Research Notes

Coyne, J. Crossing-over within a closely linked group of genes near locus 40.4 of Chromosome 3.

Preliminary tests for the location of M 33j (Demerec, x-ray induced) showed it to be between hairy(26.5) and thread (42.2) in the third chromosome. With the intention of using Dichaete as a marker M 33j was crossed with Ly/D $. The combination Ly/M33j proved lethal, since there were no Ly/M33j survivors in a total of 2416 flies. This led to testing M 33j as a possible deficiency for all available genes in the 40.4 group around D. Those tested included Glued, Minute-h, Lyra and Dichaete itself. The combination with Lyra was the only one proved to be lethal. The Mh/M33j combination is also probably lethal, but further tests must be made. Mh is not lethal with either Ly, D, or Gl. This evidence made it seem more unlikely that all five of these genes are uni-local. Crossing-over tests for all viable combinations of the five genes gave the following results: (Gl/D was not tested since Plough's evidence (Gen.20: 42-60) shows that there were no crossing-over between these two genes in 5000 test flies).

Mating	No. of crossovers	Total No. of flies
D/se Mh x seple	D-Mh=0	484
Ly/se Mh x seple	Ly-Mh=2	1335
Ly/se Gl x seple	Ly-Gl=10(.61%)	1933
Gl/se Mh x seple	Gl-Mh=24(1.4%)	1046
se D/M33j x seple	D-M33j=0	298
se Gl/M33j x seple	Gl-M33j=0	772
Ly/D x M33j/Payne	Ly-D=0	2416

Since the 10 crossover flies between Gl and Ly were of the two phenotypes se and Ly Gl, it is probable that Ly lies to the left of Gl, unless these 10 were all double crossovers, which is unlikely. Since there is no crossing-over between Ly and D or between D and Gl, it is likely that D lies between the two. As the combination M33j/Ly is lethal, M33j may be a small section deficiency to the left of and including the Ly locus. Similarly, Mh may be a deficiency just to the left of and including part of the M33j deficiency. This possibility is further confirmed by the large crossover percentage of 1.4 betwen Gl and Mh, which places them at the two extremes of the region under observation. From these experiments the probable order from left to right of these five mutatnts would be Mh, M33j, Ly, D, Gl, covering a total map distance of 1.4 units. Further tests will be made to get larger numbers for D/M33j and D/Mh crossovers, and to prove definitely the inviability of Mh/M33j. My, an allelomorph of Mh recently received, will also be investigated.

Dunn, L. C. Effects of minutes on developmental rate and on eye size of mutants.

Supplementing report in DIS-3, M1 has retarding effect on larvae, similar to Mw. Mw, M1, M33j, Mz and $M1^2$ act as minus modifiers of L/+ and L^4/+ in order given, also as minus modifiers of L/L. Mw, M1 and Mz are lethal in pupal stage when combined with L^2/+. Mw, M33j and $M1^2$ are minus modifiers of B/+ and B/B. Mw, M1, M33j and $M1^2$ are lethal or of very low viability with ey^D/+; Mw, M1 and $M1^2$ are of very low viability in combination with Dfd/+.

L and L^4 prove to be very sensitive to changes in larval growth rate brought about by culture conditions (temperature, crowding). Retardation of early growth results in increase in size of Lobe eye; effect especially marked when a Minute is also present, often resulting in eyes larger than wild-type.

Bar and Lobe have cumulative effects in reducing eye size; Bar Lobe flies often show Bar in one eye, Lobe other.

Neither L or L^2 has any marked effect on developmental rate.

Gottschewski, G. Eine deficiency ohne einen genetisch bzw. cytologisch nachweisbaren Ausfall eines Chromosomenstuckes.

Nach Hitzeexposition eines ss/ss ♀ (18-24 Std. in 35-36°) wurde von Goldschmidt mehrere Male eine Mutation gefunden, die vor allen durch Kerben an den Flügelspitzen gekennzeichnet waren (phanotypisch = cut-Beaded-Kombination). Die mutation wurde von mir $Notch^G$ genannt. N^G/+ ♀ x fa ♂ (34j23): F_1 ♀ N^G fa 56 ♀ + 61; ♂ + 71. N^G/+ ♀ x spl ♂ (35f12): F_1 ♀ N^G spl 41; ♀ + 53; ♂ + 59. N^G/+ ♀ x ec ♂ (34j23): F_1 ♀ N^G ec 166; ♀ + 163; ♂ + 157. N^G/+ ♀ x ec ♂ (35e18) F_1 ♀ N^G 9; ♀ + 7; ♂ + 9. Demnach liegt eine Df für fa und spl vor. Ob die Df immer den Locus ec einschliesst, ist noch zu entscheiden. Ein Verlust einer Chromosomenstrecke erscheint nach den genetischen Befunden unwahrscheinlich, da der Faktorenaustausch zwischen Genen, die Df einschliessen, nicht kleiner, sondern grösser ist (Vgl. Linkage data). Der cytologische Befund: Carmin-Eisessig-Quetschpraparate zeigt eindeutig, dass die Banden in der fa-region unverandert sind. In keinem Praparat hat sich eine Abanderung von der für Deletionen bzw. Chromosomen-deficiencies typischen Struktur nachweisen lassen.

Hoover, Margaret E. Salivary limits of delta-49 inversion.

Delta-49 (d1-49) inversion which has been found and analyzed at the Austin Laboratory is a useful x-chromosome balancer since it is not lethal when homozygous and it prevents entirely crossing over from cv to g and reduces it greatly in other regions of the chromosome. A study of good salivary chromosome preparations seems to indicate that cytologically d1-49 extends from 4D2 to and including 11F3. A well spread figure was found in material heterozygous for d1-49 in which the x was split from the left end of the

inversion through to the chromocenter, one half of the split being the normal and the other, the inverted half. In such a figure the bands could be carefully followed along the length of the chromosome to the end of the inversion where the matching bands were found in corresponding positions. This figure was also checked by C. B. Bridges. Although the similarity in size and shape of 4D1 and 12A1 make it possible that this interpretation is incorrect, evidence seems to indicate that 4D1 and 12A1 are the outside limits of d1-49.

Karp, M. L. The distribution of mutant genes affecting the number of sternital bristles in chromosome 3 of D. melanogaster. In chromosome 3 of D. melanogaster the presence of at least six mutant genes affecting the number of sternital bristles, independently of the possible effect of the gene markers, has been shown. These genes possess a considerable power of action, approximately 5 to 15 per cent of the manifestation of the character. Being opposite in tendency and alternately located, they are more or less balanced, not only along the whole length of the chromosome, but within its small regions as well. In the chromosome, causing the reduction of 5-6 bristles on 2 sternites of the abdomen, were detected genes which determine conjointly the reduction of 18-21 bristles on the same 2 sternites, and on the other hand there were found genes which together intensify the character by 12-20 bristles. Hence the genic balance of the chromosome examined offers the possibility of a considerable change as to the extent of the manifestation of the character.

Kaufmann, B. P. Drosophila ananassae (D. caribbea) In the autumn of 1933, D. caribbea was collected in the vicinity of Tuscaloosa, Alabama, which is considerably north of the range of distribution of the species as indicated by Sturtevant. Male flies of this stock have a J-shaped Y-chromosome, whereas the stock used by Metz (1916) had a rod-shaped Y. Recently a Nipponese stock, secured through Dr. W. P. Spencer, has been examined. This also has a J-shaped Y. Additional material, especially from America, is desired for further study.

Kerkis, J. Sex-Linked vestigial like mutant in Drosophila simulans On May 28, 1935 a single male was found in a normal mass culture of D. simulans which was like a vestigial of D. melanogaster. This male was crossed with normal simulans v. The F_1 was normal. Flies from F_1 were inbred and in F_2 there were 269 normal ♀♀, 105 normal ♂♂, and 81 vestigial ♂♂. There were no vestigial ♀♀. Males from F_2 were crossed to their sisters and in F_3 homozygous flies were produced from which a stock has been propagated. One of the ♂♂ was mated to a yellow white attached ♀♀ of D. simulans and gave in F_2 308 vestigial

♂♂ and 254 yellow white ♀♀, 2 normal ♀♀ and 3 yellow white ♂♂. The latter two classes were produced by separation of the attached X's of the yellow white ♀♀.

The data on the location of the new mutation show that it is located in the right end of the X-chromosome.

Kikkawa, H. Systematics of Drosophila. While examining the salivary chromosomes of various species of Drosophila I realized that there are (at least) two different groups with respect to the ratio of the total length of autosomes to length of X-chromosome, viz., the one giving the ratio of about 4:1 and the other, about 1.8:1. D. melanogaster, virilis, funebris, ananassae, repleta, etc. belong to the former group, while D. pseudoobscura, affinis, miranda, etc. belong to the latter. Morphologically, there is also a distinct difference between the two groups in the shape of testis. These characteristics may be worthy of dividing the genus Drosophila into two subgenera. My inference proposed in Proc. Imp. Acad. Tokyo, 9, 1935, may be applicable only to the former group. Full investigation in connection with genetics is now underway.

Parker, D. R. Locus of wy^2 (formerly cx_b). Crossover counts on the male offspring of females y v f/y^2 wy^2 g^2 were made in order to determine the locus of wy^2 more accurately. The results are given: v f - 1163; wy^2 g^2 - 1111; v wy^2 g^2 - 151; f - 126; v g^2 - 27; wy^2 f - 38; v - 208; wy^2 g^2 f - 180; v wy^2 f - 1; g^2 - 0; v wy^2 g^2 f - 5; + - 5; v g^2 f - 1; wy^2 - 1; v wy^2 - 1; g^2 f - 0; Total 3018.

These data place wy^2 about 2 units to the left of garnet. 100% of the F_1 females of a cross of wy^2 x wy were phenotypically wy; there was no crossing-over observed between wy and wy^2 in 1328 offspring from wy/wy^2.

Stark, M. B. Varieties of tumors. Selected stocks heterozygous for lethal-7, where the l-7 males die from the development of melanotic growths, show that the tumors occur in characteristically different tissues. A preliminary description of the stocks follows:

& ♀ 1 Carcinoma or melanonoma of salivary gland
& ♀ 2 " " " of stomach region
& ♀ 3 " " " of lower intestine
& ♀ 4 Lympho-sarcoma
& ♀ 5 Pigmented lipoma

The third-chromosome "benign" tumor is found to involve connective tissue.

Stone, Wilson. Allemorphic phenomena. y^{35a} An allele, phaenotypically like y^1, induced in the inversion, 99b, by x-rays.

y^{31e} (y^{303h}) A mutation accompanying a long inversion, probably y^{3P} as designated by Muller, for it gives the same males hypoploid for y and ac by crossing-over with sc^8. This mutation

is between y^2 and gray in phaenotype, but gives occasional patches of y^1 bristles and microchaetes.

y^{31d} sc^8 w^a(5 k). An x-ray induced allele in sc^8, phaenotypically like y^2.

The following crosses differentiate between these alleles:

Cross			Phaenotype of F_1 ♀
y^{35a} 99b	x	y^1	y^1
y^{35a} 99b	x	y^2	gray (♂)
y^{35a} 99b	x	y^{31e} (303 h)	31e with y^1 spots
y^{35a} 99b	x	y^{31d} sc^8 w^a	y^2
y^2	x	y^1	y^2
y^2	x	y^{31d} sc^8 w^a	y^2
y^2	x	y^{31e} (303 h)	y^{31e} -- no y^1 spots,

for in y^2, all bristles and microchaetes are black. y^{31e}/y^2 appears darker than y^2/y^1, although this could not be determined accurately, since y^{31e} is so nearly normal in color.

Although y^{35a} and y^1 are phaenotypically alike, as are also y^2 and y^{31d}(sc^8 w^a), their gene action is not identical for all steps of pigment formation. Thus, the action of these yellow alleles show them to be qualitatively as well as quantitatively different.

f^{34b}: An allele of forked, induced by x-radiation, which is phaenotypically normal in both males and females. When crossed to the original forked f, it gives a few weakly forked bristles, but shows more pronouncedly when crossed to f^5. (f^5 seems to be an amorph (Muller)). This allele is not a hypomorph, but is not strongly hypermorphic, since one dose is not sufficient to compensate for an absence and form normal bristles in the f^{34b}/f^5 condition, although it often does so in the f^{34b}/f heterozygote. It is interesting to note that the action of f^{34b} is equivalent to the "position effect" action induced in the normal allele of cubitus-interruptus by translocation involving chromosome 4, as found by Dubinin and Sidorov.

Timoféeff-Ressovsky, N.W. Induction of mutations by alpha-particles in D. melanogaster. The penetration-power of alpha-particles of radium is so low that it is impossible to induce mutations by external irradiation of flies. Thus, the following method was employed with sucess: The flies were put in a bottle with a cork-stopper containing a radonator. The flies (males) were breathing the emanation produced by the radonator for about 24-40 hours. After this treatment, the males were mated to ClB-females, and in F_2 the per cent of sex-linked lethals and visibles was determined. The mutation-rate was 1% in the treated series, as compared with 0.12% in the controls, the difference being statistically significant. Exact measurements of the radioactivity of the treated flies showed that the energy of the alpha-particles, emited by the emanation and the radioactive precipitate in the fly-tissues, is equivalent to an X-ray dosage that would produce about 1.5% of sex-linked mutations. The energy of the beta-particles and gamma-rays within the same flies is so low (about 1% resp. 0.01% of the alpha-energy) as to

be negligeable. Thus, the effect produced must be ascribed to the action of alpha-particles emitted by the radioactive precipitate in the tissues of flies breathing emanation of radium.

Gershenson, S. Possible role of the genetically-inert region of the X-chromosome in equational divisions. When bobbed-deficiency (sc^4 - sc^8) males are crossed with yy females, patroclinous females are found in the offspring with a much higher frequency than usual. It seems, therefore, probable that the genetically-inert region of the X plays a role not only in the conjugation of chromosomes during synapsis, but in the equational divisions as well. In two cases among seven, several patroclinous females were found in the offspring of one bb-def. male. Such a coincidence could hardly have been accidental and probably means that equational non-disjunction of the X's took place several cell-generations before maturation.

Technical Notes

Beadle, G. W. Collection of eggs. For the collection of eggs for measures of egg or larval-pupal mortality, small paper spoons containing food have commonly been used. They have the disadvantages of giving a food mass of unequal thickness and usually with a rounded surface. Detection of all the eggs is often difficult. Small nickel boxes made of sheet material about 0.3mm. thick and of the dimensions 15 x 40 x 4mm. with a strip 45 x 10mm. soldered to the bottom so as to project about 30 mm. have been found to be very useful for egg counts and for collecting larvae of known ages. Standard cornmeal agar (containing animal charcoal, if desired, to increase the contrast) is pipetted into these boxes, filling them level full. They can be used in 20 x 100 mm. vials very conveniently. Examinations under a binocular can be made very rapidly. Experiments with different media with and without yeast indicate that yeast is a very important factor in stimulating rapid egg-laying. Standard food "painted" with a rather heavy suspension of yeast gives very satisfactory results. If it is necessary to have the eggs develop into adults, it is easy to slide the food mass out of the box on a cardboard strip 9 x 70 mm. It can then be transferred with eggs or larvae to a standard culture bottle containing food. With care, no eggs or larvae need be lost in the transfer.

Christie, A. L. M. Culture conditions for D. Subobscura. D. subobscura is being worked with in this laboratory and at first considerable difficulties were experienced with the culture conditions. The flies were reared in a 20-22 C incubator and on the usual D. melanogaster food medium. Of 200 single pair matings set up, 92 were sterile. The fertile matings gave on an average about 166 flies during a counting period of 19 days. The development takes at this temperature between 19 and 21 days.

It was then found that rearing the flies in a 15 C. incubator gave better results. Of 60 single pair matings set up, only 2 were sterile. The average number of flies per fertile bottle was 168 and the time for emergence, 28 days. The addition of 0.05% of Nipagin to the food was found successful in preventing the molds at the low temperature. Before being set up in the culture bottles, the flies have to be held in mating vials; the tests carried out have shown that the best results are obtained after the flies have been held in the vials for 5 days.

Parker, D. R. Method of carrying stocks. The early method of carrying stocks in this institution was to keep them in bottles, merely shaking them from the old one into the new one at each change, with occasional etherization and examination of them. Last year, however, we adopted a new method which seems to be far more efficient. The stocks are now carried in vials, keeping one old vial and mating three new ones at each change. The four are fastened together by means of a rubber band to which is attached the tag label. The flies are etherized by means of the mass method of Altenburg.

The advantages of this system are: (1)The flies are examined at each change, and (2) by making 3 new vials the chances of loss by contamination are greatly reduced. It is possible by this method to practically rid all of the stocks of mites, provided there are no adverse conditions of temperature.

This method takes a bit more time than the older one, but it will perhaps repay the loss with better stocks.

Parker, D. R. Moldex-A as a mold inhibitor. Tests were run recently to find a substance to inhibit the growth of mold. The compounds tried out were Moldex-A, Nipagin-M, and Nipagin-T. These were added to our regular banana food in the ratio of .15 grams of anti-mold substance to 100 c.c. of food. Twenty vials were made of each of the above compounds, as well as twenty vials of plain food.

One half of the vials were inoculated heavily with mold, and the other half left uninoculated. One pair of flies was placed in each vial. Moldex-A was the most efficient in the prevention of mold. However, in the uninoculated series, the Moldex vials gave a slightly lower yield of flies than did the plain food. Egg counts were then run to see the possible effect that Moldex might have on hatchability. Our of approximately 3000 eggs, 98.7% reached the adult stage. This is about 7% higher than the ususal hatch on plain food.

Not only is Moldex more efficient that Nipagin-T and Nipagin-M, but it has also the additional advantage of being much more economical. It may be obtained from the Glyco Products Co., 949 Broadway, New York, N. Y.

Schweitzer, Morton D. Collecting eggs. During the past year various techniques of collecting eggs have been tried. The following method has regularly yielded 100-600 eggs per

culture per four hour period, with an average of 300. Not infrequently, on the first day of collection, the yield has been as high as 800-1300 in a four-hour egg-laying period. (D. melanogaster, pseudo-obscura, and to a small extent affinis and miranda)

The important precautions to be observed for optimum yield of eggs are:

(a) The females should not be etherized at any time prior to use for this purpose.
(b) The medium should be seeded with yeast at least 6 hours and not over 24 hours before use.
(c) The surface of the medium should be slightly roughened just before being placed with the flies.
(d) The surface on which eggs are to be collected must be ventral to the flies.

The details of the procedure I have followed are as follows: Young flies, not over 24 hours old, are transferred to fresh food without etherization (20-40 ♀♀ and ♂♂). Two or three days later they are transferred to fresh food. At this time the medium on which the eggs are to be collected is prepared. It consists of ordinary cornmeal-molassas-agar with lampblack added to give contrast to the white eggs. The cornmeal is sifted before cooking. The food mixture is poured onto the ordinary type of paper milk bottle caps, leaving a margin of 1 cm. all around. When cool, the surface is uniformly seeded with fresh yeast. (Caps for 24 hours are prepared at one time.) The next morning the surface of the food on the caps is scraped with a metal tissue lifter. The flies are transferred to empty half-pint bottles which are capped with the prepared paper caps. The bottles stand with the caps down. New caps are substituted at appropriate intervals.

Eggs have been collected by this method continuously for a week or more at intervals of 2,4,6,8,12 hours. If the rate of oviposition falls off after a few days it may sometimes be renewed by transferring the flies to regular food bottles for 2-3 days. Strains that do not reach their optimum rate of egg-laying as early as the fourth day may be kept on regular food longer before beginning the experiment. (D. Pseudo-obscura does well after 7-10 days from hatching, affinis and miranda even later.)

If properly fitting caps are used (diam.=1.625" for Bridges-type bottle, and 1.640" for most others) they may be washed and reused indefinitely.

Schweitzer, Morton D. Handling eggs and larvae. When eggs are collected in the manner outlined above the usual high mortality due to handling and yeast overgrowth may be minimized by several precautions. After counting, the entire slab of food (or a segment containing an appropriate number of eggs) may be transferred to the surface of regular unyeasted food. If the surface of the food on the cap is sliced off with a scalpel just before use, the danger of yeast overgrowth is much reduced. An alternative method of transfer, that has given high percentages of imagines, is to allow the eggs to hatch of the food while it is still attached to the cap. The young larvae are transferred with a fine scalpel. In transferring larvae, an efficient method is to gently touch the

scalpel to a larva, then touch the larva to a second one, etc. until 25-75 are adhering to each other. In this way the larvae are subject to a minimum of direct handling.