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TWO NEW EYE COLORS IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER.

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In the cultures of *Drosophila melanogaster* which I have been breeding at Indiana University, two new eye colors have lately appeared. One of these, rose, closely resembles pink and peach of the third chromosome, and is allelomorphic to them. The other eye color, scarlet, is a bright red much like the vermilion of the first chromosome in appearance, but quite distinct from it genetically, for it is located on the third chromosome.

Both eye color mutations occurred in the winter of 1916. Scarlet appeared (November 18, 1916) in a wild stock which was being used in a temperature experiment. Rose appeared (January 9, 1917) after scarlet had been crossed to eyeless, and later was found in the eyeless stock, so that the mutation evidently had occurred there.

A series of temperature experiments was undertaken in the fall of 1916 in the effort to determine whether it would be possible to adjust a strain of *Drosophila* to a temperature abnormally high for that species. The flies were bred in pairs, the temperature of the incubator usually being maintained at 27–31°. Each generation of flies was raised, during the greater part of its period of development, at this higher temperature. The flies would not lay, however, under the abnormal conditions; they were allowed to mate, therefore, at room temperature, after which they were transferred to the incubator. The larvæ having pupated, the cultures were again removed from the incubator, for the heat proved fatal to the pupa stages. Even with these precautions all of the flies died if the temperature was allowed to rise above 33°.

Although from the standpoint of its original purpose the experiment, due to inadequate apparatus, was not entirely successful, a number of interesting things resulted from it. Abnormalities of various kinds appeared in the second generation of incubated flies. In one case, after the first generation had been developed in the incubator, some of the progeny (F_1) were bred at room temperature, and among the F_2 so obtained arose the scarlet eye color.

The temperature experiments were carried on for seven generations; and in each variations appeared. Most of these were peculiarities of the wings, but there were also modifications of eye and leg characters. Among the wing peculiarities were the following: short wings, wing edges turned up, wings folded at the tip, wings bent at abnormal angles, wings spread similar to "spread" of the third chromosome, and wings of odd shapes. Still other flies had weak legs, bent between the joints, and one fly had small eyes.

In each case the variant was allowed to mate with a wild fly at room temperature. The F_1 were divided into two lots, one of which reproduced at room temperature and the other in the incubator. It was hoped in this way to perpetuate the variations, should any of them be germinal. Many of the flies were non-viable or non-fertile, but in those cases where F_2 were obtained they were usually normal, and the abnormals which did appear were not like those of the original parent.

Only one color variation appeared. It proved to be germinal in character, and has given rise to the strain called scarlet.

Scarlet is a bright color, like the vermilion of the first chromosome, in flies that are newly hatched. As the flies get older the color gradually changes, and in old flies it is almost indistinguishable from the wild red type. In all crosses where scarlet is used it is highly important, therefore, to count the flies immediately after hatching.

Scarlet was crossed with a member of each of the four groups of linked genes in order to determine its linkage. Crosses were made with blood of the first chromosome, with vestigial of the second, with sooty of the third, and with eyeless of the fourth. The results of these crosses demonstrated that scarlet is a member of the third group of linked genes. When scarlet was crossed to blood, vestigial, or eyeless, all possible combinations were formed in F_2, showing that independent assortment occurs between these genes and scarlet. However, when scarlet was crossed to sooty, no scarlet sooty flies were obtained in F_2. The crosses gave the following results:

1. Scarlet ♂ × Blood ♀ (first chromosome character).

F_1 Wild-type ♀, blood ♂

F_2 {
- Wild-type ♂s and ♀s 178
- Blood ♂s and ♀s 156
- Scarlet ♂s and ♀s 70
- Blood scarlet ♂s and ♀s 50

2. Scarlet × Vestigial (second chromosome character).

F_1 Wild-type

F_2 {
- Wild-type 312
- Vestigial 93
- Scarlet 109
- Vestigial scarlet 38

This is evidently a 9 : 3 : 3 : 1 ratio.

3. Scarlet × Sooty (third chromosome character).

F_1 Wild-type

F_2 {
- Wild-type 611
- Scarlet 265
- Sooty 284
- Scarlet sooty 0

4. Scarlet × Eyeless (fourth chromosome character).

F_1 Wild-type

F_2 {
- Wild-type
- Scarlet
- Eyeless
- Scarlet eyeless

Among the F_2 of this cross appeared the new eye color which has given rise to the strain called rose. This new eye color was later found in the eyeless stock itself.

The exact linkage relations of scarlet were next determined by mating scarlet to sooty and to dichæte, and back crossing the

F_1. Genes for these two characters are located quite far apart in the third chromosome.

Scarlet × sooty

F_1 Wild-type

F_1 ♀ back crossed to scarlet sooty ♂ gave the following:

F_2	non cross overs	Scarlet	1230
		Sooty	1511
	cross overs	Wild-type	695
		Scarlet sooty	623

Percentage of crossing over was 32.4

Dichæte × scarlet. Dichæte is a dominant character, non-viable in the homozygous condition. The dichæte to which scarlet was crossed was therefore heterozygous. The F_1 dichæte females were back crossed to scarlet males and the percentage of crossing over thus immediately obtained in the F_2.

Non cross over classes	Scarlet	781
	Dichæte	952
Cross over classes	Scarlet dichæte	23
	Wild-type	45

Percentage of crossing over is therefore 3.2.

Scarlet is thus placed at a locus of 3.2 from dichæte, and 32.4 from sooty.

According to unpublished work from the laboratory at Columbia University, the approximate loci for these third chromosome genes are the following:

Dichæte 11
Pink and peach 16
Sooty 34

Since such a large per cent. of cross over was obtained for scarlet sooty (32.4), scarlet is probably at the left of dichæte (on the sepia side), sepia being at 0. Since it is 3.2 from dichæte, it is at the approximate locus of 7.8, (11 – 3.2).

Since dichæte is 23 to the left of sooty, (34 – 11), we should expect the value of scarlet sooty to be 3.2 + 23 or 26.2, instead of 32.4. The larger value obtained in this work may have been due of one of several causes. Genetic factors which influence the amount of crossing over have been found by Sturtevant and Muller for the third chromosome group. Plough has also shown that in some cases external conditions (temperature) change the amount of crossing over.

Since the appearance of scarlet, the same mutation has been found at Columbia University by D. E. Lancefield. Lancefield's paper will be found in this journal. The two strains arose quite independently, in laboratories remote from each other, and in stocks that were absolutely distinct. They are identical in appearance and in genetic constitution, and are due to the mutation of the same gene, but the process of mutation occurred at different times and in different localities. That they are due to mutation of the same gene is demonstrated by crossing the two stocks together, as I have done, using the stock kindly sent me from Columbia and my own strain. The F_1 flies from this cross were exactly like the parents. This origin of the same mutation in two widely separated laboratories is of particular interest, for here there is no possibility of contamination.

The mutation rose occurred in the eyeless stock, but was not observed until eyeless was crossed with scarlet. In color rose, which is somewhat lighter than pink, is a shade lighter than peach in older flies, but is practically identical with the latter at the time of hatching. When rose was first observed there was no peach stock kept in the laboratory. The three colors are very similar when the flies are first hatched, but rose does not become as dark with age as do the others.

At the time when rose appeared, Dr. F. Payne had running in the same laboratory stock of a new eye color identical in appearance with rose. At that time it was thought that rose had been derived from this stock by contamination. The gene for Dr. Payne's eye color, called salmon, and as yet unreported, had already been located in the sex chromosome. When salmon was crossed with rose, the following results were obtained.

Cross 1. { Salmon ♂ × rose ♀
F_1 wild-type.

Cross 2. { Rose ♂ × salmon ♀
F_1 Salmon ♂ and wild-type ♀

These results indicated at once that the two eye colors are not the same genetically, since rose is not sex linked. The F_1 when inbred produced only the two eye colors, wild-type and salmon (or rose). Since rose and salmon are indistinguishable phenotypes, the results correspond closely with the expectation as will be seen in the following table. In these results the classes rose, salmon, and the double recessive rose salmon are all alike in appearance and therefore could not be separated.

	Classes Expected.[1]	Percentage Expected.		Percentage Obtained.	Actual Numbers Obtained.
	Cross 1.				
	Red ♀		37.5	37.3	590
	Rose ♀		12.5	14.4	228
	Red ♂		18.7	18.4	291
Rose	Salmon ♂	18.7			
Rose	Rose ♂	6.3	31.3	29.9	474
Rose	Salmon rose ♂	6.3			
	Cross 2.				
	Red ♀		18.8	20.9	322
Rose	Rose ♀	6.2			
Rose	Salmon ♀	18.8	31.2	31.4	483
Rose	Salmon rose ♀	6.2			
	Red ♂		18.8	17.7	273
Rose	Rose ♂	6.2			
Rose	Salmon ♂	18.8	31.2	30.0	462
Rose	Salmon rose ♂	6.2			

This is another case similar to the well known one of the white sweet peas, in which two different genes produce the same phenotype.

Since rose was not linked to the first chromosome group, it was crossed to vestigial of the second, and to sooty of the third group. The first cross gave a 9 : 3 : 3 : 1 ratio, and the second a 2 : 1 : 1 ratio, as follows:

Rose × vestigial

F_1 Wild-type

F_2 Wild-type 1046, vestigial 257, rose 345, vestigial rose 104.

Rose × sooty

F_1 Wild-type

F_2 Wild-type 727, rose 278, sooty 330, rose sooty 0.

These results show that there is free interchange between the genes for rose and for vestigial, but that rose and sooty are in the same chromosome pair. Rose is therefore a member of the third chromosome group. Its linkage relations were determined

[1] Expectation is calculated by the checkerboard method, on the basis of 16 F_2 individuals.

by mating the F_1 female from the cross sooty $\times$ rose, to a rose sooty male fly. The F_1 female was used instead of the F_1 male for the reason that there is no crossing over in the male of this species. The double recessive rose sooty had been obtained by mating F_2 sooty by F_2 rose flies. The percentage of cross over from the back cross was 21.1. This therefore is the strength of the linkage between sooty and rose.

F_2	Non cross overs	Rose 647
		Sooty 634
	Cross overs	Rose sooty 173
		Wild-type 170

Percentage of cross over, 21.1.

Rose was next crossed to pink, a third chromosome character of known linkage and with locus 16. The F_1 were intermediate between the two parents, and when inbred gave only F_2 of the same eye colors. This showed conclusively that pink and rose are allelomorphic.

The linkage relations of rose should of necessity be the same as those of pink. According to previous work pink had been placed at a distance of about 18 units from sooty, whereas the present data indicate a distance of 21.1 units between these factors. This small difference in the percentage of crossing over may be due to one of a number of causes.

Another allelomorph of pink, peach, had previously been identified. When peach is crossed to rose, flies with intermediate eyes resulted. Rose is therefore the third of a series of allelomorphs at the approximate locus of 16 on the third chromosome.

Summary.

In a series of temperature experiments, during which cultures of *Drosophila melanogaster* were incubated, many new variations appeared. Only one, scarlet eye color, similar in appearance to "vermilion" of the first chromosome group, was found to be a true mutation. Scarlet is a third chromosome character whose gene is on the sepia side of dichæte at a locus of 3.2 from the latter. The same eye color has arisen as an independent mutation at Columbia University in the cultures of D. E. Lancefield, whose paper is also in this journal. When crossed, the two strains give F_1 like the parents.

Rose, an eye color appearing in another stock, is the third of a series of allelomorphs. It occupies the same locus on the third chromosome as do pink and peach. Rose is identical in appearance with salmon, a new sex linked mutant found by Dr. F. Payne. This case furnishes another example of different genes producing the same phenotype.

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