A Study of High Mutability Involving Two Loci in Drosophila Melanogaster

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ABSTRACT

Data are presented which strongly indicate that the locus occupied by the mutant \( w^* \) lies to the left of, or on the same locus of apricot \( (w^a) \). The fact that \( w^* \) shows a non-suppressor effect in combination with ade and as a typical phenomenon in mutants of sites 1, 2, and 3 of the white locus, also supports that \( w^* \) is a mutant at or left of apricot (site 2). A "model" for the genetic fine structure of the mutants \( (w^{-}\text{v}, \ w^{-}\text{d}, \ w^{-}\text{y}) \) is proposed and discussed to account for the mutability among these three mutants. Analysis of \( w^+ \) leads to the hypothesis that it is the result of an inversion (sites 3 and 4) at the white locus, by two mechanisms which are discussed briefly here.

INTRODUCTION

Recent work has shown that when very large populations are observed, derived from parents in which small segments of chromosomes bear suitable marker genes, recombination can be proved to occur between what had been assumed to be alleles.

Studies of recombination between various mutants of the white series in Drosophila melanogaster have shown that the white region is a complex pseudoallelic system. Lewis (1952), and Mackendrick and Pontecorvo (1952) demonstrated that apricot \( (w^a) \) and white-I \( (w^I) \) occupy two loci in this region and are position pseudoalleles. Lewis has also shown the position of white-spotted \( (w^{sp}) \). The work of Judd (1958) has given evidence that another locus is occupied by Brownex \( (w^{ex}) \). The further work of Judd (1959) and Green (1959b) established the existence of at least five subloci or recombination sites within the white locus. Each of these, with the exception of Brownex \( (w^{ex}) \), has a series of "true" alleles, as shown in Figure 1. The loci represented by these mutants are located at about 1.5 on the X chromosome.

Because of the large number of shades, ranging from white to nearly red, in this series, investigators have been intrigued by it. In addition some of these mutants do not show the same phenotype in the male as they do in the female. Mutants, such as eosin \( (w^*) \), played an important role in the study of dosage compensation, which along with the effect of various suppressors and interactions with other eye-color genes serve to subdivide the mutants into functional groups (Figure 1).

One of the characteristics of the white-locus pseudoalleles is that no complementation seems to occur either between the members in different recombination groups, or within groups, except between the spotted whites \( (sp-w) \) and the others. Another peculiarity is that asymmetrical exchange seems to occur regularly within the groups. The white recombinants \( (w^*) \) are small deficiencies in the white
region resulting from unequal crossing over (Judd, 1959; Green, 1959a). It was possible to demonstrate that the expected complementary exchange products, a duplication and a deficiency for a portion of the white locus, were produced (Judd, 1961).

On the other hand, the zeste(z) locus in D. melanogaster is located at 1.0 on the same chromosome and female flies homozygous for z have yellowish eyes (Gans, 1953). The white pseudoalleles may be differentiated functionally to some extent, as well as by recombination, by combining them with zeste. It has been found that all w alleles in site 4 (Figure 1) and the sp-w alleles suppress zeste, but others do not. Judd (1961) was able to show that zeste may be considered closely related to the white locus, not only because of the suppressor effect of some of the white alleles, but also because heterozygous z/z⁺ flies, which are also heterozygous for a small white duplication, w⁺/w⁺w⁺, give a mutant phenotype. There is no position effect associated with the enhancement of zeste by duplication; the arrangement of z w⁺/w⁺w⁺ is identical to z⁺ w⁺/w⁺ w⁺.

The mutants z w⁺z (zeste—mottled), which causes a mottled eye color in D. melanogaster, was found by Green (1959b) as a single male from the cross sc z ec ct/w⁺f x sc z ec ct. He considered this mutant to be an allele of z. Later w⁺f (zeste—light) was found by Becker (1959) as a single male in the sc z w⁺m stock. In analyzing the genetic structure of z w⁺m, Judd (1963) hypothesized that it does not represent a change at the zeste locus, but is the result of an asymmetrical exchange at the white locus. Recombination experiments by Judd have shown that a portion (site 4 or 5) of the white locus has been duplicated. It is also postulated that w⁺f arose as a similar asymmetrical exchange from w⁺m.

The new mutant w⁺ from w⁺m, in other words, z w⁺ (white eye—color) from z w⁺m was found by Judd, from a z w⁺m stock and has since been recovered several times from crosses involving the z w⁺m and z w⁺l chromosomes. It is interesting to note that the genotype z⁺ w⁺ is white eye color in phenotype as is z w⁺, but z w⁺m is reddish-brown mottled eye while z⁺ w⁺l is wild-type.

The mutability of those mutants (w⁺m, w⁺l, and w⁺) is high and somewhat complex as shown in Figure 2. It should be noted that mutations between w⁺m and w⁺l also between w⁺m and w⁺, occur with high frequency (1/1,000) in females of particular genotypes, but only rarely in males. It is not known whether the mutation between w⁺l and w⁺ happens only in females or in both.

The work presented here is attempted to

Fig. 1. Fine structure map of the white locus. From Judd (1963). For descriptions of the mutants see the text and Bridges and Brehme (1944)

Fig. 2. The relationships among the three mutants (w⁺m, w⁺l, and w⁺). From Judd (personal communication)

z w⁺m = zeste—mottled.

z w⁺l = zeste—light.

z w⁺ = white eye color.

(z is not necessary to w⁺ to be pheno-typically white)
locate and characterize \( w^* \), and shows the relationships among those mutants (\( w^{m}, w^{z}, \) and \( w^x \)).

**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 maker genes (as an aid in simplifying the interpretation of the experimental results) located on either side of the white region. Descriptions of most of the mutants employed in this research may be found in Bridges and Brehm (1944). The mutant zeste, \( (z) \), was described by Gans (1953). The list, among with symbols and map positions, is given in Table 1.

The flies were reared at 24°C on a standard Brewer's yeast-cornmeal-molasses-agar medium containing 0.5% propionic acid as a mold inhibitor.

The experimental procedure generally followed was to obtain virgin females heterozygous for two of the pseudoalleles and maker genes located on either side of the white region, and mate them to males carrying \( y w \) spl sn. The females were also made heterozygous for the second chromosome (SMI Cy/+) and the third chromosome, (Ubx 130/+). Such an autosomal condition 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.

In order to test a suppressor effect of \( w^z \) in combination with zeste, \( z w^z \) males were mated to zeste females.

**RESULTS AND DISCUSSION**

The crosses and any exceptional offspring recovered are given in Tables 2 and 3. From the result of Table 2, the following conclusion can be made.

First of all, non-recovery of \( sc z w^+ \) spl ec individuals among approximately 143,000 offspring examined indicates strongly that \( w^* \) is not located to the right of \( w^z \), but at or to the left of \( w^z \), because if \( w^* \) is located to the right of \( w^z \) or \( w^z \) one might expect that about 30 of \( sc z w^+ \) spl ec from cross No. 1 and about 5 from cross No. 2 would appear by crossing over based on the fine structure map of the white locus in Figure 1. This conclusion coincides with the work of Judd (unpublished, cross No. 4 in Table 3) which indicates \( w^* \) is left of \( w^z \), either at or left of \( w^z \).

Second, the appearance of \( y^*+w^*++ \) individual from the cross No. 2 may indicate that the locus occupied by \( w^* \) lies to the left of \( w \), as indicated above. However, it most likely indicates that a \( w^{m} \) appeared from \( w^z \) by a mutation in the gonial cluster of the female.
Table 2. Exceptional recombinant types recovered from heterozygous females

<table>
<thead>
<tr>
<th>Cross No.</th>
<th>Heterozygous Females</th>
<th>Exceptional Recombinant Types Recovered</th>
<th>No.</th>
<th>Total Offspring Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(sc\ z \ w^+ \ + +)</td>
<td>(y^+ w^+ \ + +)</td>
<td>1* **</td>
<td>107,640</td>
</tr>
<tr>
<td></td>
<td>(y^+ w^+\ spl \ ec)</td>
<td>(sc \ z \ w^* + +)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(y + w \ spl \ ec)</td>
<td>(y + w^+ \ + +)</td>
<td>3**</td>
<td>35,011</td>
</tr>
<tr>
<td></td>
<td>(sc \ z \ w^* + +)</td>
<td>(sc \ z \ w^* + +)</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>142,651</td>
</tr>
</tbody>
</table>

* a sterile female.
** probably phenotype \(w^-w^*\) as the two fertile \(y+w^++\) proved to be \(y+w^*++\).

followed by a cross over between \(z\) and \(w^+\) during meiosis, because of the fact that all the \(y+w^++\) do not randomly originate but came from a culture line in which also \(sc\ z\ w^*++\) appeared. In other words, \(y+w^++\) seems to be \(y\ z\ w^*++\).

The exceptional normal eye-color fly from cross No. 1 seems to be the same case of \(y+w^++\) above mentioned. It looked like a triploid, but it was impossible to determine since it was sterile.

Under the hypothesis (Judd, 1963) that \(w^m\) is a result of the duplication of the white locus, which is localized within the two farthest right recombination sites of the locus, the following hypothetical assumptions for genotypes of three mutants \((w^m, w^l, \text{and } w^i)\) can be made:

1. \(w^m\) genotypically seems to be a duplication of site 4 (herein indicated as 123445) rather than site 5, since \(w^+\) may be closely related to \(w^m\). This is based on the fact that the duplication of \(w^+, \ z\ w^+, \text{and } w^i\), looks like \(z\ w^m\).

2. \(w^i\) probably is an inversion of sites 3 and 4 (conversion between sites 3 and 4, 123445) from \(w^m\) or \(w^i\), because of the fact that the \(w^+\) is at or left of \(w^+\) (site 3), and that it is frequently mutated from \(w^m\) or \(w^i\).

3. \(w^l\) may result from an inversion of sites 4 and 5 (123445) from \(w^m\). This assumption comes from the fact that \(w^l\) is easily converted from \(w^m\) and is a \(z\) suppressor, as a mutant of site 4 or 5 in the white locus.

Since mutations at sites 4 and 5 act as zeste suppressors, it is logical to expect that a duplication on the conversion of sites 4 and 5 would also suppress zeste while those of site 3 or left of it do not.

On the basis of these assumptions, one may be able to postulate that they \((w^m, w^l, \text{and } w^i)\) can easily reverse one another by asymmetrical exchanges or inversions between subloci. The model for asymmetrical exchanges was discussed by Judd (1961). A model of the inversions is proposed to account for the "exceptional" types of the data in Tables 2 and 3. The inversion here seems to be due to the loop of unpaired sites, in other words, the extra loop rather frequently may physically cause an inversion.

As shown in Figure 3, this hypothetical model for the inversion may result from two possible mechanisms: one is a "chromosomal inversion" in the chromosomal level, and the other a "miscope" mechanism in DNA level. For the sake of explaining the scheme in Figure 3, the proposed models of inversions are classified into three cases: namely, Case 1, \(w^m\rightarrow w^i\); Case 2, \(w^m\rightarrow w^i\); and Case 3, \(w^i\rightarrow w^m\).

The fundamental mechanisms of all cases are similar to one another, with a slight exception in Case 3 which is caused by a double inversion.
In order to account for the model, it is assumed that a breakage-and-refusion (180 degree inverted) of two points in terms of a chromosomal level, or a "mis-copy" of DNA strands for the replication during the meiosis may be able to occur spontaneously because of physical conditions. In Case 1, for example, \( w^+ (123\#56) \) would be mutated to \( w^- (12\#545) \) as a result of an inversion of sites 3 and the unpaired 4 by a breakage-and-refusion mechanism because the unpaired extra loop (of site 4) may frequently cause an inversion by a physical rotation of the loop plane with site 3. In other words, there a physically "twisting" happens to form a coiled loop.

On the other hand, in DNA level, it is possible to postulate that a "mis-copy" mechanism would occur, by which a new strand of DNA is copied by mistake. However, it should be noted that the opposite polarities of the two strands of DNA must be maintained (Figure 4). The mechanism mentioned above can also interpret Cases 2 and 3, but it seems necessary to expect that Case 3 occurs more rarely than other cases, because the former is caused by double inversions while the latter is by single.
Table 3. Exceptional recombinant types recovered from heterozygous females (From Judd, unpublished) and possible interpretation

<table>
<thead>
<tr>
<th>Class No.</th>
<th>Heterozygous Female</th>
<th>Male Used to</th>
<th>Exceptional Non-</th>
<th>Rec. Total</th>
<th>Possible Interpretation*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sc ( z^w ), ( w^{go} )</td>
<td>( z^w ), ( x^+ )</td>
<td>( z^w ), ( x )</td>
<td>60,000</td>
<td>sc ( z^w ), ( x ) \rightarrow ( z^w ), ( x )</td>
<td>by crossing over.</td>
</tr>
<tr>
<td>2</td>
<td>sc ( z^w ), ( w^{go} )</td>
<td>( z^w ), ( x^+ )</td>
<td>( z^w ), ( x )</td>
<td>5,000</td>
<td>sc ( z^w ), ( x ) \rightarrow ( z^w ), ( x )</td>
<td>by double inversions or “non-copy” mechanism.</td>
</tr>
<tr>
<td>3</td>
<td>sc ( z^w ), ( w^{go} )</td>
<td>( z^w ), ( x^+ )</td>
<td>( z^w ), ( x )</td>
<td>76,000</td>
<td>sc ( z^w ), ( x ) \rightarrow ( z^w ), ( x )</td>
<td>by crossing over; there are two possible sites; sc ( z^w ), ( w^{go} ) \rightarrow ( w^{go} ), ( w^{go} ) is not recognized.</td>
</tr>
<tr>
<td>4</td>
<td>sc ( z^w ), ( w^{go} )</td>
<td>( z^w ), ( x^+ )</td>
<td>( z^w ), ( x )</td>
<td>12,000</td>
<td>sc ( z^w ), ( x ) \rightarrow ( z^w ), ( x )</td>
<td>by crossing over.</td>
</tr>
</tbody>
</table>

*Based on the assumption which is explained in the text and Fig. 2

The model for inversion can logically interpret the data in Tables 2 and 3, with exceptions of cases of the symmetrical and asymmetrical exchanges. This model is based on the postulate that, even though the inversion is usually more are than crossing over (exchange) in this region, it is very possibly due to the extra unpaired loop of the duplication sites by asymmetrical exchange. The appearance of this unpaired element (loop) also coincides with the “model” for synapsis in females heterozygous for the cherry sublocus duplication in D. melanogaster, and with the fact that the pairing of salivary gland preparation of a deletion heterozygote is seen like the model.

It is difficult to explain why these mutations (inversions of 3 mutants) probably occur frequently in females and rarely in males. It seems, however, necessary to postulate that these inversions happen spontaneously at pachytene of meiosis, as a crossing over occurs at that time and only in females in Drosophila. These speculations point up the difficulty of the duplication hypothesis as an explanation for these results.

Evidence for a mechanism of a polarized effective pairing has been obtained from the analysis of recombinants produced from alternative pairing arrangements in duplication/normal heterozygotes (Judd, 1964). The classical account of synapsis as cytologically seen at zygote implies that at meiosis chromosome pairing proceeds in zipper-like manner from the proximal to the distal end of the pairing partners. However, it is not surprising to find that during the polarized pairing, the unpaired loop causes an inversion of adjacent sites, because the pairing would occur nearly simultaneously in the small region (subloci of white region).

In addition to the interpretation mentioned above, the fact that a recombinant, \( y^2 z^w w^{go} \), from sc \( z^w \), \( y^2+ \), \( w^{go} \) spl ec, looks like wildtype supports the assumption that \( w^{go} \) is a duplication of two furthest right parts of the white locus; likewise the duplication of right part of wild-type in phenotype. Even though the rearrangement of \( z \) and a duplication of site 4 shows similarity to the zeste-mottled eye, but not identical, it is possible to expect that duplications of site 4 may be different from each other in terms of nucleotides in the DNA level of the chromosome, so a combination of
Table 4. Test for suppression effect: Results of $F_1$ females from $z \times z$.

<table>
<thead>
<tr>
<th>Phenotypes in eye color</th>
<th>Total examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>zeste($z^+/z^+ w^+$)</td>
<td>919</td>
</tr>
<tr>
<td>normal (zeste suppression)</td>
<td></td>
</tr>
</tbody>
</table>

and certain duplication of site would be a zeste-mottled. The $F_1$ females, ($z^+/z^+ w^+$), from the cross $z^+ \times z^+ w^+$ expressed phenotypically zeste eye-color as shown in Table 4. It indicates that $w^+$ shows a non-suppressor effect in combination with zeste; likewise apricot or Brownex (Judd, 1959). The fact that $w^+$ is a non-suppressor of $z$ also supports the evidence that $w^+$ is located at $w^+$, and not at sites 4 or 5 which are $z$ suppressors.

As pointed out by several investigators, there are five recombination sites, at the white series in *D. melanogaster*, between which recombination may be detected. Within this region, which may be defined by the noncomplementary 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.

The classical gene was defined as both a unit of function and of recombination, and different forms of the same gene were called "alleles." Allelic mutations consequently did not recombine or complement each other. It has, however, been shown that chromosome breakage could occur at more than one site within the gene "scute" in *D. melanogaster* (Raffel and Muller, 1940). This brought a conclusion that the gene might have different dimensions according to the criterion used to define it. A fundamental revision of the classical model of the gene has come largely as a consequence of the use of nutritional mutants of microorganisms, first introduced by Beadle and Tatum (1941).

First, in *D. melanogaster*, Lewis (1945, 1951, and 1955) supported the concept that each gene is not only a unit of recombination, but also a unit of mutation and a unit of single specific function.

Second, Pontecorvo (1952) suggested that a functional unit, the gene, contained several sites capable of mutation which were separable by recombination. On this basis a functional unit was larger than a recombinational unit. This hypothesis has been confirmed by the work of Benzer (1955, 1957) in bacterio-phage, and of Demerec (1955) and co-workers using transduction in Salmonella.

Should a unit of function or a unit of recombination be called a gene? The answer to this question can be found by defining the term allele. 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 pseudalleles present a unit of function which is considerably different from the unit of recombination. However, whether the recombinational phenomenon recorded in microorganisms is the equivalent of that found in Drosophila is at present a matter of conjecture.

It is clear that genes as functional units are large and contain many smaller mutable units separable by recombination. However, it is interesting to note that the unit of mutation in this system may involve at least two adjacent recombinational units and two nearby functional units.

**LITERATURE CITED**


