## Research Notes

Goldschmidt, R. Correction of statements in Bridges-Brehme, The Mutants of Drosophila Melanogaster.

(1) Bkd: Blackoid (page 25) to be changed to bB = Df(2L)35CD. (2) The translocation called poi (page 150) is actually a silver allele called svrpoi combined with the pseudo-translocation 1-2, the latter having no effect and not being necessarily combined with the silver allele.

Hsu, T. C. On the racial differentiation of Drosophila montium.

That the so-called two races of D. montium differ in the number of teeth of the sex-combs, as formerly reported by Kikkawa, was subjected to more crucial analysis by studying the character in those individuals isolated from the hybrids. The flies so segregated from the hybrids did not show any significant difference in the number of teeth with respect to the corresponding chromosome types. Moreover, experiments were also attempted to test preferential sexual selection between the two races. The results obtained were also negative. This is in agreement with the fact that some individuals collected from the wild were definitely hybrids as demonstrated by the metaphase chromosome plates. In view of the negative sexual isolation and the absence of any distinct morphological or physiological dissimilarities between them, the so-called two races of D. montium can hardly attain the rank of two separate races and may as well be considered as only two different chromosome-type strains, as has been suggested by Dr. C. C. Tan.

Lamy, R. Chromosome 2 in D. pseudoobscura and D. persimilis.

Crossing over in pseudoobscura/persimilis hybrid females is 34.5 between Stubble and Pale, zero between Pa and Eyeless-2, and 7.9 between Ey-2 and arr. In pure pseudoobscura the corresponding figures are 33, 24 and 28 respectively. In the hybrids, 3% of double crossing over between Sb and arr was recorded on 544 flies. After the hybrids had been backcrossed for several generations, crossing over in the Sb-Pa region was reduced to about 21(267). These figures supply an approximate genetic boundary to the "racial inversion" in 2, which may be taken to extend from a short distance to the left of Pa to a point much nearer to arr than to Ey-2, and so probably including the cinnabar locus. This would explain why other workers have found it impossible to transfer cn from one species to the other, since the whole inverted region would have to be transferred with it. (Dobzhansky 1936).

By repeated backcrossing the persimilis inversion has been transferred into the pseudoobscura genotype. The stock carries the composite 2 in heterozygous condition, the persimilis region being unmarked and the pseudoobscura 2 containing the homozygous lethal Minute (62), which acts as a balancer. Both males and females homozygous for the composite 2 are perfectly viable, but only the females are fertile.

In the reciprocal arrangement, rather more than the inverted region of pseudoobscura has been transferred into the persimilis genotype, only some portion to the left of the Pa locus being of persimilis origin. Moreover, the chromosome carries the pseudoobscura markers, Pa Ey-2 arr. Heterozygous males and females are fertile. Homozygotes are viable but weakened by the markers. The females may be fertile.

Females of either of these stocks are easily crossed to pure males of the other species, and give the two expected types of hybrid sons: full hybrids with

large or medium-size testes (according to the cross), and partial hybrids homozygous for the inversion in 2 peculiar to one species and containing the X of the other species. The latter have very small testes. Males heterozygous for either composite chromosome are extremely difficult to cross with females of the opposite pure species. The reason is obscure. Some offspring have been obtained, however. Males receiving the composite 2 from their fathers are of course homozygous for the racial inversion, which corresponds in origin with their X. Where the mother is of persimilis type, such males have considerably larger testes than their full hybrid brothers. When the mother is of pseudoobscura type the testes of full hybrids are expected to be normal in size; in some crosses however, the partial hybrids (homozygous for the 2 inversion of pseudoobscura) have been observed to have larger testes even than their full hybrid brothers. This point needs confirmation.

The left portion of 2, containing the gene Stubble and probably 20-25 chromosome units, has also been successfully transferred from pseudoobscura to persimilis. Homozygous males and females are both fertile in spite of the strong handicap of Sb/Sb. This supports the idea that the more important points of divergence between the species exist within the regions where the gene sequence has been altered, as would be expected. Hybrid males homozygous for the inverted regions of both 2 and 3 would possibly be fertile. An attempt is being made to mark off genetically and transfer the inversion in 3.

Nolte, D. J. White of y w an interaction-product. The unexpected appearance of certain eye colors in our y w stock, appearing in crosses with this stock and reported in DIS-17, has now been proved to be due to the interaction of two sex-linked factors which have been called: (1) o - orange -- the males have the eye color of cadmium orange and the sex-differentiated females a reddish orange to nasturtium red; (2) dr - dark red -- males and females have the eye color a brickdust or chrysanthemum on pupating, darkening to a maroon or garnet resembling ras$^2$.

In both cases the ocelli are pale and the malpighians colorless; homozygous o dr has white eyes with colorless ocelli and malpighians. Both o and dr thus appear to be dominant alleles of w. It appears likely, however, that this y w stock, which was obtained from Germany several years ago, never contained w or does not now contain it, although the data obtained in the different crosses made do not eliminate the possibility that w may be present in addition to o and dr.

The laboratory has no w stock to compare with the interaction-product, and the deficiency of stocks has limited the determination of the c.o.v. of the two factors to that with the y of the original "y w" stock; this shows the location of o at $\pm$.5 and of dr at $\pm$ 4.3. No aberrations have been found in salivary-gland chromosomes.

Pontecorvo, G. Synchronous mitoses and differentiation, sheltering the germ track. It is rather puzzling that it takes 15 days or more, after irradiation, to begin to obtain a drop in the % of Drosophila spermatozoa carrying recessive (Muller, 1928) and dominant lethals (Kaufmann and Demerec, 1942). Even allowing for arrest of mitosis, spermatozoa mature 10 or more days after irradiation must have been in premeiotic stages at the time of irradiation. In most insects the cells derived from a single primary spermatogonium divide synchronously a number

of times, fixed in each species; in D. melanogaster, 4 times. The 16 spermatogonia undergo meiosis and spermateleosis, again synchronously, and form bundles of coeval spermatozoa (64). Owing to the unsuitability of Drosophila for detailed histological investigation, I have used Pediculus corporis, which has the advantage of having meiosis pushed back in the germ track followed by six "spermatogonial" divisions, during which, of course, the germ cells are haploid and hence supposedly exposed to drastic germinal selection. The results are, very surprisingly, that whilst irradiation (4000 r.) kills a high proportion of pre-meiotic germ cells, and many in early meiosis, and stops for five days entrance of new germ cells into meiosis, it has no immediate harmful effect on all stages ranging from late meiosis to mature spermatozoa, a span including six mitoses and spermateleosis. It should be noted that all usual chromosome rearrangements are produced and can be seen (testing of spermatozoa for dominant lethals confirms it); but the cells carrying these rearrangements, many of which are "lethals", go through six mitoses and spermateleosis undisturbed. Apart from the direct observation of no cells individually killed, counting of spermatozoa in each bundle (easily done in cross sections) confirms that no germinal selection takes place in all these stages. The only exception is that occasionally a whole cyst is killed; but this is almost certainly a secondary effect of some injury to non-germinal tissue.

What histological observation is possible in Drosophila confirms that the same situation, mutatis mutandis, holds here too. Irradiated testes show necrosis of the small number of apical cells (homologous with the pre-meiotic stages in the louse) and no necrosis of cells in the spermatogonial divisions, meiosis and spermateleosis, again with the exception of occasional cysts degenerating as a whole.

What characterizes in Pediculus the span from late meiosis to mature spermatozoa, and in Drosophila the span from spermatogonia to mature spermatozoa, is the fact that all cell activities (division and differentiation, etc.) take place with perfect synchronization between cells of a cyst. The cyst functions as a syncytium and the nuclei of different cells cooperate. A harmful mutation (in the broad sense) produced in one chromosome at the beginning of the synchronized span will be, at most, in the same condition as a harmful mutation one-fourth heterozygous in a tetraploid.

The facts reported here confirm and extend the finding by Barber (1940) of the sheltering effect of synchronized pollen grain mitosis in certain Orchidaceae. The conclusion as regards Drosophila is that the part of the germ track in which no germinal selection takes place extends from the beginning of the spermatogonial divisions to mature spermatozoa. The evolutionary implications of the sheltering of the germ track are interesting, but cannot be gone into here. The results summarized here were reported at the December 1943 meeting of the Genetical Society in London. -- July, 1944.

Sheng, T. C. A case of high frequency of nature-occuring phenocopies of Drosophila.

In a restricted locality in the vicinity of Meitan, Kweichow, China, about 1400 D. melanogaster flies were caught within a period of three weeks in the Fall of 1941. Among them 184 individuals showed various kinds of simulating mutants affecting such bodily parts as bristles, wing, eye, and others. The individual abnormal fly was allowed to inbreed for two or three generations, and in no case was it found to breed true, though abnormal ones of one kind or another might be obtained. There were, however, 20 males showing yellow-colored body, which was found to be inherited as a sex-linked recessive, presumably homologous to the well-known yellow-body gene of the species.

As regards these simulating mutants, which may be referred to also as phenocopies, some of them bore close resemblance to the expression of some of the known authentic mutants. What causes the occurrence of such a high frequency of phenocopies in nature is as yet unclear. The plausible explanation may be that its inherent special genetic constitution renders this strain of D. melanogaster more susceptible to environmental changes.

Stern, Curt. Irradiation of hairy males of D. melanogaster. Among translocations involving chromosomes 3 and 4 of D. melanogaster four new cases (in addition to the one described by Dubinin and Sidorov) were found in which the normal allele of hairy (h,3,26.5) in combination with the recessive allele showed a position effect. Since it has been shown that a changed position of recessive allele cubitus interruptus frequently results in a dominant expression of this allele, a corresponding experiment was planned for hairy. Males homozygous for this recessive allele were X-radiated at 5000 to 6000 r, mated to normal females and the scutellum of the $F_1$ inspected for cases of dominance of hairy. No such cases were found among 17,922 flies. Two individuals which showed small patches of hairy on the scutellum, but nowhere else, did not transmit to their offspring the ability to show hairy. The origin of the two patches were probably due to somatic crossing over and unrelated to the radiation of the P- males.

Sturtevant, A.H. Crossing over in 4. Following a suggestion of Dr. J. Schultz, crossing over has been tested in chromosome 4 in 3n females. Preliminary tests (from triplo-4 females) indicate that such crossing over is very frequent; the technique may be useful to those working with 4. A gvl $sv^n$ chromosome was easily obtained, and is now available.

Muller, H.J. (1) Failure of desemination by nitrogen. An easy, effective means of what may be called the "desemination" of females would be a very important advance in Drosophila breeding technique. Following the report, personally communicated by some Drosophila workers, that females placed for half an hour in nitrogen atmosphere retain their fertility but have the spermatozoa in their genital tracts killed, an extensive trial was made of this method. Treatments up to several hours in length were given to numerous inseminated females as well as, separately, to males, by keeping the flies in bottles through which humidified nitrogen was circulated. The flies were not killed, but neither were the spermatozoa. There was no indication that the females laid fewer fertilized eggs, or that the males had lowered fertilizing capacity.

Earlier experiments by the author (1927) had given some indication, however, that mature sperm are killed more easily by "heat shock" -- i.e. by a temperature of $37^{\circ}$-$40^{\circ}$ -- than are earlier stages of spermatogenesis, or any stages of oogenesis; and it may be that a satisfactory heat dose could be discovered for this purpose, for most stocks. Stocks vary considerably, however, in the resistance of their early germ cells to sterilization by heat, so that it might be advisable to test out any given stocks in this respect, before large-scale application.

Muller, H. J. (2) High primary non-disjunction of the insertional double-X. The double-X chromosome denoted yf:-, produced by insertion of the central euchromatic region of a scute-8 X into another X (containing dl-49 but no long inversion), described in DIS-17, has been found usually to give between 1 and 2% of non-disjunction with a sc. $Y^{l}_{s}$ chromosome in females having these chromosomes. Presumably it would give a similar amount of non-disjunction with a normal Y, since this sc. $Y^{l}_{s}$ chromosome has been found otherwise to disjoin normally. The non-disjunction is evidently caused by the presence of the heterochromatin of the scute-8 chromosome at points distant from the heterochromatin and centromere of the recipient X chromosome, thus tending to divert the pairing of the latter chromosome region and of the Y from each other. It is noteworthy that in scute-8 males, on the other hand, there is very little primary non-disjunction of the X and Y, despite the displacement of a considerable part of the heterochromatic region of the X to the distal end. These effects are being studied further, with special reference to the blocks.

Muller, H.J. (3) Reddish-- a new near-normal allele of white. This new allele of white does not appear to differ from the normal "red," at least in bar-eyed males. (It has not yet been obtained in non-bar flies, or in homozygous females, although crosses for this purpose are under way.) In compounds with apricot the female (heterozygous bar) shows a maroon-colored eye considerably darker than homozygous coral or wine. The mutation arose spontaneously from apricot in a scute-8 Bar Inversion-S apricot chromosome of a female whose other X was non-inverted and also contained apricot.

Muller, H.J. (4) Tandem attached X's producing ring chromosomes. A stock of attached X chromosomes has been obtained in which one of the X's is of normal structure while the other contains the scute-8 inversion and the smaller, included, Inversion-S. The centromere is attached between the eucentric regions of the two X's, near what is ordinarily the "proximal" end of each. Thus the 2 X's are not arranged in the usual mirror-image fashion but (to use Bridge's expression for repeats of this kind) "in tandem." Mutant genes present are yellow-2, in the non-invered X, and Bar and apricot in the scute-8 X. As can readily be seen on making a diagram, single crossing over between a non-inverted X chromatid and that scute-8 chromatid with which it is joined (not the sister scute-8 chromatid) gives rise, on account of the tandem arrangement with included centromere, to a monocentric ring X chromosome which is not double but single, and which is deficient for the left-hand tip of the X chromosome up to and including achaete. (This result follows only if this crossing over is outside of Inversion-S.) In our present stock, males with the deficient ring are viable, though not fertile, because the free Y chromosome present is sc. $Y^{l}_{s}$, which contains the missing part of X.

Similar deficient rings (Xc3) were obtained at the Institute of Experimental Biology in Moscow in 1936 (by Sokolov, if my recollection is correct), by means of the previous X-ray synthesis of attached X's of the above kind for that purpose. In the present case, the attached X's arose spontaneously, by exchange between the proximal heterochromatic regions of a scute-8 chromosome and an X chromosome which had a short arm of the Y attached to it. It seems likely that this exchange involved crossing over between the $Y^{s}$ and the homologous region of scute-8 lying just to the left of the centromere, so that the centromere of the attached X's was derived from that of the X.$Y^{s}$ chromosome.

This exchange took place either in the original zygote nucleus of the fertilized egg from which the variant individual was derived, or in a nucleus of a cleavage stage so early that all the nuclei of both gonads were derived from it, since all daughters of the variant individual received a derivative of the

attached X chromosomes. As the scute-8 X was of maternal origin and the X.Y$^{s}$ paternal, this exchange must have taken place at a stage at which it has often been thought that the chromosomes of flies still maintain "heterogony,"--i.e., fail to undergo a mixture of maternal and paternal groups. This evidence that the two groups can be intimately intermingled even at this stage supplements that of Sidky's, in which there was a translocation between an irradiated paternal and an unirradiated maternal chromosome.

The present stock is useful in giving a constant supply of rings (Xc4); these are produced in a fairly high proportion (4 to 5%) of the progeny.

Muller, H.J. (5) Use of males with defective Y's to promote the laying of unfertilized eggs.

In some experiments it is desired to have females which are in effect virgin lay their eggs in abundance from the beginning of their potential egg-laying period (i.e., after the first day of imaginal life), instead of retaining most of them for the first week after hatching as actual virgins usually do. Tests with males having deficient Y chromosomes show that the young females after copulation with these males lay eggs in abundance like properly inseminated females, although these eggs are, as is known, unfertilized. Although some immotile sperm may be passed in these copulations it seems probable that it is some nervous reaction attendant upon the act of mating of the female which here leads to ovulation (and oviposition), as occurs in the rabbit when vasectomized males are used. This conclusion can be check by similar tests with Drosophila males known to have normal copulatory behavior but to produce no sperm (e.g. homozygous simulans-IV melanogaster males).

## Technical Notes

Spencer, W.P. A device for pouring food into creamers or vials.

With the scarcity of agar, various media are in use which are not easily poured into vials or creamers. The device described below obviates this difficulty. A rectangular base metal support is fastened rigidly near one end of a board about 24 inches by 8 inches. Cleats are nailed to the underside of this board at the two ends, raising it off the table sufficiently so that a large Petri dish may be slipped under it. An extension clamp is fastened by a clamp holder to the metal support. In the extension clamp is placed a plastic funnel, one-pint size or larger. A hole slightly smaller than the creamer or culture vial is bored through the board and the funnel set directly above this hole. A wooden dowl pin is beveled down so that it fits into the stem of the funnel. Two small strips of wood, forming a V-shaped guide, are tacked to the board on either side of the hole. With the stick, or dowl pin, which should be about 8 inches long, in place in the funnel stem, the hot medium is poured into the funnel until it is full. Then a vial or creamer is placed under the funnel stem, using the V-shaped guide for placing it quickly and accurately. The stick is then raised and pushed back into the funnel stem, delivering the desired amount of medium. With one hand the operator raises and lowers the stick and with the other handles the vials or creamers. Any drip from the funnel stem drops through the hole into the Petri dish placed beneath the board. Even when medium becomes too stiff to pour, it can readily be pushed through the funnel stem with

the stick. With a little practice it is possible to pour 100 creamers in five minutes with this device. The only parts to be cleaned are the plastic funnel and the stick.