SOME OBSERVATIONS ON THE STRUCTURE OF THE SCUTE-8 CHROMOSOME OF DROSOPHILA MELANOGASTER

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STRUCTURE OF THE SCUTE-8 CHROMOSOME

We have been interested in the structure of the X chromosome of the stock known as scute-8 apricot, found by SIDOROV (1931). This stock has been used extensively in our laboratory, and it early became a matter of importance to have exact information on the nature of this chromosome. It was of course known that it contained a long inversion, but its exact limits had not been fully worked out. The fact that the locus of bobbed was inverted was shown in 1932 (PATTERSON 1932b, p. 154), and a diagram of the scute-8 apricot chromosome, based on all of the evidence then available, was published in 1933 (PATTERSON 1933, p. 34).

In this diagram it was indicated that the inverted section extends from a point lying just to the right of the locus of scute (scute-8), to some point lying between the locus of bobbed and the fiber-bearing end of the chromosome. The genes in the inverted section appear in reverse order, with the locus of bobbed lying near to that of yellow, and followed by the loci of such genes as carnation, forked, garnet, and so on, to white, finally ending with the so-called gene for viability (PATTERSON 1932a). We now have evidence which shows that the left-hand break occurred between achaete and scute, so that the locus of scute-8 is also inverted.

In a paper dealing with mosaic formation (PATTERSON 1933) experiments were described in which scute-8 apricot flies had been X-rayed and mated to untreated yellow white crossveinless miniature forked-5 flies. Among the F_1 flies were found 146 yellow apricot hypoploid females, the so-called aberrant females. These were interpreted as having resulted from the breaking off and elimination of the left end of the treated scute-8 chromosome, thus creating a deficiency at the locus of yellow.

If the locus of bobbed is inverted and lies near to that of yellow, then a certain percentage of these yellow aberrant females should also be deficient at the bobbed locus. In order to determine this point, 61 of these yellow females were tested to echinus forked bobbed males. The results show that eight of the 61 were also deficient for bobbed, as indicated by the appearance of bobbed females among their offspring. The conclusion to be drawn from this test is that the locus of bobbed lies near to that of yellow, and is therefore involved in the inversion.

The fact that 53 of the 61 females tested to bobbed showed that the

break had taken place between the loci of yellow and bobbed, and that only 8 showed that the break had occurred to the right of the bobbed locus, would indicate that there must be present between yellow and bobbed a considerable piece of the "inert region" of the X chromosome.

THE POSITION OF THE SCUTE-8 LOCUS

We may now consider the genetic evidence which has a bearing on the locus of the scute-8 gene in the inverted section. As stated above, it was formerly assumed that this locus was not inverted, but lay at its original position, just to the right of yellow. The first evidence that this might not be the case was obtained in an experiment in which treated scute-8 apricot males were mated to females having attached X chromosomes, homozy-

TABLE 1

Results of crosses between sc^1 f car bb males and F_1 v g f females, which were derived from X-rayed sc^8 wa males mated to females with attached XX's, and homozygous for y v g f.

CASE NUMBER	SEGREGATION TYPES		NON-DISJUNCTION TYPES	
	y v g f FEMALES	HYPERPLOID MALES	*v g f* FEMALES	*sc^1 f car bb* MALES
606j	6	1, $\frac{sc^1}{sc^8}$ *f car bb*	1	0
600k	2	4, $\frac{sc^1}{sc^8}$ *f car*	0	0
601c		8, $\frac{sc^1}{sc^8}$ *f car*	0	0
603f	12	3, $\frac{sc^1}{sc^8}$ *f car*	0	0
605a	13	2, $\frac{sc^1}{sc^8}$ *f car*	0	0
605g		3, $\frac{sc^1}{sc^8}$ *f car*	0	0
605m	14	7, $\frac{sc^1}{sc^8}$ *f car*	0	0
606i	17	7, $\frac{sc^1}{sc^8}$ *f car*	0	0
603a	1	1, $\frac{sc^1}{sc^8}$ *f*	2	0
606b	11	3, $\frac{sc^1}{sc^8}$ *f*	2	2
606e	5	6, $\frac{sc^1}{sc^8}$	0	0
603c	6	3, *sc^1 f*	0	0
606q	9	3, *sc^1 f*	1	0

gous for yellow vermilion garnet forked. A number of F_1 females carrying a duplication was obtained from this cross. Such females were non-yellow vermilion garnet forked. They had been produced by a long deletion eliminating the middle part of the X chromosome. These F_1 females were tested to scute-1 forked carnation bobbed males. In thirteen cases the hyperploid males were found to be viable, and this gives an opportunity to determine more exactly where the two breaks in the chromosome, responsible for the deletion, had taken place. These thirteen cases are listed in table 1.

The results obtained in the first eleven cases do not give any decisive evidence with reference to the position of scute-8; it could as well lie at the left as at the right end of the inverted section. But they do show where the left-hand break had taken place. Thus in the first case (606j), the single hyperploid male reveals the "compound" of scute-1 plus scute-8, and the mutant characters forked, carnation, and bobbed. The left-hand break therefore occurred at some point lying between yellow and bobbed. In the next seven cases (600k, 601c, 603f, 605a, 605g, 605m, 606i) the hyperploid males reveal the compound of scute-1 plus scute-8, and the mutant characters forked and carnation. Here the left-hand break in each case must have taken place between the loci of carnation and bobbed. In the next three cases (603a, 606b, 606e) the hyperploid males again reveal the compound of scute-1 plus scute-8, and the single mutant character forked. The left-hand break therefore occurred between the loci of forked and carnation. All that can be said about the right-hand break in each of these cases, is that it must have occurred at some point lying between the locus of forked (inverted position) and the right or fiber-bearing end of the chromosome.

The results obtained in the last two cases (603c, 606q) give very definite evidence as to the position of the locus of scute-8. Here the hyperploid males showed scute-1 only, and the mutant character forked. Obviously, the locus of scute-8 had been eliminated in the deleted section. In each case the duplication fragment covers yellow, bobbed and carnation, and the left-hand break must have occurred between the loci of carnation and forked. If the locus of scute-8 lies next to that of yellow, it would be necessary to make the very highly improbable assumption that a double deletion had taken place; the first of these eliminating the locus of scute-8, the second eliminating a section lying between the locus of forked and the right end. A much simpler and more probable assumption is that the locus of scute-8 is inverted and lies near the fiber-bearing end. On this basis, the right-hand break took place between the locus of scute-8 and the right end. In the first eleven cases, then, the right-hand break must have occurred at some point lying to the left of the locus of scute-8.

It is possible to devise an experiment to test the validity of the conclusion that the locus of scute-8 does not lie at the left end of this X chromosome. This can be done by X-raying scute-8 males and mating them to yellow scute-1 females. The F_1 yellow aberrant or hypoploid female will show whether the locus of scute-8 has been eliminated along with that for yellow. If such a female reveals the character scute-1, then the locus of scute-8 must have been eliminated, and this would indicate that this locus must be situated close to that of yellow. If, however, the female shows the combined effect of scute-1 and scute-8, then the locus of scute-8 must not lie near the left end of the chromosome. This test was recently carried out by treating scute-8 apricot males and crossing them to yellow scute-1 vermilion carnation females. The F_1 cultures yielded sixteen yellow hypoploid females, and it was found that without exception these females showed the compound effect of scute-1 and scute-8. These results are listed in table 2.

TABLE 2

Shows effects of scute-1 and scute-8 and their compound.

TYPES OF FLIES	FIRST ORBITAL	SECOND ORBITAL	OCELLAR	POST-VERTICAL	ANTERIOR SCUTELAR	POSTERIOR SCUTELAR	FIRST NOTOPLEURAL
Scute-1	0.00	0.52	0.15	0.01	0.46	0.27	0.14
Scute-8	0.98	0.72	1.00	1.00	0.78	0.77	1.00
Scute-1+8 compound	0.89	0.90	1.00	0.92	0.58	0.45	0.98
Yellow hypoploid	0.93	0.84	1.00	0.84	0.43	0.56	0.75
621·a	2	2	2	2	1	2	0
621·b	2	2	2	2	2	2	2
621·d	1	1	2	1	0	0	0
621·h	2	2	2	2	0	0	1
621·k	2	1	2	2	2	1	2
622·a	2	0	2	0	0	1	2
622·c	2	2	2	1	1	2	2
622·f	2	2	2	2	1	2	2
622·g	2	2	2	2	2	2	1
622·j	2	2	2	2	1	2	2
622·k	1	1	2	2	0	0	0
622·m	2	2	2	1	0	0	2
622·t	2	2	2	2	0	0	2
622·v	2	2	2	2	2	2	2
622·w	2	2	2	2	1	1	2
622·z	2	2	2	2	1	1	2

It is desirable to explain the tabulated data presented in this table. The scute allele of the parent females, designated as scute-1, was found to affect the following major bristles located in the upper parts of the head and thorax: the first and second orbitals, ocellars, postverticals, anterior and posterior scutelars, and first notopleurals. The figure given

for each of these seven bristles, in the first (horizontal) line of the table, represents the average number of bristles present on one side of the fly. This was determined by examining one hundred parent females and recording the presence or absence of any of these bristles. From the data thus obtained, the average numbers are easily derived. These numbers really represent the percentage of presence for the several bristles. The figures show that in scute-1 females the first orbital is always absent, while the remaining six bristles are present to a variable extent.

The number for the same set of bristles for scute-8 were obtained in the same manner, by studying one hundred scute-8 males. An examination of these numbers, as given in the second line of the table, shows that scute-8 does not affect the ocellars, postverticals, or first notopleurals, for these are one hundred percent present. It only slightly affects the first orbitals, and has less effect than scute-1 on the second orbitals, the anterior and posterior scutelars.

When a scute-1 female is crossed to a scute-8 male the F_1 females show the combined effects of these two alleles. This phenotypic expression has been called the "compound" by the Russian workers. In the case of the present experiment, the percentages were determined from data obtained in examining one hundred F_1 non-variant females. These figures are shown in the third line of the table, and from the numbers there given, it can be seen that the F_1 compound female can easily be distinguished from the scute-1 female.

There is, however, considerable variation in the phenotypic expression of the two scute alleles in the compound. Some of the F_1 non-variant females show no bristles missing, while others may have several bristles absent. The most consistent effect is on the first orbitals, the ocellars, the postverticals, and the first notopleurals. These four pairs are almost always absent in scute-1 flies, but are usually present in the compound. They therefore constitute the best criteria by which to judge whether a given yellow hypoploid female represents a compound or only a scute-1 fly.

The average for the seven pairs of bristles of the yellow hypoploid females obtained in the experiment are given in the fourth line of the table. These averages compare very favorably with those for the corresponding ones for their gray non-variant sisters, especially in view of the fact that they had to be computed from only sixteen instead of one hundred flies. The individual records of these sixteen yellow females are listed in the fifth to twentieth lines of the table. The records show that without exception these yellow hypoploid females reveal the compound of scute-1 plus scute-8.

Genetic tests carried out on the yellow hypoploid females further support the conclusion reached above. These females were crossed to

scute-1 forked carnation bobbed males. Fourteen of them proved to be fertile, and gave results which showed that one of the fourteen was deficient at the locus of bobbed (inverted position) as well as at the locus of yellow. Hence, the locus of scute-8 cannot lie between the loci of yellow and bobbed.

The cultures yielding the sixteen yellow hypoploid females also gave fifteen hyperploid males, each carrying a duplication produced by a long deletion in the treated scute-8 chromosome. These males were all gray vermilion carnation flies, but with respect to the effect of scute, they belonged to two classes. Eleven of them showed scute-1 only, while the other four were compounds of scute-1 plus scute-8. We know from the results given in table 1, that the order of the genes in the scute-8 chromosome is, from left to right, yellow, bobbed, carnation, forked, etc.; hence, since the deletion producing the type of these four males eliminated the loci of both carnation and vermilion, and not that of scute-8, the latter must lie some place to the right of the locus of vermilion. Unpublished crossover data show that the locus of scute is in its normal position with reference to apricot, echinus and vermilion. We conclude, therefore, that the scute-8 locus lies near the spindle-fiber end of the chromosome.

THE POSITION OF THE ACHAETE LOCUS

The test for the locus of achaete was made possible by the use of an eversporting gray-yellow mosaic stock of scute-8, obtained in 1932 (PATTERSON 1933, p. 44). In view of the fact that this stock (carried balanced with yellow white) never gives scute-8 apricot males, spotting must be due to a spontaneous elimination of the left or gray end of the chromosome during somatogenesis.

Proof of this is shown by the results obtained in the following experiment. If scute-8 apricot females, heterozygous for yellow white are crossed to yellow white crossveinless forked-5 males, the yellow areas of the F_1 spotted females show none of the other recessive genes for which they are heterozygous. However, when these yellow spotted females are mated to yellow achaete males, the yellow spots of their daughters invariably show the character achaete, whenever such spots fall upon an area affected by this gene. This demonstrates that the normal allele of achaete lies next to that of yellow, and is consequently eliminated along with that of yellow when the end breaks off.

We conclude from these observations that the breaks which produced the long inversion found in the scute-8 chromosome, occurred between achaete and scute on the left and between bobbed and the spindle-fiber end on the right, the entire section between these points being inverted.

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