few instances in which this process was actually observed, it was seen that usually the bristles proximal to the costal cell begin to fold back, and the other bristles follow suit throughout the length of the vein. In some instances, however, only the bristles located on the middle third or those on the distal part of the vein are affected.

The same reaction can be obtained (1) if the wings are detached from etherized flies and dropped directly into oil (but then a smaller number of wings is affected) or (2) if the flies are mounted in balsam according to the method described by Timothy Prout.

Oddly, populations of *Drosophila* differ in the incidence of this reaction. The Syosset and Oregon-R population cages maintained by Dr. J. C. King at the Biological Laboratory, Cold Spring Harbor, New York, for several years were repeatedly sampled and treated under the standard conditions described above. Each sample consisted of 50 flies of each sex from each of the population cages. Two points are worthy of mention in these tests: (1) females exhibited the reaction more frequently than males in both population cages; (2) the Syosset population exhibited the character less frequently than the Ore-R population.

*Van Valen, Leigh. Refrigerator boxes as population cages.

Polyethylene refrigerator boxes are inexpensive and convenient for maintaining *Drosophila* populations (Jack

Bennett, DIS-30). They are easy to construct, maintain, and clean. The beginning genetics lab at Columbia has since 1957 used 12" x 8" x 4" boxes (\$2.49 from Palo Plastics, New York City). Ventilation holes are covered with 60-mesh wire screening; holes for food cups are punched in the bottom, and others are punched in the sides. We use bottle #5901, Will Corp., Rochester, which has a good flange. Food containers are not placed in the side holes because the food tends to run out, even over planed-off corks reaching higher than the food surface.

Because of the pliable nature of the plastic, no more than 10 food cups can safely be placed in the bottom; 8 are used here. This sets a limit on population size disproportionately below that expected by the number of cups. In both *D. willistoni*-*D. melanogaster* and *D. persimilis*-*D. pseudoobscura* cages, the adult population size ranges between 200 and 800, as compared to 1000-3000 in 16-cup lucite cages. The difference may be due to the lower frequency of introduction of fresh cups, with adverse effects on adults.

Removal of flies from the cages is rather more difficult than with conventional cages, also. Here, and in other cages, it can be facilitated by the following apparatus: A small funnel or the equivalent (e.g., wax in a tube) is put into a cork that has a hole bored through it, and a piece of glass tubing projects from the other end of the cork. A short large-diameter glass tube can be fastened to the large end of the funnel if desired. The cork is placed in a hole in another cage or elsewhere, and the funnel or tube snug to the surface of the original cage in or around a hole. The flies move as readily as normally in the desired direction but virtually never return.

Yamada, Y. A simple technique for identifying sex at the pupal stage. A very simple but 100%-accurate method of identifying sex in D. melanogaster at the pupal stage has been used in our laboratory. It should be especially useful to researchers who must work at night or on Sunday morning to secure enough virgin females. The criterion for identification is the sex comb. Through the ventral surface of the pupal case one can easily recognize the sex comb in males, using a binocular microscope at a magnification of 30X, but only after the bristles have become pigmented. Care must be taken (1) not to injure the pupal case when lifting it from the vial, (2) not to allow the pupa to dry out under the microscope (a wet filter paper on the glass plate is recommended).

TEACHING NOTES

Hinton, Claude W. Use of the w^{VC} chromosome in class laboratories.

Several phenomena rarely encountered in undergraduate genetics laboratories can be easily demonstrated in simple crosses involving the unstable w^{VC} ring chromosome (Genetics 40: 951-961), for example, w^{VC} f/y w females by y v f/Y males. As a consequence of elimination of the w^{VC} chromosome, gynandromorphs are frequently encountered among the progeny; those gynandromorphs mosaic in the head may be used to illustrate gene hormones. Exceptional males are also abundant among the offspring; and, although many of these are the result of w^{VC} loss, others are due to primary nondisjunction. This phenomenon is also responsible for exceptional (non-forked) females occurring in the progeny. Position-effect variegation is manifested by the eye pigment of the parental females but not in the F_1 w^{VC} females; the effect of the Y chromosome in suppressing position-effect variegation is demonstrated by the exceptional females' phenotype. The writer will be pleased to supply unstable w^{VC} stocks for this purpose upon request. To insure maintenance of instability, multiple lines of the stock must be carried and selected frequently.

Seto, Frank. Styrofoam population cages for the classroom.

Inexpensive and simply constructed population cages made of styrofoam and glass have been used successfully in this laboratory for the past year.

These cages are easily made, relatively durable, usable under most population conditions, and disposable without undue loss in materials and cost. The construction of a typical small cage is as follows. The box frame of the cage is made from a block of styrofoam, about 3" x 6" x 6" in dimension, from which an inner block core is cut out leaving a box with open top and bottom and with walls 3/4"-1" thick. The inner core can be cut out with a thin-blade coping saw or a long sharp cutting blade. The two open ends are

then closed off with 6"-square glass plates, which are held securely in place with masking tape. Eight holes to insert food vials are bored into the walls at an angle to support them with the food ends lowermost. The holes can be made easily with a large-diameter cork borer or by carefully twisting a culture vial into the wall. One of the openings should be plugged with cotton and used for ventilation.

For the best use of the cage, the culture vials should be replaced in rotation, one every other day, so that each vial remains in position for 12-14 days. With continual use, the holes will become larger and support the vials loosely. To correct this situation, masking tape can be bound around the open end of the vial to give a snug fit. A sample of flies can be obtained by removing a vial in which flies have congregated or by collecting newly emerged adults from a 12-day vial. If a total count of the cage population is to be made, the colony can be anesthetized by placing a vial, containing a cotton plug saturated with ether, into the ventilation hole. Then the glass plate is removed by stripping off the masking tape and the flies are swept onto a counting plate. If, after considerable use, the cage becomes too dirty, the flies can be transferred to another cage and the old one discarded.