

## Research Notes

Cooper, K.W. Detachment of XX chromosomes.

Since heterozygous autosomal inversions may increase the rate of crossing over in X chromosomes, an

attempt was made to discover whether they increase the rate of breakage of XX chromosomes through crossing over with the Y.  $y^2 w^a cv v f$  females and Canton-S males were used throughout, and the results are as follows: XX/Y; Cy I<sup>4</sup>/+; Cx, D/+ : 4 of 53 cultures gave detachments; XX/Y; Cy I<sup>4</sup>/+; +/+ : 2 of 49 cultures gave detachments; XX/Y; +/+; Cx, D/+ : 2 of 45 cultures gave detachments; XX/Y; +/+; +/+ : 5 of 46 cultures gave detachments. Apparently there is no pronounced effect of these heterozygous autosomal inversions on detachment of XX. In all, there were 14 detachments (9 ♀♀; 5 ♂♂) in a total of 18,654 flies, giving a frequency of detachment of about 1 in 1300, which is the same as that reported by E.G. Anderson (Genetics 10: 415) for a series of 9,146 offspring. Two triploid females and one intersex also occurred in the experiments with inversions.

Falbo, Homero O.B. and Rubens, R. Re A new sex-linked lethal affected by temperature.

The lethal effect is clearly expressed in cultures kept at 23 degrees C. or below. At temperatures above 26.5 degrees C., the lethal effect disappears.

I. Cultures at 23 degrees C. Cross:  $(w^e + m/+ 1 +) ♀ \times (w^e + m) ♂$ . ♀, 435. ♂: ++, 0; +m, 9;  $w^e+$ , 48;  $w^em$ , 118; total, 175. Cross:  $(w^e + cx/+ 1 +) ♀ \times (w^e + cx) ♂$ . ♀, 6,376. ♂: ++, 7; + cx, 149;  $w^e+$ , 126;  $w^ecx$ , 2,788; total, 3,070.

II. Cultures at 26.5 degrees C. Cross:  $(w^e + cx/+ 1 +) ♀ \times (w^e cx) ♂$ . ♀, 413. ♂: ++, 119; + cx, 10;  $w^e+$ , 16;  $w^ecx$ , 194; total 339.

III. Males raised at 26.5 degrees C. and crossed by  $(w^e cx/w^e cx) ♀$  transmitted the lethal at 23 degrees C. or below. (1) Cultures at 26.5 degrees C. Cross:  $(w^e + cx/w^e + cx) ♀ \times (+ 1 +) ♂$ . F<sub>1</sub>: ♀++ , 14; ♂- $w^e cx$ , 12. F<sub>2</sub>: ♀-++ , 66; + cx, 5;  $w^e+$ , 1;  $w^e cx$ , 37; total, 109. ♂-++ , 15; + cx, 3;  $w^e+$ , 0;  $w^e cx$ , 50; total, 68. (2) Cultures at 23 degrees C. Females  $(w^e + cx/+ 1 +)$  from F<sub>2</sub> x  $(w^e cx) ♂$ . ♀: ++, 45; + cx, 5;  $w^e+$ , 3;  $w^e cx$ , 42; total, 95. ♂: ++, 0; + cx, 1;  $w^e+$ , 0;  $w^e cx$ , 46; total, 47.

This new sex-linked lethal is located approximately at locus 8.

Gloor, H. Phenocopy of a lethal mutant (crc).

The new lethal crc (2-55+1) suppresses head eversion. The head develops and remains within the thorax. But

in few cases it is partially everted. In addition to this most striking fact there are other abnormalities: bristles, wings, and legs are shortened, whereas the abdomen is much longer than normally. Imaginal development is completed in most organs, but the flies are not able to hatch. In an analysis of the developmental processes which possibly could give rise to the manifold effects in the crc mutant, an attempt was made to reproduce the same abnormalities in an experimental way. By exposing normal prepupae from a wild stock of *Drosophila melanogaster* for a short time to a temperature of 40° C., we obtained, in a considerable percentage, lethal imagoes of exactly the same type as the crc monsters. Heat treatment is not only followed by more or less complete lack of head eversion, but also the minor characteristics of the mutant type are induced. Thus we have produced a real phenocopy of a lethal mutant character. Experiments apparently leading to the same developmental disturbances may also be performed by refrigerating or by etherizing normal prepupae.

Kalmus, H. The cure of carbon-dioxide-susceptible flies by low temperature.

resistant flies, if they are exposed for several days to temperatures below 16 degrees C. or to sublethal concentrations of carbon dioxide. Some of these cures proved to be permanent; i.e., not only the flies that emerged from the treated pupae or larvae were resistant, but also their offspring. In other experiments a recovery of the strain could be observed.

Kalmus, H. and A. N. Mitchison  
Transplantation of ovaries from and to larvae susceptible to carbon-dioxide.

By the technique of Ephrussi and Beadle, ovaries from a stock bearing genes for ebony and showing the susceptibility for carbon-dioxide described by L'Heritier and Teissier were transplanted into wild-type larvae of female resistant type. A small percentage of those Anlagen joined up with the rest of the genital tract during pupation and became functional. By mating such females to males homozygous for ebony, but not showing the carbon-dioxide susceptibility, two kinds of offspring were produced; phenotypically wild-type flies from the ovary of the host, and phenotypically ebony flies from the implanted ovary. The former were never susceptible to carbon-dioxide, the latter always.

Offspring from flies developed from susceptible ebony larvae carrying an implanted wild-type ovary mated to resistant males homozygous for ebony, were also of 2 phenotypes: ebony, which were partly susceptible and partly resistant, and wild-type, which were all resistant. The causes of the partial loss of susceptibility are not yet understood. The main result of the experiments seems to be that the susceptibility to carbon-dioxide cannot be induced in resistant tissue even under the favorable conditions of histolysis during pupation. Therefore the material basis underlying the susceptibility is perhaps better described as a plasmagene, rather than as a virus.

Kaufmann, B.P. Spontaneous mutation rates.

Ten years ago N.W. Timofeeff-Ressovsky presented data showing that the percentage of mutations occurring in the chromosomes of *Drosophila* varies with the age of the spermatozoon. Similar experiments carried out during the past year have given parallel results. Lethals arising spontaneously in the X chromosome at 22 degrees C. were about one-half as frequent in 16-day-old sperm, and one-fourth as frequent in 32-day-old sperm as in sperm transferred by males 1 day after emergence. Differences between the 1-day and 32-day material were statistically significant.

Lamy, R.

A case of unexpectedly high occurrence of gonadic mosaicism has been observed incidentally in a recent experiment designed primarily to measure the spontaneous mutation rate. The ♀♀ used were  $scar/sc^{Sl}(InS)w^a sc^0$ . Three out of the 1700 (whose progeny were inspected through the glass in the ordinary way for the presence of sex-lined lethals) carried cut on about 1/8 of their X chromosomes; in two cases the  $sc^{Sl}(InS)w^a sc^0$  chromosome of paternal origin was affected, and the maternal  $scar$  chromosome in the other case. Another ♀ who showed a marked deficiency of  $w^a$  sons and whose progeny were in consequence tested individually, was proved to have been mosaic for a lethal on the  $w^a$  chromosome (derived from the father).

Philip, U. and Spurway, H.  
Equational primary ex-  
ception in D. subobscura.

A female homozygous for  $cy^2$  was discovered in the  $F_2$  from  $ctcp +/ctcp +$  female  $x ++ cv^2$  male. She produced  $XXY$  sons.

University of British Columbia  
Report on Research

Monochromatic ultraviolet radiation of Urbana-S *Drosophila* at different stages of the life-history produced numerous wing abnormalities in 4 to 6-day larvae. The results so far appear to verify Epstein's experiments on wing sensitive areas.

#### Technical Notes

Altenburg, Edgar A.  
Method of mite control.

Consists of rearing flies from shelled eggs. This completely rids a stock of mites in one generation, but of

course it does not prevent reinfestations of the stock.

Mainland, G.B. (1) The use  
of the Waring Blender in food  
making.

In the preparation of various food media, bananas or dried yeast is commonly used. Both bananas and dried yeast are normally difficult to pro-

cess so as to give uniform, finely dispersed suspensions in the media. The Waring Blender (obtainable from Cenco) obviates these difficulties. Ripe, peeled bananas may be placed in the blender without the addition of water, and within several minutes one has a smooth paste which may then be added to the other ingredients; the paste should be used immediately however, as it tends to gel upon standing. In the case of dried yeast, it seems best to use about 200 cc. of water to each 100 gr. of yeast for an easily poured mixture. Place about  $2/3$  of water in the blender, start the blender, and then slowly add the dried yeast. When the dried yeast begins to collect on top, add a part of remaining water. If all the water is used in the beginning, often sticky masses of yeast will cling to the sides of the blender. In addition the blender may be used for preparing fungus, malt sprouts, fruit, etc. for addition to base media if desirable.

Mainland, G.B. (2) The use  
of low temperatures to inhibit  
the emergence of adult flies.

At times it is inconvenient to collect virgin *Drosophila* females at the required intervals, especially in those forms mating four hours after emer-

gence. By storing the bottles or vials containing pupae about ready to emerge in a refrigerator at 2 to 5° C., it is possible to prevent the emergence of adult flies. Upon warming to about 21° C., normal adults usually emerge rapidly, especially if they are stored for several days. If the pupae are stored at 7 to 10° C., some adults will emerge while in the refrigerator; such adults while virgin often display various wing and body abnormalities, phenocopies of various mutants. Caution is advised against freezing, since this is lethal to the eggs, larvae, pupae, and adults of D. melanogaster and various other species; however, freezing of pupae is non-lethal to many nearctic (northern temperate) species although lethal to their eggs, larvae, and adults. Pupae of D. melanogaster may be stored at 2 to 5° C. several weeks without adults emerging; however, some of the larvae present may die during this interval, especially if the food is somewhat soft and flows slightly. Adult virgins may be stored at 4 to 7° C. for periods upward of a month with very little aging.

Mainland, G. B. (3) The prevention of suicides of newly emerged adults.

after emerging from the puparia. Becoming entangled in the cotton and being unable to progress forward or backward, they die. In some species more than 90% of the flies may die in this manner. To prevent this, one may use cardboard milk bottle-tops instead of cotton in bottles; however, bottle tops are very ineffective in preventing mite contaminations. Another method, which is mite-proof, is to cut a square of paper toweling, place it over the top of the vial or bottle, firmly band the paper toweling in place with a rubber band, and finally gently draw the toweling downward until it is taut. Cultures develop normally under such toweling tops.

Various species of *Drosophilids* have a characteristic manner of committing suicide by crawling up between the cotton stopper and glass vial or bottle shortly

Mainland, G. B. (4) Freeing cultures of pink mites.

ready to emerge from the puparia. The method is as follows: (1) remove and destroy the stopper, (2) remove and kill adults, (3) invert the bottle and thoroughly swab the inside to within 1.5 to 2 cm. of the food with a cotton swab liberally dipped in ether (ethyl ether), (4) blow the ether fumes out of the bottle, (5) replug the bottle with a new stopper, (6) collect emerged adults every 12 hours, and then repeat all steps except (2). It should be noted that all paper toweling projecting upward should be removed or pushed into the food for this technique to be effective. The efficacy of this method results from two facts. First, liquid ether is an efficient contact lethal agent for arthropods, and it does not produce after-effects subsequent to the removal of its fumes. Second, white mites undergoing metamorphosis into the pink contagious stage crawl upward during metamorphosis, and although present in early pink stages at 12 hours after leaving the food, they will not attach themselves to flies.

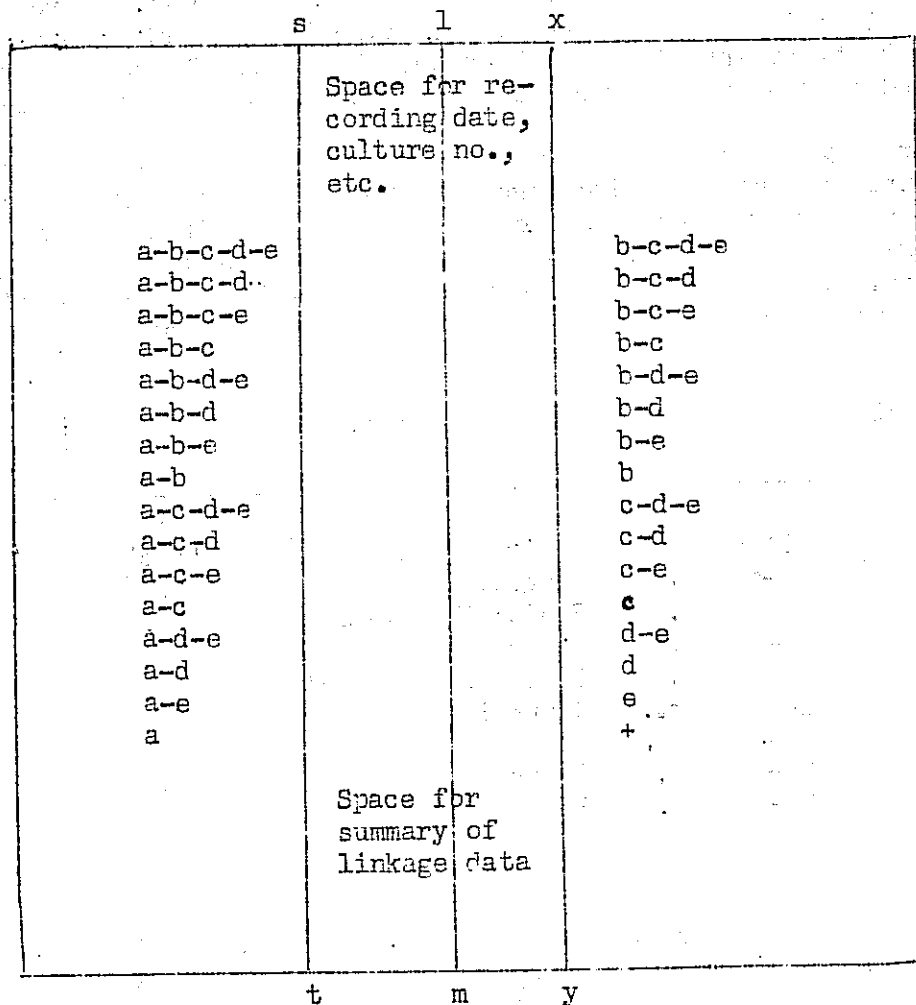
Mite-free adult *Drosophila* may be obtained easily from bottle cultures contaminated with pink mites at the time adults are

Spencer, W.P. A method of recording linkage data.

six-point crosses, and is equally applicable to experiments where fewer loci are followed. The first page in a ruled spiral stenographer's note book is divided into equal parts by two lines drawn from the top to the bottom of the page, the lines s-t and x-y in the sample page shown below. If the linkage experiment involves five loci, the 32 possible combinations of mutant and wild-type characters are arranged for the greatest convenience in recording and not according to the position of loci in the chromosome map. Thus if the mutant genes are a-b-c-d-e and their wild-type alleles A-B-C-D-E, the classes will be recorded as shown in the diagram. Only the mutant symbols or names need be written in. The symbol of the most easily and quickly separable mutant should be substituted for a, that of the next most easily separable mutant for b, and so on. The symbol of the mutant most difficult to separate should be substituted for e. Flies from a culture are etherized and separated first into a and A groups. The A group is placed in a vial and the a group separated into b and B groups and so on. The most difficult separations are made when there are only a few flies left in a group. The initial number of flies and the difficulty of the separations will determine the number of sub-groups which must be placed in vials for re-etherization. Generally 4 to 8 classes of flies can be handled on the counting plate and recorded after the first separations have been made. The center area between the lines s-t and x-y is used for recording the flies by check marks. The blue line l-m separates all classes containing a from those containing A. The arrangement of the classes on

The following method has been found convenient in recording data in large-scale linkage experiments involving five- or

the page facilitates recording. Enough room is left at the top of the page for recording culture number, date, and other pertinent data and at the bottom of the page for a summary of linkage data. The page is then cut along the lines s-t and x-y and turned back. The next page is ruled in the same way with two vertical lines, but the 32 classes on the two strips remaining in place from page one are again used for recording the flies on the center strip of page two. Notes or summary of linkage data may also be written on the back of each center strip. Once the order of the genes is known, appropriate masks can be cut from light cardboard, which when laid over the center strip will allow only the crossover classes between a certain pair of genes to be seen and the counts can be made very quickly for determining their linkage value. A sixth point may be followed by marking the two alleles in red and black respectively. Linkage data involving 20,000 to 25,000 flies and five- or six-point crosses can be recorded in one such notebook and filed away in compact and easily accessible form.



Hill, David L. Chemical removal of the chorion from *Drosophila* eggs.

Slifer (1945) has reported that sodium hypochlorite can be used for removing the chorion from the eggs of the grass hopper, *Melanoplus differentialis*. This

substance does not affect the chitinous cuticle of these eggs. It was felt that chemical rather than manual methods would be useful tool for geneticists and embryologists using *Drosophila* as experimental material. The results of applying this technique are reported herein.

Using eggs of *Drosophila virilis* it was found that the optimum conditions of exposure to sodium hypochlorite were of the same order as those found by Slifer (1945) for the egg of the grasshopper. Thus an exposure of 2 minutes in a 3% solution of sodium hypochlorite (commercial preparations such as Chlorox or Dazzle are satisfactory) was found to completely remove the chorion without subsequent harm to the embryo or delay in the time required for hatching.

The eggs were washed, prior to removing the chorion, to free them from the medium in which they had been laid. Likewise careful washing with water followed the treatment with hypochlorite. For ease of observation the eggs were placed in rows on 1% agar which had been blackened with India ink.

Apparently the stage in development at which this treatment was applied was of little importance, since eggs treated before the sixth hour responded in the same way as eggs treated between the 24th and 30th hours. The temperature was not rigidly controlled, being only approximately 25° C.; however, this variable was compensated for by subjecting untreated eggs to the same temperature conditions and comparing the percentage hatching in each.

The following table shows the results obtained:

No. eggs	Stage	Treatment	% Hatch
100	0-6 hrs.	Control	92
100	0-6 hrs.	Sodium Hypochlorite	85
100	0-6 hrs.	Control	83
100	0-6 hrs.	Sodium Hypochlorite	83
100	24-30 hrs.	Control	86
100	24-30 hrs.	Sodium Hypochlorite	88
100	24-30 hrs.	Control	88
100	24-30 hrs.	Sodium Hypochlorite	94

This technique has certain advantages: (a) It makes it possible to dechorionate any number of eggs simultaneously in the time it take to dechorionate only a few by hand. (b) Subsequent development can be observed through the cuticle without further disturbance to the embryo. (c) It affords an opportunity to study the relative importance of the chorion and the cuticle to the egg as a whole.

Reference: Slifer, Eleanor H., 1945, Removing the shell from living grasshopper eggs. *Science* 102: 282.