* Paper No. 823 from the Department of Genetics, University of Wisconsin. This work was supported by grants of the American Cancer Society and the U.S. Public Health Service (RG-7119) to Dr. Ernst Freese.

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*FORMATION OF DUPLICATION-DEFICIENCY PRODUCTS BY ASYMMETRICAL EXCHANGE WITHIN A COMPLEX LOCUS OF DROSOPHILA MELANOGASTER**

BY B. H. JUDD

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF TEXAS, AUSTIN

Communicated by Wilson S. Stone, February 13, 1961

The possibility that pseudoalleles may represent closely linked genes having similar functions such that a position effect may exist between them, and the cytological evidence that such genes may occupy regions representing duplications, has led Lewis[1] to postulate that such cases may deal "with genes which were once (or are still) identical." The hypothesis states that before a gene with a new function can arise, there must first exist a pool of extra genes established through chromosomal duplication. One might expect to find pairing homologies within such a series of "old" and "new" genes in a manner comparable to such known duplications as Bar and Beadex-recessive, where unequal crossingover is a common feature. As yet, however, no clear case of other than strictly symmetrical pairing within complex loci has been demonstrated despite extensive studies of such loci in several organisms. Several examples of recovery of single products from a crossover event have been reported;[2-4] it is assumed in these cases that crossingover occurs in association with asymmetrical pairing and that the failure to recover one product is due either to its lethality or to a lack of distinctive phenotype necessary to distinguish it from one of the parental types.

Indication that asymmetrical pairing occurs within the white locus of *Drosophila melanogaster* first came from results obtained by MacKendrick,[5] who reported that w^{bl}/w^{aE} heterozygotes yielded both wild type and white recombinants, the latter through two different directions of crossingover. Judd[6, 7] also reported unexpected white crossover products from several heterozygous combinations of white mutants. On the assumption that the reciprocal product was not being recognized the author attempted to use attached-X chromosomes to recover both

products of a reciprocal exchange. This first attempt met with failure. Green[8] presented evidence that the white crossover class obtained from w^a/w^{a4} heterozygotes represented a cytologically undetectable loss of a portion of the white locus. This suggested that this class had its origin in crossingover within an asymmetrically paired portion of the locus. Green also failed, however, to find the product reciprocal to the deficiency type, and interpreted his findings as a putative nonreciprocal crossover event.

The present paper reports the recovery and partial analysis of reciprocal products resulting from crossingover within a nonhomologously paired portion of the white locus.

Recovery of Reciprocal Crossover Products.—To accomplish the recovery of exchange products known to be reciprocal, attached-X chromosomes were constructed after the method of Muller.[9] These chromosomes were made heterozygous at the white locus[10] (*w*, at 1.5 in the X chromosome) for w^a (apricot) and w^{bf} (buff) and carried the markers yellow (*y*, at 0.0; yellow body and bristles), yellow-2 (y^2; allelic and dominant to yellow, with yellow body and black bristles), scute (*sc*, 0.0+; missing scutellar bristles), zeste (*z*, $1^{\pm}$; brownish-yellow eyes), split (*spl*, 3.0; split bristles and rough eyes), echinus (*ec*, 5.5; enlarged eye facets). The constitution of the attached-X females is shown in Figure 1. The attached-X

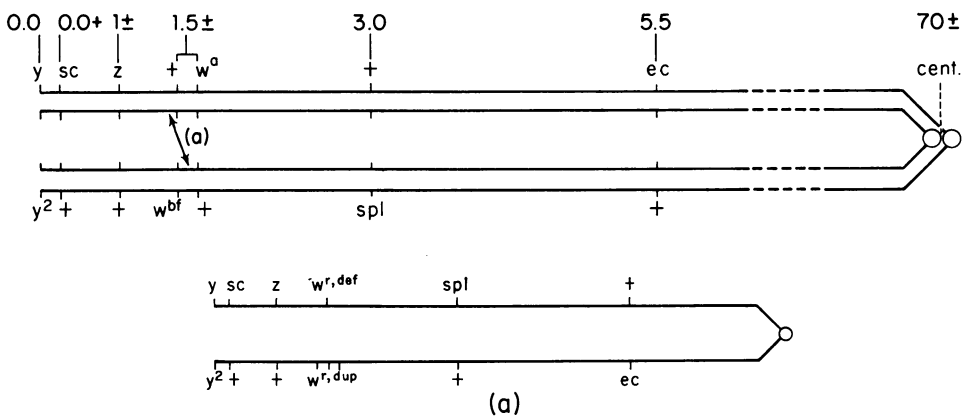

FIG. 1.—The upper figure is a diagram of the attached-X chromosomes used for detection of reciprocal products from an asymmetrical exchange. This asymmetrical exchange is indicated by (*a*) in this figure, with the resultant products diagrammed in the lower figure.

offspring from such a female are predominantly three types: (1) yellow-2 "dilute-apricot," which generally are the same genetic constitution as the parental female, (2) yellow scute "light-apricot" echinus (*y sc z* w^a *ec*), and (3) yellow-2 buff split (y^2 w^{bf} *spl*). Types 2 and 3 arise through certain types of exchanges between *ec* and the centromere, causing homozygosis for each group of linked mutants. These two rather frequent homozygous classes make it possible to determine the constitution of a single attached-X female simply by examination of her offspring.

Attached-X females of the constitution shown in Figure 1 were next made heterozygous for the second and third chromosome rearrangements, *SM1* and Ubx^{130}. These rearrangements increase the amount of crossingover in the *y-spl* region of the X chromosome approximately four times. These females were then mated

individually in food vials containing a standard yeast, cornmeal, molasses, agar medium, and the female offspring were examined for eye-color phenotypes which differed from those of the three predominant classes given above.

From approximately 31,000 female offspring, two yellow-2 white females were recovered in separate cultures. Examination of their sisters showed both had come from attached-X mothers of the type shown in Figure 1. These two exceptional females in turn produced offspring which allowed the determination of their genetic constitution. The two predominant homozygous classes produced were phenotypically (1) yellow scute white split and (2) yellow-2 "light-buff" echinus. Subsequent crossover studies showed the mutant zeste to be linked to yellow and scute. This linkage of *y*, *sc*, and *z* with *spl* and of y^2 with *ec* indicates that both females arose by a reciprocal exchange in the region between *z* and *spl*. Such a crossover is shown at point (*a*) in Figure 1. Evidence is presented below which indicates quite clearly that the yellow scute white split chromosome has a small, cytologically undetectable, deficiency for a part of the white locus and that the yellow-2 "light-buff" echinus product is the reciprocal duplication. These two products will henceforth be designated as $w^{r,\,def}$ (white-recombinant, deficiency) and $w^{r,\,dup}$ (white-recombinant, duplication). The "light-buff" phenotype of $w^{r,\,dup}$ may be surprising in view of the fact that the phenotype of the heterozygote, $y\ sc\ z\ w^{r,\,def}\ spl/y^2\ w^{r,\,dup}\ ec$, is completely white. The phenotype of the heterozygote is due to an interesting interaction of $w^{r,\,dup}$ with the mutant *z* on the homologous chromosome. The recognition and recovery of the reciprocal exchange products is based on this interaction, the significance of which will be discussed below.

Having determined that the exceptional types arose as a reciprocal exchange in or near the white locus, various tests were set up to determine the nature of the changes brought about by this event. The two crossover products first were detached from the attached-X chromosomes using *T* (*1;4*) B^S. Each of the types as hemizygous males proved to have the same phenotype as the corresponding homozygous females.

Analysis of Recombinant Chromosomes.—Males of each type were mated to Oregon-R females and the salivary gland chromosomes of the heterozygous female offspring were examined. Neither recombinant chromosome showed a detectable cytological change in structure. Both $w^{r,\,def}$ and $w^{r,\,dup}$ males were mated to *sp-w* females to test for a white locus deficiency. Green[8] showed that *sp-w/w-deficiency* females exhibit a phenotype inseparable from homozygous *sp-w* (eye light yellow with small reddish spots), while *sp-w* compounded with mutants of the white series located to the left of *sp-w* gives a uniform brown phenotype distinctly darker than either *sp-w* or the mutant with which it is compounded. Work in this laboratory confirms this test, and it appears to be a very reliable one for demonstrating white locus deficiencies. When females heterozygous for *sp-w* and the recombinant chromosomes were obtained, the $w^{r,\,def}/sp\text{-}w$ females showed a phenotype identical with that of homozygous *sp-w*, while the $w^{r,\,dup}/sp\text{-}w$ females showed a uniform brown eye color. This test clearly indicates that the $w^{r,\,def}$ chromosome is deficient for some portion of the white locus. It is also clear from the method of recovery that the $w^{r,\,dup}$ chromosome must be the reciprocal duplication.

Further evidence to support this hypothesis comes from the test to determine

the effect of $w^{r,\,def}$ and $w^{r,\,dup}$ on the mutant zeste. It has been shown by the work of Gans[11] that white deficiencies as well as some mutants of the white series act as dominant suppressors of *z*, i.e. females *z* w^+/*z w-deficiency* are wild-type in eye color. Green[12] showed that the white mutants which suppress zeste are those occupying the two rightmost recombination sites of the five sites now defined in the white locus,[13] i.e. those mutants apparently homoallelic to *sp-w* and w^{ch}. The heterozygote, *z* $w^{r,\,def}$/*z* w^+ is wild type in phenotype, showing that $w^{r,\,def}$ is a suppressor of *z*. Neither w^a nor w^{bf}, the two mutants carried in the parental chromosomes, act as suppressors. In this respect they are equivalent to w^+. On the other hand, the combination of z^+ $w^{r,\,dup}$/*z* w^+ shows a slightly mottled, reddish-brown phenotype. This is similar to the phenotype described by Gans[11] for z^+ w^+ /*Dp (1;f)* z^9/sc^{J4R}, which essentially is z^+ w^+/*z* w^+/w^+ (*z*, 1 dose; z^+, 1 dose; w^+, 3 doses). It is clear from this comparison that $w^{r,\,dup}$ is indeed a duplication for a portion of the white locus equal to 2 doses of w^+ with regard to its action on *z*. That $w^{r,\,dup}$ is acting as an enhancer of zeste is further shown by some combinations involving $w^{r,\,def}$, w^{11E4}, and w^1 (all are suppressors of zeste). Females z^+ $w^{r,\,def}$/z^+ $w^{r,\,dup}$ and z^+ w^1/z^+ $w^{r,\,dup}$ are light-buff in phenotype, while *z* $w^{r,\,def}$/z^+ $w^{r,\,dup}$ and *z* w^{11E4}/z^+ $w^{r,\,dup}$ females are white in phenotype. There can now be little doubt remaining that the reciprocal crossover products recovered from the described attached-X chromosomes are complementary deficiency and duplication products produced by a single exchange event.

The duplication and deficiency changes which occurred by recombination can be localized to the salivary gland chromosome doublet 3C2-3 by utilizing the white locus duplication *Dp* w^{+51b7}, described by Ratty.[14] This duplication which extends from 3C2-3 to 3D2 covers both $w^{r,\,def}$ and $w^{r,\,dup}$, i.e. $w^{r,\,def}$/*Dp* w^{+51b7} and $w^{r,\,dup}$/*Dp* w^{+51b7} males are wild-type in eye color. *Dp* w^{+51b7} does not cover a deficiency of salivary gland chromosome band 3C1.

Recombinant Types Recovered from Free-X Experiments.—Considerable work has been done in this laboratory on recombination products recovered from non-attached-X w^a/w^{bf} heterozygotes. Three classes of crossovers are recognized from y^2 w^a *spl ec* /w^{bf} females. Phenotypically they are yellow-2 white and white split echinus, with the yellow-2 white being divided into two groups on the basis of their phenotype when compounded with *sp-w*. One of these appears to be a deficiency product strictly comparable to the *y sc z* $w^{r,\,def}$ *spl* described here. The other nondeficient yellow-2 white and the white split echinus classes from the free-X experiment have not yet been recovered from the attached-X experiment. The analysis of these products will be reported elsewhere when more is known concerning their origin and structure.

Exceptional white recombinants have also been recovered from w^a/w^{Bwx}, w^h/w^{Bwx}, w^{bf}/w^{Bwx}, w^h/w^e, and w^a/w^a females. These have all been tested against *sp-w* and all appear to be deficiencies by this test; all are male viable, however, and the salivary gland chromosomes are normal. The recombinants recovered from the first three heterozygotes listed above have also been tested for action on zeste. All act as zeste suppressors. Since the parental eye colors are quite different for each of the heterozygous combinations above, one can only assume that if the reciprocal duplication class is appearing, its phenotype must closely resemble one of the parental types in each case. If the results reported here may be extended

to these cases, one would predict that the reciprocal class does occur in each case and probably is similar to the lighter parental eye color in phenotype.

Discussion.—This demonstration of asymmetrical pairing and crossingover within the white region is of interest because of the information it gives to the study of chromosome organization and gene evolution. It is clear that pairing homologies exist between the subunits of this locus. This is most readily explained on the basis that these units were at one time (or still are) identical and arose through some mechanism leading to small chromosomal duplications. Such a process may account for the structural and functional complexity that has been demonstrated for the white locus.

If we visualize the five recombination sites now defined as points 1 2 3 4 5 along the chromosome, we can assign w^{bf} and w^{a} to points 2 and 3, respectively. It is not at all clear which of the sites are involved in the asymmetrical exchange, but a brief consideration of what is known about the functional subdivisions of the region may cast some light on this point. Green[12] has shown that with regard to the suppressor action on zeste, the mutants occupying the five sites fall into two groups. Sites 1, 2, and 3 contain nonsuppressor mutants while 4 and 5 contain zeste suppressors. The mutants in sites 1, 2, and 3 also show dosage compensation (i.e. hemizygous mutant ♂ exhibits as much or more pigment in the eye than homozygous ♀). With the exception of w^{h} which is located in site 4, all mutants occupying 4 and 5 fail to show dosage compensation. On this basis sites 1, 2, and 3 seem functionally distinct from 4 and 5. This is of interest when one considers the two exchange products in these respects. The $w^{r,\,def}$ product is a *z* suppressor; since it is white in phenotype no measure of dosage compensation can be made. The $w^{r,\,dup}$ product on the other hand is essentially a zeste enhancer and it does show dosage compensation. These observations could be interpreted as attributable to a loss of all or part of the nonsuppressor portion (sites 1, 2, and 3) of the locus in $w^{r,\,def}$ and the duplication of this portion in $w^{r,\,dup}$. Such duplication-deficiency products could arise by crossingover within the white locus if pairing homology exists between any two of the nonidentical sites. For example, pairing may take place in the following way:

1 2 w^{a} 4 5 (mutant w^{a} assigned to site 3).

1 w^{bf} 3 4 5 (mutant w^{bf} assigned to site 2).

If a crossover occurs between sites 1 and 2 in the w^{a} bearing chromosome and between sites 2 and 3 in the w^{bf} bearing chromosome the resultant products would be 1–3 4 5 (deficient for site 2) and 1 w^{bf} 2 w^{a} 4 5 (duplicated for site 2). Asymmetrical pairing and crossingover may of course occur at other points, resulting in duplication-deficiency products for other sites as well. Some evidence for this comes from the report by Green[8] that the white recombinant deficiency obtained from w^{a}/w^{a4} (both apparently located in site 3) does not show a suppressor effect on *z*. This may mean that this product represents a deficiency for site 4 or 5 (or both). In any event the recombinant-deficiency product reported by Green is certainly different from those reported here with respect to suppressor action on the mutant zeste.

One other point can be made regarding the type of gene function in the w series. The white mutants, in general, are considered to be hypomorphs after

the system of Muller.[15] Green[8] reports that a tandem duplication for the whole w region which contains the mutants w^a and w^{a4} is distinctly more nearly normal than either mutant separately. However, when two pseudoalleles of the white series are coupled in the same chromosome the resultant phenotype is usually comparable to that of a deficiency for the white region (the exceptions to this rule are those combinations involving the mutant w^{Bwx} at site 1). These two observations are not compatible unless one invokes a position effect to account for the phenotype of the double mutant. In the case reported here, $w^{r,\,dup}$ is distinctly lighter than w^a and slightly lighter than w^{bf}. It is not equivalent to a white deficiency but it is less normal than either w^a or w^{bf}. It would seem that these observations are most readily explained on the basis that the recombinationally discrete units are *not* functionally discrete. The $w^{r,\,def}$ would then supply no gene product or an inactive one, while $w^{r,\,dup}$ would supply one which is less normal than from either w^a or w^{bf} but presumably more effective than one from $w^{bf}\,w^a$ coupled in a conventional manner in the same chromosome. Of course, one could again invoke a position effect to account for these phenotypes and thus maintain the view that the units defined by recombination are discrete functional units as well. The results presented here still do not allow a clear decision concerning this problem of what the recombination sites represent in terms of the functional genetic unit involved. One might view the hypothesized evolution by duplication process as involving the whole functional unit, or, just as logically, as involving only small segments of it. This point can be answered only when more is known concerning the structure and function(s) of the white region.

Summary.—A method for recovery of reciprocal products resulting from asymmetrical exchange within the white locus of *Drosophila melanogaster* is described. The analysis of the two complementary exchange products indicates they represent a duplication and a deficiency for a portion of the locus.

The significance of such an exchange is discussed with regard to gene evolution and with regard to the structure and function of this complex locus.

* This investigation was supported (in part) by PHS research grants C-3648 and RG-6492 from the National Institutes of Health, Public Health Service, and by a grant from the Rockefeller Foundation.

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