

THE "PLEXUS" CHROMOSOME OF *DROSOPHILA*
PSEUDO-OBSCURA RACE A.

BY F. A. E. CREW AND ROWENA LAMY.
(*Institute of Animal Genetics, Edinburgh University.*)

(With Plates I and II and One Text-figure.)

AMONG the progeny of a purple female by a spread male several flies with abnormal wings were observed. The longitudinal veins were irregularly thickened and formed deltas at the margin; the posterior cross-vein lay at an abnormal angle, and extra branches were present in the region of the anterior cross-vein. In addition to these characteristics there was some degree of branching or forking of the longitudinal veins such as occurs in the well-known plexus of *D. melanogaster*. For this reason this abnormality was named after that mutant. Breeding tests, however, showed it to be sex linked and partly dominant, though its appearance in the heterozygous condition was somewhat erratic, and it was found best to use it as a recessive in the experiments described in this paper.

The expression of the character varies considerably as shown in Plate I, and there is also some degree of overlapping with wild type. It is anticipated that further study will reveal genetical differences between the strong and the weak forms.

An attempt to locate Plexus with regard to yellow vermilion singed showed that crossing-over in that region was considerably reduced by the presence of Plexus. Table I shows the figures obtained from a 10-day count of seven pair matings. Instead of the normal 10 per cent. of recombination to be expected between yellow and vermilion, only 1 per cent. was obtained. No crossing-over took place between vermilion and singed unless this coincided with crossing-over between yellow and vermilion. This double cross-over, producing the reciprocal classes of vermilion and yellow singed, occurred four times among the 533 flies examined.

These facts suggested the presence of an inversion of the region between yellow and singed, but the figures were not sufficient to give an accurate measure of the length of chromosome inverted. Neither was it clear whether the Plexus character was the result of the abnormal position of the genes in the region studied or whether it was also associated

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with a gene mutation—a question which could only be decided when every class of cross-over fly, both *Plexus* and non-*Plexus*, had been tested for the presence of the inversion. Accordingly the cross *Plexus* male × yellow vermilion singed female was again made, and the F_1 females mated singly to yellow vermilion singed males.

Thirty-one pair matings gave a total of 5769 flies (Table II). Since the females were not classified for *Plexus*, they appear in only half the cross-over classes, and as many as possible were tested for the presence of *Plexus*. The results obtained accorded perfectly, so far as they went, with the male classes.

Thus, of three yellow females tested, one carried *Plexus* and two did not;

Of two yellow singed tested, neither carried *Plexus*;

Of four vermilion singed tested, two carried *Plexus* and two did not;

Both vermilions were tested, and both carried *Plexus*;

In all classes in which *Plexus* was present crossing-over was reduced, and in those in which it was not present crossing-over was normal.

The above results were all consistent with the idea that the *Plexus* character was a direct effect of the inverted order of the genes in a region extending from a point to the left of yellow to a point to the left of singed. Hence, there could be two classes of yellow cross-overs, one resulting from a single cross-over between yellow and the right break of the inversion (giving the reciprocal recombination classes: yellow *Plexus* (inversion) and vermilion singed), the other resulting from double crossing-over within the inversion occurring between vermilion and the right break and between singed and the left break (giving the reciprocal recombination classes: yellow and vermilion singed *Plexus* (inversion)). But, on the same hypothesis, all vermilion and all yellow singed flies would be the result of crossing-over within the inversion on both sides of vermilion; hence all the vermilions would be *Plexus* (inversion) and none of the yellow singed would be *Plexus*.

There were two classes, however, the testing of which gave rather less satisfactory results, and these were the supposed + + + + and *y v sn Px* ♂♂. If these flies were actually what they seemed, the *Plexus* character would have had to be referred to a gene outside the inversion and capable of being separated from it; the *y v sn Px* class should have been free from the inversion and the + + + + class should have retained it. Unfortunately, both these classes of flies proved difficult to breed, the non-*Plexus* ones especially being feeble and abnormal in many respects. Only one of the latter was actually bred to a *y v sn* female;

the offspring were mated *inter se*; crossing-over was reduced in F_2 , but the Plexus character also reappeared in half the males. It is possible, therefore, that these supposed non-Plexus flies represent a degree of overlapping with the wild type.

The $y v sn Px$ flies were unlike other Plexus flies in that they showed not even in a slight degree the blurring of the longitudinal veins which is the most common characteristic of Plexus. Their sole abnormality consisted in a clean extra branch connecting the second and third longitudinals in the region of the anterior cross-vein. Two of these were successfully bred to wild-type females from stock, and their offspring mated *inter se*. The Plexus character did not appear in the F_2 , and crossing-over was normal. One of these males, however, was the father of a mosaic individual and is discussed in the next section of this paper. In the light of these results it seems likely that the Plexus character is not separable from the inversion, and in considering the crossing-over data, it is here provisionally regarded as identical with the inversion.

The total amount of recombination between yellow and vermilion, using males and females, is 1.6 per cent., and between yellow and singed 1.24 per cent. The normal values for these points are approximately 11 and 13.

In this and in the subsequent experiments single exchange between v and sn was never observed, hence sn certainly lies within the inversion. If the presence of Px is taken to indicate the presence of the inversion, the crossing-over between yellow and the right point of breakage may be estimated from the number of single exchanges between y and v ; that is, the $y Px$ and $v sn$ classes. The males only can be used in these calculations, since the constitution of all the females with regard to Px was not ascertained. Thus, for the y/Px distance we get the value 0.035 per cent.

Within the inversion only two types of double exchange occurred: one taking place on both sides of v (between y and sn), and the other including both v and sn (between y and a point beyond sn). For the former the value obtained is 0.03 per cent., and for the latter 0.04 per cent.

The absence of the $sn Px$ and $y v$ classes in Table II, indicating the absence of crossing-over on both sides of sn , was puzzling in view of the fact that pairing was certainly effected beyond the singed locus. It seemed possible, however, that it did not extend far enough for double crossing-over between v and the left point of breakage to come within the limit of interference. In that case it might be assumed that there was less map distance between sn and the left break than between v and

the right break. There was one other marker available as a measure of interference within this region, and this was white⁵, a very viable and fertile allelomorph of Lancefield's white. This gene is situated four units to the right of vermilion, and would thus give some indication of the distance covered by double crossing-over between *sn* and *y*. Accordingly, fourteen females of the constitution $\frac{Px\ v\ sn}{w^5}$ were mated to *y v sn* males from stock. The results are given in Table III.

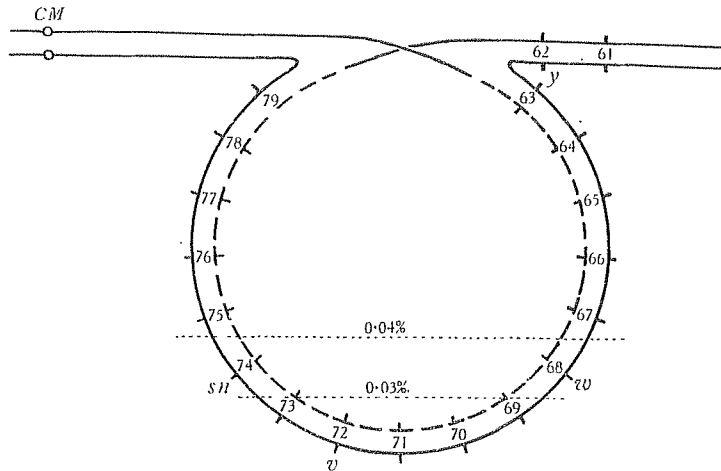
The figures obtained accord completely with those of the first experiment. No double crossing-over took place between vermilion and the left break. Moreover, it could be demonstrated here that none took place between vermilion and the right break. Those between *sn* and the right break did not include the white locus, whilst those which covered both vermilion and singed also covered the white locus.

Since white is epistatic to vermilion, the *Px w* males were tested for the presence of vermilion, and the vermilion females for the presence of white as well as Plexus. Of the three vermilion females only one was successfully bred, and proved to carry a white vermilion non-Plexus chromosome. She was therefore the result of double crossing-over between *sn* and *w*⁵. Similarly, of the two singed females, one was successfully bred and proved to carry the reciprocal combination, *Px sn*, on one of her *X*-chromosomes. Of the two *Px w* males, one was bred and proved not to carry vermilion. This male was the result of double crossing-over which covered the three loci, *w*, *v* and *sn*, the reciprocal combination, *v sn*, being also represented by one male. This male, however, failed to breed, so it was not proved that he was not an instance of overlapping of *Px* with wild type.

Text-fig. 1 shows diagrammatically the paired loop of the inversion with the positions of the loci studied, relatively to the points of breakage as these are suggested by the genetic data. If, as postulated by Koller (1935), the pairing of an inverted section begins at the centre, it would be expected that interference would be lowest at this point and would increase as pairing proceeded upwards towards the points of breakage. On this hypothesis the centre of pairing (and therefore of the inverted region) should be very close to the vermilion locus, since here interference is lowest. It has been observed, moreover, that the effect of interference in the Plexus inversion is to restrict crossing-over to points that are equidistant from this centre, an effect which may be due to the small size of the loop formed, for in the larger inversions such as Grüneberg's (Grüneberg, 1935) and *sc8* (Stone and Thomas, 1935), interference

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appears not to be so influenced by the pairing centre. However, this figure must be regarded as theoretical until the position of the yellow locus with regard to the inversion is more clearly established.



Text-fig. 1.

THE MOSAICS.

An interesting feature of the Plexus inversion was the high proportion of mosaics found; seven were observed among the 7000 flies examined in these experiments. A description of them in detail follows.

Mosaic No. 1.

This mosaic (Plate II, fig. 1) was found among the progeny of a female of the constitution $\frac{y v sn}{Px}$ by a *y v sn* male. The head, the right half of the thorax including the right wing and legs, and a small area below the scutellum were yellow and singed, the rest of the body being wild type in appearance. Though a normal female, she was not very vigorous and lived only about 10 days. She produced the following offspring by a *y v sn* male from stock:

+ ♀♀	<i>y v sn</i> ♀♀	<i>Px</i> ♂♂	<i>y v sn</i> ♂♂
13	8	13	7

Mosaic No. 2.

The second mosaic was also a female and resembled the first in every respect, except that the yellow singed region involved the left side of the thorax instead of the right, and the yellow wing was slightly warped

and shorter than the other. The head was yellow and singed. She was mated to a $y v sn$ male from stock and produced the following offspring:

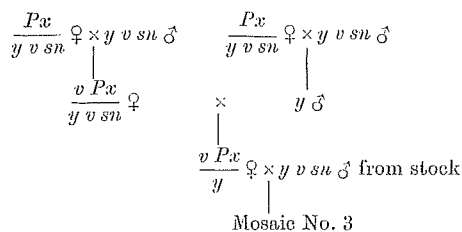
$+ \text{♀♀}$	$y v sn \text{♀♀}$	$+ \text{♂♂}$	$y v sn \text{♂♂}$
17	7	7	7

also 1 $v sn \text{♀}$; 1 $v sn \text{♂}$; 1 $y \text{♀}$; 3 $y \text{♂♂}$; 1 $v \text{♀}$.

The mother of this mosaic was a wild-type female from stock; her father was a $y v sn \text{♂}$ out of the cross $\frac{y v sn}{Px} \text{♀} \times y v sn \text{♂}$. He was one of the eight $y v sn$ males, observed among 5500 flies, which showed a small extra branch between the second and third longitudinal veins in the region of the anterior cross-vein, the remainder of the venation being quite normal. As mentioned above, it was not certain whether this small extra branch represented a variation of the Plexus character or not, since the Plexus type of vein generally includes some thickening or blurring of the veins, at least in spots. In order to test their constitution, these males were bred to wild-type females, and the offspring, which were perfectly normal save for the mosaic described above, were mated *inter se*. The Plexus character did not appear in F_2 where crossing-over was normal.

Mosaic No. 3.

This was a fertile male out of the cross $\frac{v Px}{y} \text{♀} \times y v sn \text{♂}$. The origin of the $v Px$ chromosome carried by the female is shown below:



This male was yellow except in the left half of the thorax including legs and wing, which were wild type. The wild-type wing, however, had Plexus venation. Breeding tests showed him to be pure yellow in constitution.

Mosaic No. 4.

This mosaic was of the "fore and aft" type and a female; the head and thorax were $y v sn$ and the abdomen wild type. She was rather small and weak, only survived a few days, and produced no offspring. Her

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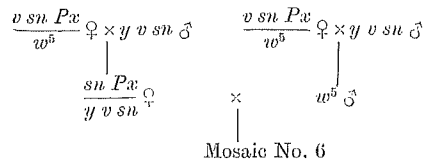
pedigree is unfortunately incomplete, but it is almost certain that she was derived from the same stock as Mosaic No. 2.

Mosaic No. 5 (Plate II, fig. 2).

This was a female and the offspring of a $\frac{v\ sn\ Px}{w^5} \text{♀} \times y\ v\ sn\ \text{♂}$. She appeared quite normal except for the patchy eye, but was unfortunately over-etherised.

Mosaic No. 6 (Plate II, fig. 3).

The pedigree of this mosaic, a sterile male, was as follows:



As shown in Plate II, fig. 3, he was normal $y\ v\ sn$ except in the right half of the head which showed wild-type colour and bristles and white eye. The white eye was rough and slightly smaller and narrower than the vermilion eye.

Mosaic No. 7.

This mosaic was a sterile female. The head and most of the abdomen were wild type; the left half of the thorax, the left wing, and left foreleg were yellow; there were also two light patches on the abdomen. Both wings showed the Plexus venation. This mosaic was in fact found in a culture homozygous for Plexus, the females of which were heterozygous for yellow.

It will be seen, therefore, that four of the mosaics had a heterozygous Plexus mother, one arose in a homozygous Plexus culture, and the remaining two were derived from a Plexus grandparent but did not carry an inverted chromosome. This latter fact suggests that there is a tendency to fragmentation in the inverted chromosome, and that the fragments are transmitted in the germ cells. It is, moreover, remarkable that in two out of the seven mosaics, the additional fragments carried by them clearly consist of that combination of genes which would result from crossing-over on both sides of the vermilion locus. In three others (2, 5 and 6) this is probably also the case, though the distribution of the mosaic tissue is unfavourable to complete analysis. Since only one marker was present in No. 7 this observation could not be made at all;

while of No. 4 nothing can be said since the constitution of her parents was unknown. There is at present little to suggest the actual length of the fragment carried, but in Nos. 1, 2 and 6 it certainly included both the singed and the yellow loci which are normally separated by a distance of at least 12 crossing-over units. The others are less conveniently marked for measurement.

The question arises, which is the hyperploid portion of these mosaics, and is the remaining portion diploid or hypoploid? Though the obvious suggestion is that the fragment is present in those minor portions of the animal which exhibit exceptional characters, closer observation shows that this hypothesis is not tenable in every case. The alternative hypothesis, that the major portion of the fly which shows the true phenotype corresponding to its genotype is hyperploid, and the remainder hypoploid, seems to be applicable to all but one, and the only one applicable to No. 6. (Nos. 2 and 7, for reasons given above, are outside the scope of this analysis.)

Thus the genetic constitution of mosaic No. 1, as shown by her breeding performance, was $\frac{Px}{y v sn}$. It is suggested that in the grey parts of the body she carried, in addition to two complete chromosomes, a portion which reduplicated at least the $y \dots sn$ region, and that in the yellow singed portions only the $y v sn$ chromosome and the fragmented chromosome were present, giving the yellow singed phenotype. The same distribution of chromatin could apply equally well to mosaic No. 2. It must be said, however, that since nothing is known of the cumulative effects of the yellow and singed genes in relation to their wild-type allelomorphs, nothing can be concluded from the expression of these characters which was in every case perfectly normal; from this point of view, therefore, either hypothesis will accommodate these two cases.

Mosaic No. 3, the yellow male with grey Plexus quarter, must have been hyperploid in his grey portions; he carried one normal chromosome with the yellow gene on it and a fragment which contained the wild-type allele of yellow, and, since the Plexus character was also visible, possibly the whole inverted portion as well. This fragment could not possibly have been present in the yellow non-Plexus parts, hence it must have been eliminated at an early cleavage division.

The patchy white eye of No. 5 must be attributed to elimination of the normal $y v sn$ chromosome from the cells of the white part. Crossing-over on both sides of vermilion in her mother would produce a white vermilion non-Plexus chromosome or fragment which would be inherited

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along with the normal chromosome carrying white. Again, it cannot be said that the wild-type allele of white which was present on the *y v sn* chromosome would or would not give a wild phenotype, but it is certain that if the *y v sn* chromosome had been eliminated from the white cells, the constitution of that tissue would be $\frac{+w}{v w}$, which would certainly be phenotypically white.

Finally, the white eye and grey head parts of No. 6 are impossible to account for except on the hypothesis of elimination of the normal chromosome from those regions. Since the mother of this male carried a chromosome which had undergone crossing-over on both sides of vermilion it may be presumed that the fragment which was present in him carried the recombinant loci resulting from that event, in this case vermilion white, with the normal alleles of yellow and singed. If both this fragment and the *y v sn* chromosome were present, the constitution of the grey white region would be $\frac{y v + sn}{+ v w +}$, and this could certainly account for the grey not-singed portion of the head, but not for the white eye. On the other hand, elimination of the *y v sn* chromosome would alone account for the whole.

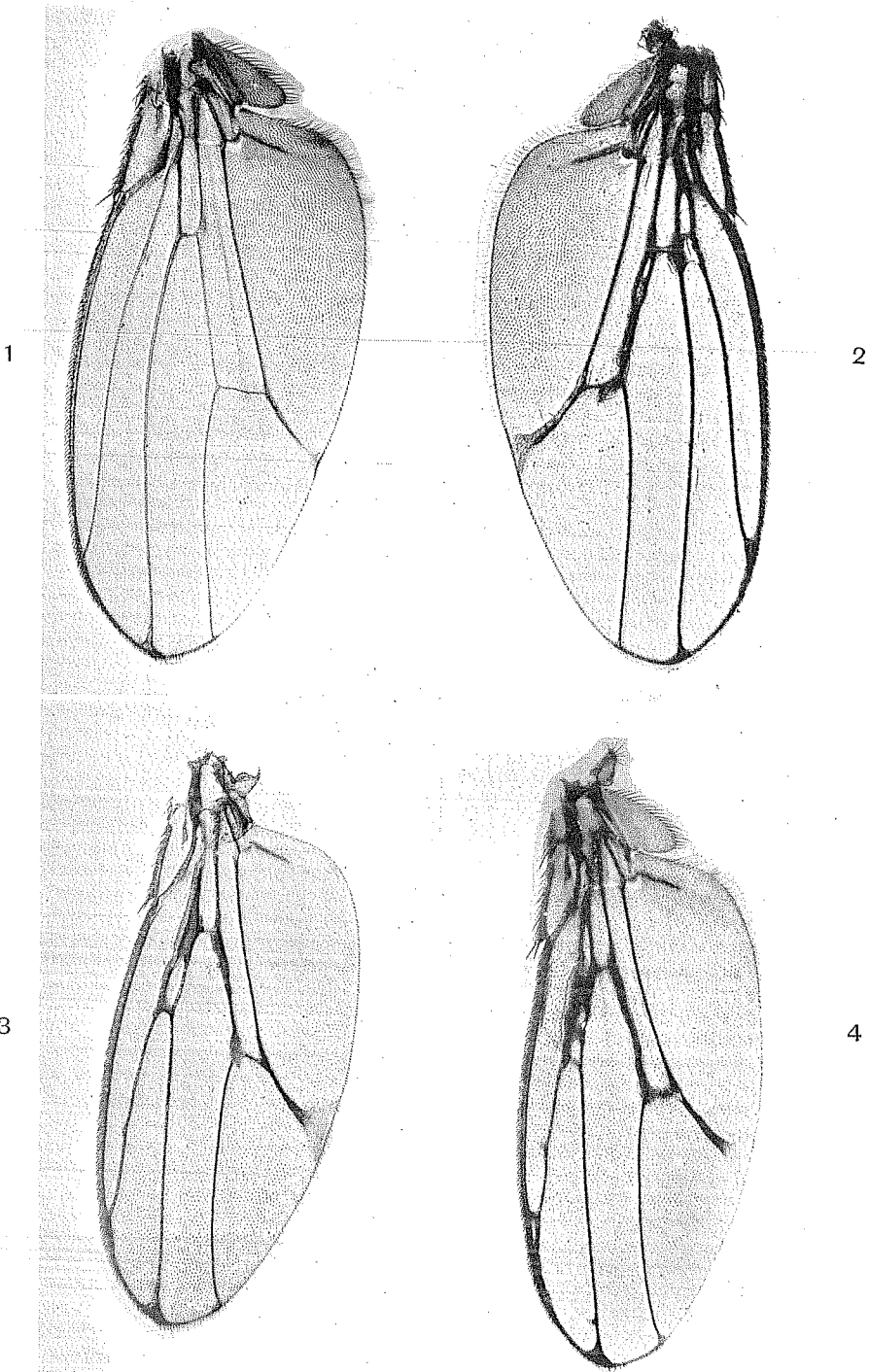
If, as is suggested by the above hypothesis, the unbroken chromosome is eliminated as often as the fragmented one, it seems likely that the fragment possesses a centromere and may undergo normal disjunction.

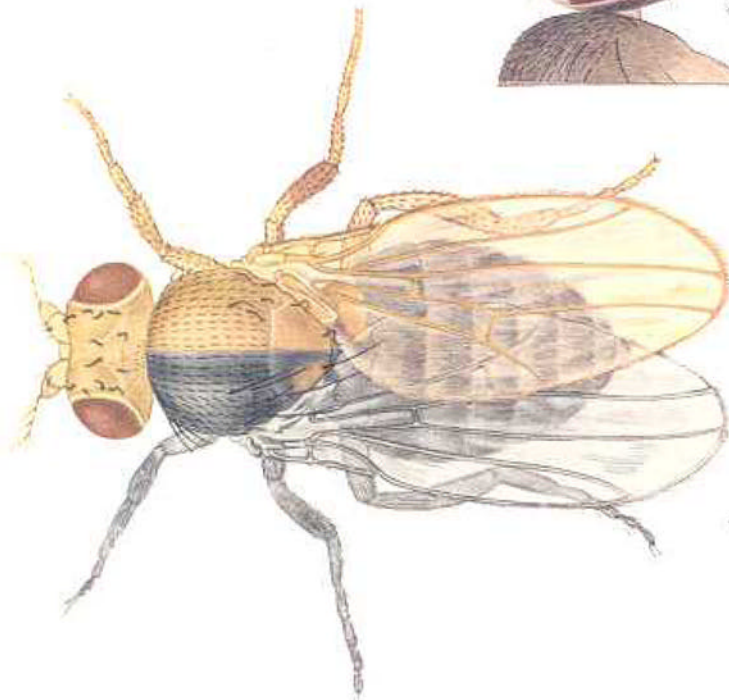
It is hoped that a close study of the salivary gland preparations of Plexus, in conjunction with further genetic analysis, will throw more light on the nature of the mechanism responsible for the formation of these mosaics.

SUMMARY.

The character Plexus is described and shown to be based not on a single gene but on the inverted order of some of the genes in the X-chromosome. The resultant alteration in crossing-over is discussed.

A series of mosaics arising in the Plexus stock is described and the origin and nature of the condition discussed.





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EXPLANATION OF PLATES I AND II.

PLATE I.

- Figs. 1, 2. Types of weak Plexus.
Figs. 3, 4. Types of strong Plexus.

PLATE II.

- Fig. 1. Mosaic No. 1.
Fig. 2. Mosaic No. 5.
Fig. 3. Mosaic No. 6.