SOMATIC RECOMBINATION WITHIN THE WHITE LOCUS OF
DROSOPHILA MELANOGASTER

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THE X-linked white locus of Drosophila melanogaster is a complex region of the chromosome which has been subdivided into a number of sites (LEWIS 1952; MACKENDRICK and PONTECORVO 1952; GREEN 1959, 1964; JUDD 1959, 1964). Heterozygotes for two mutant alleles mapping at different sites in the white region (except for compounds with the right-most site, for white-spotted) do not show complementation and at a low frequency produce gametes containing a recombinant of the two sites. Such gametes combine either the two mutant sites on the same chromosome or the two normal sites. The former transmit a mutant chromosome, the latter a normal one. To take an example, females have white eyes if they carry a mutant allele, leading to white eyes, at a left site in one chromosome, and in the other, another mutant for white located at a right site. Among the eggs produced by these females are some which carry the left and right mutant white determinants and thus transmit whiteness and some which carry the two normal determinants and thus transmit red eye color.

Meiotic recombination within the white locus is a reciprocal process presumably of the same type as meiotic crossing over in general. In Drosophila, as well as in some other organisms, recombination is not restricted to the meiotic phase, but occurs also in somatic cells. Recombination between separate loci in somatic cells has been termed "somatic crossing over" and it appears that this type of crossing over is a reciprocal process like meiotic crossing over. Whether this applies to recombination within a locus is not known for Drosophila but reciprocal intragenic recombination has been demonstrated in the fungus Aspergillus nidulans (ROPER and PRITCHARD 1955; PRITCHARD 1955). Figure 1 of this paper is based on a reciprocal crossing-over model, but most of the text will use the neutral term recombination rather than crossing over.

A priori the probability of discovering somatic recombination within a locus is low. Each eye of Drosophila, however, has approximately 750 facets any one of which might give evidence for recombination. Thus, as few as 1000 flies represent as many as 1,500,000 facets! Cases of somatic recombination within the white region are reported herewith.

MATERIALS AND METHODS

Two white mutants were obtained through the kindness of Professor B. H. JUDD of the University of Texas. One of these, w¹, which may be a derivative of the first occurrence of this mu-

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Figure 1.—Reciprocal somatic crossing over within the white locus and two alternative types of segregation (above and below) resulting in genetic twin spots. Left: the initial \( y^+w/y^+w^{65}+ \) genotype. Right: the genotypes and phenotypes after crossing over and segregation. Note that both types of segregation may lead to a pigmented spot. This is unlike the situation in which spots homozygous for a recessive allele are produced by one type of segregation only. (Compare the occurrence of homozygous yellow solely in the upper segregation type with the occurrence of eye spots in both segregation types.)

The chromosomes bearing one or the other of the white alleles also carried other mutant genes. The two chromosomes were \( y^+w^l\) and \( z \). None of these other mutants played a role in the analysis and with the exception of \( y \) (yellow body and bristles) will not be considered further. (For additional information on these mutants see Lindsley and Grell 1968.) Also, for simplicity’s sake, \( w^{62}w^{65} \) will be designated as \( w \) and \( w^l \) as \( w \). When it is desirable to refer to the two sites of the white locus which are occupied by \( w^{65} \) and \( w \), respectively, \( w^{65}+ \) will be written and \( w \) will be written \(+w\).

The eyes of females carrying the “compound” genotype \(+w/w^{65}+\) were inspected under a dissecting microscope at a magnification of 25×. Ordinarily these eyes are “white” lacking any eye pigment. If, however, during the development of the eye primordia, somatic recombination would take place which recombined the two \(+\) sites, one of the two resulting cells and its derivatives would have the constitution \(+w/++\) or \( w^{65}w/++\) (Figure 1). If autonomous in expression, both constiutions would lead to a pigmented patch in the otherwise unpigmented eye.

tant (Morgan 1910), has been localized to the right of the \( w^n \) site within the white complex. The other, \( w^{62}w^{65} \), maps to the left of \( w^n \). It is a descendant of a mutant found in Dr. Judd’s laboratory by Dr. H. M. LeFever (personal communication) in an experiment in which flies with a normal allele had been X rayed.
Compound females were obtained in three sets of crosses. The first involved \( w \) females and \( w^{65} \) males. The second, initiated several weeks later than the first, used the same types of parents. The third set, initiated simultaneously with the second, consisted of the reciprocal cross, \( w^{65} \varnothing \times w \delta \) (Table 1).

The first set yielded 1884 \( F_1 \) females, three of which each had a pigmented red area in one eye. The second set yielded 1530 \( F_1 \) females, none of which had a colored eye spot. The third yielded 2723 \( F_1 \) females, one of which had one eye spot. In sum, four spots were found among the eyes of 6,137 flies. It is not known whether the differences in incidences of spots among the three sets of data were solely due to chance or whether environmental variables were also involved. Each of the four flies with a pigmented eye spot came from different parents but there was less crowding in the cultures in the first as compared to the two later sets.

The sizes of the four pigmented areas cannot be given exactly. In terms of numbers of eye facets involved, the largest spot consisted of about 16 facets, the next largest of about 6 and the two smallest spots of 2, and of 1–3 facets, respectively. The uncertainties of these numbers derive from the distribution of pigment in the Drosophila eye. In wild-type eyes the relevant pigment is present in the form of coarse granules in the two distal primary pigment cells of each ommatidium which surround the pseudoconus, a structure located directly below the cornea of the facet, and in the elongated secondary pigment cells which form a sheath around most of the remaining length of each ommatidium (JOHANNSEN 1924; HERTWECK 1931; MILLER 1950). In addition to the primary and secondary pigment cells, some authors describe the presence of one or two additional types of pigment cells, basal cells that are located above the basement membrane of the compound eye (WADDINGTON and PERRY 1960; PERRY 1968) and post-retinal cells in the outer optic ganglion (SCHULTZ 1935; NOLTE 1950). According to SCHULTZ the pigment occurs "in three places, in the primary and secondary cells and beneath the basement membrane." He further states that most of the pigment in the wild-type eye is contained in the primary and secondary cells (loc. cit. p. 33).

It is not known for the four mosaic eyes obtained in the present experiments which specific cells carried the eye pigments in colored facet areas of the other-

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<thead>
<tr>
<th>Genotype</th>
<th>( \varnothing \varnothing )</th>
<th>( \delta \delta )</th>
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<th>Number of spots</th>
<th>( N )</th>
<th>Number of spots</th>
</tr>
</thead>
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<tr>
<td>( +w/w^{65}+ )</td>
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</tr>
<tr>
<td>( +w/w^{65}+ )</td>
<td></td>
<td></td>
<td>1530</td>
<td>0</td>
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<tr>
<td>( w^{65}+/+w )</td>
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<td>2723</td>
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<tr>
<td>( +w/+w )</td>
<td></td>
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<td>5748</td>
<td>0</td>
<td>6200</td>
<td>0</td>
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<td>( w^{65}+/w^{65}+ )</td>
<td></td>
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<td>7711</td>
<td>0</td>
<td>7898</td>
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wise unpigmented eyes. Presumably, in the two larger spots both primary and secondary cells carried pigment granules. A view of one of the two smaller spots taken perpendicularly to the eye surface at the location of the spot seemed to show two fully pigmented facets suggesting pigmentation of at least the primary cells. A similar view of the other one of the smaller spots seemed to show either one or two fully pigmented facets while some side views indicated the presence of a third such facet. Very likely the uncertainty is caused by the presence of both primary and secondary pigmented cells, the latter being shared by two or three ommatidia. No satisfactory histological preparations of the four mosaic eyes have been obtained. As to the type of pigmentation it is believed that the pigmented spots had normal, wild-type coloration but the optical conditions provided by the transparency of most of the eye area into which the colored spots were inserted excluded a valid comparison with whole red eyes.

Genetically the cells making up the pigmented spots may be regarded as heterozygous for an X-chromosome in which the wild type alternatives of both the \( w^{65} \) and \( w \) sites were coupled: +\( w^{-65} + w \). Such a chromosome could originate by either of two methods: by somatic recombination between the two sites in a +\( w/w^{65+} \) parent cell (Figure 1) or by reverse mutation of either of the mutant sites \( w^{65} \) or \( w \) in the parent cell of a colored spot.

The second alternative concerning the origin of a wild-type allele, by mutation, can be excluded by an analysis of control experiments in which no colored spots were found among 5748 homozygous \(+w/+w\) females and 6200 hemizygous \(+w\) males nor among 7711 \( w^{65+}/w^{65+} \) females and 7898 \( w^{65+} \) males. These 27,557 control flies were from cultures whose parents were \(+w/\) \( +w \) \( \times +w \) \( \delta \delta \) or \( w^{65+}/w^{65+} \) \( \varphi \varphi \) \( \times w^{65+} \) \( \delta \delta \) except for 1386 \( +w \) males which were brothers of the heterozygous \( +w/w^{65+} \) females listed in line 2 of Table 1 as well as 1963 \( w^{65+} \) males which were brothers of the heterozygous females listed in line 3.

Although the presence of colored spots in the experimental heterozygotes and their absence in homo- and hemizygous controls is fully accounted for on the assumption of somatic recombination between the two sites of the white locus which can lead to normal recombinants in heterozygotes only, it must be shown in addition that the absence of colored spots in the controls is not simply a chance phenomenon due to inadequate numbers of flies. This possibility is investigated in the following paragraphs. Before proceeding to the analysis it should be mentioned that a refined treatment of the data would include adjustments for the facts that the number of ommatidia is smaller in males than in females, that the presence of the mutant \( spl \) (split) in homozygous and hemizygous \( w^{65} \) flies tends to decrease eye size below wild type in both sexes and that the presence of \( ec \) (echinus) in homozygous and hemizygous \( w \) flies tends to increase eye size in both sexes. For the present purposes no adjustments were made.

If the heterozygous females owed their colored spots to reverse mutation, then a number of different possibilities may be distinguished, making the assumption in each case that the frequency of mutations is alike in female and male cells:

1. The mutations occur only in the \( +w \) allele at its \( w \) site. In this case one must compare the frequency of 4 spots from 6,137 \( +w \) alleles present in the heter-
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ozygous females with the frequency of no spots from 17,696 +w alleles (being the sum of two alleles in each of the 5,748 homozygotes plus the 6200 alleles in the hemizygotes). If we accept the frequency of 4 spots from 6,137 alleles, \( p = \frac{4}{6137} = 0.000,652 \), as the probability of observing a spot, then the probability of observing no spot from 17,696 alleles is \((1-0.000,652)^{17,696}\). This probability is \( P \sim 0.000001 \). Thus, the finding of no spots in the +w controls cannot be regarded as a chance deviation from the findings in the heterozygous females.

(2) The mutations occur only in the \( w^{ss} + \) allele at its \( w^{ss} \) site. In this case the comparison is between the 4 spots from 6,137 \( w^{ss} + \) alleles in heterozygotes and no spots from 23,320 alleles in control \((2 \times 7711 + 7898 = 23,320)\). In view of the significant deviation from chance shown for the 17,696 +w alleles, the deviation is even more significant for the 23,320 \( w^{ss} + \) alleles: \( P < 0.0000003 \).

(3) The mutations occur in either allele. This possibility will be analyzed for the case that both alleles have equal probabilities of reversion. Under these conditions a heterozygous female is equivalent to a homozygous female in providing opportunity for mutation but each female provides twice as much opportunity for mutation than a hemizygous male. The comparison, therefore, rests on 4 spots in 6,137 flies vs. no spots in 20,508 flies \((5748 +w/+w \) females plus 7711 \( w^{ss}+/w^{ss} \) females plus \( 1/2 \times 6,200 +w \) males plus \( 1/2 \times 7898 w^{ss}+ \) males = 20,508). Again the finding of no spot is not compatible with a chance deviation from the null hypothesis: \( P < 0.000002 \).

Since the value of \( p = 0.000,652 \) for the proportion of spots rests on only 4 spots among 6,137 flies, the lower limit of \( p \), at the 0.01 level, was determined by consulting Stevens' (1942) table, “Binomial and Poisson distributions: fiducial limits of the expectation.” The lower limit of \( p \) is \( p_L = 0.000,134 \). Even using this value one would expect more than 2 spots in each of the groups treated under (1), (2) and (3) instead of the observed zero spots. In summary, it is concluded that the absence of spots in the controls is not a result of chance in the occurrence of reverse mutations. Such mutations cannot account for the difference in occurrence of spots between the heterozygous +w/\( w^{ss} + \) flies and the controls. It may be added that germinally too, no reversions have ever been found in either of the two stocks containing the \( w^{ss} \) and \( w \) alleles (Judd, personal communications). It is assumed therefore that the spots in white compound females originated by somatic recombination.

As stated in the introduction, it is not known whether the recombination process is reciprocal and thus is of the typical crossing-over type or whether a non-reciprocal process is involved. Some relevant evidence could have been obtained if it could have been shown that the pigmented eye areas were homozygous for yellow body and bristle color as expected in one half of the segregation types after somatic crossing over in the white region (Figure 1). Unfortunately, no difference was discernible between wild-type and yellow coloration of the fine setae that are located between the corners of the facets. Nor did any one of the four eye spots extend to an area outside of the eye where the coloration of a larger bristle could have been recognized.
A normal sized eye of *D. melanogaster* consists of between 700 and 800 facets (Sturtevant 1925). If it is assumed preliminarily that each eye developmentally begins as a single cell, and that the pigment cells associated with each facet form a unit of immediately preceding common descent, then the sum of all mitoses resulting in $2^n$ pigment units is $\sum_{0}^{n-1} 2^x$. For example, in order to obtain $2^5 = 512$ pigment units, $\sum_{0}^{5} 2^x = 511$ mitoses are required, and to obtain $2^{10} = 1024$ units, $\sum_{0}^{10} 2^x = 1023$ mitoses are required. Thus, the sum of all mitoses occurring during development of an eye up to the formation of the pigment units is very close to that of the number of these units. If, instead of assuming that each eye developmentally begins as a single cell it is assumed that from 12 to 20 independent cells form the eye anlage (see Becker 1956), then the difference between number of pigment units and that of the mitoses involved in their formation is still small and may for purposes of discussion be regarded as negligible.

The number of pigment cells which constitute a pigment unit has been a matter of uncertainty. All authors agree that there are two primary pigment cells but the number of secondary pigment cells has been variously given or diagrammed as 6 (Hertweck 1931; Fuge 1967), 8 (Clayton 1954), 9 (Nolte 1950; Shoup 1966) and 12 (Johannsen 1924). Miller (1950) speaks of "a sheath of about twelve secondary pigment cells which are shared by adjacent ommatidia", and Johannsen expresses himself in a similar way. Fuge who agrees with Hertweck in the opinion that six secondary pigment cells belong to each ommatidium points out correctly that the hexagonal arrangement of adjacent ommatidia results in each ommatidium being surrounded by 12 secondary cells.

If one accepts the numbers of primary and secondary cells as 2 and 6, respectively, and disregards the uncertain situation regarding basal and post-retinal pigment cells, then the question still remains as to the cell-lineage relations of the primary and secondary cells. Are these 8 cells derived from a single cell and if so are they the product of three successive mitoses or are there differential divisions that multiply some cells more than others? The answer to this question has a bearing on considerations of the frequency of discernable spots resulting from somatic recombination within the *w* locus. If a whole pigment unit were needed in order to recognize a facet as pigment, then the total number of mitoses available for recombination would be that corresponding to the total number of facets. If on the other hand, a single pigmented cell would suffice to give the facet a recognizable colored tinge, several further mitoses would be available for each facet, leading to a recognizable colored spot due to recombination.

An attempt can be made to estimate very approximately the frequency of somatic recombination within the *w* <sup>65</sup> – *w* region of the white cistron. If it is assumed that the probability of somatic recombination in the white region is the same for each mitosis, and if a whole pigment unit needs to be colored in order to
be recognized as a spot, then each of, say 750 units, provides a sample of nearly 750 mitotic events which as a result of recombination may lead to a colored spot. The two eyes of the more than six thousand $+w/w^{65}+$ females which were inspected for the occurrence of pigmented spots would represent more than 9,000,000 mitoses ($2 \times 750 \times 6000 = 9,000,000$). Four of these mitoses led to colored spots.

Apart from the developmental assumptions made the estimates of frequencies of recombination depend on whether somatic recombination within the white cistron is a reciprocal crossing over event or whether it is non-reciprocal. If reciprocal, each $++$ recombinant would be accompanied by a $w^{65}w$ recombinant and the four $++$ recombinants plus their non-recognizable $uF5w$ partners would correspond to four mitotic events. If recombination is non-reciprocal and if it leads to $w^{65}w$ recombinants as frequently as to $++$, the number of recombinant mitotic events would be twice that of the colored spots. The frequency of somatic recombination in the white region between $w^{65}$ and $w$ is therefore 4 or 8, respectively, in $9 \times 10^6$ mitoses, i.e. approximately either less than 1 or less than 2 in 2 million mitoses. If a single pigmented cell results in a recognizable spot, the frequency of recombination could be as much as nearly one order of magnitude smaller.

A comparison of the somatic recombination rates between the $w^{65}$ and $w$ sites of the white locus with that of germinal crossing over shows that the latter is much more frequent. According to Judd (1964) the meiotic crossing over frequency between the intermediately located $w^s$ and $w$ is 0.01%. The frequency between $w^{65}$ and $w^s$ is roughly estimated as 0.004 (LeFever, personal communication). The combined frequency of germinal crossing over in the $w^{65} - w$ interval is, therefore, greater than 1 in 10,000. Since only one half of single meiotic crossovers result in recombinant strands the frequency of germinal crossing over events is actually twice as large as the frequency of recombinants, or greater than 1 in 5,000, as contrasted to the estimate of somatic recombination as at most 1 or 2 in 2,000,000. Most likely this reflects a general low frequency of recombination in somatic tissue, but in part it may be related to the facts that spontaneous somatic crossing over in the X-chromosome of D. melanogaster is concentrated in the proximal region and that the $w$ region is located close to the opposite, distal end of the chromosome.

If the frequency of somatic recombination in the $w^{65} - w$ segment could be taken as typical for the whole chromosome complement of Drosophila, then one could estimate the total frequency of mitoses in which a recombination event occurred somewhere among and along the chromosomes. Taking the combined germinal map distance of all chromosomes except chromosome 4 as 280 and the value for the white interval as 0.014, one finds $2 \times 10^4$ as many crossovers in the whole complement as in the white interval. Multiplying this value with the frequency of less than 1 or 2 in $2 \times 10^6$ obtained for somatic recombination in the white interval, one obtains a frequency of somatic recombination somewhere in the complement of less than one percent of all mitoses. As uncertain as this value is, it suggests that the frequency of somatic crossing over is not negligible.
I am grateful to Drs. B. H. Judd and H. M. LeFever for providing me with the white stocks and information about them. I acknowledge with appreciation the critical comments on an early draft of the manuscript by J. Claxton, M. M. Green, D. Jeffrey, Wm. Welshons and G. Williams. I thank particularly Dr. C. C. Li who suggested the statistical treatment of the problem of reverse mutations.

SUMMARY

Females of *Drosophila melanogaster* heterozygous in *trans* arrangement for two white eye alleles located at different sites of the complex *w* locus have white eyes. Four specimens out of 6137 were found each of which had a red-colored eye area. These pigmented spots, of varying sizes, are considered to be the result of spontaneous somatic recombination in a cell of the developing eye disc. No colored spots were observed in the eyes of 27,557 homozygous or hemizygous controls. Given certain assumptions, the 6137 heterozygous females provided an estimated minimum of $9 \times 10^6$ mitoses in which somatic recombination would have led to an observable spot. The four observed spots suggest a frequency of recombination in the tested white region of less than 1 or 2 in $2 \times 10^6$ mitoses.

LITERATURE CITED


