NON-HOMOLOGOUS PAIRING AND CROSSING OVER IN DROSOPHILA MELANOGASTER

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THE conclusion that crossing over occurs invariably in association with the two-by-two pairing of homologous chromosomes is supported by two independent lines of evidence. First, cytological observations of chromosome behavior during pachytene of meiosis show that the homologous chromosomes, almost without exception, are regularly and intimately paired in a two-by-two fashion. Second, genetic studies of crossing over in a wide variety of plants and animals have demonstrated that whatever the mechanism, the complementary products of a crossover are formed without increasing or decreasing the number of loci per chromosome, a result consonant with two-by-two pairing. In short, most facts agree that the crossing over event occurs without alteration in the over-all continuity or integrity of the participating chromosomes.

Exceptions to regular pairing behavior in the form of non-homologous or "illegitimate" associations which if accompanied by crossing over would lead to aberrant chromosomes, have been recorded in several plants and animals. Cytological detection of non-homologous chromosome pairing have not infrequently been observed in plant material e.g., by Mc CLINTOCK (1933) in maize, etc. By inference the spontaneous occurrence in Drosophila of long deficiencies, e.g., Notch-8 (MOHR 1919) and Notopleural (BRIDGES, SKOOG, and LI 1936) and extended tandem duplications, e.g., Bar (STURTEVANT 1925; BRIDGES 1936; MULLER, PROKOFYEVA-BELGOVSKAYA, and KOSSIKOV 1936), the Beadex-recessive mutants (GREEN 1953a,b) and the Star-duplication (LEWIS 1941) in *Drosophila melanogaster* occur as a result of crossing over between paired non-homologous chromosome segments. However, the data suggest that crossing over within paired non-homologous chromosome parts is, at best, rare and sporadic.

It is the purpose of this report to describe the regular occurrence of exceptional crossover products which apparently result from the crossing over within paired segments which appear to be, genetically speaking, non-homologous. The events to be described were uncovered as part of a study of pseudoallelism at the white (*w*) locus in *D. melanogaster* described in detail elsewhere (GREEN 1959).

MATERIALS AND METHODS

As an aid in simplifying the presentation of the experimental results, the common laboratory mutants used have been listed in Table 1. Where special stocks were derived and used, they will be described in the text. The usual Drosophila culture methods utilizing a standard corn meal-Brewer's yeast-

TABLE 1

Synopsis of mutants used in text. Symbols and map position generally after BRIDGES and BREHME (1944)

Mutant gene	Symbol	Map position
yellow body color	y	X–0.0
yellow-2 body color	y^2	allele of y
scute bristles	sc	X–0.0
suppressor of apricot	$su\text{-}w^a$	X–0.05
Hairy wing	Hw	X–0.±
zeste eye color	z	X–1±
white eye color	w	X–1.5
apricot	w^a	pseudoallele of w
apricot-2	w^{a2}	allele of w^a
apricot-3	w^{a3}	allele of w^a
apricot-4	w^{a4}	allele of w^a
coral	w^{co}	allele of w^a
colored	w^{col}	pseudoallele of w^a
split bristles	spl	X–3.0
echinus eyes	ec	X–5.5
singed-3 bristles	sn^3	X–21
miniature-2 wings	m^2	X–36.1
garnet-4 eye color	g^4	X–44.4
forked bristles	f	X–56.7
Bar eye	B	X–57
Curly wings	Cy	Inversions, chromosome 2
Ultrabithorax-130	Ubx^{130}	Inversions, chromosome 3

molasses-agar medium were employed throughout. Flies were raised in a room whose controlled temperature fluctuated between 22–24°C.

In the genotype notations used herein the mutant gene designations as listed in Table 1 will be employed. Absence of a gene symbol means that the standard or wild type allele is present. Symbols are those found in BRIDGES and BREHME (1944).

EXPERIMENTAL

Exceptions occurring in association with crossing over: In the study of pseudoallelism at the white locus, crossing over results were obtained showing that the mutants w^a and w^{a2} are pseudoalleles of the mutant w^{ch}. Both are localized to the left of w^{ch}. Experiments were therefore undertaken to determine whether w^a and w^{a2} occupied the same or separate loci. For this purpose females of the genotype y^2 $su\text{-}w^a$ w^a spl/w^{a2}; $Cy/+$; $Ubx/+$ were obtained and crossed to y w spl sn^3 males. Among the progeny of this cross listed in Table 2, no wild type exceptions were obtained. However, five females all w spl in phenotype were found. Each female exception was separately progeny tested by crossing to y w spl sn^3 males. All females produced identical progeny consisting of females y w spl (sn^3) and w spl and males y w spl (sn^3) only. The sex ratio in each case was approximately 2 ♀ : 1 ♂. These results were interpreted to mean that the w spl chromosome was

TABLE 2

The occurrence of white-eyed females exceptions in association with crossing over. All parental females heterozygous for Cy *and* Ubx^{130} *inversions unless otherwise noted and crossed to* y w spl sn^3 *males*

Genotype parental females	Total white males y or y^2	*spl* or *ec*	Total male progeny
y^2 *su-*w^a w^a *spl*/w^a	1	2	43,237
y w^{a2} *spl*/w^{a2}	9	.	95,062
y^2 *su-*w^a w^a *spl*/w^{a2}	.	5	31,970
y^2 *su-*w^a w^a *spl*/w^{a2}*	.	.	45,411
y^2 *sc* w^a *ec*/w^{a2}	.	2	46,746
y^2 *su-*w^a w^a *spl*/w^{col}	.	.	51,077
y *ac* w^{col} *spl*/w^{a2}	2	.	83,381
y^2 *su-*w^a w^a *spl*/w^{a3}	.	.	55,965
y w^{a3} *spl*/w^{a2}	.	6	48,469
y^2 *su-*w^a w^{a4} *spl*/w^{a2}	.	.	50,007
sc w^{co} *ec*/w^{a2}	.	.	44,919

* No autosomal inversions.

male lethal and the recovery of only exceptional females was not due to chance. In a repeat cross where autosomal inversions were omitted, no exceptions were found.

A parallel cross was made utilizing an w^a chromosome of diverse origin. Here females y^2 *sc* w^a *sc*/w^{a2}; *Cy*/+; *Ubx*/+ were crossed to y^2 *sc w ec* males. As noted in Table 2 white-eyed female exceptions carrying the *ec* marker were obtained. On progeny testing, the *w ec* chromosome similarly proved to be male lethal.

Subsequently a number of different crosses were made in which various *w* mutants were tested to both w^a and w^{a2}. The results of crosses are listed in Table 2 and they show that associated with crossing over, additional white-eyed female exceptions were recovered from females w^{a3}/w^{a2} but not w^{a3}/w^a; from w^{col}/w^{a2} but not from w^{col}/w^a.

Finally tests among females homozygous w^a and w^{a2} were carried out using markers and heterozygous inversions as in other crosses and again, as noted in Table 2 associated with crossing over, white-eyed female exceptions were found. Two female exceptions were found in a stock w^{a2}; *Cy*; *Ubx/Xa*, one in an attached-X stock homozygous for y^2 *su-*w^a w^{a2}.

In summarizing these results of Table 2 the following conclusions can be made. From a variety of crosses involving either the w^a and/or the w^{a2} mutants, white-eyed exceptional female progeny were found. The female exceptions always occur in association with crossing over. All exceptional chromosomes are male lethal. A complementary crossing over class was not detected since no other exceptions were found.

The cytogenetic nature of the exceptional chromosomes: Before a reasonable explanation for the origin of the exceptional types can be attempted, one pertinent question requires answering. Are all exceptional chromosomes identical or do they represent a variety of cytogenetic types? Since in each case the exceptional

chromosomes were male lethal and white-eyed, a tentative hypothesis was adopted that each is associated with a chromosome loss whose limits include in the very least the *w* locus. A cursory salivary gland chromosome examination of two of the exceptional types of independent origin kindly made by PROFESSOR E. B. LEWIS showed that each was associated with a deficiency of the X chromosome in the neighborhood of the *w* locus in which most of section 3A and all of section 3B are deleted, thereby supporting the hypothesis. Therefore the exceptional types will be referred to in all subsequent considerations as w^{-r} or white deficiencies arising by recombination. Since a precise cytological description of each w^{-r} exception appeared to be a laborious undertaking, analysis of the limits of the deficiencies was sought by genetic means. For such a program the *w* locus is uniquely suited since a number of duplications and deficiencies of this locus have been described.

As a first step in defining the limits of the w^{-r} chromosomes, each was tested for coverage by four separate duplications of the *w* locus *viz.* Dp w^{+51b7}, Dp w^{+51c20}, Dp w^{m59a} and Dp $w^{v,co}$. These will be referred to for simplicity as Dp-1, Dp-2, Dp-3 and Dp-4 respectively. In each duplication a segment of the X chromosome including the w^+ locus has been translocated to an autosome. The cytological limits of each are included in Table 3. Detailed descriptions are given by RATTY (1954) and BRIDGES and BREHME (1944). Stock cultures of each duplication were maintained in males by combining through appropriate crosses a white deficient (w^-) X chromosome carrying the marker *y* and a duplication covering the deficiency. These males were crossed to double-X females of phenotype *y w f*, thereby establishing the stock. Only males carrying both the deficiency and the duplication are viable in this stock; females may or may not carry the duplication.

After each of the w^{-r} chromosomes was combined into a balanced lethal stock in which the balancer *In(1) sc*^{S1} dl-49, *v f B* was used, tests for coverage were made as follows. Balanced females were crossed to duplication males and viable

TABLE 3

Tests for coverage of two w *deficiencies and representative* w^{-r} *chromosomes by four duplications of the* w *locus. (Cytological limits of each duplication included in parentheses.) 0= duplication does not cover deficiency, += duplication covers deficiency*

Deficiencies	Duplications Dp–1 (3C2–3D2)	Dp–2 (3C2–3C6)	Dp–3 (3B1–3E2)	Dp–4 (2C1–3C4)
w^{-1}	0	0	+	+
w^{-2}	+	+	+	+
$y^2\ w^{-r}$ (from w^a/w^a ♀ ♀)	0	0	0	+
$w^{-r}\ spl$ (from w^a/w^a ♀ ♀)	0	0	0	+
$w^{-r}\ spl$ (from w^a/w^{a2} ♀ ♀)	0	0	0	+
$y^1\ w^{-r}$ (from w^{a2}/w^{a2} ♀ ♀)	0	0	0	+
$y\ ac\ w^{-r}$ (from w^{a2}/w^{col} ♀ ♀)	0	0	0	+
$w^{-r}\ spl$ (from w^{a2}/w^{a3} ♀ ♀)	0	0	0	+

exceptional males sought among the progeny. The following cross will illustrate the principle.

♀♀ w^{-r} *spl/In(1)* sc^{S1} dl-49, *v f B* × ♂♂ *y* w^{-}/Dp-1

If Dp-1 covers the w^{-r} loss, two types of F_1 males are expected, those being *v f B* and w^{+} *spl*. If Dp-1 fails to cover the w^{-r} chromosome only *v f B* males emerge.

In Table 3 the results of tests for coverage by each of the four duplications with representative w^{-r} types and with two previously described independent *w* deficiencies are listed. These results show that only Dp-4 covered all w^{-r} types, a fact which permits a tentative definition of the right-most limit of these deficiencies. Cytologically the *w* locus has been delimited to salivary chromosome bands 3C1, 2 and 3. Since Dp-4 which extends just beyond 3C3 to 3C4 covers the w^{-r} deficiencies while the other three duplications which extend beyond 3C3 do not, it is concluded that the right-most limit of the w^{-r} deficiencies is band 3C4. In comparing the cytological lengths of Dp-3 and Dp-4 it will be noted that the former includes the region of the X chromosome from beyond the *w* locus to bands 3B1,2 while the latter extends to band 2C1. Since Dp-3 does not cover the w^{-r} chromosomes whereas Dp-4 does, it follows that the chromosome losses associated with these deficiencies must extend beyond 3B1–2 to somewhere before 2C1. Thus at a minimum the whole of section 3B and part of 3C are missing in the derived deficiencies.

A more precise definition of the right-most limits of the w^{-r} deficiencies was obtained by testing them for allelism to a number of *w* deficiencies of known cytological length. The following four deficiencies, the extent of their cytological losses following in parenthesis, were used as testers: w^{258-45}-(3C1); a *w* deficiency derived from crossing over between *In(1)* w^{m4} and *In(1)* rst^{3}-(3C2,3); w^{258-48}-(3B1–3C1) and w^{258-11}-(3A1,2–3C4). These deficiencies will be referred to simply as w^{-1}, w^{-2}, w^{-3} and w^{-4} respectively. All deficiencies were tested *inter se* and to representative w^{-r} chromosomes obtained from each of the crosses noted in Table 2. The method used in each case and typical results obtained are illustrated by the following crosses:

♀♀ w^{-2}/*y Hw, In(1)*dl-49, $m^{2}g^{4}$ × *y* w^{-1}/Dp-1 ♂♂ (cross 1)

♀♀ w^{-r} *spl/In(1)*sc^{S1}dl-49, *v f B* × *y* w^{-1}/Dp-1 ♂♂ (cross 2)

For each cross the phenotype, number and presumed genotype in parentheses of all female progeny are listed below.

(cross 1)	♀♀	*Hw*	161	(*y* w^{-1}/*y Hw In(1)* dl-49 $m^{2}g^{4}$ with or without Dp-1)
	♀♀	+	39	(w^{-2}/*y* w^{-1}; Dp-1)
	♀♀	*w*	38	(w^{-2}/*y* w^{-1})
(cross 2)	♀♀	*B*/+	98	(sc^{S1} dl-49 *v f B*/*y* w^{-1}; with or without Dp-1)
	♀♀	+	39	(w^{-r} *spl*/*y* w^{-1}; Dp-1)

Of special interest in comparing these two crosses is the occurrence of white-eyed females among the progeny of cross (1) and not in cross (2). The genotype of the white-eyed females which, in comparison to their sisters were delayed in

eclosion by about two days, was interpreted as noted above to be a compound of both deficiencies. On progeny testing these white-eyed females to *B* males, the assumed genotype was confirmed since no males and only heterozygous *B* females were produced. This means that a deficiency for 3C1 compounded to a deficiency for 3C2,3 is viable, a situation not unlike that reported in maize by McCLINTOCK (1944) for the chromosome 9 losses *py* and *wd*. Since in cross (2) no white-eyed females emerged it is concluded that female zygotes compounded of the two deficiencies are lethal because they are, in part, homozygous for the loss of the same region.

From the results of the allelism tests listed in Table 4 the following conclusions are warranted. (1) Females compounded of 3C1/3C2,3 deficiencies are viable and white-eyed. (2) All w^{-r} deficiencies are viable in compound with the 3C2,3 deficiency, none with those that include the loss of 3C1. (3) All w^{-r} deficiencies are lethal when tested *inter se*. Therefore, it is concluded that all w^{-r} deficiencies have one feature in common, the loss of band 3C1 and the presence of bands 3C2,3.

The evidence from the coverage by duplications placed the left-most limits of the w^{-r} losses between salivary chromosome bands 2C1 and 3B1,2. On the basis of the elegant analysis of the zeste (*z*) locus (GANS 1953) it was possible to define with greater precision the limits of the w^{-r} losses by determining whether *z*, localized to band 3A3, is or is not included. For this purpose GANS has provided

TABLE 4

Allelism tests among white deficiencies and representative w^{-r} *chromosomes*

	w^{-1}	w^{-2}	w^{-3}	w^{-4}	$\frac{y^2\ w^{-r}}{(w^a/w^a)}$	$\frac{w^{-r}\ spl}{(w^a/w^a)}$	$\frac{w^{-r}\ spl}{(w^a/w^{a2})}$	$\frac{y\ w^{-r}}{(w^{a2}/w^{a2})}$	$\frac{y\ ac\ w^{-r}}{(w^{a2}/w^{col})}$	$\frac{w^{-r}}{(w^{a2}/w^{a3})}$
w^{-1}	L	w	L	L	L	L	L	L	L	L
w^{-2}		L	w	L	w	w	w	w	w	w
w^{-3}			L	L	L	L	L	L	L	L
w^{-4}				L	L	L	L	L	L	L
$\frac{y^2\ w^{-r}}{(w^a/w^a)}$					L	L	L	L	L	L
$\frac{w^{-r}\ spl}{(w^a/w^a)}$						L	L	L	L	L
$\frac{w^{-r}\ spl}{(w^{a2}/w^a)}$							L	L	L	L
$\frac{y\ w^{-r}}{(w^{a2}/w^{a2})}$								L	L	L
$\frac{y\ ac\ w^{-r}}{(w^{a2}/w^{col})}$									L	L
$\frac{w^{-r}\ spl}{(w^{a2}/w^{a3})}$										L

L = lethal, w = viable white-eyed females. See text for description of deficiencies and methods. For w^{-r}, *w* mutants of parental females follow in parentheses.

a simple, straight forward genetic test which utilizes a duplication designated by her as Dp (1–1) z^4. She found that the eye phenotype of females compounded of Dp (1–1) z^4 and a w deficiency depends upon whether the z^+ locus is or is not included in the deficiency. For example, females Dp (1–1)z^4/w^{258-11} (loss extending from 3A1 and therefore including z^+) are zeste in eye color, whereas females Dp (1–1)z^4/w^{258-14} (loss extending from 3A4 and therefore not including z^+) are wild type in eye color. Accordingly representative w^{-r} chromosomes derived from each of the crosses tabulated in Table 2 were tested to Dp (1–1)z^4 and the eye colors scored. Without exception females Dp (1–1)z^4/w^{-r} proved to be wild type demonstrating that in each w^{-r} chromosome the z^+ locus is not deleted.

Taking into consideration all tests, it can be concluded that at a maximum all w^{-r} losses lack bands 3A4–3C1 inclusive.

In evaluating the known phenotypic interrelationship between the z and w loci and the nature of the w^{-r} chromosomes described, two questions arose. Are the mutants w^a, w^{a2}, etc. necessary for the occurrence of the w^{-r} chromosomes? Is the genetic state at the z locus important in the derivation of the w^{-r} deficiencies? Significant answers to these questions could, it was felt, provide a basis for interpreting the origin of the w^{-r} losses.

Examination of the results in Table 2 shows that w^{-r} losses were recovered from females possessing at least one X chromosome carrying either w^a or w^{a2}. However, in at least two cases, parallel results were not obtained. From females w^{a2}/w^{a3}, w^{-r} recombinants were obtained, while none was found among a comparable number of progeny of w^a/w^{a3} females. Similar results were noted for females w^{a2}/w^{col} and w^a/w^{col}. These facts suggested that it would be worthwhile to know whether the w^{a2} and w^a mutants are necessary for the origin of w^{-r} chromosomes. In one test an apparent back mutation of w^{a2} to wild type (designated $w^{a2}+$) was used. It arose as a single male $w^{a2}+$, $Ubx^{130}/+$ among the progeny of ♀♀ w^{a2}/y^2 *su-w*^{a} w^{a4} *spl*; *Cy*/+; $Ubx^{130}/+$ × ♂♂ *y w spl* sn^3. The $w^{a2}+$ chromosome was compounded in females with w^a and w^{-r} exceptions were sought among the progeny of the cross ♀♀ $w^{a2}+/y^2$ *su-w*^{a} w^a *spl*; *Cy*/+; *Ubx*/+ × *y w spl* sn^3 ♂♂.

The results listed in Table 5 show that in association with crossing over, w^{-r} exceptions were found demonstrating that the presence of the mutant w^{a2} is not prerequisite to the origin of the w^{-r} deficiencies.

The requirement for the w^a mutant was tested by using two independent partial back mutations of w^a which differ from each other in phenotype. One back mutant, w^{aM}, made available through the kindness of MRS. J. C. MOSSIGE, arose in the so-called *Basc* chromosome and is intermediate between w^a and + in phenotype. The second, w^{a57i}, arose as a single male in a y^2 *su-w*^{a} w^a *spl*; *Cy*; *Ubx/Xa* stock and is nearly wild type in appearance. Each back mutant was tested to w^{a2} and the results listed in Table 5. These results show that while w^{-r} deficiencies were recovered among the progeny of w^{a2}/w^{aM} females, none were found among the progeny of w^{a2}/w^{a57i} females. Since w^{a57i} arose in a w^a chromo-

TABLE 5

Effect of substitutions at the w^{a2}, w^{a} *and* z *loci on the occurrence of* w^{-r} *chromosomes. All parental females heterozygous for* Cy *and* Ubx^{130} *inversions and crossed to* y w spl sn^{3} *males*

Genotype parental females	Total w^{-r} females y^{2}	*spl*	Total female progeny
y^{2} *su-w*^{a} w^{a} *spl*/w^{a2+}	1	4	36,022
y^{2} *su-w*^{a} w^{aM} *spl*/w^{a2}	1	2	41,706
y^{2} *su-w*^{a} w^{a57i} *spl*/w^{a2}	.	.	49,197
sc z w^{a} *spl*/w^{a2}	.	4	34,644
y^{2} *su-w*^{a} w^{a} *spl*/*z* w^{a2}	1+1?	.	42,503
y^{2} *su-w*^{a} w^{a} *spl*/z^{+o} w^{a2}	1	.	29,736
y^{2} *su-w*^{a} w^{a} *spl*/z^{+c} w^{a2}	1	.	45,927

? =♀ y^{2} *w* in phenotype but sterile.

some which in combination with w^{a2} produced numerous w^{-r} losses, it appears that either w^{a} or a related mutant is needed for the w^{-r} losses to occur.

To test the *z* locus, the mutant *z* was introduced into both w^{a} and w^{a2} bearing X chromosomes. These were then tested, in the presence of the usual heterozygous *Cy* and Ubx^{130} inversions, to w^{a2} and w^{a} respectively. The results listed in Table 5 show that the introduction of the mutant *z* had contrary effects in the two X chromosomes. In the w^{a} chromosome the *z* mutant apparently had no effect in altering the production of w^{-r} deficiencies, for these losses occurred in a frequency and with a marker distribution precisely as though no *z* mutant was present. On the other hand the presence of the *z* mutant in the w^{a2} chromosome had a significant effect, for only one (possibly two) w^{-r} chromosome was recovered and it was in association with the *y* rather than the *spl* marker gene. That this substitution of *z* into the w^{a2} chromosome was not a chance observation was demonstrated in another way. In place of *z* in the w^{a2} chromosome the z^{+} loci from the Canton-S and Oregon-R wild type stocks were substituted. These chromosomes, designated z^{+c} w^{a2} and z^{+o} w^{a2}, were separately tested to w^{a} and w^{-r} losses were sought. The results of these tests, enumerated in Table 5, show that in effect the introduction of z^{+c} and z^{+o} in the w^{a2} chromosome paralleled precisely the introduction of *z*. In both cases there was a reduction in the frequency of w^{-r} deficiencies and a switch in the marker association. These facts mean that the genetic state of the *z* locus in the w^{a2} chromosome has a paramount role in the origin of the w^{-r} losses.

What explanation can be offered for the origin of the w^{-r} deficiencies? Obviously crossing over between regularly paired chromosomes provides no satisfactory explanation. By postulating, however, that single crossing over occurs concomitant to a pairing association between noncontiguous segments of homologous X chromosomes, a satisfactory mechanism for the origin of the w^{-r} losses is provided. Since the w^{-r} losses include at a maximum the salivary chromosome bands 3A4 to 3C1, pairing and crossing over must occur so as to yield such a loss. The simplest pairing scheme which would yield a w^{-r} loss would involve the

"illegitimate" pairing of band 3A3 (the *z* locus) with 3C1 of the homologous chromosome as follows:

3A1,2,3,4, ... 9 3B1,2 . . . . 3C1,2,3
. . . . . 3A1,2,3,4 . . . . 9 3B1,2 . . . 3C1,2,3

If within the paired region a crossover occurs between 3C1.2 of one chromosome and 3A3,4 of the homologue, the two products of the crossover event will be: (1) a chromosome in which the region extending from 3A4 to 3C1 has been lost and whose salivary chromosome band complement can be written as 3A1,2,3 C1,3,4 and (2) a chromosome in which the deleted region of (1) has been duplicated in tandem and which can be designated as 3A1,2,3,4 . . . . B1,2 . . . C1 A4 . . . B1,2 . . . C1,2,3. The deletion coincides with the limits of w^{-r} but the duplication has not been uncovered.

Assuming the pairing and crossing over scheme described does in fact occur, the question may be raised as to why the duplication has not been recovered. Since the w^{-r} chromosomes represent the only detectable exceptions, it is clear that the duplication, if it occurs, has no distinctive phenotype with which it can be associated. This is in a sense surprising since the w^{-r} chromosomes behave phenotypically as losses of the *w* locus, and it would follow that a duplication equivalent to w^{-r} would act phenotypically as a duplication of the *w* locus. From this line of reasoning it would follow that since the loss derived e.g., from w^a/w^{a2} females behaves as a *w* locus loss, the reciprocal would be a duplication of the *w* locus and be phenotypically distinctive from either w^a or w^{a2}.

There are a number of reasons which suggest that while the scheme proposed for the origin of the duplication is plausible, the anticipated phenotypic effects are not. The available facts support the idea that genic mutants associated with the *w* locus are localized to the doublet 3C2,3, and that duplications and deficiencies of band 3C1 do not involve the *w* locus *per se*. The recombination analysis of the white mutants shows that four loci are indicated. Mutants representing each of the loci, including all four at the left-most locus, have been tested for coverage by Dp-1 and Dp-2 both of which include the doublet 3C2,3 and lack band 3C1. These duplications cover all mutants tested. Tests with *w* deficiencies considered earlier show that Dp-1 and Dp-2 cover the deficiency for the 3C2,3 doublet but not the deficiency for band 3C1. Two conclusions follow from these data: first, the *w* gene mutations are localized to the doublet 3C2,3; second, since a duplication of 3C2,3 fails to cover an alteration associated with 3C1, it is not unexpected that a duplication of 3C1 will not affect phenotypes associated with mutations in 3C2,3. Therefore it is not unreasonable that the duplication reciprocal to the w^{-r} losses is without phenotypic effect on mutants of the *w* loci.

Two additional exceptions were recovered among the progeny of homozygous w^a females which differed from the w^{-r} losses. From the homozygous w^a females listed in Table 1, a single y^2 *spl*+ male was recovered whose eye color was distinctly darker than that associated with the *su-*w^a w^a genotype. A single male of similar phenotype was found in a stock of genotype y^2 *su-*w^a w^a *spl*; *Cy*; *Ubx/Xa*

and is assumed to have arisen by crossing over. Both exceptional types proved to be identical and will be considered together. Cytological examination kindly provided by PROFESSOR E. B. LEWIS showed that both exceptions are associated with tandem duplications involving regions 3B and 3C of the X chromosomes. These duplications, which will be called Dp w^a, are somewhat shorter than those found by LEWIS (1956) to be associated with the white locus. Genetic information substantiated the tandem duplication nature of the exceptions and showed that in all probability the w^a mutant together with the other w loci is duplicated. In one test of females $y\ ac\ w^+\ spl$/Dp w^a, 34 $y\ ac\ w^a$ and 4 $w^a\ spl$ males were recovered among 11,915 male progeny. These results fit the assumption that in Dp w^a the duplicated region to the left of the w^a locus, e.g., section 3B, is genetically much longer than the duplicated region to the right of the w^a locus. If the duplicated left segment is represented for simplicity by the letters *a,b,c* and the duplicated right segment by the letter *d*, then the duplication can be written as a,b,c,w^a,d, a,b,c,w^a,d; the normal chromosome as a,b,c,w^+,d.

In female $y\ ac\ w^+\ spl$/Dp w^a when pairing occurs as:

$$\begin{array}{ll} & a,b,c,w^a,\ d,a,b,c,w^a,d,e,f \\ y\ ac & a,b,c,w^+, d \ldots\ldots\ldots\ldots spl \end{array}$$

a crossover between w^a and *d* in the paired segment will ultimately yield a male $w^a\ spl$. Alternatively when the pairing occurs as:

$$\begin{array}{l} a,b,c,w^a,d\ a,b,c,w^a,\ d \\ y\ ac. \ldots. a,b,c,w^+, d \ldots\ldots spl \end{array}$$

crossovers to the left of w^a and w^+ in the paired segment, e.g. between *a,b* etc., will produce $y\ ac\ w^a$ males. On the assumption that the two pairing schemes occur with equal frequency, the recovery of 34 $y\ ac\ w^a$ males compared to four $w^a\ spl$ males demonstrates that genetically the duplicated chromosome segment to the left of w^a is greater than that to the right of w^a.

Since it is conceivable that the crossover product complementary to the w^{a-r} loss is a duplication carrying both w^a and w^{a2}, the w^{a2} mutant was substituted for one w^a in Dp w^a in order to assess the phenotype of this combination. This substitution was rather laboriously carried out by constituting females of the genotype $y\ ac$ Dp $w^a\ spl/w^{a2}$ and selecting $y\ ac$ non-w^{a2} male progeny for further testing. Such males, all phenotypically Dp w^a, could arise either by a crossover inserting w^{a2} into the right segment of the duplication or as a crossover outside the duplication between w^a and *spl*. The latter represents an unaltered Dp w^a. A number of crossovers were progeny tested by crossing to *Cy*; *Ubx/Xa* females, selecting the F_1 *Cy*; *Ubx* females and scoring the F_2 males. If the $y\ ac$ males carried the unaltered Dp w^a then only $y^+\ ac^+\ w^a$ crossover F_2 males should occur. On the other hand, if w^{a2} had been inserted into Dp w^a, then F_2 $y^+\ ac^+\ w^{a2}$ crossover males should occur. Two cases of the latter type were found. This means that the Dp with w^a in its left and w^{a2} in its right segment is phenotypically equivalent to Dp w^a. Since males of this phenotype are readily separable from

either w^a or w^{a2} males, they would hardly escape detection if they occurred as the reciprocal to the w^{-r} losses. Their absence means that the duplication carrying both w^a and w^{a2} cannot represent the crossover concomitant to the occurrence of w^{-r}.

How frequently does the "illegitimate" pairing occur in w^a/w^{a2} females? Before an estimate can be attempted, information is needed on the crossing over frequencies in the interval *y* or *sc-z-w* or from the distal end of the X chromosome to the *w* locus. GANS (1953) localized *z* at a point genetically equidistant between *sc* and w^{co}. In studying the genetic interactions between *z* and several *w* mutants (GREEN 1959) an estimate was made of the genetic distance for the interval *sc-z-w* in the following way. For each of 35 independent *w* mutants, males *sc z w* were sought among the progeny of the cross ♀♀ *sc z ec ct/w* × *sc z ec ct* ♂. F_1 *sc w* males were progeny tested for the presence of *z* by crossing to homozygous *sc z ec ct* females. After progeny testing 214 males, 139 or 65 percent proved to be *sc z w*, 75 or 35 percent *sc* z^+ *w*. This means that for the genetic interval *sc-w*, 35 percent of the genetic distance separates *sc* and *z* while remaining distance separates *z* and *w*. Comparable results were obtained by measuring the genetic interval *y-z-*w^a as influenced by the heterozygous inversions *Cy* and Ubx^{130}. For this purpose females *y z ec ct/*w^a; *Cy*/+; *Ubx*/+ were crossed to *y z* males. Since the eye color of *z* w^a males is distinctly more dilute than that of w^a males, and thereby permits a perfect separation, crossing over was estimated by enumerating males w^a (noncrossover), *y* w^a (crossover between *y* and *z*) and *y z* w^a (crossover between *z* and w^a). Among 1661 males, 41 or 2.47 percent were *y* w^a and 57 or 3.43 percent were *y z* w^a or the *y-z* and *z-*w^a intervals constitute 42 percent and 58 percent respectively of the entire interval.

It is of interest to note here the relationship between salivary gland chromosome map distance and genetic distance for the loci *y-z-*w^a. These mutants have been localized to bands 1A5–8, 3A3 and 3C2,3 respectively. In terms of the number of salivary gland chromosome bands, the *y* and *z* loci are separated by a minimum of 65 bands whereas the *z* and w^a loci are separated by only ten bands. Thus the salivary chromosome distance for this region conveys a misleading view of the relationship between cytological and genetical length, a fact already pointed out by others, and the region with the fewer bands and gene loci has the greater genetic distance.

An estimate of the frequency of non-homologous pairing can be obtained from the following considerations. The frequency of w^{-r} exceptions was found to be approximately 1/10,000 females. Since the reciprocal crossover is presumed to occur although was undetected, the total crossover frequency is 1/5000 or 0.02 percent. For simplicity, it will be assumed that (1) crossing over occurs between bands, and (2) the frequency of crossing over between any pair of contiguous bands is equal whether or not pairing is two-by-two. For the interval 3A3–3C2 crossing over can occur between any one of twelve band pairs. On the basis of the crossing over frequency between *z-*w^a as influenced by autosomal inversions, the frequency of crossing over between a band pair in this interval is

about 0.3 percent. Granting the postulate that the exceptions occur when a crossover occurs between bands 3C1,2 of one chromatid and 3A3,4 of the nonsister chromatid, it follows that 1/15 (0.02/0.3) of all the crossovers which occur between each band pair take place when 3C1,2 is so paired with 3A3,4 as to produce either the w^{-r} loss or the undetected duplication. While it is conceded that the assumptions underlying this estimate are unverifiable and probably not realistic, they do strongly suggest that the frequency of non-two-by-two pairing must be high indeed, higher than reflected by the frequency of exceptions recovered.

DISCUSSION

To explain the occurrence of the w^{-r} chromosomes the following scheme was proposed. First, during meiosis pairing occurs between bands 3C1,2 of one chromatid and 3A3,4 of the nonsister chromatid. Second, a crossover occurs within the paired region yielding a deficiency extending from bands 3A4 to 3C1 inclusive and a reciprocal tandem duplication of the equivalent chromosome segment. On the basis of the crossing over marker genes recovered in association with the w^{-r} chromosomes, two pairing situations have been encountered. Those w^{-r} chromosomes derived, for example, from female y^2 *su-w*^{a} w^a *spl*/w^{a2} carried the *spl* marker. Their occurrence is explained by assuming that pairing occurs between 3C1,2 of the w^a chromosome and 3A3,4 of the w^{a2} chromosome as follows.

$$\begin{array}{l} y^2 \ldots\ldots 3A3,4 \ldots . C1,2,3 \ldots\ldots . spl \\ \qquad\qquad\qquad\qquad\quad 3A3,4 \ldots . C1,2,3 \end{array}$$

The appropriate crossover produces a deficiency carrying *spl*.

Alternatively those w^{-r} chromosomes recovered, for example, from females y w^{a2} *spl*/w^{a2} carried the y marker. Here pairing is assumed to occur according to the following scheme.

$$\begin{array}{l} y \qquad \ldots 3A3,4 \ldots\ldots C1,2,3 \qquad spl \\ \quad 3A3,4 \ldots C1,2,3 \end{array}$$

The appropriate crossover produces a deficiency carrying the y marker.

The type of "illegitimate" meiotic pairing associations invoked here is not without precedent especially if one extrapolates from comparable pairing associations observed in the salivary gland chromosomes of Drosophila and Sciara. BRIDGES (1935, 1938) recorded in *D. melanogaster* the occurrence in the salivary gland chromosomes regions of "confused clumping due to synapsis of homologous bands along the chromosomes." These he attributed to pairing of "repeats" or putative duplicate gene loci. METZ (1947) likewise has been impressed with the cytological evidence for the occurrence of "repeats" in Sciara and has figured the occurrence in *S. ocellaris* a triple "repeat" in the salivary gland X chromosome which imposed concurrent synapsis upon three nonadjacent chromosome regions.

In BRIDGES' view the pairing associations of "repeats" could provide a means for obtaining duplications and deficiencies of the types described here. This ex-

planation is not unlike that submitted by CATCHESIDE (1947) to explain the occurrence of a duplication and deficiency in *Oenothera blandina.*

While there is no convincing evidence for the occurrence of duplicate genes in Drosophila, data are nevertheless available to show that the *z* locus (band 3A3) and band 3C1 manifest an intimate genetic relationship. This despite the absence of a clear-cut demonstration that 3A3 and 3C1 are cytologically "repeat" loci. GANS (1953) clearly demonstrated experimentally that genotypic *z* males are wild type in phenotype unless bands 3C1,2 and 3 are duplicated. In duplication males the mutant *z* eye color develops. Additional evidence shows that these genetic affinities can be more specifically attributed to band 3A3 and 3C1. As described above, the loss of band 3C1 inhibits the phenotypic effects of DP (1–1) z^4. In addition females homozygous for the mutant *z* but heterozygous for a deficiency which includes band 3C1 but not 3C2,3 are wild type in phenotype (GREEN, unpublished observations). While these facts do not establish an homology between 3A3 and 3C1, they do support the interpretation of their fundamental genetic interdependence. As such the occurrence of pairing between 3A3 and 3C1 is not a completely surprising event.

Needless to say a number of experimental facts described herein defy an unequivocal explanation at this time. Data were presented which show that the z^+ locus in the w^{a2} X chromosome has a marked pairing affinity with 3C1 in the w^a and w^{a3} carrying chromosomes. This is supported by the fact that this pairing configuration is reversed when the mutant *z* or z^+ loci from Canton-S and Oregon-R wild type stocks are substituted for the z^+ locus of the w^{a2} stock. On the other hand the z^+ locus of the w^{a2} stock appears to have a marked pairing impotency for band 3C1 carried by the *y ac* w^{col} *spl* and *y* w^{a2} *spl* chromosomes. Does this mean that the 3A3 and 3C1 bands allelic differences occur which manifest varying pairing affinities toward one another? Clearly this is a question of fundamental nature bearing upon the factors and forces which bring chromosomes together in what is recognized as meiotic pairing. The last word on this subject has yet to be written. In fact critical data relevant to this phenomenon are meager indeed and no claim is made that data submitted here add significantly to this subject.

In a general way the occurrence of non-homologous pairing and crossing over as described herein has a bearing on the mechanism of crossing over. It is difficult to reconcile the regular occurrence of the w^{-r} and Dp w^a chromosomes to a crossover event which demands that two nonsister chromatids be broken at dissimilar positions simultaneously followed by rejoining to produce the proper crossover products. Seemingly too many coincidental events are required to account for the crossover event. Perhaps the occurrence of deficiencies and duplications finds a better explanation in terms of a crossing over event which involves errors in chromatid reduplication imposed by non-homologous pairing. Such errors in reduplication would be consistent with the described copy-choice mechanism of crossing over.

SUMMARY

The regular occurrence in association with crossing over of a deficiency (w^{-r}) extending from bands 3A4–3C1 of the X chromosome of *D. melanogaster* is described. The occurrence of two independent identical tandem duplications associated in part with the white locus is also described.

A detailed analysis of the genetic conditions which influence the occurrence of the w^{-r} chromosomes has been presented. Pairing of bands 3A3 and 3C1 followed by crossing over represents the simplest explanation for the origin of the w^{-r} chromosomes.

The genetic relationships between bands 3A3 and 3C1 are discussed together with a brief discussion of the problem of crossing over.

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