STUDIES ON SOME POSITION PSEUDOALLELES AT THE WHITE REGION IN DROSOPHILA MELANOGASTER[1]

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POSITION pseudoalleles are noncomplementary mutants which may be divided into separate groups by recombination. Presently found in the literature are two contrasting interpretations of this phenomenon. The first, held by PONTECORVO (1952) maintains that the different pseudoalleles represent alterations at different sites of a single functional unit, the gene. The second, supported by LEWIS (1951, 1955) views the mutants at the different pseudoallelic loci as alterations in different units each of which should be called a gene.

The first interpretation assumes there are several mutation sites on a gene showing position pseudoallelism, and that in each case of mutation the inactivation of the gene results. On this basis, recombination between the mutation sites may be visualized as occurring within the functional unit.

The second interpretation accounts for the noncomplementarity of the mutants at two loci by invoking a position effect involving the products of two genes acting on different steps of a synthetic pathway. From this point of view, recombinant types arise by crossing over between functional units.

As has been pointed out by several investigators, one of the main difficulties in interpreting data obtained from pseudoallelic systems is the inadequate criteria for recognition of a functional unit. BENZER (1957), in his work with bacteriophage, points out the complexity of a functional unit and the difficulties involved in defining it in terms of recombination groups. BENZER's work, along with that of DEMEREC (1955) and co-workers (for review see HARTMAN 1957) using transduction in Salmonella, indicates that the unit of function in these microorganisms may be subdivided by recombination. Whether the recombinational phenomenon recorded in microorganisms is the equivalent of that found in Drosophila is at present a matter of conjecture.

From work with *Drosophila melanogaster* it is clear that the white region is a complex pseudoallelic system. LEWIS (1952) has shown that apricot (w^a) and white–1 (w^1) occupy two loci in this region and may be considered position pseudoalleles. The work of MACKENDRICK and PONTECORVO (1952) supports this interpretation. LEWIS (1956) has also described the position of another gene, white-spotted, (w^{sp}), which may be considered as occupying a third locus in the white region. The loci represented by these three mutants, apricot, white-1 and white-spotted, are located, from left to right in the order named, at about 1.5

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on the X chromosome. The work presented here gives evidence that another locus, occupied by Brownex, (w^{Bwx}), lies to the left of apricot. Unexpected crossover types also recorded in this study serve to emphasize again the difficulties involved in the definition of the functional units in this system.

MATERIALS AND METHODS

The establishment of the spatial relationships of the mutants within this pseudoallelic series is based entirely on the recovery of exceptional (nonparental eye-color) offspring from females heterozygous for the pseudoalleles being tested and for appropriate marker genes located on either side of the white region. The markers employed were yellow (y, 0.0; yellow body and bristles); yellow-2 (y^2, dominant to y; yellow body and black bristles); scute (sc, 0.0+; missing scutellar bristles); split (spl, 3.0+; split bristles and rough eyes); echinus (ec, 5.5; enlarged eye facets); singed-3 (sn^3, 21.0; gnarled bristles). The pseudoalleles studied were: apricot, (w^a); blood, (w^{bl}); Brownex, (w^{Bwx}); buff, (w^{bf}); honey, (w^h); white-1, (w^1). Descriptions of all these mutants with the exception of Brownex may be found in BRIDGES and BREHME (1944). Brownex was described by MOSSIGE (1953) as a member of the white series which in heterozygous females, w^{Bwx}/w^+, exhibits a slight dominant effect. This is easily classifiable when such females are made homozygous for the autosomal eye color scarlet. MOSSIGE also reports that crossing over is reduced in the neighborhood of Brownex, with 0.4 map units between scute and Brownex and 1.0 units between Brownex and facet. Crossover analyses done in this laboratory are in close agreement with these figures. Examination of the salivary gland chromosomes shows nothing unusual, although in general the white region is difficult to analyse.

The experimental procedure generally followed was to obtain virgin females heterozygous for two of the pseudoalleles and marker genes located on either side of the white region, and mate them to males carrying $y\ w\ spl\ sn^3$. The females were also made heterozygous for the second chromosome rearrangement *SM1* (LEWIS and MISLOVE 1953), carrying the markers aristaless (al), Curly (Cy), and speck-2 (sp^2), and the third chromosome rearrangement Ultrabithorax-130 (Lewis 1952) which is associated with the dominant marker Ultrabithorax (Ubx^{130}) and carries ebony-sooty (e^s). Such an autosomal constitution is known to be effective in causing an increase in the amount of crossing over in the X chromosome. The offspring resulting from this type of mating were examined for eye colors which differed from either of the pseudoalleles carried by the heterozygous female.

RESULTS

The crosses and any exceptional offspring recovered from each are given in Table 1. There are several results of interest. First, the appearance of y^2w^+ individuals from cross #1 indicates that the locus occupied by Brownex lies to the left of apricot. This may be questioned however since there also appear

TABLE 1

Exceptional recombinant types recovered from heterozygous females

Cross number	Heterozygous female	Exceptional recombinant types recovered	Number	Total offspring examined
1	+ w^{Bwx} + +	y^2 + + +	3	
	═══	+ + + +*	1	43,442
	y^2 w^a *spl* *ec*	y^2 *w* + +	6	
2	+ w^{bf} + +	+ + *spl* *ec*	1	
	═══			11,000
	y^2 w^{Bwx} *spl* *ec*	+ *w* *spl* *ec*	2	
3	+ w^h + +	+ + *spl* *ec*	2	
	═══			14,223
	y^2 w^{Bwx} *spl* *ec*	+ *w* *spl* *ec*	1	
4	*y* w^1 *spl* sn^3			
	═══	*y* + + +	2	9,000
	+ w^{Bwx} + +			
5	+ w^{bl} + +			
	═══	None		50,250
	y^2 w^{Bwx} *spl* *ec*			
6	+ w^{bl} + +			
	═══	None		11,909
	y^2 w^a *spl* *ec*			
7	+ w^{bl} + +			
	═══	None†		76,684
	y^2 w^{bf} *spl* +			
8	+ w^{bf} + +	+ *w* *spl* *ec*	4	
	═══			43,025
	y^2 w^a *spl* *ec*	y^2 *w* + +	4	
9	+ w^h + +	+ + *spl* *ec*	4	
	═══			13,425
	y^2 w^a *spl* *ec*	y^2 *w* + +	1	
			Total	196,375

* Single male carrying Ubx^{130}.
† For exceptional types not associated with crossing over see JUDD (1957).

y^2w and y^+w^+ classes. The latter of these does not show recombination of the markers on either side of white and may be explained either as a double cross over or as a reversion of Brownex to wild type. It is felt that this single male is not the result of contamination since he carried the third chromosome rearrangement marked with the dominant Ubx^{130}. The y^2w class, on the other hand, presents a problem since it arises from crossing over in the same direction as the y^2w^+ class. In an effort to resolve this complication, attached-X chromosomes heterozygous for apricot and Brownex and suitable markers were synthesized

after the method of MULLER (1936). Attached-X females of the constitution shown in Figure 1 were made heterozygous for the *SM1* and *Ubx*^{130} rearrangements and mated individually to *y w spl sn*^{3} males. The progeny from the females which proved to be of the type shown in Figure 1 were examined for individuals showing exceptional eye-color phenotypes. Among approximately 22,000 offspring examined, four females with wild type (red) eye-color appeared. Phenotypically two were **y**^{2} **sn**^{3}, resulting from the crossover designated as (b) in Figure 1; one was **ec sn**^{3}, which could arise as crossovers at points (a) and (c) or at points (b) and (c) as shown in Figure 1; a fourth was the result of a reciprocal crossover shown at point (a) in Figure 1, and phenotypically was **sn**^{3}. Since the distance from the marked region to the centromere of these X chromosomes is greater than 50 units, the two homozygous classes are rather frequent and the genetic constitution of each female could be readily determined by examining

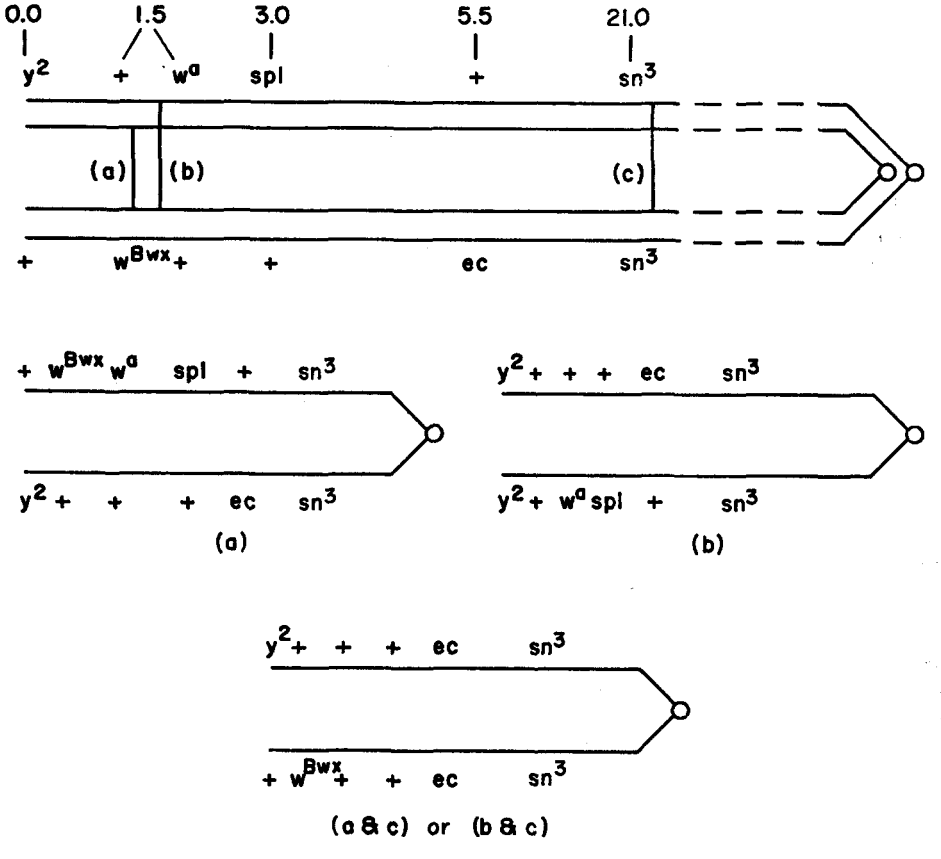

FIGURE 1.—The upper diagram shows the attached-X constitution of the parental females from which crossovers between w^{a} and w^{Bwx} were detected. Below are shown the three resultant crossover classes which arose from crossing over at point (a) (reciprocal), point (b) (nonreciprocal) and at points (a) and (c) or (b) and (c).

her offspring. Each of the four exceptional females proved to be of the constitution predicted from the crossover types shown in Figure 1.

Examination of the offspring from the female which arose by the reciprocal crossover, (point a), showed that the homozygous double mutant, $w^{Bwx}w^{a}$, is very similar to w^{a} in phenotype. On aging, however, the $w^{Bwx}w^{a}$ darkens to a brownish-pink color while w^{a} does not. Furthermore, $w^{Bwx}w^{a}/w^{+}$ shows the dominant effect exhibited by Brownex; this has not been confirmed by testing with scarlet however. The similarity between the phenotypes of the double mutant and apricot accounts for the fact that the double mutant was not recognized as an exceptional type in the experiments using free-X chromosomes.

Attached-X females homozygous for w^{Bwx} w^{a} *spl* sn^{3} were used to establish a stock culture, and from this a detachment was obtained using the T(1;4) B^{S} duplication. Females of constitution w^{Bwx} w^{a} *spl* sn^{3}/*y sc; SM1;* Ubx^{130} were then synthesized and mated to *y w spl* sn^{3} males. Among the progeny of these heterozygous females there appeared a female which was Brownex in phenotype and proved to be w^{Bwx}/*y w spl* sn^{3}. This gives proof of the existence of the mutant Brownex in the chromosome resulting from the recovered reciprocal crossover, and justifies the conclusion that Brownex occupies a separate locus at the left of apricot.

The use of the attached-X chromosomes does not clarify the origin of the y^{2} *w* class obtained from the w^{Bwx}/y^{2} w^{a} *spl ec* free-X heterozygotes. It may well be that this class would not be recognized in the attached-X, since w/w^{Bwx} is very similar to w^{a}/w^{Bwx} in phenotype and w/w^{a} cannot always be separated from w^{a}/w^{a}; thus, the white class would be recognized only if its complementary crossover type is markedly different from apricot or Brownex in phenotype.

In an attempt to define the exceptional white type, which for brevity will be referred to as w^{72}, several crosses were made with it. These crosses are given in Table 2. It is of interest to note that w^{72} does not cross over with white-1, apricot, or Brownex, though in the latter case the number of offspring examined is not large enough to rule out the possibility that recombination could occur. Furthermore, w^{72} apparently does not represent a double mutant type since no exceptional individuals were recovered from w^{+}/w^{72} heterozygotes.

Further examination of Table 1 will show that exceptional offspring with white phenotype are recovered from several crosses other than the w^{Bwx}/w^{a} combination. Crosses numbers 2 and 3 in Table 1 involving Brownex and either honey or buff also give a white class which arises from crossing over in the same direction as the wild type exceptions. These pseudoallelic combinations have not yet been placed in attached-X chromosomes nor have the exceptional types been studied to any extent. Cross number 8 involving apricot and buff offers some results which are also of interest. In this case, only exceptions with white phenotype have been found (w^{86a} and w^{86b}). From the recombination of the markers, it appears that the two may be complementary types. It was considered possible that one of them might prove to be the double mutant, that is, having both apricot and buff together in the same chromosome. To test this assumption, both of

the white types, $y^+ w^{86a}$ *spl ec* and $y^2 w^{86b}$, were crossed to wild type and to white-1 to find whether the original mutants, w^a and w^{bf}, could be recovered from one of them. These crosses and the results are given in Table 2. Neither w^{86a} nor w^{86b} proved to be the double mutant, and the origin of both types remains to be determined.

Early in the course of this investigation, the occurrence of the exceptional white type seemed to be associated with those crosses which involved the mutant Brownex. Working on the assumption that Brownex might represent changes at

TABLE 2

Exceptional recombinant types recovered from heterozygous females

Cross number	Heterozygous female	Exceptional types recovered	Total offspring examined
10	y^2 w^{72*} + + + w^+ + +	None	19,912
11	y^2 w^{72} + + y w^1 *spl* sn^3	None	12,996
12	y^2 w^{72} + + y^2 w^a *spl ec*	None	25,888
13	y^2 w^{72} + + + w^{Bwx} *spl ec*	None	5,402
14	+ $w^{86a\dagger}$ *spl ec* + w^+ + +	None	14,707
15	y^2 $w^{86b\dagger}$ + + + w^+ + +	None	10,030
16	+ w^{86a} *spl ec* y w^1 + *ec*	None	24,602
17	y^2 w^{86b} + + y w^1 *spl* sn^3	None	7,144
18	y^2 w^{Bwx} *spl ec* + w^+ + +	None	26,177
			Total 146,858

* Derived from cross number 1, Table 1.
† Derived from cross number 8, Table 1.

two points located very close together in the X chromosome, offspring from $y^2\ w^{Bwx}\ spl\ ec/w^+$ females were examined. If Brownex is a double mutant, exceptional offspring should also arise from this heterozygous combination. As is shown in cross number 18, Table 2, examination of 26,177 individuals from such a cross failed to yield any exceptional types. The results of this cross also indicate that the derived white class is not the result of unequal crossing over, for if it were, it should arise with comparable frequency from any of several heterozygous combinations tested, including w^{Bwx}/w^+.

The unusual nature of some of the recombinational events which apparently give origin to the recovered exceptional offspring is further emphasized by spatial relationships assumed by the four mutants, apricot, blood, Brownex and buff. Table 1 shows that Brownex-buff and Brownex-apricot combinations give rise to essentially the same classes of exceptions, while apricot-buff heterozygotes give the two unusual white types discussed previously. Blood, on the other hand, does not show recombination with any of these three. It is possible that blood is a small rearrangement in the region of white, but this is not likely since work now in progress shows that blood does recombine with eosin, and that its locus lies to the left of eosin.

The only exceptional types which have been rigorously tested to eliminate the possibility of modifiers are those which arose from the w^a/w^{Bwx} and w^a/w^{bf} females. Stocks of each of the exceptions were established, however, and it can be stated that if modifiers are responsible for the unusual results, they almost always appear in those gametes showing crossing over in the y-spl region of the X chromosome. The appearance of exceptional types not associated with crossing over reported previously (JUDD 1957) from w^{bf}/w^{bl} heterozygotes are presently under study.

An analysis of the wild type exceptions derived from w^a/w^{Bwx} females is now under way. Several of them have now been placed in combination with the mutant zeste, (z), (GANS 1953) and in each case their action with respect to z is indistinguishable from the w^+ derived from the Oregon-R strain. It should be noted however that neither apricot nor Brownex show a suppressor effect in combination with zeste.

DISCUSSION

As more is learned about gene action, it becomes increasingly clear that observable phenotypic effects assigned to a locus may be the result of gene action at any one of several levels. To equate phenotypic change with any particular change in the genetic structure may, in specific cases, be impossible with our present knowledge. The magnitude of a genetic change which will be registered as a change of function may depend entirely on the level at which function is measured. The detection of mutation and thus the definition of the unit of genetic change is of course directly dependent on this measure of function. Similarly, the definition of the unit which can not be further subdivided by recombination

is dependent on the recognition of phenotypic change. In other words, the genetic unit can be properly delimited only when phenotype can be measured at the same level as genotype. From this point of view it is not surprising to find that position pseudoalleles present a unit of function which is considerably different from the unit of recombination.

In the white region of the X chromosome of *Drosophila melanogaster* there are three or four points between which crossing over may be detected. Within this region, which may be defined by the noncomplementarity of the eye-color mutants localized in this part of the X chromosome, there may be several undetected or only partially detected sites which can show recombination with each other or with those sites already known. Whether the $y^2\ w^{72}$ class derived from $y^2\ w^a\ spl\ ec/w^{Bwx}$ females and either or both of the white classes arising from $y^2\ w^a\ spl\ ec/w^{bf}$ females can be explained on the basis of additional loci is not settled.

It is possible to consider the mutant Brownex as a small rearrangement, possibly an insertional duplication for all or part of the white region. It is still difficult however to account for the exceptional white types. Possibly they also are small rearrangements which will not recombine with some or all of the mutants of the white series. It is hoped that this possibility may be subjected to critical test. Even if Brownex is considered to be abnormal with regard to the number and/or arrangement of the components comprising the white region, the data obtained regarding its relationships in space and function to the other mutants of the white series are of value. Whether these data may be explained in terms of conventional recombination events or whether a reconsideration of generally accepted views is called for must necessarily await further work with other members of the white series, other suitable pseudoallelic loci and analysis of the resultant exceptional types.

SUMMARY

Recovered recombination classes from females heterozygous for the mutants Brownex and apricot show that Brownex occupies a separate locus at the left of apricot. The possibility that Brownex represents a small rearrangement in the white region is discussed in the light of the appearance of the unexpected crossover class of white phenotype.

Data are presented which indicate that neither Brownex nor the derived white class is due to mutation at more than one site in the white region, and furthermore, that the exceptional white class probably does not arise as the result of unequal crossing over. The possibility that the unusual white class represents a small rearrangement is considered.

The unusual spatial relationships of the mutants apricot, blood, Brownex and buff, as determined by the exceptional classes obtained from some of the heterozygous combinations, are recorded and discussed briefly.

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