Research Notes

Barigozzi, C. Report of research at the Zoological Institute, University of Parma.

During 1947, research on Drosophila was carried on in two main directions: (a) genetical analysis of the reaction of the flies to light, and (b) detection of Y-chromosome effect on quantitative characters. (a) The light reaction was studied in D. melanogaster and D. pseudoobscura with the technique already used for a previous investigation (see Barigozzi, C. and Tonissi, G., Zool. Inst., Univ. of Milano). It was found that wild stocks differ in degree of phototropism and that D. pseudoobscura shows very different behavior. In this latter species selection for phototropism and photophoby has been carried out for six generations to date. In the case of photophoby a variation in the stock was found, proving that the stock was originally heterozygous for the genes responsible for the light reaction. This research is still in progress. (b) The role played by the genome in determining cell size was studied in D. melanogaster. This investigation will appear in the "Rendiconti dell 'Istituto Lombardo di Scienze e Lettere, Class Scienze," probably in 1948. It was shown that the size of the cells of the wing surface, measured as frequency of unicellular hairs. depends on the genes and that there is a maternal effect. In a further step it was proved, making the studied stocks (two wild stocks from Italy) isogenic for X and autosomes, that the Y chromosome influences cell size both in the wing and in the eye corneolae. This investigation was communicated to the VIIth Congress for Exp. Cytology in Stockholm.

Bertani, G. Action of mutagenic substances.

Adult males (Oregon stock) injected with phenol, at the most effective concentration used by Hadorn and Niggli, have been tested for lethals (ClB method). Chromos. tested, 1954: lethals, 4; % lethals, 0.20 ± 0.10. Controls: chromos. tested, 546; lethals, 1; % lethals, 0.18 ± 0.18. It must be concluded that injection into adult males is an ineffective method of introducing mutagenic substances. Negative results were obtained also in tests of the progeny of males treated during larval life with methylene blue (dissolved in the food at a concentration of 0.001%) and of other males treated with a vaporized solution of 0.05 M KJ for about 40 hours.

Bertani, G. (2) Diapause in Drosophila nitens.

D. nitens undergoes winter diapause at the adult stage, also under constant conditions of temperature and lighting. Selection of individuals homozygous for a newly occurred recessive character (occhi rossi), made on adults entering diapause, has been 100% effective. This shows that at the beginning of diapause the adults have not reached mating ability (or, alternatively, that sperms in females are reabsorbed). A temperature shock may "break" the diapause (see Bibliography). After this treatment our stocks reproduced uninterruptedly for nine generations.

Bertani, G. (3) Frequency of abnormal spermathecae in Drosophila melanogaster.

The frequency of females with abnormal spermathecae (three or four spermathecae with single or subdivided ducts) has been studied in some wild melanogaster stocks:

Stock	No. normal females	No. abnormal females	% abnormal
Oregon	2759	56	1.99 ± 0.26
Tavira	1510	0	0
U. Kulm Aargau	588	1	0.17 ± 0.18
Barce	469	0	0
Dauro	551	2	0.36 ± 0.25
Salsomaggiore	364	1	0.27 ± 0.27

Some differences between stocks are significant. The Oregon stock, which has been studied most, shows variations in time, as to the frequency of abnormal females. This may depend on slight changes in environment or on random changes in the genetic basis of the character, which must be considered of a polygenic nature (see Mather's correlated responses to the selection). The behavior of the character in crosses of different wild strains is now under study.

Bertani, G. (4) Method of searching for visible mutants present in natural populations. A backcross method (wild male x laboratory virgin female; a number of F_1 virgin females x the same P male; progeny of each F_1 female bred in single cultures.) is more useful than the usual inbreeding method in the search for visible mutants heterozygous in wild individuals. By the suggested method, 7 F_2 (backcross) cultures are necessary if all ($P > 0.99$) heterozygous mutations must be revealed in the segregation; the inbreeding method (Buzzati, Ricerca Sci. 13: 448, 1942) requires 16 F_2 cultures for the same probability level. The only disturbing factor is the introduction into the crosses of a non-wild genotype (that of the P laboratory virgin female). On the other hand, this method allows greater precision in calculating gene frequency in natural populations than does inbreeding from wild females, which usually have already mated with a number of unknown wild males before being collected.

Bird, M. J. Additional notes on the X chromosome of Drosophila subobscura. New genes: Four new genes have arisen spontaneously, on the X chromosome, and have been located. burnt is phenotypically similar to singed but not allelomorphic with it. rimy (white hairs between the ommatidia and a crumpled wing effect) and a lehtal l(1)47d are both located between plum and cut; and have lengthened the chromosome map, which now is as follows: 0 miniature; 10.9 plum; 18.5 rimy; 30± l(1)47d; 44.2 cut; 48.7 burnt, etc. Beadex, which is probably homologous with Beadex in D. melanogaster is 0.23 units from vermilion.

Further investigations of the testes abnormalities in crossveinless[2] suggest that they are due to rather small changes in the genital anlage. A time factor is probably involved, as in one set of experiments 16% more abnormals were found in the late-hatching males. Outcrosses indicate that different laboratory stocks contain enhancers and partial suppressors of the genital abnormality, without altering the wing effect. Within the inbred stok the frequency of genital abnormality in uncles and nephews was also correlated. On the other hand neither the expression nor penetrance of the wing effect was variable.

roughener. When Notch ♀s are crossed with certain + ♂s their Notch progeny have large, bulging, and very rough eyes. The facets are disarranged and brown pigment is distributed to form irregular dark and light patches. Six of 12 laboratory stocks tested carried the roughener, 5 did not, while one segregated for it.

All surviving sons and all normal daughters of rough-eyed Notch ♀s carry roughener; as it does not cross over with Notch it appears to be a gene at a

locus in or very near the Notch deficiency. It has no visible effect when hemizygous or homozygous, but is made visible by exaggeration in presence of Notch.

Demerec, M. Chemical induction of mutations in Drosophila by the aerosol method.

Using a suitable nebulizer it is possible to produce a fine aerosol having a particle size of diameter 1.5 microns or less It has been found that mutationsare induced in the sperm of Drosophila males if the flies are kept in an atmosphere containing aerosols of certain chemicals. In our experiments the following set-up is used for treatment. The aerosol is generated, from either a water or an oil solution of the desired chemical, in a De Vilbies-40 glass nebulizer by an air flow of 6 liters per minute, produced by an electric air pump. The aerosol is piped through plastic tubing into a half-pint milk bottle, where the large droplets occasionally thrown out by the nebulizer are retained. From this bottle the aerosol is piped into a similar bottle containing fly food and the flies to be treated. Excess aerosol is discharged into the exhaust. Aerosol generation is started by turning on the pump. Treatments are usually of long duration--up to 200 hours or longer, depending on the toxicity of the chemical used. As a rule, flies are kept in the same bottle throughout treatment, but the bottle is changed if it becomes too moist or sticky. The aerosol is generated periodically, every 30 minutes for 30 seconds. Since this is regulated by an electric time switch, treatment can be carried on without any attention except for occasional examination of the flies to make certain that they are surviving the treatment. By means of this sytem, flies are kept in an atmosphere containing an aerosol of a chemical solution, and this atmosphere is renewed every 30 minutes.

Tests have been made for X-chromosome lethals and for translocations. The following chemicals gave significant increases in the numbers of lethals: methyl-bis(betachloroethyl)amine, 1,2,5,6-dibenzanthracene, 20-methylcholanthrene, 3,4-benzpyrene, p-hydroxyazobenze, p-aminoazobenzene, sodium desoxycholate, and acriflavin. The following showed no increase: phenanthrene, pyrene, alpha-naphthylamine, azoxybenzene, p-diethylamino-azobenzene, p-dimethylamino-azobenzene, 2-amino-5-azotoluene, and acetylaminofluorene. The chemicals underlined with a solid line are carcinogenic, and those underlined with a broken line did not show carcinogenicity in tests made with them. Considering the important role played by biological factors in the expression of both mutagenicity and carcinogenicity, and considering the biological differences between the materials in which these two effects were studied (Drosophila and mammals), the observed correlation between the two effects appears to be quite striking. From this correlation it seems unavoidable to infer a common causative relation. This inference is further strengthened by the behavior of all known nonchemical carcinogens, such as X-rays and related radiations, ultraviolet rays, and heat, all of which are mutagenic. The most obvious and most probable relation between mutagenicity and carcinogenicity is the one suggested by the hypothesis that cancer may originate through a gene mutation occurring in a somatic cell.

Fox, Allen S. Mutation of bt^D. During the use of a Cold Spring Harbor stock of bt^D/ci^D, it was observed that in no cross which should have produced the heterozygote $bt^D/+$ was any visible effect of bt^D apparent. The following series of matings was made (at room temperature) to test these observations further:

4 reciprocal matings (bt^D/ci^D x +)

	+		ci	
	♂	♀	♂	♀
F_1	153	198	177	163
	351		340	

Backcross F_1 + x + $ (1 mating)

	+		ci	
	♂	♀	♂	♀
	59	72	0	0
	131		0	

Backcross F_1 ci x + $ (4 matings)

	+		ci	
	♂	♀	♂	♀
	73	102	96	79
	175		175	

F_2 ci x ci (1 mating)

	+		ci	
	♂	♀	♂	♀
	22	22	43	42
	44		85	

To test the possibility that the temperature was responsible for the absence of visible effects of bt^D, the following matings were made:

(At 18°) 4 reciprocal matings (bt^D/ci^D x +)

	+		ci	
	♂	♀	♂	♀
F_1	70	68	67	93
	138		160	

(At 30°) 4 reciprocal matings (bt^D/ci^D x +)

	+		ci	
	♂	♀	♂	♀
F_1	35	39	36	33
	74		69	

Since the Cold Spring Harbor stock remains balanced, the most plausible explanation is that the gene bt^D had mutated, losing its visible effects. The recessive lethal effect, whether due to this same gene or a closely linked one, is retained. The genotype of the stock is, therefore, $ci^D/\underline{1}(4)$. The results of numerous crosses using the Pasadena stock of bt^D/ci^D indicate that this stock is probably identical with that of Cold Spring Harbor.

Fox, Allen S. (2) Derivation and description of isogenic stock. Chicago stocks Nos. 72 and 73 were derived by the following series of crosses:

sc lz/C1B ♀ x Cy-RNS/Pm^2; Sb/Payne Dfd ca ♂

F_1 +/C1B; +/Cy-RNS; +/Payne Dfd ca; +/+♀ x own father

+/+; Cy-RNS/Pm^2; SB/Payne Dfd ca; +/+
+/Y; Cy-RNS/Pm^2; SB/Payne Dfd ca; +/+ } ♀ No. 72

+/+; +/Cy-RNS; +/Payne Dfd ca; +/+ mating brother and sister
+/Y; +/Cy-RNS; +/Payne Dfd ca; +/+

+/+; +/+; +/Payne Dfd ca; +/+
+/Y; +/+; +/Payne Dfd ca; +/+ } ♀ No. 73

By means of this series of crosses, all chromosomes except the 4th trace to a single source. Barring crossing over in F_1 ♀ and the occurrence of spontaneous mutations, stock 73 is isogenic in chromosomes 1 and 2, and stock 72 carries the same first chromosome as the former. Since stock 73 remains balanced, its third-chromosome genotype is Payne Dfd ca/1(3)F, as listed. To date this stock has been maintained through 6 generations of brother-sister matings, thus reducing the chances of heterozygosis in chromosome 4. Viability of both stocks is excellent.

Gowen, John W., and Stadler, Janice In recent inbreeding experiments we have uncovered a genetic situation similar to that reported by Dr. Redfield some years ago. The females carrying this condition show a large excess of males in their progeny. This condition appears to be partially recessive and probably confined to the second chromosome. It is not sure that it is a single gene.

Hinton, Taylor and Dibble, F. Negative evidence on reverse mutations. y ct^6 ras^2 v f flies were irradiated with 3000 r of X-rays in an effort to obtain reverse mutations of the recessive genes. 6259 offspring were examined and no reverse mutations were found.

Lamy, R. Two small X-chromosomes deficiencies were found among spontaneous lethals. One occurred in a scar male and was carried by 9 out of 146 of his tested daughters. Salivary studies by Dr. Slizynski showed approximately 4 bands to be missing, including the cut locus. (Stock list, No. 61). The second deficiency occurred in a female and was observed in one out of 143 of her tested daughters. The chromosome involved was the sc^{S1}InS ct w^a sc^8, and the deficiency covers yellow. Salivary studies not yet completed. Now balanced against y w loz (Stock list, No. 62).

Milani, R. Lethality in so stock. Research on lethality in so stock has proved that death of lethal pupae is due to malformation of femurs, which are found folded and contracted. This malformation is nearly always limited to the legs of the first pair. Affected pupae are unable to hatch. Experimentally disengaged, they will briskly waver their legs, yet they cannot walk. They can creep for a few centimeters.

Lethality, according to research still going on, has been found to be independent of the ss factor, but subject to the action of complementary or modifying factors. In fact, individuals have been obtained from pairing with other stocks, wild and mutant, both ss and with developed eyes, who are able to hatch but unable to walk on the culture ground, or able to pull out from the puparium with their heads and part of the thorax (first pair of legs) only. In two different crosses I have found two headless individuals--extracted from the puparium while still living--with their chitin already melanic. Cephalic parts were inside the thorax, which was imperfectly chitinized. One of these was dissected and found to have spherical testes and ripe sperms.

Milani, R. (2) Wasp parasites of Drosophila, and the possibility of utilizing them for genetical research. At the end of June, this year, I observed some little parasite wasps breeding upon Drosophila pupae in an open container exposed at a window of my laboratory. They seem to correspond to Pachineuron vindemmiae, briefly described by G. Martelli in 1909 (Boll. Lab. Zool. di Portici, Vol. 4, p. 169). All pupae collected (about 200) were found infected. I began breeding with wild ♀ and individuals hatched from infected pupae. Breeding is successful both in glass cases--in the presence of Drosophila free to reproduce in open containers--and in the containers commonly used for Drosophila (vials and bottles) with some small drops of water and diluted honey on their walls. Pupae to be infected are then introduced therein. Old Drosophila cultures are not suitable for breeding, because wasps will stick eventually to the walls. However, I have obtained under these conditions several F_1 individuals. The average life span is 18 days at 25° C.

Isolated females or pairs seem to live longer than individuals in mass cultures. Females kept in isolation have lived about one month, sometimes reaching an age of 50 days. They can lay up to 30-40 eggs daily. Unmated females are as fertile as mated ones and will give numerous haploid male offspring.

Mutability or heterozygosis must be very low, since I have observed no mutants on about a thousand individuals examined (I considered only the following characters: shape and color of body, wings, antennae, eyes, and legs). Variable characters were only body size (1 to 2 mm.) and leg color (ranging from bright yellow to dark brown, through all intermediate shades). The first of these two characters (perhaps the second also, but it is difficult to find a method of evaluating color degrees) is much influenced by development conditions of the larvae. Larger individuals are generally obtained from large pupae of funebris, similis, and immigrans than from melanogaster. Very small individuals are obtained from pupae exposed for a long time to the wasps' stinging. Under these conditions a high percentage of host pupae will die, without parasite larvae being able to develop.

These wasps, easy to breed, may become a good material for genetical research, especially if cytological analysis, which has not yet been attempted, should reveal a limited number of chromosomes.

Moree, Ray Reversion and elimination of Bar eye. A few wild-type flies appeared in an old culture of a B stock. After the stock was recultured, the reverted wild-type flies replaced and finally eliminated the entire B stock. It is known that the productivity of B is less than that of wild-type; in the present case the reverted wild-type stock had a productivity greater than that of B but less than that of other regular wild-type stocks, as could be determined by simultaneous culturing of corresponding numbers of both kinds of

flies. The reason for this hasn't been determined (inexact reversion?). As concerns elimination, the case parallels the elimination of y females by detached w^e females (DIS-20, p.88)

Peterson, Peter A. A sex-linked character expressed as ether-sensitive (es).

In the course of chemical-aerosol treatments of D. melanogaster Ore-R males with acriflavine (see research note of M. Demerec), a sex-linked semilethal was picked up (47i). The semilethal wild-type males from the cross $+/sc^8\ w^a\ B$ x $sc^8\ w^a\ B$ (the + coming from the treated male) were characterized by extreme sensitivity to ether. After normal exposure to ether these + males showed the fatal symptoms of overetherization--i.e., wings folded back and abdomen at an acute angle. All other flies of the cross--namely, $+/sc^8\ w^a\ B$ and $sc^8\ w^a\ B/sc^8\ w^a\ B$ females, and $sc^8\ w^a\ B$ males-- were normally etherized.

This ether-sensitive wild-type male had a very poor viability and was not so husky as the normal wild-type males. All efforts to mate it with $+/sc^8\ w^a\ B$ resulted in failure, owing to its short life. Nevertheless, four homozygous wild-type females were found in stock bottles. These showed the same reactions to ether as the wild-type males--i.e., folded wings and abdomen at an acute angle. Most of the wild-type males and females observed were relatively newly hatched as compared to the non-wild-type flies; this may be taken as evidence of their inviable nature. Out of the total number of males, only 21% were wild-type.

The location on the chromosome is either 5 or 3 units to the left of echinus--that is, between .5 and 2.5. The position could not be determined more exactly because of low numbers and several doubtful cases.

Poulson, D. F. The pole cells of D. melanogaster.

Sonnenblick (1941) and Aboim (1945) have shown that only a part of the original large number of pole cells actually reaches the lateral mesoderm and becomes incorporated in the gonad as germ cells. In a detailed study of the embryology of D. melanogaster it has been found that the pole cells which do not reach the gonad remain associated with the posterior rudiment of the mid-gut and subsequently are incorporated as a mid section of the mid-gut. This later becomes the acid section of large mid-gut cells described by Marie Strasburger in her account of the larval gut. In other Diptera this section has been shown to lack proteolytic enzymes. In view of these findings it is necessary to revise previous ideas of the pole cells as completely predetermined germ cells and of the posterior polar plasm as a germ-cell-determining region of the egg.

Poulson, D. F. (2) Iron-accumulating cells in the larval gut of Drosophila species.

Using the technique of Waterhouse, who has studied metal-accumulating cells in the larval gut of blowflies, observations have been made on ferric iron distribution in the mid-guts of four species of Drosophila (melanogaster, funebris, pseudo-obscura, and gibberosa). In each species (all raised on the same medium without added iron) the iron-accumulating cells are readily demonstrated by the production of prussian blue on exposure to a

solution of potassium ferricyanide in dilute HCl. The pattern of distribution of the iron-accumulating cells is different in each of the species studied, sufficiently so that these species can be distinguished on this basis. The observations are now being extended to a considerable series of species, to see how far differences can be established within species groups in the several subgenera.

Suley, A. C. E. Transplantation of eye rudiments between Drosophila subobscura and D. melanogaster.

Scarlet, poppy, and cherry implants from Drosophila subobscura have previously all appeared to develop autonomously in D. melanogaster wild-type hosts. As this implies that none is homologous with cinnabar D. melanogaster, the work was repeated using double recessives with maroon. ma and ch ma developed autonomously. The color of pp ma implants however was not autonomous, though paler than ma. pp might therefore correspond to cn or bri D. melanogaster. pp ma implants to cn and bw on D. melanogaster are autonomous and have no effect on the bw cn host eye. pp is therefore more probably honologous with cinnabar. Implants from s D. subobscura cause slight formation of brown pigment in bw v D. melanogaster host eyes. Here it resembles st rather than cd D. melanogaster, but the effect is less strong than that of st implants, though certainly not a starvation effect. Implants to bw v hosts from other red-eyed D. subobscura mutants are not yet available.

Waddington, C. H. Regulation in bithorax "hemi-thorax."

In a stock of bithorax, a fair proportion of flies (some 2-3%) were "hemi-thorax" owing to the failure of the mesothoracic disc to evert. In these, the body-parts derived from the metathoracic disc had shifted forwards into the space normally occupied by the mesothoracic derivatives, and were usually larger and better developed than those on the nondisplaced side. In a few cases, the metathoracic derivative on the "hemi-thorax" side had fused laterally with the mesothoracic derivative on the normal side, and had developed into a region much more similar to the missing part of the mesothorax than to the usual bithorax metathoracic derivative. This regulation towards normality presumably takes place after, and as a consequence of, the eversion of the thoracic discs.

University of British Columbia Report on research.

Investigation of a new mutation "Synpalpi" in which the upper portions of the antennae are fused. Viability poor. Produced by a chromosomal aberration, probably a deficiency in the terminal bands of the X chromosome. Locus undetermined.

Technical Notes

Callan, H. G. Collection of dechorionated Drosophila eggs.

Hill (DIS-19, p. 62) has made use of sodium hypochlorite solutions for removing the chorion from Drosophila eggs. We have found that a degree of mechanical agitation is also required if Hill's method is to be successfully adopted. Eggs are laid on agar-plated watch glasses. After laying, eggs and agar are roughly scraped off with a scalpel and placed in a 3% (or slightly stronger) solution of sodium hypochlorite contained in a centrifuge tube. The contents of the tube are shaken and left to stand for two or three minutes. They are then violently shaken once more and centrifuged for a few seconds in a hand centrifuge. The dechorionated eggs come to the surface of the liquid and can be readily removed with a section-

lifter. 100% of the eggs originally present can be recovered if the shaking and centrifuging are repeated two or three times. Such eggs develop normally if washed subsequently in tap water.

Crow, James F. Preventing the spread of mites. A mixture of roughly equal parts of mineral oil and benzyl benzoate has been used very successfully. This is applied with a small brush in a ring around the top of the container (just under the lip in the case of milk bottles) and remains in a condition to trap and kill mites for several weeks.

Hsu, T. C. A rapid method of making permanent mounts for smear preparations. In making a permanent mount for a smear preparation, the trouble in the past has been loss of the material in the course of separating the cover slip from the slide. We have now developed a new technique, which does not require the removal of the cover slip. Instead of using 95% alcohol, we put the slide carrying the temporary smear preparation, either sealed with wax, or unsealed, into a copling jar containing a mexture of xylol and absolute alcohol in equal parts plus a few drops of glacial acetic acid. After the sealing wax is dissolved, in the case of the sealed mounts, we apply very dilute balsam drop by drop on the edge of the cover slip, allowing it to diffuse into the material gradually. Preparation of permanent mounts in this way not only prevents shrinkage and loss of the aceto-carmine smear but also saves the trouble of separating the cover slip from the slide. The preparations look as good as fresh ones.

Hsu, T. C. (2) An improved method for salivary-gland smear preparations. In the ordinary aceto-carmine smear preparations for salivary-gland chromosomes, the differentiation of chromosomes is often obscured by the stained cytoplasm. An improved method, which is aimed to render the cytoplasm colorless in contrast to the stained chromosome, consists of the pretreatment of the gland in 1/N hydrochloric acid for about 10 minutes at room temperature. The glands must be completely submerged in the acid, however, for otherwise the chromosomes would undergo disintegration. After being taken out of the acid and before being put into the aceto-carmine solution, the glands should be placed on a piece of blotting paper for a while in order to remove the excessive HCl. Usually one to two minutes in the aceto-carmine solution is sufficient to effect a good preparation. This has been found so successful that almost every such preparation gives excellent well-stained chromosomes against colorless cytoplasm. Furthermore, the glands can be more easily squashed than those untreated with HCl.

Mittler, Sidney. The amount of propionic acid necessary to inhibit mold growth. It has been shown recently that the inhibition of mold growth depends on concentration of at least .012 M of the undissociated fraction of propionic acid (Olson & Macy, J. of Dairy Science 2:9: 173-180, 1946). Since the undissociated portion = $\frac{H}{H + K}$ at a pH of 7, the undissociated fraction = $\frac{1 \times 10^{-7}}{1 \times 10^{-7} \times 1.4 \times 10^{-5}}$ = .0071, pH 6 = .067, pH 5 = .415, pH 4 = .875. Hence at lower pH values less propionic acid need be added to the food mixture to obtain the

necessary amount of undissociated propionic acid. At a pH of 5, only 2.1 cc. of propionic acid is required per liter, while at a pH of 6, 13.2 cc. is needed to give .012 M of undissociated propionic acid. In our laboratory we have had excellent results in using propionic acid to inhibit mold growth in a cracked wheat-agar mixture (pH 6). Since the proteins in food mixture act as a buffer, it is recommended that pH be taken just after boiling the food mixture and then stirring in the necessary amount of propionic acid. A concentration of .04 M undissociated propionic acid was found not to be harmful to D. melanogaster and D. macrospina.

Mittler, Sidney and Bartha, A. Brilliant cresyl blue as a stain for salivary chromosomes.

Excellent results have been obtained by staining the salivary chromosomes of Drosophila with a mixture of three parts 3/4% brilliant cresyl blue in 45% acetic acid and 1 part of 1% toluidine blue in 45% acetic acid. The excess of stain is washed out of the whole gland with 45 % acetic acid after 1/2 hour, and then the nuclei are broken. We find that this stain gives a sharper differentiation in the bands than the aceto-carmine method. A blue band on a light-grey background is more distinct than a red band on the same background when viewed through the microscope. Another modification is the use of 2% brilliant cresyl blue in 50% propionic acid. This combination penetrates more rapidly and causes less distortion.

Moree, Ray A convenient label for experimental cultures.

A new design for gummed labels has proved to be time-saving and convenient, especially for class use. The number of the experiment is written on the line after Expt.; description of the cross is written in the space below; the date on which the cross is made is written after Make; the cross is dated to be broken 7 days later, this date being written after Break (+7) (by breaking a cross it is only meant that the parents are removed); date of emergence is noted after Emerg.; the culture is dated to be discarded 8 days later, this date being written after Disc. (+8). The labels measure about 60 mm. x 20 mm.

Expt._____	Make:_____
	Break(+7):_____
	Emerg.:_____
	Disc.(+8)_____

Whittinghill, M. A method of recording multi-point linkage testcrosses.

The sample data sheet shown here has proved to be handy for linkage studies. Space and time may be saved by using this form, because all crossovers are recorded on one page, and because the sheet may be quickly prepared. It is usually made on graph paper or other data paper, allowing several lines and columns for each of the squares drawn on the sample checkerboard.

All data may be recorded in a checkerboard having one more square on each side than there are regions of detectable crossing over. Noncrossovers may be scored in the 0-0 square, putting one class in the upper half and the complementary class below. The single crossovers may be recorded on the 0 line under the column numbered for the particular region of exchange. All double crossovers may be entered at the intersections of the columns and lines numbered for the two regions of exchange; hence all doubles occupy a triangular part of the checkerboard, here the lower left. Triple and higher-multiple crossovers may be entered in the remaining large triangle as they are encountered in the

family. Each kind should be recorded at the intersection natural for any two of the three or more regions of exchange, provided the designation of all the regions of that crossover be written in the center of the square. Plenty of spaces are available for those few kinds of multiple crossovers which are apt to be found in any one culture or small family.

The division of each square into halves for each of the complementary classes of crossovers is essential; but still other subdivisions can denote the date of classifying, horizontally, and even successive subcultures from top to bottom within the large square set aside for each class of offspring. It is not too difficult to make entries in the proper places with a little concentration and a definite system of sorting specimens. Dates of classifying may be written in part of a spare square (or below in the sample table) in the position corresponding to the location of that day's counts throughout the table.

The sample table shows, for example, that 1 rucuca and 13 wild non-crossovers were obtained in the first day's count, Sept. 29, when 61 Moire' flies were discarded; that the single crossovers for region 4 were 2 ru h th st flies on the third day of counting and 1 cu sr e ca fly in the second count; that 4 h th st cu sr e flies were double crossovers in regions 1 and 7; that 1 triple crossover for regions 1-5-7 of constitution ru sr e was found; and that 1 th st sr e fly was a quadruple crossover in regions 2-4-5-7. Reference is made to the linkage map of the heterozygote given at the top of the page. No complements appeared in some cases, as shown by the empty halves of those boxes marked for multiple crossovers.

Aside from simplicity of preparation and ease of use this data sheet makes it possible to check quickly on all exchanges in a particular region of the chromosome regardless of how crossing over may be involved in single, double, and multiple exchanges. Such record forms are about to be used again in a search for oögonial crossing over.

Sample Data Sheet for Recording Crossovers

Testcross 2 ru h th st cu sr e ca by 3 rucuca/Mé

1 2 3 4 5 6 7

Regions: 0	1	2	3	4	5	6	7
1,1,4,1 0 13,4,3,2,	1,2,2,1 2,	3, 1,1 3,1	Singles	2 1,	2,1,1,1	1,1,2,1	2, 1,2 3,5,2,3
1		1-2-7 1,		1-4-5-7 1,	1, 1-5-7		
2		0		2-4-5-7 1,			
3 D o			t				
4 u b	1,	1,		h			
5 l e		2,			e	1, 2-5-6	
6 s	2,	1,		1,		r	
7	1, 2 3, 1,	2, 1, 2,		1 2,			s

Legend: Above, ru class; below, non-ru class; left to right, date classified.

S.	Oct.
29	1,3,5
61,62,46,39	

Moire' (discarded)