Research Notes.

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

This year some investigations on Drosophila have been carried on. Barigozzi and Dr. Semenza began a series of cultures in boxes of D. melanogaster for the study of competition between isogenic stocks, different for one locus. The work is in progress. A new Notch was discovered, where both hemizygous and homozygous conditions are viable. Only the sex ratio shows that the males are more numerous than the females (♂♂ 128.8 : ♀♀ 100.0). Apparently the N/N condition is less viable than N/Y. No deficiency present. Salivary chromosomes normal. Barigozzi went on collecting data on the influence exerted by the Y chromosome on cell size in D. melanogaster (publ. Nature 162: 38, 1948). Miss M. G. Castiglioni (unpublished data) began systematically to transplant imaginal disks of the eye in D. melanogaster in order to determine the action of internal environment of the size of the facets. This work is still in progress.

Buzzati-Traverso, A. D. bifasciata, a systematic note.

In Pomini's description of five species belonging to the "obscura group" (see DIS-14: 63), a systematic account of the new species. D. bilineata is given. This name is used by Pomini throughout the paper, but in the determination key (p. 163) the same fly is referred to as D. bifasciata. Since the name bilineata has been previously used by Williston for a quite different species (see Sturtevant's North American Species of Drosophila), the species described by Pomini and belonging to the European "obscura group" should be named D. bifasciata Pom.

Buzzati-Traverso, A.(2) Larvae of D. subobscura found in nature.

In order to improve the breeding technique for this species an attempt has been made to obtain data on its natural food. Previous attempts were not successful. During the month of September, 1948, adult individuals of this species as well as of D. phalerata were found congregating on fruits of cornel trees (Cornus sp.) decaying on the soil of mixed woods. A few larvae were to be seen in the fermenting fruit pulp. When put into bottles these larvae eventually matured, and from them were recovered numerous adults of the two species named. Similar results were obtained in Isola Madre, Lago Maggiore, Northern Italy, and in woods 300 kilometers apart, in the neighborhood of Belluno, Dolomites region, Northern Italy.

Buzzati-Traverso, A. (3) Wild yeasts from D. subobscura adults.

With the purpose of obtaining some information about the wild yeasts present in the natural food of D. subobscura, half-pint bottles with a layer of fermented figs were used as baited traps. The bottle's mouth was covered with a double layer of sterilized cheesecloth, in order to prevent the entrance of attracted flies into the bottle, while permitting the outflow of the fermentation scent; the outer walls of the bottle were sterilized with a Bunsen flame. After a few hours' exposure in the wild, subobscura flies were congregating on the bottle's neck and on the cheesecloth. Some of these were captured directly by means of tubes with sterlized worth-agar slants; on this culture medium several colonies of yeast developed. Such yeasts, probably present on the legs and proboscis of the flies, look morphologically alike, even when isolated in fifferent regions of Northern Italy. Studies are under way to test the preference of subobscura for these wild yeasts, as compared with the usual baker's yeast, and to determine them.

Gans, Madeleine A new, sex-limited mutation in D. melanogaster

"Z" (zeste). Spontaneous in Cy/Bl sp^2 stock. Eye color of females lemon-like, of males wild-type. Males can transmit

the gene but are always wild-type. Sex-linked, locus at 1+. The absence of manifestation in males is not due to the presence of a normal allele in the Y chromosome.

Gardner, Eldon J. A case of genetically controlled cytoplasmic influence in Drosophila melanogaster. An abnormality expressed as irregular growths on the surface of the head of D. melanogaster is being studied at the University of Utah. The trait appeared in a stock at the University of Texas which had been collected from the wild and was received at the University of Utah through the courtesy of Dr. Wilson Stone. The name "tumorous head" has been used to identify the trait and the stock which carried it. The evidence indicates that a substance in the cytoplasm interacts with an autosomal gene in expressing the abnormality. The pattern observed in the results of reciprocal outcrosses was characterized by about 30% abnormal flies in the F_1 when a tumorous-head female was crossed with a male from another stock. Less than 1% of abnormal flies was obtained when a tumorous-head male was crossed with a female from another stock. The F_2 results are comparable for reciprocal crosses, and the percentage of abnormality does not exceed 4% except in the presence of modifiers. These data are interpreted to mean that a cytoplasmic substance interacts with a genetic mechanism in the expression of the trait. Oregon-R females have been shown to carry a cytoplasmic substance comparable to that of the tumorous-head females, but the Oregon stock does not carry the gene with which the cytoplasmic substance interacts to produce the abnormality. F_3, F_4, and F_5 results show evidence of segregation of a recessive sex-lined gene which controls the production of the cytoplasmic substance, and a gene, intermediate in inheritance, with which the cytoplasmic substance interacts. Data from crosses with markers indicate that the gene with which the cytoplasmic substance interacts is in the third chromosome.

Gloor, H. Transplanatation of aristopedia and bithorax. In search of another character with non-autonomous development, imaginal discs of the middle third larval instar of the two mutants ss^a and bx have been transplanted into normal larvae of similar age, and vice versa. Both ss^a antennal and bx dorsal metathoracic transplants developed their respective mutant characters typically. The transplants also did not disturb the wild-type character of the host.

Gloor, H. (2) Dominant mutant affecting imaginal discs. From a stock, fes lt/Cy, a mutant has been isolated in which various disturbances in imaginal development take place. The most frequent type of manifestation is unilateral suppression of the ventral or dorsal or both mesothoracic imaginal discs. Less frequent are reduplications of various degrees, and only occasionally are "homoeotic" modifications found. The dominant mutant "Krüppel" (K; 2-113 approx.) overlaps wild-type (penetrance 10% +). Homozygous lethal.

Hadorn, E. Call for larval and pupal lethals. Since our laboratory is engaged in the developmental analysis of lethals, I would appreciate it very much if colleagues could send me larval or pupal lethals of Drosophila which they do not intend to analyze themselves.

Hadorn, E., Bertani, G., and Gallera, G. Results of a developmental analysis of the male genital imaginal disc of Drosophila melanogaster. The differentiations of transplanted fragments of larval male genital discs show that this disc is a patterned segregate consisting of many regions, each of which is able to regulate completely the

development of a definite part of the genital apparatus (ejaculatory bulbs, claspers, etc.). In order to determine the region responsible for a given element of the apparatus, it is necessary to divide the respective region. Thus by cutting the disc in the median plane, we always obtain two ejaculatory bulbs and two penes. If a disc becomes divided by two paramedian cuts into a central part and two lateral parts, then we get only one penis and one ejaculatory bulb (from the central part), but now the number of claspers and anal plates increases so that each one of the three parts can form two of these elements. The structures are of normal shape and reach the same size as normal elements of an undivided disc. We find, for instance, that the mean number of bristles per anal plate or per clasper is the same whether the material of a disc forms two or six of such elements. Thus in the regulating regions the cellnumber increases till the specific normal size of an element is reached.

Herskowitz, Irwin H. Sexual behavior of an anterior-posterior gynandromorph.

A gynandromoroph of Drosophila melanogaster was found that was male in the anterior half and female in the posterior half of its body. This fly was isolated with males and females alternately. When males started courting, including wing vibrations and extensions, the gynandromorph at first stood still, then decamped. The only activity it showed with females was to decamp quickly if a female came close. Even though the gynandromorph did not behave "properly" in the presence of either males or females, it did lay several sterile eggs. Since males of D. melanogaster copulate with etherized females (unpublished work of Herskowitz quoted in Streisinger, G., Evolution 2: 187-188, 1948), failure to copulate was the responsibility of the gynandromorph. Thus, although oviposition occurred, a female copulatory behavior pattern was not produced; and similarly, a male copulatory pattern was not imparted by the male genotype. This suggests that various parts of the nervous system need integration for the establishment of a copulatory behavior pattern.

Ives, Philip T., and Scott, Barbara A. A new allele of brown.

In the past two summers the Cy bw sp^2 chromosome has been used for analyzing collections of second chromosomes from the South Amherset wild populations. In 1947 a new allele of bw was found which, like bw^4, is normal phenotypically when homozygous. When crossed to several different brown stocks present in our laboratory, all nominally brown-one, but representing three slightly different phenotypes, and to bw^{2b}, it gave strikingly different results from bw^4. The only recessive bw with which it appears brown in phenotype is the bw of the Cy bw sp^2 chromosome. (It did not show bw with the Cy sp^2 chromosome from which Cy bw sp^2 arose by mutation.) The bw^4 allele shows a brown or modified brown with all the browns used except this new allele. In 1948 collections of July, August, and September from South Amherst, particular attention was given to the presence of this allele as indicated by the first-generation offspring from wild male x Bl/Cy bw sp^2 female. It was found to be present in about 2% of the chromosomes and in varying strengths from a good bw to a barely noticeable off-color when heterozygous with Cy bw sp^2. No attempts have been made to produce coisogenic or even completely homozygous stocks of the browns used in these tests; but it seems clear that this new allele, bw^{47j}, is different from bw^4 and that it represents a new addition to the many interesting and puzzling alleles known at this locus.

Kikkawa, H. Chromoplasts (plastids) as enzyme producers.

As shown by many investigators, pigment granules (chromoplasts or plastids) found in Malpighian tubes of Drosophila and Bombyx are different in shape and color according to eye mutations. Assuming that those chromoplasts are regarded as producers of enzymes concerned in tryptophane and pyrimidin metabolism, which are controlled by eye-mutant genes,

the whole system of pigment formation may be easily explained. Many experimental results support the view, and this may be applied to the mechanism of pigment formation in other organs of insects.

Kikkawa, H. (2) Kynurenine as a precursor of riboflavin. The yellow pigment having a strong fluorescence which is found in cells of Malpighian tubes of various insects, including Drosophila, is riboflavin (vitamin B_2). From various experiments, there is little doubt that this yellow pigment is derived from kynurenine. Therefore, riboflavin may be produced from the combination of a kynurenine derivative with a pyrimidin one, by functions of enzymes active in chromoplasts.

Kikkawa, H. (3) Eye pigments of insects as media of phototaxis. From a simple experiment comparing the phototaxis of bw flies with that of v bw flies, one may find that the former is always stronger than the latter. The artificial v^+ bw flies which are obtained from breeding in culture medium containing kynurenine also show a strong phototaxis as compared with the control v bw flies. The eye pigment of insects shows a strong absorptive band near the wave length 360 mu in cases where the range is extended to ultraviolet rays. As shown by Bertholf and others, the wave length 360 mu is the most effective for phototaxis of insects. The parallelism of these phenomena may throw light upon the nature of phototaxis.

King, R. C. The flight and hopping abilities of certain mutant stocks of D. melanogaster. A stock formerly called ct (Bishop) in the lists sent to DIS proved to have the genotype bi ct^6 g^2. Flies of this stock attempt to fly only under strong stimulation, and under such conditions exhibit only weak and erratic powers of flight. Such flies normally travel by walking and hopping. Flies of the constitutions ct^6/ct^6, ct^{46l}/ct^{46l}, ct^6/ct^{46l}, and ct^n/ct^n are normal in these respects, while bi/bi flies behave as do bi ct^6 g^2 flies. The restricted flying ability is therefore probably due to bi, rather than to ct as had been supposed previously. These findings led to a search through some of the stocks at Yale for flies exhibiting related behavior. All flies examined were raised on the standard corn meal, molasses, agar food at room temperature. The findings are listed as follows: (1) al b c sp^2: can beat wings jerkily; cannot fly; travel by walking and hopping, al and b flies behave normally; effect probably due to c. (2) bc (Kiil, DIS-20): can beat wings; cannot fly; behavior resembles c. (3) bs^2 and bs^{cy}: can beat wings and take off. Flying ability varies from absent or weak to nearly normal, probably depending on condition of wing surface, which is quite variable; strong hoppers. (4) dp and dp^{dr}: do not fly, but have been observed beating wings feebly while cleaning themselves; travel by walking and hopping. (5) Cy/+: can beat wings and take off; flight restricted to glides; travel by long gliding hops and walking. (6) Gl Sb/LVM: can beat wings, but unable to fly; experiments under way indicate inability to fly not due to either Gl or Sb. (7) m and m^2: can beat wings; flight restricted or absent; travel by walking and hopping. (8) Rvd/+: beat wings feebly if at all; flight absent; travel by walking and hopping. (9) w^a; ru h th st cu sr e^s ca: can twitch wings very feebly; unable to fly; weak hoppers; travel by walking. Not due to w^a or e^s; probably due to cu. (10) stw^D (Kiil): can beat wings; unable to fly; travel by walking and hopping.

Lewis, E. B. Location of c3G in the salivary-gland chromosomes. A new X-ray-induced deficiency, Df(3) sbd^{105}, behaves as a deficiency for c3G, sbd, and Sb, but not for kar, ss, or bx. The deficiency extends from the end of section 88F up to and including 89B4 (Bridges' 1935 map). Opposite a normal chromosome, sbd^{105} has a slight dominant effect of shortening or twisting the postvertical bristles; opposite sbd, the effect is

like homozygous sbd; opposite Sb, it is lethal. Small-scale tests show that $sbd^{105}/c3G$ females have the same disjunctional and crossing-over-suppressing properties as homozygous c3G females. Since on the basis of other deficiency evidence sbd and Sb are found to lie in the region of 89B3,4, the genetic locus of c3G (formerly 55+) must lie between cv-c and Sb--that is, between 57.9 and 58.2.

MacFarlane, Jean, and Brink, R. F. An orange-eye mutation. A recessive orange-eye mutation arose spontaneously in an Xple stock carrying vermilion. It has been found to involve the V locus on the first, and a third-chromosome aberration. Investigation is incomplete.

Mittler, S. Breeding populations of D. melanogaster in a large metropolitan area. Analysis of second-chromosome lethals and visible mutants of wild populations of D. melanogaster collected during the summer of 1947 in several localities of the Chicago area indicates that D. melanogaster breeds in small populations in large cities. This assumption is based on the large variation in percentage of second-chromosome lethals in the collections from one locality to another.

Moriwaki, D. Exceptional males by nondisjunction fertile in D. ananassae. Out of 29 exceptional males which appeared as the result of nondisjunction of X chromosomes, 11 flies were fertile in D. ananassae, unlike the case in D. melanogaster. It is very likely, I suppose, that the genotype of these males might be "XO triplo-IV," and that the surplus IV substitutes for Y, making the XO males fertile. An intimate relation between X and IV has been known as a characteristic of this species, along with the fact that IV resembles the Y chromosome. It has not yet been proved exactly, as the investigation was interrupted by the loss of all the stocks. This is a resumé of the following two papers. 1943, Non-disjunction of the X-chromosome in Drosophila ananassae (in Japanese), Jap. Jour. Gen. 19: 108-110. 1944, Ibid. (a continuation), Jap. Jour. Gen. 20:88.

Muir, Irene, and Brink, R. F. A new dumpy-like mutation. Investigation of a new dumpy-like mutation arising from X-rayed wild stock proved it to be an allele of dumpy. Wing shape and truncation range from typical dumpy to almost wild-type, so that the presence of modifiers is suspected.

Muller, H. J. The construction of several new types of Y chromosomes. It was thought desirable to have a chromosome consisting of the short arm of the Y, with a marker attached, to parallel the $sc.Y^{l}$ which had been found useful in many experiments. For this purpose, as well as for further analysis of the point of breakage in the scute-VI mutation described in DIS-21, males containing the X chromosome having this pericentric inversion and a normal Y chromosome were crossed with yellow forked females with doubly attached X's and a normal Y, and non-yellow forked females were looked for among the progeny. One of these was found and proved to be of the desired type, namely, a crossover having the short arm of the Y with the right-hand terminus of the X, including the locus of scute-VI and of non-yellow, attached to it beyond its centromere. This chromosome therefore has a perfect short arm, with all its fertility genes, and the other arm is no longer than the tiny right arm of the X chromosome. It is designated as $sc^{VI}.Y^{s}$.

In order to get a Y chromosome having the left end of the scute-8 chromosome attached to it, a similar cross was made, using however males whose X

chromosome carried the scute-8 inversion. A non-yellow daughter was obtained which proved to have a Y chromosome comprising both long and short arms, but with the left-hand terminus of the scute-8 chromosome, including the genes y^+ and ac^+, attached in place of the end of one of these arms. Cytological study of mitotic figures by Dr. A. M. Hannah indicated that the long arm had been lengthened so as to be about the size of an X and therefore had that portion of the heterochromatic region of the X which in the scute-8 chromosome is at the left end, attached to the long arm of the Y.

Before the cytological study was made a genetic analysis was undertaken to determine to which arm of the Y the scute-8 piece was attached. This was done by crossing males having the sc^8.Y, as we term it, and an X having the short arm of the Y attached, with yellow forked females having doubly attached X's, and in this case looking for daughters which were yellow. Three of these were found, and stocks containing the re-formed Y's were established. Flies from these stocks were then crossed to flies of two other stocks, having respectively the short arm of the Y and the long arm of the Y attached to their X, in order to determine, from the fertility of the male offspring, which arms of the Y were represented in the newly formed Y chromosomes. Two of them proved to have only the short arm of the Y. It was concluded that these, having occurred by crossing over between the sc^8.Y and the heterochromatic region of the X that had the short arm of the Y attached, consisted of two short arms of the Y attached to one another at their centromere. These chromosomes are designated as Y^s.Y^s#2 and #3 respectively, since the first such case was found by Stern. This at the same time implied that the sc^8 piece had been attached to the long arm, not represented in these crossover chromosomes. As for the third case, the fertility tests showed it to have all the necessary fertility genes of the long arm of the Y but not to have those (or not all of those) of the short arm. At the same time, this chromosome behaved as though it either lacked the locus of bobbed or had only a very extreme allele of bobbed. It was concluded from this genetic evidence that this chromosome had not resulted from crossing over between X and Y but from breakage of the long arm between the fertility genes and the sc^8 piece and breakage of the short arm of the Y basally to $bobbed^+$ and to all or some of its fertility genes, followed by union of both proximal broken ends with each other to form a ring chromosome. Cytological examination of mitotic figures by Dr. Hannah then showed conclusively that this Y chromosome is in fact in the form of a ring. Its mitotic length seems about equal to that of an X. Considering, however, that practically all of this mitotic length is due to a very few blocks, it is noteworthy that a ring chromosome with so few genes can survive and multiply.

This new "closed" $Y^{\underline{1}}$ is designated as $Y^{c\underline{1}}$. It is kept in stocks in which the males, in order to be fertile, have a Y^s attached to their X. It is to be noted that this is a very good way to keep a stock having the short arm of the Y attached to the X, since it can never be lost by crossing over with the Y, for such crossing over would produce a dicentric chromosome. This is also a useful way to keep stocks of attached X chromosomes of the ordinary, singly attached type. For crossing over between such an X.X and the ring-shaped Y would again form a dicentric chromosome that would be lost. Hence we are in process of converting all our ordinary attached X stocks to this form.

These Y's are to be studied for their effects on segregation, and on the expression of heterochromatic displacements.

Nolte, D. J. A graded series of eye colors due to interaction of genes.

Since the paucity of stocks of this laboratory has been rectified during the course of this year, espcially through the courtesy of Dr. M. Demerec

of Cold Spring Harbor, the interaction-product of a white eye color in our "y w" stock, as reported in DIS-18, has been determined to be conditioned by the compound w^e rb. With this stock as example, a series of compounds of the multiple alleles w^{sat}, w^{bl}, w^{co}, w^{ch}, w^e, and w^a with each of the mutants rb, cm, g and car has been built up, and has yielded graded series of eye colors. The last named four genes have a dilution effect on each of the multiple alleles, with a decreasing power of dilution of the four in the named order.

Comparison of phenotypes with the Munsell Color Atlas has indicated discrete grades of color ranging from a dark red through the browns, oranges, and yellows to white. In addition, compounds of rb, cm, g, and car are being built up in an attempt to determine the relations between these more-or-less-similar mutant types. Histological sections of the different mutants and compounds have indicated the reality of the dilution effect, but this cannot be based on the concentration of pigment in the four dilutors since these do not show a decreasing concentration in the named order.

Concentrations of pigment will now be determined spectrophotometrically in order to ascertain the possible roles of the red and the brown components, or of other factors, in this dilution series.

The mutant ras^2 has very little effect in compounds with the w-series, but histologically our ras^2 stock seems to have an eye color effect correlated with a derangement or abnormality of the secondary pigment cells of the eye.

Novitski, E., and Rush, G. Tolerance of Drosophila melanogaster to sub-zero temperature.

In conjunction with experiments on the effects of X-rays combined with low temperatures, information was obtained on the tolerances of Drosophila to sub-zero temperatures during the time intervals encountered in the usual X-ray exposures. For this purpose, a heating unit, thermostat, and blower were placed in a deep-freeze unit so that accurately controlled sub-zero temperatures could be maintained. The individuals to be treated were placed in size 00 gelatin capsules punctured at both ends to facilitate rapid cooling when placed in the cold air blast.

The observations may be summarized as follows. At -5°C. about 50% mortality of the Canton-S individuals used throughout these tests was reached after two hours; at -10°C. a 20-minute exposure kills very few whereas a 25-minute exposure is almost completely lethal; at -15°C. about 50% will survive exposures less than 10 minutes long, an exposure of 13 minutes or longer is completely lethal; at -20°C. all the flies are killed within 2 minutes, which thermocouple tests show is shorter than the time required for the flies to reach the temperature of the surrounding air blast. Males and females react similarly. Various combinations of pretreatments have been tried in an attempt to prolong life at low temperatures, with no success. It has been noticed, however, that complete recovery from etherization is essential for maximum recovery from these exposures.

Novitski, E., and Rush G. (2) Desemination by low-temperature shocks.

Fertilized Drosophila melanogaster females may be deseminated (see Muller, DIS-18) by exposure to sub-zero temperatures. About 16% of such deseminated females produce single offspring; these apparently represent eggs already fertilized and in the oviduct at the time of treatment, because their frequency cannot be decreased by two successive exposures and because they always hatch from eggs laid during the first 24-hour period after treatment. Cold shocks of -5°C. for 75 and 90 minutes and of -10°C. for 5 to 20 minutes, applied in the

manner described in the preceding note, have been 100% effective, except for the sporadic progeny. This treatment has been used with very good results in experiments where large numbers of virgins have been needed.

Ogaki, M., and Oshima, C. Phenocopies induced by photodynamic dye and near infrared rays.

Third-instar larvae of the Oregon strain of Drosophila melanogaster and of some strains of D. virilis are being tested for induction of phenocopies by photodynamic dye (Illuminol R II) and near infrared rays. Some results obtained so far are reported here. For irradiation, the larvae were placed in a glass tube, with about 1 cc. of 0.01% dye solution, and exposed to the light from a 60- or a 500-watt projection lamp at a distance of 15 cm. Between the light source and the tube, a water bath and Riken VR3 filter were placed. The glass tube was cooled with an electric fan, and the temperature of the atmosphere and of the liquid in the tube was kept at not more than one degree above the room temperature throughout the experiments. After irradiation the larvae were washed with distilled water, and placed in shell vials to complete development. Three hundred larvae, 60-100 hours after hatching (Oregon strain), were exposed for 300 minutes to the beam of a 60-watt lamp. About 58% became flies, and of these 5.8% showed abnormalities. Of 250 larvae treated with 500-watt radiation (without VR3) for 30 minutes, about 67% became flies, and of these 3% showed abnormalities. Larvae of the ebony strain of D. virilis were also tested. Irradiation for 30 minutes with a 500-watt lamp induced 12.5% abnormalities of all 40 flies. Eggs from a strain of D. virilis dechorionated by sodium hypochlorite were immersed in the dye solution. These eggs were exposed for 30 minutes to the beam of a 6-watt lamp. Of 96 eggs treated, about 10% were stained by the dye which entered through the micropiles. From these eggs no flies emerged. Among 45 flies obtained from 96 eggs, two females had no scutella. Many kinds of "phenocopies"--abnormal abdomen, white, missing bristles, folded wings, no mouth, etc.--appeared after these treatments. No indication was found, however, that irradiation at a particular developmental stage tended to produce a particular kind of change, as was found by Villee and Lavin (1947) with visible light and photodynamic dye (cyanin). All abnormal individuals are being tested for their heredity. It seems that abnormal flies as a rule have very low fertility.

Oshima, C. Low fertility of hybrids between D. virilis and D. americana which have X^{v-a} (compound X of virilis-americana), Y^{v} (Y of virilis), and A^{v} (autosomes of virilis)

D. americana females were crossed with virilis mt-we males, and the F_1 were mated with virilis y-ap males. The females produced were then mated with mt-w^{e} males. Three equational nondisjunctional y females were found among the females in the next generation. These hybrid females were found, by observation of the salivary-gland chromosomes, to have X^{v-a}, X^{v}, Y^{v}, and A^{v}. In the X was the americana overlapping inversion. The gonial metaphase showed one V-shaped chromosome (X^{v-a}-4), eight rod- and two dot-shaped chromosomes. These females were crossed with virilis bb males and yielded:

♀ X^{v-a} X^{v}(y/y-ap) x ♂ X^{v} Y^{v}(bb)

F_1 ♀ (+)	Exc.(y)	(bb)	♂ (y)	(y-ap)	Exc.(+)	Exc.(bb)	T.
3077	388	3	888	1426	371	9	6162

Thus the females producded very high secondary nondisjunction--about 12.5% and the F_1 y males (X^{v-a} Y^{v}) were less in number than y-ap males (X^{v} Y^{v}) in all vials. The fertility of the former was much lower than the latter when tested

by means of the competitive cross with y-ap females. The low fertility of the heterozygous males is probably due to a factor (or factors) existing in americana's X which disturbs the genic balance of X and Y, something like in the case of montana and texana hybrids, though it is apparently less effective. The unexpected bobbed females were probably due to crossing over between X^{v-a} and X^{v}, at a locus between ap and bb. The americana's inert region probably has a deficiency in bobbed locus, and the factor (or factors) in americana's X mentioned above possibly has some relation to this deficiency.

Slizynski, B. M. Cytological limits of In-S. Bridges and Brehme's book describes, under sc^{S1}, an inversion in the X chromosome for which analysis by Muller and Prokofieva showed that besides the large inversion and inside of it there is also an inner inversion, smaller than In(1) dl-49 and having its breaks inside those of In(1)dl-49. Demerec (Genetics 33: 337-348) quotes Dr. Bruce Wallace, who determined the break points of the inner inversion as: first break in region 6AB, second break in section 10F. Incidentally, I have found a figure in which the limits of the small inversion may be determined down to bands. The first break occurred just after the 6A1,2 capsule, the second break after 10F10 but before 11A1,2. The fate of the 10F11 faint band is not known. The new chromosomes is-----6A1,2/10F10-----6A3,4/11A1,2-----.

Strømnaes, Øistein The production of dominant lethals with X-rays in aged Drosophila melanogaster sperms. Male flies from an Oregon-R-C stock were stored alone for from 1 to 23 days and then given a dose of 2300 r of X-rays. The treated males were mated to virgin females. The percentage of dominant lethals induced was determined as the percentage of eggs that failed to hatch. The experiments showed an increase in dominant lethals induced correlated with an increase in age of the sperms at time of irradiation. Thus, 1-day-old sperms gave ca. 45% dominant lethals, and 17-day-old sperms ca. 69%. In the control series, with untreated males, no increase in dominant lethals was observed with an increase in age of the sperms. When the males, before irradiation, had been stored with an excessive number of females, no increase in dominant lethals was observed until the 15-day male group. Then, however, a drastic increase was observed, which is attributed partially to the exhaustion of sperm due to the high level of sexual activity of the males, and partially to their radiation of very young and immature sperms. An experiment was carried out chiefly to determine whether early or late development would make any difference in sensitivity to exposure to X-rays. Early-developing males, when irradiated at 1 day of age, gave about 68% dominant lethals, and late-developing males gave about 49%. Both groups gave about 65% when irradiated at 15 days of age. The interesting hypothesis is suggested that only sperms from late-developing males show an age effect with regard to sensitivity to induction of dominant lethal mutations by X-rays. Further experiments are in progress to test the hypothesis.

Sulcy, A. C. E. Modifiers of the expression of the gene grandchildless. All the offspring of a gs/gs (chr.3) female are sterile and have reduced gonads (Spurway, DIS-20). When two lines segregating for the gene were crossed together, abnormal cultures in the resulting stock contained only 2% - 20% of sterile flies in the offspring of paired matings. It was now possible to breed from sibs of sterile flies instead of from their cousins. But after about nine generations of this type of stock-keeping (paired matings), the stock has three times become so sterile that it was only saved by outcrossing. There is evidence that once, just before this happened, the stock had become homozygous. The condition appears to be due not to a mimic gene, but to maternally acting modifiers, which reduce the proportion of offspring that are

affected. This complex of modifiers appears to be recessive to those in the normal gs stock. The laboratory stock of nt does not contain modifiers which can restore the normal gs expression by the F_3 (test of F_2) when the new stock is outcrossed to nt. This outcross also suggests that the modifiers in the new stock can very occasionally produce sterile flies when the gs gene is absent.

Suley, A. C. E. and Milani, R. Transplantation of gonads between wild-type D. subobscura and the offspring of homozygous grandchildless females. Third-instar larvae of gs/gs females have gonad discs very slightly smaller than discs of wild-type larvae. Transplants to wild-type larvae develop autonomously. That is, the gonad implant, three days after the eclosion of the host, appears like the minute gonad of the sterile offspring of a gs/gs female. Therefore, either the effect of a gs/gs female on her offspring is directly on the germ line, or any general effect, through the soma of the offspring, takes place before the third instar is reached. Gonad implants from wild-type larvae to the offspring of gs/gs females also develop autonomously.

Whittinghill, M. Lethals in chromosome 3 wanted. I would like to be informed of the availability of recessive lethal stocks having any lethal near locus 40 or near locus 58 in chromosome 3.

Wolfe, Leslie, and Brink, R. F. Susceptibility to X-ray radiation at different stages of development. X-ray radiation of 7080 R. U. was applied under constant conditions to 50 representatives of each 24-hour period in the life of the pre-adult Drosophila. The egg and larval stages proved to be highly susceptible, with 100% lethability. but the lethal effect was delayed until after pupation, 80-98% forming pupa. The X-rayed pupae showed a variable susceptibility, 60% emerging from the 144- to 168-hour group, 76% from the 168-to 192-hour group; and 60% from the 192- to 216-hour group. This difference in susceptibility is closely correlated with changes in physiological activity occurring during these periods of development. The differential fertility existing in surviving flies and the structural abnormalities occurring in the imagos could be correlated in many cases with developmental changes which were proceeding at the time of irradiation.

TECHNICAL NOTES.

Clark, Carl A flotation method for the collection of insect eggs.

During a recent review of Drosophila embryology, a flotation method was developed for the rapid collection of large numbers of eggs from the food on which they were deposited. Cups of fresh food were put into a Drosophila population cage containg about 2000 flies, for a specified time. The cups were then removed, and the surface layer of food, containing the eggs, was scraped into a 100-cc. graduate cylinder containing 75 cc. of saturated salt water. The cylinder was shaken and stirred, causing the food to sink to the bottom of the salt water and the eggs to float to the top. The cylinder was then filled to the top with salt water and the floating eggs, free of food, were poured off. The eggs do not float in fresh water. For other insects than Drosophila perhaps other fluids of other specific gravities will be required for the separation of eggs from food.

The embryos are still alive. A chemical study might well be made of this copious material. If the food cups are put into the population cage for, say, one-half hour and then removed, a series of closely isochronic embryos can then be collected for study. If all the flies in the population cage represent a particular genetic cross, the large numbers of collected embryos are sibs.

For the embryological work, the eggs were poured directly from the cylinder into a 25-cc. Stendor dish containing 15 cc. of 5.25% sodium hypochlorite (as the commerical Chlorox, Veedox, etc.) for chemical dechorionation. (Slifer, Eleanor H., Removing the shells from living Drosophila eggs. Science 102:282, 1945.) With care, the eggs can be decanted from the cylinder with less than 10 cc. of water, giving a final hypochlorite solution powerful enough to remove the chorions in two minutes. The eggs float in this solution; any food still with the eggs sinks to the bottom. Eggs about four minutes in sodium hypochlorite are damaged, as shown by the black border they develop in hematoxylin-stained sections. The eggs and hypochlorite solution are poured from the Stendor dish into a 100-cc. graduate, which is then filled with distilled water, in which the eggs sink. This water is changed, and the clean dechorionated eggs are ready for further study.

The embryos are still alive. (Hill, David, Chemical removal of the chorion from Drosophila eggs. DIS-19: 62, 1945.) Certain organological changes in the dechorionated living embryo can be observed with a microscope. Dark-field illumination and phase microscopy should be of help here. The whole eggs may be stained.

For fixation and staining, the embryos are transferred to 3.5-cc. vials. Even with mechanical dechorionation, Huettner recommends that the eggs be punctured with a fine needle for proper fixation. (Huettner, Alfred, Origin of the germ cells in Drosophila. J. Morph. 37: 385-423, 1923.) It was suggested by this work that satisfactory fixation was obtained for the chemically dechorionated material without puncturing. FAA fixative (5 parts 37% formaldehyde : 15 parts 70% alcohol : 1 part glacial acetic acid) was used for eight hours. The further procedures employed for staining and sectioning are standard. (Rabinowitz, Morris, Studies on the cytology and early embryology of the egg of Drosophila melanogaster. J. Morph. 69: 1-50, 1941.)

Rifenburgh, S. S. Ridding Drosophila stocks of mites.

The cultures and shelves in our laboratory were badly infested with mites, which were apparently similar to those reported on by Hughes in DIS-20. After trying various procedures and several messy acaricides, a method was developed which has kept our stocks free from

mites for more than two years.

In getting rid of mites, two steps much be successfully accomplished: (1) start mite-free cultures, and (2) prevent mites from migrating into such cultures. The first step was done by successive transfers at five-day intervals. A slight change was made in the technique of transferring stocks, in order to prevent shaking mites off the old plug into the new culture bottle. Under the conditions obtaining in our laboratory, five days was long enough for most of the hypopi to transform into adults and settle down, but not long enough for the next generation to appear. The second step was finally worked out successfully by sprinkling the shelves where cultures are kept with a coat of powdered sulfur, and by making sure that the culture bottles--including the cotton plugs--were far enough apart to prevent them from touching each other.

Slizynski, B. M. An improved method for squash preparations. In making permanent squash preparations the most troublesome point is that very frequently the cover slip does not go off. It has been noticed that in such cases, when the cover slip is forced away (with razor blade, etc.), most of the material does not stick to the albuminized slide and is in the majority of cases completely lost.

The new technique is as follows. Dissection in 45% acetic acid (with carmine, lackmoid, or orcein stain); staining 10-20 minutes. Afterwards the glands are transferred onto a clean slide and placed there in a small (2- to 3-mm. diameter) drop of stain. An albuminized slide is now placed over the clean slide, with its albuminous layer facing down. The albuminized slide is rested by one end on the clean slide (about 1 cm. from its end), the other end being lifted at an angle of about 45°. By forcibly letting the lifted end fall down on the preparation, necessary crushing of nuclei is obtained. After that some additional pressure is applied with a finger tip. The two slides are then submerged in 45% acetic acid, in which--in about half an hour--the albumized slide, together with all the material sticking to it, slides down and may be carefully transferred to alcohol for further procedure.

Strømnaes, Øistein Egg-counting made easy. The author uses a small creamer (i.e., a miniature milk bottle of 20 cc. capacity) to store the egg-laying female, and places the food on the cap covering the creamer. The food consists of equal parts of mashed banana (the pulp filtered away) and a 4% agar solution. If desired, molasses can be added to darken the solution. Small rectangles (12 x 17 mm.) of black filter paper are dipped in the culture medium and placed on wet paper towels. After a few minutes they are dipped again and then placed on the cap. To facilitate the egg count, a line can be drawn with a knife, in the medium, along the middle of the rectangle. The caps with fresh food are kept moist by placing them on wet paper towels between two glass plates until they are used. Experience has shown that the flies lay all their eggs on the culture medium. A drop of very dilute yeast suspension is added to the food on those caps used for egg counting, and a heavy yeast suspension on those used when only storing the females. In the latter case the author found it more convenient to add the culture medium directly on the caps, using an eye dropper. More medium is needed if the caps are not parafinized. This method makes it easy to obtain samples, which can be readily counted and stored for later observation of the hatchability of the eggs.