STUDIES ON GENE CONVERSION AND ITS RELATIONSHIP TO LINKED EXCHANGE IN *DROSOPHILA MELANOGASTER*[1]

ARTHUR CHOVNICK, GRAEME H. BALLANTYNE[2] AND DAVID G. HOLM[3,4]

Genetics and Cell Biology Section, Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268

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GENE conversion and its relationship to linked recombination has been the subject of extensive investigation in fungal systems, and the subject of recent reviews (EMERSON 1969; WHITEHOUSE 1970). In higher eukaryotes, indications of conversion were first reported in the earliest study of intracistronic recombination in Drosophila, involving the garnet locus (CHOVNICK, LEFKOWITZ and McQUINN 1956; CHOVNICK 1958, 1961; HEXTER 1958, 1963). Additional observations suggestive of conversion have since been reported with other Drosophila genetic systems (BAILLIE, ASTELL and SCHOLEFIELD 1966; GREEN 1960; WELSHONS and VON HALLE 1962), and in maize as well (NELSON 1962; SALAMINI and LORENZONI 1970). However, rigorous confirmation of conversion in higher eukaryotes has come only recently in association with the development of suitable genetic systems for systematic investigation (BALLANTYNE and CHOVNICK 1971; CARLSON 1971; CHOVNICK *et al.* 1970; FINNERTY, DUCK and CHOVNICK 1970; SMITH, FINNERTY and CHOVNICK 1970).

The prior work with Drosophila, based entirely upon random strand and half-tetrad analysis of meiotic products in crosses involving very close linkage (i.e., mutants within a cistron) provides a series of parallels between classical crossing over and conversion. Like crossing over; (1) conversion is seen in mutant heterozygotes, and not in homozygotes (CHOVNICK 1961; CHOVNICK *et al.* 1970); (2) conversion occurs in meiosis in females, but not in males of *Drosophila melanogaster* (CHOVNICK *et al.* 1970); (3) it may be suppressed by heterozygous rearrangements with breaks immediately flanking the region of interest (CHOVNICK 1961); (4) the wild type alleles generated by conversion in various mutant allele heterozygotes are identical to each other, and indistinguishable from apparent classical wild-type crossovers, as well as a control stock wild-type allele. in terms of gene product function (CHOVNICK *et al.* 1970; FINNERTY, DUCK and CHOVNICK 1970). These parallels between crossing over and conversion, taken together with the fact that conversion is seen only in cases of exceedingly tight linkage, suggest that meiotic products identified as conversions represent observations on the products of linked exchange, when examined in the immediate

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[2] Present address: Department of Microbiology and Genetics, Massey University, Palmerston North, New Zealand.

[3] Trainee of the Institute of Cellular Biology, supported by Training Grant 5T1 GM317 from the Public Health Service.

[4] Present address: Department of Zoology, The University of British Columbia, Vancouver, British Columbia.

region of the exchange event. The present report represents continuing progress in the experimental analysis of conversion events in *Drosophila melanogaster*. The results of these studies, taken collectively, provide the basis for a formal model of linked exchange in which a single process, always non-reciprocal in the immediate region of the event, leads to the production of both apparent crossovers and conversions, in contrast to the dogma of classical crossing over which required that chromosomes break and exchange at precisely homologous points.

MATERIALS AND METHODS

The Genetic System: The rosy cistron in *Drosophila melanogaster* (*ry*:*3*–52.00) is a solitary unit concerned with the enzyme xanthine dehydrogenase (XDH), located within an intensely mapped short region of the right arm of chromosome *3* (salivary section 87D). Of the various genetic systems used in studies of genetic organization in Drosophila and other multicellular organisms, the rosy cistron stands as an example of the simplest type. Strong genetic and biochemical evidence argues that it is a structural gene for XDH (GRELL 1962; YEN and GLASSMAN 1965). Mutations restricted to the rosy cistron are homozygous viable, and fall into three groups: (1) a class of "wild type isoalleles" which produce electrophoretic variants of the enzyme (YEN and GLASSMAN 1965); (2) a "leaky" mutant which has very much reduced enzyme activity (HUBBY 1961); (3) and a large group of mutants which are enzymatically inactive and exhibit a brownish mutant eye color phenotype resulting from a reduction in the red (drosopterin) pigments (CHOVNICK *et al.* 1964). Study of this last class of mutants failed to find any evidence of allele complementation (SCHALET, KERNAGHAN and CHOVNICK 1964), and investigation of allele recombination has been restricted to this class of mutants.

Figure 1 presents a map of the centromere proximal region of the right arm of chromosome *3* of *Drosophila melanogaster* indicating the location of rosy, the centromere, and other markers used in the study (LINDSLEY and GRELL 1968). In addition, Figure 1 presents a summary map of separable sites within the rosy cistron obtained from prior random strand mapping experiments (CHOVNICK 1966). These earlier mapping experiments utilized a system which selected for survival only those progeny receiving a single (or odd-numbered multiple) meiotic exchange product between markers flanking the rosy cistron, and consequently suppressed observations on conversion.

Selective system matings–random strand analysis: Most of the experiments described in the present report involve scale crosses of free-third chromosome strains. Rosy mutant hetero-

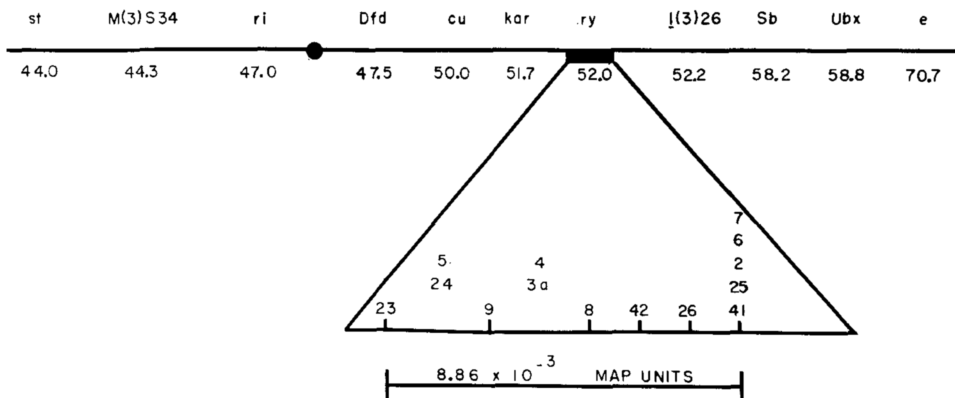

FIGURE 1.—A genetic map of the rosy region of chromosome 3. Various mutants used in this study are indicated, and the genetic fine structure of the rosy cistron is summarized.

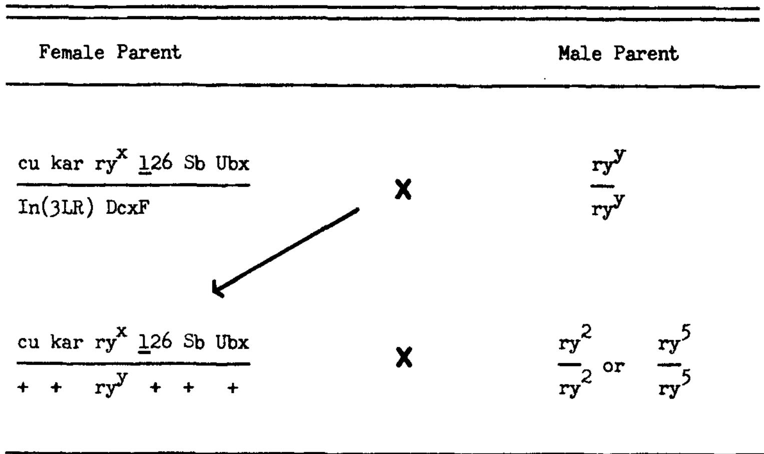

FIGURE 2.—Production of rosy mutant heterozygous females, and subsequent test cross on selective medium.

zygous females are generated from preliminary crosses and mated as indicated in Figure 2. Large scale crosses of such heterozygotes involve matings of 15 pairs of parents per half-pint milk bottle, on standard Drosophila medium, maintained at 23°–26°C. Following an initial 3 day incubation, the parents are transferred to fresh culture at 2-day intervals, through a total of four breeds of progeny representing nine days of egg laying. Experiments are run in numbered trays, each containing thirty (30) numbered bottles. Brood transfer of parents is to numbered bottles and trays such as to permit identification of possible clusters of exceptional progeny. Immediately after each transfer, 1.4 ml of 0.2% aqueous purine (Sigma Chemical Co.) is added to each developing culture which contains approximately 50 ml of standard medium. Prior experiments revealed that such supplementation effectively kills all mutant zygotes lacking XDH activity, without significantly influencing either hatchability or survival of zygotes possessing this activity (FINNERTY, BAILLIE and CHOVNICK 1970; FINNERTY, DUCK and CHOVNICK 1970). Estimation of the total number of zygotes sampled in each cross is accomplished, as in the prior work, by omitting purine from a sample of the cultures in each experiment, and counting total progeny in those bottles.

Such purine treatment leads to the production of cultures rich in larval growth, but only rare individuals complete development and emerge as adults. The surviving exceptions are ry+ in eye color phenotype, and occur as single individuals of either sex, distributed at random among the matings.

Genetic tests of exceptional progeny–random strand analysis: Surviving progeny of the selective system crosses (Figure 2), invariably (A) ry+Sb or (B) ry+Sb+ in phenotype, are subjected to the following crosses to tester stocks in order to determine completely the distribution of flanking markers present on their respective *ry+* chromosomes. Class A exceptionals are mated individually to $kar^2 l26d/In(3LR)DcxF$ flies. The absence of Sb,D+ flies among the progeny indicates the presence of the *l26* flanking marker on the *ry+Sb* exceptional chromosome. The presence of Sb,D+ progeny, indicating the absence of *l26* on this chromosome, permits determination of the presence or absence of the *kar* marker by examination of the eye color phenotype of the Sb,D+ offspring. In either case, three Sb,D male progeny are mated individually to $cu\ kar\ ry^{23}/cu\ kar\ ry^{23}$ females in order to confirm the presence of *ry+* on the *Sb* bearing chromosome as well as to classify the chromosome with respect to the *cu* and *kar* markers. Class B exceptionals are mated individually to $cu\ kar\ ry^{26}\ l26\ Sb/In(3LR),\ cu\ kar\ Ubx^A$ flies. The presence of ry+Sb progeny confirms the presence of *ry+* on a *Sb+* bearing chromosome, indicates the absence of the *l26* marker on that chromosome, and permits determination of *cu* and *kar*

markers from the phenotype of these flies. The absence of ry+Sb progeny indicates the presence of *l26* on the *ry+* chromosome. In such cases, examination of the Ubx^A progeny permits determination of the *cu* and *kar* markers, and matings of the Ubx^A progeny to *cu kar* ry^{23}/*cu kar* ry^{23} permit confirmation of the diagnosis with respect to *cu, kar,* and *ry*. All mutants and chromosomes discussed in this section are described elsewhere (LINDSLEY and GRELL 1968) with the exception of *l26d*. Produced in this laboratory from X-ray treatment of a kar^2ry^+ chromosome, *l26d* is a short deletion of part of salivary chromosome section 87E (LEFEVRE 1970), lethal with *l26*, and deficient for another gene complex presently under study in this laboratory.

Half-tetrad experiment: Construction, maintenance, selective system mating and detachment of exceptional half-tetrads as well as their genetic analysis follow the procedures described in prior reports (CHOVNICK *et al.* 1970; BALLANTYNE and CHOVNICK 1971.

RESULTS

General features of the analysis: Table 1 summarizes rosy mutant homozygote reversion frequency experiments carried out over the several years of this investigation. Except for the fact that mutant homozygotes were used as parents, these experiments followed the selective system protocol described above. No *ry+* progeny were recovered from any of the reversion experiments, indicating reasonable stability of these mutants in homozygotes. On the basis of these data, estimation is made of the maximum spontaneous reversion frequency for each allele, and these estimates, included in Table 1, serve as a reference control for the results described below.

Figure 2 illustrates the basic cross involving free third chromosome rosy mutant heterozygote females, marked as indicated, with a series of outside flanking markers. Exceptional ry+ progeny are produced, and it is inferred that they arose from events which occurred in the rosy mutant heterozygous female parents. Justification for this inference is based upon (1) extensive documentation of this point presented in prior reports (CHOVNICK *et al.* 1970; BALLANTYNE and CHOVNICK 1971), as well as (2) the homozygote spontaneous reverse mutation control data of Table 1. This study was carried out as a series of crosses using ry^2 homozygotes as male parents, and then repeated using ry^5 homozygotes as male parents. There being no difference in the pattern of results obtained, the two series of experiments were pooled. We admit that one, or as many as several,

TABLE 1

Homozygous reversion tests of the indicated rosy mutants

Mutant	Total chromosomes samples	Maximum estimated reversion frequency*
ry^1	2.0×10^6	1.5×10^{-6}
ry^2	1.9×10^6	1.6×10^{-6}
ry^5	2.0×10^6	1.5×10^{-6}
ry^8	0.84×10^6	3.6×10^{-6}
ry^{26}	1.6×10^6	1.9×10^{-6}
ry^{41}	0.92×10^6	3.3×10^{-6}
ry^{42}	1.4×10^6	2.1×10^{-6}

* Upper limit of the 95% Poisson Confidence Interval (STEVENS 1942).

reversions of ry^2 and/or ry^5, each arising as independent events in spermatogenesis, might be entered into the results of this study. However, we submit that such "contaminants" cannot possibly represent a significant factor in the present data. In any case, major conclusions drawn from these data are based upon the relationship of classical crossovers to conversions of the ry^x allele (Figure 2), both clearly marked to identify them as products of oogenesis.

Returning to the basic cross of Figure 2, ry^+ chromosomes, recovered as products of the mutant heterozygous females, fall into three categories on the basis of the immediate flanking markers, *kar* and *l26*. Thus, *kar* ry^+ *l26* chromosomes are classed as conversions of ry^x, kar^+ry^+ $l26^+$ classifies a conversion of ry^y, and apparent crossovers are identified by exchange for the flanking markers. Such crossovers are either *kar* ry^+l26^+ or kar^+ry^+l26. For any pair of *ry* mutants in an experiment, only one of the two crossover types is obtained, and this crossover type provides a left-right positioning of the rosy mutants, which is consistent in all cases with the prior mapping studies (Figure 1). This classification of ry^+ exceptional chromosomes is used in subsequent tables and discussion. Additionally, the ry^+ exceptional chromosomes are classified with respect to the more distant flanking markers, *cu, Sb* and *Ubx*. Consider the ry^+ exceptional chromosomes, all clearly identifiable as members of the three classes defined by analysis of the immediate flanking markers *kar* and *l26* (crossovers, ry^x conversions, ry^y conversions). Some of these also exhibit an exchange in the region *cu–kar* or, more commonly, in the region *l26–Sb*. No exchanges have been seen in the region *Sb–Ubx* among the ry^+ exceptionals. The relative frequencies of these extraneous exchange events roughly reflect their standard map lengths (Figure 1), and no special significance is placed upon them. If the immediate flanking markers, *kar* and *l26,* are omitted from consideration, classification of the exceptional ry^+ chromosomes becomes more complex. In addition to the two convertant classes, one finds a major and minor recombinant class, reminiscent of results reported in fungal studies, and in one case in Drosophila where the immediate flanking markers are an order of magnitude further apart than in the present study (CARLSON 1971).

The question of clusters and mitotic events: In the earlier reports dealing with conversion events at the rosy cistron, it was noted that all ry^+ exceptional chromosomes arise as rare single surviving individuals scattered at random among the matings. This observation, coupled with other features of the earlier work, permitted the conclusion that the exceptional ry^+ chromosomes arose as the result of rare, independent, events taking place in oogenesis. In our most recent report (BALLANTYNE and CHOVNICK 1971), similar observation is made on the distribution of ry^+ individuals with the exception of one cluster of three ry^+ exceptionals which arose from the four broods of a single mating (15 pairs of parents/mating). The reported data (summarized in Experiment A, Table 2) involved 1,050 matings, and produced 46 tested ry^+ exceptionals in an estimated sample of 1.9×10^6 progeny. Since the flanking marker analysis of the three members of the cluster indicated that they were members of the same conversion class, it was inferred (BALLANTYNE and CHOVNICK 1971) that these three

TABLE 2

The effect of Df(3)kar^{31} *upon number and frequency of crossovers and convertants in the rosy cistron*

Experiment	Female parent	Total ry$^+$ progeny	Analysis of ry^+ chromosomes: Crossovers	Conv-ry^{41}	Conv-ry^5	Total progeny
A	*Dfd kar*^{31} *ry*^{5} + + + / *cu kar ry*^{41} *l26 Sb Ubx*	45	15	26	5	1.89×10^6
		24.3×10^{-6}	7.9×10^{-6}	13.8×10^{-6}	2.6×10^{-6}	
B	+ + *ry*^{5} + + + / *cu kar ry*^{41} *l26 Sb Ubx*	52	21	20	11	1.259×10^6
		41.3×10^{-6}	16.7×10^{-6}	15.9×10^{-6}	8.7×10^{-6}	
C	*cu kar ry*^{5} *l26 Sb Ubx* / + + *ry*^{41} + + +	78	40	30	8	1.34×10^6
		58.2×10^{-6}	29.8×10^{-6}	22.4×10^{-6}	6.0×10^{-6}	

ry^+ chromosomes derive from a single conversion event which took place in a premeiotic gonial mitosis.

The present random strand analysis, ten times the size of the prior work,

yielded additional clusters of ry+ exceptionals, thereby providing the opportunity to question the validity of our prior suggestion concerning mitotic conversion in Drosophila. A total of 12 additional clusters are included in the present data. They arose in crosses scattered throughout the study. Eleven were clusters of two, and one was a cluster of three ry+ exceptionals. If such clusters are reflections of premeiotic gonial mitotic events, one would expect concordance in the marker analysis (crossover, conversion of ry^x, conversion of ry^y) for members of a cluster. Of the eleven clusters of two exceptionals each, five were concordant, and six were combinations of differing diagnoses. The one cluster of three was not concordant. On the basis of these observations, it is concluded that the present data do not support the argument that members of a cluster reflect a single event. All clusters are included in the data presented below, and are given no special treatment.

The effect of kar^{3l} *on the frequency of recombination events within the rosy cistron:* In our most recent report (BALLANTYNE and CHOVNICK 1971), rosy mutant heterozygous female parents, of the specific genotype noted in experiment A of Table 2, gave rise to the indicated distribution of exceptional ry^+ meiotic products. An earlier half-tetrad analysis, involving the same female genotype (CHOVNICK *et al.* 1970), gave rise to a similar distribution of ry^+ exceptionals. Of particular interest is (1) the relative frequency of ry^{41} conversions to ry^5 conversions, (2) the relative frequency of conversions to crossovers, (3) as well as the total frequency of events leading to the production of ry^+ meiotic products.

If conversion events are, indeed, reflections of the mechanism of recombination, as indicated by the prior studies of conversion in Drosophila (see Introduction), then factors known to influence recombination frequency must be considered in any comparative study of conversion frequencies. Of immediate interest is the inequality in conversion frequency for ry^{41} and ry^5 seen in the prior random strand analysis (Row 1, Table 2), as well as in the half-tetrad analysis (CHOVNICK *et al.* 1970). In all of the prior work, the immediate marker to the right of rosy is *l26*, a recessive lethal mutation in the sixth known cistron to the right of the rosy cistron. Despite extensive study of this region (DELAND and CHOVNICK 1970), there is no evidence to indicate that *l26* is a rearrangement which might have an influence on effective pairing for recombination in the rosy cistron. In contrast, the region to the left of rosy was marked using two different mutant alleles of karmoisin (kar^1 and kar^{3l}), the sixth known cistron to the left of rosy. Thus, heterozygotes were marked with one chromosome carrying kar^1, a fully viable recessive allele, while the other chromosome carried kar^{3l}, a homozygous lethal allele which shows a kar phenotype with other viable *kar* mutants. The mutant kar^{3l} has been shown to be a short deletion, located to the left of rosy, extending from salivary section 87C2–3 to 87D1–2 (LEFEVRE 1971), and involves at least four cistrons including *kar*. Since the mutant, ry^5, is located at the left end of the rosy cistron, and ry^{41} at the right end, question is raised concerning the possibility that the heterozygous deletion, kar^{3l}, disturbs effective pairing in the immediate region of the deletion (LEFEVRE and MOORE 1968), and that this

effect extends in a polarized fashion through the rosy cistron to provide a basis for the discrepancy in conversion frequency seen for the two alleles.

Two experiments were carried out, involving ry^5/ry^{41} heterozygous females, constructed according to the mating protocol of Figure 2, and which do not use kar^{3l} as a flanking marker. Exceptional ry^+ progeny were recovered from both sets of matings, and the analysis of exceptional ry^+ chromosomes is summarized in experiments B and C of Table 2. Comparison of the data of the several experiments reveals the following features: (1) The upper limit of the 95% Poisson Confidence Interval constructed about the frequency of total ry^+ progeny of experiment A (32.51×10^{-6}) is significantly less than the observed frequencies of total ry^+ seen in experiments B and C. (2) This significance extends to the crossover class of experiment A (upper limit of the 95% Poisson Confidence Interval $= 13.09 \times 10^{-6}$), as well as to the total conversions in experiment A (upper limit of the 95% Poisson Confidence Interval $= 23.32 \times 10^{-6}$), but does not extend to each of the conversion classes, taken separately. (3) Despite the fact that the conversion frequencies seen in the prior study (experiment A) are lower than in experiments B and C, the discrepancy between ry^{41} conversion frequency compared to ry^5 conversion frequency persists in experiments B and C. It is inferred from these observations that kar^{3l} a known deletion of a small region to the left of rosy, does reduce recombination in the rosy cistron. While this reduction manifests itself in the form of a reduction in the frequency of convertants as well as crossovers, the inequality in conversion frequency seen for ry^{41} and ry^5 cannot be attributed to the presence of kar^{3l}.

Another point of interest is raised by a comparison of the number and distribution of ry^+ exceptionals in experiment B with those seen in experiment C. The total frequency of ry^+ exceptionals recovered in experiment C is significantly greater than that recovered for experiment B. If one compares the two experiments class by class, only the crossover frequencies differ significantly (upper limit of the 95% Poisson Confidence Interval for the experiment B crossover class $= 25.52 \times 10^{-6}$). Although the difference between experiments B and C may relate somewhat to small differences in the viability of ry^+ exceptionals brought about by the differing combinations of heterozygous markers, our experience with this experimental system does not encourage strong support for this argument. For, if this were the case, differences between experiments B and C (Table 2) should be greatest in the convertant classes where differences in the heterozygous recessive mutants are greatest. Moreover, if this argument were valid, then one would expect to find similar discrepancies as a uniform feature of other crosses involving the same alleles. Compare the results of the cross involving $\frac{cu\ kar\ ry^5\ l26\ Sb\ Ubx}{+\ +\ ry^{42}\ +\ +\ +}$ females (Table 5) with the results of the cross involving $\frac{cu\ kar\ ry^{42}\ l26\ Sb\ Ubx}{+\ +\ ry^5\ +\ +\ +}$ females (Table 6). There are no significant differences between the two experiments in any possible comparison that might be considered (total ry^+ exceptionals, crossovers, conversions of ry^5, conversions

of ry^{42}, total conversions). Consider the two convertant classes where differential viability effects should be maximized. Thus, for the cross in Table 5, the weight of heterozygous recessive mutants should operate against the frequency of ry^5 conversions as compared to the cross of Table 6, while ry^{42} conversions should be reduced in the latter experiment. In point of fact, the reverse is the case in both comparisons. Still other comparisons are evident from an examination of the data, and these do not point to differential viability effects as the answer to the discrepancy between the results of experiments B and C in Table 2.

Another factor may be of greater importance. If one compares the parent stocks which were used to generate the females of experiment A, they differ by one stock from that used to produce the females of experiment B. In contrast, experiment C females were constructed from two entirely different stocks when compared with both experiments A and B. The stocks used in these experiments were constructed nine to ten years ago, are not coisogenic, and may possess background genotype differences that might influence crossover and conversion frequencies in the rosy cistron.

The effect of a heterologous rearrangement on the frequency of recombination events within the rosy cistron: A long known, but inadequately understood fact of recombination studies in Drosophila, is the interchromosomal effect associated with chromosome rearrangements (LUCCHESI and SUZUKI 1968). Indeed, the use of specific rearrangements on one chromosome to significantly increase recombination on another, non-homologous chromosome is a standard procedure for students of Drosophila genetics. This point assumes particular importance in comparing conversion frequency data based upon crosses involving stocks with possibly differing genetic backgrounds, as suggested in the previous section.

Table 3 summarizes the results of experiments designed to question this point. Experiments A and B (Table 3) represent replicate experiments following the mating protocol of Figure 2, and assessing the frequency of apparent crossovers and conversions in the heterozygote, ry^{41}/ry^{42}. The mutant ry^{42}, like ry^5 of the prior experiments, is located to the left of ry^{41}, but closer to the center of the rosy cistron map (Figure 1). The replicate experiments are similar in results, and the data are pooled. Of interest is the fact that the proportion of crossovers to the total of ry^+ exceptions seen in these experiments (A and B pooled) is significantly lower than that seen for ry^{41}/ry^5 crosses.

The breeding protocol for experiment C (Table 3) differed from the standard procedure in that one of the parent stocks, ry^{42}/ry^{42}, was crossed to the multiple rearrangement stock, *SM1, Cy; Ubx*^{130}*/T (2;3)ap*^{Xa}, and phenotypically Cy flies were then crossed to the other parent stock to introduce the heterologous rearrangement, *SM1,Cy*, in all of the female parents of experiment C. Comparison of the results of experiment C with the pooled result of experiments A and B dramatically reveals the effect of such heterologous rearrangements. The upper limits of the 95% Poisson Confidence Intervals about the frequencies for the pooled results of experiments A and B reveal that total ry^+ exceptions (32.62×10^{-6}), crossovers (8.88×10^{-6}), total conversions (28.12×10^{-6}), and ry^{41} con-

TABLE 3

The effect of the second chromosome rearrangement, In(2LR)SM1, Cy, *upon the number and frequency of* ry$^+$ *crossovers and convertants produced in females of the genotype*
$\frac{\text{cu kar ry}^{41}\ \text{126 Sb Ubx}}{+\ +\ \text{ry}^{42}\ +\ +\ +}$ *mated to males of either* ry^2/ry^2 *or* ry^5/ry^5

Experiment	Chromosome 2 genotype	Total ry$^+$ progeny	Analysis of ry$^+$ chromosomes			Total progeny
			Crossovers	Conv-ry^{41}	Conv-ry^{42}	
A	+/+	16	3	9	4	623,000
B	+/+	14	2	9	3	691,000
A + B	+/+	30	5	18	7	1,314,000
		22.8×10^{-6}	3.8×10^{-6}	13.7×10^{-6}	$5.3 \times ^{-6}$	
C	*SM1,Cy/+*	19	5	11	3	355,000
		53.5×10^{-6}	14.0×10^{-6}	31.0×10^{-6}	8.5×10^{-6}	

versions (21.66×10^{-6}) are significantly lower than those seen in experiment C. Although the frequency of ry^{42} conversions also is lower for experiments A and B pooled than that seen in experiment C, the difference is not significant.

TABLE 4

Number and frequency of ry+ exceptional progeny resulting from crosses of the indicated females to homozygous mutant males of either ry^2/ry^2 *or* ry^5/ry^5

Female parent	Total ry+ progeny	Analysis of ry+ chromosomes: Crossovers	Analysis of ry+ chromosomes: Conv-ry^{41}	Analysis of ry+ chromosomes: Conv-ry^{y}	Crossovers / Conv-ry^{41}	Total progeny
cu kar ry^{41} *l26 Sb Ubx*	9	0	8	1	0	2.473×10^6
+ + ry^2 + + +	3.64×10^{-6}	....	3.24×10^{-6}	0.40×10^{-6}		
cu kar ry^{41} *l26 Sb Ubx*	16	3	10	3	0.30	0.712×10^6
+ + ry^{26} + + +	22.5×10^{-6}	4.2×10^{-6}	14.1×10^{-6}	4.2×10^{-6}		
cu kar ry^{41} *l26 Sb Ubx*	30	5	18	7	0.28	1.314×10^6
+ + ry^{42} + + +	22.8×10^{-6}	3.8×10^{-6}	13.7×10^{-6}	5.3×10^{-6}		
cu kar ry^{41} *l26 Sb Ubx*	23	11	10	2	1.10	0.818×10^6
+ + ry^1 + + +	28.1×10^{-6}	13.4×10^{-6}	12.2×10^{-6}	2.5×10^{-6}		
cu kar ry^{41} *l26 Sb Ubx*	40	11	18	11	0.60	1.234×10^6
+ + ry^8 + + +	32.4×10^{-6}	8.9×10^{-6}	14.6×10^{-6}	8.9×10^{-6}		
cu kar ry^{41} *l26 Sb Ubx*	52	21	20	11	1.05	1.259×10^6
+ + ry^5 + + +	41.3×10^{-6}	16.7×10^{-6}	15.9×10^{-6}	8.7×10^{-6}		

Relationship between crossovers and conversions in random strand tests of rosy mutant heterozygotes: The random strand experiments summarized in Tables 4 and 5 were carried out following the procedures described above. Table 4 sum-

TABLE 5

Number and frequency of ry+ exceptional progeny resulting from crosses of the indicated females to homozygous mutant males of either ry^2/ry^2 *or* ry^5/ry^5

Female parent	Total ry^+ progeny	Analysis of ry^+ chromosomes			Crossovers	Total progeny
		Crossovers	Conv-ry^5	Conv-ry^y	Conv-ry^5	
cu kar ry^5 *l26 Sb Ubx*	9	0	2	7	0	0.75×10^6
+ + ry^8 + + +	12.0×10^{-6}	....	2.7×10^{-6}	9.3×10^{-6}		
cu kar ry^5 *l26 Sb Ubx*	10	3	5	2	0.60	0.681×10^6
+ + ry^1 + + +	14.6×10^{-6}	4.4×10^{-6}	7.3×10^{-6}	2.9×10^{-6}		
cu kar ry^5 *l26 Sb Ubx*	20	10	7	3	1.43	0.776×10^6
+ + ry^{42} + + +	25.8×10^{-6}	12.9×10^{-6}	9.0×10^{-6}	3.9×10^{-6}		
cu kar ry^5 *l26 Sb Ubx*	39	28	10	1	2.80	1.604×10^6
+ + ry^2 + + +	24.3×10^{-6}	17.5×10^{-6}	6.2×10^{-6}	0.6×10^{-6}		
cu kar ry^5 *l26 Sb Ubx*	78	40	8	30	5.0	1.34×10^6
+ + ry^{41} + + +	58.2×10^{-6}	29.8×10^{-6}	6.0×10^{-6}	22.4×10^{-6}		

marizes the results of a series of crosses in which ry^{41}, located at the right end of the rosy cistron (the ry^x allele of Figure 2) was tested against a series of ry^y alleles. The results are presented in Table 4 in order of increasing distances of

the ry^y alleles to the left of ry^{41}. Similarly, Table 5 summarizes the results of a series of crosses in which ry^5, located at the left end of the rosy cistron is the ry^x allele of Figure 2, and was tested against a series of ry^y alleles. Table 5 presents the results in order of increasing distances of the ry^y alleles to the right of ry^5. Two points should be noted about the alleles, and their ordering in these tables. The mutant, ry^2, was never separated from ry^{41} in our prior mapping experiments, and has been considered to be a member of the rightmost cluster of rosy alleles (Figure 1). The second point deals with the location of the mutant, ry^1. Prior mapping studies (CHOVNICK *et al.* 1964) had placed ry^1 to the left of ry^{26}, but a series of tests against several separable mutants in the left part of the map failed to yield crossovers, and the suggestion was offered that perhaps ry^1 was an intracistronic rearrangement involving the left end of the rosy cistron. Its position in Tables 4 and 5 reflects the pattern of data obtained in the present report.

Examination of the data presented in Tables 4 and 5 reveals the following general features: (1) The frequency of total ry^+ progeny, as well as the frequency of crossovers is, in general, consistent with the relative distance between the rosy mutant alleles tested. (2) In Table 4, the frequency of ry^{41} conversions appears to be the same in all genotypes tested, with the exception of ry^{41}/ry^2, the closest alleles tested (Row 1, Table 4). The pooled mean for ry^{41} conversions in all tests excluding Row 1 is 14.24×10^{-6}. Construction of the 95% Poisson Confidence Interval about this mean reveals that all values for ry^{41} conversion are quite homogeneous, with the exception of Row 1 value which is far below the lower limit of the 95% Confidence Interval. (3) Similarly, the frequency of ry^5 conversions (Table 5) appears to be the same in all genotypes tested, with the exception of ry^5/ry^8, the closest alleles tested in Table 5. The pooled mean for ry^5 conversions in all tests excluding Row 1 of Table 5 is 6.82×10^{-6}. Construction of the 95% Poisson Confidence Interval about this mean includes all values for ry^5 conversion except that seen in Row 1, which is far below the lower limit. (4) In summation, as one progresses down Tables 4 and 5, from tests involving the closest alleles to more distant alleles, the ratios, crossovers/conversions—ry^{41} (Table 4) and crossovers/conversions—ry^5 (Table 5) exhibit a trend to increasing values.

Two points should be noted about the recombination data of Table 5, and its relationship to the prior mapping studies (CHOVNICK *et al.* 1964). The mutant, ry^1, had been localized to the left half of the rosy cistron map. The present crossover data provide localization of this mutant to the right of ry^5 (Row 2, Table 5). Moreover, the ry^5/ry^8 heterozygote did yield a low frequency of crossovers in the prior study, which permitted establishment of their relative positions in the rosy cistron (Figure 1). The present data (Row 1, Table 5) are consistent with this prior localization.

Table 6 summarizes the results of crosses in which ry^{42}, a central allele in the rosy cistron was tested against a series of ry^y alleles. The pattern exhibited by the data of Table 6 conforms, in general, with that seen in Tables 4 and 5. Beginning with Row 1 of Table 6, the results are ordered in terms of tests with the right-

TABLE 6

Number and frequency of ry+ exceptional progeny resulting from crosses of the indicated females to homozygous mutant males of either ry^2/ry^2 *or* ry^5/ry^5

Female parent	Total ry+ progeny	Analysis of ry^+ chromosomes: Crossovers	Conv-ry^{42}	Conv-ry^y	Crossovers / Conv-ry^{42}	Total progeny
cu kar ry^{41} *l26 Sb Ubx* / + + ry^{42} + + +	30	5	7	18	0.71	1.314×10^6
	22.8×10^{-6}	3.8×10^{-6}	5.3×10^{-6}	13.7×10^{-6}		
+ + ry^2 + + + / *cu kar* ry^{42} *l26 Sb Ubx*	4	0	4	0	0	0.584×10^6
	6.85×10^{-6}	......	6.85×10^{-6}	......		
+ + ry^{26} + + + / *cu kar* ry^{42} *l26 Sb Ubx*	14	1	5	8	0.20	0.697×10^6
	20.1×10^{-6}	1.4×10^{-6}	7.2×10^{-6}	11.5×10^{-6}		
cu kar ry^{42} *l26 Sb Ubx* / + + ry^8 + + +	9	2	4	3	0.50	0.755×10^6
	11.9×10^{-6}	2.6×10^{-6}	5.3×10^{-6}	4.0×10^{-6}		
cu kar ry^{42} *l26 Sb Ubx* / + + ry^5 + + +	14	5	4	5	1.25	0.641×10^6
	21.8×10^{-6}	7.8×10^{-6}	6.2×10^{-6}	7.8×10^{-6}		

most alleles of the cistron reading down to the leftmost allele, ry^5. The major features of this table are: (1) The frequency of crossovers reflects distance of the ry^y alleles from ry^{42}. (2) The frequency of conversion of ry^{42} appears to be

the same in all tests. None are excluded by the 95% Poisson Confidence Interval constructed about the pooled mean for ry^{42} conversion (6.01×10^{-6}). (3) These points are summarized by a consideration of the ratio, crossovers/conversions—ry^{42}, which is minimal for tests involving mutant alleles close to ry^{42}, but increases with distance in both directions (Table 6).

Separation of ry^{41} *and* ry^{2} *in half-tetrad experiment:* Following the procedures described earlier, large scale crosses of *C(3L)RM,P2,ri/ri; C(3R)RM,SH9,Dfd* $kar^{3l}ry^{2}$*/cu kar* ry^{41}*l26 Sb* females to *C(3L)RM,P2,ri/ri; C(3R)RM,SH3,*ry^{2}/ry^{2} males were carried out making use of the purine enriched selective medium. From this experiment, which sampled an estimated 50,000 progeny, one Dfd kar ry^{+} Sb male was produced. A detachment experiment produced 12 detachments of the chromosome arms present in the exceptional male, and the subsequent marker analysis revealed that the genotype of the exceptional male was $\frac{Dfd + kar^{3l}\ ry^{+}\ l26\ Sb}{+\ cu\ kar\ \ ry\ \ +\ +}$. On the basis of the marker analysis, we infer that the exceptional ry^{+} male arose from a meiotic event in the female parent which gave rise to an apparent reciprocal exchange for the flanking markers in association with the production of the ry^{+} chromosome. Moreover, the distribution of flanking markers indicates separation of the two alleles with ry^{2} located to the right of ry^{41}. Subsequently, an identity test of the rosy mutant bearing chromosome was carried out to determine the rosy mutant composition of this chromosome following the logic and protocol of prior identity tests (BALLANTYNE and CHOVNICK 1971). This test indicated that the rosy mutant bearing chromosome was *cu kar* ry^{2}, and did not have a ry^{41} allele present. Thus, we are able to classify the original ry^{+} exceptional fly as the result of a non-reciprocal conversion event associated with exchange for the flanking markers.

Relationship between conversion frequency and mutant allele map position: As noted in our prior studies, and throughout the present investigation, a sharp difference exists in conversion frequency for ry^{5}, at the left end of the rosy cistron, compared to that seen for ry^{41} at the right end. Since conversion frequency inequalities related to map position of the mutant alleles (polarity) have been important in consideration of the mechanism of conversion, it is pertinent to question this point in the present data. Two approaches to the question are possible. The first argues that if the mutants exhibit polarity of conversion frequency, then mutants located very close to each other should exhibit similar conversion frequencies. Thus, the mutants ry^{41} and ry^{2} both map in the right end of the rosy cistron and are very close to each other. Table 7 summarizes the results of crosses in which ry^{2} was tested against several mutant alleles. The distribution of flanking markers in the ry^{2}/ry^{1} heterozygous female parent (Row 3, Table 7) differs from that used in all other crosses. Consequently, the classification of ry^{+} exceptional progeny from this cross, while based upon the same test crosses, deviates appropriately from that described above (See MATERIALS AND METHODS). Despite its proximity to ry^{41}, the mutant, ry^{2}, exhibits in all tests a conversion frequency very much lower than that seen for ry^{41} and all other alleles in this study. Figure 3 provides a summary of conversion frequencies for

TABLE 7

Number and frequency of ry+ *progeny resulting from crosses of the indicated females to homozygous mutant males of either* ry²/ry² *or* ry⁵/ry⁵

Female parent	Total ry+ progeny	Analysis of ry+ chromosomes			Total progeny
		Crossovers	Conv-ry^2	Conv-ry^y	
$\frac{+ \; + \; ry^2 \; + \; + \; +}{cu \; kar \; ry^{41} \; l26 \; Sb \; Ubx}$	9	0	1	8	2.473×10^6
	3.64×10^{-6}	....	0.40×10^{-6}	3.24×10^{-6}	
$\frac{+ \; + \; ry^2 \; + \; + \; +}{cu \; kar \; ry^{42} \; l26 \; Sb \; Ubx}$	4	0	0	4	0.584×10^6
	6.85×10^{-6}	....	....	6.85×10^{-6}	
$\frac{cu \; kar \; ry^2 \; + \; + \; +}{+ \; + \; ry^1 \; l26 \; Sb \; Ubx}$	26	20	0	6	0.961×10^6
	27.1×10^{-6}	20.8×10^{-6}	....	6.2×10^{-6}	
$\frac{+ \; + \; ry^2 \; + \; + \; +}{cu \; kar \; ry^5 \; l26 \; Sb \; Ubx}$	39	28	1	10	1.604×10^6
	24.3×10^{-6}	17.5×10^{-6}	0.6×10^{-6}	6.2×10^{-6}	

all other alleles. The ry^{41} estimate derives from Table 4, the ry^5 estimate is that of Table 5, and the remaining estimates utilize the data presented in Tables 4 through 7. The ry^2 conversion frequency of Table 7, not shown in Figure 3,

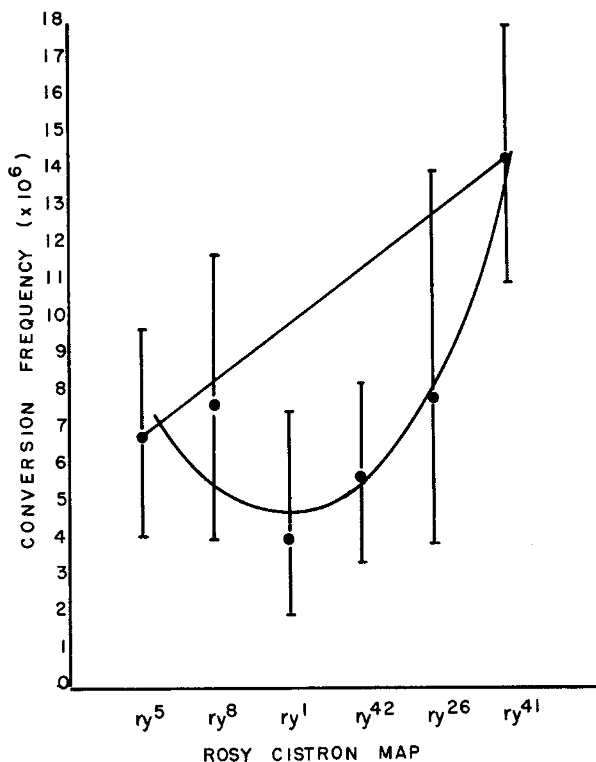

FIGURE 3.—Summary of rosy mutant allele conversion frequencies as a function of relative map position in the rosy cistron.

ranges from one to two orders of magnitude lower than other alleles in the present investigation. One may question the possibility that the presence of ry^2 has a general effect of suppressing conversion, including conversion of the other mutant allele present in mutant heterozygotes. Examination of the conversion frequency for each of the ry^y alleles in the experiments of Table 7, and comparison of these frequencies with the summary conversion frequency data of Figure 3 provides no support for this suggestion. The conversion frequencies of ry^{42}, ry^1, and ry^5 in heterozygotes with ry^2 (Table 7) are not significantly different from the mean frequencies of conversion for these alleles throughout the study (Figure 3). As noted earlier, in consideration of the data of Table 4, the frequency of conversion of ry^{41} is, indeed, significantly reduced in heterozygotes with ry^2 while it appears to be quite constant in tests with other, more distant, alleles. Taken together, the data of Tables 4 and 7, as well as Figure 3, indicate that: (1) The mutant ry^2 has an intrinsically low conversion frequency. (2) The presence of ry^2 has no general effect on the conversion frequency of other mutant alleles in heterozygotes. (3) The reduction in conversion frequency of ry^{41} when heterozygous with ry^2, is a function of the proximity of these alleles, as suggested earlier.

In view of these facts, the nature of the ry^2 lesion assumes some import. Is it

possible that ry^2, a spontaneous mutant, is a deletion (or other rearrangement) restricted to the right end of the rosy cistron? Such a lesion might well exhibit a reduced conversion frequency in all tests, and lead to a significant reduction in conversion frequency of a close neighbor allele, as well as a reduced crossover frequency in heterozygotes with such close neighbors (ry^{41}). Evidence on this question has come from other experiments (unpublished work of this laboratory) which have shown that certain of the null enzyme activity rosy mutants (including ry^2) direct the production of an altered XDH molecule. Under appropriate *in vitro* conditions, extracts of these mutants have XDH activity which exhibits a single band in the same location as the wild type XDH upon electrophoresis in acrylamide gel. This observation suggests that the ry^2 lesion is a very small one, and is quite likely to be a single site mutant.

We may turn next to a consideration of other mutant allele pairs in this investigation. Thus, ry^8/ry^1 heterozygotes have never yielded crossovers, despite extensive sampling in prior investigation (CHOVNICK *et al.* 1964). Comparison of ry^8 and ry^1 mean conversion frequencies (Figure 3) reveal differences which are of borderline significance. All other pairs of neighboring alleles are considered to be more distant from each other by virtue of their separation by crossovers. Nevertheless, all combinations of neighboring allele pairs have been examined, and there are no significant differences.

Figure 3 presents a summary of mean conversion frequencies as a function of map position for all rosy mutant alleles in the study, omitting ry^2 which falls far below all others (see above). The order of alleles along the abscissa reads from the left end of the map beginning with ry^5 to ry^{41} at the right end. By virtue of the low frequency of crossovers in this and prior experiments, relative distance estimates have large 95% Poisson Confidence Limits. Consequently, the alleles have been ordered arbitrarily at equal intervals along the abscissa. The 95% Poisson Confidence Intervals are shown for each estimated mean conversion frequency, and a straight line connects the mean estimates for ry^5 and ry^{41} conversion frequencies. Clearly, all alleles located between the end points exhibit means that fall below the line, and a U-shaped curve, as indicated, would better fit these data. However, the large Poisson Confidence Intervals do not encourage strong support for any specific geometry.

Postmeiotic segregants and hybrid DNA: Various models of gene conversion have proposed that the basic event in conversion involves the repair of breaks on either complementary or identical chains of two "non-sister strand" DNA double helices (see reviews by EMERSON 1969; WHITEHOUSE 1970). Such models suggest that the repair event involves the production of an intermediate "hybrid" DNA sector which, upon correction, produces the converted DNA double helix. The chief source of evidence for the hybrid DNA intermediate, apart from its similarity to molecular models for phage recombination, bacterial transformation and repair of damaged DNA (see review by BODMER and DARLINGTON 1969), is the observation of postmeiotic segregants found commonly in studies of gene conversion in Sordaria and Ascobolus. In these systems, the postmeiotic segregants are taken to be instances of hybrid DNA formed as an intermediate in the

exchange process, but that some hybrid sectors remained uncorrected into the first postmeiotic mitosis, and consequently produced mitotic segregants.

The present study provides opportunity to question in Drosophila, the extent of uncorrected hybrid DNA reflecting this proposed intermediate stage in the conversion process. Let us assume that, at least for the rosy region of chromosome *3*, the genetic contribution from each parent consists of a single double helix of DNA. The male parent contributes a double helix of DNA containing rosy mutant information. Consider next the contribution of a female parent in which a non-reciprocal meiotic exchange event had occurred in the rosy cistron such as to produce a hybrid double helix with one strand carrying mutant information, while the other strand carries ry^+ information. If the hybrid double helix is corrected, then a standard conversion may be produced. If it remains uncorrected, DNA replication followed by somatic segregation at the first mitosis after fertilization would lead to the production of two nuclei, one heterozygous ry^+/ry, and the other a mutant homozygote. Subsequent mitoses would produce a somatic mosaic individual. In view of the nonautonomous nature of the rosy mutant eye color phenotype, and the fact that flies with low levels of XDH can survive growth on the purine enriched selective medium (FINNERTY, DUCK and CHOVNICK 1970), such mosaic individuals would be expected to survive and be scored as ry^+ exceptionals. The present analysis of the ry^+ exceptionals is competent to determine the extent of such mosaicism. Consider such a mosaic individual. If all or most of its gonadal tissue derived from the ry^+/ry heterozygous nucleus produced at the first mitosis, then such a mosaic individual would not be distinguished from other ry^+ exceptionals by the present system of analysis. However, one would anticipate that equal numbers of such mosaic individuals would have gonadal tissue derived largely from the homozygous mutant nucleus. Such individuals would be identified by the present analysis as ry^+ exceptionals which reproduced as rosy mutant homozygotes. Not included in the totals of ry^+ exceptionals reported above, was one individual that fell into this classification. Upon analysis, this individual had one rosy mutant paternal chromosome as well as a *cu kar ry l26 Sb Ubx* rosy mutant, non-crossover chromosome. No individuals of this classification occurred in the experiments leading to our most recent report (BALLANTYNE and CHOVNICK 1971), but there was one exceptional individual in the prior halftetrad analysis (CHOVNICK *et al.* 1970) that fell into this category. In summary, the total data, including the present analysis as well as prior random strand and half-tetrad data involved analysis of 498 ry^+ exceptionals, two (2) of which fell into the category of possible postmeiotic segregants. Clearly, the extent of uncorrected hybrid DNA remaining at the time of the first postmeiotic mitosis in this system is insignificant.

DISCUSSION

The present report, as well as prior reports from this laboratory result from an effort designed to examine, perhaps more closely than ever before, the phenomenon of linked exchange in *Drosophila melanogaster*. The results of these

studies indicate the need for revision of classic notions of the mechanics of linked exchange, and a beginning at such revision is offered in the form of a model describing the genetic consequences of such exchange. The basis for the model derives largely from the present study, as well as prior reports on studies of genetic fine structure and conversion in Drosophila, and its key features are elaborated in the following paragraphs.

We start with the classic notion that exchange takes place at a four strand stage, with each exchange involving two non-sister strands of the tetrad. Figure 4 presents a diagrammatic representation of an exchange event, in which only the non-sister strands involved in the exchange are shown (S_1 and S_2). Although each strand should be considered to consist of a single DNA double helix, Figure 4 represents each strand as a single line (solid or dotted).

As a point of departure from classical thinking about linked exchange, we assume that (1) breaks occur at random along both chains of the non-sister strand DNA double helices and that (2) for the most part, isolated breaks are restituted by a repair process without genetic consequence. (3) Among the population of such breaks, there will occur instances where two breaks have taken place within a restricted region on different chains of the non-sister DNA double helical strands. In contrast to the classic notion of crossing over, such breaks are not likely to be located at identical sites on the two chains, but are restricted to a

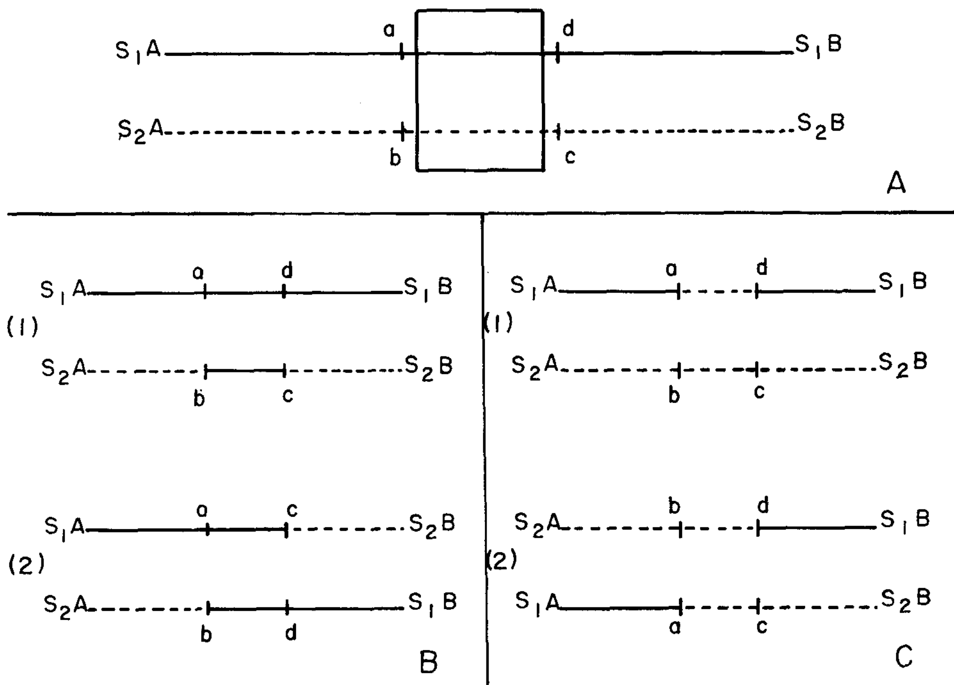

FIGURE 4.—(A) Diagrammatic representation of a repair event leading to reciprocal conversions (B and C) taking place with and without exchange for adjoining segments. See text description of Case 1.

region described by the "black box" contained within the points *abcd* which, in turn, may be considered as sites along the two non-sister strands outside the restricted region (Figure 4A). It is the repair of such breaks which may lead to an event of genetic consequence. Ignoring the molecular details of the repair event taking place within the "black box", let us focus attention upon the genetic consequences of such a repair event. (4) As a result of the process of repair, the segments of the two non-sister strands located within the "black box" of Figure 4 come to be identical in genetic composition. The repair process which gave rise to this genetic outcome is referred to as conversion, and it should be noted that the converted segment has been shown to be substantially greater than a single site in Drosophila (CARLSON 1971) as well as in fungi (FOGEL, HURST and MORTIMER 1971; STADLER and KARIYA 1969). Although the converted segments within the "black boxes" of the several figures are illustrated to be of equal length, no such restriction should be inferred. Rather, we envision a population of converted segments as exhibiting a continuous frequency distribution with the larger and smaller segments being reduced in frequency. The reduced frequency of small segments reflects the low probability of appropriate breaks within short intervals, while the reduced frequency of larger converted segments may reflect a limitation placed upon the system by one or more physico-chemical features of the molecular repair system. (5) That either strand may be converted is indicated by Figures 4B and 4C. In Figure 4B, both segments within the "black box" become solid lines, while in Figure 4C both segments become dotted lines. We shall refer to these conversions as reciprocal conversion events. Moreover, the model requires that the reciprocal conversion events be equally likely to occur. Exceptions to this rule, are known, and will be discussed in a subsequent section. (6) Either conversion event (Figure 4B or C) may occur with or without exchange of markers immediately outside the segment. Thus, in Figures 4B and 4C, diagram 1 illustrates the reciprocal conversions within the "black box", with the outside markers *ad* on strand S_1 and *bc* on strand S_2 remaining unchanged. In contrast, diagram 2 illustrates the conversion event within the "black box", and associated with the conversion event is an exchange for the immediate markers. It should be noted that diagram 2 of Figure 4B illustrates conversion accompanied by flanking marker exchange, and the conversion as well as the marker exchange are the reciprocal of diagram 2, Figure 4C. The model further asserts that given a conversion event, the probability that it will involve marker exchange (diagram 2, Figures 4B and C) is itself a variable which is a function of the length of the converted segment. The probability that a conversion will involve marker exchange increases from zero for exceedingly small segments to frequency values approaching 1 for the rare largest conversions, with an average value of 1/2. This point is discussed in greater detail in a subsequent section.

Case 1: Consider the diagram in Figure 4A as representing a length of two non-sister strands differentially marked at their ends S_1A—S_1B and S_2A—S_2B. The strands additionally are marked at a point to the immediate left (*a* and *b*) and immediate right (*d*–*c*) of an exchange event described by the "black box". In this case, there are no further genetic differences distinguishing the two non-

sister strands. Consequently, the exchange event which occurs in the region limited by the "black box" (Figure 4A) leads to reciprocal conversion events (Figure 4B and C) which are identical with respect to the converted sector, and the only result of genetic significance is the reciprocal exchange of markers flanking the converted region (Figure 4B and C, diagram 2). The resulting strands, in this instance, are identical to the products expected as a result of classical crossing over occurring at points within the "black box" of Figure 4, and would provide left-right positioning of the markers identical to that of the classical crossover model. Now consider the entire length of the two strands of Figure 4A, and these are differentially marked at their ends. Since breaks may occur anywhere along the length of these strands, repair in "black boxes", as described above, takes place along the entire length of the strands. We envision the situation as an array of overlapping "black boxes" enclosing overlapping sectors which might be involved in repair events leading to conversion. In such case, the results described for Case 1 clearly provide for mapping as a function of distance between the markers, just as does classical crossing over.

Case 2: Figure 5 illustrates the results of an exchange between non-sister strands marked with mutants (m_1 and m_2) located at some distance apart within a single cistron. The strands are marked additionally with flanking markers shown at their ends in Figure 5A. An exchange which occurs in a sector between the markers (Figure 5A) will result in reciprocal conversions of the segments

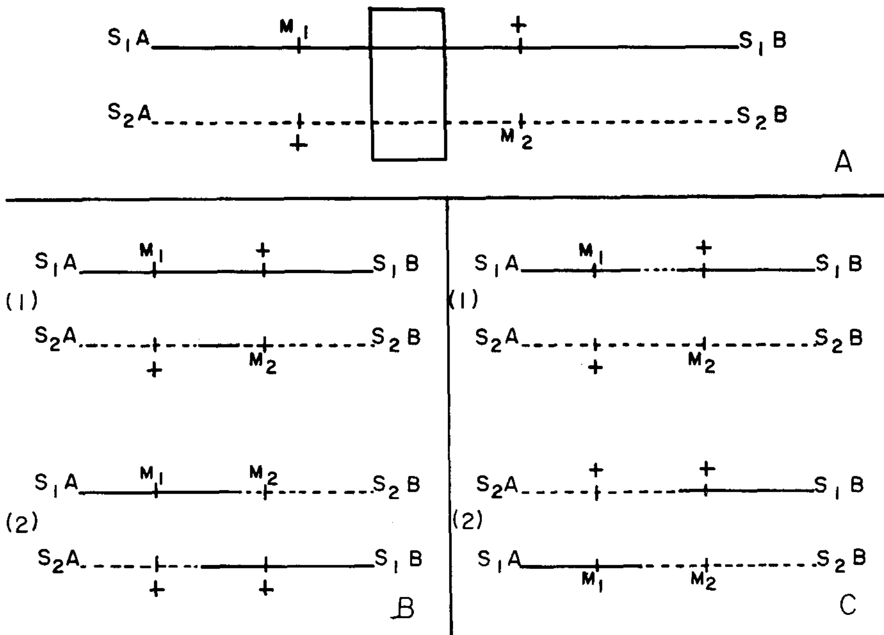

FIGURE 5.—(A) Diagrammatic representation of a repair event taking place in a region located between the intracistronic markers, m_1 and m_2. (B and C) represent the reciprocal events taking place with and without exchange for adjoining segments. See text description of Case 2.

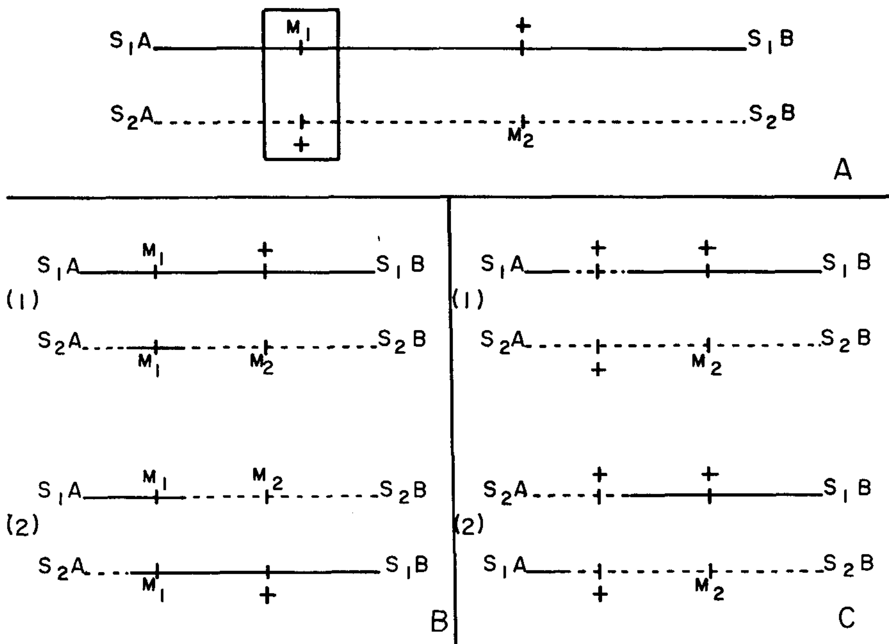

FIGURE 6.—(A) Diagrammatic representation of a repair event taking place in a region that includes the intracistronic marker, m_1. (B and C) represent the reciprocal events taking place with and without exchange for adjoining segments. See text description of Case 2.

within the "black box", and these are shown in Figures 5B and C. Since there are no genetic differences in the parent sectors involved in the conversions, the only changes of genetic consequence are (as in Figure 4B and C) the reciprocal exchanges for the regions flanking the conversion event (diagram 2 of Figures 5B and C). If one were following the markers m_1 and m_2, the results, shown in diagram 2 of Figures 5B and C, are indistinguishable from those predicted by classical crossing over as seen in random strand, tetrad or half-tetrad experiments. Reciprocal products are produced for the intracistronic markers, and these are accompanied by exchange for the outside flanking markers noted at the ends of the strands. Such event provides unambiguous left-right positioning of the mutants within the cistron.

Figures 6 and 7 diagram the results to be expected when the "black box", describing the region of a conversion event, includes one or the other of the intracistronic markers, m_1 and m_2. Thus, Figure 6 describes the conversion events involving the m_1 region, while Figure 7 illustrates conversion events in the m_2 region. The rules elaborated above reveal the following features: (1) All events are non-reciprocal with respect to the converted region. (2) Conversion leads to the production of $+ +$ strands as well as double mutant m_1m_2 strands. (3) These events occur with and without the exchange of flanking markers. (4) When following the direction of flanking marker exchange in association with conversions leading to the production of wild type, $+ +$ strands, both con-

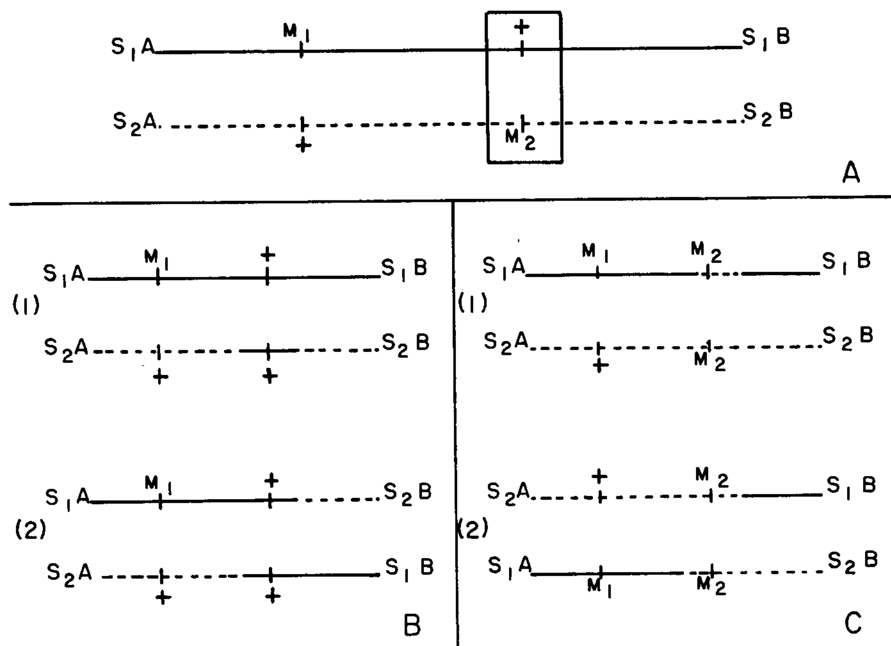

FIGURE 7.—(A) Diagrammatic representation of a repair event taking place in a region that includes the intracistronic marker, m_2. (B and C) represent the reciprocal events taking place with and without exchange for adjoining segments. See text description of Case 2.

version events (Diagram 2, Figure 6C and Diagram 2, Figure 7B) produce + + strands with the same direction of flanking marker recombinants. Moreover, the reciprocal flanking marker recombinants are found in association with the conversions leading to the production of double mutant strands, m_1m_2 (Diagram 2, Figure 6B and Diagram 2, Figure 7C). (5) Finally, a comparison of the convertant + + strands and m_1m_2 strands produced in association with flanking marker exchange (Figures 6 and 7) exhibit the same direction of flanking marker exchange seen in Figure 5.

In summary, the formal model presented above describes the results of linked exchange in terms of a single event, the repair of breaks within a restricted region involving a pair of non-sister DNA double helices. Consider the situation involving a pair of closely linked markers such as the mutants ry^5 and ry^{41} at opposite ends of the rosy cistron. Exchange events occurring between the markers lead to the production of exchange products which are indistinguishable from that expected by classical crossing over either in random strand, tetrad or half-tetrad analysis. Events which directly convert one or the other mutant intracistronic marker are always non-reciprocal for the converted segment, and are thus distinguishable from exchanges between the markers by tetrad and half-tetrad analysis. However, if one follows the production of random strand wild-type products, as in the present investigation, then one finds such wild types, with and without apparent recombination for the flanking markers. Presumably, the

wild types associated with marker exchange result from direct conversion of the mutant alleles as well as from conversion events involving segments between the intracistronic markers, and the direction of marker exchange, in both classes of events, provides unambiguous mapping of the mutant sites within the cistron.

Case 3: If we next consider the case where the mutant allele markers, m_1 and m_2 (Figures 5, 6 and 7), are much further apart than in the present investigation, the frequency of conversion events between the markers becomes overwhelmingly greater than direct conversion of one or the other mutant marker in the study. Thus, in the study of the lozenge mutants (GREEN and GREEN 1949; 1956) where map distances between members of the complex are an order of magnitude further apart than is the total length of the rosy cistron, or in the case of the bithorax region (LEWIS 1967), where separable mutants are two orders of magnitude further apart, relatively small sampling provides for recovery of large numbers of "classical crossovers". The exceedingly rare direct conversion event in such studies may never be noted, or if recovered without flanking marker exchange, may have been scored as a spontaneous reversion.

Case 4: We may next consider the situation involving two mutant allele markers, m_1 and m_2, very closely linked, such that the interval between them is quite reduced. In such instance, repair of breaks which lead to exchange events almost always involves conversion of one or the other mutant allele in the experiment. The low frequency (or absence) of apparent "classical crossovers" reflects the short interval between the markers used. Nevertheless, those conversions associated with marker exchange provide for consistent mapping of the mutants in the study. Just such a case, involving the maroon-like mutants, has been investigated in this laboratory. A large scale half-tetrad analysis following exchanges involving the most distant markers in the cistron revealed that all events were non-reciprocal conversions involving one or the other mutant allele, and that they occurred with equal frequency (SMITH, FINNERTY and CHOVNICK 1970). On the other hand, a random strand analysis following events leading to the production of *ma-l*$^+$ meiotic products from mutant allele heterozygotes revealed that such *ma-l*$^+$ products were produced, and that approximately 1/2 of them exhibited exchange for the closely linked outside flanking markers. Indeed, by utilization of the flanking marker exchanges, an internally consistent linear map of mutant sites was constructed which conforms to the complementation map of these mutants (FINNERTY, DUCK and CHOVNICK 1970). Thus, even in a study where all of the events involve direct conversion of one or the other allele, consistent mapping is facilitated by following those conversions associated with flanking marker exchange. Moreover, on the basis of such mapping, an experiment was conceived, and successfully conducted to select random strand double mutant products (FINNERTY and CHOVNICK 1970). These products now may be understood as reciprocals to the conversion events that produced *ma-l*$^+$ strands. Their flanking marker exchanges are the reciprocal of the flanking marker exchanges associated with the *ma-l*$^+$ convertants, and the frequency of the events leading to the production of such double mutant strands, are not different from the frequency of production of *ma-l*$^+$ convertants.

Case 5: Let us next consider cases where the interval between the markers, m_1 and m_2, is so short that (1) there are never repair intervals that do not include one or the other marker, and (2) a significant portion of the repair intervals include both markers m_1 and m_2. Consider an interval enclosed by a "black box" which is destined to lead to conversion of one or the other strand. If both strands are mutant (i.e., one strand carries m_1, and the other is m_2), then a single conversion event cannot lead to the production of either a wild type or double mutant product. Thus, when the interval between the markers is sufficiently reduced, the apparent frequency of conversion is reduced. This is the case seen for ry^{41} conversion in ry^{41}/ry^{2} heterozygotes (Table 4) as well as ry^{5} conversion in ry^{5}/ry^{8} heterozygotes (Table 5). This feature of conversion may, in fact, be the basis for map expansion, and has been interpreted in a similar fashion by HOLLIDAY (1964) as well as by FOGEL, HURST and MORTIMER (1971).

Case 6: Finally, we consider the question of multiple conversion events. In our most recent report (BALLANTYNE and CHOVNICK 1971), an intracistronic three-point half-tetrad experiment is described which examined half-tetrad meiotic products of females of the genotype $\frac{Dfd + kar^{31}\ ry^{5}\ + ry^{41}\ l26\ \ Sb}{+\ cu\ kar\ \ +\ ry^{42} +\ \ +\ \ +}$. Of seven exceptional ry+ progeny recovered, five reproduced, and the analysis of their chromosomes following detachment revealed that all five exceptionals were nonreciprocal conversions of the ry^{42} chromosome, which occurred without exchange for the immediate flanking markers. Part of the analysis involved testing the rosy mutant bearing chromosomes of each of the 5 ry^{+} exceptional half-tetrads to determine if ry^{42} were present on one or more of these chromosomes, as might be expected if negative interference were involved in the origin of the exceptionals. Recombination tests against ry^{42} produced ry^{+} exceptions in each case, thus indicating that the rosy mutant bearing arms did not carry ry^{42}. From these tests, however, 3/9 of the ry^{+} exceptions were apparent conversions of the detached presumptive double mutant $ry^{5}\ ry^{41}$, which would require two conversion events to produce each such ry^{+} exception. These presumptive double conversions were produced in tests involving the mutant bearing arms of two of the five half-tetrad ry^{+} exceptionals. Since these mutant bearing arms were never retested for the presence of ry^{5} or ry^{41}, one might argue that, in fact, they carried one or the other single mutant. However, such argument requires that they arose as double conversions in the original three-point half-tetrad experiment. In either case, one is forced to the conclusion that multiple conversion events do take place within short chromosomal regions in Drosophila and only require competent genetic systems to assess their significance. In this case, each apparent double conversion is either a two-strand or three-strand double convertant, using classical genetic terminology. In attempting to incorporate the observations on conversion in Drosophila into a single, general, descriptive model of linked exchange, we are compelled to face the fact of high negative interference, in terms of multiple conversion events in short segments, with the fact of positive interference seen in traditional genetic analysis. In this context, we offer the following additional suggestion. Effective pairing for exchange is never complete

for entire chromosome pairs in meiosis. Rather, for any given chromosome pair, there are short regions of effective pairing, and these effectively paired regions vary from meiotic cell to meiotic cell. Multiple exchanges take place in such effectively paired short segments following the model described above, and such events are largely unrecognized in the absence of appropriately marked genetic systems. Positive interference in traditional linkage experiments then merely reflects the discontinuous nature of effective pairing.

One feature of the present model is the requirement that conversion events occur both with and without the exchange of flanking markers. Moreover, we assert that the probability that a given conversion involves marker exchange is itself a variable which is a direct function of the length of the converted segment, and has a mean value of 1/2. This assertion derives from the following features of the maroon-like and rosy studies: (1) The maroon-like cistron is at least an order of magnitude smaller than the rosy cistron. This comparison is based upon recombination studies involving rosy mutants in standard chromosomes (CHOVNICK 1966), while the maroon-like map length is based upon recombination data collected in a homozygous inversion system, specifically used to remove the maroon-like cistron from any possible centromere effect that might reduce recombination (FINNERTY, DUCK and CHOVNICK 1970). A further point in support of this contention stems from our experience in mutagenesis studies. Although controlled comparative mutation studies were never carried out, our efforts to produce maroon-like mutants free of rearrangements were only modestly successful compared to similar experiments which produced overwhelmingly large numbers of rosy mutants. (2) As noted in the discussion of Case 4, a large scale half-tetrad analysis following exchanges involving *ma-l* mutants at opposite ends of the *ma-l* cistron map revealed that all events were non-reciprocal conversions of one or the other mutant allele (SMITH *et al.* 1970). Moreover, a random strand analysis following the production of *ma-l*$^+$ meiotic products from mutant allele heterozygotes revealed that such *ma-l*$^+$ products were produced, and that approximately 1/2 of them exhibited exchange for the flanking markers which provided the basis for the construction of an internally consistent linear map of mutant sites which conformed to the complementation map of these mutants (FINNERTY, DUCK and CHOVNICK 1970). (3) Examination of the random strand mapping data reveals that the frequency of flanking marker exchange among the *ma-l*$^+$ products produced from heterozygous involving very closely linked alleles is lower than 1/2, and that tests involving more distant alleles produced *ma-l*$^+$ exceptionals with a higher frequency of flanking marker exchange. (4) A consideration of the present data involving the close rosy alleles tested (Tables 4, 5, 6 and 7) reveals that the frequency of *ry*$^+$ exceptionals associated with flanking markers exchange is considerably less than 1/2. Indeed, for the closest alleles tested, *ry*^{41} and *ry*^{2}, the random strand analysis yielded no apparent crossover wild types, but as indicated, the one *ry*$^+$ exceptional recovered from a half-tetrad experiment was clearly a conversion of *ry*^{41}, associated with reciprocal exchange for the flanking markers.

Considerable present thinking about the mechanism of conversion derives

from allele conversion frequency inequalities seen as a function of map position. This phenomenon, termed polarity, is seen commonly in fungal systems, and has served as the basis for the polaron model of conversion (LISSOUBA and RIZET 1960). Essentially a "copy-choice" model in which the primary points of breakage occurred at fixed points or linkage structures at the ends of replicating units called polarons, the model fell due to the weight of evidence favoring recombination as the result of breakage and rejoining after replication (see review of WHITEHOUSE 1970). The WHITEHOUSE and HASTINGS (1965) model for conversion, based upon the repair of breaks through the formation of hybrid DNA segments, solves the problem of polarity by arguing that there exist fixed pairing regions or fixed points of initiation of hybrid DNA formation (MURRAY 1969).

The present report attempts to describe the results of allele recombination studies in Drosophila in terms of a model based upon the notion of exchange as the result of the repair of spontaneous breaks within a limited region on two "non-sister" DNA double helices. In the presentation of results, we have indicated how a polarity might be established in a trivial fashion, albeit unsubstantiated by the experiment which was carried out. Nevertheless, factors which are surely secondary to the mechanism of recombination do influence the frequency of exchange in given regions (Tables 2 and 3), and dramatic chromosome regional differences in response to such factors exhibit well established polarities (see review of LUCCHESI and SUZUKI 1968). That there exist allele-specific factors which influence allele conversion frequency is clear (ROSSIGNOL 1969), and these may relate to the specific DNA bases involved in the exchange (DRAPEAU, BRAMMER and YANOFSKY 1968). In view of the unknowns yet to be resolved, and in the absence of strong evidence for polarity of allele conversion frequencies in Drosophila, we are compelled to remain silent on this issue.

At this time, we additionally resist the temptation to fill in the "black box" surrounding the exchange event with a specific detailed description of the repair process leading to exchange. Rather, we direct the reader to the several ingenious efforts in this direction (HOLLIDAY 1964; HOWELL and STERN 1971; PASZEWSKI 1970; STAHL 1969; WHITEHOUSE and HASTINGS 1965), and discussion of the implications and limitations of the various proposals (EMERSON 1969; GUTZ 1971; HOLLIDAY and WHITEHOUSE 1970; PASZEWSKI 1970; WHITEHOUSE 1970).

SUMMARY

A study of recombination between separable mutants of the rosy cistron in *Drosophila melanogaster* was carried out utilizing free-third chromosomes in most experiments. One half-tetrad experiment is described. The progeny of rosy mutant heterozygous females, whose chromosomes were additionaly marked with a double set of flanking markers, were grown on a purine enriched selective medium which selects for survival only those offspring receiving a ry^+ meiotic product. Analysis of exceptional ry^+ survivors permits their classification as either classical crossovers or convertants. Experiments are described which demonstrate: (1) The effect of a heterozygous deletion in a region adjacent to

the rosy cistron is to reduce both crossovers and conversions within the rosy cistron. (2) The effect of a heterologous, multiple break rearrangement, is to substantially increase both crossovers and conversions within the rosy cistron. (3) A specific relationship exists between apparent crossovers and convertants in any cross which directly relates to the relative distance between the rosy mutants in that cross. The data are considered with respect to polarity of conversion frequency, as well as the question of postmeiotic segregants. A model is presented which describes crossing over and conversion in Drosophila as the result of a single event. Emphasis is placed upon setting forth, in model form, the consequences of such events, in terms of the present as well as prior results of studies of genetic fine structure and conversion in Drosophila.

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