

Research NotesBarigozzi, C.

A new form of melanotic disgeny (generally indicated as tumor) has been found in D. melanogaster (see New Mutants). The dark masses appear clearly through the abdominal wall of the imago, as irregularly located black spots. Histological examination proves that the disgenetic masses are located near the most different organs (intestine, Malpighian tubes, etc.); their structure shows small cells surrounding pigmentary masses, which remain inside the cell cloth. Pubb. Compt. Rendus Int. Congr. Cancer, Paris 1950 (in press).

Bertani, G. A case of hereditary somatic elimination of X chromosomes.

The following observations were made in 1948 at the Zoology Department of the University of Zurich with the collaboration of Miss Z. Blankart. Although their analysis is incomplete, these results may interest other workers in the field. A few exceptional cases either of females showing patches of male tissue or of males and females lacking patches of tissue, were accidentally observed in the wild-type stock "Oerlikon" of D. melanogaster. This stock had originally been obtained from a cross between a wild-type individual collected in nature and a Cy/L stock, and was homozygous for chromosome 2 of the wild parent. 1.4% exceptional flies were found among 4248 scored in cultures of the Oerlikon stock. After a few generations of selection their frequency rose to 6.1%. The frequency of exceptional flies increases with the temperature: 2.6% at 18° C., 3.8% at 25° C. (2501 and 3163 flies scored, respectively). The abnormalities observed were of the following types: (a) females with male patches of tissue--male eyes, male wings, one or more abdominal segments showing dark male pigmentation, sex comb present on foreleg, genitalia partly male; (b) males and females showing abnormalities due to loss of some areas of tissue--loss or reduced size of eyes or antennae, loss of some parts of the thorax, loss of a wing, a haltere, or a leg, loss of part of one or more abdominal segments. The abnormal areas were generally small. All the abnormalities listed above can be explained by assuming that one or both of the X chromosomes were lost at some time during the development of the exceptional individuals. The factor that causes this loss acts as a dominant, since abnormal individuals are always found in the F<sub>1</sub> of crosses of Oerlikon with other stocks. In the F<sub>1</sub> of crosses between y cv v f females and Oerlikon males, 7 gynandromorphs had y male patches and 8 had + male patches. This shows that either one of the X chromosomes may get lost and not necessarily the Oerlikon one. The factor that causes this loss is not in chromosome 2, since, in the F<sub>2</sub> of crosses between Oerlikon and b pr c px sp, homozygous b pr c px sp individuals showing abnormalities were found.

Bird, Myrtle J. Mutagenic chemicals.

The following compounds have been tested for mutagenic action in D. melanogaster:

(a) ethylene oxide, dissolved in saline solution; (b) 1:2,3:4-diepoxybutane, dissolved in saline solution; (c) 2:4:6-tri(ethyleneimino)-1:3:5-triazine, dissolved in saline solution; (d) NN-di-(2-chloroethyl)-p-anisidine, dissolved in arachis oil. A comparison has been made of various methods of administration; i.e.--mixing the compound with the food of the larvae, injecting the imagines, and exposing the imagines to aerosols. In all experiments, wild-type (Oregon-K) males were used, and after treatment they were tested for sex-linked recessive lethals by the Muller-5 technique. A summary of the results is given below, and shows that all four compounds are mutagenic and that the injection method is the most satisfactory for both oil-and water-soluble compounds.

Compound tested	No. Chromosomes tested	No. lethals	% lethals
a) Injection--0.5%	713	10	1.4
0.8%	198	9	4.5
b) Feeding--0.01%	809	8	1.0
Injection--0.1%	1288	133	10.3
c) Aerosol--0.5%--2 hours	1323	81	6.1
2.5%--1 hour	957	83	8.7
Injection--0.005%	206	29	14.1
0.008%	266	48	18.0
d) Aerosol--10%--1 hour	164	0	0
2 hours	490	3	0.6
5 hours	1081	8	0.7
Feeding--0.07%	1644	20	1.2
0.09%	465	7	1.5
Injection--1.0%	900	20	2.2

Brncic, D. Mutability rate in the Chilean stock of D. melanogaster, Santiago.

With the Muller-5 method it was found that the wild-type stock, Santiago, originated from flies found in Santiago, Chile, had 0.84% sex-linked

lethals. Under the same experimental conditions the Oregon-R-c stock presented only 0.088% lethals.

Burla, H., Ernest, F., Gloor, H., and Hadorn, E. Collecting wild Drosophila in southwestern Europe.

During September and October, 1950, a collecting trip was made to France, Spain, and Portugal. In 27 different places, far from human habitations, we collected a total of 21,000 Drosophila

individuals. Twenty-six known species and four or five new species were recorded. A few preliminary faunistical data are mentioned here. D. subobscura Collin was found to be the most widespread species, and in most places the commonest one. D. pallida Zetterstedt occurred with relatively high frequencies in the region of the Pyrenees. D. fenestrarum Fallen was captured in small numbers in Portugal, where both of the color varieties mentioned by Duda (1943) occurred. This species, exceedingly rare in Switzerland, seems to be limited to coastal regions. More detailed data on systematics and faunistic and ecological observations will be published later.

Castiglioni, M. C.

Eye discs of the mutant so (sine oculis of Milani), have been found nearly

normal until pupation, and then where transplanted into normal larvae. Development of the transplanted disc generally does not take place. The reciprocal transplantation shows a full development of the normal disc in the so larva. The development of the transplanted and that of the normal eye seem then to be autonomous. An investigation is in progress with a new mutant affecting both eye development and the number of the spermathecae, although not allele of spt. *Pubb. Boll. Soc. it. Biol. Sperim.* (in press).

Chiyoko, Tokunaga Genetic studies on Aphiochaeta sp.; reverse mutation found in short arista mutant. During the course of genetic studies of Aphiochaeta sp. the author obtained twenty some mutants. Some occurred

naturally and others as the result of X-ray irradiation. The mode of inheritance is more or less irregular in most cases. Some of these mutated genes have been definitely located on the chromosomes--for instance, short arista ( $sa^0$ ) and Delta, on the third chromosome, and bar and truncate (main gene) on the second.

$Sa^0$  appeared during selection experiments (minus direction) with truncate. Genetic studies with  $sa^0$  indicated the presence in this mutant of modifiers for this gene. Furthermore, normal individuals appeared from time to time in the  $sa^0$  stock. From genetic tests it was found that the majority of the reverted individuals are heterozygous for  $sa$  whereas others are homozygous for its wild allele. In three cultures ( $sa^0 \times sa^0$ ) the offspring was all phenotypically wild. This is an extreme mass reverse mutation. Some of those reverted individuals were also Abrupt ( $Ab$ ; sex-linked, homo lethal) and bar. Inbreeding of those reverted wild flies soon gave rise to several mutants, such as  $Ab$ -low,  $Ab$ , truncate, purple and bar. Short arista reappeared also after several generations.

When a reverted wild male is crossed to a  $sa$  female, the  $sa$  character behaves, in  $F_1$  and  $F_2$  generations, as if it is sex-linked. Furthermore, when an Abrupt female is crossed to a reverted wild male, and  $Ab$  character segregates as if it is no longer sex-linked.

Genetic studies indicated that these unusual phenomena are due to the Y chromosome of the reverted wild individuals. Some other mutants such as  $sa^1$  (a derivative of  $sa^0$ ),  $sa$  reverted back from the reverted wild flies ( $sa^0 - + - sa$ ), and some  $sa^0$  individuals have a similar type of Y chromosome. Most of the flies in  $sa^0$  and the derivatives of  $sa^0$ , such as  $sa^{0120}$ , bar<sup>67</sup> and  $Ab$ <sup>67</sup>, were found to have normal Y chromosomes. The lethal (or semi-lethal) on the X chromosome of  $Ab$  b and  $sa$  becomes ineffective in the presence of the above-mentioned peculiar Y chromosome.

The unusual genetic phenomena mentioned above are explained on the basis of the following assumptions. (1) The Y chromosome in this species has a strong male-determining factor. (2) A translocation between the Y and the third chromosome gave rise to a third chromosome with a part of Y containing the male-determining gene.

Intense cytological studies were made in an attempt to find if chromosomal aberrations are present in  $sa^0$ , reverted wild,  $sa^0$  derivatives, and  $Ab^{2b^0}$  males having unusual Y chromosomes. Unfortunately, salivary-gland chromosomes are totally unusable and therefore chromosomes of the larval ganglion and of the gonad were studied. Although no aberrations were detected, it is perfectly possible that undetectable chromosomal rearrangements exist. A very strong somatic pairing was recognized during the prophase of the gonial and ganglionic mitosis. Furthermore, each of three pairs of chromosomes has short achromatic sections at constant locations.

Studies on environmental effects on the  $sa$  mutant were also made.  $Sa^0$  and re-reverted  $sa$  individuals were reared from the larval stage at 25°C. (control) and at 18°C., and it was found that the expressivity of the  $sa$  character was lowered at 18°C. without changes in the genotype.

It is to be emphasized that the mass reverse mutation of  $sa^0$  and the various types of mutation of the reverted wild which have been frequently encountered in the present studies cannot possibly be due to point mutations, for a number of strong reasons, and these phenomena are considered by the author as due to more-or-less minute rearrangements of the chromosomes.

Di Pasquale, A. Corneal irregularities have been studied in D. melanogaster, connected both with  $\text{In}(3\text{R})\text{Me}$  and with the Y chromosome (see Barigozzi, DIS-23). Heterozygous with a wild stock (Oerlikon) showed a different manifestation rate at the distance of one year, not only for that character, but also for the frequency of the unicellular wing hairs. The finding seems related with the presence of polygenic systems in the Y chromosome. Publ. Rend. Ist. Lomb. Sci. Lett. (in press).

Fraile, A., and Sang, J. H. Induction of mutation with  $\text{Na}_3\text{PO}_4$  and with Harmine. Milani (DIS-20) reported that he was able to induce lethal mutations to the extent of nearly 3% by feeding larvae on a medium containing about 2%  $\text{Na}_3\text{PO}_4$ . This work has been repeated, using Pearl's S101 medium as a base instead of cornmeal-molasses. The  $\text{NH}_3$  content of the medium was altered, and the mutagenic effect of Harmine hydrochloride (which produces mutations in yeasts) was also tested, with the following results:

Treatment	No. larvae treated (Ore-K)	Chromosomes tested	No. lethals
Control $\text{PO}_4^{3-} = 0.045\% ; \text{NH}_4^+ = 0.054\%$	150	427	0 (1 semi-lethal)
$\text{PO}_4^{3-}$ increased to 1.54% as $\text{Na}_3\text{PO}_4$ = Milani	150	396	0
Ditto, but $\text{NH}_4^+$ reduced to 0.013%	150	447	0
1/10,000 Harmine HCl	150	649	1 (1 semi-lethal)

This confirms Buzzati-Traverso's finding (Ricerca Scientifica E Ricostruzione 8: 3, 1947) that addition of phosphate to the food does not increase mutation rate. This work is being repeated using a sterile, synthetic medium.

Fung, Sui-tong Chan The Hermaphrodite phenotypes in D. melanogaster developmental effects of a resulting from the developmental action of a sex gene in D. melanogaster mutant gene are described. The gene is dominant, located in the third chromosome. It is sex-limited in its effect. The males carrying it show no morphological or physiological character changes. The phenotypic effect produced by this gene is first visible in the third-day larvae. Morphologically, the hermaphrodites have two sets of genital ducts and two sets or combinations of gonads. All have sex combs. No fertile triploids carrying the hermaphroditic factor were obtained. They all have sex combs. The hermaphroditic gene does not seem to affect the sexual organs of the intersexes to any noticeable degree. Salivary-gland chromosomes of the hermaphroditic larvae are cytologically normal; no chromosome rearrangements such as deletions, duplications, inversions, translocations, or fusions were observed. Anatomical and morphological studies suggest that the normal allele of the hermaphroditic factor must be a gene guiding normal development of the sexual organs. Development of the hermaphrodite sex pattern is a dual development of both male and female organs taking place simultaneously. There is no evidence for the initial development of one sex system, then a period of transition followed by the development of organs of the other sex.

Gall, Joseph Effects of X-  
raying copper-fed *Drosophila*.

The spontaneous back-mutation rate at the *mt-3a* locus of *D. virilis* was demonstrated to be significantly decreased when copper sulfate was incorporated into the medium in the experiments of Zamenhof (J. Genet., 47, 1945). Since copper is an essential part of several oxidative enzymes, this effect is of very considerable interest in view of the more recent findings of Wyss, Clark, Haas, and Stone (J. Bact. 56, 1948) that organic peroxides produce mutations in bacteria. The experiment reported here was performed to see what effect increased copper content of flies might have on the rate of induction by X-rays of sex-linked lethal mutations in *D. melanogaster*. Females rather than males were irradiated, since it was thought that any effect of copper might be greater in eggs than in sperm because of the larger amount of cytoplasm of the former and their known high copper content. The Muller, M-5, technique was used.

Two groups of Canton-Special inbred females were simultaneously X-rayed with a dose of about 4650 r (300 r/min., 5 ma, 50 KV). One group was a control raised on the standard cornmeal-molasses-agar medium; the other was raised on the standard medium plus 5 mg/ml.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . The irradiated females were mated to M-5 males. Virgin  $F_1$  M-5/wild-irradiated females were collected as soon as they emerged. The latter were tested by mating to M-5 males, the appearance of any red-eyed males in the  $F_2$  or  $F_3$  being the criterion of nonlethality of the tested X chromosome.

No significant difference was found between the two series, the rate of induction being  $5.85 \pm 1.00$  per cent in the controls and  $6.38 \pm 1.08$  per cent in the experimental cultures (32/547 and 33/517 respectively). However, in both series it was noted that the rate was much higher for the first  $F_1$  females emerging, about 8 per cent, than for those collected approximately a week later, about 2 per cent. This may be due in part to selective elimination of lethals associated with chromosome rearrangements induced in earlier oocytes, and suggests that "mutation rates" for chromosomes treated in eggs must be determined under carefully specified conditions.

Gersh, Eileen Sutton Pigment  
cells in the white-mottled eye  
of  $T(1;4)_w^{258-16}$ .

Histological study of the eyes of flies of this stock shows that whereas the primary pigment cells are of two types (strongly pigmented or unpigmented)

there are three types of secondary pigment cells (strongly pigmented, unpigmented, and intermediate). Fixation by freezing-and-drying is necessary to reveal the intermediate type of pigmentation.

Gloor, H. Polyploidy in *Drosophila* larvae induced by cold treatment.

Nearly full-grown larvae of *D. hydei* were exposed to a low temperature ( $8^\circ$ - $12^\circ$  C.) for 10 days. When examined after treatment, some of these

larvae were found to contain a considerable number of polyploid ganglion cells, mostly in metaphase, with chromosome numbers up to about one hundred. Of two different wild strains (Morocco and Zurich), both of which have been treated repeatedly in the same manner, only one (Morocco) appears to be sensitive and regularly yields a number of larvae with polyploid cells.

Kikkawa, H. Tryptophane metabolism in *Drosophila*.

Since the report of Butenandt, Weidel, and Becker (1940), alpha-hydroxytryptophane was assumed to be an intermediate substance between tryptophane and kynurenine. But I have recently found that alpha-hydroxytryptophane, which was synthesized by Professor T. Sakan of

Osaka City University, was quite negative as a  $v^+$ -substance. A similar conclusion was obtained for Bombyx. On one hand, formyl-kynurenine, which was synthesized by Dr. T. Amanó of Osaka University, was positive for vermilion-brown mutant of *D. melanogaster*. Thus, the tryptophan metabolism in *Drosophila* may be shown as follows: tryptophan  $\rightarrow$  formyl-kynurenine  $\rightarrow$  kynurenine  $\rightarrow$  3-hydroxykynurenine  $\rightarrow$  pigments. Of course, it is supposed to exist another intermediate between tryptophan and formyl-kynurenine. The cardinal mutant of *D. virilis* seems to lack the enzyme which converts formyl-kynurenine into kynurenine, but this assumption is not conclusive.

Koref, S., and Brncic, D.  
Influence of diethyl stilbestrol on *D. melanogaster* tumors.

The influence of diethyl stilbestrol on the tumoral incidence of three tumor stocks: bw(tu),  $se^{11}(tu\ 49h)$ , and st sr; tu36a, was studied. The substance was added in a proportion of 1/100 to shell vials containing Birsh's synthetic medium. A total of 2243 flies was examined. It was observed that the estrogenic substance had a marked influence in reducing the number of melanotic tumors in the stocks in which the incidence was normally high: bw(tu) and  $se^{11}(tu\ 49h)$ . The reduction was not statistically significant in the stock in which the incidence is low, st sr; tu 36a..

Lamy, R., and Slizynska, Helena  
A spontaneous ring-shaped deletion in the Muller-5 chromosome.

Among the offspring of a  $y\ w\ sn$  female mated to an M-5 male, one  $y\ w^a\ sn$  female was found. Breeding tests showed that this female was non-disjunctional and had received a deleted X chromosome from the father, containing the  $w^a$  locus but not the  $y$  or  $ac$  loci. Salivary studies showed it to be a ring-shaped fragment including the bands 1B9, 10 (incl.) to 3E1,2 (incl.). The fact that  $y^+$  and  $ac^+$  are not included makes it probable that one break--between the  $ec^+$  and  $fa^+$  loci (which on this chromosome are of course at the right end, near the centromere)--occurred to produce the deleted X. A second break in the small right arm of the X would explain the formation of the ring and the survival of the fragment without the telomere.

Preliminary studies show that the M-5 deletion in the males segregates almost invariably with the X, and is easily kept with  $y\ w\ f$  females. In females heterozygous for M-5 or chromosomes of similar composition, the fragment has not been observed to segregate with the inverted chromosome. Phenotypically, males containing the deleted M-5 are small and rather inviable, with abnormalities of wings, legs, and bristle number. The wing veins are usually strongly Confluens (owing, presumably, to the duplication of the 3C7 band). The appearance of Co in females is very variable, and may overlap wild-type, but there are indications that within a given genotype the degree of expression is fairly constant. The  $w^a$  eye color appears somewhat darker than that of ordinary  $w^a$  stocks. Females containing two M-5 deletions occur and are very strongly Confluens; they are rather inviable and usually sterile.

Lefevre, George, Jr.  
Effects of repeated subculturing of irradiated males.

Canton-S wild-type males were irradiated with 5000 r at the rate of 650 r per minute. After exposure they were mated to  $y\ w\ f$  females. All parents were subcultured in fresh bottles after 4 days, again after 5 more days, and a third time after 7 more days. Male progeny were examined for sex-linked visible mutations. Nineteen such mutants were found in the first 3 cultures among 4300 flies examined. Only in the last subculture, made 16 days after irradiation, did mutant frequency decline. Just one mutant, a white-

mottled, was found among the 1071 males that hatched. At the same time the frequency of hyperploid females was recorded. Three classes were found: (1)  $y^+ w^+ f^+$ , (2)  $y^+ w^+ f$ , and (3)  $y^+ w f$ . (One  $y w f$  fly occurred.) The first class contains triplo-X flies as well as ones containing deletions of the irradiated X chromosome. The other classes of hyperploids possess only deleted X chromosomes. The first class,  $y^+ w^+ f^+$ , showed no significant change in frequency throughout, comprising about 5 per cent of all the females examined. The other classes,  $y^+ w^+ f$  and  $y^+ w f$ , were constant in the first two cultures (41 hyperploids in 2714 females) but, unlike the visible mutations, dropped off sharply in the third and fourth cultures (9 days after irradiation), where only 8 hyperploids appeared among the 1054 females examined. In addition  $In(1)w^{m4}$  males were irradiated, mated to  $y w f$  females, and successively subcultured. With one exception only  $y^+ w^{m4} f^+$  hyperploid females were found. The exception, one  $y w^{m4} f$  female, proved fertile, and the X-chromosome fragment carrying  $w^{m4}$  does not permit survival of hyperploid males.

Lindsley, D. L. The occurrence of inverted attached X chromosomes.

From a cross similar to that described by Lindsley and Novitski (see below) except that the males carried  $f$ ,  $y^2 su^{wa} w^a bb/o x In(1)EN sc$ ,  $y^+ f y$ ,

several  $f$  females were recovered. Crosses of these females to Muller-5 males yielded only Muller-5 males and  $f$  females in the next generation, indicating that an attachment of the X chromatids had occurred in the male. In ganglion metaphase figures this chromosome appears as a large V and has therefore been designated  $In(1)sc^8 EN$ ,  $y f y$ . Such an attached X chromosome probably arose through a crossover between the short arm (Novitski, DIS-23) of  $In(1)EN$  at the base of one chromatid and the proximal heterochromatin of  $In(1)EN$  at the base of the other.

Also, an attached X homozygous for the Muller-5 chromosome has been picked up.

Lindsley, D. L., and Novitski, E. The synthesis of an attached XY chromosome.

The study of crossing over between the X and Y chromosomes depends upon the analysis of Y fragments produced by such crossing over. Ordinarily the

recovery and perpetuation of these fragments presents a problem, since males carrying them are sterile, and the addition of compensating fragments to produce complete fertility is not practical, since the composition of the newly derived fragments is not known. For this purpose, a chromosome carrying all of the X-chromosome genes plus the Y-chromosome fertility factors has been synthesized.

Crossover products between the  $In(1)sc^8$ ,  $sc^8$  chromosome and  $In(1)EN$ ,  $y$  were recovered, which contained the tip of  $In(1)sc^8$  with  $y^+$  and the base of  $In(1)EN$  with  $y$ . Males carrying such an X chromosome were crossed to attached X females. Occasional yellow males were recovered; such males are products of crossing over between the distal heterochromatin of the  $sc^8 EN$  inversion and an arm of the Y, in which all of the distal genes of the X ( $y^+$  and  $ac^+$ ) have been replaced by part of the Y. The loci removed, however, are duplicated at the base of the X chromosome which is derived from  $In(1)EN$ , and the new chromosome is viable in the male. Of the yellow males, some appear to be  $Y^S X$ ,  $In(1)EN$ ,  $y$ , since they are fertile upon addition of  $Y^L$  only but not upon addition of  $Y^S$  only. Males with this chromosome and  $Y^{sc^8}$  (a normal Y chromosome with the distal uninverted segment of  $In(1)sc^8$ , carrying  $y^+$ , attached to the tip of  $Y^L$ ; Muller, DIS-22) were crossed to  $y/Y^H$  females; only nondisjunctional males should be fertile



in the progeny of such a cross, and they should be non-yellow. Large numbers of the  $F_1$  of the above mating were put into bottles without examination, and from each of four bottles showing larvae one non-yellow male was recovered. Three of these males when crossed to  $y^2 su^{wa} w^a bb/o$  females gave sterile sons, which showed that they were simply nondisjunctive; the fourth, however, always gave fertile male offspring upon backcrossing on successive generations to  $y^2 su^{wa} w^a bb/o$  females. It was, therefore, a crossover product between the base of the  $Y^{SX}$  chromosome and the Y such that  $Y^{Lsc8}$  had become attached to  $Y^{SX}$ , giving rise to a chromosome that we have designated as  $Y^{SX.Y^{Lsc8}}$ , In(1)EN, y .  $y^+$  (abbreviated  $Y^{SX.Y}$ ). This chromosome is carried in the following way:  $y^2 su^{wa} w^a bb/o$  X  $Y^{SX.Y^{Lsc8}}$ , In(1)EN, y .  $y^+/o$ .

In neuroblast metaphase figures the attached XY chromosome appears as a large J with a long arm approximately the length of an autosome and a short arm approximately one-third as long. No Y fragments are seen.

Preliminary results indicate that females homozygous for  $Y^{SX.Y^{Lsc8}}$ , In(1)EN, y .  $y^+$  are about half as viable as heterozygotes and quite fertile while the males show essentially normal viability.

The reciprocal attached XY chromosome,  $Y^{LX.Y^S}$ , In(1)EN, y, has also been recovered, but it has not yet been balanced or its exact constitution determined. In this case a normal Y was used in the place of  $Y^{sc8}$ .

Loh, Sing Yuan Early testing Progeny from three strains of D. melanogaster as a means of evaluating  $F_1$  heterosis between inbred lines of D. melanogaster.  
 Progeny from three strains of D. melanogaster were used as foundation stock to establish the inbred lines utilized in these experiments. Matings throughout were full brother x sister in single pairs. A synthetic stock derived from 8 inbred lines was used as the tester parent in the crosses. A 3-day laying period, 5th, 6th, and 7th day after emergence, was standard throughout these experiments. Three sets of data were collected: (1) the hybrid egg-laying performance from different-generation inbreds test-crossed to the synthetic stock; (2) egg yields from pure-line flies after more than 20 generations, and (3) egg yields of single-cross progeny resulting from mating individual inbred lines in all possible combinations.

Real differences in combining ability between strains were found in first-generation inbreds crossed to synthetic testers. Ames 1947 had the highest average egg record,  $178.8 \pm 2.5$  per fly; Ames 1943 was second with  $176.2 \pm 2.7$ ; Amherst was last with  $166.2 \pm 2.8$ . The average standard deviation was around 60 eggs. The average coefficient of variation was about 35 per cent. The distributions for the egg productions of the three strains' hybrids were continuous and symmetrical. Inbreeding does not stabilize the egg productions of the different synthetic-inbred crosses. Instead, the egg productions of these test hybrids decrease steadily at the rate of about 2.4 eggs per generation. The degree of heterosis exhibited by inbred line crosses increases with the generation of inbreeding from the 15th, 24th, and 34th generation. The average percentage increases of the hybrids over the parents are 17.2%, 30.2%, and 62.4% in the 15th, 24th, and 34th generations respectively. The inbreds showed lowered vigor and productiveness as inbreeding advanced, but this loss of vigor is not detrimental to the egg productions of the hybrids made from these inbreds. Inbred lines with more than 20 generations of brother-sister matings show a general downward trend in productivity. The average strain egg production is reduced 4.3 eggs or 3%-4% per generation of inbreeding.



Uncontrolled variations contribute most of the variations in egg production. Differences between reciprocals appear due to the synthetic females' being poor mothers. Line differences are small and irregular. Strain differences are large in the first generation. Continued inbreeding has caused an increasing separation of the strains' egg productions. Within inbred lines, after 20 generations of brother-sister matings, the uncontrolled variances are similar in the successive generation tests. The pure inbreds have 68% more line-within-strain variance than was observed for the inbreds x synthetic over the period covered by the same generations. The strain differences are not significant. No trend was found in the correlation coefficient between successive tests on the same top crosses. The top-cross tests thus contributed no information of value for predicting subsequent performance in later generations.

General combining ability, maternal influences, specific combining ability, and the effects of reciprocal crosses freed of additive, specific, and maternal effects show small effects on line-cross performance. The uncontrolled portion of the total variance,  $\sigma^2_e$ , contributes most to the variance, 60%. Taking  $\sigma^2_e$  as a standard for comparison,  $\sigma^2_g$  is 23%,  $\sigma^2_m$  is 11%,  $\sigma^2_s$  is 9% and  $\sigma^2_r$  is 46%. Correlation coefficients between general combining ability,  $g$ , values of three different generations are negative and small. Correlations for the maternal effects are small and inconsistent. Values for specific combining ability are positive and small. Those for  $r$  are negative and small. The lack of stable estimates for the performance of the different inbred lines suggest strong environmental-genotypic interactions of direct significance to successive yields of the same line crosses.

Idlers, H. Raising *Drosophila* on a medium containing DDT.

Studies concerning the genetic basis of the development of resistance to insecticides have been originated with

*D. melanogaster*. In selection experiments adult flies have been exposed to insecticidal sprays of DDT in Petri dishes. Expecting differences in the speed of the evolution of tolerance or resistance, and for copying conditions in treated farms, selection experiments were arranged in which all developmental stages were exposed to the insecticide. In normal culture bottles the surface of a cornmeal-agar-molasses medium is slightly and regularly powdered with a 5% DDT powder. Then the parental flies are set in. Selection begins at this stage, it continues in the developing larval stages, and from the pupae varying percentages of imagoes are completely unable to emerge or emerge only partially. Flies that do emerge show different degrees of DDT poisoning. Some of them die after about 24 hours even if put on normal food. The rest lose all symptoms of poisoning after 24 hours, behave normally, and are subjected to the same treatment.

Makino, Sajiro, and Mizurō, Tsuruo Distribution of some *Drosophila* species in Hokkaido.

Collection of *Drosophila* has been continued in various localities of the island Hokkaido. *D. melanogaster*, *D. virilis*, *D. immigrans*, *D. nigromaculata*, *D. histrio*, *D. funebris*, *D.*

*soldidula*, *D. coliforae* and *D. suzukii* were collected during the summer time in the vicinity of Sapporo City. *D. funebris* seems to show a much wider distribution than others: it was found abundantly in Imagane, Sapporo, Takasu, Obihiro, and Akkeshi. Thus this species shows a distribution ranging from South Hokkaido northwestward to East Hokkaido. Further, we have obtained seven other species whose names are now unknown.

Makino, Sajiro, and Mizuno, Tsuruo. Some experiments to induce morphological aberrations with dinitrophenols and menthol in D. virilis.

The culture medium used was the following: corn meal (22.4%), sugar (3.6%), agar (0.5%), and water (73.5%). In the experiments this culture medium was mixed with saturated solutions of dinitrophenols and menthol. The culture of larvae was made at 23°-24° C. The flies for experiments were wild-type from Hokkaido. The results of this preliminary treatment are shown in the following table:

Reagents	No. of Individ.	Abnormalities Induced	% Abnormalities
Control	483	Wing deformation, abnormality in sternite	0.62%
beta-dinitrophenol	385	Assymetry in wing length, wing deformation, ski, partial deficiencies of longitudinal vein in different grades, abnormal arrangements of hairs on mesinotum, deformation of scutellum, abnormalities in scutellar bristles, excess of posterior crossveins, and deficiencies of anterior crossveins.	6.7%
gamma-dinitrophenol	284	The same as above.	9.5%
menthol	245	Deformation of sternite, abnormal shape of wing, complete and partial deficiencies of anterior crossveins.	4.9%

After the experiment, by means of reciprocal crosses between a female of normal type and a male having no anterior crossveins induced by the treatment with dinitrophenols, it was found that the abnormality induced is a sole permanent modification.

Milani, R. Interspecific courting behavior in subobscura, ambigua, bifasciata, (tristis) obscuroides.

Reciprocal pairs have been observed for almost all the combinations of these species. Obscuroides seems to be completely debarred. All the others court and attempt copulation; however, the attraction seems to vary. The reciprocal crosses seem to be in agreement. Ambigua x bifasciata court freely and insistently, subobscura x ambigua rather well, bifasciata x subobscura very poorly. Male bifasciata seem to break court when their antennae are near the nonconspecific female genitalia. The behavior of male obscuroides, with non-conspecific females is exactly comparable to their behavior with the dummies (see notes 3 and 5, next page).

Milani, R. Recording sexual behavior.

I have found it very useful, in recording large numbers of observations on behavior; to use a shorthand system based on symbols that vaguely suggest the movements performed by the fly. The recording can be done while watching, and so one need neither depend on memory nor interrupt observations to take notes. Repeating the symbols.

with a known rhythm, or just following the movements of the fly, can give a good idea of the time spent in performing them.

Milani, R. Release of courtship display in subobscura males stimulated with dummies. Experiments have been done using little ovoidal balls of wax connected by a thin copper wire to a handle, and moved near the males. The size varied

from that of a Drosophila to that of a house fly. Males have been induced to complete display and attempt copulation and mounting. Color and size seem to be unimportant; however, the bulk of the experiments have been done with black dummies. Males kept in mass culture in the absence of females did not react to the model when isolated, and gave poor responses the next day (only a few reacted) but better results afterwards. Males isolated in their first day of life and examined later and repeatedly gave a very high number of positive responses, with consistent evidence of individual behavior both in particulars of the display and in general reactivity. Males reacted also to dummies moved outside of the vials.

Milani, R. Researches on sexual behavior in D. subobscura. The sexual behavior of D. subobscura has been studied by direct inspection. Attempts have been made to check

inter- and intra-male variability, both in courtship patterns and in length of copulation. Individual males tend to spend a given time in copulation if a suitable period of rest is provided. Broadly speaking, the length of copulation in subobscura increases, if the interval between subsequent matings is short, but a one-day rest for males who have had one copulation, or two-three days for males who have had more, restores them completely. Some males have four and five copulations with an interval of a few minutes. The frequency distributions of the length of copulation for individual averages, as well as for first, second, etc. copulations, are not unimodal. The shortest observed has been .3 minutes, the longest 75 minutes. The data on the variability of courtship display have not yet been fully analyzed; but it is possible to say that statistical differences in the male display have been observed in courting performed by single pairs, by one male with several females, or by two males with one female. These differences are not in the elements of the display, but in their coordination and frequency of use. British strains have been seen to display occasionally a wing movement which has not been observed in Italian strains and which suggests the incorporation of a frightening display in the courtship. Females have been seen accepting copulation after the protruding of the vagina, which is usually considered a repelling movement, and which is done by both virgin females and females that have had a previous copulation. The latter seems to be repelling to the males.

Milani, R. Sexual behavior of D. subobscura, ambigua, bifasciata, tristis, obscuroides. The above-mentioned species have fully distinct display patterns, particularly obvious in elaborated courtship.

The differences are mostly due to coordination and emphasis of movements and in smaller degree to the nature of the movements themselves. The average times of copulation are: 5-10 minutes for subobscura, 17 minutes for ambigua, 7 minutes for bifasciata, 10 minutes for tristis, 17 seconds for obscuroides. The last is the shortest on record for Drosophila species (as far as I know). All these species but tristis have been tested with the wax dummies. The response of subobscura males is described separately (see above); males of the other species reacted positively--sensing, orientating, following, tapping. Occasionally some displayed and circled to the rear, but the courtship broke off when they touched the model. The movement of the model appar-

ently must be adapted to the species. The reactivity varied with the species, but there was no way to determine whether these differences were of a specific nature or depended on the batches of males. It is interesting to note that these species are able to mate in the dark. Preliminary observations of obscuroides and bifasciata showed that the mating frequency in the dark is much lower than when the flies are exposed to light. Subobscura, which gives full response simply to optical stimuli (as proved by moving the dummy outside of the vials) is the only one that does not mate in the dark. The ones that are attracted by the model but do not court it completely have higher mating frequencies when exposed to light.

Neidhardt, Frederick C. Ether susceptibility, phototropism, and activity of several stocks of D. melanogaster.

Investigation has determined that there is a wide range of susceptibility to ether among twelve mutant stocks of D. melanogaster. Assuming the wild-type (Gambier-K) as a standard, the susceptibilities to ether of these stocks were found to be the following: Gambier-K = 1.00, Iy/D<sup>3</sup> = 1.13, bi ct<sup>6</sup> g<sup>2</sup> = 1.18, al b c sp<sup>2</sup> = 1.20, dp = 1.23, bx<sup>34</sup>e/Payne, Dfd ca = 1.27, m = 1.31, w<sup>a</sup>; ru h th st cu sr e<sup>s</sup> ca = 1.42, L<sup>2</sup>/sp<sup>2</sup> Cy = 1.55, bi = 1.57, cu kar = 1.70, ss<sup>a</sup> = 1.89. It was further observed that the relation between the resistance of a fly to ether and the time required for that fly to recover is in the nature of a direct proportion.

Nine of the above stocks, plus vg, were tested for phototropism, using apparatus suggested by Brown and Hall (J. Exp. Zool. 76: 205-220, 1936). All the different stocks showed a positive phototropism of 95 ± 5 per cent, the mutants' reactions showing no appreciable difference from that of the wild-type.

When these same ten stocks were tested for activity, using apparatus suggested by McEwen (J. Exp. Zool. 25: 49-105, 1918), marked differences were observed. The following values were found for the activity indexes of the various stocks (based on the wild-type): Gambier-K = 1.00, w<sup>a</sup>: ru h th st cu sr e<sup>s</sup> ca = 0.67, cu kar = 0.35, bi = 0.22, vg = 0.15, ss<sup>a</sup> = 0.15, dp = 0.13, bx<sup>34</sup>e/Payne, Dfd ca = 0.13, al b c sp<sup>2</sup> = 0.09, bi ct<sup>6</sup> g<sup>2</sup> = 0.07. McEwen's apparatus was originally intended to measure phototropism, yet it actually measured phototropism plus activity. Since the phototropic responses were practically equal, as determined above, we can assume that McEwen's apparatus measures activity alone.

It was also found that operations that reduce the wing area of the fly do not affect its phototropic response, but do reduce its activity.

Newby, W. W., and Thelander, R.P. Early development of the head in normal and tumorous head D. melanogaster.

Embryological studies have demonstrated clearly that the primordia of the eyes and antennae are present in the newly hatched larva. These primordia appear as a pair of flattened pouches extending from the posterior ends of the dorsal pouches of the larval pharynx. Optic stalks connect them with the brain lobes. At hatching, each primordium contains about 95 cells (on the basis of nuclei count in serial sections) and is about 55 microns long. They double in length by the 16th hour of larval development and quadruple by the 32nd hour. However, the cell number increases over five times by this latter stage. Separate eye and antennal discs are recognizable at 32 hours. The head primordia of Canton-S wild type and tumorous-head larvae show no definite differences before the 32-hour stage. At 32 hours tu-h primordia are frequently asymmetrical and

are associated with peculiar cells containing large chromatic inclusions not seen in conjunction with wild-type primordia.

Nolte, D. J., and Stoch, Zelda G.  
A new South African species,  
D. opisthomelaina.

This new species was recently discovered amongst batches of mixed Drosophilids collected in the north-eastern parts of this country. It appears to have been confused with D. melanogaster and/or D. simulans, which species it greatly resembles. It appears, indeed, to belong to the same subgroup as the last-named two species, and thus to the melanogaster species group of the Sophophora. Both sexes, however, differ from those of the two species in having brighter red eyes. The males differ further in two main criteria. First, the single sex-comb on each anterior tarsus has an average of 7 bristles only; second, the anal plate is prolonged ventrally into a flat-toed plate covered with bristles, and the primary clasper is short, with about 12 primary teeth arranged in two convex rows and 1 secondary tooth anterior to the clasper. The female differs further in the abdomen, in that the fifth tergum also is black; this darker tip of the abdomen, however, is not a constant characteristic in laboratory stocks, since in crowded cultures it greatly resembles that of the other species. The eggs resemble those of the other two species, and so do the mitotic chromosomes, though preliminary study of salivary-gland chromosomes shows an apparently longer arm for the small fourth chromosome. In preliminary tests D. opisthomelaina has proved to be cross-sterile with both D. melanogaster and D. simulans.

Novitski, E., and Lindsley, D. L.  
Construction of tandemly attached  
X chromosomes.

Previous work on crossing over in attached X chromosomes heterozygous for an inversion appears to be inconsistent with the expectations based on a tetrad analysis. In order to repeat this experiment with a genetic set-up such that the distinctive crossover product--a ring chromosome--would not be subject to undue viability complications, a method was devised for constructing tandemly attached X chromosomes whose derived ring chromosome would be viable in the male, that is, would have no appreciable deficiencies or duplications.

A triploid stock with free X chromosomes was successively crossed first to males with an X chromosome of normal sequence carrying y Hw, then to an X chromosome with inverted sequence, the one used having the left end of the sc<sup>8</sup> chromosome and the right end of In(1)EN, and carrying f, and finally to males with a ring chromosome, X<sup>c2</sup>, with the markers cv v f. Triploid females with these three different X chromosomes were then mated to B males, and the progeny examined for females that were Hw f but not B. Several kinds of crossovers in the triploid, along with subsequent nondisjunction, will give rise to F<sub>1</sub> females of this phenotype. The type desired, however, was one in which the ring had crossed over with the normal chromosome in one direction and the same strand of the ring had crossed over with the inverted chromosome in the other direction, thus opening the ring out into a large V, with each arm of the V a complete X chromosome. Since the X chromosomes would be tandemly attached, the pairing configuration would be the same as with an attached X chromosome heterozygous for an inversion, and one of the single crossover products would be a ring chromosome that must structurally be identical to X<sup>c2</sup>.

Of fourteen Hw f females tested from the above cross, three proved to be of the desired type structurally, but the one finally selected for analysis carried both cv and v in the heterozygous state, as a result,

apparently, of the occurrence of both crossovers in the cv-v region. The mutant genes in the arm in normal order with respect to the centromere are y, Hw, v, and f; and those in the arm in reverse order with respect to the centromere are cv and f.

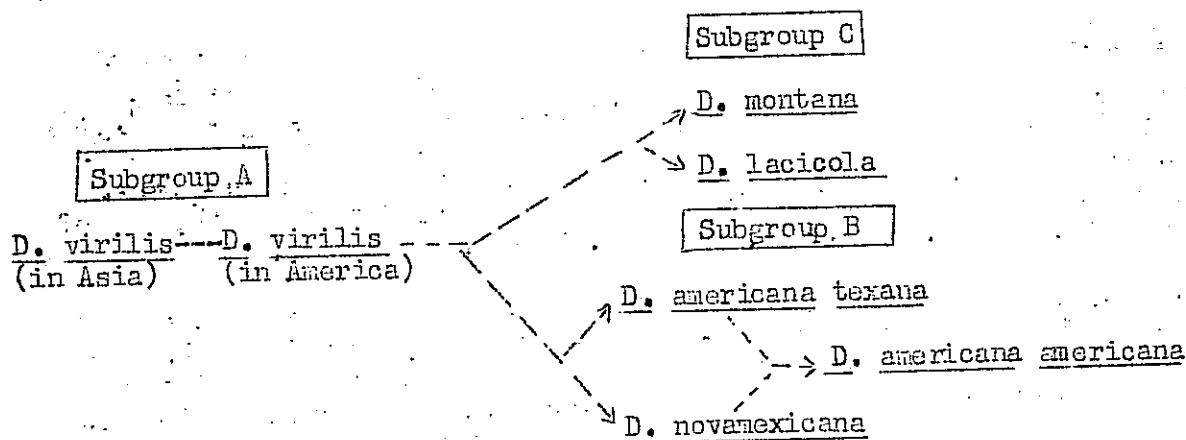
Oshima C. On the inter-relationships of the virilis group.

From the facts regarding geographical distribution, taxonomic distinctness, and breeding tests, the virilis group may be divided into three subgroups.

Patterson and Stone (1949) are of the opinion that D. novamexicana belongs to the same subdivision as D. americana texana, D. americana americana, and D. virilis. According to my experiments, however, D. virilis seems to belong to a subgroup (A) different from that which includes texana, americana, and novamexicana (subgroup B). Among the hybrid males between subgroups A and B, which have the Y chromosome of virilis, those with the X chromosome of subgroup A are always superior in viability and fertility to the hybrid male having the X chromosome of subgroup B. (DIS-22, 23). In addition to this relation between X and Y, Patterson and his collaborators have observed that there is a Y-autosome relation for the fertility of the hybrid males; thus, subgroup B is perhaps differentiated mainly as regards the sex chromosomes.

D. laticola and D. montana are the most related species, and they belong to the other subgroup, C. Though the hybrid males between laticola and virilis are always sterile, the hybrid females show very slight fertility, when they are backcrossed to virilis males. About one-third of the hybrid males among the progeny of the backcrossed females have a rudimentary testis; and they show sterility in the test-cross. However, their sterility has no relation to their sex chromosomes, and it is probably due to autosomal incompatibility. The hybrid males between subgroups A and C do not show any such relation, showing that subgroup C is probably differentiated mainly as regards the autosomes.

From these results, the inter-relationships of the virilis groups may be shown as follows:



Poulson, D. F. Influence of medium uptake and accumulation by larvae.

In our investigations on mineral accumulation by gut cells of larvae of various Drosophila species, it has become increasingly apparent that certain constituents of the medium may markedly influence the uptake and retention of particular mineral elements. In the case of copper, the difference in

In our investigations on mineral accumulation by gut cells of larvae of various Drosophila species, it has become increasingly apparent that certain constituents of the medium may markedly influence the uptake and retention of particular mineral elements. In the case of copper, the difference in

results between Cu-supplemented standard cornmeal-molasses-agar medium and Cu-supplemented Kalmus medium is strikingly demonstrated both by histochemical localization with sodium diethyl-dithiocarbamate and by radioisotope techniques. In the Kalmus medium tartaric acid acts as a chelating agent and markedly interferes with the uptake of Cu. If tartaric acid is omitted the effect on accumulation is removed, although the pattern differs somewhat from that on standard medium. Such effects are less striking in the case of iron. In the case of silver, which DiStefano (Am. Nat. 77, 1943) found to be tolerated in concentrations as high as 1 mg.  $\text{AgNO}_3$  per gram of food, the toxicity is vastly greater in the Kalmus medium. At this concentration larvae die within a few hours of being placed on such a medium, presumably through the immobilization of the chloride-regulating mechanism as described by Gloor.

Ratty, Frank J., Jr. Covering sex-linked lethals with a duplication showing position effects.

The w-N region of the X chromosome has been inserted as a duplication in the heterochromatin of chromosome 3 ( $w^{msp}$ , described by Lefèvre, DIS-23, page 59). A survey of lethals in the w-N region

was made in order to determine whether they are covered by the duplication, which shows mottling for w, rst, and spl. Only one lethal, a white deficiency produced by crossing over between  $\text{In}(1)w^{m4}$  and  $\text{In}(1)\text{rst}^3$ , is covered and yields fertile males. Two Notches are covered, but give sterile males. Many other lethals, including Notches with no cytological deficiency, are not covered. A few cases of erratic coverage have been noted, in which some males may be produced in one test, but not in a retest. Cell-lethality tests of all lethals and a comparison of results using a similar duplication, N264-58, are in progress. It is possible that most of the lethals are being expressed in a mosaic fashion during development when "covered" by the duplication, thus killing the individual; whereas in a few cases the duplicated loci act as normal dominant alleles except for fertility. However, in the one white deficiency that is fully covered, the eye shows a strong white-mottling effect of the duplication.

Semenza, L.

melanogaster has been accomplished: vg. The results show that the coexistence in the genome of  $\text{fa}^n$  and  $\text{blt}^S$ , and of vg and  $\text{blt}^S$ , produces a combined manifestation of both mutations. The same has not been obtained in the coexistence of  $\text{N}^B\text{-S}$  and  $\text{blt}^S$ , and of sd and  $\text{blt}^S$ . Publ. Boll. Soc. it. Biol. Sperim. (in press).

The investigation of interrelations among the following genes of D.  $\text{blt}^S$  (see new mutants),  $\text{fa}^n$ ,  $\text{N}^B\text{-S}$ , sd, and of vg and  $\text{blt}^S$ , produces a combined manifestation of both mutations. The same has not been obtained in the coexistence of  $\text{N}^B\text{-S}$  and  $\text{blt}^S$ , and of sd and  $\text{blt}^S$ . Publ. Boll. Soc. it. Biol. Sperim. (in press).

Strømnaes, Øistein The sensitivity of different strains of D. melanogaster to induction of dominant lethals by X-rays.

Experiments have been carried out in which 366 males, from 31 stocks representing 18 different strains, were treated with 2300 r-units of X-rays.

The irradiated males were mated to virgin females. The percentage of dominant lethals induced in the sperms by X-ray treatment was calculated as that proportion of the eggs that failed to hatch. A total of 90,036 eggs were counted. It was found that there was a significant variation between sperms of different stocks in their sensitivity to the induction of dominant lethals. The lethal range was from 42.54 per cent to 64.78 per cent. The four stocks giving the highest percentages of lethals had a mean of 61.12 per cent eggs that did not hatch, whereas the controls for the same stocks had 4.45 per cent. Four of the stocks giving the lowest percentages of lethals had a mean of 44.05 per cent, and the control series had 3.20 per cent. The variation between the controls for the series giving a high percentage of lethals and the controls



for the low-percentage series was insignificant. The males were stored with the females during the whole egg-laying period, which was never longer than one week.

A detailed analysis of the data shows that the variation in hatchability of eggs (fertilized by different males of the same strain) laid during the fourth to sixth days after irradiation was much greater than that of eggs laid during the first three days. The hatchability during the first three days was significantly different from the hatchability during the last three days in 14 stocks and insignificant in another 14 stocks. An explanation of this was made difficult by the fact that of the 14 stocks with significant differences between the first and second three-day periods, nine showed an increase and five a decrease in percentage of dominant lethals.

Valencia, J. I. A case of double position effect involving the forked and Bar loci.

A forked mutant female was found containing an irradiated  $sc^{S1}$  B In49  $sc^8$  Y chromosome from the father and the "plwx" chromosome, marked with 15 recessives, including f, from the mother. The  $F_1$  of this female gave a number of narrow-Bar, mosaically forked males plus other males that were Bar non-forked. The mutant female was apparently a fractional. This was confirmed after establishing stocks by crossing separately B  $f^+$  males and narrow-B  $f^{M}$  (mosaic) males to y  $f:=$  females. Furthermore, the cross narrow-B  $f^{M}$  males to y  $f:=$  females produced yellow non-forked females and yellow forked-mosaic females in addition to yellow forked; all males were narrow-B  $f^{M}$  or narrow-B  $f^+$ . It thus appeared that a suppressor for forked had arisen somehow in the Y chromosome. Further genetic tests showed that this suppression was not covered when in the presence of an X with a  $Y^L$  or a  $Y^S$  attached.

Study of the salivary chromosomes revealed the presence of a small deficiency in the treated X, extending from 15E7 to 16A3.4(a). All of this chromosome piece had been inserted in the Y chromosome. Thus the irradiated sperm had been a nondisjunctional one containing both an X and a Y.

The mosaic forked may overlap with wild-type but never appears as an extreme forked; any of the bristles from the head, scutellum, or thorax may be affected. This suppressing effect is of interest since it might help in explaining the nature of certain other dominant suppressors. This Y chromosome may be used as a marked Y when used with a forked-containing X chromosome.

The narrow-Bar position effect given in this case is only observed in the presence of the deleted X.

Valencia, J. I. Variegation of the rb locus due to additive heterochromatic influence.

In the course of our investigations on induced mutations at individual loci, numerous variegation ruby mutants have been detected. In all previous cases one of the breaks had been near the ruby region (4C7.8 - 4D1.2) and always to the left of it, the other break being a heterochromatic one. No variegated position effects had been observed from breaks at the right of ruby.

The break furthest to the left of the ruby region, involved in a heterochromatic rearrangement with mottling effect, had been placed at 4C2.4. Another break, which placed 4B3.4 near the heterochromatin of the X, showed no variegation effect. It seems reasonable to think that breaks

had been induced on either side of the rb region, further to the left than 4B2.4 and also at or to the right of at least 4D3.4, and had been involved in heterochromatic displacements, but that they were not detected because there was no position effect.

In the present case, however, a piece derived from the X chromosome sc<sup>51</sup> B In49 sc<sup>8</sup>, extending from 3F6.7 to the 7D7 - 10F7-10 inversional junction of In49 and from this junction on to 11B 16.17, had been deleted, and inserted into the heterochromatic region of the left arm of the third chromosome, near the centromere. Despite the fact that both breaks are far removed from the rb locus, a weak position effect upon ruby is observed. Apparently this is due to the fact that the influence spreading from the heterochromatin is being exerted upon the locus from both sides at once. It would seem that the two influences operate additively. If such position effects are due to some substance emanating from the heterochromatin and spreading along the chromosome to the affected locus, it is obvious that in such a case as this there would be a larger amount of the substance reaching the locus.

Wallace, Bruce An experiment on sexual isolation.

New species arise as the result of a fourfold process: (1) the isolation of a population from the remainder of

the species; (2) development in the isolated population of an integrated polygenic system, which is no longer coadapted to that of the rest of the species; (3) after removal of the isolating barrier, the development of reproductive isolating mechanisms that prevent the formation of ill-adapted hybrids; (4) the development of sexual isolation as a means of conserving gametes. It is possible that (3) and (4) may result from (2) automatically, or that (4) may follow (2) directly. It is known, however, that sexual isolation can arise through the action of selection (Koopman, K. F., *Evolution* 4: 135-148, 1950).

The rapidity with which isolation had arisen between the sibling species *D. pseudoobscura* and *D. persimilis* in laboratory experiments suggested that a similar phenomenon might occur between two strains of one species. An attempt to demonstrate this has failed; the data are presented here because the problem is an obvious one and useless repetition of experimental technique may be avoided.

Two strains, Oregon-R and Florida-19, and two mutants, se and stw<sup>3</sup>, of *D. melanogaster* were used in this experiment. Sepia flies were mated with flies of the Florida strain, se was recovered in the F<sub>2</sub>, se F<sub>2</sub> flies were remated with Florida flies, and se was recovered once more. These se-Fla flies carried genetic material of Florida origin (75%) and se origin (25%). A similar series of matings between stw and Oregon-R flies gave a stw-Ore-R stock in which 75 per cent of the genes were of Oregon-R origin and 25 per cent stw<sup>3</sup>.

Approximately 200 males and 200 virgin females (aged 3-7 days) of each mutant strain were placed in a Lucite box (3" x 4" x 8") into which one food cup could be inserted. Each day for 4-5 days a fresh cup of food was exposed to the flies. The eggs collected each day were subdivided among 10 culture bottles containing cornmeal-molasses-brewers' yeast-agar medium. The adults were destroyed after the egg sampling was completed. All phases of the experiment were done at 25° C.

When the egg samples hatched, full counts were made of all bottles, and approximately 200 males and virgin females of each mutant type were

collected as parents for the next generation. Three types of flies were recovered in the egg samples: wild flies, which arose from heterogamic matings; and stw and se flies, which resulted from homogamic fertilizations. By discarding the wild flies and using only homozygous mutants to continue the population, a selective advantage was bestowed upon any incipient sexual isolation of genetic origin that may have existed between the two strains.

The results of the matings are given in the table below. The data given include numbers of parents, frequencies of +, se, and stw flies among the egg samples, the total flies sampled (n), and the "isolation ratio"—the ratio of frequency of wild flies observed to frequency of wild flies expected. The latter frequency was calculated by taking liberties with Hardy's equilibrium: half the frequency of wild flies observed in the sample was added to the frequency of each of the mutant types observed; the expected frequency of wild flies, then, was twice the product of the frequencies of the two mutants. An adjustment of this sort was required because the "effective" frequency of each mutant among the parental flies may have differed from the numerical frequency as a result of dissimilarities in sexual activity, fecundity, or viability. (The method of calculation of this ratio is not necessarily correct, but its general soundness is indicated by the uniform ratios obtained despite large fluctuations in the frequencies of the three classes of flies.)

Generation	Parents		Offspring			n	Isol. Ratio
	se	stw	+	se	stw		
1	400	400	41.2%	56.9%	1.9%	3,802	1.18
2	400	500*	53.9	32.2	13.9	17,578	1.12
3	381	492	49.7	24.8	25.5	13,245	1.06
4	400	500	53.1	27.4	19.5	10,161	1.07
5	332	366	44.9	36.0	19.1	8,968	.92
6	250	250	59.8	22.9	17.3	11,561	1.20
7	358	371	42.2	15.4	42.4	10,152	.91
8	256	300	40.5	25.1	34.4	11,888	.81
9	250	300	43.7	21.5	34.8	12,638	.89
10	376	400	60.0	20.4	19.5	11,289	1.20
11	396	400	40.7	42.6	16.7	4,854	.87

\* stw-Ore-R flies from culture bottles

The results of 11 generations of selection are obviously not significant. The regression of the isolation ratios is given by  $Y = 1.15 - .02X$  (S.E. slope = .02). Either no sexual isolation developed, or it developed so slowly that it was not detected. Experimental methods that will intensify the action of selection are now being considered—suggestions are welcome.

In conclusion, the fluctuations of the isolation ratios may be discussed. These fluctuations resulted primarily from the random matings of the parental flies. Essentially, 200 females had a choice of equal numbers of two classes of males; the 95 per cent confidence interval extends from 43 per cent to 57 per cent for a sample of this size. The isolation ratio can vary from .86 (high frequencies of homogamic matings) to 1.14 (high frequencies of heterogamic matings) by chance. The actual range of error would tend to be enlarged because of death of flies, failure of females to

mate or oviposit, the limited number of males available, and the error in the egg samples themselves. The isolation ratios calculated fell well within the range of error expected if the true ratio were 1.00.

Whittinghill, M. Another complex mosaic.

From a testcross of a "res"/+ female an offspring was found which was both a gynandromorph and a mosaic for the "res" characters. The left side of the specimen was female and wild-type, and some wild-type tissue continued into the right eye. The rest of the right side was male and "res" to the following extent: sex comb, smaller wing, smaller body, male pigmentation of abdomen and external genitalia; thread antenna, roughoid scarlet peach in majority of the eye, hairy wing (but not curled), stripe, and sooty. It did not breed with males or with females, although the abdomen filled with eggs. The specimen is believed to have come from a binucleate egg.

#### TECHNICAL NOTES

Carpenter, John M. A new semi-synthetic food medium for *Drosophila*.

The following easily prepared medium, developed by the author and used in this laboratory very successfully during the past three years for experimental and stock culturing of *Drosophila* species, may be of interest to other investigators in the fields of *Drosophila* genetics, cytology, and ecology.

This food is semisynthetic (ingredients used are C.P. chemicals, except agar and yeast) and has been found to be especially useful in experimental research where a high degree of ingredient constancy is required. It has been found to be highly nutritious and capable of producing from two to three times the number of offspring obtained from the usual types of banana or corn meal food. The use of paper toweling strips or Kleenex as extra surface for pupation has not been necessary, ordinarily. It is an excellent medium for the growth of the yeast necessary for adult nutrition of the flies. The growth of molds is almost completely inhibited by the propionic acid used.

#### Solution A

Water (dist.).....	700 cc.
Agar (granular or shredded)...	15 gms.
Sucrose .....	50 gms.
Dextrose .....	50 gms.
Yeast (dried Brewers) .....	80 gms.
KH <sub>2</sub> PO <sub>4</sub> .....	1 gm.

Add the agar to 500 cc of the water and bring to a boil, stirring occasionally until the agar is dissolved. Add the sucrose, dextrose, and KH<sub>2</sub>PO<sub>4</sub>. To the remainder of the water (200 cc.), add yeast, and mix for several minutes in a Waring Blendor (or similar type of apparatus). Add to previous mixture.

## Solution B

Water (dist.).....	300 cc.
KNaC <sub>4</sub> H <sub>4</sub> O <sub>6</sub> (Rochelle salt) .....	8 gms.
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2 gms.
CaCl <sub>2</sub> .....	0.5 gms.
NaCl .....	0.5 gms.
MnSO <sub>4</sub> .....	0.5 gms.
MgSO <sub>4</sub> .....	0.5 gms.
FeSO <sub>4</sub> .....	0.5 gms.

Add salts to water in order indicated. Mix thoroughly in Waring Blender. Add to solution A, mixing thoroughly. Finally, add 5 cc. of propionic acid, stirring thoroughly. Pour into bottles or vials when food has reached a temperature of approximately 40° C. and cool rapidly. (This prevents settling of the suspended yeast.) Spray very lightly with fresh yeast suspension before using. The pH of this food is 4.5 at the above temperature. This formula will produce approximately 30 bottles or 100 vials of food at a cost of approximately 50 cents for the ingredients used.

King, James C. Parasitic hymenoptera in population cages.

identified as Pachycrepoides dubius (Ashmead), family Pteromalidae. The wasps were first discovered crawling over the pupae in the exhausted food cups, which are subjected to a routine examination for mites. Some wasps were then discovered on the screen of the cages (30 x 30 mesh, opening .0248 in.), which they were small enough to pass through without difficulty. The two populations were transferred to new cages on which the screen had been covered with fine voile, and although six weeks have passed no wasps have been found there since. Large numbers of wasps have emerged from the pupae that were in the cups in the old cages.

This species of parasite is unlikely to become a serious problem in populations of D. melanogaster because of the length of its life cycle. Crandall found the period from egg to adult to be about eighteen days; but from pupae in which we have seen wasps oviposit and which were kept at room temperature, no wasp has emerged until at least thirty-seven days later. Since the eggs are laid only in pupae, no wasp could possibly emerge during the time that a cup is customarily left in a population cage. The wasps apparently establish themselves in our cages by ovipositing in pupae that lay on the floor and were not removed in the exhausted cups. Three other population cages in another room of the same laboratory have not become infested although there have been free wasps in the building. An excellent monograph by H. A. Crandall on the biology of P. dubius is available in Ann. Ent. Soc. Am. 32: 632-54, 1939.

Mickey, George H. Furfural as a mold inhibitor.

have found furfural to be superior to Moldex, Tegosept M, etc. A 10 per cent solution in alcohol is used in the same way as Moldex was formerly used.

Parker, D. R. Temperature control in a small laboratory

An infestation of parasitic chalcidoid hymenoptera was recently found in two of our population cages containing D. melanogaster. The parasites have been

In a search for more effective mold inhibitors, which will not interfere with the development of Drosophila, we

It is possible to maintain a room temperature as low as 22° C., with variation of less than ± 0.5° C., at very

little expense. It has been accomplished in this laboratory by using a basement room with a refrigeration-type room cooler (window unit) in series with a Fenwal thermostat (cat. no. 17551). All steam pipes have been insulated and an electric heating system substituted. A fan-type electric heater (1.5 kw) is used with a Fenwal thermostat (cat. no. 17550). This has been satisfactory in controlling the temperature in a room 14 x 18 feet. An advantage of the type of thermostat used is that expensive relays are not needed, although the thermostats are quite sensitive and will carry a load up to 25 amperes. These may be obtained from Fenwal, Inc. Ashland, Mass., for about \$18 each. Electric heaters may be obtained from any appliance store for around \$15, and satisfactory room coolers are supplied by a number of manufacturers for around \$300 to \$350 for half-ton units.

Rizki, M. T. M. A method of preparation of *Drosophila* eggs for micro-manipulation.

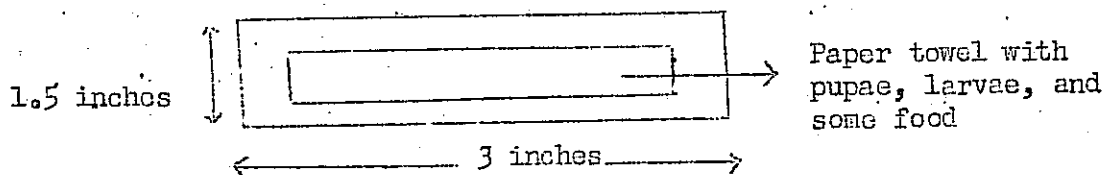
The difficulty of handling dechorionated eggs of *Drosophila* can be overcome by embedding the eggs in agar in order to avoid their collapse while performing operations.

For this purpose the following procedure has been found useful. (1) Collect the eggs and dechorionate them either by peeling off the chorion or by using sodium hypochlorite (Hill, DIS-19, p. 62; Callen, DIS-21, p. 89; Clark, DIS-22, p. 79). Keep these eggs in a watch glass on the right-hand side of a binocular microscope. (2) Place a few drops of melted agar solution (2.5-5% depending upon hardness of block desired) in a syracuse watch glass #0 under the binocular scope. Use washed agar. (3) Pick up an egg with a blunt glass needle. Meanwhile test the consistency of the agar solution with another glass needle held in the left hand. When the agar is about to set, quickly insert the needle with the egg into the agar. Withdraw the needle immediately with a slight jerk. The egg will remain in the solidifying agar. (4) Quickly place the syracuse watch glass into a fingerbowl of ice-cold water for thirty seconds. (5) Remove the solidified button and trim with a sharp razor blade on all sides. The egg can be oriented as desired while trimming the sides. Trim the agar as close to the micropyle as possible, since this will make it easier for the larva to crawl out of the agar. Leave the block in a wet chamber. *D. melanogaster* larvae hatch in 24 hours at  $23 \pm 0.50$  C. when embedded in 2.5% agar. The survival of the eggs is 90-95%. This technique has been developed during an attempt to try microinjections on *Drosophila* eggs. One can also observe the developing embryo in agar blocks under a high-power microscope.

Wedel, Marta, and Andres, J. M. An easy and inexpensive way to mail *Drosophila* cultures.

Mailing of *Drosophila* cultures is not easy in many countries because of the cost of air-mail shipment. To overcome this difficulty we have successfully used a small board that may be sent with a letter in an air-mail envelope.

This consists of a pasteboard framework one-fifth of an inch thick wrapped with an inner cellophane sheet and an outer adhesive tape in order to avoid desiccation.



In an ordinary air-mail envelope it is possible to send as many as four *Drosophila* samples, which keep perfectly well for several days. Some of the pupae arrive as adults, any many of the larvae grow to pupae during the trip.

Wortman, Bernard A simple method for the collection of large numbers of eggs from D. melanogaster.

The complete food used in the Drosophila laboratory for the maintenance of stocks is increased from 2% to 5% agar and autoclaved for 15 minutes at 15 pounds pressure. The food is poured into

sterile Petri plates, allowed to harden, and stored for future use. One to two hundred flies, three to six days old, are placed in a clean, empty half-pint bottle, and a 5% food-agar plate is placed over the opening. Both plate and bottle are inverted and allowed to stand for four to five hours. The plate and bottle are then reinverted, and the plate is removed. The weight of the bottle will leave an impression showing the area from within which the eggs must be taken. The food is hard enough to allow feeding of the flies but not to permit extensive working of the surface. The eggs are laid on the hard surface. A wire-loop needle is used to brush the eggs quickly together and to remove them to a sterilizing solution. The wire-loop needle may be flamed to reduce the carrying over of contaminants.