STUDIES of recombination in Drosophila involving closely linked markers (i.e., separable sites within a complex locus) have yielded results which may be grouped into at least two classes. Thus, if one considers investigations of random products of meiosis, one class of observations is characterized by: (1) unidirectional regular exchange for the closely linked flanking markers which permits the construction of a unique, and internally consistent, linear map of sites within the complex. (2) Positive interference is seen in that all products of exchange between the marker sites within the complex are classifiable as products of single-exchange tetrads. An example of such data are those seen in the study of the lozenge complex (GREEN and GREEN 1949, 1956). In contrast, the garnet mutants (CHOVNICK 1958, 1961), maroon-like mutants (FINNERTY, DUCK and CHOVNICK 1970) and Notch mutants (WELSHONS and VON HALLE 1962; BAILLIE, ASTELL and SCHOLEFIELD 1966) yield meiotic products which appear to reflect negative interference in conjunction with exchange between the mutants within the complex. Nevertheless, if one restricts attention to the apparent single-exchange products in these systems, unambiguous linear mapping of sites within the complex obtains.

Although full tetrad analysis is not possible in Drosophila, investigation of the reciprocity of recombination events at complex loci is afforded by use of compound chromosomes such as the attached-X chromosome and compound autosomes, which permit recovery of half-tetrad products of meiosis. Recombination studies with such chromosomes in Drosophila provide complete confirmation of the classical principles of exchange for relatively distant chromosome markers (WELSHONS 1955; BALDWIN and CHOVNICK 1967). However, studies of the half-tetrad products of exchange involving very closely linked markers, like the random strand analyses, may be divided into two categories of results. One class of observations on exchange events between mutant members of a gene complex is best exemplified by the bithorax system of mutants (LEWIS 1967). In this system, exchanges are reciprocal, and positive interference appears to be the rule. On
the other hand, half-tetrad analysis of recombination events involving garnet mutants have yielded evidence that both reciprocal and nonreciprocal events are taking place, and negative interference may be inferred from the reported data (Chovnick 1961; Hexter 1963).

Similar observations have long been associated with intracistronic recombination in fungi. Nonreciprocal exchange observed in tetrad analyses (Mitchell 1955), high negative interference associated with random strand and half-tetrad analyses (Pritchard 1955), and frequency inequalities associated with mutant allele map position (Lissouba and Rizet 1960; Murray 1963) were early key observations in the study of allele recombination, now referred to as gene conversion. Efforts to understand these deviations from classical notions about linked recombination have led to provocative models (Boon and Zinder 1969; Hastings and Whitehouse 1964; Holliday 1964; Paszewski 1970; Whitehouse 1963) which interpret these observations on recombination in molecular terms (See review by Meselson 1967). Although there is a rich literature of ingenious experimental effort in fungi designed to provide an understanding to this phenomenon (Emerson 1969; Emerson and Yu-Sun 1967; Fogel and Mortimer 1969; Kitani and Olive 1967, 1969; Murray 1969, 1970; Stadler and Kariya 1969), the mechanism of gene conversion and its relationship to linked recombination is not yet fully understood. Although the occurrence of a conversion-like event in Drosophila melanogaster may be inferred from the prior studies, none of the genetic systems used in those investigations provided an opportunity for systematic investigation as characterized by studies with the fungal systems. The present report presents an attempt to develop such a system for the investigation of gene conversion in Drosophila. In addition to providing a convincing demonstration of gene conversion in Drosophila and its relationship to linked recombination, this approach may provide additional versatility to the experimental pursuit of questions concerning the mechanism of recombination.

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 classes: (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 (Hurry 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 reveal any evidence of allele complementation (Schalet, Kernaghan and Chovnick 1964).

Figure 1 presents a map of the centromere proximal third of the right arm of chromosome-3 indicating the map position of various mutants used in the present investigation (Lindsley and Grell 1967), and focusing upon the results of prior genetic fine structure mapping of enzymatically inactive rosy cistron mutants (Chovnick 1966). The centromere position is based upon
Figure 1.—A genetic map of the rosy region of chromosome 3. The map positions of various mutants used in this study are indicated, and the genetic fine structure of the rosy cistron is summarized.

Holm et al. (1969). The rosy cistron fine-structure map, presented in Figure 1, emerged from a random strand analysis of meiotic products. In order to permit large-scale sampling of such meiotic products, the experiments utilized a system of recessive lethal mutants flanking the rosy region, which selected for survival only those progeny receiving a meiotic product which had undergone a single (or odd-numbered multiple) exchange between the flanking lethal markers. Although these earlier experiments succeeded in demonstrating the existence of intracistronic recombination in higher organisms, the selective system was most effective in suppressing observations on gene conversion.

The construction of compound autosomes: All compound-3 autosomes constructed at Storrs (identified by code letter S) were produced by X-ray treatment (3,000r) of virgin females carrying standard chromosomes, and mating them to differentially marked C(3L); C(3R) males. Except for occasional nondisjunctional progeny, all viable and fertile offspring inherit one paternal compound autosome, and a newly generated complementary compound chromosome from the treated mother. Such synthesis of new compounds may be envisioned as resulting from the rejoining of a pair of induced breaks straddling the centromeric region of the treated chromosomes, thereby producing a reversed metacentric compound for either the left or right arms of the chromosome (Figure 2). While a group of such compound chromosomes may differ in their
proximal heterochromatic regions by virtue of the independence of the events which led to their production, differences between them are limited to the extent that they were selected as producing viable and fertile progeny with a preexisting complementary compound. Consequently, all of the compounds produced in this manner relate to the synthesis of the first compound-3 chromosomes (RASMUSSEN 1960). The symbolism adopted by LINDSLEY and GRELL (1967) is used to describe specific compound chromosomes. Thus, \( C(3L)RM, P2, ri/ri \) describes a reversed metacentric compound 3L chromosome, designated P2 (Pasadena-2), reflecting its origin, and homozygous for the recessive mutant radius incompletus (\( ri \)). Heterozygous compounds are selected as newly generated single males, and maintained in stock by mating to differentially marked homozygous females in each generation. Thus, males of the genetic constitution, \( C(3L)RM, P2, ri/ri; C(3R)RM, SH1, Dfd \ kar 31 \ ry^s/cu \ kar \ ry^s \) \( l26 \ Sb \) are crossed, in each generation, to virgin females from the homozygous stock \( C(3L)RM, P2, ri/ri; C(3R)RM, SH3, ry^s/ry^s \).

Selective system matings: By virtue of the absence of recombination in males, large populations of progeny males and females may be produced with each individual possessing a \( C(3R)RM \) chromosome heterozygous for a given pair of rosy mutants, and a specific distribution of markers flanking the rosy region. Large-scale matings of such heterozygotes to appropriately marked testers involve matings of 30 pairs of parents/half-pint milk bottle, on standard cornmeal molasses medium, maintained at 23°-26°C. Following an initial 3-day incubation, the parents are transferred to fresh cultures at 2-day intervals, through a total of four broods of progeny representing nine days of egg laying. Immediately after each transfer, 1.5 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 the activity of the enzyme XDH 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.

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 invariably \( ry^+ \) in eye color phenotype, and occur as single individuals of either sex, distributed at random among the matings. Essentially, the experiment involves large scale production of zygotes, each representing a half-tetrad, and the experimental system selects only the interesting half-tetrads for survival. Exceptional \( ry^+ \) survivors are placed in stock by crossing to \( C(3L)RM, P2, ri/ri; C(3R)RM, SH3, ry^s/ry^s \) homozygotes, and maintained by selective mating of homozygous \( ry \) females to \( ry^+ \) males in each generation.

Detachment of exceptional half-tetrads: Aged virgin females carrying an exceptional \( ry^+ \) half-tetrad are X-rayed (2,000r), and mated to standard chromosome males carrying \( In(3LR) DczF \). (LINDSLEY and GRELL 1967), an effective balancer for the rosy region of chromosome 3. Usually, fifteen matings of 20 pairs of parents/half-pint milk bottle with six days of egg production suffice to produce 15 to 25 surviving and fertile offspring, each carrying a detached member of the half-tetrad of interest, balanced over \( In(3LR) DczF \).

Genetic tests of detachments: Each detachment experiment survivor is crossed to \( l26/In(3LR)cu \ kar \ Ubx^A \). This cross tests for the presence of \( l26 \) and \( cu \), permits confirmation of the presence of a \( kar \) allele, and provides progeny carrying the detached arm balanced over the \( Ubx^A \) balancer chromosome (LINDSLEY and GRELL 1967). In addition to establishing stock for each detached chromosome, subsequent matings are made to the following stocks: (1) \( ry^s/ry^s \); (2) \( kar 31/In(3)MRS, M(3)S34 \ ry^s \) \( Sb \); (3) \( Dfd \ kar/In(3LR)cu \ kar \ Ubx^A \). \( In(3)MRS \) is an X-ray induced rearrangement which completely suppresses recombination in heterozygotes for the region between scarlet (\( st \)) and ebony (\( e \)), and which has been used as an effective balancer for the rosy region in prior studies (CHOVNICK et al. 1962). Since the dominant deformed eye phenotype (\( Dfd \)) overlaps wild type at ordinary culture temperatures, the presence of this marker was routinely verified by its lethality in homozygotes in subsequent tests. In fact, all of the exceptional \( ry^+ \) half-tetrads successfully analyzed did carry \( Dfd \) on one of its arms. Analysis of the progeny of matings to the tester stocks described above permits complete diagnosis of the
genetic composition of each detached chromosome. Thus for each \( ry^+ \) half-tetrad survivor of the purine selective system experiment, the group of detached arms, upon analysis, identifies the genetic composition of the half-tetrad.

**Further tests of detachments; XDH assay:** For each surviving \( ry^+ \) half-tetrad, a series of detached arms falls into two classes with respect to the rosy cistron, and these reflect the two strands of the half-tetrad. One strand is \( ry^+ \) with respect to eye color, and the other invariably is mutant. The XDH activities associated with detached \( ry^+ \) chromosomes were examined in heterozygotes carrying a deficiency for the rosy cistron, \( Df(3)ry^{75} \) (LINDSLEY and GRELL 1967) by use of a modification of the method of GLASSMAN (1962). Unless otherwise indicated, all procedures were carried out at \( 0^\circ \text{-} 5^\circ \text{C} \). Enzyme is prepared as follows: 30 flies (15 males and 15 females) are homogenized in 1 ml of 0.1M phosphate buffer (pH 7.6) containing 2 mg/ml of bovine serum albumin (Sigma Chemical Co.). The homogenate is centrifuged at 35,000 \( \times g \) for 45 min, and 10 mg of Norite-A is added to the decanted supernate, and permitted to stand for 30 min. The mixture then is filtered through a 0.45 \( \mu \), Type HA, Millipore filter, the filtrate serving as enzyme. Activity is measured as the change in fluorescence associated with the conversion of 2-amino-4-hydroxypteridine (AHP, Calbiochem) into isoxanthopterin (ISO) at \( 30^\circ \text{C} \) using a Photovolt Model 540 fluorometer equipped with a 335 nm primary filter and a 412 nm secondary filter. At maximum sensitivity, the instrument is standardized at 100 fluorescence units with 1.6 \( \times 10^{-4} \)M quinine sulfate in 0.1M \( \text{H}_2\text{SO}_4 \), and zeroed with a blank containing the reaction mixture omitting enzyme. Reaction tubes (8 \( \times \) 8 \( \times \) 50 mm cuvettes) contain 1 ml of albuminized phosphate buffer, .01 ml of \( 10^{-3} \)M AHP and .01 ml of \( 10^{-3} \)M methylene blue to which is added 0.1 ml of enzyme. Following this procedure, the increase in fluorescence is linear for 30 minutes and is proportional to the amount of enzyme added between 0 and 0.4 ml.

**RESULTS**

The progeny of large-scale reciprocal crosses of \( C(3L)_RM,P2,ri/ri; C(3R)RM,SH1,Dfd\ kar^{q1}ry^{41}/cu\ kar\ ry^{41}\_{126} Sb \) to \( C(3L)_RM,P2,ri/ri; C(3R)RM,SH3,ry^{y}/ry^{2} \) were grown on purine enriched medium which permits the survival only of individuals with significant XDH activity, and consequently a \( ry^+ \) eye color phenotype. The results of these crosses are summarized in Table 1. A total of 26 phenotypically \( ry^+ \) progeny were recovered from crosses involving rosy mutant heterozygous females (Experiment 1), while none was observed from the reciprocal cross (Experiment 2). There were no clusters observed, and their phenotypes with respect to other markers involved in the cross point to the origin of these \( ry^+ \) half-tetrads as meiotic products of tetrads involving the \( C(3R)RM,SH1,Dfd\ kar^{q1}ry^{41}/cu\ kar\ ry^{41}\_{126} Sb \) chromosome. In addition to the striking difference in reciprocal cross results (Experiments 1 and 2, Table 1), a recombinational rather than mutational basis for the origin of the exceptional \( ry^+ \) half-

<table>
<thead>
<tr>
<th>Expt #</th>
<th>Female parent</th>
<th>Male parent</th>
<th>Phenotypes of ( ry^+ ) progeny</th>
<th>N</th>
<th>System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( ry^{y}/ry^{41} )</td>
<td>( ry^{y}/ry^{2} )</td>
<td>( \text{Dfd} ) ( \text{ker} ) ( ry^+ \text{Sb} ) ( ry^+\text{Sb}^* )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>( ry^{y}/ry^{41} )</td>
<td>( ry^{y}/ry^{2} )</td>
<td>21 5</td>
<td>520,000</td>
<td>C(3R)</td>
</tr>
<tr>
<td>2</td>
<td>( ry^{y}/ry^{41} )</td>
<td>( ry^{y}/ry^{41} )</td>
<td>0 0</td>
<td>950,000</td>
<td>C(3R)</td>
</tr>
<tr>
<td>3</td>
<td>( ry^{y}/ry^{41} )</td>
<td>( ry^{y}/ry^{41} )</td>
<td>0 0</td>
<td>400,000</td>
<td>Free 3</td>
</tr>
<tr>
<td>4</td>
<td>( ry^{y}/ry^{41} )</td>
<td>( ry^{y}/ry^{41} )</td>
<td>0 0</td>
<td>535,000</td>
<td>Free 3</td>
</tr>
</tbody>
</table>
MEIOTIC EVENT PROGENY HALF-TETRAD PROGENY PHENOTYPE

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Figure 3—Classical crossing over between rosy mutants in half-tetrads.

tetrads is supported by the homozygous controls of Experiments 3 and 4. No ry+ exceptions arose in 800,000 free third chromosomes sampled in progeny of homozygotes of cu kar ry4'/cu kar ry41, nor did any arise in 1,070,000 chromosomes sampled in progeny of ry4'/ry41 homozygotes.

Let us assume that all ry+ survivors derive from classical single exchanges between the two mutants, ry4 and ry41, taking place in oogenesis in the heterozygote. Figure 3 illustrates the two classes of single exchanges, which are expected to occur with equal frequency on this model, and the resulting ry+ survivors. Class A exchanges involve two non-sister chromatids destined to be attached to the same centromere at the completion of meiosis, while Class B exchanges involve non-sister chromatids destined to be attached to different centromeres at the completion of meiosis. It should be noted that while the simple crossover model in fact predicts the two phenotypic classes of ry+ progeny observed, their observed frequencies (Table 1, Experiment 1) deviate sharply from the expected 1:1 ratio (BALDWIN and CHOVNICK 1967).

Analysis of exceptional (Dfd)kar ry+ Sb half-tetrads: Of the 21 exceptions recovered in this category (Table 1), only 18 were subjected to further analysis. Those omitted were: (1) one female died without reproducing, (2) one male died without reproducing, and (3) one female transmitted only rosy mutant half-tetrads. In view of the nonautonomous nature of the mutant phenotype, and since flies with low levels of XDH activity can survive on the purine selective medium (FINNERTY, DUCK and CHOVNICK 1970), it is inferred that the phenotype of this fly reflected a somatic event. Thus, somatic reverse mutation, suppressor mutation, recombination, or even somatic segregation from a hybrid DNA double helix produced as a result of a meiotic event, are feasible explanations for the origin of this fly.

The remaining 18 exceptional Dfd kar ry+ Sb progeny half-tetrads (originally appearing as eight female and 10 male exceptional survivors) were subjected to the protocol described above for detachment and analysis of exceptional half-tetrads. The resulting data are summarized in Table 2.
The 18 exceptional Dfd kar ry+ Sb half-tetrads fall into 4 genotypic classes with respect to the distribution of markers involved in the experiment. While six of the half-tetrads (first row, Table 2) are classified most simply as Class A, classical, single exchanges (Figure 3), the remaining 12 ry+ half-tetrads defy explanation in terms of classical single exchanges within the rosy cistron. We shall refer to the process which gave rise to the latter group of half-tetrads as gene conversion, and the specific product of such an event will be referred to as a convertant. For the moment, let us restrict our definition of conversion to operational terms. Thus, we are dealing with an unspecified event which leads to the "conversion" of a rosy mutant allele to a ry+-like allele. The event occurs in heterozygotes, and not in homozygotes, and takes place when such heterozygotes are passed through meiosis in females, but not in males. At this point, it is distinguished from classical allele recombination in that it takes place unaccompanied by exchange for the closely linked flanking markers.

One group of 10 ry+ half-tetrads, diagnosed as ry41 convertants (row 2, Table 2) exhibits the parental distribution of all markers flanking the rosy cistron. In each half-tetrad of this group, the ry+ chromosome bears the specific array of flanking markers originally associated with the ry41-bearing chromosome, while the ry mutant member exhibits the markers associated with the ry5-bearing chromosome. The last two ry+ half-tetrads (rows 3 and 4, Table 2) are classified as ry5 convertants. One (row 3, Table 2) exhibits no change from the parental distribution of markers flanking the rosy region. The ry+ chromosome carries the markers originally associated with the ry5-bearing chromosome, while the rosy mutant-bearing chromosome carries the distribution of markers originally present on the ry41 parent chromosome. The last ry+ half-tetrad (row 4, Table 2), also classified as a ry5 convertant, exhibits the parental distribution of markers immediately flanking the rosy cistron, but is a recombinant with respect to the more distal flanking markers.

**Analysis of exceptional (Dfd) kar ry+Sb+ half-tetrads:** All five of the ex-

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### Table 2

<table>
<thead>
<tr>
<th>Half-tetrad genotype</th>
<th>Number</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dfd + kar ry+ Sb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ cu kar + + + +</td>
<td>6</td>
<td>Class A (Figure 3) exchange</td>
</tr>
<tr>
<td>Dfd + kar ry+ Sb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ cu kar + + +</td>
<td>10</td>
<td>Conversion-ry41</td>
</tr>
<tr>
<td>Dfd + + + + +</td>
<td>1</td>
<td>Conversion-ry5</td>
</tr>
<tr>
<td>Dfd + + + Sb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ cu kar + +</td>
<td>1</td>
<td>Conversion-ry5 Crossover, 126-Sb</td>
</tr>
</tbody>
</table>
ceptional progeny recovered in this category (Table 1) were males that could not be further analyzed. They either failed to reproduce at all, or reproduced poorly. All were kar ry+ Sb+ in phenotype, and four of them were diagnosed as carrying the Dfd marker. Loss of the Sb marker in association with the ry+ phenotype suggests a Class B (Figure 3) exchange event which would produce half-tetrads rendered homozygous for the chromosome-3 arm distal to the exchange. Consider that a recessive mutation, perhaps induced by the same X-ray treatment that led to the production of the C(3R)SH chromosome, was present distal to the rosy cistron on the Dfd kar+ ry+ Sb+ arm of the SHI compound-3 chromosome. A Class B (Figure 3) exchange would then produce a ry+ half-tetrad rendered homozygous for the mutation. In order to pursue this point further, a small-scale repeat of Experiment 1 (Table 1) was carried out, and progeny were reared on standard medium without purine supplementation. Examination of the progeny of this cross revealed that (1) there was no disturbance in the sex ratio of (Dfd) kar ry Sb+ progeny, but that (2) both males and females of this class were highly infertile. These observations support the notion that the five (Dfd) kar ry+ Sb+ exceptions (Table 1), in fact, represent Class B (Figure 3) exchanges. Since the number of half-tetrads for Class A has been determined as six (row 1, Table 2), a 1:1 ratio of the two classes of exchanges, predicted on the classical crossover model (Figure 3), does obtain.

**Further tests of detachments; rosy mutant identity tests:** As indicated in prior discussion, one arm of each exceptional ry+ half-tetrad (Table 2) is mutant with respect to the rosy cistron. An important question concerns the nature of the specific mutant allele(s) present on these arms. Thus, one wonders if: (1) the Class A reciprocal exchanges (row 1, Table 2) in fact, do carry both parental rosy mutant alleles on the rosy mutant arm? (2) the mutant-bearing arms of the convertant half-tetrads do carry one, or the other, or both, parental mutant alleles? Experiments designed to answer these questions are in progress, and will be reported separately.

However, one series of experiments bearing on these questions is completed. This experiment deals with one exceptional half-tetrad, I-B62, which arose early in the study. It was subjected to the protocol described above for detachment and analysis of detached arms, and fell into the category of a Class A exchange (row 1, Table 2). One of the detached rosy mutant-bearing arms of this half-tetrad, I-B62-16, carrying the markers Dfd kar+ ry l26 Sb, was subjected to experiments designed to identify the specific rosy mutant allele(s) present, and the data are summarized in Table 3. Large-scale testcrosses involving free third chromosome females heterozygous for the I-B62-16 chromosome and a chromosome bearing either ry+ or ry1 were carried out following the purine selective system protocol described above. As indicated, (Table 3), these experiments failed to yield ry+ progeny in approximately 10⁶ chromosomes assayed from each heterozygous genotype. In contrast, a control experiment on the frequency of ry+ chromosomes (both convertants and recombinants) produced by free third chromosome heterozygous ry+ females provides dramatic support for the conclusion that the I-B62-16 chromosome is a double mutant, ry+ ry1.
**TABLE 3**

*Test of detachment, I-B62-16, Dfd kar^31 ry l26 Sb*

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>ry⁺ progeny</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-B62-16</td>
<td>ry⁺</td>
<td>ry⁺/ry⁺</td>
<td>0</td>
</tr>
<tr>
<td>cu kar ry₄¹</td>
<td>ry⁺</td>
<td>ry⁺/ry⁺</td>
<td>0</td>
</tr>
<tr>
<td>Dfd + kar +</td>
<td>ry⁺</td>
<td>ry⁺/ry⁺</td>
<td>13</td>
</tr>
<tr>
<td>cu kar +</td>
<td>ry₄² l26 Sb</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I-B62-16 = Dfd kar^31 ry⁺ ry₄¹ l26 Sb

An intracistronic three-point half-tetrad experiment: Utilizing the I-B62-16 chromosome, a compound-3 chromosome was constructed (see MATERIALS AND METHODS) which is designated C(3R)RM,SC2, and which possesses arms of the genotype, Dfd + kar + ry⁺ + ry₄¹ l26 Sb, (Figure 1). Large-scale crosses to C(3L)RM,P2,ri/ri; C(3R)RM,SH3,ry⁺/ry⁺ males were carried out utilizing the purine selective system, and the results are summarized in Table 4. Seven phenotypically kar ry⁺ Sb progeny (5 males, 2 females) were recovered. There were no clusters, and their phenotypes indicate their maternal origin from the C(3R) RM,SC2 chromosome. Two males died without reproducing, but the remaining five ry⁺ exceptionals were established in stock and confirmed to be Dfd kar ry⁺ Sb in phenotype. Subsequent detachment and genetic analysis (see protocol above) revealed that all five half-tetrads were identical, and were most simply classifiable as ry⁺ revertions or conversions. Each half-tetrad possessed a ry⁺ arm bearing the specific array of flanking markers originally associated with the ry₄²-bearing chromosome, as well as a rosy mutant-bearing arm carrying all of the flanking markers originally associated with the I-B62-16 parental chromosome. Evidence against the notion that the exceptional half-tetrads arose by reverse mutation of ry₄² derives from the following considerations: An estimate of

**TABLE 4**

*Three-point half-tetrad experiment*

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>ry⁺ progeny</th>
<th>Total progeny sampled</th>
<th>Total ry⁺ chromosomes sampled</th>
<th>System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dfd + kar +</td>
<td>ry⁺</td>
<td>ry⁺/ry⁺</td>
<td>7</td>
<td>251,000</td>
<td>C(3R)</td>
</tr>
<tr>
<td>cu kar +</td>
<td>ry₄² l26 Sb</td>
<td></td>
<td></td>
<td>125,500</td>
<td></td>
</tr>
<tr>
<td>cu kar ry₄²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cu kar ry₄²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cu kar ry₄²</td>
<td></td>
<td></td>
<td></td>
<td>Free 3</td>
</tr>
</tbody>
</table>
the control reversion rate in free third chromosome homozygotes of ry^{4z} may be derived from the failure to recover ry^{+} progeny in experiments which assayed 1,376,000 free third, ry^{4z}-bearing chromosomes (Table 4). The maximum reverse mutation frequency based upon construction of a 95% Poisson confidence interval is $2.2 \times 10^{-6}$ (Stevens 1942). Estimating that $\frac{1}{2}$ of the total progeny sampled in the compound-3 experiment were carrying $C(3R)RM,SC2$ chromosomes, each of which possesses one ry^{4z}-bearing arm, then the observed frequency of ry^{4z} reversion (or conversion) is one order of magnitude greater ($3.9 \times 10^{-5}$) when assayed in $C(3R)$ heterozygotes passed through oogenesis.

Further tests of detachments; XDH activities associated with the ry^{+} chromosomes: Individuals with low levels of XDH activity exhibit a wild-type eye color phenotype (Chovnick et al. 1969), and are capable of surviving the purine selection procedure (Finnerty, Duck and Chovnick 1970). In view of these facts, it is pertinent to question the XDH activity levels associated with the ry^{+} chromosomes generated in this investigation. One would anticipate that the ry^{+} alleles produced by exchange events (as classically envisioned) should possess wild-type genetic information. Might the ry^{+} alleles generated by conversion be distinguishable from a standard wild-type ry^{+} allele? Are there differences in the ry^{+} alleles produced by conversion of different rosy mutants? Are there differences between ry^{+} alleles produced by conversion and those classified as recombination products?

For each exceptional ry^{+} half-tetrad subjected to detachment and further analysis, a series of ry^{+}-bearing free third chromosome stocks exists. To characterize the ry^{+} alleles associated with these exceptional half-tetrads, the XDH activity associated with one ry^{+} chromosome from each exceptional half-tetrad was assayed, and the results are summarized in Figure 4. The experiment was repeated by choosing another ry^{+}-bearing free third chromosome derived from each exceptional half-tetrad, and the results of the two experiments are indis-

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**Figure 4.** XDH activity levels associated with exceptional ry^{+} chromosomes. Ordinate: XDH activity measured as the average change in fluorescence/10 minutes. Abscissa: Origin of exceptional ry^{+} chromosomes. (A) Convertant of ry^{41}, (B) Convertant of ry^{4k}, (C) Classical recombinant between ry^{5} and ry^{41}. Each exceptional ry^{+} chromosome was examined in heterozygotes with $Df(3)ry^{75}$. 

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tinguishable. Each $r_y^+$-bearing chromosome was assayed in heterozygotes over a deficiency for the rosy cistron following procedures described above (see MATERIALS AND METHODS). Fluorescence readings were taken at 0, 10, and 20 min, with XDH activity recorded along the ordinate of Figure 4 as the average change in fluorescence/10 min. The mutant allele, $r_y^a$, served as a null activity control, while the $r_y^+$ allele present on $In(3)Ubx^+$ served as an independent wild-type control. Both controls were assayed as extracts of flies heterozygous for the rosy deficiency. The $r_y^a$ mutant control invariably registered zero level activity in repeated assays, while the wild-type control registered a mean of 40 units of activity in four assays which ranged from 36 to 44 units at the extremes.

The results of the experiment are summarized in Figure 4. The horizontal line located at 40 units of XDH activity represents the activity associated with the standard $r_y^+$ allele. Each dot represents the activity measurement associated with a $r_y^+$-bearing chromosome recovered from a half-tetrad which had been classified as a convertant of $r_y^{41}$ (A), convertant of $r_y^{42}$ (B) or a classical recombinant between $r_y^a$ and $r_y^{41}$ (C). The number of dots in each class of Figure 4 reflects the number of half-tetrads completely analyzed at the time of this experiment. Only two $r_y^{41}$ convertants (A) and one recombinant (C) were not included. Examination and comparison of the distribution, and the 95\% confidence intervals of XDH activities associated with each class of $r_y^+$ alleles clearly leads to the conclusion that the convertants and recombinants are indistinguishable from each other, and from the standard $r_y^+$ allele present on the $In(3)Ubx^A$ chromosome.

DISCUSSION

Prior to the availability of compound autosomes, half-tetrad analysis in Drosophila was restricted to experiments utilizing sex-linked markers and compound-X chromosomes. While the compound-X system indeed provides for the recovery of half-tetrads, large-scale sampling of rare recombinational events making use of mass matings and selective procedures is severely restricted. Following the synthesis, in a single female, of a compound-X chromosome heterozygous for a series of markers with a specific chromosomal distribution, recombination during oogenesis will lead to the production of a genotypically heterogeneous population of compound-X females, identical in phenotype. Analysis of subsequent experiments which use such females, requires single-female matings and complete scoring of all offspring phenotypes to determine the specific chromosomal distribution of markers for each female parent. In contrast, half-tetrad analysis using compound autosomes may be conducted on a mass-mating scale (BALDWIN and CHOIVNICK 1967), and selective procedures may be imposed upon the experimental system to provide for selective recovery of rare classes of progeny, as exemplified by the present investigation.

The present report describes experiments leading to the selective recovery and analysis of rare $r_y^+$-like half-tetrads produced in mass matings involving rosy mutant half-tetrads. The following features of the analysis indicate their origin as products of a recombinational event: (1) They exhibit an unclustered distri-
bution as products of oogenesis, and never arise from spermatogenesis. (2) They arise only from mutant females heterozygous for recombinationally separable rosy mutants, and not from mutant homozygotes. (3) The \( ry^+ \) alleles generated in these experiments are indistinguishable from each other, as well as from a standard wild-type allele (Figure 4). One might envision a model of conversion which involves a special mutagenesis occurring in heterozygous females. On such a model, the array of \( ry^+ \) alleles generated from various mutant alleles of rosy might be expected to differ somewhat in levels of restored XDH activity. In contrast, a recombinational model would suggest uniform restoration of wild-type levels of XDH activity.

Restricting attention to the first group of experiments (Tables 1 and 2), the analysis indicates approximately 50% of the \( ry^+ \) half-tetrads result from events associated with apparent single exchange (Figure 3). Unidirectional exchange is seen for the markers immediately flanking the rosy region, and these data confirm the prior positioning of the rosy mutants (Figure 1). The remaining \( ry^+ \) half-tetrads do not exhibit recombination for the markers immediately flanking the rosy cistron. Since the present data do not determine the mutant allele(s) present on the mutant-bearing arm of each of these nonrecombinant \( ry^+ \) half-tetrads, explanations of their origin involving reciprocal events and high negative interference are not precluded. In this context, three points about the present data should be noted: An inequality exists in the relative frequencies of conversion of \( ry^4 \) and \( ry^{4+} \), and this inequality is inconsistent with one feature of such a model. The map distance from \( kar-ry \) is twice that from \( ry^{-126} \). If one considers that the convertant classes involve classical double exchanges, each involving one exchange between the separable \( ry \) mutants as outlined in Figure 3, then one would expect more \( ry^4 \) convertants than \( ry^{4+} \) convertants. In fact, there were five times more \( ry^{4+} \) convertants than \( ry^4 \) convertants. Such frequency inequalities might be indicative of a polarized pattern of conversion, and this matter is currently under investigation. (2) An extremely high intracistronic negative chromatid interference is required to explain the results of the intracistronic three-point test (Table 4) on a classical multiple exchange model. (3) The present data on conversion in Drosophila are entirely consistent with the fungal data, and are interpretable in terms of nonreciprocal events involving breakage and DNA base correction.

A pattern of facts about conversion and classical reciprocal recombination in Drosophila is apparent from current investigations which directly relate these events to the length of the genetic interval between the markers under study. A large-scale half-tetrad analysis of recombination between site mutants in the \( \text{ma-l} \) cistron reveals that such events are predominantly, and possibly exclusively, nonreciprocal in nature (Smith, Finnerty and Chovnick 1970 a,b). If we assume, for the moment, that the convertants of the present report (Tables 1 and 2) reflect nonreciprocal events, while the classical recombinant classes are reciprocals, then the results of the rosy cistron analysis stand in contrast to that obtained for \( \text{ma-l} \). For approximately 50% of the events leading to the generation of \( ry^+ \)
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half-tetrads are classical recombinant classes (Figure 3). In both the ma-l and ry experiments, the specific mutants used in the experiment were chosen because of their location at opposite ends of their respective intracistronic maps. It is more than curious that the total map length of the rosy cistron (Figure 1) is an order of magnitude greater than that of the maroon-like cistron (FINNERTY, DUCK and CHOVNICK 1970). Returning now to the cases of complex loci in Drosophila which have exhibited positive interference in random strand analysis and only reciprocal exchanges in half-tetrad analysis (see INTRODUCTION), one is struck by the fact that map distances between members of the lozenge complex are an order of magnitude greater than the total length of the rosy cistron (GREEN and GREEN 1956). Moreover, separable members of the bithorax system are two orders of magnitude further apart than the length of the rosy cistron (LEWIS 1967). It is tempting to speculate that these exceedingly large complex genes may be clusters of cistrons, perhaps related in function as members of an operon. Indeed, LEWIS (1964) has shown how the phenotypic interactions exhibited by mutants of the bithorax system may be explained on such a model. Alternatively, systems such as lozenge and bithorax may well represent giant cistrons which code for exceedingly long polypeptides. For example, in vitro studies of polyribosomal synthesis of various peptide subunits provide strong evidence for an array of monocistronic messages varying in length over one order of magnitude from that of rabbit hemoglobin (WARNER, RICH and HALL 1962) to chick myosin (HEYWOOD, DOWBEN and RICH 1967; HEYWOOD and RICH 1968). Indeed, still longer peptide subunits are recorded (KLOTZ and DARNELL 1969) which provide credibility for the range of cistron sizes suggested in this discussion.

SUMMARY

A study of gene conversion and recombination between separable mutants of the rosy cistron in Drosophila melanogaster was carried out in half-tetrads utilizing compound-third chromosomes. The progeny of mass matings were grown on a purine-enriched selective medium which permits survival only of offspring with significant xanthine dehydrogenase activity. Analysis of exceptional ry+ survivors permit their classification as either classical recombinants or convertants. Both classes share features which implicate a single process as their mode of origin. A three-point intracistronic half-tetrad analysis was carried out utilizing a mutant heterozygous half-tetrad of the type $a^+ c/ b^+ +$, and possessing flanking markers. A high frequency of ry+ half-tetrads were recovered, and all of these, upon analysis were convertants of the middle allele. The data are entirely consistent with the extensive studies of gene conversion and allele recombination in fungal systems, and are interpretable in terms of nonreciprocal events involving breakage and repair synthesis of DNA as the mechanism of recombination. Moreover, a consideration of recent research on allele recombination in Drosophila suggests that the relative frequency of nonreciprocal to reciprocal events is directly related to the length of the genetic interval between the markers under study.
LITERATURE CITED


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