GENETIC LIMITS OF THE XANTHINE DEHYDROGENASE STRUCTURAL ELEMENT WITHIN THE ROSY LOCUS IN *DROSOPHILA MELANOGASTER*[1]

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Experiments are described that provide an opportunity to estimate the genetic limits of the structural (amino acid coding) portion of the rosy locus (3:52.0) in *Drosophila melanogaster*, which controls the enzyme, xanthine dehydrogenase (XDH). This is accomplished by mapping experiments which localize sites responsible for electrophoretic variation in the enzyme on the known genetic map of null-XDH rosy mutants. Electrophoretic sites are distributed along a large portion of the null mutant map. A cis-trans test involving electrophoretic variants in the left- and right-hand portions of the map leads to the conclusion that the entire region between these variants is also structural. Hence most, if not all, of the null mutant map of the rosy locus contains structural information for the amino acid sequence of the XDH polypeptide. Consideration is given to the significance of the present results for the general problem of gene organization in higher eukaryotes.

THE genetic unit in higher organisms may be a much larger entity than its counterpart in prokaryotes. Support for this statement emerges from a comparison of genetic and chemical observations on *Drosophila melanogaster* and *Escherichia coli*. *D. melanogaster*, having only two- to threefold more genes than *E. coli*, has roughly 50-fold more DNA per haploid genome. The Drosophila estimates are based upon an extrapolation from the saturation mapping of small genetic regions (JUDD, SHEN and KAUFMAN 1972; HOCHMAN 1973) and genomic DNA estimates by cytophotometric (RASCH, BARR and RASCH 1971) or reassociation kinetic (LAIRD and MCCARTHY 1969; LAIRD 1971) techniques. Estimates of the number of cistrons in *E. coli* comes from TAYLOR (1972) and the physical size of the genome from CAIRNS (1962).

Coupling the above observations to the facts that (1) eukaryote mRNA is monocistronic (KUFF and ROBERTS 1967; PETERSON and MCLAUGHLIN 1973), and (2) the size range of eukaryote polypeptide gene products is similar to that of prokaryotes (LAIRD 1973), we are led to the conclusion that only a small portion of the DNA of the higher organism genetic unit, in fact, bears structural information.

Through renaturation kinetic analysis, Drosophila DNA, like other higher organism DNA, has been shown to include highly repetitive, middle repetitive

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and unique sequence fractions (see LAIRD 1973 for a review of this work). While the highly repetitive DNA is associated with centric heterochromatin (GALL, COHEN and POLAN 1971), the euchromatic regions of the *Drosophila melanogaster* chromosomes consist largely of unique sequence DNA interspersed with middle repetitive sequences (WU, HURN and BONNER 1972). Because of the obvious association with the sites of most known genes, considerable interest is directed to the structural and functional relationship between the latter two DNA components. Clearly, the great excess of DNA to be found in *Drosophila melanogaster* consists largely of unique sequences. Because of considerations discussed above, we presume this excess not to code for proteins. However, much of it may be transcribed. TURNER and LAIRD (1973) have observed that at least 34% of the unique sequence DNA is transcribed. While the sizes and functions of these transcripts are unknown, it is conceivable that they represent the Drosophila counterpart of the HnRNA, described in several higher eukaryotes (DARNELL, JELINEK and MOLLOY 1973).

Several intriguing models of gene organization have been proposed to account for these and other observations (BRITTEN and DAVIDSON 1969; GEORGIEV 1969; CRICK 1971). A common feature of these models is that they postulate a large regulatory segment, an order of magnitude larger than the contiguous structural element under its control.

For the purpose of examining the validity of this generalized model of gene organization, the rosy locus is an appealing system. The genetic information coding for the primary structure of xanthine dehydrogenase (XDH) has been localized to rosy (GRELL 1962; YEN and GLASSMAN 1965); fine structure analysis has identified several map sites within the locus (CHOVNICK 1966; CHOVNICK, BALLANTYNE and HOLM 1971); limits of the locus are known in terms of identifying adjacent genetic units (SCHALET, KERNAGHAN and CHOVNICK 1964; DELAND 1971); the cytological location of rosy has been restricted to an undefined portion of five specific polytene bands (LEFEVRE 1971a and personal communication).

Prior to this investigation, the genetic map of the rosy locus consisted solely of XDH-null alleles. Since none of these alleles have a detectable altered XDH product, they provide no information as to their structural (amino acid coding) or control (transcription and translation regulating) roles.

Our present strategy, then, is to identify variants of standard ry^+ alleles for which structural or control categorization is possible. These variants will be used to partition the locus into its structural and control components. Several classes of such variants are presently under investigation in our laboratory. They include wild-type isoalleles which exhibit variation in electrophoretic mobility, heat stability or level of XDH activity, as well as mutants which exhibit interallelic complementation or altered sensitivity to purine, an inhibitor of XDH. In addition to distinguishing between models of gene organization, these classes of mutants will serve as raw material for investigations of the genetic control of XDH during development.

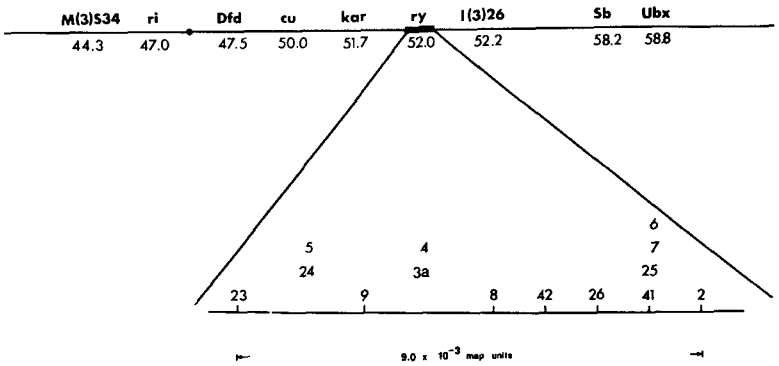

FIGURE 1.—A genetic map of the rosy region of chromosome 3. The map positions of various mutants used in this study are indicated, and the genetic fine structure of the rosy locus is summarized.

The present report describes the identification of sites within the rosy locus responsible for variation in the electrophoretic mobility of XDH. Such sites surely are responsible for differences in charged amino acids in the XDH peptide. By superimposing these sites on the known genetic map of rosy, we will have a minimum estimate of the structural portion of the locus. At face value, the general class of models discussed above would predict that the electrophoretic sites will cluster in a small portion of the rosy map. Such prediction is based upon the assumption that the genetic map of X-ray-induced XDH-null mutants (Figure 1) includes the entire rosy functional unit. However, the results we present support the idea that most, if not all, of this map encodes structural information.

The rosy genetic unit has been demonstrated to be a cistron by classical criteria (CHOVNICK *et al.* 1964). However, because the term cistron implies analogy with prokaryotic genetic units, we will refrain from further use of it. Instead, we will refer to the rosy locus, deeming this a term which does not presuppose any particular idea of gene structure.

MATERIALS AND METHODS

The genetic system: The rosy locus in *Drosophila melanogaster* (*ry*:3–52.0) is a genetic unit controlling xanthine dehydrogenase (XDH) activity, located on the right arm of chromosome 3 within polytene bands 87D8–12. The locus was originally defined by a set of brownish eye color mutants deficient in drosopterin pigment. These mutants exhibit no detectable XDH activity. Two observations place the structural information for XDH in or near rosy: (1) heterozygotes possessing one dose of *ry*+ exhibit 50% of normal enzyme activity while flies carrying three doses of *ry*+ have 150% activity (GRELL 1962); (2) isoalleles differing in their XDH electrophoretic mobility map to rosy or its immediate vicinity (YEN and GLASSMAN 1965).

The rosy eye color mutants have been the subject of intensive fine structure analysis (CHOVNICK 1966; CHOVNICK, BALLANTYNE and HOLM 1971). Figure 1 presents a map of the centromere-proximal region of the right arm of chromosome 3, indicating the location of rosy, the centromere, and other markers used in the study (LINDSLEY and GRELL 1968). In addition, Figure 1 contains a summary map of separable sites within the rosy locus obtained from prior fine structure analysis.

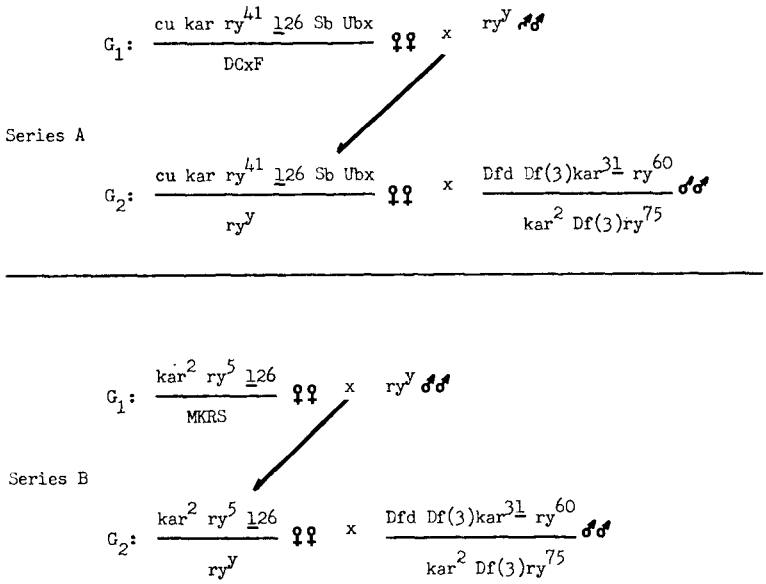

FIGURE 2.—Crosses constructed for fine structure analysis of the rosy locus. G_1 crosses generate the heterozygous females which in turn generate rare *ry+* recombinants among their progeny. The *ry*^{y} allele represents mutants of any of the following series: *ry*^{100}, *ry*^{200}, *ry*^{300}, *ry*^{400}, *ry*^{500}. The progeny of the G_2 cross were reared on purine-enriched medium.

Selective system matings: The experiments involve two series of matings of rosy mutant heterozygous females to tester males, as indicated in Figure 2. Progeny were reared on purine-supplemented medium which effectively kills before eclosion all individuals lacking XDH activity. The protocol for conducting large scale crosses is described previously (CHOVNICK 1973). Unless otherwise noted, all mutants and rearrangements are listed in LINDSLEY and GRELL (1968). *Tp(3)MKRS* is a complex multiple break rearrangement which is an effective balancer of the proximal region of the third chromosome, including the region from *M(3)S34* to *Ubx* (see Figure 1) (LEFEVRE, personal communication).

Genetic tests of exceptional progeny: Surviving individuals of the selective system crosses (Figure 2), all of which are ry+ in phenotype, may be classified immediately with respect to the markers *kar*, *Sb* and *Ubx*. Such individuals are crossed singly to *kar*^{2} *Df(3)ry*^{75}*/Tp(3)MKRS, M(3)S34 kar ry*^{2} *Sb* flies. Progeny phenotypes confirm the diagnoses with respect to *kar*, *ry*, *Sb* and *Ubx*. Since *cu* is not a marker immediately flanking the rosy locus, no test is performed to diagnose this marker. The progeny are further tested in two ways: (1) males carrying an ry+ exceptional chromosome are crossed to an appropriate *l26* tester stock (several have been used) to diagnose the presence or absence of the *l26* marker, and (2) *ry+/kar*^{2} *Df(3)ry*^{75} progeny are used to determine XDH electrophoretic mobility.

Mutagenesis: New mutants *ry*^{102}, *ry*^{103}, *ry*^{106}, *ry*^{110}, *ry*^{201}, *ry*^{203}, *ry*^{204}, *ry*^{205}, *ry*^{402}, *ry*^{501}, *ry*^{502} *and ry*^{506} were recovered as independent events from among the progeny of X-ray-treated aged males of a known *ry+* constitution, using a Cs-137 source (3000r in the case of *ry*^{102}–*ry*^{110}; 4500r in the others). Treated males were crossed to *kar*^{2} *Df(3)ry*^{75}*/Tp(3)MKRS, M(3)S34 kar ry*^{2} *Sb* or *cu kar ry*^{41} *l26 Sb Ubx/In(3LR)DCxF* virgin females. The new rosy mutants were recovered as rare, brown-eyed F_1 individuals carrying the newly-induced mutant heterozygous with a marked rosy chromosome. The mutants were confirmed by subsequent testing and were placed in balanced stocks. These mutants are not associated with deficiencies extending into the rosy locus.

The new mutant ry^{501} was recovered after EMS treatment. The EMS was applied according to the protocol of LEWIS and BACHER (1968). Other details are identical to those for X-ray mutagenesis.

Enzyme preparation and electrophoresis: The procedures followed are previously described (MCCARRON, GELBART and CHOVNICK 1974).

EXPERIMENTS AND RESULTS

Our immediate object is to localize genetic sites responsible for differences in the electrophoretic mobility of XDH. Since direct mapping of such sites is extremely impractical, an indirect approach, taking advantage of selective procedures, is utilized. The basic approach has been described (MCCARRON, GELBART and CHOVNICK 1974), and involves three steps. (1) Electrophoretic variants of XDH must be identified. Several electrophoretic variants of XDH have been isolated from natural populations and laboratory strains (Figure 3). Five discretely different mobilities of XDH have been identified. They are designated by Roman numeral superscripts in order of increasing mobility, XDH^{I} through XDH^{V}. The variants ry^{+6} and ry^{+10} are currently under investigation, but will not be discussed in this report. The heavy and narrow bands diagrammed for ry^{+4} and ry^{+10}, respectively, represent observed differences in activity levels in these two variants. The genetic bases for these variant activities will not be pursued in this report. For each of two mobilities, XDH^{III} and XDH^{V}, two alleles are included in the present investigation. These alleles are derived from different sources and might well have different coding sequences leading to the same net charge on the XDH molecule. (2) Rosy eye color mutants were derived from each wild-type strain according to procedures described above. We have adopted the convention of identifying rosy mutants by three- or four-digit superscripts. The first number(s) identify the ry^{+} isoallele from which the mutant is derived and are followed by two digits which indicate the order in which the mutants of that particular isoallele were recovered. Thus, for example, the first mutant derived from ry^{+5} is ry^{501}. Table 1 summarizes the sources and designations of the various mutants used in these experiments. The mutants ry^{1} through ry^{81} were recovered prior to the present investigation and are not homogeneous in isoallelic background. Those mutants pertinent to the present study (Figure 1) were re-

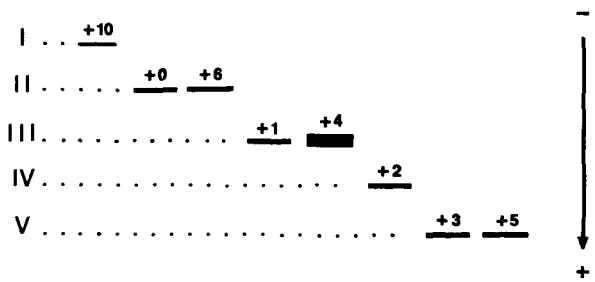

FIGURE 3.—The $ry+$ isoalleles used in this study, diagrammatically arranged according to increasing electrophoretic mobility.

TABLE 1

The rosy null activity alleles used in the present experiments, listed according to their isoallelic origin and mode of induction

Isoallele	Mobility	Mutants	Induced by
$ry+^{0}$	XDH^{II}	ry^{5}, ry^{8}, ry^{41}	X-ray
$ry+^{1}$	XDH^{III}	ry^{102}, ry^{103}, ry^{106}, ry^{110}	X-ray
$ry+^{2}$	XDH^{IV}	ry^{201}, ry^{203}, ry^{204}, ry^{205}	X-ray
$ry+^{3}$	XDH^{V}	ry^{301}	EMS
$ry+^{4}$	XDH^{III}	ry^{402}	X-ray
$ry+^{5}$	XDH^{V}	ry^{501}, ry^{502}, ry^{506}	X-ray

covered from X-ray mutagenesis of a *cu kar* $ry+^{0}$ chromosome. These include ry^{3a}, ry^{4} through ry^{9} and ry^{23} through ry^{54}. (3) Mutants derived from different isoallelic backgrounds were crossed according to the selective system procedures described above (see MATERIALS AND METHODS). The ry^{+} survivors were progeny tested for flanking marker distribution and electrophoretic mobility, all of which serve as unselected markers in the crosses. Three classes of ry^{+} survivors are recovered with respect to *kar* and *l26* flanking marker distribution. The nature of these three classes has been described in detail elsewhere (CHOVNICK, BALLANTYNE and HOLM 1971). One and only one of the two possible classes of crossover chromosomes (either *kar l26*$^{+}$ or *kar*$^{+}$ *l26*) is recovered in any cross; and these crossover chromosomes permit unambiguous relative positioning of the two rosy eye color mutants. In addition, two classes of ry^{+} survivors, each nonrecombinant for flanking markers, are recovered. The *kar* ry^{+} *l26* class represents conversions of either ry^{5} or ry^{41} (depending on the input allele) to ry^{+}, while the *kar*$^{+}$ ry^{+} *l26*$^{+}$ class represents conversions of ry^{y} to ry^{+}. These various classes of ry^{+} survivors are further subdivided according to electrophoretic type.

From such experiments, electrophoretic sites will be located on the genetic map as rosy variants. The sites are named according to the aforementioned rules governing the naming of rosy eye color mutants, except that the superscript is preceded by an ^{e}. We will describe the XDH moieties produced by the ry^{+} exceptions by the mobility designations I through V (see Figure 3). Note that identity in mobility (that is, net charge) does not presume identity in the amino acid sequence producing that net charge. Considering the fact that these isoalleles were all collected from nature or laboratory stocks, the net variations between them might well be due to a veritable host of differences. We will infer the minimum number of sites consistent with the recombination data. Obviously, single sites might represent several closely linked sites. The results of the electrophoretic site localizations are presented in the following section.

ry^{+0} *vs.* ry^{+1}: Crosses of four ry^{100} series mutants to ry^{8} and ry^{41} generate data demonstrating the existence of an electrophoretic site (ry^{e111}) near ry^{41}. The first step in the localization of this site was reported earlier (MCCARRON, GELBART and CHOVNICK 1974). The ry^{100} series mutants were mapped against ry^{8}, a centrally located site mutant of the ry^{+0} isoallele. Two mutants, ry^{103} and ry^{110},

TABLE 2

Mapping ry^{e111}: *Results of crosses of* cu kar ry^{41} l26 Sb Ubx/ry^{y} ♀ ♀ *to tester* Dfd Df(3)kar^{31} ry^{60}/kar^{2} Df(3)ry^{75} ♂ ♂

Zygotes were reared on purine-supplemented medium, permitting only rare ry+ progeny to survive.

	Crossovers *kar ry+*		Conversions *ry^{y} ry+*		Conversions *ry^{41} kar ry+ l26 Sb Ubx*		Total *ry+* survivors	Zygotes sampled ($\times 10^{-6}$)
ry^{y}	XDH^{II}	XDH^{III}	XDH^{II}	XDH^{III}	XDH^{II}	XDH^{III}		
ry^{102}	0	16	0	12	7	5	40	1.56
ry^{103}	0	22	0	13	8	1	44	1.10
ry^{106}	0	5	0	6*	14	9	34	2.46
ry^{110}	0	17	0	16	14†	5	52	1.91
Total	0	60	0	47	43	20	170	7.03

* One of the conversions was *kar+ XDH^{III} l26+ Sb Ubx*, due to a crossover in the l26–Sb interval.

† One of the conversions was *kar XDH^{II} l26 Sb+ Ubx+*, due to a crossover in the l26–Sb interval.

fell to the left, while *ry^{102}* and *ry^{106}* fell to the right, of *ry^{8}*. In addition, the pattern of recombination data placed *ry^{e111}* to the right of *ry^{8}*.

In order to further localize *ry^{e111}*, the four *ry^{100}* series mutants were mapped against *ry^{41}*, the rightmost rosy allele of the *ry+^{0}* isoallele. The crosses were of the series A type (Figure 2) and the results of these experiments are summarized in Table 2. In all, approximately 7×10^{6} zygotes were screened in these experiments and a total of 170 ry+ survivors were recovered. These survivors fall into three classes, based on flanking marker distribution. All sixty crossovers are *kar ry+ l26+ Sb+ Ubx+*, thereby placing all four *ry^{100}* series mutants to the left of *ry^{41}*. The crossovers are all of the XDH^{III} mobility, as is the ancestral *ry+^{1}* isoallele. Thus, *ry^{e111}* is to the right of all sixty recovered crossovers. *ry^{e111}* resides in either the crossover interval very near to *ry^{41}*, or to the right of *ry^{41}*.

Further resolution of the location of *ry^{e111}* is obtained from observations of simultaneous conversion of the electrophoretic site with mutant sites (co-conversions). The frequency of co-conversion relative to single-site conversion in yeast appears to be inversely related to the physical distance between two sites (Fogel, Hurst and Mortimer 1971). We have observed the same relationship in Drosophila (manuscript in preparation). The mutant sites in our experiments are selected markers while the electrophoretic sites are unselected. Hence, we can only recover conversions of the mutant sites to *ry+* and determine whether the electrophoretic site is co-converted; coversions of the electrophoretic sites which are not co-conversions of the mutant site will produce rosy zygotes which do not survive purine treatment. The sixty-three conversions of *ry^{41}* to *ry+* form two groups (Table 2). The forty-three XDH^{II} conversions do not include *ry^{e111}* within the converted segment, while the twenty XDH^{III} conversions are, in fact, co-conversions of the slower variant of the electrophoretic site to the faster one. We take the frequent co-conversion of these two sites to indicate their proximity.

Since two-thirds of the conversions of ry^{41} to ry^{+} are not co-conversions, the two sites are indeed separable. The absence of co-conversion of the ry^{100} mutants and ry^{e111} is consistent with our placement of the electrophoretic site. Perforce, we conclude that ry^{e111} and ry^{41} are very closely linked but separable sites, at the right end of the rosy locus, whose relative positions are not yet defined.

According to the model under test, we expect clustering of the remaining electrophoretic sites in the vicinity of ry^{e111} and hence ry^{41}. Consequently, in all subsequent tests to identify and localize new sites, recombination experiments are carried out initially against ry^{41}.

ry^{+0} *vs.* ry^{+4}: Only one cross was necessary to localize the electrophoretic site (ry^{e408}) distinguishing ry^{+0} and ry^{+4} (Table 3). This cross, ry^{41}/ry^{402}, produced results very similar in pattern to those of the ry^{100} series mutants/ry^{41} crosses. The logic that was used to position ry^{e111} near ry^{41} can be employed to place ry^{e408} in a similar location. In fact, ry^{e111} and ry^{e408} might represent the same site.

ry^{+0} *vs.* ry^{+5}: The results of crosses of ry^{500} series mutants to ry^{41} (Table 4) and to ry^{5} (Table 5) serve to identify at least two electrophoretic sites (designated ry^{e507} and ry^{e508}), one near each end of the rosy locus. The existence of multiple electrophoretic differences between these isoalleles follows from an examination of the results of the cross of ry^{502} to ry^{41} (Figure 2, series A). This cross produced

TABLE 3

Mapping ry^{e408}: *Results of the cross of* cu kar ry^{41} 126 Sb Ubx/ry^{402} ♀ ♀ *to tester* Dfd Df(3)kar^{31} ry^{60}/kar^{2} Df(3)ry^{75} ♂ ♂

Zygotes were reared on purine-supplemented medium, permitting only rare ry+ progeny to survive.

Crossovers *kar ry+*		Conversions ry^{402} *ry+*		Conversions ry^{41} *kar ry+ 126 Sb Ubx*		Total *ry+* survivors	Zygotes sampled ($\times 10^{-6}$)
XDH^{II}	XDH^{III}	XDH^{II}	XDH^{III}	XDH^{II}	XDH^{III}		
0	13	0	8	6*	2	29	1.11

* One of the conversions was *kar XDH^{II} 126 Sb+ Ubx+*, due to a crossover in the 126–Sb interval.

TABLE 4

Mapping ry^{e507} *and* ry^{c508}: *Results of crosses of* cu kar ry^{41} 126 Sb Ubx/ry^{v} ♀ ♀ *to tester* Dfd Df(3)kar^{31} ry ^{60}/kar^{2} Df(3)ry^{75} ♂ ♂

Zygotes were reared on purine-supplemented medium, permitting only rare ry+ progeny to survive.

	Crossovers *kar ry+*			Conversions ry^{y} *ry+*			Conversions ry^{41} *kar ry+ 126 Sb Ubx*			Total *ry+* survivors	Zygotes sampled ($\times 10^{-6}$)
ry^{y}	XDH^{II}	XDH^{III}	XDH^{V}	XDH^{II}	XDH^{III}	XDH^{V}	XDH^{II}	XDH^{III}	XDH^{V}		
ry^{501}	0	0	0	0	0	0	0	0	0	0	1.37
ry^{502}	1*	23	0	0	6	1†	9	0	0	40	1.48

* This chromosome was *kar XDH^{II} 126+ Sb Ubx*, due to a double crossover: one in the rosy region and the other in the 126–Sb interval.

† This conversion was *kar+ XDH^{V} 126+ Sb Ubx*, due to a crossover in the 126–Sb interval.

TABLE 5

Mapping ry^{e507} *and* ry^{e508}: *Results of crosses of* kar^2 ry^5 126/ry^y ♀ ♀ *to tester* Dfd Df(3)kar^{31} ry ^{60}/kar^2 Df(3)ry^{75} ♂ ♂

Zygotes were reared on purine-supplemented medium, permitting only rare ry^+ progeny to survive.

	Crossovers ry^+ 126			Conversions ry^y ry^+			Conversions ry^5 kar^2 ry^+ 126			Total ry^+ survivors	Zygotes sampled ($\times 10^{-6}$)
ry^y	XDH^{II}	XDH^{int}	XDH^{V}	XDH^{III}	XDH^{int}	XDH^{V}	XDH^{II}	XDH^{int}	XDH^{V}		
ry^{501}	2	13	0	0	2	2	3	3	0	25	0.84
ry^{506}	1	3	0	0	0	0	3	0	0	7	0.90
Total	3	16	0	0	2	2	6	3	0	32	1.74

40 ry^+ exceptions in a sample of approximately one and one-half million zygotes. The twenty-four crossovers are all *kar* ry^+ *126*$^+$, locating ry^{502} to the left of ry^{41}. Almost all the crossovers (23/24) are XDH^{III} while the other one is XDH^{II}. Since XDH^{III} is a nonparental mobility, intermediate to the ry^{+0} (XDH^{II}) and the ry^{+5} (XDH^{V}) isoalleles, we must conclude that the difference in mobilities of these two isoalleles is due to at least two sites. One site (ry^{e507}) is to the left of all the crossover points while the other site (ry^{e508}) is to the right of all but one crossover. Moreover, these data place ry^{e508} near and to the left of ry^{41}. Confirmation of the separation of ry^{e508} and ry^{41} comes from the fact that none of nine conversions of ry^{41} to ry^+ include ry^{e508} within the converted segment.

Turning to the leftmost electrophoretic site, ry^{e507}, we infer that it must be very near ry^{502}, since (1) no crossovers separate them, and (2) six of seven conversions of ry^{502} are co-conversions of both sites. Moreover, the one conversion of ry^{502} that does not include the electrophoretic site demonstrates their separability. To determine the position of ry^{e507} on the standard rosy map, it was necessary to cross ry^{500} series mutants to a mutant of the ry^{+0} isoallele which is located in the left-hand portion of the locus. The mutant ry^5 was chosen because the ry^{502}–ry^{41} map distance is similar to the ry^5–ry^{41} distance (CHOVNICK, BALLANTYNE and HOLM 1971). Since ry^{502} was probably near ry^5, a cross of these two mutants would not have produced many ry^+ survivors. Instead, ry^{501} (inseparable from ry^{41}, Table 4) and a previously untested mutant, ry^{506}, were crossed to ry^5 (Figure 2, series B). The results are summarized in Table 5, and provide evidence for the proximity of ry^5 and ry^{e507}. A number of ry^+ exceptions (Table 5) are designated XDH^{int} (intermediate). This designation indicates that their mobilities are clearly distinguishable from, and intermediate to, the parental mobilities (XDH^{II} and XDH^{V}). However, we have had difficulty assigning them to one or the other of the intermediate mobility classes (XDH^{III} and XDH^{IV}). This difficulty may reflect the resolution limit of the present electrophoretic assay system. The ultimate resolution of these mobilities may permit us to separate ry^{e507} into two or more components, but it will not alter the basic localization of the electrophoretic site(s). The crossover data (Table 5) confine ry^{e507} to the left of both ry^{501} and ry^{506}, and to the right of ry^5. Since three of nine con-

versions of ry^5 are co-conversions of ry^{e507} as well, these two sites must be located close to each other.

Let us return to the relative localization of the right electrophoretic site, ry^{e508}, which we have already placed just to the left of ry^{41}. Further information about the relative map position of site markers at the right end of the map can be gleaned from the ry^5/ry^{501} cross. None of the fifteen crossovers separate ry^{e508} from ry^{501}. However, since two of the four conversions of ry^{501} are co-conversions of ry^{e508}, we conclude that these two sites are close to each other, but separable. This observation, taken together with the fact that none of the nine conversions of ry^{41} are co-conversions of ry^{e508} (Table 4), suggests that ry^{e508} is closer to ry^{501} than it is to ry^{41}. Since ry^{e508} is to the left of ry^{41}, ry^{501} must be similarly situated. Thus, despite the absence of crossover data separating ry^{501} from ry^{41} (Table 4), we are able to define their relative position. Clearly, co-conversion data provide a sensitive tool for the ordering of closely linked sites in Drosophila, in a manner formally analogous to recombination analysis in bacterial transformation and transduction.

Let us now consider two other isoallelic comparisons. While the observations are admittedly preliminary, they serve to augment the data already presented, and emphasize the distribution of electrophoretic sites relative to the standard map (Figure 1).

ry^{+0} vs. ry^{+3}: The isoallele, ry^{+3}, exhibits the same mobility (XDH^V) as ry^{+5}. Just as ry^{+5} possesses two electrophoretic site differences with respect to ry^{+0}, one close to each end of the locus, ry^{+3} has two similarly located sites. These observations are drawn from the results of the cross of ry^{301}/ry^{41} (Table 6). All thirty-six crossover chromosomes are *kar* ry^+ *l26*$^+$ *Sb*$^+$ *Ubx*$^+$, thus locating ry^{301} well to the left of ry^{41}. Additionally, all crossovers are XDH^{III}, a non-parental mobility. Hence, there must be at least two electrophoretic sites of difference between ry^{+0} and ry^{+3}. One, ry^{e302}, can be located close to but separable from ry^{41} by the same logic that has been used to position ry^{e111} and ry^{e402}. Furthermore, the same data argue that the second site, ry^{e303}, is close to the left end of the rosy locus. In the absence of conversions of ry^{301}, we are unable at this time to define further the location of ry^{e303}.

ry^{+0} vs. ry^{+2}: Crosses of four ry^{200} series mutants (ry^{201}, ry^{203}, ry^{204} and ry^{205}) to ry^{41} provide results (Table 7) which establish the existence of an electro-

TABLE 6

Mapping ry^{e302} *and* ry^{e303}: *Results of the cross of* cu kar ry^{41} l26 Sb Ubx/ry^{301} ♀ ♀ *to tester* Dfd Df(3)kar^{31} ry^{60}/kar^2 Df(3)ry^{75} ♂ ♂

Zygotes were reared on purine-supplemented medium, permitting only rare ry$^+$ progeny to survive.

Crossovers *kar ry*$^+$		Conversions *ry*^{301} *ry*$^+$		Conversions *ry*^{41} *kar ry*$^+$ *l26 Sb Ubx*		Total *ry*$^+$ survivors	Zygotes sampled ($\times 10^{-6}$)
XDH^{II}	XDH^{III}	XDH^{II}	XDH^{III}	XDH^{II}	XDH^{III}		
0	36	0	0	11	7	54	2.53

TABLE 7

Mapping ry^{e217}: *Results of crosses of* cu kar ry^{41} l26 Sb Ubx/ry^{y} ♀ ♀ *to tester* Dfd Df(3)kar^{31} ry^{60}/kar^{2} Df(3)ry^{75} ♂ ♂

Zygotes were reared on purine-supplemented medium, permitting only rare ry+ progeny to survive.

	Crossovers *kar ry+*		Conversions ry^{y} *ry+*		Conversions ry^{41} *kar ry+ l26 Sb Ubx*		Total *ry+* survivors	Zygotes sampled ($\times 10^{-6}$)
ry^{y}	XDH^{II}	XDH^{IV}	XDH^{II}	XDH^{IV}	XDH^{II}	XDH^{IV}		
ry^{201}	8	0	3	0	10*	0	21	0.74
ry^{203}	0	0	0	1	1	0	2	0.69
ry^{204}	6	0	7	1	6	0	20	0.68
ry^{205}	4	0	0	6	5*	0	15	0.66
Total	18	0	10	8	22	0	58	2.77

* One of the conversions was *kar* XDH^{II} *l26 Sb+ Ubx+*, due to a crossover in the l26–Sb interval.

phoretic site (ry^{e217}) some distance to the left of ry^{41}, and clearly distinct from the cluster of electrophoretic sites which have been located at the right end of the standard map (Figure 1).

Consider the crossovers resulting from those tests which place the alleles ry^{201}, ry^{204} and ry^{205} to the left of ry^{41}. All eighteen crossovers are XDH^{II}, *kar* $l26^{+}$ Sb^{+} Ubx^{+} and hence require that ry^{e217} be to the left of each crossover point, as well as to the left of ry^{41}. Indeed, ry^{e217} must be very near ry^{201} and ry^{204}, since the electrophoretic site exhibits exceedingly high co-conversion frequencies with these alleles. Further localization of ry^{e217} cannot be extracted from the present data.

Figure 4 presents a map of the rosy locus in which the null enzyme mutants ry^{5}, ry^{8} and ry^{41} represent reference points for the left, center and right of the map. Attention is focused upon the relative positions of the electrophoretic sites elaborated in the present report. Clearly, structural sites within the rosy locus are not confined to a small portion of the map. Since we know that rosy contributes two subunits to the XDH enzyme (see DISCUSSION), we are tempted to conclude that most of the standard rosy map (Figure 1) represents a single structural element coding for an XDH peptide which is represented twice in each XDH molecule. (i.e., a homodimer). However, a comparison of the distribution of the seven identified electrophoretic sites (Figure 4) with the distri-

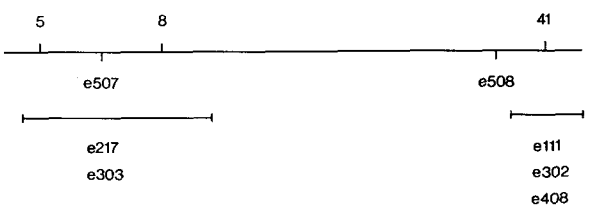

FIGURE 4.—The positions of the known electrophoretic sites relative to the standard null-XDH mutants ry^{5}, ry^{8} and ry^{41} (See Figure 1).

bution of known null activity mutant alleles (Figure 1) suggests that the genetic bases for electrophoretic variation in XDH may be restricted to two quite separable sectors of the standard map. The possibility exists that the structural information for XDH may reside in two elements, perhaps separated by a control region of undefined length. Here we envision that each structural element would code for a different peptide, which we designate as the α and β subunits of XDH. An active molecule would then be a heterodimer consisting of one molecule of each subunit. Let the leftmost group of electrophoretic sites (Figure 4) reside in the coding element for the α subunit, and the rightmost group reside within the β coding element. Let α^S and α^F represent subunits of slower and faster mobilities, respectively, and let β subunits be similarly designated. For consideration of this model, the XDH moiety produced by ry^{+0} would then be $\alpha^S \beta^S$, ry^{+1} and ry^{+4} are $\alpha^S \beta^F$, ry^{+2} is $\alpha^F \beta^S$ and ry^{+3} and ry^{+5} are $\alpha^F \beta^F$. As we have demonstrated herein, ry^{+0} and ry^{+5} differ by two electrophoretic sites, ry^{e507} and ry^{e508}. By proper selection and examination of ry^{e507} and ry^{e508} allelic combinations, we can generate a test distinguishing these two models (Figure 6).

The XDH electrophoretic pattern of ry^{+0}/ry^{+5} heterozygotes consists of a heavy band of intermediate mobility and light bands corresponding in mobility to ry^{+0} (XDH^{II}) and ry^{+5} (XDH^{V}) homozygotes. On the homodimer model, in order of increasing mobility, these bands correspond to: XDH^{II}/XDH^{II}, XDH^{II}/XDH^{V} and XDH^{V}/XDH^{V} molecular species. On the heterodimer model, the slowest migrating form is $\alpha^S \beta^S$, the intermediate is a combination of $\alpha^S \beta^F$ and $\alpha^F \beta^S$ molecules and the fastest form is $\alpha^F \beta^F$. Thus, ry^{+0}/ry^{+5} heterozygotes exhibit identical electrophoretic patterns on either model.

The heterodimer model predicts that $\alpha^S \beta^S/\alpha^F \beta^F$ and $\alpha^S \beta^F/\alpha^F \beta^S$ individuals will generate identical three-banded hybrid electrophoretic patterns. On the homodimer model, these same heterozygotes would be designated XDH^{II}/XDH^{V} and XDH^{III}/XDH^{int}, respectively; the XDH^{II}/XDH^{V} heterozygote would be expected to produce the three-banded hybrid electrophoretic pattern while the XDH^{III}/XDH^{int} individual would produce a single band of XDH^{int} mobility. Essentially, the homodimer model predicts a *cis-trans* difference, whereas the heterodimer model predicts no such difference (see Figure 5).

For the sake of this discussion, the ry^{+0}/ry^{+5} heterozygous genotype may be rewritten as $ry^{e507S}\ ry^{e508S}/ry^{e507F}\ ry^{e508F}$. This represents the *cis* configuration of

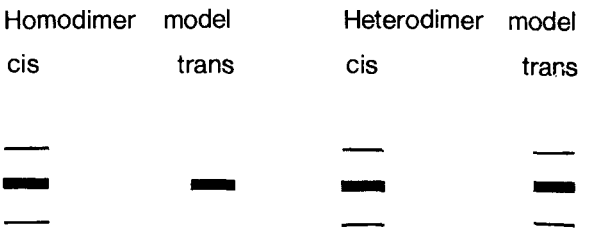

FIGURE 5.—The predictions of the homodimer and heterodimer models. Diagrams of the electrophoretic patterns expected on either model are included. The *cis* configuration is $ry^{e507S}ry^{c508S}/ry^{e507F}\ ry^{e508F}$; *trans* is $ry^{e507S}\ ry^{e508F}/ry^{e507F}\ ry^{e508S}$.

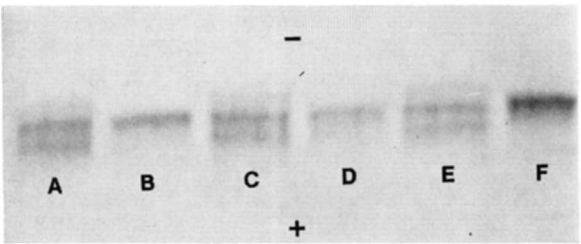

FIGURE 6.—XDH electropherogram of alternating *cis* (A,C,E) and *trans* (B,D,F) heterozygotes. The *cis* configuration is ry^{e507S} ry^{e508S}/ry^{e507F} ry^{e508F}; *trans* is ry^{e507S} ry^{e508F}/ry^{e507F} ry^{e508S}.

electrophoretic sites. From chromosomes recovered as co-conversions of ry^{502} (Table 4) and ry^{501} (Table 5), we are able to generate the trans heterozygote, ry^{e507S} ry^{e508F}/ry^{e507F} ry^{e508S}. The *cis* and *trans* heterozygotes were constructed and their electrophoretic patterns examined. Figure 6 is an electropherogram of these heterozygotes. The *trans* heterozygote produces a single electrophoretic band of XDH^{int} mobility, while the *cis* heterozygote produces three distinct bands. Clearly, this result contradicts the heterodimer model. Hence, we conclude that the entire region from ry^{e507} through ry^{e508} represents a single continuous XDH-coding element.

DISCUSSION

The results described in the present report lead to the general conclusion that most, if not all, of the standard genetic map of the rosy locus (Figure 1) represents a single uninterrupted DNA sequence which is the XDH structural element. This structural element consists of a unique DNA sequence of some 3×10^3 nucleotide pairs in length. The unique nature of the structural element is documented by our total experience with fine structure recombination experiments involving some 2000 analyzed ry^+ recombinants. We have never seen evidence of the "unequal" crossing over that would occur if repeat sequences were present. The physical length of the structural element is derived as follows: The molecular weight of Drosophila XDH has been estimated at 250,000 daltons (GLASSMAN *et al.* 1966). SDS gel electrophoresis reveals that Drosophila XDH consists of a single subunit of 130,000–150,000 daltons (CANDIDO, BAILLIE and CHOVNICK 1974). From these observations, we are able to infer that the active XDH molecule consists of two subunits of similar size. That both subunits are derived from rosy structural information may be inferred from YEN and GLASSMAN (1965). In the present report, we have demonstrated that both subunits of a single XDH molecule are peptide products of one structural element. By this logic, we conclude that the rosy structural element codes for a polypeptide of 130,000–150,000 daltons or approximately 1000 amino acids, requiring a coding sequence of some 3×10^3 nucleotide pairs. It is of interest to note that this estimate of the physical length of the XDH structural element is strikingly similar to an estimate based upon recombination data. LEFEVRE (1971b) correlates 0.01 map units with a

length of 3.7–3.8 $\times$ 10^3 nucleotide pairs in *Drosophila melanogaster*. The standard map of the rosy locus (Figure 1) extends over 0.009 map units and thus 3.3–3.4 $\times$ 10^3 nucleotide pairs.

Let us now consider the rosy locus as a cytogenetic entity. We have already noted the evidence in support of the 1 gene:1 chromomere (or polytene band) hypothesis (see INTRODUCTION). Indeed, we believe that this evidence far outweighs the objections to the hypothesis (O'BRIEN 1973). Moreover, the 1 gene:1 chromomere relationship appears to hold for the rosy region (DELAND and CHOVNICK, unpublished). The rosy locus has been restricted to the five-band chromosomal region 87D8–12 (LEFEVRE 1971a), a group of bands of average dimensions. Given that an average band contains a haploid amount of DNA of 2–3 $\times$ 10^4 nucleotide pairs (RUDKIN 1965; LEFEVRE 1971b; LAIRD 1973), then the rosy locus, occupying one of these five bands, has available to it at least ten times as much DNA as is needed to code the amino acid sequence of its polypeptide chain.

Return to the problem of gene organization and the general model under test. Clearly, the present data do not support the model, which anticipated a cluster of electrophoretic sites localized to a short sector of the total map. In formulating this model, we tacitly assumed that the distribution of mutants would directly reflect the relative sizes (both physical and recombinational) of the postulated control and structural elements. With the failure of the present data to support the model, we must either reject the general model or question the validity of our underlying assumptions.

Let us first examine our assumptions. (1) *The distribution of mutants within the control and structural elements directly reflects the relative lengths of their nucleotide sequences*. Consider the nature of rosy eye color mutants which comprise the standard map of the rosy locus. Since even low levels of XDH activity allow expression of the ry^+ phenotype, the visual selective procedure requires that these mutants lack any residual activity. Clearly, single base-pair alterations in the structural element have the potential to eliminate all XDH activity. In the control element, as CRICK (1971), PAUL (1972) and others perceive it, such alterations generally should have a less drastic effect on the enzyme activity. These workers intimate that control specificity resides in extensive nucleotide sequences, in contrast to the triplet codon specificity of structural regions. Thus, while most single base-pair alterations in control elements might cause slight quantitative changes in XDH activity, they would not be expected to produce the complete disruption of activity necessary for expression of the mutant eye color. In contrast to prevailing scientific folklore, we believe single site alterations to comprise the bulk of intragenic events produced by X-irradiation, the agent that generated these rosy mutants. Our assertion is best documented by the work of MALLING and DE SERRES (1973), examining 101 X-ray-induced *ad-3B* mutants in *Neurospora crassa*. Based upon the reversion properties characterizing these mutants, they determined that only six of these 101 mutants were multisite lesions. Additionally, three of the five X-ray-induced maroon-like mutants in *Drosophila melanogaster* are inferred to be base substitutions since they partici-

pate in interallelic complementation (CHOVNICK *et al.* 1969). Extrapolating from these observations, we presume most of the rosy mutants also to be single base-pair alterations. Inadvertently, the conspiracy of X-ray mutagenesis and the visual selection of null-XDH eye color mutants restricted the lesions recovered to the rosy structural element. Thereby, we are able to account for the concordance of the XDH-null and electrophoretic maps. If this explanation is correct, the use of alternative mutagens and more sensitive selective procedures may yet permit us to examine variation within the control element.

(2) *The recombination rates within control and structural elements are identical per unit of nucleotide length.* In sooth, most exchange may occur within the structural element, producing the biased distribution of mutant map sites which we have observed. Why would a mechanism limiting exchange in this manner evolve? Control elements have been implicated as regions containing middle repetitive DNA. If so, recombination in these regions will be characterized by unequal exchanges. Assuming an optimal size and sequence for a particular control element, we envisage a selective advantage favoring the evolution of mechanisms which inhibit exchange in this element, thereby stabilizing it. Analogous disadvantages accorded unequal exchange may have participated in limiting the positions of highly redundant loci (18S and 28S rRNA [bobbed] genes—RITOSSA and SPIEGELMAN 1965; histone gene—PARDUE *et al.* 1972) to centric regions, where the frequencies of exchange are reduced. Recall also that most rapidly annealing DNA localizes to centric regions (GALL, COHEN and POLAN 1971). Moreover, transposition of the bobbed locus to a more distal position via inversion of the *X* chromosome results in increased recombination and the unequal exchange associated with such redundancy (ATWOOD 1969; SCHALET 1969). We note, as an exception to the above pattern, that the redundant 5S rRNA genes are located in the distal polytene region 56E–F on the right arm of chromosome 2 (WIMBER and STEFFENSON 1970). Despite this exception, we believe that mechanisms exist for regional control of recombination frequency. Similarly, on the intralocus level, exchange within a control element may be inhibited, thereby creating the illusion that most of the locus is structural. The pertinence of this argument for the present study of the rosy locus is obvious, and assessment of this possibility is under investigation.

Let us next consider the possibility that, in fact, the assumptions underlying the present experiment are valid, and that the general model is incorrect. We are then left in the unhappy situation of having a genetic unit associated with a chromomere containing an order of magnitude more DNA than is needed as structural information. We must search elsewhere for the *raison d'être* of this excess DNA.

Other functions for it may involve control of DNA replication and chromosome behavior during cell division cycles. Both of these processes involve the interactions of large numbers of chromosomal sites (PLAUT, NASH and FANNING 1966; BAKER and CARPENTER 1972). Certainly, a variety of initiator, binding and receptor sites must be required for these processes to operate normally; the excess DNA may serve in these capacities.

Our long-range interest is in the organization of a genetic unit in higher organisms, and in particular, the control mechanisms underlying differential gene function. Establishment of the limits of the rosy structural element is a necessary prerequisite to the identification of mutants in the control process. The work reported herein represents our progress in delimiting said structural element. Further localization of electrophoretic and other classes of structural variants will aid in clearly defining these limits.

Note added in proof: Since preparation of this manuscript, all XDH^{int} recombinants have been determined to be of the XDH^{IV} mobility class (see RESULTS).

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