

## RESEARCH NOTES

Annan, Murvel E. Effects of dehydration on X-ray-induced damage to *Drosophila* females.

of this treatment, they were exposed to 5000, 2500, or 0 roentgens of X-rays. Immediately after exposure, each female was placed in an individual container with two males. Every day following treatment (for 10 days), two individual females from each of the six treatment groups (2 degrees of dehydration and 3 degrees of irradiation) were selected randomly to be sacrificed for ovarian examinations. The ovaries were examined for quantitative enumerations of stages 5 and 10 of oögenesis (after King, et al., GROWTH, 1956) for 10 days following treatment.

The data are too few to permit drawing general conclusions on the effects of X-rays on frequencies of stages of oögenesis. In none of the data were there any significant deviations due to the dehydration treatment. Herskowitz (DIS, 1956) found that dehydration increased X-ray-induced egg mortality. Although the deviations due to dehydration in our results were not statistically significant, the mean values were in the opposite direction; that is, dehydration tended to decrease egg mortality. This failure to duplicate Herskowitz's results may have been due to our use of a different degree of dehydration or different-aged females. Further work is under way to find possible effects of degree of dehydration on X-ray-induced damage to *D. melanogaster* females.

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Aslaksen, Erik. Pleiotropic pattern of the mutant red (red Malpighian tubules) in *D. melanogaster*.

The mutant red used in this experiment was received from I. Oster, and the investigations were carried out together with E. Hadorn in Zürich. We found that the red eye pigment and the content of isoxanthopteryne are decreased in this mutant compared to what is found in the wild type. This is compensated for by an increase of other pterines. The red pigment in the Malpighian tubules is found to be an ommochrome, but is not the same as that found in the eyes. In experiments on transplantation of eye discs, the mutant red exhibits autonomous development with respect to the amount of red pigment.

Auerbach, C. The production of visible mutations by chloroethyl methane sulphonate (CB 1506).

In view of the personal factor involved in the scoring of visible mutations, it seems important to obtain independent information of the claim that some alkylating agents produce very high frequencies of visible mutations, many of which have not occurred previously. In Muller-5 experiments by the Fahmys, CB 1506 produced an over-all ratio of 4 visible to 10 lethal recessives, and in later broods from sensitive spermatogonia this ratio increased to near unity. In the experiments to be presented here, 1- to 3-day-old OrK males were injected with a  $10^{-2}$  M solution of CB 1506. They were then mated to a succession of virgin females at intervals of 3-4 days. In broods d (days 11-14) and e (15-18), half the

*Virgin D. melanogaster* (Oregon-R) females, 24 to 48 hours old, were placed either in a desiccating chamber or in a moist chamber. After 8 hours

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males were mated to 3 M-5 females each and half to 3 XX= females each; males which had been given one type of female in d were given the other type in e. The sons by XX= females were carefully examined by myself at 10x magnification, and all deviants were progeny tested. The F<sub>2</sub> vials of the Muller-5 tests were examined without etherization through a binocular. This was done by a laboratory assistant, who was told to look carefully for visibles. She was chosen for the job because she has had over 10 years' experience with flies, uses a higher magnification for scoring lethals than is customary in our laboratory, is unusually observant and as a rule detects more visibles in M-5 tests than I do. A summary of the results is tabulated. The figures in brackets refer to males which for some reason or other could not be progeny tested. The column "Exp. 1" states the number of lethals which, on the basis of the parallel M-5 test, would be expected in a progeny of the size sampled.

| ♀♀ M-5   |     |    |      |        | ♀♀ XX= |        |        |           |
|----------|-----|----|------|--------|--------|--------|--------|-----------|
| Brood    | n   | l  | Vis. | % Vis. | n      | Exp. 1 | Vis.   | % Vis.    |
| <u>d</u> | 386 | 21 | 2    | 0.5    | 864    | 47     | 6 (8)  | .7 (1.6)  |
| <u>e</u> | 379 | 50 | 2    | 0.5    | 878    | 115    | 27 (3) | 3.2 (3.4) |

In the Muller-5 tests the ratio of visibles to lethals was about 1 : 17, that is, within the range found in X-ray experiments in which similar scoring methods were used, including experiments carried out by us. It is very much below the 4 : 10 ratio found by the Fahmys. In the XX= tests the percentage of visibles was higher, presumably for two reasons. (1) Even very slight abnormalities could be detected. (2) Many of the detected mutants had poor or very poor penetrance in progeny tests, and these would have escaped notice in M-5 tests. In brood e, only 10 of the tested mutants were fully penetrant, and if these alone are used the percentage of visibles drops to 1.1. My results, therefore, do not confirm the finding by Fahmy and Fahmy. This does not, of course, invalidate their observations; but it shows that definition of what constitutes a visible mutation, methods of scoring, and powers of observation differ considerably between laboratories and workers. Judged by my results in X-ray tests, my standards are roughly similar to those used by the earlier X-ray workers. That the Fahmys used higher standards is shown by the fact that in X-ray experiments by the Muller-5 method, in which the F<sub>2</sub> cultures were "scored under a low-power binocular without etherization rather early after emergence," they found the same ratio of 2 visibles to 10 lethals which Spencer and Stern obtained in experiments in which every culture was etherized and examined under the binocular. Incidentally, it will be of interest to see the detailed data on which the Fahmys base their conclusion that the ratio of 2 visibles : 10 lethals in their X-ray experiments differs significantly from the over-all ratio of 3 visibles : 10 lethals in experiments with phenyl alanine mustard. According to the table published in DIS-28, this latter ratio fluctuates between 1.8 : 10 and 3.3 : 10, when series containing less than 400 tested chromosomes are excluded. An additional source of discrepancy between the findings of the Fahmys and myself may be that I counted visibles which occurred in clusters as so many single mutations, because obviously in these broods the nonmutated germ cells may also have occurred in clusters. The Fahmys, on the other hand, counted each cluster as a single mutation and corrected the frequency of lethals "on the basis of the frequencies of

identical visibles in each brood." If this correction was done on the basis of the largest observed cluster it must have resulted in an underestimate of the lethal frequency. Moreover, lethals, many of which are subject to germinal selection, might be expected to form smaller clusters than visible viable mutations.

The nature of the visible mutations which occurred in the experiment with CB 1506 was not striking. There were alleles of *fu*, *f* and *w*, and in addition a number of unidentified mutations to rough eyes, smaller body size, and spread or drooping or incised wings. The one striking fact was that two mutations occurred repeatedly in the progeny of different males. One was a satsuma-like allele of *w*, which occurred twice. According to an analysis, kindly carried out by Dr. M. M. Green, the two mutants are indistinguishable phenotypically and in their interactions with "zeste" and with the enhancer of *w*<sup>e</sup>; they are thus probably identical. The other case was that of a mutation to rotated abdomen (not yet located accurately), which occurred twice independently in the experiment described above, a third time in another experiment with CB 1506, carried out on a different strain of males, and a fourth time in a recent experiment with mustard gas. These recurrences point to the possibility that some mutational steps occur more readily than others under the influence of CB 1506 and, possibly, of other mutagens with chloroethyl groups.

Auerbach, C., and  
Sonbati, E. M. The  
brood pattern of  
mutations after  
treatment with  
chloroethyl methane  
sulphonate (CB 1506).

Fahmy and Fahmy found that injection of CB 1506 into adult males produces very low mutation frequencies in early broods and very high ones in late broods. Possible causes of this unusual brood pattern are slow action of the chemical, long mutational delay, dependence of the mutagenic action on

cell division or on rate or type of metabolism. These possibilities have been explored in a number of preliminary experiments. (1) Treated spermatozoa were stored in males or inseminated females, and mutation rates were scored (a) in progeny obtained during the first days after treatment, (b) in progeny from stored spermatozoa, (c) in progeny from males which had been kept with females for the periods of storing used in group (b). Mutation rates in (b) were intermediate between those in (a) and (c). Slow mutagenic action and/or mutational delay are thus only contributory causes of the brood pattern. With periods of storing exceeding 12 days, mutation frequencies dropped again, presumably because of selection against males or spermatozoa which had absorbed more than the average amount of mutagen. Possibly the slight drop in mutation frequency which the Fahmys obtained in the fourth brood may have a related origin, for with their slow-breeding method (1 ♀ per ♂ every 3 days) some storing of treated germ cells would appear unavoidable. (2) Immature inseminated females were injected, and progeny was collected (a) during the first 4 days, (b) from females which previously had been kept on sugar-agar for 1 or 2 weeks. Mutation frequencies in the female germ cells were the same in all series (about 7%). Thus oögonia respond immediately to the treatment, and there is no noticeable delay in the occurrence of the mutations. (3) Immature virgin females were injected and kept on sugar-agar or maize meal-molasses food for various periods of time before mating and production of progeny. As in the previous experiment, storing on sugar-agar had no influence on mutation frequency. Storing on food resulted in an increase in mutation frequency

which after 2 weeks reached statistical significance. Thus, active metabolism seems to favor the mutagenic action of CB 1506. When successive broods were produced from the treated females, mutation frequencies tended to drop, sometimes drastically. It has not yet been decided whether this was due to lower mutation frequencies in young oögonia or to selection against more heavily affected cells. (4) If active metabolism should, indeed, promote the action of CB 1506, one would expect high mutation rates from the germ cells in well-fed larvae. This expectation was borne out in a number of small-scale tests. The most effective treatment so far consisted in keeping 72-hour-old larvae for 24 hours on food containing  $10^{-2}$  M of CB 1506. 223 X chromosomes from treated males carried 80 lethals, and 613 X chromosomes from females carried 117 lethals.

Barigozzi, C., Castiglioni, M. C., and Di Pasquale, A. Genetical analysis of a new tumorous stock, melanotic e 144 in D. melanogaster.

A new spontaneous stock carrying pseudotumors (courtesy of Dr. E. Goldschmidt, Jerusalem) has been submitted to the same investigation as several other stocks in this laboratory. It proved to have its

main group of genes controlling the production of pseudotumors on the second pair of chromosomes, although the first and third pair interact in raising the incidence. The stock, if selected for and against tumor manifestation, shows a strong homeostasis. High-incidence lines give only a small number of offspring, so that selection after 6 generations becomes difficult.

Bertram, Cl., Hohne, G., and Kunkel, H. A. Induction of sex-linked recessive lethals in D. melanogaster by high-energy electrons and conventional X-rays.

The following data are the result of new comparative investigations with 200-kV X-rays and fast electrons of a 15-MeV betatron (Fa. Siemens-Reiniger). Adult males of D. melanogaster (Berlin normal inbred), 3-4 days old, were irradiated and 24

hours later mated to Muller-5 virgin females for three days. The results shown in the table demonstrate that the rates of mutation are nearly the same after irradiation with equal doses of X-rays or of fast electrons. The mean lethal mutation rate per 1000 rep is  $2.47 \pm 0.24\%$  for X-rays and  $2.52 \pm 0.28$  for fast electrons. Under the described circumstances the relative biological efficiency of the high-energy electrons is within the range of the statistical error.


|           | Dose in<br>rep | No. chromosomes<br>tested | No. of<br>lethals | Per cent<br>mutation |
|-----------|----------------|---------------------------|-------------------|----------------------|
| 200 kV    | 4000           | 2934                      | 293               | 10.0                 |
| X-rays    | 2000           | 1373                      | 66                | 4.8                  |
| 15 MeV    | 4000           | 2289                      | 219               | 9.6                  |
| electrons | 2000           | 1019                      | 57                | 5.6                  |

Binder, R. G. Observation on the mite Histioglyphus laboratorium in Drosophila cultures.

A population study was made of the Drosophila food mite, H. laboratorium, from November 1954 to June 30, 1955. Population growth curves were recorded under conditions of light (sun or

artificial), dark, heat (up to 105° F), cold (40°-45° F), dryness (74%-86%

normal food weight), and moistness (water added to 400% normal food weight), tested singly or in combinations in 150 cultures. Observations were made with (a) reused *Drosophila* cornmeal-agar medium previously infested with mites, and (b) fresh medium inoculated with bacteria cultured from previous mite-infested food plus a few roccal-sterilized mites. The results were as follows:

(1) The mite population curve was -shaped under normal viability conditions. The early plateau may indicate the bacterial incubation period (2-6 days after start of culture). The humps may indicate relationships between mite-hatching potential and available-food potential. (2) Mite populations were generally found to thrive in the presence of healthy living bacteria, especially when autolysis occurred. Many bacterial species contributed to the mite culture. (3) Population was found highest with alternating periods of light and dark at room temperature. Cold (40°-45° F) and particularly warm (near 100° F) cultures were almost inviable, though eggs could last for months. (4) The major limiting factors were available food and living space (cultures were in test-tubes with 1 g or 0.5 g of food), with the accumulation of waste a probable secondary factor. (5) Rise in pH was generally associated with population rise. pH 6.8-9 was associated with heavy population and escaping hypopi--which, under pressure of numbers might even bore out of waxed-in cotton plugs.

It was found that *H. laboratorium* can live on peptone food products, including beef-extract; but *Drosophila* cultures are especially favorable because the fly serves as a transport host. A mechanism sensitive to the shadows of passing objects causes the mite hypopus (the 6th of 7 epimorphic stages) to spring up and attach to passing objects with its ventral suckers. This can be demonstrated by passing a small brush above a bottle cap infested with hypopi.

These observations were made while the author was a graduate student in the Department of Biology, Purdue University. (Present address: 189 Staples Street, Farmingdale, New York.)

Bonnier, G. Effects of selection pressure on irradiated populations of *D. melanogaster*.

From a common wild-type stock which was made co-isogenic and homozygous for chromosomes 1, 2, and 3, different populations were started. All populations are kept in an incubator

room at 25° C. In each of the populations the larvae are bred in vials (see below). After eclosion the adults are transferred to population cages, and thereafter all flies are given an acute X-ray dose of 1500 r. In two of the populations, named P 25 and P 200, the females then oviposit on blackened food in Petri dishes. This oviposition takes place from 5 p.m. to 9 a.m. the next morning, and, after 28 more hours, freshly hatched larvae are collected and put into 90 x 25 mm vials, with ordinary cornmeal-agar food. The amount of food is not weighed, as we think that differences necessarily must cancel each other. Twenty-five larvae are put into each of the vials of population P 25; and 200 larvae into the vials of P 200. Between 5000 and 6000 larvae are collected and transferred to the vials in this way. In order to get that many larvae it is often necessary to make the same kind of collection for two or even three days in succession. When the adults emerge in the vials the same procedure is repeated for the next generation. In this way

every generation has a length of exactly 3 weeks. Two other populations, bb 25 and bb 200, are handled in the same way as P 25 and P 200, respectively, except for the way in which the oviposition takes place. In order to have a guarantee that no single female will produce too large a number of offspring, the egg-laying is done in vials. Five females, together with males, are put into each vial, which contains a spoon with blackened food; no more than 25 larvae are collected from any one of these vials. From population P 200 a new population P 400, with 400 larvae per vial, has recently been started.

These experiments have now been going for about one year. Different kinds of tests have been made. We expect that the first group of results will be available in the middle of 1958.

Bonnier, G., Ramel, C.,  
and Jonsson, Ulla B.  
Relative growth rates of  
larvae homozygous and  
heterozygous for alleles  
of the w locus.

Strains of five w alleles, namely,  $w^+$ ,  $w^{co}$ ,  $w^a$ ,  $w^t$ , w, were made co-isogenic and homozygous for chromosomes 2 and 3 and for a part of chromosome 1 extending from a point between the loci of w and ec to a point to the right of the locus of f.

Freshly hatched larvae were transferred to vials, 200 to each vial. In a first experiment, in which the growth rates of larvae of the pure strains were compared, each vial got 100 larvae from one of the strains and 100 from another strain. On the average 83 per cent of the larvae reached eclosion in a practically 1:1 proportion between the two competing types. However, at the time when only about 25 per cent of the larvae had reached eclosion, great differences were found between the two competing types. For one thing, it was found that  $w^{co}$  grew at a slower rate when competing with  $w^+$  than when competing with  $w^a$ , and  $w^a$  grew at a slower rate when competing with  $w^+$  or  $w^{co}$  than when competing with  $w^t$  or w. In a second experiment, comparisons along the same lines were made between the growth rates of homozygous and heterozygous wild-type females (which were identified by progeny tests with regard to their genotypic constitution). It was found that in competition between homozygotes  $w^+/w^+$  and any of the heterozygotes,  $w^+/w^{co}$ ,  $w^+/w^a$ ,  $w^+/w^t$ ,  $w^+/w$ , the homozygotes grew faster than the heterozygotes. New experiments are in progress.

Brown, Wm. P., and  
Bell, A. E. Analysis  
of developmental time  
involving three isogenic  
lines of D. melanogaster.

Three randomly chosen wild-type isogenic lines, which had been isolated from a closed population that exhibited a plateau for fecundity after 47 generations of selection (Brown and Bell, DIS-29), were carried for 25

generations by sib mating without artificial selection. Subsequently, a new isogenic line was derived from each of the three lines, thus giving six lines which were utilized in the analysis reported here. Crosses between the lines were made in all possible combinations, including pure lines and reciprocals, and a 24-hour egg deposit was collected from each. All progeny from each cross were observed and classified for sex, and the time of emergence was recorded. The time of emergence was broken down into 10 periods of 12 hours each (midnight to noon, and noon to midnight), making an emergence duration of 120 hours. A mean emergence time was

obtained for each sex and cross, and a constant of 240 hours representing the time between oviposition and the emergence of the first fly was added to the average emergence time to obtain the average developmental time. An analysis of variance revealed a highly significant difference in average developmental time between the sexes, with 288 hours for females and 294 hours for males. A highly significant difference was also found between crosses. When the crosses were summarized according to source of the parents, the following relationships were obtained.

| Type of crosses according<br>to source of parents   | Average developmental<br>time in hours |
|---|--|
| 1. Among original lines . . . . .   | 287                                    |
| 2. Between original and derived lines,<br>excluding each original and its<br>derived line . . . . . | 290                                    |
| 3. Between each original and its<br>derived line only. . . . .                                      | 290                                    |
| 4. Pure original lines . . . . .  | 293                                    |
| 5. Among derived lines . . . . .  | 295                                    |
| 6. Pure derived lines . . . . .   | 296                                    |

Since the original lines were carried for 25 generations without selection, mutations no doubt occurred within each which made them no longer isogenic. Assuming that earlier emergence is an indication of vigor or heterotic effects and that in general the degree of probable heterozygosity decreases as one goes down the table, the summary suggests that vigor in terms of developmental time is directly related to the degree of heterozygosity. A more detailed analysis of these data is under way.

Burdette, W. J. Interaction  
between tumor genes in  
*Drosophila* and genoid for  
CO<sub>2</sub> sensitivity.

Evidence that germinal mutants control  
cancer susceptibility is well estab-  
lished, but examples of tumors associated  
with viruses are increasing in number.  
As part of an investigation into the

relations between the two, chromosomes bearing tumor genes were introduced into cytoplasm containing the genoid, Tr, for CO<sub>2</sub> sensitivity. The first table demonstrates a uniform distribution of the agent in individuals with and without tumors in the experimental group. The second shows a decrease in incidence of tumors in comparison with control cultures. The genoid apparently alters tumor incidence in the presence of susceptibility genes, although it neither incites tumors alone nor induces heritable changes at these loci.

CO<sub>2</sub> Sensitivity tu vg bw

|            | Males |       |     | Females |       |     | Total |       |     |
|------------|-------|-------|-----|---------|-------|-----|-------|-------|-----|
|            | Sensi | Total | %   | Sensi   | Total | %   | Sensi | Total | %   |
| With tu    | 895   | 899   | 100 | 801     | 804   | 100 | 1696  | 1703  | 100 |
| Without tu | 117   | 119   | 98  | 201     | 202   | 100 | 318   | 321   | 99  |
| Total      | 1012  | 1018  | 99  | 1002    | 1006  | 99  | 2014  | 2024  | 99  |

## Effect of Genoid on tu vg bw Tumor Incidence

|                        | Males |       |    | Females |       |     | Total |       |     |
|------------------------|-------|-------|----|---------|-------|-----|-------|-------|-----|
|                        | tu    | Total | %  | tu      | Total | %   | tu    | Total | %   |
| CO <sub>2</sub> Genoid | 1555  | 1753  | 89 | 1466    | 1803  | 81  | 3022  | 3556  | 85  |
| Control                | 604   | 608   | 99 | 546     | 546   | 100 | 1150  | 1154  | 100 |

(Aided by a grant from the National Cancer Institute.)

Burla, H., and Zürcher,  
C. Color polymorphism  
in D. melanogaster.

In females of a wild strain from Uganda, obtained through the courtesy of Dr. Dobzhansky, the dark marginal bands of the abdominal tergites are broadened

and the light anterior parts of the tergites are shadowed, a condition uncommon in the species, but similar to that in dark females of D. kikkavai. Densimetric determination of the darkness of selected parts of the tergites gave no overlapping between the two color types. The trait appears to show simple monohybrid inheritance with incomplete dominance, the heterozygotes showing intermediate darkening of the tergites. Dark homozygotes have reduced viability in comparison with light and intermediate phenotypes.

Castiglioni, M. C.  
Developmental genetics  
of pseudotumors in  
D. melanogaster.

A developmental study of pseudotumors in D. melanogaster has been carried on in close collaboration with the formal investigations of Barigozzi and Di Pasquale. (see DIS-28, -29, -30).

It has been possible to show that the presence of a polygenic tu system is not sufficient to explain the developmental behavior of the character; thus it is necessary to postulate the existence of another polygenic system, modifying or conditioning the action exerted by the tu genes. More than 20 different genotypes have been studied, and it has been concluded that the presence of melanotic masses is the result of at least two independent mechanisms: (1) A more or less apparent disruption of the first pair of lymph glands during the third instar, with the consequence that a large number of cells—previously contained in the gland—swim freely in the hemolymph. One kind of these cells, especially, tends to congregate in clumps. (2) Melanization of the clumps, and finally, disappearance of any cellular structure. These two mechanisms combine in the following way:

| <u>Disrupted</u><br><u>gland</u> | <u>Preserved</u><br><u>gland</u> |                       |
|----------------------------------|----------------------------------|-----------------------|
| a                                | b                                |                       |
| tumors .....                     | no tumors .....                  | ability to melanize   |
| c                                | d                                |                       |
| no tumors .....                  | no tumors .....                  | inability to melanize |



Condition a occurs in all tumorous individuals in every genotype. Condition b is typical in the tumorless individuals of low-incidence stocks. Condition c occurs in some stocks without tumors (e.g., +/+ +/+ Sb Me/H) and in those cases where tu genes have been replaced by their alleles through recombination. Condition d occurs in some wild stocks (Varese) as well as in heterozygotes of this type: Varese/tu stock. These observations have been made with three techniques: (a) examination of the whole gland, (b) examination of a series of microtome sections, (c) counts of the hemolymph cells smeared and stained with May-Grünwald Giemsa. The results obtained by these techniques have always been in good agreement. The polygenic system controlling the disruption or the preservation of the lymph gland during development works epistatically upon the tu system.

Crowell, Villa E. B.,  
and Herskowitz, I. H.  
Heterosexual activity  
and longevity of the  
*Drosophila* male.

Although it has been frequently noted in the literature that unmated females live longer than mated ones, the effect of mating on the longevity of males has not been sufficiently studied. Loeb and Northrup (1917) found that at 30° C

"isolated males lived a little longer than males when mixed with females," the noncelibate males living only 83.5% as long as celibate ones. To prevent isolation from being a possible factor, the effect of heterosexual activity of males on their longevity was studied as follows.

Oregon-R males less than one day old were collected and placed, one per vial, in vials containing standard yeasted food medium. In class I (110 flies), one virgin of a type easily distinguished from the wild type was also placed in each vial. In class II (114 flies), one Basc male fly was added to each vial. Every day for the first 11 days and every other day thereafter the flies were transferred to fresh vials. In class I the male was separated at each transfer, without etherizing, from the old female and put into a vial with a fresh virgin. In class II a simple transfer was made to a fresh vial with a fresh Basc male added if the old one was dead. The used vials of class I were kept for a few days to check on whether mating had taken place (by the presence of larvae). The experiment was carried out in an air-conditioned laboratory with temperature at about 24°-25° C.

The mean lengths of life for the classes of flies were: I (mated flies), 51.0 days; II (unmated flies), 56.8 days. The percentage of fertile matings decreased gradually for the first seven weeks, after which it declined rapidly. After 58 days only one fly produced offspring. The averages indicate that under these conditions the mated males lived about 90% as long as the unmated males. However, at the end of two months, 23.6% of the mated flies were alive as against 20.2% of the unmated flies. Thus, although the mated males died off earlier than the unmated ones during the first two months, toward the end of the experiment the mortality of the unmated males may have become greater than that of the mated ones. This has been indicated more strongly in two other comparable experiments in this laboratory.

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

Di. Pasquale, A.  
Reappearance of  
pseudotumors in  
tumorless isogenic  
recombinants.

From tumorous stocks of *D. melanogaster*  
(tu A2 and tu B3, see DIS-28,-29,-30)  
isogenic recombinants for the second  
chromosome were obtained, without  
tumors. Note that the involved genes  
(tu) are located mainly on the second

chromosome. The aim of making recombinants isogenic was to be able to keep them for further studies. After an interval of about 36 generations, pseudotumors began to reappear. From each stock 5 individuals were isolated, and the descendants again made isogenic, using a Cy L/Pm stock, which suppressed crossing over very well (0.5% between arms, no crossovers for the tu region, located at the right end, out of 832 individuals; the marked chromosome was cn c px). The new isogenic lines showed pseudotumors, whose frequency increased in the following generations. The reappearance of pseudotumors, as well as their increase in isogenic lines, are phenomena difficult to interpret. Excluding incomplete suppression of crossing over (because of the stock used), two possible explanations remain: (1) a cytoplasmic agent, acting like an infecting particle; (2) high frequency of mutation of the tu genes. At present, there are no reasons to prefer one of the two possibilities.

Dobzhansky, Th., Mallah, G. S.,  
Tantawy, A. O., and Mourad, A. M.  
Collection of *Drosophila* species  
in Egypt.

A survey collection of *Drosophila* fauna  
in different localities in Egypt was  
made between July 1956 and October  
1957, mainly during the summer months.

A few species, differing in their  
relative frequencies were captured. These were *D. melanogaster*, *D. simulans*,  
*D. buzzati* (repleta group), and *D. busckii*; the last species was very rare.  
Collections were made by means of fermenting banana traps from the following  
regions: University of Alexandria Farm (UF), Alexandria; Abou-Sir (AS), 40  
km west of Alexandria; Fayuom (FY), 92 km southwest of Cairo; Beni-Swef (BS),  
112 km south of Cairo; Kom-Ombo (KO), 640 km south of Cairo; Mehalla-El-  
Koubra (MK), 128 km east of Alexandria; Wadi-El-Natroon (WD), 90 km south  
of Alexandria; Noubaria (NB), 30 km southeast of Alexandria.

The frequency distribution of adults of *D. melanogaster* and *D. simulans*  
captured is shown in the table. Other species are not listed because of  
their rarity.

| Locality | Date of<br>Collection | <i>D. melanogaster</i> |         |       | <i>D. simulans</i> |         |       |
|----------|-----------------------|------------------------|---------|-------|--------------------|---------|-------|
|          |                       | Males                  | Females | Total | Males              | Females | Total |
| UF       | August 1956           | 206                    | 120     | 326   | 26                 | 30      | 56    |
|          | October 1956          | 160                    | 109     | 269   | 36                 | 20      | 56    |
|          | December 1956         | 28                     | 22      | 50    | 26                 | 19      | 45    |
| AS       | August 1956           | 1                      | 3       | 4     | 112                | 95      | 207   |
| FY       | July 1956             | 90                     | 75      | 155   | 3                  | 8       | 11    |
| BS       | December 1956         | 1                      | 9       | 10    | 246                | 277     | 523   |
| KO       | August 1956           | --                     | 3       | 3     | --                 | --      | --    |
| MK       | July 1957             | 120                    | 126     | 246   | 18                 | 50      | 68    |
| WD       | September 1957        | 49                     | 94      | 143   | 5                  | 25      | 30    |
| NB       | August 1956           | 248                    | 500     | 748   | 1                  | 20      | 21    |

Most of the collecting was done during July, August, and September from grapes, figs, and palm dates. The results clearly indicate that D. melanogaster dominates D. simulans in some regions and D. simulans dominates D. melanogaster in other regions. For instance, the NB population consists mostly of melanogaster whereas the BS consists mostly of simulans. The UF results demonstrate that collections are more successful during the summer than in the winter.

The Department will carry out such collections for all the Drosophilidae fauna in Egypt in the very near future.

Ensign, S., and Miller,  
D. D. Sexual isolation  
in the affinis subgroup.

An effort was made to study sexual isolation between various species of the affinis subgroup by placing newly emerged flies together and allowing them

to cohabit for 10 days. At the end of this time, the females were dissected and the ventral seminal receptacles examined for sperm. The following are old (ca. 1940), unpublished data of D. D. Miller, who used recessive mutant strains of the different species D. algonquin (droop wings), D. affinis (rugose), D. athabasca (vermillion and cinnabar), D. azteca (cinnabar), and D. narragansett (bubble):

| Females | Males       |             |              |             |             |
|---------|-------------|-------------|--------------|-------------|-------------|
|         | aff.        | alg.        | ath.         | azt.        | narr.       |
| aff.    | 50/51 (98%) | 0/56        | *36/52 (69%) | 0/57        | 0/50        |
| alg.    | 0/52        | 45/51 (88%) | *3/53 (6%)   | 0/52        | 5/56 (9%)   |
| ath.    | 0/102       | 0/104       | 45/53 (85%)  | *9/53 (17%) | 0/56        |
| azt.    | 0/52        | 0/61        | *45/59 (76%) | 49/53 (93%) | 0/54        |
| narr.   | 0/55        | 0/53        | 0/57         | 0/52        | 47/51 (92%) |

Recently Ensign made the following observations with D. tolteca (Nicaragua) in conspecific and interspecific crosses involving D. affinis (Nebraska), D. algonquin (Nebraska), D. athabasca (Wyoming), and D. azteca (California):

| Females | Males       |             |             |             |              |
|---------|-------------|-------------|-------------|-------------|--------------|
|         | aff.        | alg.        | ath.        | azt.        | tolt.        |
| aff.    | 39/52 (75%) |             |             |             | 16/55 (29%)  |
| alg.    |             | 54/67 (81%) |             |             | 0/71         |
| ath.    |             |             | 49/55 (89%) |             | +4/57 (7%)   |
| azt.    |             |             |             | 48/51 (94%) | *23/60 (38%) |
| tolt.   | 12/52 (23%) | 4/55 (7%)   | 31/51 (61%) | 24/55 (44%) | 53/56 (95%)  |

\*Adult hybrids, all kinds of which have already been reported elsewhere (Sturtevant and Dobzhansky, 1936; Miller, 1950; and Patterson, 1954).

+Very few larvae obtained.

Fahmy, O. G., and Fahmy, Myrtle J. Selective cell-stage response to the mutagenicity of S-chloroethyl cysteine in D. melanogaster.

Our analysis of the mutagenic cell-stage response during spermatogenesis to the alkyl-methanesulphonates (DIS-30; Fahmy & Fahmy, 1957b) has revealed a gross difference in the brood-mutation pattern of ethyl methanesulphonate as

compared to its chloro-derivative (2-chloroethyl methanesulphonate). The ethyl ester was most active on the late germ cells (spermatozoa and spermatids) and practically ineffective on the early germ mother cells, whereas the chloroethyl ester gave maximal mutagenic effect on spermatogonia. Biochemical evidence was available that the chloroethyl ester could produce in vivo a metabolite S-chloroethyl cysteine, which is a monofunctional amino acid mustard. The mutagenic effect of this mustard was therefore investigated to determine its possible role in relation to differential cell-stage response.

S-chloroethyl cysteine was administered by injection into adult males of the same age and average weight as those used in the experiments with the sulphonates, and their progeny was fractionated according to our standard technique, that is, in 3-day broods. The yield of sex-linked mutations was determined by the Muller-5 method and that of autosomal mutations by the Cy/BLL technique. The results indicate an extraordinary differential mutagenic effect on the early germ cells, particularly the spermatogonia. An injected concentration of  $0.27 \times 10^{-2}$  molar, induces a recessive lethal rate of the order of 0.5% in the sex chromosome and 1.0% in the second chromosome of sperm and late spermatids, whereas the corresponding rates in spermatogonia rise to an average of roughly 15% and 40% respectively. With the cysteine mustard the high mutation rate in spermatogonia (predominantly recovered starting from the 5th brood, as indicated by visible clusters) cannot possibly be due to longer time of treatment, since the compound is almost completely inactivated by hydrolysis within 1 hour.

The selective action of S-chloroethyl cysteine on spermatogonia adds further support to our interpretation of the pattern of mutagenic cell-stage response under the effect of 2-chloroethyl methanesulphonate. It was suggested that the high mutation rate induced in spermatogonia by the latter compound is not a function of the sulphonate itself, but of a secondary metabolite chemically related to the cysteine mustard.

Farnsworth, M. W.  
Mitochondria in normal  
and nullo-X embryos of  
Drosophila.

Boell and Poulson (Anat. Rec. 75: 65, 1939) reported the results of studies on oxygen uptake in wild-type and nullo-X eggs of D. melanogaster. They found that YY eggs respired initially

at the same rate as controls. Within several hours after egg deposition, however, the oxygen uptake of the genetically deficient embryos fell to a level one-fifth that of wild type. The time of respiratory breakdown was found to coincide with the onset of the developmental anomalies characteristic of nullo-X individuals. In view of the association of some respiratory enzymes with mitochondria, the question arose whether or not mitochondria are present in nullo-X individuals. Since the distribution of mitochondria in early cleavage and blastoderm stages of normal embryos has not been described, the present study was undertaken to determine the localization of these components in both normal and X-deficient embryos and to ascertain, if possible, whether or not these particles are active.

The stock of D. melanogaster used in the study was In(1)dl-49, ty-1 bbl/ y v f car. The attached-X females of this stock carry a free Y chromosome. They were crossed to Canton-S wild-type males and inbred for six generations before egg collections were initiated. One-fourth of the eggs produced by these females were expected to be nullo-X (YY). This class of progeny could be readily recognized in living, dechorionated eggs. The subsequent developmental history of these individuals agreed with that described by Poulson (J. Exp. Zool. 83: 271, 1940).

For the cytological demonstration of mitochondria in both normal and X-deficient eggs, Flemming's fixative without acetic acid was employed. Control embryos of both genotypes were preserved in Flemming's with acetic acid and in Kahle's fluid. In these groups, mitochondria could not be demonstrated. Approximately 400 embryos from fertilization to blastoderm stages were used. For in vivo work, Janus green B in *Drosophila* saline was employed. In an attempt to ascertain the presence of dehydrogenases, specifically succinic dehydrogenase, as an indication of mitochondrial activity, histochemical studies utilizing neotetrazolium chloride were carried out after the methods of Shelton and Schneider (Anat. Rec. 112: 61, 1952).

In normal embryos, mitochondria were found to be localized primarily in the protoplasmic islands surrounding the nuclei. The morphology of these particles was usually filamentous. At the anterior dorsal end of the egg near the region of the micropyle, a concentration of mitochondria was found outside the protoplasmic islands. This diffuse granular mass extended posteriorly about one-fourth the length of the embryo. It was most evident in early and middle cleavage stages and could no longer be observed at the time of nuclear migration. During blastoderm formation in the normal embryo, the mitochondria of the protoplasmic islands remain in close association with their respective nuclei. These particles can be identified around the periphery of the nuclear membrane. In later blastoderm stages, cell membranes form between nuclei and each cell so formed appears to contain its own complement of mitochondria.

In the nullo-X individual, the distribution of mitochondria follows the pattern of the normal individual. Mitochondria remain in association with their respective protoplasmic islands regardless of the abnormal distribution of those islands. With the onset of cell membrane formation, mitochondria were included in the cells. A comparative count of the number of mitochondria in normal and nullo-X cells could not be carried out because of the small size of the particles and the crowded condition of the blastoderm cells of both genotypes. On the basis of general appearance, however, the number of mitochondria in cells of the X-deficient embryos did not seem to differ markedly from that of the normal.

Janus green preparations of squashed blastoderms showed stained granules present in the cytoplasm of cells of both genotypes. The number of such granules was not significantly different in the two genotypes.

Reduction of neotetrazolium chloride occurred only in those eggs whose vitelline membranes had been punctured. No color reaction was obtained with material fixed in Kahle's fluid or incubated without the tetrazolium salt. The addition of succinate as substrate had no observable effect on the speed of the reaction or the depth of color obtained. Apparently, sufficient substrate is already present in the yolk of the early embryo. Under these

circumstances, it cannot be stated which substance is being utilized or what enzyme or enzymes are responsible for the reaction. In squash preparations, formazan crystals were found primarily associated with the nucleated protoplasmic islands, the site of the mitochondria.

The results of this study demonstrate the presence of mitochondria in cells of X-deficient individuals. The drop in oxygen consumption which coincides with the onset of developmental failure cannot be explained, therefore, on the basis of the absence or degeneration of these particles.

Frydenberg, Ove.  
Equilibrium of a  
lethal mutant.

A dominant mutant at the Sb locus was isolated approximately three years ago from a natural population of D. melanogaster in Wisconsin. Though the

mutant is lethal when homozygous it has been observed to persist in stocks. Investigations have shown that the mutant is linked to an inversion in III-R, the inversion being of much the same extent as the Payne inversion with which it is possibly identical. Two sets each of 10 populations of the bottle type were set up in such a way that the generations were kept separate. In one of the sets the emerging flies were allowed to start egg-laying as they emerged. In the other set the flies were collected as they emerged and stored until all flies had emerged; the whole generation was then allowed to start egg-laying simultaneously. Selection in favor of rapid development was operating in both sets, but it was obviously stronger in the first set than in the second. In the populations of the first set the mutant reached equilibrium at gene frequencies between 20% and 30%, regardless of whether the initial frequency had been 2.5% or 50.0%. The populations in the second set established equilibria around 10%, again regardless of initial frequency. In three ordinary population cages in which the generations were not kept separate the mutant has dropped in frequency to less than 1% during nine months of observation, which probably means that it is being eliminated from the populations.

The equilibria in the bottle populations seem to be maintained by superiority of the heterozygotes, which have a slightly faster larval and pupal development than the wild type and whose males exhibit a greater readiness to mate during the first 4 to 6 days of adult life than the wild-type males do. However, in the big cages where flies of all ages are present at any one time the young heterozygous males can not compete successfully with the older wild-type males.

After several generations of distinct equilibrium the mutant was suddenly completely eliminated from three of the bottle populations. This fact remains unexplained, but it seems likely that a recombination which separated the mutant from the inversion was responsible.

Glass, B., and Ritterhoff,  
Rebecca K. Radiation-  
induced sterility after  
irradiation of third-instar  
larvae.

The results of treatment of male third-instar larvae with doses of 1000 r and 2000 r, previously reported by A. F. E. Khishin (DIS-29: 128, and Z. indukt. Abst.-Vererbl. 87: 97-112), have been fully confirmed in our own

experiments. In addition, female larvae were treated, like the males at an age of  $80 \pm 1$  hours after hatching, at 25° C. When given 2000 r, the males

were completely sterile until 4 days after eclosion, and at 7 days post-eclosion only one-third to one-half of them had recovered fertility. Fertility continued to increase to a maximum of about 70% of individuals. Among the correspondingly X-rayed females, on the other hand, fertility was significantly reduced only on the first and second days after eclosion. After a dose of 1000 r in the third-instar larval period, the females recovered fertility about as quickly and completely as after 2000 r; but the males showed a more rapid and more general recovery after the lower dose. Some males (ca. 3%) were fertile on the second or third day after eclosion; on the fourth day the percentage reached nearly one-half; by the seventh day 80% of individuals were fertile. These results indicate either that oögonia and spermatogonia, and young primary oöcytes and spermatocytes, differ considerably in sensitivity to X-rays at comparable stages, or, as Khishin has suggested, that much of the infertility induced in males irradiated as larvae is not attributable to killing of germ cells or induction of dominant lethals, but is due to induction of physiological changes that render the spermatozoa ineffective.

Glassman, E. Studies  
on maroon-like eye  
color mutant.

Previous work (Science 124: 725, 1956;  
Rec. Genetics Soc. 26: 372, 1957) has  
shown that both maroon-like (ma-1) and  
rosy (ry) eye color lack the enzyme

xanthine dehydrogenase, and as a result accumulate the substrates of this enzyme (hypoxanthine and 2-amino-4-hydroxypteridine) and do not form the products (uric acid and isoxanthopterin) formed from these compounds. Ma-1 is a sex-linked recessive recently relocated to the left of Bx (see below), and ry is on the third chromosome at 51 $\frac{1}{2}$ .

Hadorn and Schlink (Nature 177: 940, 1956) have shown that ry is non-autonomous when appropriate transplantations of tissues and anlage are carried out. Since ma-1 resembles ry, autonomy of ma-1 was tested by crossing aged y ec ma-1; st females to X<sup>co2</sup>, cv v f; st males, according to the method of Hamah. One gynandromorph was produced which exhibited y, ec, and a smaller male-like wing and sex combs on the left side; the right side was wild-type (or female) with respect to these characters (external genitalia were male-like). However, the color of the eyes on both sides was scarlet (ma-1; st is yellow-orange), indicating that ma-1, like ry, is nonautonomous.

In most crosses ma-1 acts as a typical sex-linked recessive. However, ma-1/ma-1<sup>+</sup> females do not produce phenotypically ma-1 progeny until the bottle is 16 to 20 days old. For example, if m ma-1/FM6; st females are crossed to m ma-1; st males, the non-FM6 males and females are phenotypically st, although progeny tests indicate that they are genetically ma-1 (the difference between ma-1 and ma-1<sup>+</sup> is more pronounced if st/st is present). A similar result is obtained if y f:=; st females are crossed to m ma-1; st males. This maternal effect from females containing ma-1<sup>+</sup> continues through the first 6 to 10 days of hatching; after this time the adults that hatch are typical orange-eye ma-1; st progeny. This reversal indicates that the maternal effect is also affected by a food factor, which either is taken up by older larvae, or is an inhibitor accumulating as a result of larval action. That the female parents themselves do not exhaust some chemical in their bodies is evidenced by the fact that seven successive transfers of y f:=; st females (crossed to m ma-1; st males) to new food bottles after 5 to 7 days



produced maternally affected male offspring (i.e., st eye) in the first 5 to 6 days of hatching, and typical orange-eye  $ma-1$ ; st males thereafter in each bottle. Cross-feeding from  $ma-1^+$  to  $ma-1$  larvae has been eliminated by raising  $ma-1^+$  and  $ma-1$  flies in the same bottle; no effect of  $ma-1^+$  on  $ma-1$  was observed. It is interesting that  $ry^+$  does not have a maternal effect in the  $ry/ry^+$  heterozygote. Studies on the biochemical changes associated with the maternal effect and the factor in the food may increase our understanding of the biogenesis of the red eye pigment.

Graf, G. E., and  
Hadorn, E. Chromato-  
graphic studies on  
*Drosophila* testes.

Paper chromatographic studies have been carried out on the testes of several species of *Drosophila*. Preliminary results indicate that the red pigments in the testes of *D. subobscura* and *D.*

*pseudoobscura* are identical with the red pigments (drosopterines) found in the eyes of *D. melanogaster*. The pattern of other fluorescent substances is qualitatively similar but there are marked quantitative differences. The testes of both *D. pseudoobscura* and *D. melanogaster* are very rich in isoxanthopteryne, whereas *D. subobscura* has relatively little of this compound. The testes of *D. hydei* resemble those of *D. melanogaster*, both qualitatively and in the relative amounts of the various fluorescent compounds.

Hexter, W. M. A mosaic  
resulting from double  
fertilization of a  
triploid female.

Single triploid females which contained attached-X chromosomes homozygous for  $g^{53d}$  sd and a free X which had the genes  $y^{3ld}$   $sc^8$   $wa$   $lz^s$  B (hereafter referred to as FMI) were fertilized

by  $wy^2$   $g^3$  males. From one such mating a female resulted whose left eye was  $g^{53d}$  (orange) and the left wing scalloped. The right eye was typical of a Bar heterozygote. This female, which was virgin, was mated by  $sn^3$  males to test the possibility that her ovaries were genetically mosaic. She yielded the following progeny: females, 346 heterozygous Bar and 417 wild type; males, 421  $wy^2$   $g^3$ , 298 FMI, and 2  $sn^3$ . The  $sn^3$  males proved to be sterile and presumably were nondisjunctional males. The heterozygous Bar females when mated yielded FMI males, and the wild-type females when mated yielded  $wy^2$   $g^3$  males. These results indicate that this mosaic female had nonmosaic ovaries which were genetically FMI/ $wy^2$   $g^3$ .

This mosaic female can best be explained by assuming that a double fertilization occurred. One female nucleus contained the FMI chromosome and was fertilized by the  $wy^2$   $g^3$  X chromosome of the male. This fertilization accounts for the right eye of the mosaic female and for her ovaries. The left eye and left wing most certainly resulted from a female nucleus containing the  $g^{53d}$  sd attached-X chromosomes fertilized by the Y chromosome. Another explanation, considered most unlikely, is that the egg nucleus contained both the attached-X and the FMI chromosome (owing to nondisjunction) and was fertilized by the  $wy^2$   $g^3$  chromosome. Then one would have to make the improbable assumption that as a result of the first cleavage one daughter cell lost the attached-X chromosomes and the other daughter cell lost both the FMI and  $wy^2$   $g^3$  chromosomes.



Hexter, W. M.

Gynandromorphs probably resulting from double fertilization of attached-X females.

Single attached-X females, one X which was  $g^{53d} sd$ , the other  $wy^2 g^3$ , were mated by males which were  $y^{3ld} sc^8 wa^1 lz^s B$  (hereafter referred to as FMI). From this mating two independent gynanders resulted. One was an almost perfect bilaterally symmetrical fly

whose left side was yellow; the left eye was  $B lz^s wa^1$ ; and the front left leg had a sex-comb but no tarsal claw; the genitalia of the left side were male-like. The right side was non-yellow; no sex-comb was present on the front right leg although a tarsal claw was present; the eye was  $g^{53d}$  and the wing was scalloped; the genitalia of the right side were female-like. This gynander probably resulted from a double fertilization. One egg nucleus contained the attached-X chromosomes homozygous for  $g^{53d} sd$  (resulting from a crossover of the attached-X) and was fertilized by a Y-bearing sperm, and the other egg nucleus contained the maternal Y chromosome and was fertilized by the FMI sperm. Another explanation, considered improbable, would be the assumption that the gynander originated as a triplo-X and that as a result of the first cleavage one daughter cell lost the attached-X chromosomes and the other daughter cell simultaneously lost the FMI chromosome.

From the same original cross another gynander was found whose genitalia were male although rotated 180 degrees. The left eye was garnet and the left front leg had no sex-comb but had a tarsal claw. The right front leg had a sex-comb and no tarsal claw. The right eye was  $B lz^s wa^1$  with two small patches of apparently garnet facets. This gynander presumably arose as a double fertilization in the manner already described. Simultaneous elimination at an early cleavage of two different chromosomes, although unlikely, is not excluded.

Hiraizumi, Y., and

Crow, J. F. The

amount of dominance of "recessive" lethals from natural populations of D. melanogaster.

Newly arisen lethal mutants have been shown by Stern et al. (Genetics 37: 413) and by Muller and Campbell (unpublished) to cause about 4%-5% reduction in viability of heterozygotes. However, those mutants with the greatest heterozygous effect would be most rapidly eliminated from a population, so

that the average effect of those lethals found in a natural population would be less. It can be shown (Muller, Morton, and Crow, PNAS 42: 855) that the mutants remaining in a population would have an average dominance equal to the harmonic mean of the original values, and in Muller and Campbell's data this was estimated to be roughly 2%.

Tests were made of 53 second-chromosome lethals, 64 semilethals, and 60 control chromosomes isolated from natural populations near Madison, for heterozygous effects on viability in crosses of  $cn bw$  females x  $cn/+$  males. Comparison of the ratio of wild-type and  $cn$  offspring for the three classes gave a measure of the heterozygous effects. There was no significant difference between lethals and semilethals, the average selective disadvantage as compared with lethal-free chromosomes being .030. Since some chromosomes may carry more than one deleterious gene, a correction was made assuming a Poisson distribution; this led to an estimate of .026 for the selective disadvantage in the heterozygote of a recessive lethal or semilethal.

Horikawa, M. Growth, differentiation, and tryptophan metabolism in eye discs of D. melanogaster in tissue culture.

Eye-antennal discs and cephalic complexes obtained from mature third-instar larvae (95 hours after hatching at 25° C) were cultured in vitro in a synthetic medium (see DIS-30: 161), to investigate the effect of the metamorphic hormone upon growth, differentiation, and

tryptophan metabolism in the eye discs.

When the eye discs of Oregon and bw were cultured in the synthetic medium containing 5 mg/ml L-tryptophan, together with the cephalic complex of the same body, brown pigment was deposited in the eye discs after culturing for about 72 hours. On the other hand, when the eye discs were cultured in the same medium together with the cephalic complexes of ten bodies, brown pigment was deposited in the eye discs after culturing for only about 24 hours. Furthermore, when eye discs were cultured together with ten cephalic complexes in medium not containing tryptophan for about 48 hours, and then transferred to medium containing tryptophan, the eye discs showed more pronounced growth, differentiation, and pigmentation than in any of the other cases mentioned above, after culturing for only about 16 hours.

In hanging-drop cultures, ten cephalic complexes gave the optimum concentration of the metamorphic hormone in the culture medium; more or less than ten cephalic complexes proved to have a less favorable effect on growth, differentiation, and pigmentation of the eye discs.

Various combinations of the eye discs of one strain and the cephalic complexes of another strain, or the metamorphic hormone extracted from pupae of the silk worm according to the method of Butenandt, were cultured in the same medium. According to the results, the cephalic complexes of some strains seem to be classifiable into three groups on the basis of qualitative and quantitative difference of the metamorphic hormone. The first group possesses normal hormone activity affecting growth, differentiation and tryptophan metabolism in the eye discs. Oregon, bw, v, and cn belong to this group. The second group possesses normal hormone activity as regards growth and differentiation, but not tryptophan metabolism. The mutants w, v, bw, and cn, bw belong to this group. The third group possesses normal hormone activity on tryptophan metabolism, but not on growth and differentiation. B, bar-3, and Dp/In (3L)P, In (3R)C, Sb e 1(3)e belong in this group. The last group is divided into two subgroups. The eye discs of B and bar-3 showed more pronounced growth and differentiation with the addition of ten cephalic complexes of Oregon and the metamorphic hormone extracted from the silk worm. The eye discs of Dp/In(3L)P, In(3R)C, Sb e 1(3)e showed no better growth and differentiation when these substances were added.

Jacobs, M. E. Influence of desiccation on dopa oxidizing activity and amino acid levels in D. melanogaster.

When late larvae, just before pupation, were exposed to low humidities for four hours, dopa oxidizing activity was accelerated, and the levels of glutamic and aspartic

acids were increased and that of alanine decreased as compared with siblings kept in moist containers.

Khishin, Aziz F. Studies on heat tolerance in Egyptian populations of D. melanogaster and other Drosophila species.

Wild stocks of D. melanogaster as well as other species found in Egypt are constantly being collected. The present study is being carried out with populations collected mainly during the summer months. Summer temperatures in

Egypt vary according to localities, and range from about 30° C. in Alexandria to about 40° C. or more in Upper Egypt (daytime temperatures). Some collections have been made from localities which are more or less isolated; therefore the degree of inbreeding is expected to be higher in some strains than in others. The object of the study is to find out how high temperatures affect the flies, and whether or not there are differences in heat tolerance among strains from different localities. Experiments are being carried out in controlled incubators. Flies from an Oregon-K stock are used as controls.

Preliminary results show that at 40° C. flies are not killed after exposure for 85 minutes. At this temperature flies become almost inactive and look as if trying to recover from etherization. When taken out into room temperature (25° C.) they eventually recover completely. Males were examined for sterility, and most of them were fertile. In another experiment flies stood the treatment for 130 minutes. Flies can be kept at 35° C. for about 3 days and sometimes more. They deposit no eggs, however, and usually die after that period. Larvae of all instars can stand 35° C. for 24 hours. Late-third-instar larvae usually pupate, but no adults emerge. Eggs, if exposed for more than 9 hours, do not hatch. Flies seem to tolerate well a temperature of 33° C. They are active and more or less normal. They deposit eggs; eggs hatch in almost the normal percentage; and larvae grow and pupate; however, no adults have been obtained. It is observed that adults attempt to emerge but only succeed in dragging the head and thorax out of the pupal case. They are found dead with their posterior half inside the puparium. The experiments are still under way.

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

Among various strains of D. melanogaster, Hikone-R and WMB (selected by Hiroyoshi for DDT resistance) showed the highest resistance to parathion. The LD50 for larvae of these strains was about 2 ppm,

whereas that for other nonresistant strains was about 0.08 ppm. Genetical analyses showed that the major gene for parathion resistance was at a locus near 64.5 (to the left of sca and vg) on the second chromosome.

Various mutant strains, which were originally nonresistant but obtained the chromosome segment responsible for parathion resistance in Hikone-R by means of double crossing over, showed high resistance. Cross resistance to various insecticides such as DDT and BHC was also found in these resistant strains. Presumably, the major gene located on the second chromosome may have a common mechanism for resistance to various insecticides, that is, it may be included in the category "vigor tolerance" of Hoskins (1956).

Kim, K. W., and Paik, Y. K. Keys to species of Family Drosophilidae occurring in South Korea.

The following six keys will undoubtedly be modified as the collections progress.

(1) Key to the species of Genus Amiota

1. Mesonotum uniformly dark brown, without markings; legs not banded; 5X index about 1.5; middle orbital bristle about 2/3 size of first.....alboguttata Wahlberg.  
Mesonotum with dark markings; legs distinctly banded; 5X index about 0.8; middle orbital bristles about 1/3 size of first; arista with three or four branches above near base, none below.....variegata Fall.

(2) Key to the species of Genus Leucophenga

1. Arista with dorsal rays only.....argentosa Okada.  
Arista with both dorsal and ventral rays.....2
2. Wings clear.....3  
Wings with cloudy markings.....5
3. Palpi very large and black (but in male, palpi small).....magnipalpis Duda.  
Palpi moderate in size.....4
4. Third to fifth abdominal tergites with caudal bands.....concilia Okada.  
Second to sixth abdominal tergites with black spots, mesonotum silvery pollinose in male.....maculata Dufour.
5. Postvertical bristles much smaller than orbitals...ornatipennis de Meijere.  
Postvertical bristles well developed, nearly as large as middle orbitals.....quinquemaculipennis Okada.

(3) Key to the species of Genus Mycodrosophila

1. Wings clear; mesonotum uniformly black; thoracic pleura with black patches; second to sixth tergite with black caudal bands, second to fourth tergite bands broadly interrupted at middle; male fore tibia and tarsi with long recurved upright hairs along anterior margin.....poecilogaster Loew.  
Wings clear; mesonotum uniformly black; thoracic pleura without black patches; second to fifth tergite with black caudal bands, second to third tergite bands broadly interrupted at middle.....Mycodrosophila sp.

(4) Key to the species of Genus Microdrosophila

1. Orb<sub>1</sub> about 1/5 as far from orb<sub>2</sub> as from verticals; mesopleura with a distinct black longitudinal stripe; third costal section with heavy bristles on basal 4/5.....Microdrosophila sp 1.  
Orb<sub>1</sub> about 1/2 as far from Orb<sub>2</sub> as from verticals; third costal section with heavy bristles on the entire length.....2
2. Abdomen black; mesopleura without black longitudinal stripe.....congestus Zetterstedt.  
Abdomen black; mesopleura with black longitudinal stripe.....Microdrosophila sp 2.

(5) Key to the species of Genus Scaptomyza

1. Body yellowish gray; acrostichal hairs in 2 rows; one prominent humeral.....graminum Fall.  
Body grayish brown or black; acrostichal hairs in 4 rows; two humerals.....2

2. Body grayish brown; costal index about 3.5; third costal section with heavy bristles on basal  $2/5$ .....disticha Duda.  
 Body grayish black; costal index about 3.0; third costal section with heavy bristles on basal  $1/3$ .....polygonia Okada.

(6) Key to the species of Genus Drosophila

1. Preapical bristles are small or absent on the first and second tibiae...2  
 .....2  
 Preapical bristles evident on all three tibiae.....8
2. Mesonotum with distinct longitudinal stripes.....3  
 Mesonotum without stripes.....6
3. Acrostichal hairs in 8 rows; mesonotum with five narrow longitudinal stripes, the median longitudinal stripe is bifid posteriorly.....busckii Coquillett.  
 .....4  
 Acrostichal hairs in 6 rows.....4
4. Crossveins clouded; mesonotum with 4 longitudinal stripes; oral margin and cheeks snowy white.....alboralis Momma & Takada.  
 Crossveins not clouded.....5
5. Mesonotum with 3 pairs of narrow longitudinal stripes..sexvittata Okada.  
 Mesonotum with 4 black longitudinal stripes, inner pair broader caudally and outer pair interrupted at suture.....quadrivittata Okada.
6. Arista with one branch below in addition to terminal fork; abdominal dark bands not interrupted at middle.....nokogiri Okada.  
 Arista with a few branches below; abdominal dark bands interrupted at middle.....7
7. Orb<sub>2</sub> minute, about  $1/5$  size of first.....D. sp. close to histrion.  
 Orb<sub>2</sub> about  $1/3$  size of first.....D. sp. of Hirtodrosophila.
8. Prescutellar bristles present.....9  
 Prescutellar bristles absent.....11
9. Body yellowish; wings somewhat brownish along costa...puncticeps Okada.  
 Body blackish; wings clear.....10
10. Acrostichal hairs in 8 rows; third costal section with heavy bristles on basal  $4/5$ .....coracina Kikkwa & Peng.  
 Acrostichal hairs in 6 rows; third costal section with heavy bristles on basal  $2/3$ .....rufifrons Loew.
11. Abdominal dark bands never broken in mid-dorsal line.....12  
 Abdominal dark bands usually narrowed or broken in mid-dorsal line...20
12. Body blackish.....13  
 Body yellowish.....15
13. Acrostichal hairs in 6 rows.....helvetica Burla.  
 Acrostichal hairs in 8 rows.....14
14. Orb<sub>2</sub> about  $1/3$  size of first.....bifasciata Pomini.  
 Orb<sub>2</sub> about  $1/2$  size of first; abdominal tergites entirely black.....  
 .....D. sp. close to bifasciata.
15. Acrostichal hairs in 6 rows.....16  
 Acrostichal hairs in 8 rows.....17
16. Orb<sub>2</sub> minute, about  $1/5$  size of other two; third costal section with heavy bristles on basal  $2/5$ .....magnipunctinata Okada.  
 Orb<sub>2</sub> about  $1/3$  size of first; third costal section with heavy bristles on  $1/2$ .....auraria Peng.

17. Pulpus with a few prominent setae.....melanogaster Meigen.  
Pulpus with only one prominent seta.....18
18. Costal index about 4.0; male wings apically with black spots.....  
.....suzukii Matsumura.  
Costal index about 2.0; male wings apically without black spots.....19
19. An indistinct very small blackish spot present in the groove located on  
the posterior base of fore coxa; posterior paramere with basal  
branch very long.....lutea Kikkwa & Peng.  
No such blackish spot present; posterior paramere with basal branch  
very short; body is of small size.....takahashii Sturtevant.
20. A pair of presutural bristles present.....testacea van Roser.  
No presutural bristles.....21
21. Yellowish or yellowish brown species.....22  
Blackish or dark brown species.....31
22. Abdominal tergites with varieties of black spots.....23  
Abdominal tergites without spots, usually with bands.....24
23. Crossveins and wing tip clouded.....nigromaculata Kikkwa  
& Peng.  
Wing tip not cloudy, posterior crossveins slightly clouded.....  
.....transversa Fall.
24. A row of short stout bristles on lower apical part of each fore femur.  
.....25  
Fore femur without such a row of bristles.....26
25. Posterior crossveins and tip of longitudinal veins cloudy; one prominent  
bristle at apex of first costal section.....immigrans Sturtevant.  
Tip of longitudinal veins not cloudy; two prominent bristles at apex  
of first costal section.....D. sp. of immigrans  
group.
26. Second oral over  $3/4$  size of vibrissa.....27  
Second oral  $1/3$  or less size of vibrissa.....30
27. Crossveins deeply clouded; third costal section with heavy bristles on  
basal  $1/3$ .....D. sp. close to kuntzei.  
Crossveins clear or slightly clouded.....28
28. Acrostichal hairs in 8 rows; abdominal bands become narrow and indistinct  
as they approach the lateral margin.....histrio Meigen.  
Acrostichal hairs in 6 rows.....29
29. Third costal section with heavy bristles on basal  $1/4$ ; abdominal bands  
of two basal segments are darker and broader than those of other  
segments.....bizonata Kikkwa & Peng.  
Third costal section with heavy bristles on basal  $2/5$ ; abdominal black  
bands broaden laterally.....D. sp. of quinaria  
section.
30. Third costal section with heavy bristles on basal  $1/3$ ; acrostichal hairs  
in 6 rows; arista with about 7 very short branches including minute  
fork.....makinoi Okada.  
Third costal section with heavy bristles on basal  $1/2$ ; acrostichal hairs  
in 8 rows.....D. sp.
31. Mesonotum with black spots; carian sulcate; costal index about 3.0.....  
.....repleta Wollaston.  
Mesonotum almost dark brown.....32
32. Abdominal dark band broadly interrupted at middle..D. sp. of Drosophila.  
Abdominal tergites uniformly dark brown, band interruption is obscure.  
.....33
33. Costal index about 2.5; palpus with only one prominent apical bristle.  
.....virilis Sturtevant.

- Costal index about 3.0 or more.....34
34. Posterior crossveins slightly clouded; genital arch black, anterior and posterior margin of lower portion symmetrically convex, clasper with invariably 9 teeth arranged in a straight row..cheda Tan, Hsu, and Sheng.
- Crossveins clear.....35
35. 5X index about 1.5; genital arch black, anterior margin of lower portion slightly concave, clasper with about 12 black teeth arranged in a shallowly concave row.....lacertosa Okada.
- 5X index about 1.0; genital arch brownish black, anterior and posterior margin of lower portion symmetrically convex, clasper with about 10 black teeth arranged in deeply concave row.....sordidula Kikkawa & Peng.

King, R. C. Experiments  
on the alkaline earth  
requirements of *Drosophila*.

Even the most highly purified agars contain large amounts of Ca and Mg. The cellulosic polymer "Methocel" (U. S. P. grade), produced by Dow Chemical, is

free of these contaminants. Ten grams of 4000 centipoise viscosity type Methocel are added to 100 ml of boiling-hot distilled water. The resulting suspension is cooled rapidly to 2° C and poured into sterile containers, where it will form a thixotropic gel. *Drosophila* larvae can now be fed on nutrients pipetted onto this gel.

The yeast *Candida monosa* (strain Y-96 Northern Regional Research Laboratory, Peoria, Ill.) grows well in an aerated fluid medium of the following composition (weight of components in g): dextrose, 5; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1; KH<sub>2</sub>PO<sub>4</sub>, 1; KCl, 0.4; MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 0.06; CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.06; Inositol, 0.025; FeCl<sub>3</sub>, 0.0025; MnSO<sub>4</sub> · 1H<sub>2</sub>O, 0.0025; pyridoxin HCl, 0.0005; thiamine HCl, 0.0005; biotin, 0.000025; distilled water, 100. It should be pointed out that reagent grade MgSO<sub>4</sub> contains 1 Ca atom per 800 Mg atoms, and CaCl<sub>2</sub> contains 1 Mg atom per 1000 Ca atoms. *Candida monosa* will grow if Mg or Ca is omitted from the medium. *Drosophila* will complete its life cycle when fed on such Ca-deficient yeast, but not when fed Mg-deficient yeast. *Drosophila* will complete its life cycle when fed Mg-deficient yeast on an agar gel, since it can extract the alkaline earth metals bound in the agar.

King, R. C. Oogenesis  
in female-sterile mutants.

A study was made of Feulgen-stained whole mounts of ovaries of females homozygous or hemizygous for any one

of 23 female-sterile mutations. The mutant ovaries may be grouped into various categories on the basis of their cytological appearance. A. "Normal": (1) oc, (2) lz<sup>s</sup>, (3) na, (4) ap<sup>56f</sup> (not sterile), (5) bf, (6) sv<sup>de</sup>. B. Ovaries tumorous: (7) fes, (8) mw<sup>2</sup>, (9) fu, (10) fuff, (11) fu<sup>57a</sup>, (12) fu<sup>57f</sup>. C. Synthesis of Feulgen-positive material in nurse-cell nuclei retarded: (13) sn<sup>36a</sup>, (14) ras<sup>4</sup>, (15) rn, (16) fs 2.1. D. Yolk synthesis retarded or abolished: (17) dm, (18) ty, (19) ap<sup>4</sup>, (20) mi. E. Mature eggs stored, not laid: (21) sta. F. Certain mutants are characterized by exceptionally high frequencies of fusions between adjacent egg chambers in an ovariole [(22) og] or between germaria and adjacent chambers [(23) cmp]. Fusions of adjacent chambers occur less frequently in ras<sup>4</sup>, mi, and the fu alleles; rarely in dm, fs 2.1, rn, and oc.



King, R. C., and  
Burnett, R. G. Effect  
of etherization upon  
oögenesis.

Under our conditions a 15- to 20-  
second etherization is sufficient to  
anesthetize *Drosophila* so that they  
remain unconscious for several minutes.  
We were interested to see whether pro-

longed etherization of young females would disturb oögenesis. We used Oregon-R females which were less than 1 hour old, and etherized them for 45-60 seconds. More prolonged exposures were lethal. Approximately half the flies had unexpanded wings. Records were kept of daily egg production for days 5 through 10. The flies laid about 30 eggs daily. No significant difference was noted between the 45- and 60-second series or between flies exposed while their wings were expanded or unexpanded. However, etherization caused about half the flies with unexpanded wings to fail to expand their wings within 10 days. Feulgen-stained whole mounts of ovaries of flies killed 5 or 10 days after ether treatment were examined. The ovaries appeared normal. Pycnotic egg chambers were observed very rarely (1/90). It thus appears that etherizations normally used in *Drosophila* work produce no permanent damage to the female reproductive system.

Kuroda, Y.  
Comparisons of tyrosinase  
activity in tumorous and  
nontumorous strains of  
*D. melanogaster*.

Tyrosinase activity in the body fluid  
of third-instar larvae (95 hours after  
hatching at 25° C) of some tumorous  
strains of *D. melanogaster* was  
measured by the manometric technique,  
and compared with that in nontumorous

strains. Sixty larvae were dissected in 3 ml distilled water, setting the body fluid free in the medium. This diluted body fluid was chilled for 20 hours at 4° C., incubated for one hour at 25° C, and centrifuged. The supernatant was used as the enzyme solution. Tyrosinase activity was measured by following in a Warburg respirometer its oxygen uptake of enzyme preparation in M/10 phosphate buffer (pH 6.8) at 25° C, using M/100 dopa as the substrate.

The results of comparisons between the tumorous strains st tu, v tu, and tu-h, and the corresponding nontumorous strains st, v, and Oregon (wild) are shown below.

O<sub>2</sub> uptake (µl/hr/larva)

| st tu | st   | v tu | v   | tu-h | Oregon |
|-------|------|------|-----|------|--------|
| 18.3  | 16.0 | 9.3  | 8.5 | 14.9 | 10.6   |

Tyrosinase activity was higher in the body fluid of the tumorous strains than in that of the nontumorous strains.

Lewis, Bonny Morgan.  
Formaldehyde-induced  
crossing over in *D.*  
*melanogaster* males.

Larvae in the first or second instar  
were transferred onto either 0.20% or  
0.25% formalin food in a total of five  
different experiments. Controls were  
also transferred, but onto fresh medium

without formalin. The larvae completed development in the same culture bottles



without further transfer. When adult males of the constitution  $My\ Gl + +/+ + Sb\ bx^D$  emerged, they were mated individually to untreated virgin females of the constitution  $My + Sb +/+ Gl + bx^D$ , and crossovers in the middle region in sperms were studied and compared with spontaneous crossovers in eggs. This followed the crossover-selector system of Whittinghill (Science 11: 377, 1950). At the end of 3 days, the males were transferred and remated to fresh virgin females for a period of 6 days.

Fertile test crosses were completed on 150 treated males and 111 control males. Male crossing over was found only in the middle or  $Gl-Sb$  region. Since any one of the four markers is lethal in the homozygous state, viable offspring would occur by one of two means: a wild crossover sperm in combination with any egg, and noncrossover sperm in combination with certain crossover eggs. The origin of these viable offspring could be diagnosed correctly both by phenotype and by subsequent breeding tests.

Of the 150 treated males, 15 produced a total of 52 crossover offspring, representing an estimated frequency of .00306 crossover sperm. From control males, one crossover offspring was produced, representing an estimated frequency of .00003 crossover sperm. Comparison of the control and treated series shows that both the percentage of males with any crossover offspring at all and the estimated frequency of crossover sperm were significantly increased after the formalin-food treatment. From the brood data, a wide range of germ-cell sensitivity was suggested. The recovery of clusters of recombination offspring indicated that crossing over probably occurred in spermatogonial cells. However, the single recombinations might have had either a spermatogonial or a spermatocytic origin.

Lewis, H. W., and Lewis, H. S. Thermostability of tyrosinase from Canton and sable adults.

Young adult flies of the Canton-S strain grown at 25° and 15° C and sable flies grown at 25° C all have the wild-type phenotype, whereas sable flies grown at 15° C express the sable phenotype. Tyrosinase activity in

these four classes of flies differs markedly. Assigning a value of 100 to the tyrosinase activity of sable flies grown at 15° C, the value for sable flies grown at 25° C is 77, the value for Canton flies grown at the lower temperature is 71, and that for Canton flies grown at the higher temperature is 17. Since the effect of temperature during development is greater on the wild-type tyrosinase than on the enzyme system under the control of the sable locus, it seems possible that the enzyme from wild-type flies is more thermolabile than the enzyme from sable flies. It also seems possible that flies grown at the lower temperature produce a more thermostable enzyme, independently of the genotype. To test these ideas, heat inactivation experiments were performed on tyrosinase extracted from young adults of the four classes of flies. These tests showed that the heat inactivation pattern is the same in all classes. That is, with regard to the property of thermostability, the enzymes of all four classes of flies are identical.

Lindsley, D. L., and Edington, C. W. A screening method for recovering sex-linked recessive lethals whose expression is influenced by the Y chromosome.

For every four sex-linked recessive lethals recovered by the Muller-5 technique there is one that is missed because its expression is suppressed by the Y chromosome and the X-irradiated/Y males survive. These XO lethal, XY viable changes are of considerable interest and the following series of crosses is designed

to facilitate their recovery. Three essentially new chromosomes are used in the crosses, and they will be described first.

1) Ins (1)sc<sup>8</sup>L, dl-49, sc<sup>8</sup>LR, y<sup>3</sup>ld B f v: The essential features of this chromosome are that it is multiply inverted, marked with B, and deficient for sc. It is similar to the Binsy stock in the Indiana list except that it is sc<sup>-</sup>; it might well be symbolized Biny.

2) y 1<sup>259</sup> w m f: The essential features of this chromosome are that it has a normal sequence, is recessively marked, and lethal in combination with a normal Y but viable in combination with sc<sup>8</sup>.Y since 1<sup>259</sup> is in the region of yellow.

3) Ins(1)sc<sup>4</sup>L, S sc<sup>8</sup>R, y sc<sup>4</sup> B wa sc<sup>8</sup>: The essential features of this chromosome are that it is multiply inverted, marked with y, B, and wa, and deficient for the bulk of the proximal heterochromatin. As a consequence of the heterochromatic deficiency, X-Y pairing is reduced and primary nondisjunction frequent; X-, Y-, O-, and XY- bearing sperms are formed roughly in the ratio 45:35:15:5. This chromosome is essentially y sc<sup>4</sup> M-5, which we symbolize scumy-5 = S-5. A similar chromosome has been used by Oster (DIS-30).

y/Y males are irradiated and crossed to Biny/y 1<sup>259</sup> w m f virgins (from Biny/y 1<sup>259</sup> w m f x y 1<sup>259</sup> w m f/sc<sup>8</sup>.Y stock). The F<sub>1</sub> consists of: y/Biny (phenotypically y B) female; y/y 1<sup>259</sup> w m f (phenotypically y) female; Biny/Y, which is phenotypically sc<sup>-</sup> and dies; and y 1<sup>259</sup> w m f/Y, which dies. Since the F<sub>1</sub> males die, the females will be virgin if the parents have been dumped. y/Biny females are selected and crossed individually to S-5/sc<sup>8</sup>.Y males. The expected progeny from this cross are Biny/S-5 (phenotypically y sc B/B) female; Biny/S-5/sc<sup>8</sup>.Y (phenotypically sc B/B) female; Biny/sc<sup>8</sup>.Y, which is phenotypically sc<sup>-</sup> and dies; and Biny/O, which is phenotypically sc<sup>-</sup> and dies; also y/S-5 (phenotypically y B/+) female, y/S-5/sc<sup>8</sup>.Y (phenotypically B/+) female; y/sc<sup>8</sup>.Y (phenotypically +) male; and y/O (phenotypically y) male. The cultures are examined for the presence of + (XY) and y (XO) males. The presence of both classes indicates a nonlethal-bearing X chromosome; the absence of both classes indicates an orthodox sex-linked recessive lethal. Two additional classes of lethal cultures are possible, those in which the XO but not the XY males appear and those in which the XY but not the XO males appear. y/S-5 (or y/S-5/sc<sup>8</sup>.Y) females from each lethal culture can be re-crossed to S-5/sc<sup>8</sup>.Y males to check the original classification. These females will be virgin in every case except the XY viable XO lethal cases where, if the XY males are fertile, the inviability of XO males can be confirmed by crossing + males to XX/O females. Finally, lethals in the bb region can be detected immediately by the absence of y B females (y/S-5) from the culture, since S-5 is deficient for most of the proximal heterochromatin, including the locus of bb.

Lindsley, D. L., and  
Edington, C. W. Failure  
to close the YSX.YL  
chromosome.

For several years we have been interested in obtaining a ring-shaped XY chromosome. To this end we have constructed a chromosome of the constitution y<sup>+</sup>YSX.YL<sup>+</sup>; this was done by

detaching a reversed acrocentric compound-X chromosome, which had YL<sup>+</sup> (derived from sc<sup>8</sup>.Y) as a second arm, with sc<sup>8</sup>.Y:bw<sup>+</sup> (=y<sup>+</sup>YS.bw<sup>+</sup>YL, see Baker DIS-29). Males carrying the y<sup>+</sup>YSX.YL<sup>+</sup> chromosome and no free Y were irradiated and crossed to y<sup>2</sup> su-wa wa bb/O females. Simultaneous loss of y<sup>+</sup> from each end

of the  $y^{+Y^S X.Y^L y^+}$  chromosome, which results in recovery of a  $y$  male, was considered strong presumptive evidence of ring formation. Retention of both  $Y^L$  and  $Y^S$  fertility factors is required for fertility of the  $y$  males; on the basis of Baker's observations on fractionation of  $sc^8.Y:bw^+$  we expected roughly 1% of these males to be fertile. To date we have recovered 155  $y$  males, all of which have been sterile.

Makino, S., Takada, H.,  
and Lee, J. J.  
Drosophilidae from Kongju  
in South Korea.

Several drosophilids were reported in Korea by Kikkawa and Peng, 1938; Nakayama and Okamoto, 1940; and Chung et al., 1955, 1956. A collection was attempted in Kongju and its vicinity

during the period from September, 1956 to August, 1957. Twenty-four species were collected, by means of small jars baited with fermenting fruits, and with a sweep net. It was found that twelve of them were new to the fauna of Korea. They are as follows: Amiota variegata, Leucophenga quinquemaculata, Mycodrosophila basalis, Drosophila angularis, D. auraria, D. bifasciata, D. brachynephros, D. lacertosa, D. melanissima, D. nipponica, D. testacea, and D. unispina.

Mead, C. G. The effect  
of Bar, Enhancer of Bar,  
and changes in their  
positions on the free  
amino acids and peptides  
of D. melanogaster.

Stocks were constructed of car, B car, BB car (by unequal crossing over), En-B car, and B En-B car, co-isogenic with one another for their autosomes and their X chromosomes except for the B--En-B region. (Original material kindly provided by G. Bonnier.) The

free amino acids and peptides of each genotype were analyzed by two-dimensional chromatography of twenty whole, squashed flies, five days old; and the resulting ninhydrin-positive spots were measured densitometrically. Ten chromatograms were made of each genotype, and the concentration of each spot was expressed as the mean proportion of the total free ninhydrin-positive material measured. The amino acids identified by co-chromatography were aspartic acid, glutamic acid, cystine, serine, glycine, threonine, taurine, lysine, glutamine, alpha-alanine, beta-alanine, tyrosine, proline, histidine, arginine, and methionine and/or valine and/or tryptophan, of which aspartic acid, glutamic acid, cystine, serine, glycine and taurine (measured together), threonine, alpha-alanine, beta-alanine, glutamine, and histidine and arginine (measured together) were measured quantitatively. A peptide, "pupine" (probably corresponding to Chen and Hadorn's  $P_2$ ), and a front peptide were also identified, of which pupine was measured quantitatively.

The Bar position effect was demonstrated to be exhibited in terms of free amino acids by comparing the results of the analyses of B car/B car and BB car/car, which differ significantly in their proportions of pupine, aspartic acid, glutamic acid, cystine, serine, glycine and/or taurine, beta-alanine, and histidine and/or arginine. The Enhancer of Bar position effect was also demonstrated, but proved to be less extreme than the Bar position effect. This was shown by comparing the results of the analyses of En-B car/B car and B En-B car/car, which differ significantly with respect to serine, alpha-alanine, glutamine, and histidine and/or arginine. It is also of interest that the presence of Enhancer of Bar results in a decrease in the proportion of threonine and alpha-alanine and an increase in the proportion of the peptide, pupine. This result suggests that pupine may possibly be a

peptide consisting largely or entirely of the two amino acids, threonine and alpha-alanine. This possibility is being tested by elution of the pupine spot and subsequent hydrolysis.

These results demonstrate that the position of Bar and Enhancer of Bar not only have an effect on eye facet number but also have an effect, although different, on the constitution of the free amino acids and peptides in the hemolymph and tissues of the adult.

(Supported by a research grant, C-2440, from the National Institutes of Health, administered by Allen S. Fox.)

Meyer, Helen U. Induction of autosomal lethals by ultraviolet treatment of male first-instar larvae of D. melanogaster.

Though the embryonic polar cap has proved to be the most suitable stage for ultraviolet treatment of the germ cells of D. melanogaster, it is occasionally desirable to use a different phase in the life cycle.

Irradiation of adult males, successfully carried out by several investigators, is limited to certain genotypes with little pigmentation. E. Altenburg (1934) irradiated 2- to 3-day-old larvae with U. V. over a period of several days, but concluded from the low yield of sex-linked lethals (.14% as contrasted with .03% in the controls) that this stage is almost as impervious to U. V. as the adult stage.

Lately we have treated first-instar larvae during the first hour after hatching; at this time they are still quite transparent, and the gonads of males are close to the dorsal surface and fairly unobstructed, whereas those of females are embedded in fat tissue. The larvae were placed in water, and held in place by slight pressure between a glass and an overlying quartz plate, through which the radiation was given.

Two series of experiments were performed, both of which utilized similar stocks and the same genetic methods to score recessive lethals in chromosome 2. In both instances, an increase of lethals was found in treated males as compared with treated females and untreated controls. Some of these lethals appeared as allelic clusters among the offspring of a given treated male, indicating that mutation occurred during early germ-cell development, the stage when radiation had been applied. The first, pilot experiment used germicidal U. V. (mainly 2537 Å; surface dose, 585 ergs/mm<sup>2</sup>), whereas the main experiment used longer-wave U. V. (mainly 2900 to 3100 Å; surface dose, 112,000 ergs/mm<sup>2</sup>). Though much less effective mutagenically, this longer ultraviolet has greater power of penetration and is less detrimental to the survival of the larvae to adulthood (50.8%). The following results were obtained:

| Experiment                  | Treated males                              | Treated females     | Controls, both sexes |
|-----------------------------|--|---------------------|----------------------|
| Pilot expt.<br>(2537 Å)     | 9 leth./341 chroms.<br>2.6 ± 2.1% lethals  | 1/200<br>0.5 ± 0.5% | 1/1045<br>0.1 ± 0.1% |
| Main expt.<br>(2900-3100 Å) | 30 leth./1269 chroms.<br>2.3 ± .6% lethals | 1/492<br>0.2 ± 0.2% | 9/2306<br>0.4 ± 0.1% |

Thus, a significant increase in the lethal rate from males was found in the main experiment; the germ cells of treated females remained unaffected, no doubt because they failed to be reached by the irradiation for reasons discussed above. Though the mutation rate obtained is comparatively low (corresponding to about 1% for sex-linked lethals), this technique of treating males is practicable in large-scale experiments.

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

Milani, R. Housefly genetics: linkage of *kdr* (knock-down resistance to DDT) with *bwb* and *dv*.

Two-point and three-point tests showed linkage between the genes *dv* (divergent wings), *bwb* (brown body), and *kdr* (knock-down resistance to DDT). The tests have been carried with the markers

in coupling and in repulsion. Backcross tests gave the following approximate recombination ratios. Heterozygous females (consistent results in different series): *bwb-dv*, 40%; *bwb-kdr*, 49%; *dv-kdr*, 45%. Heterozygous males (obviously different data from two different series have not been pooled): *bwb-dv*, 0.00% and 2.40%; *bwb-kdr*, 0.42% and 1.12%; *dv-kdr*, 0.58% and 3.46%. The recombination for *bwb* and *dv* (syn. *div*) are consistent with that already published (DIS-30: 138); male recombination--varying between series--has been confirmed.

Milani, R., and Palenzona, D. Hatched larvae in the uterus of *D. melanogaster*.

In dissection of *D. melanogaster* females from old, overcrowded bottles of a strain originally collected in nature and kept in captivity for five years, it

was observed that most of them were carrying in the uterus an egg at a level of development just before the emergence of the larva. In some females, however, a hatched larva was already present, moving above the chorion distended on the ventral surface of the uterus; in all cases the head of the larva was directed toward the ovaries. The larvae taken out by dissection behaved normally on fresh culture medium, without signs of suffering. Attempts to observe parturition of living larvae were unsuccessful. However, active larvae were observed a few minutes (from three to five) after the introduction of fresh food into a population cage; one was obviously wounded. Among the normally hatched chorions were others that were clearly abnormal and crumpled; counts of chorions and larvae showed correspondence between the number of larvae and the number of normal-looking chorions. This suggested retention (or resorption) of larvae hatched in the uterus. In one single case a female clearly showed difficulty in laying what proved to be an egg with a partially hatched dead larva. This female made short runs, and stopped to perform unsuccessful oviposition movements alternated with lifting and perhaps repulsive turning of the abdomen. During these antics, which lasted some five minutes, it ate "nervously." After parturition it moved away with a sudden run, and behaved normally thereafter.

Miller, D. D. Variation of the Y chromosome in *D. athabasca*.

As originally described (Sturtevant and Dobzhansky, 1936), *D. athabasca* has a J-shaped Y chromosome. This has been verified in recent strains of both

eastern and western localities. However, a strain from Ontario (Algonquin Park) was found to have a small V-shaped Y (smaller than the large V-shaped

X). Two other strains, one from northern Michigan (Iron Mountain) and one from New York (Cold Spring Harbor), were found to have a large V-shaped Y (similar to the X) associated with the rod-shaped autosome, which was present only once in males, thus indicating a translocation between this autosome and the Y. One of these aberrant strains (C.S.H.) has been used extensively in crosses with a Wyoming strain having a normal J-shaped Y, and these crosses have yielded fertile hybrids; hence, the unusual sex-chromosome relationship of the Michigan and New York strains does not indicate a new species.

Miyoshi, Y. Physiological significance of yellow pigment.

The yellow pigment extracted from the mutant strain sepia of D. melanogaster, which does not seem to occur in recog-

nizable amounts in the wild strain Oregon R-S, is very much like flavin in its chemical composition. The pigment, however, is not precipitated by NaCl, whereas flavin makes an insoluble compound with it. This fact suggested that the yellow pigment might play some important role in lieu of flavin in the presence of high concentrations of NaCl in the body of the mutant. Therefore, these two strains were tested for differences in susceptibility to excess amounts of NaCl. The eggs were allowed to hatch on, and fed with, Carpenter's semisynthetic medium (DIS-26, p. 132) containing NaCl in concentrations of 0.5 M, 1.0 M, and 2.0 M. The cultures were kept at  $25^{\circ} \pm 1^{\circ} \text{C}$ , and survivors were counted at each stage of development. The results obtained were as follows:

| Conc. of NaCl<br>in medium (M) | Strain | No. of<br>Eggs | Unhatched<br>% | 2nd<br>instar<br>% | 3rd<br>instar<br>% | Pupae<br>% | Emerged<br>No. | %    |
|--------------------------------|--------|----------------|----------------|--------------------|--------------------|------------|----------------|------|
| 0<br>(control)                 | se     | 500            | 5.6            | 72.8               | 65.0               | 59.8       | ♂ 125<br>♀ 132 | 51.4 |
|                                | +      | 500            | 7.6            | 88.4               | 77.8               | 75.6       | ♂ 172<br>♀ 196 | 73.6 |
| 0.5                            | se     | 500            | 6.0            | 57.6               | 43.6               | 39.6       | ♂ 90<br>♀ 71   | 32.1 |
|                                | +      | 500            | 10.4           | 51.2               | 37.6               | 36.0       | ♂ 81<br>♀ 84   | 33.0 |
| 1.0                            | se     | 450            | 10.4           | 29.1               | 22.2               | 21.3       | ♂ 33<br>♀ 42   | 16.7 |
|                                | +      | 500            | 10.6           | 2.2                | 2.0                | 2.0        | ♂ 8<br>♀ 2     | 2.0  |
| 2.0                            | se     | 400            | 9.0            | 9.5                | 0.75               | 0.75       | ♂ 1<br>♀ 1     | 0.5  |
|                                | +      | 500            | 9.8            | 0                  | 0                  | 0          | ♂ 0<br>♀ 0     | 0    |

The data show significant differences between the strains when the NaCl concentrations were 1.0 M and 2.0 M. These were chiefly due to differences in mortality in the first and second instars. The results clearly indicate that se is more resistant than the wild stock to a high concentration of NaCl.

Momma, E. The behavior of the paranucleus in the spermioteleosis of D. lacertosa.

The morphological changes leading to the formation of the paranucleus during spermatogenesis were reported in a few words by the author in DIS-30. At first

the mitochondrial masses are two in number, and structureless. In the early spermatid stage they come together into a single body to form the nebenkern, which is considerably larger than the nucleus. It shows a rapid increase in volume to about ten or more times that of the nucleus, and assumes a lamellar, concentric structure. Presently the nebenkern undergoes a change in appearance; the lamellae seem to decrease in number and to increase in thickness by coming together at several points. The lamellae seem to be double in structure, dividing into two threads. Sometimes, several deeply stained granules become observable outside the lamellae, or within the interlamellar matrix. Meanwhile, the nebenkern is divided into two parts, which form two progressively elongating halves. The lamellae decrease still more in number, though their profile is not clear. First, each half of the nebenkern elongates in a curve within the spermatid. Then both halves elongate further as the spermatid itself elongates. A well-stained fine thread appears in the cell body. Deeply staining minute beadlike bodies appear along the thread in the late spermatid stage.

Morita, T. Purine catabolism in D. melanogaster.

It was demonstrated by Hadorn et al. that the eye-color mutant rosy<sup>2</sup> (ry<sup>2</sup>) does not contain isoxanthopterin. Sub-

stances with fluorescence or absorption at about 260 mμ have been identified by means of chromatographs and absorption spectra, in ry and Oregon-R flies.

It was found that ry did not contain isoxanthopterin at any developmental stage, but after pupation it contained more AHP (2-amino-4-hydroxypteridine) than the wild-type strain. Uric acid was not contained at any developmental stage in ry, but ry after pupation contained hypoxanthine instead of uric acid. Hypoxanthine was not detected in the wild type by the chromatographic method. The results are shown in the table.

|                 | Oregon-R |      |       | ry    |      |       |
|-----------------|----------|------|-------|-------|------|-------|
|                 | Larva    | Pupa | Adult | Larva | Pupa | Adult |
| Isoxanthopterin | ±        | ++   | ++    | -     | -    | -     |
| AHP             | ±        | +    | +     | ±     | ++   | +     |
| Uric acid       | ±        | ++   | +     | -     | -    | -     |
| Hypoxanthine    | ±        | ±    | ±     | ±     | ++   | +     |

In a double mutant se ry, which showed the se phenotype, the same result was demonstrated as in ry. From these results, it seems that in wild-type D. melanogaster AHP acts as an intermediary in isoxanthopterin formation. Although xanthine was not demonstrated in ry by the chromatographic method, in the case of the wild type uric acid could be produced along the general pathway according to the following scheme: hypoxanthine → xanthine → uric acid. It seems that the activity of xanthine oxidase is deficient in ry.



Moriwaki, D., and Nakajima, Y.  
Reciprocal effects on heterosis  
in D. ananassae.

In D. ananassae, the gene arrangements,  
A and B, resulting from the inversion  
InIII can be paired in four genotypes:  
AA, BB, AB, and BA. Comparative

studies of rate of development in these four types show the following relationship,  $AB > BA = AA > BB$ . It is clear that the two heterozygotes produced by reciprocal matings differ significantly in rate of development; that is, those derived from A mothers (AB) seem to develop faster than those from B mothers (BA), the homozygote AA being superior to the homozygote BB in rate. More rapid development resulted from a mating in which the female had the higher rate of development, at least under optimal conditions, contrary to results obtained with D. persimilis by Spiess.

Later, in a comparison of the development of offspring from AB mothers with those from BA mothers, it was found that the former developed faster than the latter regardless of the source of sperm. Consequently it can be said that the cytoplasmic character which produced these differences in reciprocal crosses is effective to at least the next generation. In spite of the cytoplasmic effect, differences in genotypes could be recognized in the two kinds of back-cross offspring of each group. However, it is not yet known whether these two effects are additive.

Mourad, A. M., Mallah, G. S.,  
and Tantawy, A. O. Frequency  
of heterozygous inversions in  
natural populations of D.  
melanogaster and D. simulans.

D. melanogaster and D. simulans,  
collected from eight different geo-  
graphical regions, were examined  
cytologically for frequency of inver-  
sions on the second and third chromo-  
somes. (For the different localities

mentioned, see research note by Dobzhansky, Mallah, Tantawy, and Mourad in this issue.) The results obtained for D. melanogaster from five of these regions are given in the table.

| Locality          | No.<br>of<br>♀♀ | % of inversions in each chromosome |      |                |       |
|-------------------|-----------------|------------------------------------|------|----------------|-------|
|                   |                 | 2nd chromosome                     |      | 3rd chromosome |       |
|                   |                 | L                                  | R    | L              | R     |
| University Farm   | 110             | 20.00                              | 4.55 | ---            | 7.27  |
| Fayuom            | 54              | 3.70                               | 1.85 | ---            | 29.62 |
| Mehalla El Kobera | 111             | 20.72                              | ---  | 0.90           | 5.40  |
| Wadi El Natroon   | 94              | 47.87                              | ---  | 8.51           | 13.82 |
| Lebanon           | 66              | 7.57                               | ---  | ---            | 3.03  |

The collection from Abou-Sir involved mostly D. simulans and contained only three females of D. melanogaster. Two of these females were found to have inversions on the second chromosome (R); the third had an inversion on the third chromosome (R). From Beni-Sweif, the flies collected were mainly D. simulans, with only nine females of D. melanogaster. Of these nine females, three carried inversions, one each, on the third chromosome (L). D. melanogaster captured in Kom-Ombo (N = 13) did not show any inversions.

The results presented in the table show that most of the inversions were found on the second (left) and third (right) chromosomes. Flies from Wadi-El-Natroon showed the highest percentage of inversions on the second chromosome,



and those from Fayoum the highest percentage of inversions on the third chromosome.

No inversions have been found so far in any of the D. simulans collected from these localities.

Experiments are still in progress, and more populations will be investigated as well as hybrids between them.

Muller, H. J. Mutation studies of chromosome 3 simplified by the "sifter-3" method.

The chief bottleneck in the detection of recessive autosomal mutations at non-specific loci, which has limited the number of chromosomes that can be

tested, has been the necessity of inbreeding with one another just those  $F_2$  individuals of each  $F_1$ - $F_2$  culture that have inherited the same specified autosome of the  $F_1$  individual being tested. This operation ordinarily requires the virtually simultaneous collection of virgin females of the specified type from each of the numerous  $F_1$ - $F_2$  cultures. For chromosome 2, several schemes to overcome this difficulty have been devised by the writer (Anat. Rec. 24: 419, 1923; Genetics 13: 279-357, 1928; DIS-25: 117-118, 1951; DIS-27: 104-105, 1953), but for chromosome 3 only one scheme (DIS-29: 147-149, 1955). The scheme now being offered for chromosome 3, called "sifter-3," has the following advantages over the previous one: its operation is simpler; it requires fewer stocks; it kills, instead of sterilizing, all  $F_2$  males of the undesired types; it gives rise only to the types of  $F_3$  desired, and requires no selection among the latter and no collection of virgin  $F_3$ .

The main principle involved in the operation of the sifter-3 method is that of the lethality of hyperploid individuals that have four doses (tetraploidy) of the region of chromosome 2 extending from about 59C to 60E. This is the region common to the Pale transposition (59A1-60E1) and the  $Y:bw^+$  insertion (about 59C-60E5+4). At the same time, hyperploids having three doses of this region (or of the slightly longer one of either of the duplications just mentioned) are viable and fertile. It is arranged that all  $F_2$  males of the undesired kind are either tetraploid for this region or killed by being homozygous for a lethal in chromosome 3. Thus the  $F_2$  females can have mated only with males of the desired kind. The  $F_2$  flies must be etherized, however, so that the desired type of nonvirgin  $F_2$  females can be picked out for making up the  $F_2$ - $F_3$  cultures.

The scheme requires that the  $F_1$  males containing a chromosome 3 that is to be tested for recessive lethal and/or visible mutations have a homologous chromosome 3 provided with inversions and a dominant marker (the combination  $ru\ h\ D\ InsCXF\ e^S$  being in most cases the most useful for this purpose), and also have a Y chromosome of type  $Y:bw^+$ , carrying the 59C-60E section of 3 inserted in its long arm. The  $P_1$  male should have had its two chromosomes 3 (if both of them are to be tested) differentiated in respect to one or more of the recessive markers (such as  $ru$ ,  $h$ , or  $e^S$ ) carried by the dominantly marked chromosome of the  $F_1$ , so that the  $F_1$  males are distinguishable with regard to which chromosome 3 of the  $P_1$  male being tested they contain. Thus, the composition of a given  $F_1$  male may be  $Y:bw^+; h\ ri/ru\ h\ D\ InsCXF\ e^S\ \delta$ , and that of a brother may be  $Y:bw^+; ve\ bv/ru\ h\ D\ InsCXF\ e^S\ \delta$ . Such  $F_1$  males can have been derived from  $P_1$  males of composition  $Y:bw^+; h\ ri/ve\ bv$  crossed by virgin females of composition  $e\ P^i/ru\ h\ D\ InsCXF\ e^S$ .  $P^i$  designates section 59A1-60E1 (of the Pale transposition) inserted into chromosome 3. The  $F_1$  males (see above) are individually backcrossed to virgin females of this same

type. In the  $F_2$  generation, all males getting the  $e P^i$ -containing chromosome 3 are killed by hyperploidy, since they also have  $Y:bw^+$  that contains section 59C-60E (except for any males which because of nondisjunction lack a Y and are therefore sterile). Of the  $F_2$  males getting the D-containing chromosome 3 of their mother, those also getting this chromosome from their father (the  $F_1$  male) will be homozygous for lethals, while only those getting the chromosome to be tested can live; these are of the desired type for breeding. The females of the same chromosome-3 type as the males are picked out for mating with them.

A sample pedigree illustrating the above is as follows:

$P_1$   $Y:bw^+; h\ ri/ve\ bv\ \delta$  x  $e P^i/ru\ h\ D\ InsCXF\ e^S\ \phi$

$F_1$   $Y:bw^+; h\ ri\ or\ ve\ bv/ru\ h\ D\ InsCXF\ e^S\ \delta$  x  $\phi$  like  $P_1\ \phi$

$F_2$  All viable males are like father ( $F_1\ \delta$ ); females (nonvirgin) having chromosomes 3 like that of  $F_1\ \delta$  are picked out for breeding.

In  $F_3$  look for presence of non-Dichaete and check their phenotypes.

(The  $F_2$ - $F_3$  cultures already constitute balanced stocks of whatever chromosome-3 lethal or other mutant it may later be desired to save.)

A minor defect in the scheme arises from the fact that if any of the females to which the  $P_1$  or  $F_1$  males are mated should contain a normal Y (or if the  $P_1$  males should contain one), one or a few viable fertile  $F_2$  males containing the  $e P^i$  chromosome may be produced by nondisjunction. They would however be recognizable by being either non-Dichaete or Dichaete ebony, and cultures containing such males may be discarded. Even if they were used, mutations would still be recognizable among the  $F_3$  by looking for (or at) the non-Dichaete non-ebony flies. In any case this situation would not cause nonlethals to be scored as lethals (a much more serious error than the converse one). A stock for furnishing the  $e P^i$ -containing females is now under construction in which this source of difficulty will be obviated by having the males supplied with a  $Y:bw^+$  instead of a  $Y^+$  chromosome; females with the  $Y:bw^+$  in addition to their  $e P^i$  would be inviable.

The  $P_1$  males of the above scheme can be obtained by crossing two " $P_0$ " stocks having the respective chromosomes 3 of the  $P_1$  and having (at least in the  $P_0$  stock that furnishes the male in the  $P_0$  cross) a  $Y:bw^+$  instead of ordinary Y. However, the chromosomes 3 of flies of any desired stock (or from nature) can be tested by crossing females containing them to males having  $Y:bw^+$  and  $ru\ h\ D\ InsCXF\ e^S$ , or by crossing males containing them to females having  $Y:bw^+$ , attached X's, and  $ru\ h\ D\ InsCXF\ e^S$ . These crosses at once provide  $F_1$  males of the type shown in the preceding scheme, but they can if preferred be used as " $P_1$ " if the mutation frequency of their own generation is to be ascertained. A stock that is being made up for this purpose has the composition  $Y:bw^+/X^+ \& X.X$  ("snoc");  $ru\ h\ D\ InsCXF\ e^S/M\acute{e}$ ,  $Ins\ ri\ Sb^1$ .

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Muller, H. J., and Edmondson, M.  
Transposition of entire 4-euchromatin into a fully functional Y.

A translocation between the Y and the fourth chromosome, with functional male-fertility genes, was found by Edmondson in 1946, in x-rayed material,

and has been carried among the Indiana stocks ever since (see DIS-26, p. 20, 1946, et seq.). Recent genetic analyses by Muller have shown that the whole

of the euchromatic portion of 4 has been inserted into the Y, leaving both chromosomes fully functional without any other parts of 4 or Y being required. Thus the case fits the definition of "transposition," a deletion from one chromosome into a nonhomologous chromosome, but it is unusual in being a whole-arm transposition.

Muller has constructed a stock of it, denoted simply by  $Tp4:Y$ , or still more simply by  $Y:4$ , in which all females as well as males contain it in diploid dose and are free from all other Y and 4 chromosomes and chromosome parts, and another stock, in which the  $Y^L$  arm of Novitski's  $Y^S.X$  InEN  $y.Y^L$   $sc^8 y^+$  has been exchanged for the portion of the  $Y:4$  chromosome that includes  $Y^L$  and 4 but not  $Y^S$ . We are not yet sure whether 4 lies on the same side of the centromere as  $Y^L$ , or whether the centromere of  $Y:4$  was derived from Y or from 4. However, four breaks would have been required if the centromere were from 4, otherwise only three.

That the inserted 4 affects the recombinational properties of this Y is indicated by the fact that in a count of 12,187 offspring from females containing ordinary attached X chromosomes homozygous for yellow, besides a  $Y:4$  and two free 4's homozygous for  $ci\ ey^R$ , crossed to males with a  $Y:4$ , a normal X, and likewise two free 4's with  $ci\ ey^R$ , not a single case of "detachment" of the attached X's was found. Ordinarily these attached X's in company with a normal Y would have given 8 or more spontaneous detachments, by exchange between the  $X.X$  and the normal Y. However, the above-mentioned case of attachment of  $Y^L:4$  to Novitski's " $X.Y$ " did involve an exchange of much the same kind as is in question here.

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Muller, H. J., and Oster, I. I.

Suppressor action effective with a subgene deficiency of a normally duplicated locus.

An X-ray-induced forked of mosaic expression (Muller, DIS-20: 88, 1946) was found to have involved a simultaneous change in two positions (Muller, DIS-21: 71, 1947): (1) a recessive

mutation to a moderately expressed forked, " $f^X$ ," which had arisen in the position usual for this character and which showed ordinary crossing-over relations, and (2) a gene having a dominant suppressor-like action on forked, of mosaic expression, located in the heterochromatin of the X near the centromere; addition of a Y chromosome increased the normalization of the forked character caused by the heterochromatically placed mutant. It was inferred from this simultaneous origin of a complementary hypomorph and hypermorph in different positions that they did not represent a mere coincidence but that, as in the case of the Pale transposition for which Altenburg in 1918 made a similar inference on the same grounds, a region had been removed from one position, here that of  $f^X$ , and inserted into the other position (a change designated as a "shift" when the deficiency is in the same chromosome as the insertion). The gene in the heterochromatic location was therefore designated " $f^{+ih}$ " (i for insertion and h for its heterochromatic position), and both its mosaic expression and the normalizing effect of extra heterochromatin upon its action were in accord with these effects in other cases of variegation.

It was thereby implied, although not expressly stated, that  $f^X$  was really a deficiency, of the piece represented in the  $f^{+ih}$  duplication. More-

over, since crossovers having  $f^X$  without  $f^{+ih}$  were found to have a viability and fertility usual for forked, along with only a moderate expression of the forked character, unlike what was known to be true for chromosomes showing a deficiency of the entire forked region, it was self-understood that the  $f^X$  deficiency involved only a portion of the region functionally concerned with forked. That is, this region must normally be made up of two or more subloci or subgenes, not all of which had been shifted to the  $f^{+ih}$  position. Direct proof of the compoundness of the forked region, by means of experiments showing that the different forked alleles tested fall into two groups, the members of either of which show crossing over with the members of the other group, was later provided by M. M. Green (P.N.A.S. 41: 375, 1955, and P.N.A.S. 42: 73, 1956).

Valencia (reported by Muller, 1946, *ibid.*) had found that the  $f^X$  deficiency is too small to be seen in salivary chromosomes, as is only to be expected of a subgene deficiency. Further evidence of the deficiency nature of  $f^X$  was obtained by Oster (reported by Muller and Oster, *Advances in Radiobiology*: 407, 1957), in the finding of no case of spontaneous or induced back mutation of  $f^X$  in counts that would have given an expectation of 6 cases if forked-1 had been used. This does not, however, imply that  $f^X$  is unable to give back mutations, as by means of duplication of the remaining region; but most back mutations of forked are not duplications, as shown by the fact that their frequency in ring X's is about as high as elsewhere (see Muller and Oster, *ibid.*). It may be concluded that in this region hypermorphic mutations of normal subgenes are either less frequent or less effective than those of mutant subgenes.

It might seem natural to assume that because  $f^X$  is a deficiency it cannot be suppressed by a modifier, such as Whittinghill's *su-f*, especially since Green (*ibid.*) found that only about half of the spontaneously arisen forked alleles tested by him (including none known to belong to his second locus-group) and none of the X-ray-induced ones (of either locus-group) were suppressible by *su-f*. In our laboratory Sara Frye has recently obtained a similar series of results on spontaneous and induced forked alleles, not yet published. However,  $f^X$  has been found by us to be definitely suppressible by *su-f*. We may conclude from this not only that some X-ray-induced forkeds are suppressible but also that suppressibility does not constitute evidence against deficiency. Conversely, the unsuppressibility of the previously tested X-ray-induced forkeds cannot constitute evidence that they are deficiencies, as might otherwise have been suspected.

This result calls for a revision of thinking concerning suppressor action. It seems to have been tacitly assumed by some students of the subject that when a forked or other mutant is suppressible, the suppressor is somehow stepping up the effectiveness of the reactions initiated by that mutant gene or subgene itself. This is impossible, however, when the given gene or subgene is actually absent, as in the case of  $f^X$ . Here, then, it is the effectiveness of the reactions initiated by the remaining subgene(s) that is stepped up, so that they are able to compensate for the deficiency. If the same general principle holds also in the suppression of nondeficient mutants, such as forked-1 and forked-5, the action of the suppressor here may really be on the sublocus that adjoins them, the one that remained normal, rather than on the mutant sublocus itself, that we had considered to be suppressed. The suppression would consist in an enhancement of the unmutated sublocus, causing it to react, in effect, hypermorphically. Thus when forked-1 was suppressed it might be the sublocus of forked-3 that was enhanced. However, it is also possible, in the case of nondeficient mutants,

that the gene-initiated reactions of the different subloci may merge at a stage preceding that at which the "suppressor" action is exerted, so that it would be improper to refer the suppressor effect to a reaction on the product of any given sublocus.

We have used the term subgene advisedly in this case, since the above-noted evidence indicates that the forked region of the normal X chromosome is compound in the sense of consisting of a number of separable parts of similar structure and function just as does the scute region of the X (viz., at least *achaete* and *scute*) and the male-fertility regions of the Y. Only in these three cases has this conclusion, to our knowledge, been well supported. The support involves evidence, in each case, indicating that when a break occurs in the given region, followed by a linear separation of its parts, both parts continue to some extent to perform the function in question.

In cases exhibiting only the more usual evidence of pseudoallelism based on crossing over, on the other hand, it is always possible to postulate, and often there is direct ground (based on the difference between *cis* and *trans* compounds) for concluding, that the similarity of action of mutants occupying nearby positions has been caused by a "position effect," a reaction occurring adequately only within a minute distance between one gene or gene product and a neighboring gene or gene product. When this is the case, however, it is usually a sufficient explanation in itself, without the postulation of a similarity in actual structure and mode of action of the cooperating genetic entities. Thus, in these very cases we lack grounds for inferring that the given region is of duplicational nature or origin.

In the case of forked, as in that of *scute* and the Y, it is the structural change that gives us evidence that each part of the region, even when separated from the other, can to some extent perform the function of the other, inasmuch as (1) the remainder present in its normal location in the  $f^X$  chromosome does not have the extreme forked effect shown by a full-fledged deficiency and (2) the shifted material (when the incapacitating effect of the adjoining heterochromatin is counteracted by an extra Y) also is able to act in bristle normalization. In other words, both parts have some "anti-forked" or bristle-straightening effect that is not dependent on a positional reaction and they therefore carry on such similar functions as to lead us to conclude that they were derived from a common ancestral gene by its linear duplication.

A less likely interpretation of the insertion,  $f^{+ih}$ , is that it involved a larger piece than the deficiency,  $f^X$ , but if this were true this inserted piece would have had to be contributed by the sister chromatid to that in which the deficiency  $f^X$  occurred, and that chromatid would have sustained a larger deficiency than  $f^X$ . The complicated nature of this interpretation reduces its probability; but if this process had occurred the case would not illustrate the similar functioning of different subgenes, since the inserted piece might contain the entire region, and the evidence that the neighboring loci were products of a duplication occurring in the past evolution would thereby disappear.

If we accept, at least as a working hypothesis, the interpretation that  $f^X$  and  $f^{+ih}$  are complementary, a curious paradox is encountered in attempting to bring this case into line with Green's finding that the loci (or subloci) dealt with by him fail to produce normal bristles except when there is a chromosome having both or all unmutated loci (or subloci) close together, that is, in *cis* arrangement. For in our case (at least with the aid of an

extra Y) the parts even when separated, as they are in the  $r^x r^{+ih}$  chromosome, are able to give rise to a normal bristle phenotype. Evidently, the parts are not just the same ones as those studied by Green, or the mode of reaction of one or more parts has been altered by their mutation or positional change.

We should be glad to have anyone who wishes to do so conduct further cytological study on this material.

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

Muller, H. J., and Schalet, A.  
Further improvements in the "Maxy" stock for detection of specific-locus mutations.

As noted in the earlier description of this stock (Muller, DIS-29: 146, 1955), the X of the male has the moderate-sized inversions In49 and B<sup>M1</sup>. It also has the minute inversion

that goes with the combination L<sup>J1</sup> sc<sup>J1</sup> (mistyped sc<sup>S1</sup> in DIS-29) and was provided with the marker miniature wings (m). The duplication of the left end of the X designated as L<sup>J1</sup>+, attached to the long arm of the Y to give the chromosome designated as L<sup>J1</sup>+.Y, allows the males to live despite the presence of L<sup>J1</sup> in their X, but it does not cover the scute or yellow loci (Muller, DIS-28: 140, 1954).

Unfortunately, both sc<sup>J1</sup> (like most scutes) and m reduce the viability and fertility of the males. Moreover, sc<sup>J1</sup> interferes with the detection of bristle mutants and m with that of wing mutants. These difficulties have now been overcome.

In place of sc<sup>J1</sup> in the X chromosome of the male we now utilize sc<sup>J1(+)</sup>, which was found by Schalet as a spontaneous back mutation of sc<sup>J1</sup> that results in a virtually normal phenotype of bristles and good viability but does not prevent the lethal effect of L<sup>J1</sup>. In place of m we now use oc, which does not interfere with the detection of any of the genes in question, and has good viability and fertility in the male, so that approximately 45% of the flies in these cultures are males. In addition, oc has the advantage of sterilizing all those females which, having acquired a L<sup>J1</sup>+.Y through non-disjunction, are viable even though homozygous for the L<sup>J1</sup>-containing X chromosome. The Bar-M1 region of this chromosome contains a nonlethal moderate bobbed allele.

The insertion of oc into the In49 of this chromosome was carried out by Muller, by utilization of heterozygous inversions in chromosomes 2 and 3 simultaneously (those associated with Oster's Curly chromosome and with the Dichaete CXF complex) to step up crossing over between the X's. This insertion of oc from the X without In49 into that with In49 required double crossing over in which both points of crossing over were within the limits of this inversion. One case occurred among about 5000 offspring that had received oc. The In49 oc combination will also be useful in other connections, as for instance in our derived stock of the composition sc<sup>S1</sup> B In49 sn<sup>x2</sup> oc ptg sc<sup>B</sup>. As usual, the oc within In49 has the nearby ptg along with it, but this has no useful function here.

As a further improvement, Schalet has obtained a representative of the multiple-recessive X chromosome of the "Maxy" stock (that carrying the specific-locus markers) which has, by spontaneous mutation, acquired a lethal.



This lies between the loci of *sc* and *w*. It prevents the appearance, even as larvae, of the multiple-recessive males, which, being unwanted, were wasteful of breeding space and scoring time, and sometimes bred. It also kills so effectively that no gynanders manifesting this chromosome have yet appeared, although in the nonlethal stocks the same number of flies had shown a noticeable frequency (1/7500 ♀) of them, resulting in mutant-like individuals. Finally, the multiple-recessive chromosome, which had been found to have lost *rb* in the process of inserting *ec*, has been repaired so that it now contains both these genes.

The stock is now constituted as follows in odd generations:  
 1Jl<sup>+</sup>.Y/1Jl sc<sup>Jl</sup>(+) In49 ptg oc B<sup>M1</sup>, In ♂ and 1Jl sc<sup>Jl</sup>(+) In49 v ptg oc B<sup>M1</sup>, In/y<sup>S1</sup> sc<sup>S1</sup>, In car odsv f g<sup>2</sup> dy v ras<sup>2</sup> sn<sup>3</sup> ct<sup>6</sup> cm rb ec w pn sc<sup>8</sup> ♀. In even generations the sc<sup>Jl</sup>-containing chromosomes, having v<sup>+</sup> and v, respectively, are interchanged between the male and female, with a resultant crisscrossing of the vermilion phenotype, as a check against the rare production of exceptions through the occurrence of nondisjunction in the mother. Nondisjunction in the father produces daughters with a 1Jl<sup>+</sup>.Y, and these in the next generation produce many homozygous oc females and a few male and female exceptions with regard to vermilion; cultures showing these characteristics are not used for further breeding. It will be seen that in this scheme only one type of female and one type of male is regularly produced in any given generation, so that attention may be concentrated on finding the mutants. Moreover, the nonvirgin females can be bred individually, like ClB females, for ascertainment of the frequency of lethals in their sc<sup>Jl</sup>-containing X; a female with a lethal produces no sons.

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Murray, C. L., and Lewis, H.  
 Studies of the effect of varying concentrations of salt on recombination in D. melanogaster.

The suggestion has been put forth by several workers in recent years that chromosomes are composed of particulate units linked through divalent cations and that electrostatic attraction plays

an important role in chromosome stability. This suggestion has come from experiments in which excess or deficient amounts of divalent cations are correlated with altered recombination frequency. Altering the chemical environment of chromosomes by varying ionic strength may permit the detection of some clue as to the importance of electrostatic forces in chromosome stability. The experiments reported in this note were performed to detect such clues. This was a pilot study, and no attempt was made to determine the extent of alteration of ionic strength in the cells of the various tissues.

Larvae heterozygous at the y ec ct v w y f loci were bathed in NaCl solutions of various concentrations for 24 hours during the last day of the third-instar stage. It has been shown that treatment by immersing larvae in test solutions is as effective as injecting the test solution into the larvae. Both hypo- and hypertonic salt solutions were used as test solutions (0.0, 0.5, 0.75, 1.0, and 1.2% NaCl). Control groups were untreated flies. All work was carried out at 24±1° C. If chromosomal instability was induced by this treatment it could be reflected as disturbed development of imaginal tissues or altered recombination frequency among the offspring of test-crossed treated females. Neither of these effects was observed. Nearly all the treated flies pupated and subsequently eclosed into normally developed adults. The recombination frequencies in the segments of the X chromosome



tested are not significantly different between any of the treated groups and the controls. There are a number of possible explanations of why the treatment does not affect the frequency of recombination, the most likely being that the sodium ions do not penetrate into the cells of the gonads.

Nawa, S., Taira, T., and Oshima, C.  
Nonenzymatic conversion of the  
yellow pigment found in D. melanogaster.

As a result of incubation of the yellow pigment obtained from se with methylene blue at near neutrality, AHP (2-amino-4-hydroxypteridine) was produced. This result was confirmed

by means of two-dimensional paper chromatography. It is concluded that DPN (diphosphopyridine nucleotide) cannot serve as a hydrogen acceptor. The other pterins found in D. melanogaster, such as 2-amino-4-hydroxypteridine-6-carboxylic acid and 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine, were not affected by incubation with methylene blue.

Nawa, S., Taira, T., and Oshima, C.  
Pterin dehydrogenase and its  
coenzyme in D. melanogaster.

An enzyme which was found in our laboratory in a homogenate of D. melanogaster can catalyze the oxidation of AHP (2-amino-4-hydroxy-

pteridine) to isoxanthopterin, and it may be capable of converting xanthine into uric acid. The pH optimum of this enzyme is in the neighborhood of 8.5. The enzymatic oxidation of AHP to isoxanthopterin could not be catalyzed without a cofactor such as methylene blue or DPN (diphosphopyridine nucleotide), when a dialyzed preparation was used. Because of its nature this enzyme was named "pterin dehydrogenase." When an undialyzed preparation was used, however, the enzymatic oxidation progressed at an appropriate rate without the addition of methylene blue or DPN.

Our experiments showed that DPN was a more effective electron acceptor than methylene blue. Therefore it seems that DPN may be a more useful coenzyme of this enzyme in vivo.

From our evidence it appears that an enzyme obtained from mild and mammalian liver immediately catalyzed the oxidation of both pterin and xanthine by molecular oxygen. Further, a preparation from chicken liver reacted only very slowly with molecular oxygen as compared with either methylene blue or DPN. In this case, however, the ability of DPN as an electron acceptor was approximately 10 per cent that of methylene blue.

In another experiment, an enzyme obtained from the silkworm in our laboratory showed a similarity to that from D. melanogaster. The mode of action of this enzyme in insects, therefore, differs functionally from that in mammals or birds.

Ogita, Z. Resistance to phenylthiocarbamide (PTC) and phenylcarbamide (PC) in D. melanogaster.

PTC is well known not only as an inhibitor of melanin formation but also as a substance used in diagnosing "tasters" and "nontasters," the

trait being inherited as a Mendelian recessive. Effects of PTC and PC on the emergence of flies were investigated by measuring percentages of flies emerging on a dry yeast medium (agar 2 g, dry yeast powder 3 g, sugar 4 g, in 100 ml water) containing PTC in concentrations of 0.5 mM, 1.0 mM, and 3.0 mM, and PC in concentrations of 5.0 mM, 10.0 mM, 50.0 mM, 70.0 mM, and 100 mM.

The order of tolerance to PTC was as follows:  $y \dot{\div} y \ w \ f \dot{\div} w \dot{>} \text{Oregon (iso)} \dot{\div} e^{ll}(\text{coiso}) \dot{\div} B \ e^{ll}(\text{coiso}) \dot{>} w^e \dot{>} \text{Canton-S} \dot{>} e^{ll} \dot{\div} \text{Oregon} \dot{>} bw \dot{\div} \text{Hikone-R} \dot{>} \text{Oregon-R-(I)} \dot{\div} y \ \text{Oregon-R-(I)} \dot{\div} v \ \text{Oregon-R-(I)} \dot{\div} v$ . Emergence of Oregon, bw, Hikone-R, Oregon-R-(I), y Oregon-R-(I), v Oregon-R-(I), and v was inhibited markedly by the addition of 2.0--3.0 mM PTC to the dry yeast medium. On the other hand, y, y w f, w, Oregon(iso),  $e^{ll}(\text{coiso})$ , and B  $e^{ll}(\text{coiso})$  were resistant to 5.0 mM PTC.

The order of tolerance to PC was as follows:  $\text{Hikone-R} \dot{>} e^{ll}(\text{coiso}) \dot{\div} \text{Oregon(iso)} \dot{\div} \text{Canton-S} \dot{\div} y \ w \ f \dot{\div} w$ . Emergence of  $e^{ll}(\text{coiso})$ , Oregon(iso), Canton-S, y w f, w, etc. was inhibited markedly by the addition of 50.0 mM PC to the medium, whereas the Hikone-R strain could resist up to 100.0 mM PC.

It is of interest that the Hikone-R strain, which shows "cross resistance" to various insecticides such as DDT and BHC, is less resistant to PTC than the Canton-S strain (susceptible to DDT, BHC). On the contrary, the relation to these strains to PC seems to be reversed. Therefore, the mechanism of inheritance for resistance to PTC seems to be different from that to insecticides like DDT, BHC, and parathion.

Oksala, T. A. The mechanism of secondary nondisjunction of X chromosomes and autosomes.

The following types of females were tested with respect to frequency of secondary nondisjunction of the X chromosomes:

| <u>Mother</u>                                       | <u>Percentage of sec. nondisjunction</u> |
|---|--|
| 1. w/w/Y  | 7.2%                                     |
| 2. w/wm <sup>4</sup> /Y                             | 16.2%                                    |
| 3. wm <sup>4</sup> /wm <sup>4</sup> /Y              | 17.3%                                    |
| 4. wm <sup>4</sup> /wm <sup>4</sup> /Y; Cy/+        | 10.0%                                    |
| 5. wm <sup>4</sup> /wm <sup>4</sup> /Y; T(2;3)rn/+  | 8.1%                                     |
| 6. wm <sup>4</sup> /wm <sup>4</sup> /Y; Cy/T(2;3)rn | 2.3%                                     |

In case no. 6 the disjunctive progeny exhibited the following peculiarity: in the + fraction, about 90% of the flies possessed a Y chromosome, whereas in the Cy fraction only about 10% had a Y chromosome.

All the results listed above can be explained on the assumption that bouquet orientation occurs in the early meiotic stages. The distal ends of all the chromosome arms and the heteropycnotic Y chromosome are situated close to each other at the base of the bouquet, where the pairing begins, whereas the centromeric regions are raised to the top of the bouquet as a result of the pairing process. The interpretation is as follows.

When the X chromosomes are heterozygous for an inversion (case 2) they pair with a loop. Accordingly, they do not rise to the top but remain at the base of the bouquet. The Y chromosome situated there now has a better opportunity to pair with the heterochromatic blocks of both X chromosomes than is normally the case. The result is a high percentage of nondisjunction as compared with the normal situation (case 1). The fact that X chromosomes remain at the base of the bouquet is thus the primary reason for the higher percentage of nondisjunction.

Cooper's studies (Proc. Natl. Acad. Sci. 34: 179-187, 1948) have shown that the more X chromosomes differ in structure, resulting in slower and more

difficult pairing, the higher is the percentage of nondisjunction.

Case 3 shows that even when there is inversion homozygosity of the X chromosomes the percentage of nondisjunction may be exceptionally high, provided that the inversion is of the type of  $In(1)w^{m4}$ , in which the heterochromatic block of the X has broken and a considerable portion of it has been transferred to the distal end of the X. Situated thus, it remains at the base of the bouquet, and this heterochromatin of both homologues readily pairs with the Y chromosome.

The two inversions in the second chromosome of the Cy stock significantly decrease the rate of nondisjunction of the X chromosomes, as has been known since the studies of Sturtevant (Carnegie Inst. Wash. Year Book 43: 164-165, 1949). Since the two second chromosomes, which pair by making two inversion loops, remain at the base of the bouquet, their heterochromatin has an opportunity to pair with the Y chromosome. Since as a consequence the X chromosomes, in many cases, remain free and segregate normally, the result is a decrease in the percentage of their nondisjunction. The pairing of the Y with the second chromosomes obviously fairly often leads to (secondary) nondisjunction of the latter. This can be concluded from the fact that flies without a Y produce during the same period more than twice the progeny produced by flies with a Y. Obviously the latter, owing to the nondisjunction just mentioned, lay a large number of two-II and no-II eggs, which naturally give rise to lethal zygotes. Since these inviable zygotes are all disjunctive with respect to the X chromosomes, the actual percentage of nondisjunction is still lower than the 10% obtained in the experiment. The inversions of the third chromosome have a corresponding effect, but the data from these experiments are as yet insufficient.

Case 5 proves that (autosomal) translocation heterozygosity also decreases the percentage of secondary nondisjunction of the X chromosomes.  $T(2;3)rn$  is practically a whole-arm transfer between II and III (cf. Carlson, DIS-30: 109, 1956). Pairing in the heterozygote rotund thus gives a group of four, with an exchange of partners near the centromere of each chromosome. In such a configuration the pairing is slower than normal, since each arm pair forms an obstacle to the pairing of neighboring chromosome arm pairs (cf. Oksala, Hereditas 38: 449-480, 1952). Obviously, the pairing often remains incomplete, since in the rotund heterozygote the crossing-over percentages for all the arm pairs are much lower than normal. Owing to this slow and incomplete pairing, the proximal regions of the chromosomes are delayed longer than usual at the base of the bouquet, where their heterochromatic blocks offer an attractive pairing partner for the Y. Thus the X chromosomes frequently remain free and their nondisjunction rate is decreased.

Case 6 displays the strong combined effect of autosomal inversion and translocation heterozygosity. The pairing pattern of the autosomes is largely the same as in the foregoing case. The nontranslocated second chromosome, however, obviously pairs only with the distal parts of its arms, since pairing with loops is practically impossible in a group of four. It is the heterochromatic block of the Cy chromosome which, having remained at the base of the bouquet, pairs most easily (in 90% of the cases) with the Y and segregates from it, thus going to the same pole with the two translocated chromosomes.

Oksala, T. A. Pairing pattern of the chromosomes in female meiosis in *Drosophila* and the "interchromosomal effect on crossing over."

The experimental results reported in the preceding note make it appear highly probable that in the early stages of meiosis the Y chromosome, when present in the female, is able to pair with the heterochromatic blocks

not only of the X but also of the two large autosomes. Consequently, it can further be assumed that a general pairing affinity exists among the heterochromatic regions of all, even heterologous, chromosomes. In the same report it was also shown that many of the phenomena connected with secondary nondisjunction of the chromosomes are explicable on the assumption that a bouquet orientation prevails during the pairing process of the chromosomes. On these ideas is based the following hypothesis, which has been advanced to explain a number of important findings concerned with the "interchromosomal effects on crossing over."

If all the chromosomes are structurally of the wild type, all the proximal heterochromatic parts (6 blocks in all) are situated at the top of the bouquet. There each heterochromatic block has the opportunity to pair not only with its ordinary partner but with any of the other heterochromatic blocks. Each "illegal" pairing produces an exchange of partner, which greatly slows down the pairing of the euchromatic chromosome arms and is even likely to make it impossible in the most proximal regions. Thus crossing over only rarely takes place in these parts of the arms. The result is that gene loci are crowded around the centromere in the crossover maps. This state of affairs also affords an explanation of the phenomenon that interference, as a rule, does not work across the centromere, since it does not act over unpaired chromosome regions (cf. Oksala, *Hereditas* 38: 449-480, 1952).

The phenomenon of "interchromosomal effect on crossing over" implies especially the effect of inversion heterozygosity on the crossing-over frequency in heterologous chromosomes (see, e.g., the review by Schultz and Redfield, Cold Spring Harbor Symp. Quant. Biol. 16: 175-197, 1951). If one chromosome has a large enough inversion or two inversions, it pairs with its normal partner with an inversion loop(s). Such a configuration is not able to rise to the top of the bouquet but remains with its heterochromatic block at the base. In this case only four heterochromatic blocks are left at the top of the bouquet, and this gives them a better opportunity for "legal" pairing. The result is an easier and more complete pairing of the proximal euchromatin and thus an increased frequency of crossing over. If two chromosome pairs are heterozygous in regard to inversions, both remain at the base of the bouquet and the only homozygous pair alone rises to the top, having thus optimal chances for a complete pairing. Consequently, inversion heterozygosity in other chromosomes mechanically facilitates the pairing of the proximal parts of the arms of the remaining chromosomes causing in them a large relative increase in the crossing-over percentage. The other phenomena connected with this pronounced effect are explicable on the assumption that the interference pattern must be profoundly modified as a result of such a complete pairing.

Other findings related to "interchromosomal effects" are also explicable on the basis of the present theory, but they are not touched on in this limited exposition.

Okubo, S. A glucoside in D. melanogaster.

twenty pupae of various strains were extracted with 80% methanol, and the concentrated extracts were spotted on filter paper. The chromatograms were developed with *n*-butanol, acetic acid, and water (4:1:1) and then sprayed first with 2:6-dibromoquinone-chlorimide dissolved in butanol and subsequently with veronal buffer (pH 8.5). The blue spot was recognized at Rf 0.5. This spot is most evident in the claret (ca) strains, but indistinct in the ca<sup>2</sup> and other strains. A genetic analysis is in progress. As this compound is resolved into a diphenolic substance and a glucose after hydrolysis, it seems to be a glucoside similar to 3-hydroxy-4-O-( $\beta$ -glycopyranosido)-benzoic acid isolated from the cockroach (P.C.J. Brunet & P.W. Kent, 1956).

Metabolic products of tyrosine in D. melanogaster have been investigated by means of paper chromatography. About

Oster, Irwin I. Two unusual cases of white-variegation.

of their mutant character by addition of heterochromatin (usually in the form of an extra Y chromosome) have proved to exhibit this reaction. These cases were originally called "eversporting displacements" by Muller because he had found that their mosaic expression (i.e., an interspersing of mutant and non-mutant tissues) was caused by a chromosomal disarrangement; later observations by him (1935) and by Schultz (1936), independently, showed that in each case the euchromatic locus in question had been placed in the neighborhood of heterochromatin.

Thus far all known mutations resulting in the so-called variegated phenotype that have been tested for suppression

However, in 1946 (DIS-20: 88) Muller found a sex-linked recessive mutation of spontaneous origin which causes alleles of white, such as apricot, to become mottled. This mutation, designated "mottler of the white series" (mw), is located slightly to the right of cut and is not associated with a structural change of the chromosome. By using a scheme for introducing extra Y chromosomes into the male we have found that extra heterochromatin is without effect in suppressing the action of mw.

The second case was found by Mrs. Astrid Cicak, working in our laboratory, amongst the F<sub>1</sub> offspring of Y<sup>S</sup>.X InEN y.Y<sup>L</sup> sc<sup>8</sup> y<sup>+</sup> (no free Y) irradiated males mated to y<sup>2</sup> su-w<sup>a</sup> w<sup>a</sup> bb. = (no free Y) females. This fly was a male with variegated eyes (i.e., mosaic for red and white facets). When bred to females with attached-X's it yielded males which all showed mottling of the eyes. However, only about half of them were non-yellow (y<sup>+</sup>), whereas the rest had lost the sc<sup>8</sup> insertion (which carries y<sup>+</sup>) and therefore were yellow (y). This variegated mutation, designated "white mottled of Cicak" (wmC<sup>1</sup>), was found to have been caused by a change allelic to white. It was similar in appearance in both yellow and non-yellow lines. Although some double crossovers would be expected from breeding Y<sup>S</sup>.X InEN wmC<sup>1</sup> y.Y<sup>L</sup> sc<sup>8</sup> y<sup>+</sup>/y<sup>2</sup> v f car females, none were obtained in a total of 300 offspring; this indicates that wmC<sup>1</sup> is associated with a large structural change of the chromosome. Introduction of an additional free Y into the stocks did not alter the expression of the variegation.

These observations indicate that the phenomenon of variegation in D. melanogaster is not necessarily associated with heterochromatic disarrangements that are suppressible by extra heterochromatin.

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Paik, Y. K. Identification of a few uncertain species of *Drosophila* reported in DIS-30.

We have identified these as follows: *D. (Hirtodrosophila) sp.* = *D. (H) sexvittata*; *Amiota (Phortica) sp.* = *A. (P) variegata*; *Leucophenga sp.* = *L. (Trichiaspiphenga) argentosa*; *Mycodrosophila sp.--1* = *Mycodrosophila poecilogastra*.

In the last issue of DIS (p. 110) we reported a number of *Drosophila* species collected from May to October, 1956, of which some were recorded as uncertain

Paik, Y. K. Seasonal changes in *Drosophila* populations in two adjacent areas in Korea.

The purpose of the present article is to report the results of a survey of seasonal changes in population size, and of the sex-ratio balance, of wild *Drosophila* populations.

Samples were taken at two woodland areas around the foot of Mt. Mootung (1000 m in height), about five kilometers distant from the University. Collections were made, as a rule, at intervals of one week during the whole season from July, 1956, to June, 1957, by sweeping over large apple-baited trap-cans. At each of two areas four traps were placed in a row (10 m apart) at the fixed positions throughout the whole period. Baits were changed every week. All collections were done for three hours right before sunset in the late afternoon.

Our collections records show that a total of 12,918 flies were taken during the period in both areas. The area-1 collection consisted of 6082 flies, representing twenty-seven sympatric species, of which nineteen belonged to the genus *Drosophila* (including subgenera *Drosophila*, *Sophophora*, and *Pholadoris*) and eight to other genera of the family (*Amiota*, *Mycodrosophila*, *Microdrosophila*, and *Leucophenga*). The 6836 flies collected at area 2 represented twenty-five sympatric species, of which sixteen belonged to the genus *Drosophila* (including three subgenera, as in area 1) and eight to other genera (including the four found at area 1 plus *Scaptomyza*).

Changes at each of the two areas, showed two sharp seasonal maxima in size, one in the autumn (October-November) and the other in the spring (April). Total populations sank to an extremely low level, statistically zero, during the cold winter months (December-February), which can generally be considered a severe "population bottle-neck period" in our climate. Total populations also dwindled to a low level during the warm summer months (July-August). Results obtained here are in striking agreement with the pattern of seasonal changes in *Drosophila* populations of a temperate climate predicted by Professor Patterson (Univ. of Texas Pub. 4313: 203, 1943). The total population changes from month to month throughout the year were closely concordant with each other in the two populations at the two areas (correlation coefficient,  $r = 0.969$  and  $t = 12.402$ ).

Species-specific changes in the populations were also considered. Records of six species and two complexes of the genus *Drosophila* which were abundant or common throughout the year were selected for this purpose. Most of the selected species showed two yearly maxima, the rest one sharp maximum. Furthermore, monthly changes in relative frequencies were species specific. This is confirmed in some degree by computing the correlation coefficient ( $r$ ) for relative frequency of a given species in the two areas. Some of the results are summarized in the first table. The data used for figuring the correlations were the numbers of flies of a given species collected in a given month divided by the total number of flies of the genus *Drosophila* collected in the same month.

|                                   | Relative Frequency |        | Seasonal peak     | r     | t      |
|-----------------------------------|--------------------|--------|-------------------|-------|--------|
|                                   | area 1             | area 2 |                   |       |        |
| <i>D. auraria</i>                 | 8%                 | 11%    | autumn and spring | 0.964 | 11.463 |
| <i>D. transversa</i> -<br>complex | 14                 | 25     | autumn and spring | 0.928 | 7.876  |
| <i>D. nigromaculata</i>           | 3                  | 5      | autumn and spring | 0.763 | 3.439  |
| <i>D. cheda-lacertosa</i>         | 2                  | 3      | autumn and spring | 0.781 | 3.954  |
| <i>D. bizonata</i>                | 30                 | 30     | winter and spring | 0.998 | 49.921 |
| <i>D. coracina</i>                | 30                 | 16     | spring            | 0.955 | 10.177 |
| <i>D. lutea</i>                   | 6                  | 6      | autumn            | 0.991 | 23.409 |
| <i>D. suzukii</i>                 | 5                  | 4      | autumn            | 0.874 | 5.687  |

*D. bizonata* represents an interesting case. This is the only species that was present throughout the whole year. Only one female was trapped at area 1 in February, when the mean temperature was below zero centigrade; none of any other species was trapped in this month. Nevertheless, this species was trapped at the two areas in considerable numbers during the rest of the "population bottle-neck period," during which cold weather near the freezing point continued. In addition to this species, out of ten rare species collected at either one or both areas, seven, including *D. histrio*, *D. rubifrons*, *D. bifasciata*, *D. sternopleuralis* (in Okada's MS), *D. helvetica*, *D. sp.* (*quinaria* section), and *D. sp.* (subgenus *Drosophila*), were collected sporadically only in the winter months. *D. bizonata* was the most abundant of these species adapted to winter environment.

The common and abundant species were again selected for a study of sex-ratio balance in the populations. Some of the results are summarized in the second table.

| Species                           | Area | Females trapped | Males trapped | % female | % male | Chi square<br>1 d.f. | P           |
|-----------------------------------|------|-----------------|---------------|----------|--------|----------------------|-------------|
| <i>D. auraria</i>                 | 1    | 187             | 252           | 42       | 58     | 9.62                 | *           |
|                                   | 2    | 239             | 431           | 36       | 64     | 55.02                | *           |
| <i>D. lutea</i>                   | 1    | 163             | 228           | 42       | 58     | 10.81                | *           |
|                                   | 2    | 148             | 271           | 35       | 65     | 36.11                | *           |
| <i>D. suzukii</i>                 | 1    | 60              | 250           | 19       | 81     | 116.45               | *           |
|                                   | 2    | 54              | 186           | 23       | 77     | 72.6                 | *           |
| <i>D. bizonata</i>                | 1    | 1166            | 832           | 58       | 42     | 55.83                | *           |
|                                   | 2    | 1541            | 803           | 63       | 37     | 135.0                | *           |
| <i>D. cheda-lacertosa</i>         | 1    | 58              | 54            | 52       | 48     | 0.41                 | 0.8-0.7     |
|                                   | 2    | 110             | 74            | 60       | 40     | 7.04                 | *           |
| <i>D. nigromaculata</i>           | 1    | 85              | 81            | 51       | 49     | 0.1                  | 0.8-0.7     |
|                                   | 2    | 183             | 166           | 52       | 48     | 0.83                 | 0.5-0.3     |
| <i>D. transversa</i> -<br>complex | 1    | 431             | 490           | 47       | 53     | 3.78                 | 0.083-0.046 |
|                                   | 2    | 761             | 906           | 46       | 54     | 12.61                | *           |
| <i>D. coracina</i>                | 1    | 776             | 840           | 48       | 52     | 2.54                 | 0.157-0.083 |
|                                   | 2    | 443             | 528           | 46       | 54     | 7.44                 | *           |
| Totals                            | 1    | 2926            | 3027          | 49       | 51     | 1.71                 | 0.317-0.157 |
|                                   | 2    | 3279            | 3365          | 49       | 51     | 1.11                 | 0.317-0.157 |

\* Probability much less than 0.01.



The deviation from the expected 50:50 sex ratio is striking in a number of species; but in the total number of flies collected it is not significant. Furthermore, female or male preponderance in each species is not random in the two populations at the two areas, but always consistent. Whenever a discrepancy between the sexes is apparent, it seems to be due rather to a differential attraction to the bait than to a real preponderance of one sex; and the differential attraction to the bait seems to be species specific. A more critical study of this problem is being attempted.

Paik, Y. K., and Kim, K. W.

Local key to species of Drosophilidae collected so far in South Korea.

Since 1955 we have carried on field collections in several parts of South Korea, and a considerable number of species have been taken. Some of the collection records were reported in DIS-30. As

collection localities have been increased, we have attempted to survey the distribution patterns of these species, as summarized below. Roman numerals indicate (I) Mt. Moodung (located in Kwangju, Chunnam province), (II) Mt. Chiri (in Kurae, Chunnam province), (III) Mt. Hanra (in Chaeju, Quilpart Island), (IV) Mt. Sori (in Kwangnung, Kyongi province), and (V) Mt. Taepaik (in Hwangjee, Kangwon province).

| Species   | Localities |    |     |    |   |
|---|------------|----|-----|----|---|
|   | I          | II | III | IV | V |
| <i>Amiota alboguttata</i>                         | -          | +  | -   | -  | - |
| <i>A. variegata</i>                               | +          | +  | -   | +  | + |
| <i>Leucophenga argentosa</i>                      | +          | -  | -   | -  | + |
| <i>L. magnipalpis</i>                             | +          | -  | -   | -  | - |
| <i>L. concilia</i>                                | +          | -  | -   | -  | - |
| <i>L. maculata</i>                                | +          | -  | -   | -  | - |
| <i>L. quinquemaculipennia</i>                     | -          | +  | -   | -  | - |
| <i>L. ornatipennis</i>                            | +          | -  | -   | -  | - |
| <i>Mycodrosophila poecilogastra</i>               | +          | -  | -   | -  | - |
| <i>Mycodrosophila</i> sp.                         | +          | -  | -   | -  | - |
| <i>Microdrosophila</i> sp.-1                      | -          | +  | +   | -  | - |
| <i>Microdrosophila congesta</i>                   | -          | +  | -   | -  | + |
| <i>Microdrosophila</i> sp.-2                      | -          | -  | +   | -  | - |
| <i>Scaptomyza disticha</i>                        | +          | +  | +   | -  | + |
| <i>S. graminum</i>                                | -          | +  | +   | +  | + |
| <i>S. polygonia</i>                               | -          | -  | -   | -  | + |
| <i>Drosophila</i> (H) <i>alboralis</i>            | -          | +  | +   | +  | - |
| <i>D.</i> (H) <i>sexvittata</i>                   | +          | -  | +   | +  | + |
| <i>D.</i> (H) <i>quadrivittata</i>                | -          | +  | -   | -  | - |
| <i>D.</i> (H) <i>nokogiri</i>                     | -          | -  | +   | +  | - |
| * <i>D.</i> sp. of <i>Hirtodrosophila</i>         | -          | +  | -   | +  | - |
| <i>D.</i> sp. close to <i>histrion</i>            | -          | -  | +   | +  | + |
| <i>D.</i> ( <i>Dorsilopha</i> ) <i>busckii</i>    | +          | -  | -   | -  | - |
| <i>D.</i> ( <i>Pholadoris</i> ) <i>coracina</i>   | +          | +  | -   | +  | + |
| <i>D.</i> ( <i>Pholadoris</i> ) <i>puncticeps</i> | +          | -  | -   | -  | - |
| <i>D.</i> ( <i>Pholadoris</i> ) <i>rubifrons</i>  | +          | -  | -   | -  | - |
| <i>D.</i> ( <i>Sophophora</i> ) <i>suzukii</i>    | +          | +  | +   | +  | + |
| <i>D.</i> (S) <i>takahashii</i>                   | +          | -  | -   | -  | - |
| <i>D.</i> (S) <i>lutea</i>                        | +          | -  | +   | -  | - |
| <i>D.</i> (S) <i>melanogaster</i>                 | +          | -  | +   | +  | - |
| <i>D.</i> (S) <i>magnipunctinata</i>              | -          | -  | +   | -  | - |
| <i>D.</i> (S) <i>auraria</i>                      | +          | +  | +   | +  | + |

| Species                            | Localities |    |     |    |   |
|------------------------------------|------------|----|-----|----|---|
|                                    | I          | II | III | IV | V |
| D. (S) bifasciata                  | +          | -  | +   | -  | + |
| D. (S) sp. of obscura group        | -          | -  | +   | -  | + |
| D. (S) helvetica                   | +          | -  | -   | -  | - |
| D. (Drosophila) transversa complex | +          | +  | +   | +  | + |
| D. (D) nigromaculata               | +          | -  | -   | -  | + |
| D. (D) sp. close to kuntzei        | -          | -  | -   | -  | + |
| D. (D) testacea                    | -          | -  | -   | +  | + |
| D. (D) bizonata                    | +          | +  | +   | +  | + |
| D. (D) makinoi                     | -          | -  | -   | -  | + |
| D. (D) immigrans                   | +          | +  | +   | -  | - |
| D. (D) sp. of immigrans group      | -          | -  | +   | -  | - |
| D. (D) virilis                     | -          | -  | +   | +  | - |
| D. (D) sordidula                   | -          | -  | -   | +  | - |
| D. (D) lacertosa                   | +          | -  | +   | +  | + |
| D. (D) cheda                       | +          | +  | -   | +  | - |
| D. (D) repleta                     | -          | -  | +   | -  | - |
| D. (D) histrio                     | +          | +  | +   | +  | + |
| D. (D) sp. of quinaria section     | +          | +  | +   | +  | - |
| **D. (D) sp.                       | +          | -  | -   | -  | - |
| D. sp. of Drosophila               | +          | -  | -   | -  | - |

\*D. sp. of Hirtodrosophila is recorded in Okada's MS as D. histrioides.

\*\*D. (D) sp. is recorded in Okada's MS as D. sternopleuralis.

Paré, J. P., and Bell, A. E.

Lethal and sterility analysis of two populations of D. melanogaster under reciprocal selection for high fecundity.

In an experiment comparing various methods of selection for high fecundity, the population under individual and family selection was observed to plateau at generation 7 (Bell et al., 1955). A genetical analysis of this

population by the marked-inversion technique revealed that neither lethal nor sterility genes contributed to this lack of response to selection (Brown and Bell, 1955). A statistical analysis of these data indicated that the plateau was caused by an exhaustion of additive genetic variation even though nonadditive genetic variation remained (Bell et al., 1957).

A second method of selection compared in the original experiment was reciprocal selection, which is designed to exploit both additive and non-additive genetic variation by selecting for crossing or combining ability between two segregating populations. Response to selection under this method, although more modest initially, continued over a period of 40 generations, and thus the performance under reciprocal selection eventually exceeded that of the closed population under individual and family selection. Response to reciprocal selection soon plateaued, however, even though apparent genetic variation remained in the segregating lines. Since the reproduction fitness of these lines as measured by fecundity and per cent emergence was observed to decline during the course of the experiment, the possibility of deleterious recessive genes could not be ignored. In order for these genes to be maintained at frequencies higher than their mutation rates, they would be expected to possess at least one of the following properties: (1) exist as balanced lethals; (2) contribute to heterozygote superiority for within-line reproductive fitness,  $+/1 > +/+$ ; or (3) contribute to heterozygote superiority for fecundity in the cross-line progeny. This last point is significant in

view of the nature of reciprocal selection. Individuals selected to reproduce each segregating line are those whose cross-line daughters have high fecundity.

Using a marked-inversion technique similar to that described by Brown and Bell, 1955, eighty genomes were sampled from each of the two segregating lines under reciprocal selection, and carried through appropriate matings for producing "homozygous or isogenic" lines. Since some isogenic lines were derived from each segregating line, the suggestion of a balanced lethal mechanism is immediately ruled out. No sex-linked lethals were found. Tentatively, for chromosome 2, seven different (nonallelic) lethals have been identified in one line and four nonallelic lethals in the other line. Eight nonallelic third-chromosome lethals have been identified in the first line as compared with nine nonallelic third-chromosome lethals in the second line. None of the lethals found in either line were found to be allelic with any lethal from the other line. Matings are now being made to (1) measure the effect of these lethals when heterozygous on within-line reproductive fitness and (2) measure the effect of these lethals on fecundity when they are made heterozygous in cross progeny.

Plaine, Henry L., and Fradkin, Cheng-Mei. A sex-differential suppressor of erupt in the Swedish-b strain of D. melanogaster.

Practically all laboratory and wild strains of D. melanogaster tested carry the mutant gene "erupt" on the third chromosome and the suppressor of erupt on the second. The only difference in these strains is the strength of the

alleles at the two loci; nevertheless, the suppressor alleles are universally effective against the erupt alleles with which they are by nature in combination in the different strains. When the two loci of the various strains are recombined, however, the degree of effectiveness of a suppressor allele varies, depending upon its strength and that of the erupt allele with which it is in new combination. The erupt mutant also becomes manifested when the action of its suppressor is blocked by certain treatments.

Regardless of the strain tested, or of the nature of the treatment used to induce the phenotype, no differences between males and females have previously been obtained in either frequency or expression of erupt eyes. It soon became apparent that there was a considerable sex difference among the affected flies of a Swedish-b strain in which erupt spontaneously appeared. After five generations of selection for the phenotype, the culture was distributed into and continued as six sub-strains, five of them being selected for and one against the phenotype. The expression is always more extreme in the females, and there is, moreover, a significant difference between the low frequencies of affected males and the high frequencies of affected females through more than 45 generations. It was originally thought that a mutation at the suppressor locus could not account for this sex difference (DIS-29). From analyses of chromosome substitution tests and outcross tests, it is now evident that the suppressor locus, or at least the chromosome on which it is located, is solely responsible for allowing the mutant to be expressed differently in the two sexes. When the X, 2nd, and 3rd chromosomes of the erupt and suppressor-erupt strains are respectively replaced with those from the Swedish-b strain, only the Swedish-b 2nd chromosome, with its suppressor of erupt, yields a difference between the sexes. In outcrosses, the sex difference is greater when the Swedish-b chromosome is derived from the female parent.

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

Shortly after the appearance of erupt eyes in an otherwise wild-type Swedish-b strain (DIS-29 and this issue), a large number of spontaneous mutations were also obtained. To date, studies

of the mutation rate per locus for eight loci on the third chromosome have given  $2.2 \times 10^{-5}$  as the male rate. For seven loci on the 2nd chromosome, an average rate of  $2 \times 10^{-4}$  has been obtained for the male. In another series, based on twelve loci, the male rate was  $4.2 \times 10^{-4}$ . For the female, the average second-chromosome rate was  $7.4 \times 10^{-5}$ , based on twelve loci but less than 9000 flies. In the male, where repeated backcrosses may be made, there appears to be an increase in mutations as the number of generations tested increases; that is, after the initial introduction of the mutator into the heterozygote, there appears to be an increase in mutation rate per generation, per generation--at least for some loci being tested. It is striking that both the sex-differential suppressor of erupt and the high-mutating system seem limited to the second chromosome!

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

Rasmuson, B. Genetic analysis of an isoxanthopterin-determining gene in D. melanogaster.

In an investigation concerning amino acid metabolism after treatment with organo-phosphorous insecticides, chromatographic separations of free amino

acids in butanol : acetic acid :  $H_2O$  were made on some wild-type stocks of D. melanogaster. When examined in UV light, one of the fluorescent spots, the one due to presence of isoxanthopterin, was found to be almost absent in the Örebro stock. Head and abdomen were chromatographed separately for males and females of this stock and of a control stock containing normal amounts of isoxanthopterin. The difference could be attributed to the abdomen, and thus is probably due to the gonial pigmentation. It was much less pronounced in females than in males; the latter were therefore used in the following analysis. The amount of isoxanthopterin is highest in newly hatched males and decreases gradually with ageing. Animals of known age must therefore be used in quantitative estimations. The isoxanthopterin spot was eluted in  $NH_4OH$  and the intensity of the fluorescence measured with reference to a solution of kinin (0.25  $\mu g/ml$ ). For 8-day-old males the following values were obtained: Örebro stock--head 9.6, abdomen 6.8; control stock--head 7.9, abdomen 27.2. The gene responsible for the isoxanthopterin difference could be localized in the region between y and ec on the X chromosome. Among more than 200 crossovers from females heterozygous for the Örebro X chromosome and a chromosome containing y w<sup>e</sup> ec, no crossing over was found between the isoxanthopterin gene and the white locus. It can thus be supposed that this gene is an allele of white, but it does not influence the pigmentation of the eyes. A decisive test of allelism must be made with females heterozygous for the isoxanthopterin gene and the different known pseudoalleles of the white locus. Such tests are under way, but the small amount of isoxanthopterin in the females makes the technique more involved.

Rasmuson, M. Unequal crossing over in the Bar region of D. melanogaster.

Unequal crossing over in the Bar region of the X chromosome gives rise to offspring with reverted + or BB eyes. The frequency of such crossover offspring

was studied in crosses between f B od car/+ B + + females and f od car males,

with the intention of finding clusters of these phenotypes, which would indicate that crossing over had taken place in gonial cells. It was possible to raise the frequency significantly by means of high-temperature (31° C) treatment of the females for 48 hours. An increase from 0.133% to 0.206% was found in the offspring from eggs laid 5-10 days after the start of the heat treatment. It was accompanied by an increase of the crossover frequency in the region f to car. X-ray treatment (2500 and 4000 r) did not increase the frequency of unequal crossovers, nor the percentage of crossing over between f and car. The appearance of clusters of unequal crossovers was searched for by comparison with the Poisson distribution. The X-ray experiments, which were the most extensive, revealed a significant departure from the expected distribution. However, an analysis of the cases in which more than one unequal crossover appeared in a batch of offspring did not indicate any clusters that could have arisen from a single gonial crossing-over event.

Ronen, Amiram. Fluorescent substances in eyes of the Plum phenotype.

Chromatograms of heads of flies carrying the inversion Pm (dp, b, balancer) on a background of Berlin chromosomes were compared with chromatograms of wild-type

Berlin. The solvent employed for unidimensional ascending chromatography was Propanol-1% ammonia solution (2:1) and the chromatograms were developed in an atmosphere saturated with collidine. In addition, separation was improved by the following procedure. After being run for 3 1/2 hours at 26° C, the chromatograms were dried, and a narrow strip, containing the start points and Fl 1, was severed horizontally from the upper part, containing Fl 3 and all subsequent spots. The upper strip was then further developed in the same dimension for another 2 1/2 hours. The fluorescent spots were cut out and eluted in 3 cc double distilled water each, and after 24 hours their fluorescence was measured in a Farrand Fluorometer. Filters employed for Fl 1 were 436 mμ as primary and 557 mμ as secondary, and those for all other spots were 365 mμ as primary and 436 mμ as secondary. The designation of the spots which is adopted here corresponds to that of Hadorn & Mitchell (1951) and Hadorn & Schwink (1956). A bluish fluorescent spot, which appeared between Fl 3 and Fl 4, and which was particularly distinct in chromatograms of the Plum phenotype, is provisionally referred to here as Fl 4 A. (Further tests are necessary before it can be decided whether Fl 4 A and Fl 4 B of the present note are identical with the two substances described by Forrest and Mitchell, 1955, under these names and identified by these authors as 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine and 2-amino-4-hydroxypteridine, respectively.)

Correction for possible differences in eye size between the two phenotypes could be dispensed with in the present case, since planimetric measurements of camera lucida drawings of the eyes indicated almost complete identity in size (mean of 30 eyes of Berlin males, 36.17±0.45; mean of 30 eyes of Plum males, 36.33±0.47).

Besides a marked reduction in the brown (density of start-points) and the red (Fl 1) pigments, the Plum phenotype is characterized by a relative increase in the concentrations of Fl 4 A and Fl 4 B and Fl 5 (see table). Fl 7 is also greatly increased in Plum chromatograms, but could not be measured with the available filters. A study of the Plum position effect in different genetic combinations should indicate whether normalization (increase) in the concentration of Fl 1 is correlated with normalization (decrease) in the amounts of Fl 4-5.

Following are the results of a comparison of 10 chromatograms each of four male heads of Berlin with 10 chromatograms each of four male heads of Pm.

| <u>Spot</u> | <u>Berlin flies</u> | <u>Pm flies</u> |
|-------------|---------------------|-----------------|
| F1 1        | 7.1±0.5             | 1.3±0.3         |
| F1 3        | 2.5±0.4             | 2.8±0.7         |
| F1 4 A      | 2.1±0.8             | 4.5±0.3         |
| F1 4 B      | 3.9±0.8             | 8.4±0.6         |
| F1 5        | 1.2±0.2             | 2.8±0.3         |

Ronen, Amiram. Variable success of crosses between D. melanogaster and D. simulans.

It is well known that the interspecific cross between D. melanogaster and D. simulans succeeds more readily when D. melanogaster is the female parent.

Using virgin males and females of specific ages as proposed by Uphoff (1949), our mass matings in either direction have usually given results which were comparable to those of Sturtevant (1929) and of Uphoff. Thus one series of mel. x sim. matings yielded 63% of successes, whereas among the reciprocal crosses only 5% were fertile.

A strain of D. simulans collected in Tel-Aviv-Jaffa, Israel, by Mr. F. Gruber has recently given an unusual percentage of successes when utilized as female parent, and the results of the reciprocal cross are unexpectedly poor (see table). Since two different laboratory strains of D. melanogaster (Berlin and Cy L/Pm) were employed in each combination, we must conclude that the aberrant proportions of successes and failures were due to the genetic constitution of the simulans strain.

| Type of cross         | No. of "creamers" | No. fertile | % fertile | % females among hybrids |
|-----------------------|-------------------|-------------|-----------|-------------------------|
| <u>mel. x sim.</u>    |                   |             |           |                         |
| Berlin x <u>sim.</u>  | 38                | 1           | 2.6%      | 100%                    |
| Cy L/Pm x <u>sim.</u> | 33                | 1           | 3.0%      | 100%                    |
| <u>sim. x mel.</u>    |                   |             |           |                         |
| <u>sim. x Berlin</u>  | 69                | 36          | 52.2%     | 1.0%                    |
| <u>sim. x Cy L/Pm</u> | 88                | 27          | 30.6%     | 0.5%                    |

Sandler, L. Exchange in tandem compound ring X chromosomes.

A tandem compound ring X chromosome (without useful heterozygous markers) has been synthesized by Novitski (1954).

This ring spontaneously converted, by crossing over, to a reversed compound ring, data from which have also been reported by Novitski. One of the major difficulties in interpreting these data is the lack of information on the relative viability of the compound ring-bearing female class. The present writer has recently made a study of newly synthesized reversed rings that do contain heterozygous markers, and by a comparison of the results of his study with that of Novitski, can make a strong argument indicating that there is no appreciable depression in viability of classes in the random ring

experiments. This being so, it is interesting to re-examine, briefly, the results from those experiments.

Novitski's results (with all numbers corrected for meiotic loss) from a cross of females carrying a tandem ring and FR2 by YSX.YL, y B males are: regular ♂♂ = 3,012, exceptional ♂♂ = 302, matroclinous ♀♀ = 245, single ring-bearing ♂♂ = 913, single ring-bearing ♀♀ = 912.

From a tetrad analysis for the tandem ring (for details, see Novitski, 1954), it can be seen that one-eighth of all double exchanges result in equal bridges that yield exceptional males (Novitski, 1955). If there is no other source of such males, then the frequency of double exchanges in the tandem ring becomes 80 per cent ( $8 \times 302/3,012$ ); a surprisingly high value. Now, the tetrad analysis also shows that single rings produced from double-exchange tetrads come from dyads in which single rings separate either from single rings or from triple rings. In the latter case, it is probable that the single rings would be included in the functional egg nuclei with a frequency greater than 50 per cent (Novitski, 1951). It is, unfortunately, not possible to state exactly what this frequency is, but it has been shown (Novitski and Sandler, 1956) that when single rod chromatids separate from triple rod chromatids (in asymmetric dyads at anaphase II), the single rod is almost always recovered in preference to the triple rod. If this same situation obtains for the ring dyads, then it is the case that all the single rings recovered from the tandem ring are accounted for by double exchanges. It is true, however, that there is a deficiency of recovered tandem rings, the reason for which is not obvious at present.

Although it is very possible that there are other sources of exceptional males in tandem ring experiments, and it is also possible that the degree of nonrandom disjunction is less than that postulated here (either possibility would probably be indicative of some frequency of single exchanges), it is also conceivable that these estimates are reasonable, in which case the reduction (or absence) of single exchanges previously noted for reversed acrocentric and reversed ring compounds (Sandler, 1954 and in press) might extend also to tandem ring compounds.

Schalet, Abraham P. A spontaneous inter-chromatid exchange involving the Notch region.

Among the offspring of a cross of males ( $P_1$ ),  $\underline{1J1}^+.Y/\underline{1J1} \text{ sc}^{J1} \text{ In49 } B^{M1}$ , by females ( $P_1$ ),  $\underline{1J1} \text{ sc}^{J1} \text{ In49 } v B^{M1}/\text{"Maxy-1"}$  (see Muller and Schalet, this issue) a

single mosaic female was found, having one wing showing a typical Notch phenotype with respect to notching of the tip and thickened veins with deltas. The other wing was closely examined and appeared to be wild type; furthermore, no noticeable irregularity of thoracic hairs or of either eye was detected. The female had already mated with a brother ( $P_2$ ),  $\underline{1J1}+Y/\underline{1J1} \text{ sc}^{J1} \text{ In49 } v B^{M1}$ , but was in addition crossed with other males ( $P_2$ )  $\text{sc}^8.Y/\underline{y} \text{ sc}^{S1} B \text{ InS}$ .

From these matings at least two derivatives of the original X chromosome from the  $P_1$  male were recovered. Stock 1: All  $F_2$  males that survived to the imaginal stage showed a variable irregular thickening or extra veins along one or more longitudinal veins and sometimes at the distal ends of the veins. Irregular thickenings or deltas frequently were present at crossveins. Some  $F_2$  females showed the same characteristics as the males, but to a lesser degree and grading to wild type. Stock 2: Other  $F_2$  females showed a typical Notch phenotype with respect to wing characters and thoracic hairs at least.



Males from stock 1 were crossed to females from a spontaneously arisen Notch stock of independent origin in which all the females regularly show a Notch phenotype. However, all female offspring of this cross were wild type even though they carried a Notch chromosome. Females from stock 2 were crossed with split (spl) males. The pseudodominant expression of split in the female offspring receiving the Notch-type chromosome confirmed the genotypic designation of Notch. Unfortunately, stock 2 was accidentally lost soon after this confirmation was made.

These results indicate that the derivative of the original paternal X chromosome represented by the chromosome of stock 1 carries a duplication for the Notch region. This interpretation is supported by the similarity, in its phenotypic expression and suppressor action, to the published reports of Dp(1;1)Confluens found by Gottschewski and analyzed by Schultz (see Bridges and Brehme). In the present case, designated Confluens<sup>2</sup>, a crude localization has placed the duplication at  $4.5 \pm 1.1$  units from yellow. Furthermore, in addition to the duplicated chromosome being present in a portion of the gonads of the F<sub>1</sub> female, another portion of the gonads and also a part of the soma must have carried a chromosome deficient for at least part of the region represented by the duplication.

As in the original Bar case, the interchange must have arisen in a mature spermatozoon or one of the initial cleavage stages as a consequence of two nearby breaks in a chromosome before or after its formation of chromatids. If the breaks occurred before chromatid formation, the small piece between the breaks of one of the resulting chromatids could have been inserted into the sister chromatid, along with the piece proper to it, to produce a duplication in tandem or in reverse order. This would produce a complementary deficiency in the other chromatid. In the event that two very close breaks were produced in each chromatid of an already duplicated chromosome, an asymmetric rejoining of broken ends would produce one tandemly duplicated chromatid and one deficient chromatid.

Thus far no attempt has been made to obtain females homozygous for the duplicated chromosome, to test for crossing over with the recessives of the Notch region or to obtain reversions to wild type or changes to more extreme type by unequal crossing over. Confluens<sup>2</sup> is represented (as stock f28) in the current Bloomington stock list and will be available for anyone desiring to analyze the duplicated region cytologically.

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Seto, F. Delay in pupation of pupal lethals.

It was observed in the study of some balanced lethal strains that lethal pupae were found consistently in the lower part of the culture vial, near or in the food, whereas the Cy/1 pupae were found higher up the wall of the container. Furthermore it had been noticed in previous observations of crowded cultures that the earlier-emerging larvae pupated at higher levels than those that emerged later, many of the latter pupating in the food itself. This difference in pupation levels between the lethal homozygotes and heterozygotes was perhaps indicative of a difference in pupation time. The validity of this supposition was tested in the following simple manner. Eggs from the balanced stocks were collected within a 12-hour period and incubated in vial cultures at 25° C. Upon emergence of the larvae from the food, they were separated into two

groups: vial A, containing the larvae which pupated within the first 24 hours; and vial B, containing the remaining larvae. The separation was accomplished by either removing the larvae individually or scooping up the food with the remaining larvae and transferring them to a second vial (B). Since practically all the larvae of strains N1A, N55, and N25 emerged from the food during the first 24 hours, the procedure had to be modified by shortening the separation time from 24 hours to 12 hours.

In all cases vial B contained a higher percentage of lethals than vial A. This difference clearly indicates that the lethal larvae emerged to pupate later than their heterozygous culture mates. Moreover, it appears that the lethals with the earlier-acting effects show greater delay in pupation than those with effects appearing later in development. The study indicates that part of the total pattern of damage of pupal lethal factors extends to a varying degree into the "larval stage" and influences the time of emergence.

Seto, F. Effects of crowding on the time of action of some pupal lethals.

In a previous study in which the times of action of recessive lethals were determined, vial cultures of various population sizes were used to obtain the

data. It was noticed at that time that crowding tended to reduce the number of lethal homozygotes appearing at the expected stage of development. To test whether this crowding effect was important in altering the time of action and if it was equally effective for all the pupal lethals studied, the following plan was carried out. Vials containing the same amount of food but varying in the number of eggs were started. At intervals, egg-hatch counts, pupa counts, and adult counts were made. Special attention was given to the time of expression of the lethals within the pupation period. Four population sizes in replicates were studied: 25 in 30 replicates, 50 in 20 replicates, 100 in 10 replicates, and 200 in 10 replicates. Population size of 400 was started, but the excessive mortality and overcrowding made scoring difficult. In a few strains the proportion of lethals was the same in all four population sizes; in others, with an increase in number there was either a reduction in the proportion of lethals or the lethals did not develop as far as expected. The results of the study of thirteen pupal lethals are summarized in the table. (L-Pr = larval-prepupal; EP = early pupal; LP = late pupal; I = imaginal.)

| Lethals | Time of action | No effect | Reduction in no. of lethals | Earlier expressions of the lethals |
|---------|----------------|-----------|-----------------------------|------------------------------------|
| N42A    | L-Pr           | *         | -                           | -                                  |
| N61     | L-Pr           | *         | -                           | -                                  |
| X3      | L-Pr           | *         | -                           | -                                  |
| N51     | L-Pr           | -         | *                           | -                                  |
| N50     | EP             | -         | *                           | -                                  |
| N32     | EP, (IP)       | -         | -                           | Fewer LP's                         |
| N4      | EP, (IP)       | -         | -                           | Fewer LP's                         |
| Co7     | EP, (IP)       | -         | -                           | Very few LP's                      |
| Co3A    | EP, LP         | -         | -                           | Fewer LP's                         |
| N1A     | LP             | -         | -                           | Fewer LP's, many EP's              |
| N55     | LP, (I)        | -         | -                           | Many EP's                          |
| N13     | LP, I          | -         | *                           | -                                  |
| N45     | LP, I          | *         | -*                          | -                                  |

In the lethals which normally show their effects before the eversion of the cephalic complex (larval-prepupal lethals) or at the time the adults emerge, there appears to be no change due to crowding. In the others, with an increase in population size (especially at 200), there is either a reduction in the number of lethals or an earlier manifestation of lethality. In the lethals which display their effects during early and late pupation, crowding increases the early class at the expense of the late class. The apparently negative results for the three L-Pr (N42A, N61, and X3) and the one LP (N45) lethals may simply indicate inability to score the particular stage of action. It is not possible to partition the prepupal stage as can be done with the pupal stage, which is of long duration and has early, middle, and late phases that can be easily distinguished by visible changes in the developing fly. Although no details are presented here, crowding has only a minor effect. Although the number of lethals and/or the time of expression may be modified, each lethal still manifests itself within a definite ontogenetic stage.

Work is now under way to determine the effects of pupal lethals in combination. The preliminary results indicate that there is no complementary effect between the lethals taken two at a time which extends the development of the pupae. As would be expected, the time of action is determined by the earlier-acting lethal, and often the combination results in an earlier cessation of development than is manifested by either lethal singly.

Shiomi, T. Lethal effect of In(1)-T(1;4) in *D. virilis*.

This strain was obtained by Oshima through repeated crosses between *D. virilis* and *D. texana*. A distal

translocation is present between the X and fourth chromosomes, and an inversion ranging from the break point of the translocation to the proximal end of the euchromatic region is recognized on the X chromosome. The hemizygous male and the homozygous female as regards the aberrant X are lethal. The lethal effect appears at two embryonic stages; first, before blastoderm formation; and second, about the stage of tracheal formation. Approximately a quarter of the eggs do not form the blastoderm, and most of the nuclei formed inside may not be able to migrate towards the periphery of the egg. At about the stage when the tracheal system becomes almost complete, retardation of development is observed in 20% of the eggs, which fail to hatch. The few larvae which do hatch from such eggs soon die.

Sondhi, K. C. Identification of the fourth linkage group in *D. subobscura* with element E.

Flies homozygous for the mutants poppy and pointed, from the fourth linkage group, were crossed to the B and K inbred lines, both structurally homo-

zygous for all the chromosomes. From the F<sub>1</sub> two sets of backcrosses were made and fifteen cultures of each were counted. The following results were obtained:

|   | n.c.o. | c.o. | Total | Recombination |
|---|--------|------|-------|---------------|
| B | 2150   | 758  | 2908  | 26.06%        |
| K | 3160   | 1060 | 4224  | 25.09%        |

Maynard Smith, Clarke, and Hollingsworth (1955) described the chromosome orders for the B and K inbred lines. According to them, only the E chromosome, out of the four long autosomes, has the same order (alpha) for both the B and K inbred lines. The fact that pp pt yield the same recombination values with the B and K inbred lines is suggestive of their identification

with element E. As a check, the pp pt flies were also crossed to the NFS inbred line, which has a different order for element E. F<sub>1</sub> flies were backcrossed to pp pt, and fifteen cultures were counted. Out of 2820 flies, only 4 crossovers were obtained. This confirms the previous findings.

Spiess, E. B. Effect of Tegosept M on rate of development in D. persimilis.

During experiments on survival and rate of development of pre-adult stages in D. persimilis at 16° C, it was accidentally discovered that the amount of mold preventive (Tegosept M) being used was only 10% of the amount specified in the food formula (cream of wheat-molasses: Spassky, DIS-17, 1943). As soon as the correction was made and the amount of Tegosept was multiplied by ten, it was noted that a number of strains were slowed down in development. To be certain that the retardation was brought about by the Tegosept and not by some temperature or other environmental fluctuation, the following test was made at cold temperature (12° C). Two "normal" strains of homokaryotype WT persimilis were mated (about one dozen pairs per half-pint bottle) and food was provided on plastic spoons. Eggs were collected every day and planted in lots of ten, five lots per vial, on the surface of slanted food medium in glass vials (9.5 x 2 cm). Tegosept was added to the cream of wheat-molasses food in the following proportions to the Spassky formula: 0x, 0.1x, 1x, and 2x. (The Spassky medium calls for Moldex at about 0.06% of the liquid constituents by weight.) Ten vials containing 50 eggs each were made up for each proportion, and all were run simultaneously. The following data on survival and rates of development were collected. (N = average no. survivors; Days = average no. days from egg to adult.)

| 0 x      |           | 0.1 x    |           | 1 x      |           | 2 x      |           |
|----------|-----------|----------|-----------|----------|-----------|----------|-----------|
| N        | Days      | N        | Days      | N        | Days      | N        | Days      |
| 15.4±2.2 | 36.7±1.38 | 21.2±2.9 | 36.4±1.55 | 23.3±3.4 | 38.6±1.64 | 24.0±2.6 | 61.3±1.87 |

None of the differences in survivors is significant. (The largest difference, 5.8 between 0x and 0.1x, gives a "t" value of 1.606, d.f. 18, p = 0.12.) Cultures with 1x Tegosept took longer to develop than cultures with no mold preventive or only a trace (0.1x). The difference of 2.2 days is highly significant, with a "t" value of 2.51 (d.f. 18, p = 0.02.) Cultures with 2x Tegosept took about 1.6x the days to develop that cultures with 1x Tegosept took, or about 1.7x as long as cultures lacking Tegosept. Tegosept is not toxic, apparently, but must be used with care in developmental rate studies. The trend of increase of average survivors with increasing Tegosept may be real in that the mold preventive suppresses growth of micro-organism contaminants which might have antibiotic activity on the larvae.

(This work has been supported by Contract AT(30-1)-1775 with the U. S. Atomic Energy Commission.)

Strangio, V. A. Genic interaction in D. melanogaster.

The researches of Stern (1943) and House (1953) on the progressive obliteration of the distal tip of the fourth longitudinal vein in D. melanogaster have established the existence of an unidentified biochemical substance, P, responsible for the elaboration of pupal lacunae by a patterned inhibition of certain cells lying opposite one another in the dorsal and ventral laminae. Recent work in this laboratory has

centered on the behavior of P above the postulated "100%" level of the normal phenotype (House, 1954). It is suggested that excess P invades the erstwhile intervein material, producing extra venation and/or a weakening of interlaminal adhesion, with consequent blister formation during wing expansion. Investigation has shown that, in the heterozygous condition, each of three wing mutants at the extreme right end of the second chromosome (plexus, blistered, and balloon) induces a significant exaggeration of the third-chromosome dominant, Delta ( $Dl^3$ ), based on the presence or absence of large blisters in the adult wing. From a quantitative aspect, this method of estimating P-level for various markers is far from satisfactory. Further work is planned along lines likely to provide a more rigorous statistical basis by means of cancellation in plus/minus-P combinations with one group marker as a standard level (cf. the Delta-Hairless interaction).

Taira, T., Nawa, S., and Oshima, C.  
Pterin dehydrogenase in eye-color mutants of D. melanogaster.

by means of both paper chromatography and comparative fluorometrical measurements. It was found that pterin dehydrogenase had the highest activity at the pupal stage, and Oregon-R had the same enzymatic activity as v, se, bw, w, and  $w^a$ . However,  $Hnr^3$  had less coenzyme than the other strains. Furthermore, ry showed hardly any activity of the enzyme. This corresponds to the fact that isoxanthopterin was not detected in the body of ry, although AHP (2-amino-4-hydroxypteridine) was found in its body.

The activity of pterin dehydrogenase in Oregon-R and in the eye-color mutants v, se  $Hnr^3$ , ry, bw, w, and  $w^a$ , was investigated

Takada, H., and Okada, T.  
Occurrence of a new species of the virilis group in Hokkaido.

They will be reported as a new species, with the name D. ezoana, belonging to the virilis group. The phallic organs and egg-guide of this species are characterized as follows.

Twelve specimens, each of which is closely related to D. lit-toralis were collected in traps at eight districts in Hokkaido.

Phallic organs: Aedeagus pale brown, large, fusiform; darker at margin, and apically with divergent pale processes. Basal apodeme of aedeagus about half of total length. Anterior paramere small, pale brownish grey, fused to novasternum. Novasternal or hypandrial plates dark brown, pubescent, and each with a long stout submedian spine. Ventral phragma pale brown, darker at margin, nearly as long as broad, proximally narrowing with convergent margins, laterally almost straight, and rounded at the anterior tip.

Egg-guide: Lobe broadest subapically, rather rounded apically, tapering basally, and with about 18 marginal and 2 distal pointed teeth. Subterminal hair located near the tip of the lobe. Basal isthmus narrow and very short.

Tantawy, A. O. Crosses between parents of different body size, selected from random inbred lines of D. melanogaster.

each system five parallel random-mated lines were maintained. At 25, 50, and 75% coefficients of inbreeding, adults were measured and selected for long,

An inbreeding experiment (random matings) was carried out in which two different systems of matings were used, namely, brother-sister and double-first-cousins. In

medium, and short wing length. Such selected parents (large, medium, small) were crossed within each system of mating, that is, large x large, medium x medium, and small x small. The ten possible crosses were carried out, and heterosis was measured as ratio of average  $F_1$  to average parent size. The heterotic effect on wing length in such crosses is shown in the table.

| $F_x$      | <u>Brother-sister</u> |      |      | <u>Double-first-cousins</u> |      |      |
|------------|-----------------------|------|------|-----------------------------|------|------|
|            | 25%                   | 50%  | 75%  | 25%                         | 50%  | 75%  |
| Large x L  | 0.26                  | 1.64 | 4.57 | -1.14                       | 0.68 | 0.97 |
| Medium x M | 0.83                  | 1.42 | 3.98 | -1.74                       | 0.66 | 1.42 |
| Small x S  | 0.92                  | 1.44 | 3.93 | -2.15                       | 0.71 | 1.04 |

It is clearly shown that when the selected parents were crossed within each system, heterosis occurred in both cases. Heterosis of wing length increased gradually with increased homozygosity, and matings between sibs displayed greater heterosis than those between double cousins.

Selection of parents in crosses gives more heterosis than inbred lines crossed at random (DIS-29). The same results have been obtained with regard to percentage of emergence; those results are in preparation and will be published soon.

Tantawy, A. O. Limits of selection with sib matings in D. melanogaster.

Long selection experiments have been carried out to investigate limits of selection with brother-sister matings.

Two identical experiments have been maintained with four different selected lines, two of which were selected for long wings and the other two for short wings. The first experiment was carried out for twenty generations and the second for twenty-five generations. Before selection was started, heritability estimates were investigated by six progeny tests. The weighted means indicate that 43% and 45% of the total variances for wing and thorax length, respectively, are apparently due to additive genetic variance.

The results indicate clearly that selection is effective in both directions; selection for small size showed greater response than selection for large size. In all lines, selection limits were achieved at the fourth and fifth generations, respectively, in the case of long and short wings, after which there was a tendency for all lines to remain constant in size to the end of the experiments.

Phenotypic variance declined in the earlier generations of inbreeding and selection, after which it remained almost constant. When selection was relaxed, at the lower levels of inbreeding, all the characters studied returned back completely to the control level, whereas at higher levels relaxed selection had no effects.

Heritability of wing length was estimated at different levels of homozygosity, and the results are presented in the first table. It can be seen that heritability estimates declined in all the selected lines after the fifth generation, that is, 67.2% of inbreeding. In both experiments short-wing selected lines showed higher estimates than long-wing selected lines.

| <u>Generation</u> | <u>F<sub>x</sub></u> | <u>Experiment I</u> |              | <u>Experiment II</u> |              |
|-------------------|----------------------|---------------------|--------------|----------------------|--------------|
|                   |                      | <u>Long</u>         | <u>Short</u> | <u>Long</u>          | <u>Short</u> |
| 1                 | 25.0                 | 38.5 ± 5            | 42.3 ± 4     | 35.2 ± 4             | 40.5 ± 3     |
| 5                 | 67.2                 | 19.4 ± 6            | 23.2 ± 7     | 17.2 ± 4             | 25.3 ± 5     |
| 10                | 88.6                 | 6.2 ± 4             | 10.3 ± 6     | 5.3 ± 2              | 10.2 ± 3     |
| 15                | 96.1                 | 4.3 ± 4             | 7.6 ± 4      | 4.2 ± 3              | 8.3 ± 2      |
| 20                | 98.1                 | 3.2 ± 2             | 5.6 ± 3      | 6.2 ± 4              | 5.2 ± 4      |
| 25                | 99.5                 | -----               | -----        | 3.1 ± 2              | 4.3 ± 2      |

When selection was relaxed for five successive generations at the previous levels of homozygosity, the values for heritability estimates shown in the following table were obtained.

| <u>F<sub>x</sub></u> | <u>Experiment I</u> |              | <u>Experiment II</u> |              |
|----------------------|---------------------|--------------|----------------------|--------------|
|                      | <u>Long</u>         | <u>Short</u> | <u>Long</u>          | <u>Short</u> |
| 25.0                 | 40.2 ± 5            | 44.2 ± 3     | 45.2 ± 3             | 50.2 ± 4     |
| 78.5                 | 10.3 ± 4            | 16.3 ± 4     | 12.3 ± 4             | 10.3 ± 5     |
| 88.6                 | 6.2 ± 3             | 8.3 ± 4      | 4.4 ± 4              | 8.2 ± 2      |
| 98.6                 | 4.2 ± 2             | 3.0 ± 2      | 5.2 ± 3              | 5.5 ± 3      |

The results indicate that relaxed selection caused an increase in the heritability estimates at the lower level of inbreeding, that is, 25%, whereas at higher levels relaxed selection had practically no effect on the genetic variance of the selected lines.

Tantawy, A. O., and Mallah, G. S.  
Genetic resistance to heat in  
natural populations of D. melano-  
gaster and D. simulans.

Natural populations of D. melanogaster  
and D. simulans were sampled from dif-  
ferent geographical regions--one each  
from Lebanon and Uganda and three from  
Egypt. The Egyptian populations were

from: the University of Alexandria Farm; Wadi El Natroon, a desert isolated area halfway between Alexandria and Cairo; and Luxor, about 550 km south of Cairo. These five localities are designated as LB, UG, UF, WD, and LX, respectively.

D. melanogaster and D. simulans were found in all these localities except LX, where only melanogaster was found. However, a simulans population from Beni-Sweif (BS), about 112 km south of Cairo, was used.

All these ten populations were exposed to different temperatures--18°, 22°, 25°, 28°, 30°, and 31° C. The experiment is still going on, and lower and higher temperatures will be used. The characters being studied are: body size, that is, wing and thorax length; percentage of hatchability; and percentage of sterility. The results for wing length are presented in the table, as averages for both sexes, since males and females show almost the same reactions. The unit of measurement is 1/100 mm.

(see table on following page)



D. melanogaster

| Locality | 18° C  | 22°    | 25°    | 28°    | 30°    | 31°    |
|----------|--------|--------|--------|--------|--------|--------|
| WD       | 223.45 | 202.48 | 189.34 | 179.00 | 165.88 | 152.66 |
| UF       | 223.00 | 201.57 | 189.39 | 175.63 | 168.16 | 158.90 |
| IX       | 225.37 | 204.43 | 188.27 | 176.18 | 167.72 | 158.04 |
| LB       | 235.35 | 211.71 | 197.25 | 185.13 | 173.48 | 164.40 |
| UG       | 224.22 | 206.53 | 195.15 | 187.80 | 178.84 | 171.06 |

D. simulans

| Locality | 18°    | 22°    | 25°    | 28°    |
|----------|--------|--------|--------|--------|
| WD       | 204.23 | 197.30 | 174.30 | 147.99 |
| UF       | 199.12 | 204.01 | 173.57 | 158.38 |
| BS       | 201.04 | 203.21 | 174.77 | 158.99 |
| LB       | 202.82 | 205.56 | 176.20 | 159.01 |
| UG       | 214.16 | 200.73 | 186.17 | 166.55 |

The results demonstrate clearly that there are significant differences in wing length within all populations at different temperatures, that is, wing length is much greater at lower temperatures. D. melanogaster from LB has the longest wings at lower temperatures, and that from UG has the longest at higher temperatures. It can also be seen that D. melanogaster is larger than D. simulans. D.m. showed higher percentages of emergence than D.s.; this character appears to be greatly affected in all populations of D.m. and D.s. by higher temperatures. The best fertility was achieved at 25° in D.m. and 18° in D.s. Percentage of sterility increased with increase in temperature. In all the populations of D.m. studied, complete sterility was reached at between 30° and 31°. In four of the populations of D.s., it was reached at between 28° and 29°, but D.s. from the locality of UF is still fertile at 29° C.

These experiments are still under way, and the full results will be published very soon.

Thoday, J. M. Effects of stabilizing and disruptive selection.

Stabilizing selection is here defined as selection which favors the same (mean) phenotype in every generation. Disruptive selection is defined as selection

which picks out both extreme phenotypes in every generation. A population of D. melanogaster has been exposed to disruptive selection by mating opposite extremes for 35 generations (disruptive selection with negative assortative mating). Another has been exposed to disruptive selection for 23 generations by mating like extremes together and selecting both extremes from the offspring (disruptive selection with positive assortative mating). A third has been exposed to 23 generations of stabilizing selection. All are derived from the same wild stock. The character selected for measurement was number of sternopleural chaetae. Four single-pair cultures are used each generation. Heritability has been tested in these populations by taking out high and low directional selection lines and recording the divergence of their mean chaeta numbers. Divergence is greatest in the disruptive selection lines, least in the stabilized lines, and (at least in the first

generation) intermediate in the foundation wild stock. It therefore seems that disruptive selection may increase and stabilizing selection decrease the effective variety of chromosomes in a Mendelian population. Disruptive selection may therefore be capable of producing polymorphism, as Mather has suggested.

Toyofuku, Y. Further observations on chromosomal polymorphism in natural populations of *Drosophila* in Hokkaido.

In addition to those reported in DIS-30, thirteen different kinds of variations have been found by investigation of salivary-gland chromosomes. Among them were nine types of variation occurring in five strains of *D. nigromaculata*. The remainder were observed in *D. auraria*, *D. coracina*, *D. funebris*, and *D. histrioides*.

Tsukamoto, M. Cross resistance to insecticides in *D. melanogaster*.

In order to ascertain the relation between the DDT-resistance gene on the second chromosome and cross resistance to various synthetic insecticides, several series of selections with DDT, BHC, and Dipterex were carried out in the laboratory. A wild-type population established from a mixture of various strains other than the most resistant Hikone-R strain was divided into several groups, and selections were started in 1955-1956. After intensive selection with each insecticide, these stocks developed levels of resistance as high as the multiple-resistant Hikone-R strain, not only to the selected insecticide but also to DDT.

Genetic analyses suggest that DDT resistance and BHC resistance in the BHC-selected strain depend upon a second-chromosome factor, and that Dipterex resistance and DDT resistance in the Dipterex-selected strain are also linked with the second chromosome. The effect of other chromosomes, if any, is not so significant to resistance. Although an analysis for location on the chromosome has not been made, it is thought likely that these resistance factors have the same locus or closely linked loci, because of the similarity in development of resistance to the insecticides.

Ulrich, Hans. The influence of oxygen on the lethal action of X-rays on nucleus and cytoplasm of uncleaved *Drosophila* eggs.

Anterior and posterior halves of *Drosophila* eggs, 10-20 minutes old at the beginning of exposure, were X-rayed separately with various doses (50 kv, 10 ma, filter 1 mm Cellon, time of exposure constantly 3 min., target distance varied) in atmospheres of air or nitrogen. At this age the anterior half of the egg contains the nucleus (the two pronuclei) at stages varying between meiosis and first cleavage, whereas the posterior half does not contain any nucleus. The frequencies of nonhatching eggs were registered and corrected according to the rate of nonhatching eggs in non-X-rayed controls. The results of one experiment are tabulated below.

As reported previously (DIS-29, 170-171), the dose-effect curves obtained by X-raying the anterior or the posterior halves in air differ quantitatively and qualitatively. The new results show that the lethal action of X-rays is reduced by nitrogen in the case of the anterior halves as well as the posterior halves. The specific shapes of the dose-effect curves of the two halves obtained by X-raying in normal air are not modified by nitrogen.

Plotted semilogarithmically, both curves (air and  $N_2$ ) of the anterior halves are nearly straight lines. Both curves (air and  $N_2$ ) of the posterior halves, on the contrary, rise concavely in semilogarithmic plot.

| Anterior halves X-rayed         |             |                  | Posterior halves X-rayed           |             |                  |
|---------------------------------|-------------|------------------|------------------------------------|-------------|------------------|
| Dose<br>r                       | In air<br>% | In nitrogen<br>% | Dose<br>kr                         | In air<br>% | In nitrogen<br>% |
| 100                             | 11.0        | 9.8              | 20                                 | 18.0        | 19.6             |
| 200                             | 31.7        | 9.8              | 40                                 | 37.3        | 12.7             |
| 400                             | 43.7        | 16.7             | 60                                 | 39.8        | 19.9             |
| 600                             | 70.2        | 35.2             | 80                                 | 46.8        | 32.1             |
| 800                             | 70.8        | 39.6             | 100                                | 47.4        | 27.8             |
| 1000                            | 80.0        | 55.9             | 120                                | 57.9        | 37.7             |
| 1200                            | 79.8        | 60.8             | 140                                | 74.3        | 47.4             |
| 1400                            | 77.0        | 65.9             | 160                                | 85.7        | 60.4             |
| 1600                            | 85.1        | 68.4             | 180                                | 95.8        | 83.5             |
| 2000                            | 90.3        | 74.0             | 200                                | 95.3        | 78.7             |
| 2400                            | 87.4        | 78.0             | 240                                | 97.7        | 93.0             |
| 2800                            | 85.5        | 85.7             | 280                                | 98.5        | 99.9             |
| LD <sub>50</sub><br>about 500 r |             |                  | LD <sub>50</sub><br>about 95,000 r |             |                  |

Walén, Kirsten H. Unexpected mosaicism for  $y$  with four doses of  $y^+$ .

Flies of the constitution  $sc^7 w^a/sc^7 w^a$  with a centric ring fragment,  $y^+ ac^+$   $sc^+$ , and  $T(1;4) y^+ ac^+/ci ey^D$  had four representatives of the  $y^+$  locus.

Abdomen mounts showed about 21% mosaicism for yellow bristles, that is, on the average 2 yellow bristles per abdomen. This percentage is somewhat higher than that usually obtained with such mosaic-producing processes as somatic crossing over. Mosaicism for  $y$  was not found with the  $sc^7$  chromosomes alone or with the addition of either small duplication; it seems unlikely that the  $y$  spots can be attributed to position effect of the variegated type or to the somatic loss of either or both of the small duplications. The possibility of interaction of plus alleles to produce a recessive phenotype is being further investigated. Mosaicism for hairless spots, characteristic of the "shaved" appearance of  $sc^7$ , was found in 90 per cent of abdomen, and can readily be explained as loss of the centric ring fragment.

Williamson, D. L. Incidence of  $CO_2$  sensitivity in several *Drosophila* species.

Carbon dioxide sensitivity (death in  $CO_2$  at  $13^\circ C$ ) has been found in wild-caught *D. melanogaster*, *affinis*, and *athabasca*, and also in laboratory

strains of *tolteca*. The inheritance of this sensitivity in *affinis*, *athabasca*, and *tolteca* has not yet been determined but is being investigated. (The author is indebted to Dr. Nadine Plus, Laboratoire de Génétique Formelle, Gif-sur-Yvette, France, who, while at the University of Nebraska in 1953-57, instigated the author's interest in this research and kindly taught him the procedure and technique.)

Wild-caught *melanogaster* from Lincoln during August exhibited 1.97% sensitivity (84/4248) and during September .87% (18/2053).  $CO_2$  sensitivity

was found in smaller samples (October, 1957) from Beatrice (14/263 = 5.3%) and Blair (4/38 = 10.5%), Nebraska.

Wild-caught affinis from Chadron State Park, Nebraska (June and July, 1957) exhibited 25% sensitivity (41/163) and small samples from Lincoln (June, August, and September, 1957) 24% sensitivity (19/79).

Several samples of wild-caught athabasca from Alaska (July and August, 1957) have shown CO<sub>2</sub> sensitivity: 2 out of 47 (4%) from College, 38 out of 441 (8.6%) from the Matanuska Valley, and 52 out of 591 (8.8%) from Big Lake. (These were kindly furnished by Dr. D. D. Miller.)

Two laboratory strains of tolteca, Santa Maria de Ostuma (Nicaragua) and Chapulhuacan (Hidalgo, Mexico), were found to contain flies sensitive to CO<sub>2</sub>: Santa Maria de Ostuma, 24 out of 469 (5%); and Chapulhuacan, 14 out of 167 (8%). (These strains were kindly furnished by Drs. William Heed and Marshall Wheeler.)

Wolfson, M., Stalker, H. D., and Carson, H. L. A serious parasite of laboratory Drosophila.

In this laboratory recent investigations with D. parthenogenetica have resulted in the discovery of a parasitic protozoan, possibly a microsporidian, which

may result in a type of parasitic castration of males and may also drastically affect the longevity of flies of both sexes, depending upon the degree of infection. Infection may be so serious as to prevent hatching of pupae. Infected individuals can be recognized, upon dissection in saline, by the presence of spores in the tissues and body fluid. The spores are easily identified by their strikingly consistent size and shape and by an extremely thick and rigid capsule. They are ovoid in shape, 4-5  $\mu$  in length, and may occur singly in the body cavity or associated with tumor-like structures.

Spores were first noticed in the adult testis. In less severe cases, the infection may appear as growths on the surface of the testis. In more severe cases, the infection fills the lumen of the testis and is generally localized in the lower two-thirds of the testis just to the point of union with the seminal vesicle. Infection may be bilateral or unilateral and may differ in degree in the two sides. Unilateral and bilateral rudimentary adult testes were also observed and appeared to be the result of an early infection of the larval testis. Spores were also found to be associated with the adult fat bodies. Larvae from infected cultures contain spores in the lumen and wall of the intestine, and spores have also been found in the lateral oviducts of adult females; this may indicate infection per os and/or direct infection of ova.

This infection has also been noted in a strain of D. melanogaster (subgenus Sophophora), resulting in a marked mortality. Young larvae of D. paramelanica may be infected if grown on spore-containing food. Efforts are being made to determine the manner of infection and methods of control and extinction. It is hoped that this brief note will serve to alert other Drosophila workers.

Research Note - Received Late

Fritz-Niggli, Hedi. Qualitative and quantitative differences in the induction of dominant lethals in *Drosophila* by 31-MeV X-rays, 30-MeV electrons, and 180-keV X-rays.

In connection with other tests, we were able to prove the effectiveness of 30-MeV electrons and 31-MeV X-rays from a Betatron, as compared with ordinary X-rays, in inducing dominant lethals in *Drosophila*. The test consists in determining the percentage of unhatched

embryos in different periods after irradiation of the male parents. We mated 15 irradiated males (0-4 hours old), immediately after treatment, with 20 virgin females (3 days old) of an inbred stock (Sevelen). On the 4th, 7th, and 10th day the females were removed and new virgin females (3 days old) were mated with the irradiated males. Every day (28 hours after deposition of the last egg) we counted dead and living embryos hatched.

By this method we obtained the most exact radiobiological test with which we have ever worked. The experiments, repeated in six series with intervals of one month between, gave results with a variability for certain points of only  $\pm 2\%$  or less. The accuracy of this radiobiological experiment is usually found only in physical measurements. We irradiated with 30-MeV electrons (91 rad/min.) in air, with 31-MeV X-rays in a plexiglass container at a depth of 40.5 mm (93-108 rad/min.), and with 180-keV X-rays in a plexiglass container at a depth of 12.5 mm (78-102 rad/min.) (30- and 31-MeV X-rays from the Brown, Boveri Betatron). The dose measurements were done with a Victoreen condenser r-meter for 31-MeV X-rays and 180 keV, and simultaneously for electrons with an integrator (thin-walled ionization-chamber) calibrated with the Victoreen condenser r-meter.

In table 1 we can see a strong dependence of hatchability on the stage at which sperm are irradiated (1000 r ionization dose). We can see a low efficiency for all three types of radiation in the first brood (irradiated mature sperms), then an increase of sensitivity in the second brood from the 4th to the 7th day. In the first brood a sharp increase was observed at the 3rd day, but only after irradiation with rays of high energy. After the 4th to the 7th day the efficiencies of 31-MeV X-rays and 30-MeV electrons remained the same, in contrast to the higher efficiency of 180 keV.

Between the 7th and 10th days we found a different action for every type of radiation. The most effective were the 180-keV rays, then the 30-MeV electrons, and last the 31-MeV X-rays. The peak of sensitivity for 30-MeV electrons was later than the peak for the 31-MeV X-rays. The efficiencies of these two types of radiation must be qualitatively different.

Table 2 shows the relation of efficiency of 30-MeV electrons or 31-MeV X-rays to 180 keV with regard to RBE (relative biological effectiveness). A strict dependence of RBE on the age of irradiated gametes and also on dosage can be observed. In the first brood, ordinary X-rays were less efficient, the RBE was more than 1, but only at 1000 r. With 2000 r the RBE was lower than 1. From these very exact data we can conclude that comparison tests are only decisive when they are made with the same stage and the same dose, and under the same conditions.

In conclusion, we found four strictly marked stages of spermatogenesis with different reaction patterns. Stage 1 (0-3 days after treatment) is characterized by an indifference to different qualities of radiations. The same indifference is found to changes of milieu: absence of  $O_2$  (in press);

treatment with cyanide, acid, or dihydroxydimethyl peroxide (Sobels, F. H., *Nature* 177: 979-982, 1956); and fractionation of dose (Muller, J. *Genet.* 40: 1, 1940). Stage 1 involves mature sperms. Stage 2 (5th-7th day) shows a marked difference between radiations of high and low energy and also an increased dependence on milieu. Stage 3 (8th-10th day) differentiates among all three types of radiations and is very dependent on milieu. During this period sperms are used which were probably irradiated at meiosis, as we conclude from our experiments with irradiated pupae. Finally, stage 4 (11th-13th day; irradiated spermatogonia) is again indifferent. It is not easy to explain this result. As in previous experiments (inducing lethal factors with 31-MeV X-rays, killing mice, killing *Drosophila* embryos, stopping mitosis; (Fritz-Niggli, *Fortschr. Röntgenstr.* 80: 28-38, 1954), we found mostly a higher efficiency of ordinary X-rays. But in stage 3 the 31-MeV X-rays, with a great spectrum of energy and lower energy than 30-MeV electrons, have the lowest efficiency. We assume that for stage 1 (mature sperms) OH and H radicals are effective and for stage 3 (meiosis)  $H_2O_2$ -molecules, the production of which is sensitive to milieu and to linear energy transfer 31-MeV X-rays (mean energy 10 MeV) and 30-MeV electrons may have a small difference in linear energy transfer.

We hope that these experiments which have been started will furnish some solutions to the problem. The assistance of Miss H. Inauen and the measurements of 30-MeV-electrons by Dr. Sempert are gratefully acknowledged.

(Table 1 and Table 2 on opposite page.)

Table 1. Embryonic lethality in %. Each value represents the mean of six experiments with a total of 3000 eggs.

| Relation                 | Days between irradiation and insemination |      |      |      |          |      |      |           |      |      |          |      |     |
|--------------------------|---|------|------|------|----------|------|------|-----------|------|------|----------|------|-----|
|                          | Brood I                                   |      |      |      | Brood II |      |      | Brood III |      |      | Brood IV |      |     |
|                          | 1   | 2    | 3    | 4    | 5        | 6    | 7    | 8         | 9    | 10   | 11       | 12   | 13  |
| 30-MeV electrons/910 rad | 17.4                                      | 14.6 | 30.7 | 20.8 | 20.9     | 31.2 | 31.4 | 58.9      | 42.8 | 36.1 | 16.5     | 10.1 | 9.1 |
| 180-keV/930 rad          | 16.8                                      | 16.3 | 16.0 | 11.0 | 43.6     | 49.1 | 52.4 | 75.5      | 69.0 | 52.3 | 6.4      | 5.0  | 4.3 |
| 31-MeV X-rays/930 rad    | 17.3                                      | 13.9 | 28.6 | 19.9 | 19.0     | 33.9 | 34.6 | 22.3      | 22.3 | 19.3 | 10.8     | 7.9  | 7.2 |

Table 2. RBE in different days between irradiation and insemination

| Relation                 | Days between irradiation and insemination |     |     |     |          |     |     |           |     |     |          |     |     |
|--------------------------|---|-----|-----|-----|----------|-----|-----|-----------|-----|-----|----------|-----|-----|
|                          | Brood I                                   |     |     |     | Brood II |     |     | Brood III |     |     | Brood IV |     |     |
|                          | 1   | 2   | 3   | 4   | 5        | 6   | 7   | 8         | 9   | 10  | 11       | 12  | 13  |
| 30-MeV electrons/180-keV |   |     |     |     |          |     |     |           |     |     |          |     |     |
| X-rays                   | 1.0                                       | 0.9 | 1.9 | 1.9 | 0.5      | 0.6 | 0.6 | 0.8       | 0.6 | 0.7 | 2.6      | 2.0 | 2.1 |
| 30-MeV electrons/31-MeV  |   |     |     |     |          |     |     |           |     |     |          |     |     |
| X-rays                   | 1.0                                       | 1.1 | 1.1 | 1.1 | 1.1      | 0.9 | 0.9 | 2.6       | 1.9 | 1.9 | 1.5      | 1.3 | 1.3 |



## TECHNICAL NOTES

Cordeiro, A. R. Orcein with Janus Green B for better salivary-gland-chromosome staining.

A simple but valuable improvement of the classical aceto-orcein staining of *Drosophila* polytenic chromosomes is attained by adding 30 mg. of Janus

Green B for each 10 cc of aceto-orcein stain (orcein, 2 g; acetic acid, 60 cc; distilled water, 40 cc). The "Harleco" brand has been used. The dissection is made, as usual, in aceto-orcein only. The isolated glands or cerebellar ganglia are transferred to a drop of orcein with Janus Green B on a slide and then covered with a coverslide. The smear is made by the common technique. Advantages: The chromosomes are strongly stained by the dye mixture, and the cytoplasmic material remains lighter than it usually does with the aceto-orcein method. Ageing of the aceto-orcein-Janus-Green-B smears improves instead of reducing the contrast.

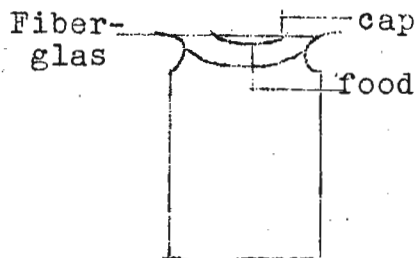
Fuscaldo, Kathryn E. A technique for the collection of bacteriologically sterile flies.

A method has been devised for the sterilization of large numbers of eggs of *Drosophila* for the purpose of maintaining stocks of sterile flies.

The method makes available the largest number of sterile eggs with a minimum of loss due to handling injuries, arrested development from chemical injuries, or mechanical loss through sterilization procedures.

Virgin males and females are collected and maintained separately for 4 days on cornmeal-molasses-agar medium. The flies are mated on the fifth day, using approximately two males to one female, for 24 hours. On the sixth day the females are separated and placed on fresh food for about 6 hours before egg laying, to allow for recovery from the effects of etherization.

The collection of eggs is accomplished as follows. A square, 2" x 2", of finely woven Fiberglas cloth is placed over a slice of solid medium about 1" square, which is put on the under surface of a half-pint-bottle cap. The cap is then inserted into the mouth of a half-pint milk bottle so that the



surface of the Fiberglas is presented to the flies. The food seeded with living yeast serves to induce the flies to lay their eggs on the surface of the cloth, which is of sufficiently close weave to prevent the eggs from falling through to the food. The fertilized females are placed in the bottle and allowed to lay eggs for 12 hours, after which the eggs are collected by removing the cloth containing them and securely tying the ends with cotton thread, making a small egg sac. This egg-containing sac can then be put through the sterilization procedure.

The sac is immersed in a 3% solution of Clorox for twenty minutes to dechorionate the eggs. After dechoriation, the eggs are rinsed in sterile insect Ringer's solution and then placed in a solution of Roccal, 1:10,000, for sterilization. This step takes about one hour. The egg sac is rinsed again in sterile Ringer's solution for 10 minutes (3 changes). After sterilization the sac is held by the bound ends over the mouth of a bottle of

sterile culture medium. It is then cut just below the binding thread and allowed to fall open onto the food. The larvae can then crawl into the food upon hatching. The sterilization procedure is carried out in a sterile transfer box equipped with three UV lamps. After 7-8 days of incubation, before eclosion of the flies, the sterility of each culture is tested by inoculating two slants of each of the following media: TGE, Sabourandes dextrose, and wort agar. Those culture bottles whose inocula produce growth on the agar slants are discarded as being contaminated.

Sterile cultures can be maintained indefinitely by subsequent transfer of adults to fresh, sterile medium. Such transfer is accomplished by shaking into new bottles in the sterile transfer box. The sterile culture medium is the standard cornmeal-molasses-agar medium without living yeast, to which 1 ml of folic acid (.036 g/100 ml 20% alcohol) has been added to each bottle after pouring. The medium is rendered sterile after preparation by autoclaving for 20 minutes at 15 lbs. The necessity of the folic acid was indicated in previous experiments, since larvae raised on unsupplemented sterile medium failed to pupate or eclose.

(Supported by a research grant, C-2440, from the National Institutes of Health, administered by Allen S. Fox.)

Hochman, Benjamin. A new counting plate.

Originated in 1956 by Dr. W. W. Newby, this counting plate embodies all the favorable features and none of the dis-

advantages of glass, paper, and painted metal plates currently in use. It is constructed of white formica, 1/16" in thickness. A sheet of formica measuring one square foot can be purchased at a builders' materials company for about one dollar. Employing a fine-toothed blade in a power saw, pieces of desired size can be cut. We find 3" x 5" makes a satisfactory plate size. The cut edges are smoothed with sandpaper. The smooth, hard formica surface provides an excellent background for examination of flies. Moreover, the surface is unaffected by ether or alcohol, is very resistant to needle scratching, and can be cleaned easily with soap and water. Unbreakable under normal laboratory conditions, some of these plates have had constant use for over a year without showing signs of wear. For reasons of economy these formica counting plates could be utilized profitably in genetics laboratory courses as well as by individual investigators.

Hollingsworth, M. J. A simple device for ensuring that *Drosophila* bottles contain an equal amount of food.

The spout is removed from a large polythene funnel and replaced by a glass tube made by removing the bottom of a 3" x 1" vial. The tube is fixed to the funnel with melted polythene from the

spout. The flow of food is controlled by two valves made from a length of glass rod and two rubber bungs. The upper bung fits snugly into the neck of the funnel when the rod is pushed down. The lower bung is tapered to ensure a good fit when the rod is lifted up. The rate of flow of the medium is controlled by adjusting the two bungs. Lateral play is eliminated by means of wire supports across the mouth of the funnel.

Jaeger, Celso P., and Jaeger, Euterpe C. Chemically defined medium for D. willistoni.

A chemically defined medium for D. willistoni was prepared by altering the Hinton et al. (1951) medium for D. melanogaster.

|                  | mg/ml |                                  | ug/ml |
|------------------|-------|----------------------------------|-------|
| L-Arginine       | 0.559 | Biotin                           | 0.020 |
| L-Cysteine       | 0.480 | Calcium pantothenate             | 16.0  |
| L-Glutamic acid  | 4.418 | Choline chloride                 | 575.0 |
| Glycine          | 1.745 | Pteroyl glutamic                 |       |
| L-Histidine      | 0.484 | folic acid                       | 3.0   |
| L-Isoleucine     | 1.260 | Pyridoxine                       | 2.5   |
| L-Leucine        | 2.345 | Riboflavin                       | 10.0  |
| L-Lysine         | 1.337 | Thiamine                         | 2.0   |
| DL-Methionine    | 0.339 | Niacinamide                      | 12.0  |
| L-Phenylalanine  | 1.008 |                                  |       |
| DL-Threonine     | 0.756 |                                  |       |
| L-Tryptophan     | 1.745 |                                  |       |
| L-Valine         | 1.355 |                                  |       |
|                  |       |                                  | mg/ml |
| Fructose         | 7.5   | KH <sub>2</sub> PO <sub>4</sub>  | 1.83  |
|                  |       | NaH <sub>2</sub> PO <sub>4</sub> | 1.89  |
| Cholesterol      | 0.3   | NaHCO <sub>3</sub>               | 1.4   |
|                  |       | Agar                             | 20.0  |
| Ribonucleic acid | 4.0   |                                  |       |

Meyer, Helen U. Obtaining completely relaxed and stretched live larvae of D. melanogaster.

It is sometimes desirable to obtain completely stretched-out and relaxed larvae for better microscopic observation *in vivo* or for other purposes.

This can be accomplished by placing young larvae, in a drop of water, into a container filled with nitrogen gas. After about 5 minutes the larvae have lost their muscle tone completely, they are limp and stretched out at full length. When brought back into air after this period, they regain their muscle tone, start the muscular contractions again, and resume their normal shape, apparently without any ill effects.

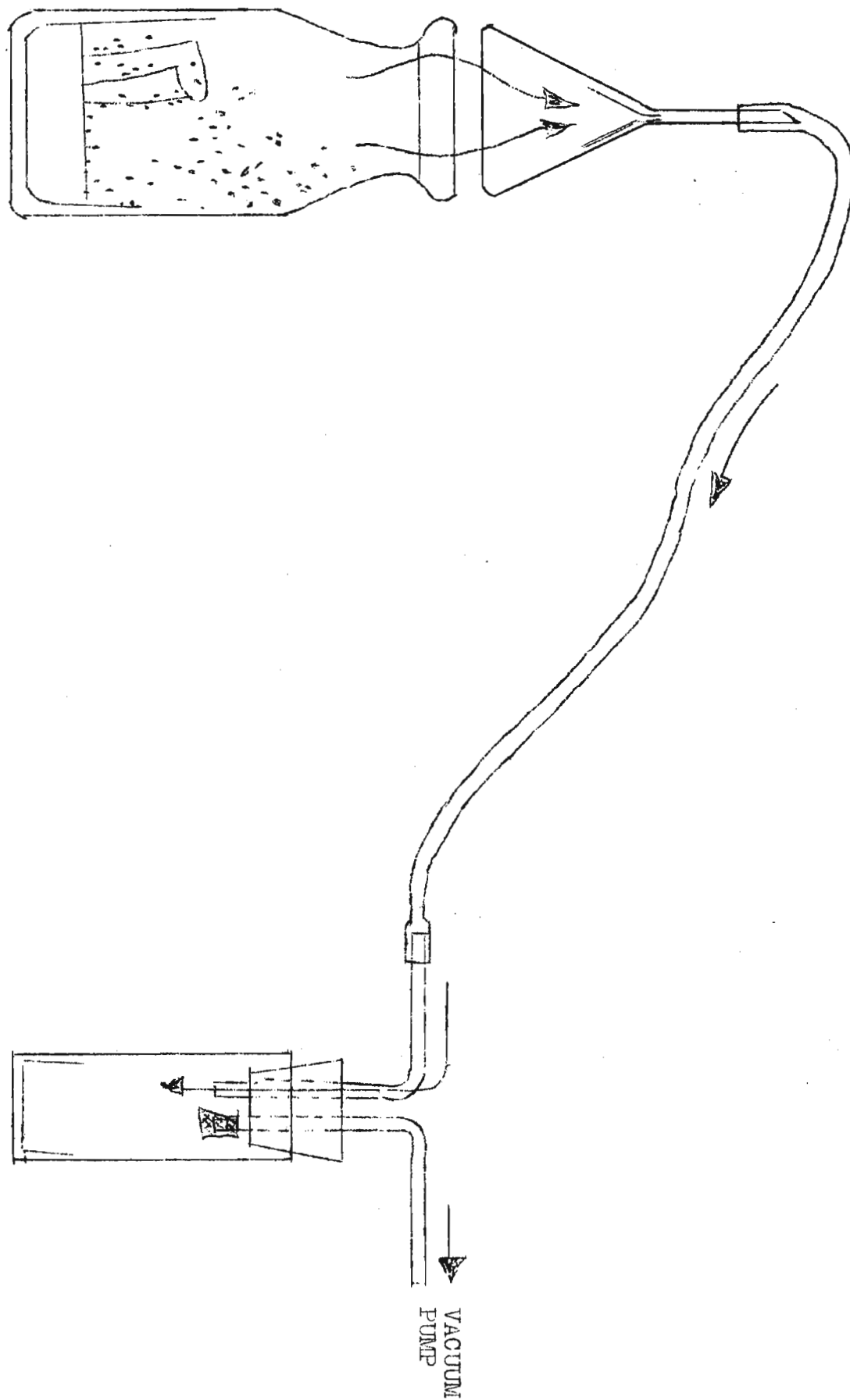
Nicoletti, B. A method for counting a large number of flies easily.

For counting a large number of flies from extremely crowded population bottles, when it is not necessary to consider the sex of the individuals,

the following system has been used. The etherized *Drosophila* are placed and uniformly distributed on the surface of a sheet (11.7 x 8.3 inches) of sensitized paper (Agfa Copyright). The paper with the flies is conveniently exposed and a positive photocopy is made (photoprint Dupleca, white light 150 volts/20 seconds). It is possible to detect the exact number of flies by the dark spots on the positive paper. The sheets are used for counting individuals, checking each mark with a colored pencil. On each sheet about 600-700 flies can be counted.

Nicoletti, B. System for collecting flies from population bottles.

The system shown in the drawing has been used successfully for collecting flies from extremely crowded population bottles. A funnel is placed on



the opening of the bottle and the flies are pulled into it by gentle impulses from the vacuum pump. The sucking produced by the vacuum carries the animals into the etherizing bottle. This prevents the standard medium from running and individuals from escaping. After the mass of the population has been transferred in this way, the funnel is replaced by a small glass tube, so that the few flies left walking on the sides of the bottle or on the paper can be collected. This latter arrangement is also useful for collecting etherized individuals without using forceps, saving a considerable amount of time.

#### MATERIALS REQUESTED OR AVAILABLE

A. R. Cordeiro requests the favor of receiving any available back issues of DIS. Address: Instituto de Ciências Naturais, Universidade do Rio Grande do Sul, Avenida Paulo Gama, Porto Alegre, Brazil.

W. M. Hexter (Department of Biology, Amherst College, Amherst, Mass.) would like a duplication covering the singed locus of D. melanogaster.

R. C. King is interested in obtaining stocks of female-sterile mutants other than those currently available at Northwestern (dor, sc<sup>Sl</sup>, sta, dm, sn<sup>1</sup>, sn<sup>2</sup>, sn<sup>3</sup>, sn<sup>4</sup>, sn<sup>5</sup>, sn<sup>34e</sup>, sn<sup>36a</sup>, sn<sup>50k</sup>, oc, gg<sup>2</sup>, lz<sup>s</sup>, ras<sup>4</sup>, ty, na, r<sup>9</sup>, fu, fuff, fu<sup>57a</sup>, fu<sup>57f</sup>, ds<sup>38k</sup>, ds<sup>52k</sup>, fes, rn, ap<sup>4</sup>, ap<sup>56f</sup>, fs2.1, cg, nw<sup>2</sup>, mi, mr, cmp, bf, and sv<sup>de</sup>).

E. Ortiz (Laboratorio de Citogenetica, Serrano 113, Madrid 6, Spain) would like to receive reprints that are available, and back issues of DIS.

Henry L. Plaine (Zoology Department, Ohio State University, Columbus) still desires to be notified of or to receive any new suppressors found in D. melanogaster. He would also like to be notified of any suppressors found in other species.

Duplicate copies of DIS numbers 3, 9, 11, 13, and 15 are available at the Department of Biological Sciences, Stanford University, Stanford, California. Laboratories requiring these particular issues to complete sets may inquire of Professor David Perkins.

## PUBLICATION AND NOMENCLATURE

Report of K. Brehme-Warren

The second edition of "The Mutants of *Drosophila melanogaster*" by Bridges and Brehme has been in preparation since March, 1957, and is well under way. The author has an extended leave of absence from college responsibilities and will be able to work full time on this volume until September, 1958. Investigators are urged again to send new material, and particularly corrections and new information on mutants already described in the 1944 edition and subsequent numbers of DIS. The deadline for such material is February, 1958.

Nomenclature: A report has been received from Dr. Ernst Hadorn of the meeting held in Zurich in August, 1957, of the International Committee on Genetic Symbols and Nomenclature. Only one departure has been made from the symbols used in the 1944 edition of Bridges and Brehme: enhancers are to be written En- or en- (examples: En-S, en-N<sup>B</sup>) instead of the shorter symbols E- or e- previously in use. This change will be adopted in the second edition. As no new terminology has been suggested for pseudoalleles, these will be described under their original names (examples: bx, Cbx; S, ast; w, w<sup>a</sup>), and their pseudoallelic relationships will be indicated in the descriptions and on the maps.

## ANNOUNCEMENTS

André Dreyfus Foundation.

Announcement of the International Genetics Prize for 1958.

In accordance with its statutes and regulations the Board of Directors of the Foundation André Dreyfus invites interested persons to register as ap-

plicants for the International Genetics Prize of 1958.

1. The International Genetics Prize for 1958 amounts to Cruzeiros 150,000 (one hundred and fifty thousand).
2. The prize is open to individual scientists or groups of research workers from any country, working on problems of genetics or related fields.
3. The International Genetics Prize is intended for the promotion of:  
(a) the development of research programs; (b) travel for purposes of research; (c) publication of the results in research or of monographic summaries.
4. Applications should be accompanied by: (a) the candidate's

curriculum vitae; (b) a list of publications; (c) a detailed plan of the research program proposed or a copy of the manuscript for publication. Note: In the case of a team of research workers, the application should be signed by one of its components.

5. In case of equality of qualifications, preference will be given to the project which may have more direct influence on the development of genetical research in Brazil.

6. Applications accompanied by supporting documents should be received by the Secretary General of the Foundation at the address below not later than the 31st of January, 1958. (Jenny Dreyfus, Secretária Geral da Fundacao-Premio André Dreyfus, Rua Belfort Roxo 40, apto. 502, Copacabana, Rio de Janeiro, D.F.)

Creighton, Harriet B. A request from the Travel Assistance Committee for the Genetics Congress.

A number of geneticists will be coming to the International Genetics Congress in Montreal in August, 1958. Undoubtedly, some of them can come before the

Congress and some can stay after the Congress. Undoubtedly, also, some would like to visit laboratories in the United States, but to do so they will need dollars. The Travel Assistance Committee for the Congress is anxious to know which laboratories would like to help foreign geneticists by inviting one or more either to give a lecture or to come as a consultant on research. If you have any funds for lectures or for consultations which you could allocate to geneticists, would you let the Committee know as soon as possible. (Harriet B. Creighton, Department of Botany & Bacteriology, Wellesley College, Wellesley 81, Mass.)

Following is a list of geneticists the Committee expects will be in Montreal:

- Professor Ivar Johannson, genetics in animal breeding (animal husbandry, particularly cattle)
- Dr. H. P. Donald, genetics in animal breeding (cattle breeding, particularly monozygotic twins)
- Dr. Francois Jacob, Institut Pasteur
- Dr. Alan Robertson, genetics in animal breeding (quantitative inheritance, both in animal husbandry and in *Drosophila*)
- Professor Hans Nachtsheim, genetics in animal breeding (genetics of rodents, particularly hereditary abnormalities from a comparative and development point of view)
- Dr. C. Syrach Larsen, cytogenetics and plant breeding (forest genetics)
- Dr. D. Lewis, cytogenetics and plant breeding (self-incompatibility, particularly in fruit trees, but also in other organisms)
- Professor H. Kihara, cytogenetics and plant breeding (wheat genetics and fruit cytogenetics)
- Professor H. Stubbe, mutation and mutagenesis (mutations, both radiation-induced and spontaneous)
- Dr. J. R. S. Fincham, physiological genetics (gene-enzyme relations in *Neurospora*)
- Dr. E. Hadorn, physiological genetics (development and biochemical genetics, particularly in *Drosophila*)
- Dr. P. Michaelis, physiological genetics (cytoplasmic inheritance in *Epilobium*)



Dr. N. P. Dubinin, genetics in evolution (evolutionary genetics in *Drosophila*)  
Dr. C. Paven, physiological genetics in *Drosophila* and physiology of the salivary gland  
Dr. H. Harris (human biochemistry)  
Professor R. Ceppellini (human blood groups)  
Dr. Maurice Lamy (twin studies)  
Dr. Cavelli-Sforza (evolutionary genetics)  
Dr. Jen Book (human genetics)  
Dr. Arne Muntzing (plant genetics)

Herskowitz, Irwin H. Proposed Plans for a *Drosophila* Research Stock Center in the Department of Biology at Saint Louis University are under way.

It will aim not only to maintain present and future stocks but to encourage their use in research. It is hoped to start the Center about July, 1958. Each worker will be notified by mail several months before the start as to how the Center will operate initially.

To help determine the desirability, scope, and mode of operation of the Center, your comments on the following plans would be appreciated.

1. The Center will be restricted, at least at first, to maintaining stocks of *D. melanogaster*.

2. Each stock sent to the Center for keeping will need to be described by the sender as to genotype, phenotype, and research purposes for which it is especially useful.

3. Workers sending the Center more than 10 stocks in any week will have to clear the sending date in advance with the Director.

4. A fee of \$5 is to be charged for each stock sent to the Center. Such a stock will be maintained for a minimum of 5 years before the Director can remove it from the stocks held, unless permission to discard the stock is granted by the worker who originally sent it in. The fee is intended to discourage flooding the Center with stocks of limited or questionable research value.

5. Stocks sent to the Center become the property thereof, and are available to all bona fide research workers. No stock may be withdrawn from the Center's stock holdings without the approval of the Director. However, he would welcome comments from any worker on the suitability of maintenance of a stock.

6. Only stocks whose mutant phenotypes are easily defined and whose mutations are localized are to be accepted for keeping.

7. Stocks which are difficult to maintain because they cannot be cultured on standard brewers' yeast-enriched cornmeal-molasses-agar medium at a temperature of 17° C are not acceptable.

8. On the day of receipt of a stock the Director will mail a post-card notice to the sender. Within two months of receipt, a notification of acceptance or rejection for maintenance will be mailed by the Director. If not accepted, \$4.50 will be refunded for each stock.

9. Any number of stocks may be obtained from the Center, as frequently as requested, by any researcher. For each stock requested, 25¢ in U.S. stamps or money must be provided.

10. A yearly list of stocks and their most desirable uses will be made by the Center, to be distributed as a part of "Drosophila Information Service" or as a separate sent together with it, or mailed independently. Workers listed in DIS will receive this listing free.

11. The Center is not designed to be a competitor of private companies which sell stocks for classroom work or nonresearch purposes.

12. The Center, once established, is planned to be partly self-supporting.

A stock sent to the Center may continue to be maintained by the sender. Thus the Center will provide a second place for maintenance as insurance against loss of valuable stocks. Or laboratories that wish to eliminate the keeping of certain stocks--for space, budget, or personnel reasons--may do so, yet be assured that they will be maintained for at least five years. Duplication of stocks maintained by various laboratories can be eliminated in some cases.

Space is available at Saint Louis University for maintaining approximately 4000 stocks.

#### TEACHING NOTES

Hexter, W. M. A two-factor sex-linked cross involving gene interaction.

The mutants  $g^{53d}$  and  $w^a$ , both sex-linked recessives 42.9 map units apart, are phenotypically indistinguishable. The phenotype of these mutants is orange,

varying somewhat with age. The class is given as parents females of one mutant and males of the other, and the cross is designated simply as "orange 1 x orange 2."  $F_1$  females are wild type and  $F_1$  males are orange.  $F_1$  are interbred to raise an  $F_2$ . The  $F_2$  females are expected to be wild type and orange in equal frequencies, and the  $F_2$  males theoretically should be approximately 60 per cent orange (parental types) and 40 per cent crossovers, half of which are wild type and half double mutant ( $w^a g^{53d}$ ). Actual class data were: females, 1530 wild type, 1402 orange; males, 690 wild type, 1420 orange, 454 white. Deviations from equality were due primarily to differential viability of the various genotypes. The student is confronted with the following facts: a wild-type  $F_1$  female; a mutant  $F_1$  male; a new phenotype (white) in the  $F_2$  but confined to one sex. From this information the student should conclude that orange 1 and orange 2 are not alleles and are recessive; that one of them is sex-linked; and that white is probably due to the combined action of orange 1 and orange 2. The student then usually assumes a second gene that is autosomal. This assumption will not account for the data. The second gene is then assumed also to be sex-linked, and the conditions of the problem

are satisfied if linkage is assumed to be about 45 per cent. In addition to the unexpected phenotypes and the challenging yet not too complicated analysis, this experiment has the advantage of simple and rapid classification.

Rizki, M. T. M. Genetics  
of behavior.

We have been doing some experiments on the response of normal and mutant strains of D. melanogaster to the smell

of food, in order to demonstrate the possible influence of inheritance on the behavior pattern of flies. A simple apparatus constructed from a cardboard box with a transparent cover is sufficient to carry out these experiments (see drawing). Air saturated with the desired mixture is blown through rubber tubing into the box. Flies are generally starved overnight and conditioned in the plastic-covered box, which contains a small vial of moist cotton. The flies will respond to the control of moist air if water is not available during the conditioning period. Dessication is also avoided. When air saturated with the odor of yeast and honey is blown into the box, the following components of behavior of Ore-R flies can be observed in an orderly sequence: (a) fluttering of wings, (b) shaking of abdomen, (c) looping or circling, (d) walking straight to the orifice of the tube which is the origin of the odor. This experiment can be modified by introducing other variables, such as different kinds of smells and different mutants. Students have found these experiments interesting and instructive, particularly those who are interested in psychology and behavior.

Control:

Moist Cotton

