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A genetic analysis of the *rudimentary* locus of *Drosophila melanogaster**

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SUMMARY

A study of the structural and functional organization of the *rudimentary* (*r*: 1–54·5) locus of *Drosophila melanogaster* has demonstrated that alleles of this gene reside in a number of recombinationally separable sites, and display a complex pattern of interallelic interaction. Data relating to interallelic interaction have been utilized to construct a linear complementation map consisting of 7 complementation units and 16 complementation groups. Comparison of the genetic fine structure map and the complementation map shows that the two maps are approximately colinear. Totally non-complementing alleles reside at both ends of the fine structure map. The *r* locus is best interpreted by the model of a single cistron whose product affects several distinct developmental processes and whose alleles display a complex pattern of interallelic complementation. Intragenic recombination within the *r* locus is accompanied by the appearance of parental and recombinant flanking marker classes not expected on the basis of reciprocal recombination. Studies with half-tetrads demonstrate that intragenic recombination can occur either by gene conversion or by a reciprocal exchange mechanism. The pattern of organization seen at the *r* locus is similar to patterns of organization found in work with fungal genes.

1. INTRODUCTION

This study is designed as an experimental examination of several of the characteristics exhibited by a 'complex gene' in *Drosophila melanogaster*. Specific attention is focused on genetic complementation, genetic fine structure, and the mechanism of intragenic recombination.

The *rudimentary* (*r*:1–54·5) locus of *D. melanogaster* was chosen for intensive analysis because the techniques described in this paper permit an experimental resolution of its structural and functional organization comparable to that achieved with fungi. Alleles of *r* were described by Morgan (1912, 1915) as sex-linked, recessive visible mutations affecting both wing morphology and female fertility. The wings of *r* flies are obliquely truncate with sparse and irregular

marginal bristles. Lynch (1919) described the expression of the female sterility phene of r by outlining its pattern of inheritance:

Parental genotype   Classes of progeny

$r/+ \; ♀ \times r/7 \; ♂ \;\; \rightarrow r/+ \; ♀; \; r/r \; ♀; \; +/7 \; ♂; \; r/7 \; ♂$

$r/r \; ♀ \times r/7 \; ♂ \;\; \rightarrow r/r \; ♀ \text{ (escaper)}; \; r/7 \; ♂ \text{ (escaper)}$

$r/r \; ♀ \times +/7 \; ♂ \;\; \rightarrow r/+ \; ♀; \; r/7 \; ♂ \text{ (escaper)}$

The presence of a wild allele of r either during oogenesis or during embryogenesis is sufficient to produce a fully viable class of progeny. The absence of a wild r allele during both of these developmental periods leads to an inviable class of progeny (cf. Counce, 1956). The inviable classes of progeny, which are termed escaper classes, occur at only very low frequencies. Escaper classes appear at approximately 1 % of their expected frequencies (cf. Carlson, 1970).

Recent studies have indicated that the r locus consists of a series of pheno-typically homogeneous alleles which exhibit a complex pattern of interallelic complementation and which are located in at least three recombinationally separable sites (Fahmy & Fahmy, 1959; Green, 1963a).

Analysis of a gene theoretically equivalent to that accomplished in microbial systems requires: (1) an adequate definition of a unit of function, and (2) a selective procedure for use with recombination studies which is capable of fine resolution of structural detail. The first section of this paper treats the delineation of a functional unit, independent of biochemical information, by means of *cis-trans* tests of interallelic interactions. The following sections describe a selective system capable of recovering rare interallelic recombinants, and the use of the selective system to construct a fine structure map and to investigate the mechanism of intragenic recombination.

2. MATERIALS AND METHODS

(i) *Culture conditions*

Experimental cultures were raised on standard corn meal molasses agar medium with yeast suspension added and incubated at $24 \cdot 5 \pm 0 \cdot 5 \, °C$. Departures from these conditions are noted in the text.

(ii) *Mutant stocks*

The 45 r alleles used in this study are listed, along with their source and complementation group, in Table 1. Alleles were designated according to their position in the r fine structure map, and their distal and proximal location in relation to the centromere. The most distal allele was termed r^1 and the most proximal r^{45}. The majority of alleles utilized in this study are a selected sample of those isolated in previous work of the Fahmys (1959) and of Green (1963a). The sample contains more complementing and fewer non-complementing alleles than would be expected from a random sample. Table 2 lists genetic markers and a special chromosome important to an understanding of this work. Further

information about the r alleles and other mutants is given in Lindsley & Grell (1967). The designation r^x and r^y indicate different r alleles in the explanation of a cross.

Table 1. *The rudimentary alleles used in this study*

Experimental designation	Complementation group	Previous designation	Mutagen*	Source†
1	VII	3719	c	F
2	VII	X917	c	F
3	VII	1996	c	F
4	VII	56k	c	F
5	VII	3484	c	F
6	I–VII	15	c	F
7	I–VII	75	c	F
8	VI	9	s	B
9	VI	39k	s	M
10	VI	2381	c	F
11	VI	61j26·1	s	G
12	VI	60k27·3	x	G
13	VI	3720	c	F
14	V–VII	68g18	c	C
15	VI	3718	c	F
16	VI	2622	c	F
17	V–VI	3721	c	F
18	VI	61	s	G
19	V–VI	55k	c	F
20	V–VI	3589	c	F
21	III–VI	58b28	s	G
22	III–VI	62j	c	F
23	V–VI	2291	c	F
24	II–VII	3722	c	F
25	IV–VI	56d	c	F
26	IV–V	57a′	x	Gl
27	IV–V	57a	x	Gl
28	I–V	60c 15·2	x	G
29	III	531	c	F
30	I–II	54d	c	F
31	II–III	55a	c	F
32	I–IV	58a	x	G
33	I–III	64k	c	F
34	I–IV	3433	c	F
35	I–VII	2696	c	F
36	I	54c	c	F
37	I–VII	54j	c	F
38	I–IV	56j	c	F
39	I–II	3455	c	F
40	I–II	3463	c	F
41	I–IV	59k23	s	G
42	I–VII	60d1	x	G
43	I–VII	60k27·1	x	G
44	I–VII	61i8	s	G
45	I–VII	MAHI13A	c	F

* s, spontaneous; x, X-rays; c, chemical.
† B, C. B. Bridges; C, P. S. Carlson; F, O. J. Fahmy and M. B. Fahmy; Gl, H. Gloor; G, M. M. Green; M, L. V. Morgan.

(iii) *Isogenic stocks*

Stocks isogenic for chromosomes I, II, and III were produced as outlined by Doane (1960a, Appendix). All r alleles were analysed in stocks isogenic for the entire genome except for that portion of I proximal to g and for chromosome IV. An isogenic line derived from an Oregon R-CH wild strain provided a superior residual genome for the expression of r, and was used exclusively in these studies. Experimental cultures were coisogenic for the same portion of the genome.

Table 2. *Genetic markers and balancer chromosomes*

Symbol	Mutant	Phenotype	Location on the X chromosome
y^2	Yellow	Cuticle yellow. Hairs and bristles black	0·0
dm	Diminutive	Bristles and body small. Females sterile	4·6
g	Garnet	Eye colour purplish ruby	44·4
tc	Tiny chaetae	Bristles short and fine	51·6
f	Forked	Forked bristles	56·7
B	Bar	Bar eyes	57·0
$FM3$	First multiple 3	A rearranged X chromosome carrying two recessive lethals and alleles of y, dm, and B	

(iv) *Assays of mutant expression*

The r wing phene was quantified by the method of Green (1963a). Green defined five classes of mutant expression ranging from typical r to wild-type. Each phenotypic assay involved scoring ten individual female flies and averaging the results. With practice such a procedure provides a reproducible indication of the wing phene for each allele or heterozygous combination of alleles. Complementation of the wing phene was examined by scoring wing expression in females heterozygous for two r alleles. A wing phene more normal than either parental r allele was scored as partial complementation, while a totally wild wing morphology was scored as complete complementation.

The female sterility phene was quantified by assaying the progeny types emerging from the cross: r/r ♀♀ × $+/�$ ♂♂. A comparison of the progeny types with the total number of eggs produced from the mating provides a measure of the proportion of the escaper class of males. Complementation of the female sterility phene was examined by mating females heterozygous for two r alleles to wild males. The progeny produced by at least 50 eggs collected from the mating were examined. The number of r males defined the degree of complementation. Less than 5 % escaper males was defined as non-complementation, from 6 to 35 % as partial complementation, and from 36 to 50 % or above as complete complementation. Care was taken to allow all slowly developing r males time to emerge before counting.

An analysis of escaper production by *r* alleles isogenic for the Oregon R-CH background indicated that each class appears reproducibly at 1 % of its expected frequency. Hence, when an iso-*r* female is mated to an iso-*r* male, approximately 1 % of the eggs yield escapers, which include both sexes in a ratio of 1:1 (Carlson, 1970).

(v) *Synthesis of attached-X chromosomes*

Attached-X chromosomes with arms carrying two different *r* alleles were synthesized as outlined by Muller (1936). Attached-X stocks were maintained with $Dp(1;4)$ r^+ of Green and $XY^L\text{-}Y^S$, *f* males.

(vi) *Synthesis of duplications for specific* r *alleles*

Duplications carrying specific *r* alleles were produced as outlined by Brosseau, Nicoletti, Grell & Lindsley (1961). Each duplication carried a specific *r* allele, all Y male fertility factors, and all X euchromatin proximal to the 14A region. The duplications showed no gross morphological effects when made hyperploid with an attached-X chromosome.

3. GENETIC COMPLEMENTATION

Previous studies with *r* (Fahmy & Fahmy, 1959; Green, 1963a) used only normalization of the wing phene to detect complementation between alleles. Such an assay system is not entirely suitable, since it is quite difficult to score the degree of complementation between alleles and since the wing abnormality is only one component of the phenotype. A more complete analysis of complementation would include an examination of the female sterility phene. A measure of allelic complementation during oogenesis would involve crossing *r* females, heterozygous for two *r* alleles, to wild males. A greater proportion of *r* males in the progeny would indicate complementation between the two alleles in the female parent during oogenesis. If the two *r* alleles do not complement, then *r* males will emerge from the same percentage of the eggs as in crosses of homozygous females for either *r* allele (i.e. approximately 0·5 % of the eggs). If the *r* alleles do complement, then the percentage of *r* males will be greater than 0·5 %.

Previous work by Green (1963a) indicated that a several fold increase in the number of *r* male progeny is associated with the smallest degree of complementation detectable on the basis of wing phenotype. Hence, the number of *r* male progeny should provide a sensitive test for allelic complementation. Experimental procedure involves the generation of all possible heterozygous combinations of *r* alleles in co-isogenic Oregon R-CH females. The wing phenotype and the relative numbers of *r* male progeny in crosses with a co-isogenic wild male defined the degree of complementation between the respective alleles. Table 3 summarizes the complementation data for all possible combinations of the 45 alleles utilized in this study. A striking feature of the data presented in Table 3 is that both the wing and female sterility phenes show identical patterns of complementation.

The complementation map presented in Fig. 1 was constructed following the rules outlined by Gillie (1966). It is a linear map composed of seven complemen-

tation units and 16 complementation groups. Eight groups are represented by only one allele. Four units are represented by at least one allele while three units are inferred from overlaps.

The map is similar to those found in other organisms (see Fincham, 1966, for a complete discussion). A similar example of allelic complementation in *D. melanogaster* is that of *maroon-like* presented by Chovnick and his associates (Chovnick *et al.* 1969). The results are consistent with the less extensive mapping of the *r* locus reported by Fahmy & Fahmy (1959), and by Green (1963*a*).

4. GENETIC FINE STRUCTURE

Previous work on the genetic fine structure of *r* (Green, 1963*a*) utilized a duplication containing a wild allele of *r* to overcome the female sterility characteristics of the mutant locus. In the present study the expression of every *r* allele is quantitatively predictable, hence the pattern of female sterility can be used in a selective system to recover wild recombinants between two *r* alleles. Since a homozygous *r* female mated to an *r* male produces only a low number of escaper progeny, and since an *r* female mated to a wild male produces almost entirely heterozygous female progeny, then a female heterozygous for two non-complementing *r* alleles mated to a non-complementing *r* male will only produce escaper progeny, which will have a typical *r* phenotype, and progeny of recombinant types which will appear as non-*r* flies. The rare escaper classes, distinguishable as *r*, will appear at a constant frequency (approximately 1.0% of the total number of eggs produced) so that their numbers can be used as an indication of total population size.

Diagramatically, mating

$$\frac{r^x}{r^y}\,♀ \times \frac{r^x}{7}\,♂$$

gives the following:

Escapers $\dfrac{r^x}{r^x}, \dfrac{r^x}{r^y}\,♀♀;\quad \dfrac{r^x}{7}, \dfrac{r^y}{7}\,♂♂,$

Recombinants $\dfrac{+}{r^x},\quad ♀♀;\quad \dfrac{+}{7}\quad ♂♂,$

if r^x and r^y do not complement.

Such a selective scheme will recover all wild recombinants between *r* alleles regardless of the flanking markers associated with the recombinant.

Since the escaper progeny appear at a frequency of approximately 1% an assay of 10^5 progeny involves counting 10^3 *r* flies as well as the crossover types. Estimates of the total population size are made by multiplying the number of escapers by 100. This estimate is verified by a knowledge of the number of heterozygous females used in a given cross. All recovered crossovers were progeny-tested to verify that they carried a wild allele of *r* and to confirm their outside marker constitution. Progeny testing eliminates the possibility that the wild expression for *r* is due to a loosely linked suppressor mutation, but it cannot eliminate the possibility of a closely linked suppressor or of a second site reversion.

Table 3. *Genetic complementation between r alleles**

Alleles	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
A 36	X	++ 32	+ 16-28	++ 40-54	++ 40-50	– 0	++ 32-45	– 0	++ 38	– 2	++ 42	– 0-2	++ 32-48	– 2	+ 12	– 0-2
B 29		X	+ 26-28	+ or ++ 14-48	++ 40-52	+ 10-22	+ or ++ 32-46	– 0	++ 46	– 2	++ 34	– 0	– 0-4	– 0	– 2	– 0-2
C 26, 27			X	+ 6-22	+ 14-28	+ or ++ 38-52	– 0-2	+ or ++ 22-26	– 0-2	+ 18-24	– 0-2	– 0-2	– 0	– 0-2	– 0-2	– 0-2
D 8-13, 15, 16, 18				X	+ 8-38	+ or ++ 18-56	– 0-2	+ or ++ 30-46	– 0-2	+ or ++ 14-42	– 0-2	+ or ++ 10-52	– 0-2	+ 12-24	– 0-2	– 0-2
E 1-5					X	++ 40-52	+ 10-44	++ 28-50	+ 14-22	+ or ++ 22-50	– 0-2	+ or ++ 14-48	+ 10-34	+ or ++ 24-50	– 0-2	– 0-2
F 30, 39, 40						X	+ or ++ 16-52	– 2	+ or ++ 10-40	– 0	++ 40-52	– 0	+ or ++ 18-40	– 0-4	– 0-2	– 0-2
G 17, 19, 20, 23							X	++ 30-54	– 0-2	+ or ++ 26-36	– 0-2	+ or ++ 8-38	– 0-2	–	– 0-2	– 0-2
H 31								X	+ 16	– 0	++ 26	– 0	– 0-2	– 0	– 2	– 0-2
I 25									X	+ 20	– 2	– 0-2	– 0-2	– 0	– 0	– 0-2
J 33										X	+ 24	– 0	– 0-2	– 0	– 0	– 0-2
K 14											X	+ or ++ 16-44	– 0-2	– 0	– 0	– 0-2
L 32, 34, 38, 41												X	– 0-2	– 0-2	– 0	– 0-2
M 21, 22													X	– 0-2	– 0	– 0-2
N 28														X	– 0	– 0-2
O 24															X	– 0-2
P 6, 7, 35, 37, 42-45																X

* This table contains complementation data for all possible combinations of the 45 r alleles. Complementation of the wing phene is indicated in the upper portion of each square as non-complementing (–), partially complementing (+), or total complementation (+ +). The lower portion of each square contains the percentage of r males of the total number of progeny of the cross: r^x/r^y ♀♀ × $r^+/7$ ♂♂. 5% or fewer males among the progeny was defined as non-complementing, from 6 to 35% males as partial complementation, and from 36 to 50% males or above as complete complementation.

The flanking markers used were tc and f (Table 2). An analysis of 5×10^4 individuals indicates that tc lies 3·29 map units distal to r while f is 1·27 units proximal.

The following scheme was devised to complete the required crosses. Stocks of the primary alleles used for the mapping of all other alleles were inserted into X chromosomes also carrying $tc\ f$ and B and were balanced over $FM\ 3$. All r alleles, including the primary alleles, were placed in X chromosomes also carrying
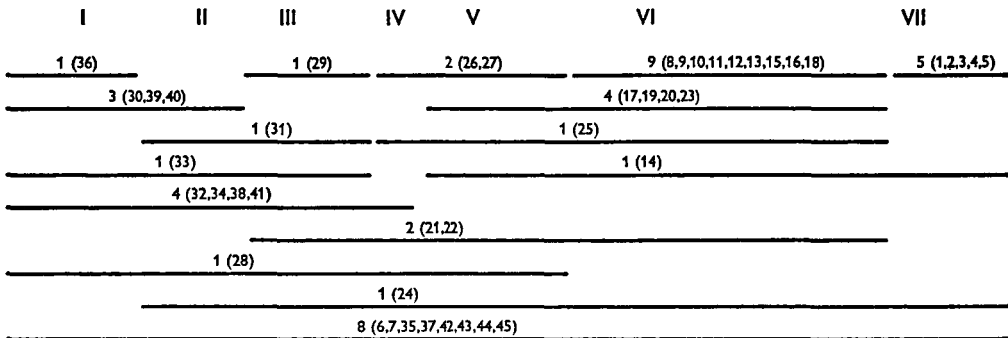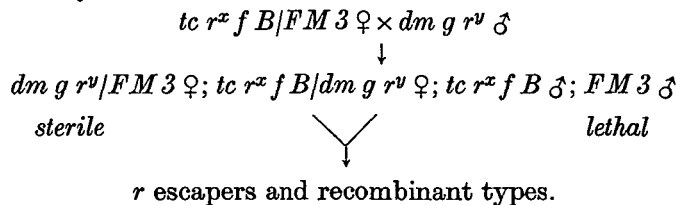

Fig. 1. Complementation map of the *rudimentary* locus. The *rudimentary* locus includes: (*a*) Seven complementation units (I–VII). (*b*) Sixteen complementation groups (solid lines), each represented by one or more alleles. Eight complementation groups are represented by only one allele. (*c*) The number of alleles in each complementation group is stated above the lines of the group followed by the allele designation of the r mutants which belong to that group.

dm and g. For any given cross, virgin females of the constitution $tc\ r^x\ f\ B/FM\ 3$ were mated to $dm\ g\ r^y$ males. Of the four classes of progeny expected from this cross only two, $tc\ r^x\ f\ B/dm\ g\ r^y$ females and $tc\ r^x\ f\ B$ males are not lethal or sterile. Females of the constitution $dm\ g\ r^y/FM\ 3$ were sterile because of being homozygous for dm; $FM\ 3$ males were lethal because of recessive lethals carried by the FM 3 chromosome. The two viable and fertile classes of progeny were collected at daily intervals and used as the parental generation for the fine structure crosses. They were permitted to produce eggs for 8 days and then discarded. Progeny emerging from these eggs provided the data necessary for the construction of a fine structure map.

Diagrammatically:

$$tc\ r^x\ f\ B/FM\ 3\ \female \times dm\ g\ r^y\ \male$$
$$\downarrow$$
$$dm\ g\ r^y/FM\ 3\ \female;\ tc\ r^x\ f\ B/dm\ g\ r^y\ \female;\ tc\ r^x\ f\ B\ \male;\ FM\ 3\ \male$$

sterile $\bigvee$ lethal

r escapers and recombinant types.

Such a scheme necessitates collecting virgin females only once during a given cross. Since virgins are needed for the production of the parental generation only, a small number (150 to 200 per cross) of them suffice.

Initially all mutant alleles were mapped with reference to one non-complementing *r* allele (no. 42, see Table 4, section 1). The spatial relations among the alleles were then confirmed by mapping all alleles with reference to a second non-complementing allele located at the opposite end of the map (no. 7, see Table 4, section 2). When the positions of the individual alleles were roughly determined, alleles in one region were mapped in relationship to other alleles within the same region (see Table 4, sections 3–11). Eleven alleles (nos. 6, 7, 14, 17, 20, 23, 28, 35, 37, 42, and 44) spaced evenly throughout the map were chosen for the completion of all possible crosses *inter se* to verify experimentally the consistency of recombination within the *r* locus (Table 4, sections 3–11). Though some of these 11 alleles are not totally non-complementing, none complement with any of the other 10 chosen alleles.
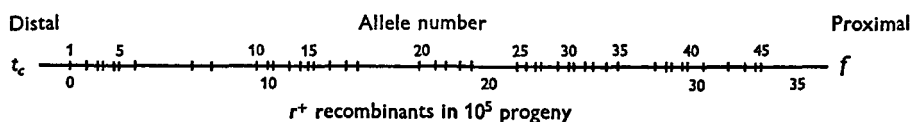

Fig. 2. Genetic fine structure map of the *rudimentary* locus. Numbers above the line are designations of each allele from 1 to 45. Numbers below the line represent the genetic distance along the fine structure map in terms of wild recombinants in 10^5 progeny. All 45 alleles reside within a genetic distance of approximately 35 wild recombinants in 10^5 progeny or of 0·07 map units.

A fine structure map consistent with the majority of the data in Table 4 is presented in Fig. 2. It was constructed on the basis of the frequency of recombination between alleles, and the proximal and distal marker criteria outlined by Catcheside, Jessop & Smith (1964). The relative order of allelic sites was deduced by data on the distribution of linked, flanking markers. Data from wild *r* recombinants carrying recombinant classes of flanking markers were treated on the assumption of reciprocal exchange. In a cross $tc\ r^x f/ + r^y +$, where *tc* is the distal marker and *f* the proximal marker, recovery of wild *r* recombinants associated with *tc* and + as the flanking markers indicates that r^y is distal to r^x.

The data from wild *r* recombinants carrying both recombinant and parental classes of flanking markers was also analysed by two further criteria. In any cross, the proximal marker associated with a majority of the wild recombinants is that marker which entered the cross associated with the more distal *r* allele. Likewise, the distal flanking marker associated with a majority of the wild recombinants is that marker which entered the cross carried by the more proximal *r* allele. The total distance between the two end alleles is approximately 35 wild recombinants in 10^5 progeny. This is equivalent to 0·07 map units. The distance between the two closest alleles separated is approximately 2·6 wild recombinants in 10^6 progeny. This corresponds to a conventional map distance of 0·00052 units.

A striking feature of the fine structure map is that it is broadly co-linear with the complementation map (consult Table 1, and Fig. 1). This finding is consistent with other examples of co-linearity in *D. melanogaster* (Chovnick *et al.* 1969; Finnerty, Duck & Chovnick, 1970) and in lower organisms (Fincham, 1966).

Table 4. *Genetic fine structure crosses**

Summary and Key

* General format of the crosses: $tc\ r^y\ f\ B/dm\ g\ r^x$ ♀♀ mated to $tc\ r^y\ f\ B$ ♂♂. For each section a different allele is used as r^y. The allele designations of r^x are stated in the individual sections. The complete data are only presented for sections 1 and 2. Though similar findings relating to the recovery of flanking markers were observed in all crosses, they are not included *in extenso*. Consult Carlson (1970) for complete details.

Section number	Allele used as r^y
1	42
2	7
3	6
4	14
5	17
6	20
7	23
8	28
9	35
10	37
11	44

Section 1

$tc\ r^{42}\ f\ B/dm\ g\ r^x$ ♀♀ × $tc\ r^{42}\ f\ B$ ♂♂

		Flanking marker genotype of r^+ progeny				
r^x	No. of escapers	P_1	P_2	R_1	R_2	Total $r^+ \times 10^{-5}$
1	1760	18	5	40	4	38·1
2	1820	22	5	29	3	32·4
3	1957	20	6	32	3	31·2
4	2170	19	5	42	3	31·8
5	2003	23	3	33	2	30·5
6	2001	21	4	34	2	30·5
7	2007	21	5	30	3	29·4
8	2491	20	6	39	4	27·7
9	1587	14	5	23	1	27·1
10	2130	11	4	36	2	24·9
11	1931	15	3	28	1	24·3
12	1769	16	3	25	1	25·4
13	1988	10	4	32	2	24·1
14	1024	7	3	13	1	23·2
15	1984	19	2	24	2	23·7
16	1948	12	3	29	2	23·6
17	2192	14	5	29	2	22·8
18	1243	9	4	13	1	21·7
19	2171	13	3	27	1	20·3
20	1599	9	3	14	1	16·9
21	2214	11	1	20	0	14·5
22	1994	7	2	19	0	14·0
23	1998	8	1	17	0	13·0
24	1943	7	3	14	1	12·9
25	1933	5	2	12	1	10·4
26	1855	6	3	10	0	10·2
27	1236	5	1	7	0	10·6

Table 4 (*cont.*)

Section 1 (*cont.*)

r^x	No. of escapers	Flanking marker genotype of r^+ progeny				Total $r^+ \times 10^{-5}$
		P_1	P_2	R_1	R_2	
28	2033	6	2	12	0	9·8
29	2177	4	2	11	1	8·3
30	1901	4	1	10	0	7·9
31	2538	4	2	13	1	7·9
32	1805	4	0	10	0	7·8
33	2112	3	2	10	0	7·1
34	1913	4	1	7	0	6·3
35	2271	4	1	6	0	4·8
36	2375	1	0	4	0	2·1
37	2307	2	0	3	0	2·2
38	2269	2	0	3	0	2·2
39	2232	2	0	3	0	2·2
40	1946	1	0	4	0	2·6
41	2462	3	0	1	0	1·6
42	2302	0	0	0	0	—
43	2477	2	0	1	0	1·2
44	2120	2	0	1	0	1·4
45	2091	2	1	3	0	2·9

Section 2

$tc\ r^7\ f\ B/dm\ g\ r^x\ ♀♀ \times tc\ r^7\ f\ B\ ♂♂$

r^x	No. of escapers	Flanking marker genotype of r^+ progeny				Total $r^+ \times 10^{-5}$
		P_1	P_2	R_1	R_2	
1	1231	2	0	1	0	2·4
2	1251	3	0	0	0	2·4
3	1583	2	0	1	0	1·9
4	1390	2	0	0	0	1·4
5	1393	1	0	1	0	1·4
6	927	1	0	0	0	1·1
7	1407	0	0	0	0	—
8	1146	2	0	0	0	1·8
9	993	1	0	1	0	2·0
10	1159	1	1	2	0	3·5
11	1016	2	0	2	0	3·9
12	925	1	0	2	0	3·2
13	1227	2	0	3	0	4·1
14	1059	2	1	2	0	4·7
15	1048	1	1	3	0	4·8
16	801	2	0	2	0	5·0
17	845	2	1	3	0	7·1
18	1097	3	0	5	0	7·3
19	935	2	1	4	0	7·5
20	814	3	2	9	0	17·2
21	902	4	1	11	0	17·7
22	1009	5	2	9	1	16·9
23	1007	4	2	12	0	17·9
24	913	6	2	10	1	20·8

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Table 4 (*cont.*)

Section 2 (*cont.*)

r^x	No. of escapers	Flanking marker genotype of r^+ progeny				Total $r^+ \times 10^{-5}$
		P_1	P_2	R_1	R_2	
25	998	6	2	14	1	23·1
26	850	4	2	13	1	23·5
27	994	5	2	13	1	21·2
28	1013	8	2	14	0	23·7
29	821	6	1	13	0	24·4
30	825	5	2	11	1	23·0
31	811	6	1	13	0	24·7
32	773	6	1	12	1	25·9
33	952	6	3	13	1	24·2
34	831	8	3	10	0	25·3
35	934	7	2	15	1	26·8
36	973	10	2	15	1	28·8
37	897	7	3	16	0	29·0
38	811	9	2	13	1	30·9
39	917	5	2	14	1	24·0
40	808	7	2	12	2	28·5
41	989	6	3	17	1	27·3
42	991	8	2	20	0	30·3
43	1020	10	4	16	1	30·4
44	884	8	3	15	1	30·5
45	720	6	2	13	0	29·2

Section 3

$tc\ r^6\ f\ B/dm\ g\ r^x$ ♀♀
$\times\ tc\ r^6\ f\ B$ ♂♂

r^x	Total $r^+ \times 10^{-5}$
1	1·2
2	1·1
3	0·93
4	0·33
5	0·26
6	—
7	0·74
14	5·1
17	7·0
20	17·0
23	18·3
28	24·2
35	28·1
37	28·2
42	28·6
44	31·4

Section 4

$tc\ r^{14}\ f\ B/dm\ g\ r^x$ ♀♀
$\times\ tc\ r^{14}\ f\ B$ ♂♂

r^x	Total $r^+ \times 10^{-5}$
6	5·8
7	3·8
10	3·0
11	2·6
12	1·7
13	0·8
14	—
15	0·65
16	1·5
17	2·4
20	7·6
23	9·3
28	14·0
35	23·6
37	22·2
42	18·7
44	26·5

Section 5

$tc\ r^{17}\ f\ B/dm\ g\ r^x$ ♀♀
$\times\ tc\ r^{17}\ f\ B$ ♂♂

r^x	Total $r^+ \times 10^{-5}$
6	9·4
7	9·2
14	1·9
16	1·6
17	—
18	1·5
19	2·2
20	5·4
23	10·9
28	14·1
35	17·4
37	20·3
42	21·5
44	24·0

Table 4 (*cont.*)

Section 6		Section 7		Section 8	
$tc\ r^{20}\ f\ B/dm\ g\ r^x$ ♀♀		$tc\ r^{23}\ f\ B/dm\ g\ r^x$ ♀♀		$tc\ r^{28}\ f\ B/dm\ g\ r^x$ ♀♀	
$\times tc\ r^{20}\ f\ B$ ♂♂		$\times tc\ r^{23}\ f\ B$ ♂♂		$\times tc\ r^{28}\ f\ B$ ♂♂	
r^x	Total r^+ $\times 10^{-5}$	r^x	Total r^+ $\times 10^{-5}$	r^x	Total r^+ $\times 10^{-5}$
6	12·4	6	18·8	6	19·3
7	14·1	7	19·4	7	20·6
14	8·0	14	13·0	14	15·1
17	4·8	17	9·7	17	12·1
19	4·5	20	3·2	20	7·1
20	—	21	0·9	23	3·0
21	2·0	22	0·6	24	0·74
23	4·0	23	—	25	0·41
28	7·5	24	3·7	26	0·36
35	13·6	28	2·9	27	0·37
37	17·0	35	10·2	28	—
42	25·1	37	9·7	29	1·3
44	18·2	42	15·1	30	3·1
		44	14·5	35	4·0
				37	9·1
				42	11·7
				44	11·8

Section 9		Section 10		Section 11	
$tc\ r^{35}\ f\ B/dm\ g\ r^x$ ♀♀		$tc\ r^{37}\ f\ B/dm\ g\ r^x$ ♀♀		$tc\ r^{44}\ f\ B/dm\ g\ r^x$ ♀♀	
$\times tc\ r^{35}\ f\ B$ ♂♂		$\times tc\ r^{37}\ f\ B$ ♂♂		$\times tc\ r^{44}\ f\ B$ ♂♂	
r^x	Total r^+ $\times 10^{-5}$	r^x	Total r^+ $\times 10^{-5}$	r^x	Total r^+ $\times 10^{-5}$
6	26·5	6	28·8	6	27·9
7	28·1	7	29·7	7	29·7
14	19·4	14	26·6	14	24·4
17	16·8	17	18·9	17	23·0
20	12·0	20	16·4	20	16·8
23	7·8	23	10·8	23	18·3
28	4·5	28	7·4	28	11·4
34	2·4	35	2·0	35	7·4
35	—	36	0·79	37	3·5
37	2·2	37	Revertant	42	1·9
42	6·0	38	0·28	44	—
44	6·3	39	0·60		
		40	0·75		
		41	2·5		
		42	1·7		
		44	2·8		

A similar observation was previously reported for *r* by Green (1963*a*), whose findings are consistent with the present data.

Another feature of the fine structure map is that it appears to be organized into several approximate clusters of alleles much as have been reported for many previous examples of pseudoalleles in *Drosophila* (Green, 1963*b*; Lewis, 1967), and

5-2

for several examples of fine structure maps in fungi (Holliday, 1964). Alleles proved separable in all instances where this was attempted. The assumption of linearity of the genetic map is borne out by the data from reciprocal interallelic crosses (Table 4, sections 3–11). It is reasonable to conclude that the r locus contains a number of mutational sites which are separable on the basis of recombination, and which are arranged as a unique linear array. It is important to note that not all 45 alleles have been mapped in relation to each other. Hence, the linear order of alleles as well as the clustering of the alleles presented in Fig. 2 should not be considered definite.

An interesting component of the recombination data is the consistent occurrence of several classes of flanking markers associated with wild r recombinants which are not entirely expected. Specifically, the observation that a sizeable fraction of the wild recombinants is accompanied by parental classes of flanking markers (P_1 and P_2 classes in Table 4) suggests that recombination between two r alleles may involve more than a single reciprocal recombinational event. These results cannot be explained by the independent behaviour of the flanking markers. Parental classes of flanking markers with wild r recombinants occur in much greater frequencies than can be explained by the assumption that a recombinant event within the r locus and crossing over between r and one of the flanking markers occurred as independent events. Evidently there is some correlation between recombination within the r locus and recombination between the flanking markers, since the recombinant classes of markers are consistently the predominant recovered classes. However, it is also clear that the generation of r recombinants is not dependent upon recombination between the flanking markers because wild r recombinants often occur without flanking marker recombination.

Wild r recombinants from the cross $tc\ r^x f/+\ r^y\ +$ would carry the following classes of flanking markers:

(1) if r^x is proximal,

$$\frac{tc\ r^x f}{+\ r^y\ +} \qquad \begin{array}{ll} R_1;\ tc\ + & P_1;\ tc\,f \\ R_2;\ +\,f & P_2;\ +\ + \end{array}$$

(2) if r^y is proximal,

$$\frac{tc\ r^x f}{+\ r^y\ +} \qquad \begin{array}{ll} R_1;\ +\,f & P_1;\ +\ + \\ R_2;\ tc\ + & P_2;\ tc\,f \end{array}$$

The expectation of reciprocal intragenic exchange and hence a flanking marker pattern of $R_1 \gg P_1 = P_2 >> R_2$ is not seen in any cross. The data is generally approximated by a flanking marker pattern of $R_1 \geqslant P_1 > P_2 \geqslant R_2$. In every cross, the major parental class of flanking markers found associated with a wild r recombinant is that class carried by the more proximal r allele. On the basis of reciprocal exchange, these observations would demand a high degree of negative interference. Recombination within the r locus should be highly correlated with an exchange occurring just proximal to r. Such a correlation would generate the expected R_1 class of flanking markers, and a P_1 class of markers which would be the parental class carried by the more proximal r allele. These observations

could also be explained on the basis of gene conversion. The parental classes of flanking markers associated with a wild recombinant would arise as a result of conversion of one of the r alleles. Recombinant classes of flanking markers would occur as a result of conversion of one of the r alleles being associated with an exchange event. The fact that the major parental class of markers is that carried by the more proximal allele would then argue that the proximal r allele is preferentially converted.

Another interesting aspect of the fine structure data is the relationship between the distance separating two alleles and the behaviour of the flanking markers in r^+ recombinants. Table 5 presents a comparison of the distance between alleles and the classes of flanking markers associated with r^+ recombinants. There appear to be several distinct effects:

(1) A progressive increase in P_1 and decrease in P_2 as allelic sites cover intervals shorter than a frequency of 5×10^{-5} wild recombinant progeny.

Table 5

Distance between alleles (frequency of r^+ in 10^{-5} progeny)	No. of cases	Classes of flanking markers associated with r^+ (in %)			
		P_1	P_2	R_1	R_2
0–1·5	34	85	0	15	0
1·5–5	190	39·5	3·2	56·9	0·5
5–10	273	29·7	7·7	61·5	1·1
10–15	330	30·9	8·8	58·8	1·5
15–20	336	31·5	11·0	55·4	2·1
20–25	759	29·1	9·0	58·5	3·4
25–30	661	30·7	10·1	55·7	3·5
30 and above	513	32·2	7·9	55·8	4·1
Total	3106	31·7	8·7	56·8	2·8

(2) A progressive decrease in R_1 over distances shorter than 5×10^{-5} wild recombinant progeny, and in R_2 over distances shorter than 15×10^{-5} wild recombinant progeny.

(3) An increase in the total parental classes (P_1 and P_2) relative to recombinant classes (R_1 and R_2) in intervals shorter than 5×10^{-5} wild recombinant progeny.

The remainder of this work is devoted to an analysis of the process of intragenic recombination, and an attempt to determine if reciprocal exchange, gene conversion, or both, are involved in the generation of r^+ recombinants.

5. EXPERIMENTS AND OBSERVATIONS RELATING TO THE MECHANISM OF INTRAGENIC RECOMBINATION

The previous section presented data which demonstrated that recombination between two r alleles is accompanied by the appearance of classes of flanking markers not explained by the reciprocal exchange hypothesis of crossing over. This section attempts to analyse further and to define the phenomenon of intragenic recombination in *Drosophila*. It is concerned with: (1) the patterns and

Table 6. *Selected reciprocal interallelic crosses*

Numbers and types of wild r recombinants from the general cross:
$tc\ r^x\ f\ B/dm\ g\ r^y\ ♀♀ \times tc\ r^x\ f\ B\ ♂♂$

Alleles		Total	Flanking marker genotype of r^+ progeny			
r^x	r^y	$r^+ \times 10^{-5}$	P_1	P_2	R_1	R_2
6	17	7·0	2	1	5	0
17	6	9·4	3	1	4	0
6	20	17·0	5	1	7	0
20	6	12·4	4	1	6	0
6	28	24·2	6	2	8	0
28	6	19·3	4	2	8	1
6	37	28·2	7	3	11	1
37	6	28·8	6	2	13	1
6	42	28·6	7	3	13	0
42	6	30·5	21	4	34	2
6	44	31·4	7	2	12	1
44	6	27·9	5	3	12	0
7	20	17·2	3	2	9	0
20	7	14·1	3	1	5	1
7	23	17·9	4	2	12	0
23	7	19·4	6	1	8	0
7	28	23·7	8	2	14	0
28	7	20·6	5	2	8	0
7	35	26·8	7	2	15	1
35	7	28·1	4	1	16	0
7	37	29·0	7	3	16	0
37	7	29·7	6	3	11	0
7	42	30·3	21	5	30	3
42	7	29·4	8	3	15	1
7	44	30·5	8	3	15	1
44	7	29·7	7	3	11	1
14	35	23·6	5	3	8	1
35	14	19·4	4	2	9	2
14	42	18·7	7	2	10	0
42	14	23·2	7	3	13	1
17	28	9·7	5	1	7	1
28	17	14·1	3	1	6	0
17	35	17·4	4	2	9	0
35	17	16·8	5	1	8	0
17	42	21·5	7	1	10	0
42	17	22·8	14	5	29	2
20	35	13·6	3	0	7	0
35	20	11·9	5	2	6	0
20	42	25·1	7	2	10	2
42	20	16·9	9	3	14	1
20	44	18·2	8	1	11	0
44	20	16·8	4	2	8	1

Table 6 (*cont.*)

Alleles		Total	Flanking marker genotype of r^+ progeny			
r^x	r^y	$r^+ \times 10^{-5}$	P_1	P_2	R_1	R_2
23	42	15·1	5	1	5	0
42	23	13·0	8	1	15	0
28	42	11·7	3	2	6	0
42	28	9·8	6	2	12	0
35	42	6·0	2	1	4	0
42	35	4·8	4	1	6	0
37	42	1·7	0	0	3	0
42	37	2·2	2	0	3	0
42	44	1·4	2	0	1	0
44	42	1·9	1	0	1	0

consistencies of flanking marker distribution after a recombinational event within the r locus; (2) evidence for map expansion (non-additive interallelic recombination frequencies); (3) an attempt to determine if gene conversion is involved in intragenic recombination at r; (4) patterns of recombination in 3-point and 4-point intragenic cross; (5) a possible case of post-meiotic segregation; and (6) patterns of interference associated with intragenic recombination.

(i) *Consistency and pattern of flanking marker distribution*

The following results verify that the patterns of flanking markers associated with a chromosome recombinant for r are consistent throughout the locus and that their appearance is predictable and reproducible. Eleven alleles spaced at approximately equal intervals along the fine structure map were crossed in all combinations *inter se*. Selected data from the *inter se* crosses are presented in Table 6. Table 6 demonstrates that recombinants between two r alleles are associated primarily with one recombinant and one parental class of flanking markers. When the position of the alleles is reversed, then the reciprocal classes of recombinant and parental markers are in the majority. This pattern is consistent throughout the locus. In a majority of the crosses, the chromosome carrying the more proximal r allele also carried the major parental class of flanking markers and the distal marker of the major recombinant class. The classes of flanking markers found associated with a wild r recombinant depend upon the relative positions of the two r alleles within the fine structure map, and not upon any other peculiar property of the r alleles themselves. The process which generates recombinants within the r locus is consistent throughout the gene, for in a majority of cases the pattern of outside marker recovery is $R_1 \geqslant P_1 > P_2 \geqslant R_2$.

An interesting feature of the data is that there is no indication of a reversal of polarity at the distal end of the locus. Polarity reversal has been found to be a common phenomenon in fungal genes (cf. Whitehouse & Hastings, 1965). Further data from three-point crosses indicate that a slight polarity reversal may occur at the distal end of the r locus (consult section 6, iv).

(ii) *Evidence for map expansion*

The data presented in Table 4 provides information on the additivity of distances between alleles within the fine structure map. More accurate map distances between each of the 11 alleles were calculated by combining the estimates of the reciprocal crosses. These values were then utilized to determine the expected distances between alleles if the frequency of intragenic recombination is additive. The additive values were calculated by summing the smallest distances which were experimentally measured between two alleles. For example: the distance
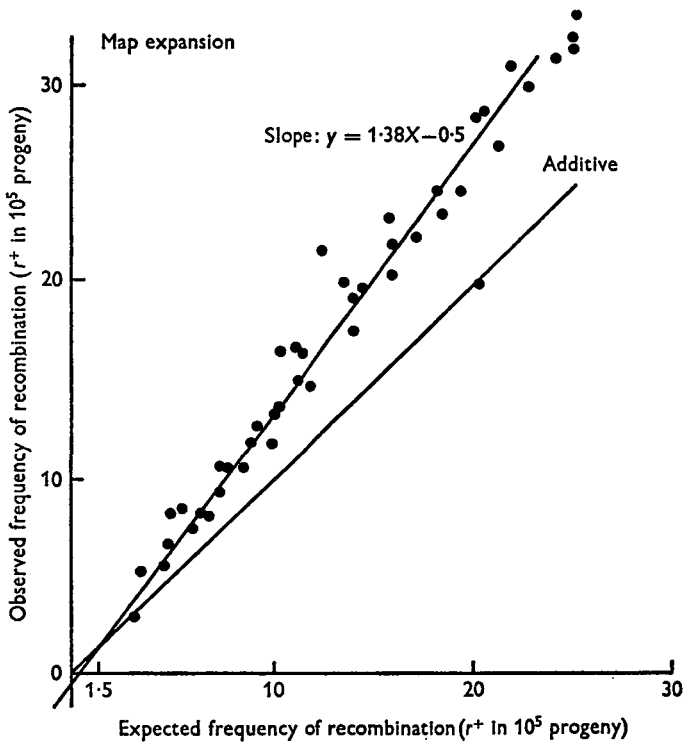
Fig. 3. Map expansion within the *r* locus.

between alleles 6 and 7 is 0·88 wild recombinants in 10^5 progeny while that between alleles 7 and 14 is 4·25. On the assumption of additivity, the distance between alleles 6 and 14 should be 5·13 wild recombinants in 10^5 progeny. In all but two cases (14/42 and 37/44) observed distances between alleles are greater than expected on the basis of additivity. A least-squares plot (Bliss, 1967) of the observed values is shown in Fig. 3. If recombination frequencies were additive, then the slope of the line would be unity. The calculated slope was 1·38 with an intercept on the Y axis at -0.5. The distance between two *r* alleles is not strictly additive. Map expansion occurs within the *r* locus. The general properties of map expansion found at *r* are similar to those found in several fine structure maps of fungal loci (consult Holliday, 1964 and 1968, for a complete discussion).

(iii) *Observations on half-tetrads*

An essential component of the analysis of intragenic recombination is verification that gene conversion is or is not occurring during a recombinational event. Extensive evidence for the occurrence of gene conversion is not available in *Drosophila*, although there are reports of high negative interference, recovery of wild-type exceptionals, and of non-reciprocal crossing over which suggest its probable existence (Welshons & von Halle, 1962; Baillie, Astell & Scholefield, 1966; Chovnick, 1961; Hexter, 1963; Green, 1960; Finnerty *et al.* 1970; and Dorn & Burdick, 1962). Since all four meiotic products cannot be isolated in *Drosophila*, data relating to the occurrence of gene conversion must come from an analysis of half-tetrads.

The *r* locus offers a favourable system for half-tetrad work because: (1) the screening system designed to recover wild recombinants in free X chromosomes can also be used with attached-X chromosomes, (2) it is possible to identify different *r* alleles unambiguously on the basis of their known patterns of complementation, and (3) the system is not based on examination of flanking markers for the analysis of intragenic events. Two *r* alleles which do not complement but which have different patterns of complementation are placed in separate arms of an attached-X chromosome. Wild recombinants between the two alleles are then selected by the screening technique. The allelic composition of both the arm carrying the wild recombinant and the arm opposite the recombinant can be analysed without detachment of the attached-X. The recombinant arm and the arm opposite are both obtained in a homozygous condition by selecting for cross-overs in the attached-X which occur proximally to the proximal flanking marker. The presence of either *r* allele or of both alleles in the opposite arm is then determined by testing the homozygous attached-X with duplications carrying *r* alleles with known complementation patterns. Since each allele in the original attached-X chromosome has a different pattern of complementation, then the pattern of complementation of *r* in the opposite arm defines its allelic composition. These complementation tests are able to distinguish each *r* allele either singly or in double mutant combination.

If the wild-type exceptional females are generated by reciprocal recombination, two types of r^+ females would be predicted to occur with equal frequency. Reciprocal exchange between two *r* alleles involving chromatids attached to different centromeres will yield an attached-X chromosome carrying an r^+ in one arm and a single *r* in the opposite arm. This single *r* allele will always be the more proximal of the two alleles in the parental heterozygous attached-X (Fig. 4, no. 2). Exchange between two *r* alleles involving chromatids attached to the same centromere will yield an attached-X chromosome carrying an r^+ allele in one arm and both *r* alleles in the opposite arm (Fig. 4, no. 1).

If the wild-type exceptional females arise from non-reciprocal conversion events, two types of r^+ female would be predicted. If the more distal *r* allele is converted to wild type, then the resulting attached-X chromosome will carry

one arm containing an r^+ allele and one arm containing the more proximal r allele (Fig. 4, no. 3). Conversion of the more proximal r allele would yield an attached-X carrying an r^+ allele in one arm and the more distal r allele in the opposite arm (Fig. 4, no. 4).

Distinction between the two models is based on their contrasting predictions for r^+ offspring. Individual reciprocal recombinational events cannot generate a singly mutant arm of an attached-X which carries the more distal r allele. Likewise, single conversion events do not yield chromosomes carrying both r alleles in the same arm.
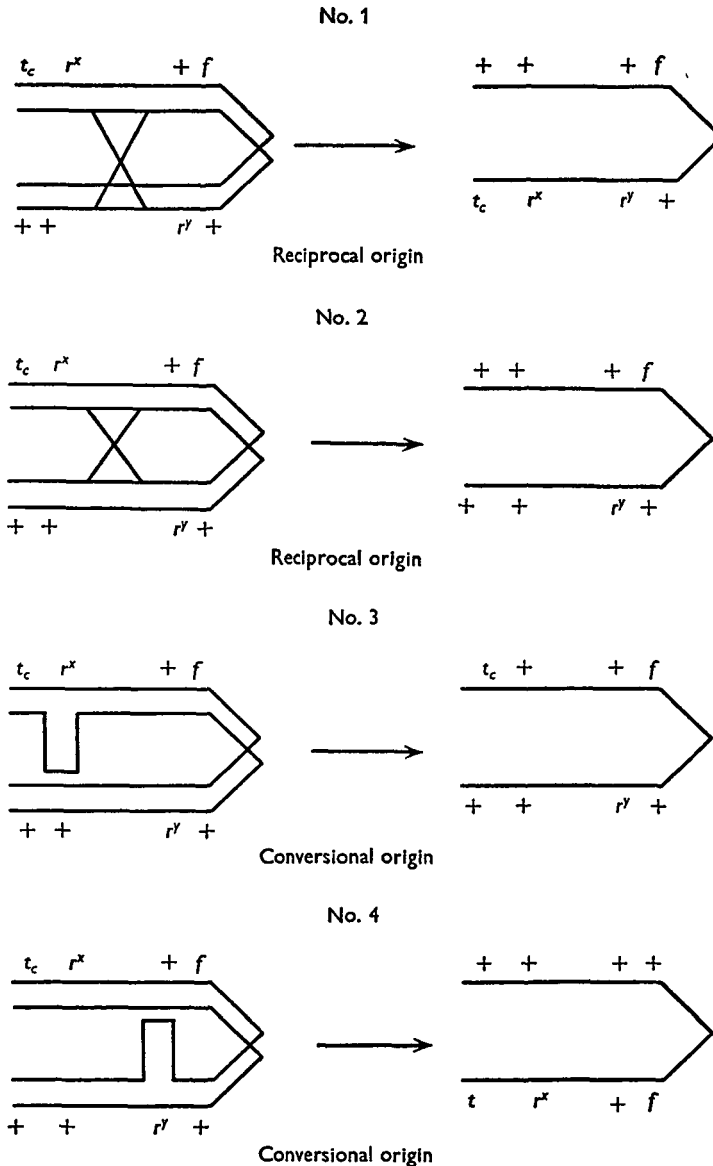
Fig. 4. Graphic representation of the r^+ products from reciprocal exchange and gene conversion in attached-X chromosomes.

The data for six combinations of r alleles in attached-X chromosomes are given in Table 7. The data demonstrate that neither reciprocal recombination nor gene conversion are exclusive mechanisms of intragenic recombination. Of 92 r^+ recombinants in heterozygous attached-X chromosomes that were analysed, 78 carried a single r allele in the non-sister chromatid while 14 carried both r alleles in the non-sister chromatid. Of the 78 single r alleles, 41 were the more proximal allele and 37 the more distal allele. It would appear as if approximately 30 % of the intragenic recombinants arose by reciprocal recombination (15 % of the recovered opposite arms carried a double mutant r locus. This number is only one-half of the recombinants which arose by this mechanism). A substantial proportion of the r^+ recombinants were generated by a conversion mechanism since more than one-third of the recovered opposite arms carried the more distal single r allele.

Table 7. *Data from half-tetrad analysis*

(Summary of the allelic composition of non-sister chromatids attached to a common centromere as an r^+ recombinant from the attached-X chromosome $y^2\,tc\,r^x f\,B\,.\,r^v\,y^2$.)

r alleles in the attached-X chromosomes	r^x / r^v						Total
	r^{27} r^{41}	r^{27} r^{34}	r^{27} r^{20}	r^{27} r^{14}	r^{28} r^{23}	r^{25} r^{38}	
Total no. r^+ recombinants analysed	28	5	5	29	16	9	92
Alleles in non-sister chromatids:							
A. Single proximal allele	13	2	3	13	6	4	41
B. Double allelic combination	5	0	0	5	3	1	14
C. Single distal allele	10	3	2	11	7	4	37

The reciprocal exchange model of recombination predicts that 14 of the singly mutant arms carrying a proximal r allele were also generated by a single exchange mechanism. Hence, of the arms carrying single r alleles which appear to be the products of gene conversion, 27 carried the more proximal r allele while 37 carried the more distal r allele. It would appear that conversion of the proximal r allele occurs more frequently than conversion of the distal.

(iv) *Patterns of recombination in three- and four-point intragenic crosses*

Patterns of recombination between three and four alleles within a locus during a single recombinational event may provide some insight into the mechanism of intragenic recombination. It is of some interest to note that the double mutant chromosomes recovered from the attached-X studies are all totally non-complementing. Even though the individual complementation patterns of the two alleles composing the double mutant would suggest that their combination should still complement a third allele, such expected complementation was never observed. All double mutants behaved as totally non-complementing (I–VII) alleles. The observation is not entirely expected since double mutant combinations of complementing alleles at other loci (e.g. *pan-2* in *Neurospora*, Case & Giles, 1958) have not demonstrated totally non-complementing behaviour. The experimental procedure for completion of the three- and four-point recombination studies was essentially as follows: A chromosome carrying a double mutant r locus was made

heterozygous for either a single r allele or another double mutant r chromosome. Wild recombinants were then recovered in the progeny.

Diagrammatically:

$$\frac{tc \; r^x \; r^y \; f \; B}{dm \; g \qquad r^z} \quad \text{or} \quad \frac{tc \; r^x \; r^y \; f \; B}{dm \; g \quad r^z \; r^{zz}} \, \female\female \times \frac{tc \; r^{42} \; f \; B}{7} \, \male\male$$

$$\downarrow$$

Escapers and wild r recombinants

Table 8. *Three- and four-point intragenic crosses*

A. Characteristics of double mutant chromosomes

Number	Alleles in double mutant	Distance between alleles (r^+ recombinants $\times 10^{-5}$ progeny)	Complementation pattern*
(1)	27 (IV–V), 41 (I–IV)	9	I–VII
(2)	14 (V–VII), 27 (IV–V)	13	I–VII
(3)	23 (V–VI), 28 (I–V)	3	I–VII
(4)	25 (IV–VI), 38 (I–IV)	7	I–VII
(5)	23 (V–VI), 39 (I–II)	11	I–VII

* Note the totally non-complementing pattern of doubles which might be expected to show complementation on the basis of their individual patterns.

B. Three-point crosses

(1) $tc \; r^{23} \; r^{28} \; f \; B/dm \; g \; r^x \, \female\female \times tc \; r^{42} \; f \; B \, \male\male$

r^+	No. of escapers	Flanking marker genotype of r^+ progeny / Progeny crossover types				Total $r^+ \times 10^{-5}$	Expected frequency of r^+ recombinants $\times 10^{-5}$
		$+ \; r^+ \; +$;	$tc \; r^+ \; f$;	$tc \; r^+ \; +$;	$+ \; r^+ \; f$		
$x = 7$ (distal)	2274	4	0	4	1	3·96	18
$x = 25$ (middle)	3739	1	0	0	1	0·54	—
$x = 42$ (proximal)	1147	4	2	0	5	9·59	10

(2) $tc \; r^{23} \; r^{39} \; f \; B/dm \; g \; r^x \, \female\female \times tc \; r^{42} \; f \; B \, \male\male$

r^x	No. of escapers	Flanking marker genotype of r^+ progeny / Progeny crossover types				Total $r^+ \times 10^{-5}$	Expected frequency of r^+ recombinants $\times 10^{-5}$
		$+ \; r^+ \; +$;	$tc \; r^+ \; f$;	$tc \; r^+ \; +$;	$+ \; r^+ \; f$		
$x = 7$ (distal)	2571	6	0	5	1	4·67	18
$x = 31$ (middle)	3070	5	0	0	1	1·95	—
$x = 42$ (proximal)	1654	2	0	1	0	1·85	2·2

C. Four-point cross

$tc \; r^{23} \; r^{39} \; f \; B/dm \; g \; r^{14} \; r^{27} \, \female\female \times tc \; r^{42} \; f \; B \, \male\male$

Total no. escapers	r^+ recomb. per 10^5 progeny	Flanking marker genotypes of r^+ progeny			
		$+ \; r^+ \; +$;	$tc \; r^+ \; f$;	$tc \; r^+ \; +$;	$+ \; r^+ \; f$
4815	0·21	0	1	0	0

Recall that in two-point crosses the more proximal r allele always contributed the major parental class of markers associated with a wild recombinant as well as the distal marker of the major recombinant class of flanking markers. The data for the three- and four-point crosses are presented in Table 8. First consider the three-point crosses. If r^z is the more proximal of the three r alleles, then the number of wild recombinants and their flanking marker constitution is essentially that expected from a two-point recombinational event between the two proximal r alleles, i.e. r^y and r^z. If r^z is located between the two opposite r alleles, then the number of wild recombinants is quite low. The number of wild recombinants is, however, greater than expected if recombination between r^x and r^z and between r^z and r^y occurred as two independent events. The flanking marker constitution is primarily the parental class carried by the chromosome containing the single allele, r^z. If r^z is the most distal of the three r alleles, then recombination is disturbed. The frequency of recombination is less than a quarter of that expected if recombination occurred only between the two distal r alleles, i.e. r^z and r^x. The primary class of flanking markers associated with wild recombinants is the parental class carried by the chromosome marked with the more distal r allele (r^z).

The data may be taken to indicate a slight reversal of polarity at the distal end of the locus. Intragenic recombination appears to proceed normally in the proximal region of the locus, but not in the distal region in three-point crosses. The data are most easily explained by assuming that the region proximal to the recombinational event is normally essential for its completion. However, less frequently, the region distal to the recombinational event becomes the essential segment of the locus. Though a reversal of polarity at the distal end of the locus is not detectable in the data from two-point crosses, the results from the three-point crosses point to its probable occurrence.

Only very scant information can be drawn from the four-point cross. The frequency of wild recombinants is greater than that expected for the three simultaneous events needed to generate a wild recombinant in such a cross if each event occurred independently. Further speculation would be meaningless.

The fact that wild r recombinants are recovered in three- and four-point intragenic crosses excludes the possibility that double mutant r loci are deficiencies. Hence, the non-complementing behaviour of the double mutants must have its explanation in a functional basis rather than that of a genetic deficiency.

(v) *A possible case of post-meiotic segregation*

During the course of the fine structure mapping an odd male appeared from the cross, $tc\ r^{42}\ f\ B/dm\ g\ r^{24}\ ♀♀ \times tc\ r^{42}\ f\ B\ ♂♂$. The right wing of the male was r while the left wing was wild in morphology. The male transmitted only sperm of the composition $tc\ r^+\ f\ B$. The right wing was typically r so that there is a minimum chance of misclassification. The wild r recombinant displayed no abnormal behaviour over the three generations in which it was progeny tested. This observation may be accounted for by post-meiotic segregation. The phenomenon might also be due to the occurrence of a back mutation, to the occurrence

of a second site mutation, or to a closely linked suppressor mutation. Other explanations (such as the occurrence of a loosely linked suppressor mutation) were eliminated by progeny testing.

(vi) *Patterns of interference associated with intragenic recombination*

Evidence from fungal systems indicates that intragenic recombination does affect recombination in more distant regions of the chromosome. Generally, different patterns of interference are associated with different types of intragenic recombination. Recombinants which carry the R_1 and R_2 flanking marker classes usually show positive interference while those which carry the P_1 and P_2 flanking marker classes show a lack of either positive or negative interference (cf. Whitehouse, 1970). Data relating to recombination between g and tc and between f and B which is associated with intragenic recombination at r is available from the results of progeny testing. Note that g and tc are 7·2 map units apart while f and B are 0·3 map units distant (Table 2). A total of 2954 wild r recombinants were

Table 9. *Patterns of interference associated with intragenic recombination at* r

Frequency of recombination (in map units)

Internal	Class of flanking marker associated with wild r recombinant	
	Parental ($n = 1189$)	Recombinant ($n = 1765$)
g–tc	6·2 ($n = 74$)	0·05 ($n = 1$)
f–B	0·25 ($n = 3$)	0 ($n = 0$)

recovered in which all flanking markers could be determined. Of this number 1189 carried parental combinations of tc and f and 1765 carried recombinant combinations of the two markers. Table 9 presents the data relating the frequency of recombination in each interval and the type of intragenic recombinant. It is obvious that different kinds of intragenic recombination are associated with different patterns of interference. Wild r recombinants carrying parental classes of markers show little or no interference. However, wild r recombinants carrying recombinant classes of markers show definite positive interference. This finding is in agreement with the work on fungal genes.

7. DISCUSSION

(i) *Functional organization of the* r *locus*

Several lines of circumstantial evidence indicate that the typical r phenotype has as its biochemical basis a single functional product. The finding that both the female sterility and wing phenes have identical patterns of complementation argues that both phenes are dependent upon a common biochemical process.

(ii) *Structural organization of the* r *locus*

A number of different lines of evidence point to the conclusion that r is best described by the model of a single cistron exhibiting a complex pattern of interallelic complementation. The following deductions can be made on the basis of the assumption that the pattern of interallelic complementation at r is due to homologous subunit aggregation of the protein produced by the r locus. The facts that (1) all 45 alleles used in this study demonstrated a homogeneous phenotype when assayed under well-defined conditions, that (2) the pattern of interallelic complementation is consistent with that expected on the basis of a single polypeptide, and that (3) the complementation map is co-linear with the genetic fine structure map, are all consistent with the model of a single simple gene. A comparison of the genetic and complementation maps demonstrates that non-complementing mutants reside at both ends of the genetic map, a fact inconsistent with any model of genetic organization except that of a single cistron exhibiting a complex pattern of interallelic complementation. Logical deduction further excludes the possibility that r consists of several linked cistrons in a complex genetic unit. The possibility that r consists of several linked cistrons which are independently transcribed and translated is excluded by the observation that the non-complementing r alleles map as point mutants. Furthermore, such an interpretation is inconsistent with the complementation map. The possibility that r consists of several cistrons which are co-transcribed and co-translated is eliminated by the fact that non-complementing mutants reside at both ends of the genetic map. Moreover, r double mutants have all proven to be totally non-complementing and show no complementation with a third allele with which they would be expected to complement on the basis of the properties of the three individual alleles.

Rudimentary appears to be best interpreted as a single cistron whose product is required for several distinct developmental processes and whose alleles display a complex pattern of interallelic complementation. Any further speculation must await a biochemical knowledge of the action of the locus.

(iii) *Validity of the pseudoallelic model*

An important problem to which this study was addressed is the general validity of the pseudoallelic model of genetic organization. Is it possible to exclude the pseudoallelic interpretation as a plausible model for the genetic organization of r? Exclusion of this model essentially requires the identification of a genetic unit of function and the demonstration that recombination occurs between allelic mutants deficient for that function. If the entire r locus is considered to be a single genetic unit of function, then obviously the pseudoallelic interpretation does not adequately describe its organization. Recombination readily occurs between functionally allelic mutants. However, final exclusion of the pseudoallelic model demands positive evidence that r is not composed of a number of similarly functioning units. Such evidence comes from the complementation results of

double mutant chromosomes. If r were composed of a number of individually functioning units, then a chromosome carrying two r alleles would be expected to exhibit the sum of the complementation patterns of the two alleles. Each allele as an independent functioning entity should retain its own characteristics. This expectation is not observed. Instead, all double mutant chromosomes have been found to be totally non-complementing, arguing that all r alleles are mutant for one and the same functional process. Hence, the pseudoallelic model is not an adequate description of the organization found at the r locus.

(iv) *Observations on the* r *alleles*

The r alleles used in this study most probably represent amorphic mutants of the r locus. The amorphic character of the mutants best explains the uniform phenotypic expression of all the alleles assayed, and the fact that $Df(1)r^{69c}$ which is cytologically deficient for a portion of the 14D 1–2 region of the salivary chromosomes has a typical r phenotype (consult Carlson, 1970).

One interesting aspect of the data presented in this work is evidence for the specificity of some mutagens for certain intragenic regions. A comparison of the position of an allele within the genetic fine structure map and the mutagen used to induce the allele (Table 1) shows that some mutagens attack only a limited number of sites. In general, there is no definite regional specificity on the part of chemical mutagens. There is, however, evidence of regional specificity of the X-ray induced and spontaneously occurring alleles. All of the X-ray induced and most of the spontaneously arising alleles map within three distinct intragenic regions (alleles 8–12; alleles 26–32; and alleles 41–45). Similar phenomenon have been observed in fungal genes (e.g. Ishikawa, 1962). Though no similar observations of interallelic specificity have been made with *Drosophila*, this finding is of importance for the interpretation of previous work on genetic fine structure. Since the majority of mutants used in previous studies were either of X-ray or spontaneous origin, the discontinuous clustering of alleles so often observed at pseudoallelic loci may well be an artifact imposed by the mode of origin of the mutants.

(v) *Comments on complex genes in* Drosophila

In a recent analysis of the *maroon-like* cistron Chovnick and his associates (Chovnick *et al.* 1969) proposed a model for the interpretation of the pleiotropic effects associated with mutants of complex genes. In most work with complex genes evidence for pleiotropic morphological and developmental mutant effects has been explained by a correspondingly complex genetic organization of the locus. However, if the pleiotropic effects associated with different mutants or alleles of a complex gene are interpreted as biological assays of differing sensitivity for the functioning of a single, unknown gene product, then a morphologically complex locus might have a single cistron as its genetic basis. Chovnick has shown that this model provides the explanation for the complex phenotypic effects associated with alleles of the *maroon-like* cistron. The model also provides an adequate explanation of the functional and structural organization of the r cistron.

(vi) *Observations on intragenic recombination*

When recombination between alleles was first observed in *Drosophila* (Oliver, 1940) it was thought to occur by the same reciprocal mechanism as intergenic recombination, since interallelic recombination was accompanied by outside marker exchange, and since it was possible to recover the expected, reciprocal products. The data presented in this work are not compatible with the interpretation that intragenic recombination occurs only by single reciprocal exchanges.

Any interpretation of the mechanism of recombination within the *r* locus must account for certain facts. Observations on the random products of meiosis indicate that (1) the *r* locus consists of a unique linear order of alleles, that (2) there is a general additivity of map distances within the locus with evidence for map expansion, and that (3) there is a consistent pattern of non-recombinant flanking markers associated with intragenic recombination within *r*. Observations on half-tetrads indicate that both gene conversion and reciprocal recombination are mechanisms which generate intragenic recombinants, and that there appears to be a preferential conversion of the more proximal *r* allele.

An interesting observation relating to the mechanism of recombination is the co-incidence of two seemingly unrelated quantities. At a distance of approximately $1\cdot5$ wild recombinants in 10^5 progeny or less, wild *r* recombinants are preferentially associated with the P_1 class of flanking markers. Also, at approximately $1\cdot5$ wild recombinants in 10^5 progeny or below, the phenomenon of map expansion is not detectable.

(vii) *Genetic organization in eukaryotes*

The structural and functional organization of the *r* locus is entirely compatible with that found in work with functionally defined fungal genes. The mechanism of intragenic recombination within the *r* locus appears identical to that operating in fungi. Intragenic recombination in *r* can be interpreted by the current models proposed to explain intragenic recombination in fungi. Similar results have recently been reported for several other genes in *Drosophila* (*rosy*, and *maroon-like*, Chovnick *et al.* 1970; Smith, Finnerty & Chovnick, 1970). Hence, genetic organization in lower and higher eukaryotes appears to be very similar if not identical.

REFERENCES

BAILLIE, D. L., ASTELL, C. & SCHOLEFIELD, J. (1966). Double crossovers within a short genetic interval in *Drosophila melanogaster*. *Canadian Journal of Genetics and Cytology* **8**, 350.

BLISS, C. I. (1967). *Statistics in Biology*, vol. I, 558 pp. New York: McGraw-Hill.

BROSSEAU, G., NICOLETTI, B., GRELL, E. & LINDSLEY, D. (1961). Production of altered Y chromosomes bearing specific sections of the X chromosome in *Drosophila*. *Genetics* **46**, 339–346.

CARLSON, P. S. (1970). A genetic analysis of the rudimentary locus of *Drosophila melanogaster*. Ph.D. Dissertation, Yale University, New Haven, Conn.

CASE, M. E. & GILES, N. H. (1958). Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. *Proceedings of the National Academy of Sciences* (*Washington*) **44**, 378–390.

CATCHESIDE, D., JESSOP, A. & SMITH, B. (1964). Genetic controls of allelic recombination in *Neurospora*. *Nature, London* **202**, 1242–1243.

CHOVNICK, A. (1961). The *garnet* locus in *Drosophila melanogaster*: I. Pseudoallelism. *Genetics* **46**, 493–507.

CHOVNICK, A., BALLANTYNE, G. A., BAILLIE, D. L. & HOLM, D. G. (1970). Gene conversion in higher organisms: Half-tetrad analysis of recombination within the *rosy* cistron of *Drosophila melanogaster*. *Genetics* **66**, 315–329.

CHOVNICK, A., FINNERTY, V. G., SCHALET, A. & DUCK, P. (1969). Studies on genetic organization in higher organisms: I. Analysis of a complex gene in *Drosophila*. *Genetics* **62**, 145–160.

COUNCE, S. J. (1956). Studies on female-sterility genes in *Drosophila melanogaster*: III. Effect of the gene rudimentary on embryonic development. *Zeitschrift Fur Vererbungslehre* **87**, 482–491.

DOANE, W. W. (1960*a*). Developmental physiology of a female sterility mutant in *Drosophila melanogaster*. Ph.D. Dissertation, Yale University, New Haven, Conn.

DORN, G. L. & BURDICK, A. B. (1962). On the recombinational structure and complementation relationships in the *m-dy* complex of *Drosophila melanogaster*. *Genetics* **47**, 503–518.

FAHMY, O. & FAHMY, M. (1959). Complementation among the sub-genic mutants of the *r* locus of *Drosophila melanogaster*. *Nature, London* **184**, 1927–1929.

FINCHAM, J. R. S. (1966). *Genetic Complementation*, 143 pp. New York: W. A. Benjamin, Inc.

FINNERTY, V. G., DUCK, P. & CHOVNICK, A. (1970). Studies on genetic organization in higher organisms: II. Complementation and fine structure of the *maroon-like* locus of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* (*Washington*) **65**, 939–946.

GILLIE, O. (1966). The interpretation of complementation data. *Genetical Research* **8**, 9–32.

GREEN, M. M. (1960). Double crossing over or gene conversion at the *white* locus in *Drosophila melanogaster*? *Genetics* **45**, 15–18.

GREEN, M. M. (1963*a*). Interallelic complementation and recombination at the rudimentary wing locus in *Drosophila melanogaster*. *Genetics* **34**, 242–253.

GREEN, M. M. (1963*b*). Pseudoalleles and recombination in *Drosophila*. In *Methodology in Basic Genetics*, p. 279. Ed. W. Burdette. San Francisco: Holden-Day.

HEXTER, W. (1963). Non-reciprocal events at the *garnet* locus in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* (*Washington*) **50**, 372–380.

HOLLIDAY, R. (1964). A mechanism for gene conversion in fungi. *Genetical Research* **5**, 282–304.

HOLLIDAY, R. (1968). Genetic recombination in fungi. In *Replication and Recombination of Genetic Material*, p. 157. Ed. W. Peacock and R. Brock. Canberra: Australian Academy of Sciences.

ISHIKAWA, T. (1962). Genetic studies of *ad-8* mutants in *Neurospora crassa*. I. Genetic fine structure of *ad-8* locus. *Genetics* **47**, 1147.

LEWIS, E. B. (1967). Genes and gene complexes. In *Heritage from Mendel*, pp. 17–47. Ed. R. A. Brink. Madison: University of Wisconsin Press.

LINDSLEY, D. L. & GRELL, E. H. (1967). Genetic Variations of *Drosophila melanogaster*. *Carnegie Institution of Washington Publication*, no. 627.

LYNCH, C. (1919). An analysis of certain cases of intra-specific sterility. *Genetics* **4**, 501–533.

MORGAN, T. H. (1912). A modification of the sex-ratio and of other ratios in Drosophila through linkage. *Zeitschrift Fur Vererbungslehre* **7**, 323–345.

MORGAN, T. H. (1915). The infertility of *rudimentary* winged females of *Drosophila ampelo-*. *phila*. *American Naturalist* **49**, 240-250.

MULLER, H. (1936). Insertion of desired genes into attached-X's. *Drosophila Information Service* **6**, 8.

OLIVER, C. P. (1940). A reversion to wild type associated with crossing over in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* (*Washington*) **26**, 452–454.

POULSON, D. F. (1950). Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster* Meign. in Biology of *Drosophila*, pp. 168–274. Ed. M. Demerec. New York: John Wiley and Sons.

SMITH, P. D., FINNERTY, V. G. & CHOVNICK, A. (1970). Gene conversion in *Drosophila*: non-reciprocal events at the *maroon-like* cistron. In manuscript.

WELSHONS, W. G. & HALLE, VON E. S. (1962). Pseudoallelism at the Notch locus in Drosophila. *Genetics* **47**, 743–759.

WHITEHOUSE, H. (1963). A theory of crossing over by means of hybrid deoxyribonucleic acid. *Nature, London* **199**, 1034–1035.

WHITEHOUSE, H. (1970). The mechanism of genetic recombination. *Biological Reviews* **45**, 265–315.

WHITEHOUSE, H. & HASTINGS, P. (1965). The analysis of genetic recombination on the polaron hybrid DNA model. *Genetical Research* **6**, 27–92.