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THE REGULATION OF THE MOLYBDOENZYME SYSTEM OF *DROSOPHILA MELANOGASTER*

By

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled 'The regulation of the molybdoenzyme system of Drosophila melanogaster ' submitted by Damu Tang in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

A number of Drosophila melanogaster cross progenies and stocks were constructed to have from 4 to 10 ry $^{+}$ gene copies. Enzymatic activity assays for xanthine dehydrogenase (XDH), aldehyde oxidase (AO), pyridoxal oxidase (PO) and sulfite oxidase (SO) were performed on these flies. The hypothesis of differential affinities of XDH, AO, PO and SO for MoCo was examined. These experiments fail to provide evidence to support this hypothesis. In one of these Drosophila stocks, designated E, the XDH activity level is 16 times that of OR wild type control flies. But these two strains of flies do not differ significantly with respect to PO, AO or SO activities.

Southern analyses of rosy DNA have been performed on OR, R310, E and T flies. The results indicate that E and T flies have more ry $^{+}$ DNA than that of R310 flies and all three stocks have more ry $^{+}$ DNA than OR. Therefore, the ry $^{+}$ DNA was amplified in E and T stocks relative to R310. In situ hybridizations to OR, R310, E and T larval salivary gland chromosomes indicate that the ry $^{+}$ DNA transposed in E and T flies and amplified about five and four times that of ry $^{+}$ DNA in R310 flies, respectively. The protein kinase-C (PKC) gene and the ribosomal protein 49 (rp49) gene of Drosophila were used for the purpose of control probes in the Southern analyses. Surprisingly, the results show that the restriction sites of the PKC and rp49 genes were altered. Recent evidence shows that the P element transposase activity

will increase during maintainence of P element transformed lines, and at the same time the P element number will increase in these Drosophila lines. It is possible that the changes of PKC and rp49 genes are due to the P element sequences in R310 flies.

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INTRODUCTION.

Much of our understanding of the mechanisms involved in gene expression has come from studies concerned with how groups of related genes interact. One approach has been to study sets of structural genes whose products are required or expressed at the same time in development or perhaps in the same tissues (Cypher et al., 1982; Dickinson and Gaughan, 1981; Spradling and Rubin, 1983). The structural genes are the DNA sequence encoding the amino acid sequence of a protein. Such coordinately expressed genes particularly lend themselves to developmental and biochemical analyses (Snyder et al., 1981 and 1982). The molybdoenzyme system of Drosophila melanogaster is an excellent example of several seemingly unrelated loci whose final functional expression is dependent upon loci separate and distinct from the structural genes (Bentley and Williamson, 1982a and b; Bentley et al., 1981; Browder and Williamson, 1976; Meidinger and Bentley, 1986; Meidinger and Williamson, 1978; Glassman and Mitchell, 1959a and b). The activities of the four molybdoenzymes are also affected by four loci, which do not encode the major polypeptide of any one of these enzymes (Bentley and Williamson, 1982a and b; Bentley et al., 1981; Meidinger and Bentley, 1986; Bogaart and Bernini, 1981). D. melanogaster has the added advantages of possessing the best defined genetic system of any higher eukaryote, developing with a relatively short generation time and being amenable to biochemical analyses. The molybdoenzyme system of D. melanogaster is one of the most extensively investigated gene-enzyme systems in eukaryotes.

Molybdenum-containing enzymes comprise a small but important class of proteins which may be divided into two groups based on the manner in which the molybdenum atom is bound to the apoprotein. The first group consists solely of nitrogenase and possesses molybdenum in a center also containing iron and acid-labile sulfur. The second group consists of xanthine oxidase, xanthine dehydrogenase (XDH, EC. 1.2.1.37), aldehyde oxidase (AO, EC. 1.2.3.1), sulfite oxidase(SO, EC. 1.2.8.1), nitrate reductase, pyridoxal oxidase(PO), and in all likelihood, formate dehydrogenase and carbon monoxide oxidase. These enzymes appear to have in common a Mo=O group bound in complex with an organic cofactor molecule which is transferable among all the enzymes of the group (Hille and Massey, 1985) except SO whose cofactor does not have a terminal sulfide ligand. In the second group, xanthine oxidase, xanthine dehydrogenase, aldehyde oxidase, and pyridoxal oxidase have a sulfur atom in the molybdenum center while sulfite oxidase does not (Hille and Massey, 1985).

The molybdoenzyme gene-enzyme system of Drosophila melanogaster is known to consist of four enzymes: xanthine dehydrogenase (XDH), aldehyde oxidase (AO), pyridoxal oxidase (PO), and sulfite oxidase (SO). These enzymes share a common molybdenum cofactor (MoCo), as demonstrated by the loss of the enzymatic activities when tungstate is administered and restoration when molybdate is supplemented (Bentley et al., 1981; Warner and Finnerty, 1981; Bogaart and Bernini, 1981). The structural

gene encoding the major polypeptide of XDH is the rosy locus (ry , 3-52; Chovnick et al., 1978). Aldehyde oxidase (Aldox , 3-57) is the structural locus for AO (Dickinson, 1970). The low-pyridoxal oxidase locus (lpo , 3-56.9) is the structural locus encoding the major polypeptide of PO (Collins and Glassman, 1969; Dickinson and Weisbrod, 1976). Pyridoxal oxidase was directly demonstrated to be a molybdoenzyme (Warner and Finnerty, 1981). Sulfite oxidase is demonstrated to be a molybdoenzyme by the experiments involving tungstate feeding (Bogaart and Bernini, 1981; Bentley et al., 1989). No variant alleles have been reported for this enzyme and therefore no gene has been identified to encode the major polypeptide. There are four other loci (cin , ma-1 , lxd , and aldox-2) in this D. melanogaster gene-enzyme system, which affect the activities of XDH, AO, PO, and SO. All of these enzymes can be expressed at the same time and in the same tissue (Cypher et al., 1982; Spradling and Rubin, 1983). XDH activity is primarily associated with Malpighian tubules and fat body (Ursprung and Hadorn, 1961). AO is present in all the major internal organs of the larvae and adults, including brain, imaginal discs, Malpighian tubules, digestive system, fat body and reproductive structures (Cypher et al., 1982; Dickinson and Gaughan, 1981). PO is present in the Malpighian tubules and the only tissue, which possesses PO but lacks AO, is the larval salivary gland (Cypher et al., 1982; Dickinson and Gaughan, 1981).

The first and most extensively studied structural gene in this

system is *rosy*, which encodes the major polypeptide of XDH. Null mutants at this locus have no XDH activity during any stage of development yet the fly is viable and fertile under laboratory conditions. However, no *ry* null alleles have been found in large studies of natural populations (Voelker et al., 1980; Langley et al., 1981). Both null alleles and sites for XDH electrophoretic variants are localized to the *ry* gene. Electrophoretic sites mapped by recombination fall into a linear order within this gene. That the *ry* locus function is nonautonomous has been demonstrated by implants of *ry* mutant larval eye discs into wild type larval hosts, which results in normal eye color in the transplant eye. Hadorn and Schwinck, (1956) showed that there is a substance transmitted from wild type tissue that is capable of producing normal red pigment in a mutant eye disc. The XDH tissue distribution in Malpighian tubules and the fat body can be demonstrated by an experiment where small pieces of *ry*$^{+}$ tubule or fat body, implanted into *ry* mutant larvae, produce wild-type eyes in the host while other tissues are not as effective (Hadorn, 1956). The *ry* gene has been cloned by Bender et al. (1983) by chromosomal walking. The *ry* locus of *D. melanogaster* has been the subject of extensive genetic and biochemical characterization, including intragenic fine structure recombination experiments as well as molecular analyses. The intragenic recombination experiments produced a genetic map, which localized many *rosy* mutants to the structural element of the *rosy* gene. The molecular analyses of *ry* created a molecular map, which was coordinated with the genetic map produced by intragenic recombination experiments and it was

found that many rosy mutants were associated with either insertions or deletions (Chovnick et al., 1976; Rushlow and Chovnick, 1984; Clark et al., 1986a and b; Reardon et al., 1987; Clark et al., 1984; Cote et al., 1986; Gausz et.al., 1986; Hilliker and Chovnick, 1981). The rosy gene carries the coding information for xanthine dehydrogenase, which is a homodimer with a subunit molecular weight of 150,000 daltons. It has been found that xanthine dehydrogenase of D . melanogaster exists as multiple forms (Glassman et. al., 1968; Yen and Glassman, 1965; Collins et. al., 1971). The multiple molecular forms of this enzyme are associated with a number of wild-type isoalleles of the rosy gene that have been found from laboratory and natural populations of D. melanogaster . These wild type rosy isoalleles are associated with distinctive electrophoretic mobilities and different levels of XDH enzymatic activities (Chovnick et. al., 1978; Barbara et al., 1983). The sites responsible for electrophoretic variation of the XDH enzyme and for other mutations which make an altered XDH peptide define on the genetic map the XDH coding region or structural element. Of alleles extracted from laboratory and natural D. melanogaster populations, the ry^{+4} strain produces twice as much XDH protein and RNA, and the ry^{+10} strain half as much XDH protein and RNA, as a standard wild-type stock. These sites responsible for overproduction or underproduction of the XDH enzyme in different wild type strains have been mapped to the left of the structural element and are called control elements (Chovnick et al., 1976; McCarron et al., 1979; Clark et al., 1984). These control elements have been demonstrated in the order of kar+i1005 i409+ry .

kar (karmoisin, 3-51.7) is the gene that is left of rosy and centromere proximal. The control element i1005 is associated with ry^{+10} for the underproduction of the XDH enzyme. The i409 allele is associated with ry^{+4} and with a large, tissue-specific increase in fat body XDH expression (Clark et al., 1984). The high and low levels of XDH enzymatic activities associated with variation in the rosy locus control element relate to differential transcription of XDH-specific mRNA and do not relate to different numbers of rosy DNA templates (Clark et al., 1984; Covington et al., 1984). By doing mutagenesis with 1-ethyl-nitrosourea (ENU), Lee et al. (1987) found that mutations in the rosy control element can affect splicing and translation of rosy mRNA. Because S. Clark found that the ry^{+4} control element, i409, lies in the first intron of the rosy locus (S. Clark. unpublished data from Lee et al., 1987), it is believed that the ry^{+10} control site, i1005, lies in the nontranscribed DNA upstream of the first intron (Lee et al., 1987). The complete rosy gene has been sequenced (Keith et al., 1987) and the DNA restriction map indicates that an 8.1 Kb SalI fragment includes the functional $rosy^{+}$ gene (Clark et al., 1984). Subsequent elegant confirmation of this point came from transformation experiments that demonstrated the association of this DNA fragment with ry^{+} locus expression (Rubin and Spradling, 1982; Spradling and Rubin, 1983). This DNA fragment encodes a 4.5 Kb ry^{+} mRNA (Cote et al., 1986). It was suggested that a control element contiguous with the right end of the structural element does not exist and that the right and left ends of the ry^{+} structural element are ry^{606} and ry

$ps5205$, respectively (Clark et al., 1986a). By a recombination study of the rosy locus, Clark et al. (1988) have found that recombination can initiate and terminate at a large number of sites within the rosy locus. This argues against the notion that recombination may be polar and may initiate or terminate at fixed sites in the DNA.

The XDH of Drosophila melanogaster is a homodimer with a molecular weight of approximately 300,000 (Andres, 1976) and can use a wide range of substrates, two of which are of special interest. The first of these is 2-amino-4-hydroxypteridine (AHP), which is converted into isoxanthopterin (IXP). Analysis of the pigments found in ry mutant fly heads reveals an accumulation of the substrate and a lack of the product (Mitchell et al., 1958). This AHP to IXP conversion appears to be involved in the synthesis of Drosophila eye pigments since flies completely deficient for XDH have brown colored eyes when compared to the wild type reddish-brown color (Nolte, 1955). Even though the role of XDH in eye pigment synthesis is still not completely understood, the association of a known enzymatic defect with a visible phenotype is very useful. Since even very low levels of XDH activity are capable of restoring wild type eye color, the visible phenotype becomes an extremely sensitive in vivo assay for XDH activity. Individuals with as little as 1% XDH activity have wild type eye color (Gelbart et al., 1976).

The second XDH substrate of genetic interest is purine. XDH catalyzes the oxidation of hypoxanthine to xanthine to uric acid. Flies, which lack XDH, accumulate hypoxanthine and completely lack uric acid, the normal nitrogenous excretion product (Mitchell et al., 1958). Selection of individuals with XDH in a population of those lacking XDH is possible with purine treatment of growing cultures (Glassman, 1965). At appropriate doses, even flies with low levels of XDH activity can be selected while flies lacking XDH die.

The second structural gene in the molybdoenzyme system is Aldox (3-57), which encodes the major polypeptide of aldehyde oxidase (Dickinson, 1970). AO is a homodimer with a molecular weight of 280,000 daltons by Sepharose 4B chromatography (Andres, 1976). The distribution of AO in the Drosophila melanogaster wing disc has been used for visualization of the formation of developmental compartments (Kuhn and Cunningham, 1977; Kuhn et al., 1983). It has been found that the distribution of AO in imaginal discs of D. melanogaster is influenced by several homoeotic genes. The tumorous-head gene of D. melanogaster is a homoeotic mutation resulting genetically from a third chromosome semidominant mutant gene tuh-3 (Gardner and Woolf, 1949, 1950). In the tumorous-head strain, the AO activity is 100% and 65% higher during embryogenesis and during metamorphosis than that in Oregon-R-C flies respectively and AO distribution in eye discs is changed in tumorous-head flies by comparison to wild type flies (Kuhn and Cunningham, 1976; Kuhn and Walker, 1978). By the study of homozygous

engrailed clones that were induced by mitotic recombination, Sprey et al. found that AO activity in imaginal discs of D. melanogaster was dependent upon the determinative state of the cell and was not a function of the compartment as a whole (Sprey et al., 1981). A cis-acting tissue-specific regulatory region has been identified for Aldox (Dickinson, 1975, 1980a; Bentley, 1986). A variant in the cis-acting tissue specific regulatory region causes overproduction of AO by a factor of two to three fold in paragonia (male accessory sex glands) while having no significant effect on the enzyme level in other tissues (Dickinson, 1978). By the use of interspecific Drosophila (grimshawi and formella) hybridization, Dickinson (1980b) found that, in addition to the cis-acting control region of AO, there may be some diffusible trans-acting factor for the regulation of Aldox (Dickinson, 1980b). AO is not synthesized until after hatching of the egg (Dickinson, 1971). AO activity is also affected by cin , lxd , aldox-2 ,and ma-1 (Meidinger and Williamson, 1978). AO catalyzes the oxidation of an array of aliphatic and aromatic aldehydes to their corresponding keto-acids. AO activity is apparently not essential for survival since null activity mutants grow as well as wild type flies. AO null mutant flies are also fairly common in wild populations (Voelker et al., 1980; Langley et al., 1981).

The third structural locus is low pyridoxal oxidase (lpo , 3-56.9), which is apparently the structural gene for pyridoxal oxidase (PO; Collins and Glassman, 1969). PO is also a homodimer with a

molecular weight of 250,000 daltons and catalyses the conversion of aldehydes to acids (Cypher et al., 1982). The lpo gene product is not essential for survival and null mutants for PO activity do not exhibit a visible phenotype. AO and PO can both catalyze the oxidation of aldehydes to their corresponding acids. A specific in vitro substrate for pyridoxal oxidase is 2,4,5-trimethoxybenzaldehyde, though this probably has no in vivo significance. This conclusion is based on the histochemical distribution of oxidase activity, the absence of enzymatic activity in the lpo strains, and the dosage dependence on the number of lpo genes present (Cypher et al., 1982).

The fourth known Drosophila molybdoenzyme is sulfite oxidase (SO), which has a molecular weight of 148,000 daltons and catalyses the conversion of sulfite to sulfate. The genetic location of its structural gene is unknown and flies with little or no SO activity do not display a visible phenotype when reared on normal media. When excess sulfite is present in the media, flies lacking SO do not survive (Bogaart and Bernini, 1981; Bentley et al., 1989; Braaten and Bentley, personal communication).

These four enzymes are also affected by four other known loci in D. melanogaster . The ma-1 locus (maroon-like , 1-64.8) is represented by a large number of alleles which depress AO, XDH, and PO below measurable levels and cause a dull brown eye color in homozygous stocks (Glassman and Mitchell, 1959a and b; Finnerty and Johnson, 1979;

Bentley and Williamson, 1982b; Browder et al., 1982b; Bogaart and Bernini, 1981). A maternal effect of the ma-1 allele can also be observed in crosses in which the female parent is homozygous. More recently, five new ma-1 alleles were described, two of which allow measurable levels of AO and XDH in young adults (Bentley and Williamson, 1982b). The typical ma-1 mutant eliminates XDH, AO and PO activity while SO activity remains nearly normal (Bogaart and Bernini, 1981). It has been found that the ma-1 mutant alleles have nearly normal levels of XDH-CRM while the level of AO-CRM is greatly reduced (Bentley and Williamson, 1982b; Browder et al., 1982a and b;).

The cinnamon locus (cin , 1-0.0) was first described as a lethal, lacking XDH activity and associated with a maternal effect on eye color (Baker, 1973). The description of the original allele was extended to include a coordinate lack of AO and PO activities (Browder and Williamson, 1976). Subsequent studies of 16 cin alleles have separated the eye color, lethality, and enzymatic defects and described allelic complementation in various developmental stages (Bentley and Williamson, 1979b, 1982a). The cin^{1} and cin^{9} mutants were found to possess decreased level of XDH-CRM (Browder et al., 1982a) compared to wild type flies. The cin^{9} mutant decreased AO-CRM (Browder et al., 1982b) compared to wild type flies. The cin^{B} and cin^{MD} lacked CRM for PO (Warner et al., 1980).

The low-xanthine dehydrogenase locus (lxd , 3-33) was originally

identified by an allele isolated from a natural population with reduced XDH activity (Keller and Glassman, 1964a). Further studies indicated a loss of PO activity (Keller and Glassman, 1964b) while AO activity (Courtright, 1967) and SO activity (Bogaart and Bernini, 1981) were greatly reduced. The approximate molybdoenzyme activity levels for XDH, AO, PO and SO are 25%, 12%, 0%, and 2% respectively in lxd flies (Schott et al., 1986). A number of new alleles were recently induced and described with similar phenotypes but quantitative differences in enzyme defects (Bentley et al., 1981; Schott et al., 1986).

The aldox-2 locus in D. melanogaster has been shown to differentially affect the molybdoenzymes, AO, PO and XDH and SO. These effects are most obvious at times surrounding the pupal-adult boundary, when the normal organism accumulates large amounts of these enzymes in their active form. The genetic location of this gene is 2-82.9. The cytogenetic location has been determined to be between 52E and 54E8, with the likelihood that it lies within the region 54B1-54E8. The aldox-2 mutant allele has no visible phenotype and is completely recessive for enzyme effects at all stages tested. The effects of this locus on AO, XDH, SO, and PO suggest that this locus may encode a product involved in the synthesis of the molybdenum cofactor common to these enzymes (Bentley and Williamson 1979a; Bentley et al., 1989; Meidinger and Bentley, 1986).

All normal XDH, AO, PO, and SO require molybdenum cofactor (MoCo).

This kind of relationship among molybdoenzymes was first elucidated by genetic studies concerning the control of XDH and nitrate reductase activity in fungi. In Aspergillus , the enzymatic expression of nitrate reductase (NR) is dependent on the wild type function of five non-structural cnx (cofactor-nitrate reductase-xanthine dehydrogenase) loci (Cove, 1979). These results of genetic studies led Pateman et al. (1964) to propose that there must be a common factor, which was essential for both XDH and NR activities and whose synthesis was mediated by the cnx loci. This proposal was supported by experiments in which incubation of extracts from a variety of sources (bacterial, plant or animal) containing molybdoenzymes with extracts of Neurospora nit-1 mutants, which lack cofactor but produce NR polypeptides restored the NR activity (Nason et al., 1974). Molybdoenzymes are also found in humans. A patient lacking SO and XDH activities shows no detectable molybdenum cofactor (Johnson et al., 1980b). It has been shown that the MoCo is a novel pterin (Johnson et al., 1980a) with an associated phosphate group. The active cofactor is presumably composed of molybdenum and a reduced form of the pterin (Johnson et al., 1980a). The presence of a pterin as a structural component of the cofactor isolated from chicken liver sulfite oxidase, milk xanthine oxidase, and Chlorella nitrate reductase has been demonstrated (Johnson et al., 1980a). It was found that the chemical properties of MoCo form B, which is an inactive form of MoCo isolated aerobically in the absence of iodine and KI, are strikingly similar to those of an unusual sulfur-containing pterin isolated from human urine more than 40 years ago and termed urothione

(Johnson and Rajagopalan, 1982). It is hypothesized that urothione is in fact the metabolic excretory product of the molybdenum cofactor (Johnson and Rajagopalan, 1982). The molybdenum cofactor has been shown to contain a pterin nucleus with an unidentified constituent in the 6 position (Johnson et al., 1980a).

In Drosophila , mutants at the non-structural loci, cin , lxd , ma-1 , and aldox-2 , appear to be analogous to the cnx loci in that they each interrupt the enzymatic expression of the molybdoenzymes. The regulatory genes, cin , lxd , and aldox-2 , affect XDH, AO, PO, and SO coordinantly with ma-1 only affecting XDH, AO, and PO. Therefore, it is assumed that these regulatory loci affect the synthesis of MoCo in some steps through which the molybdenum enzymes are affected. The ma-1 $^{+}$ allele has been shown to be involved in the sulfuration of the Moco by the demonstration that the inactive xanthine dehydrogenase and aldehyde oxidase proteins present in ma-1 mutants are identical to the catalytically inactive desulfo forms obtained by cyanide treatment of active enzymes and the cyanide-inactivated desulfo enzyme can be reactivated by anaerobic incubation with 1 mM sulfide and 1 mM dithionite (Wahl et al., 1982). The ma-1 mutants have reasonably high levels of the molybdenum cofactor and levels for any given allele can be 30% to 50% or more of the wild type levels (Warner and Finnerty, 1981). The level of MoCo activity was shown to depend on the gene dosage of wild type lxd gene copies (Schott et al., 1986). The aldox-2 mutant was found to reduce the levels of XDH-CRM and AO-CRM and is assumed to be

involved in the synthesis of the molybdenum cofactor common to molybdo-enzymes (Meidinger and Bentley, 1986; Bentley et al., 1989). It has been suggested that the aldox-2 gene product has a molybdenum binding site, which can also bind tungsten and this site is altered in the mutant strain. Therefore, the mutant strain is much more resistant to the effects of dietary Na_2WO_4 and much more responsive to the administration of Na_2MoO_4 than the OR control strain (Bentley et al., 1989).

Two kinds of gene regulation may occur when the dosage of a gene is changed. These two possible effects are dosage-dependence and dosage-compensation. The well known dosage compensation affects most X chromosome-linked genes. In Drosophila , dosage compensation of sex-linked genes is not mediated by chromosome condensation as it is in mammals; instead, the transcriptional activity of X chromosome genes is regulated to synthesize the same amount of gene product in both males and females (Stewart and Merriam, 1977). Dosage compensation of sex-linked genes in Drosophila is controlled at the transcriptional level. Devlin et al. have observed autosomal dosage compensation in trisomy 2L and trisomy 3L larvae and all three compensating alleles are active (Devlin et al., 1982, 1984 and 1985). If one of three alleles was a deficiency or null mutant, the amount of gene product was reduced further by one third (Devlin et al., 1985). They found that the compensating and non-compensating loci were not segregated into distinct regions in 3L trisomies. Heat shock protein (hsp) 27 and 26 genes are

dosage dependent while hsp 23 and 22 are dosage compensated (Devlin et al., 1985). These four small heat shock protein genes are located at 67B within a 12 Kb region with transcription oriented in both directions (Corces et al., 1980). Devlin et al. proposed that dosage compensation for hsp 83 is controlled post-transcriptionally (Devlin et al., 1985). Organisms can exploit several mechanisms to control dosage compensation. Dosage compensation in yeast can occur either at the translational level (Pearson et al., 1982) or at the transcriptional level (Osley and Herefort, 1981). Under conditions of gene dosage-dependent regulation, an increase in gene dosage is reflected by elevated levels of the gene product. Gene dosage-dependent regulation has been seen in human adenine phosphoribosyltransferase (Marimo and Giannelli, 1975), in human erythrocyte acid phosphatase (Magenis et al., 1975), in human skin fibroblasts (Kurnit, 1979), in some protein levels of mouse embryos (Klose and Putz, 1983) and in maize (Birchler and Newton, 1981). The ry $^{+}$ gene of Drosophila melanogaster is subject to dosage-dependent regulation. By making small deficiencies (87E) or small duplications, Grell showed that the XDH activity level of D. melanogaster was dosage-dependent from 1 to 3 doses of ry $^{+}$ gene (Grell, 1962).

A large number of mobile elements are known to exist in Drosophila melanogaster . In the P element family of mobile elements, P strain males crossed to M strain females will produce dysgenic progeny while all other crosses of individuals from P or M strains produce normal progeny (Kidwell and Kidwell, 1977). P-M hybrid dysgenesis includes

male recombination, sterility and chromosome rearrangements (Kidwell and Kidwell, 1977; Kidwell, 1986). P-M hybrid dysgenesis usually appears to be restricted to germ line cells of both sexes (Engels, 1983). The P strain individuals are termed to the possess the P cytotype in which P factors are quiescent and M strain individuals possess an M cytotype in which P factors are absent (Engels, 1983). The P-M hybrid dysgenesis results from the insertion of a functional P element into an M cytotype, which can then express transposition (Rubin et al., 1982; Bingham et al., 1982). P elements have been used as vectors for gene transfer. The rosy $^{+}$ gene has been introduced into different positions in the Drosophila genome by P element mediated gene transformation (Rubin and Spradling, 1983) and expression of these ry $^{+}$ genes can be affected by the flanking sequences (Spradling and Rubin, 1983).

In this investigation, flies were constructed by genetic methods, which have different numbers of ry $^{+}$ gene copies. A set of parent Drosophila stocks were then used in the construction of different genotypes of flies which have 4, 5, 6, 8, 9 and 10 ry $^{+}$ gene copies. Among these stocks is the Drosophila stock named R310 which has four tandem repeats of ry $^{+}$ inserted at the 93AB position by P element transformation (Rubin and Spradling, 1983). Enzymatic assays for XDH, AO, PO and SO were performed on these genotypically different flies to study the competitive regulation of XDH, AO, PO and SO. Southern analyses and in situ hybridizations to late third instar salivary gland chromosomes were completed to determine the genetic structure of these

newly constructed genotypes.

MATERIALS AND METHODS

DROSOPHILA MELANOGASTER STOCKS:

1. cu kar ry^{42} Ace^{126} / In(3LR)DCXF, ru h D (6380)
2. st sbd e^{s} ro ca / st sbd e^{s} ro ca
3. Ace^{126} / In(3LR)Ubx^{A}, cu kar Ubx^{A}
4. In(3LR)DCXF, ru h D e / ru tra p
5. ve h th cu bx e^{s} ro ca / ve h th cu bx e^{s} ro ca
6. R310
7. OR

All genes listed in the above stocks are on the third chromosome, which carries ru (roughoid, 3-0.0), ve (veinlet, 3-0.2), h (hairy, 3-26.5), D (Dichaete, 3-40.7), th (thread, 3-43.2), st (scarlet, 3-44.0), tra (transformer, 3-45), p (pink, 3-48.0), cu (curled, 3-50), kar (karmoisin, 3-51.7), ry ^{42} (rosy, 3-52), Ace ^{126} (Acetyl cholinesterase, 3-52.2), sbd (stubbloid, 3-58.2), bx (bithorax, 3-58.8), Ubx ^{A} (Ultrabithorax, 3-58.8), e (ebony, 3-70.7) and ro (rough, 3-91.1; Lindsley and Grell, 1967). In(3LR) is an inversion involving the left and rights arm of the third chromosome. The ru , h and D in stock 1, which is also called 6380, and the cu , kar , Ubx ^{A} in stock 3 are in the inverted region of the third chromosome. The genes notated by capital letters are dominant and the genes notated by small letters are recessive. Ace ^{126} is lethal in homozygotes or in combination with a deficiency for Ace $^{+}$ (Lindsley and Grell, 1967; Lindsley and Zimm, 1985). R310 is a stock which has

four *ry*$^{+}$ gene copies inserted in tandem at 93AB by the use of P-element mediated gene transformation (Rubin and Spradling, 1983). Oregon-R is a wild type control stock.

All stocks were maintained on standard cornmeal-yeast-sugar media (Lewis, 1960). Experimental stocks were kept at 25^{o}C and 40% relative humidity while maintenance stocks were kept at room temperature.

METHOD OF DROSOPHILA STOCK CONSTRUCTION:

Two *Drosophila* stocks were constructed to have eight and ten *ry*$^{+}$ gene copies, respectively, with recessive markers on both sides. This makes it easier to keep the stocks uncontaminated and to tell when eight and ten *ry*$^{+}$ gene copies are present. First, I inserted *cu* on the left and then, in a separate stock, *ro* , and *ca* on the right side of the four tandemly inserted *ry*$^{+}$ gene copies present in R310. Crosses were then made to put these recessive markers on both sides of the four tandem repeats of the wild type rosy genes. Based on this stock, a stock was constructed to have ten wild type rosy gene copies by inserting a *ry*$^{+}$ allele in place of the *ry*^{42} mutant allele present in the original R310 stock. The method of constructing these two *Drosophila* stocks is presented in Figures 1, 2, 3 and 4. By the use of these *Drosophila* stocks, flies with different wild type rosy gene copy numbers were constructed, as shown in Table 1.

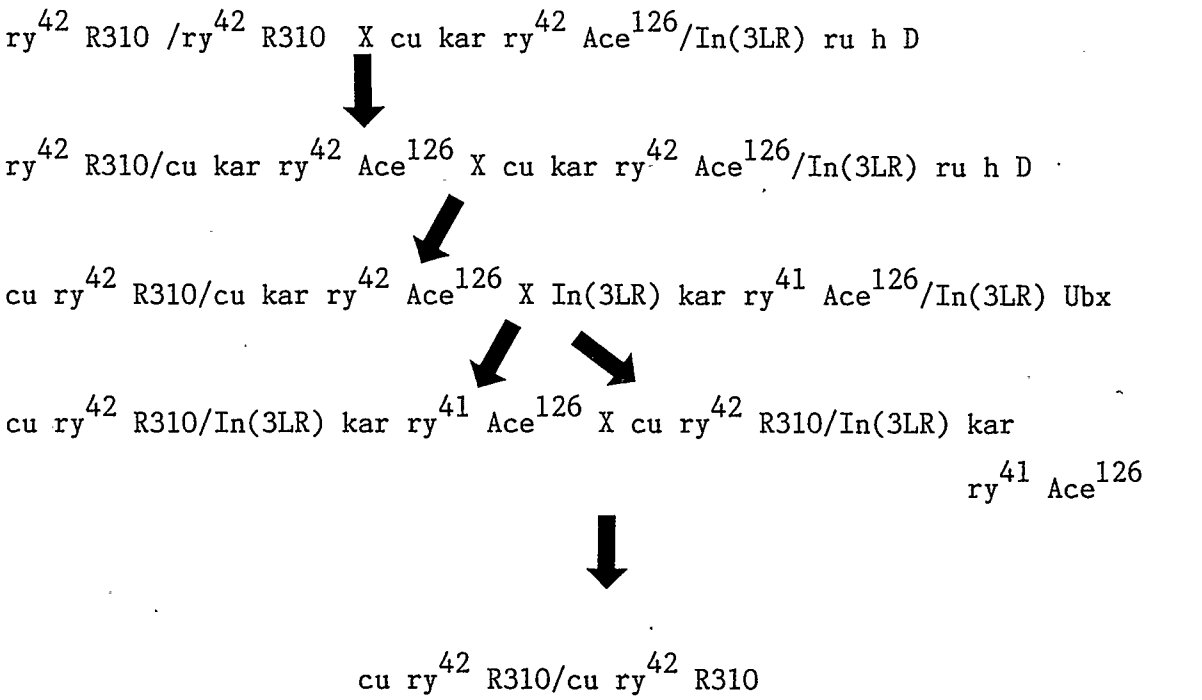


Figure 1. Mating scheme for the insertion of recessive marker to the left side of ry ^{42}.

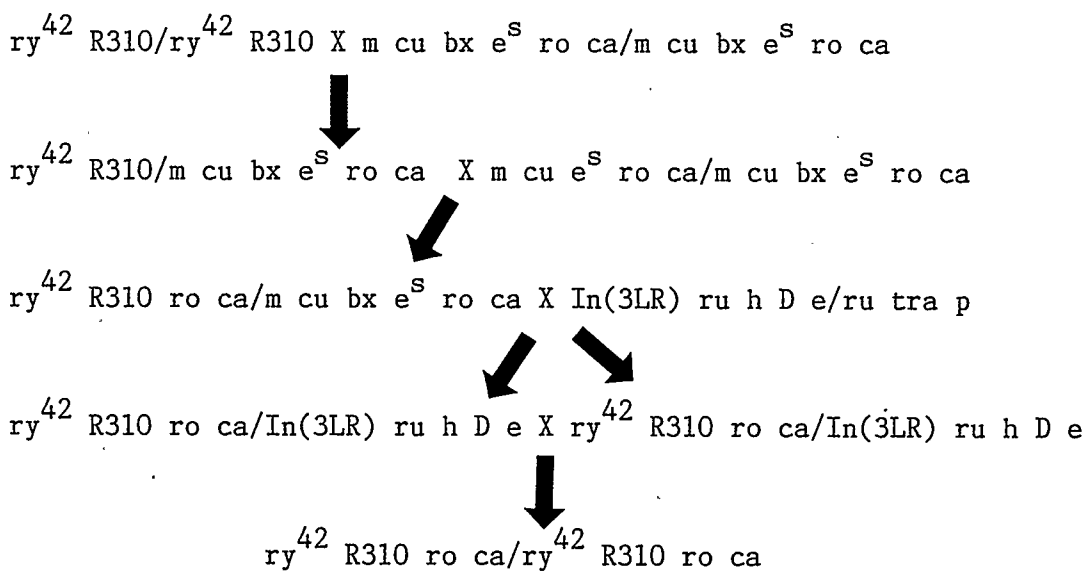


Figure 2. Mating scheme for the insertion of recessive markers to the right side of R310.

The m represents genes ve , h , and th in Figures 2 and 3.

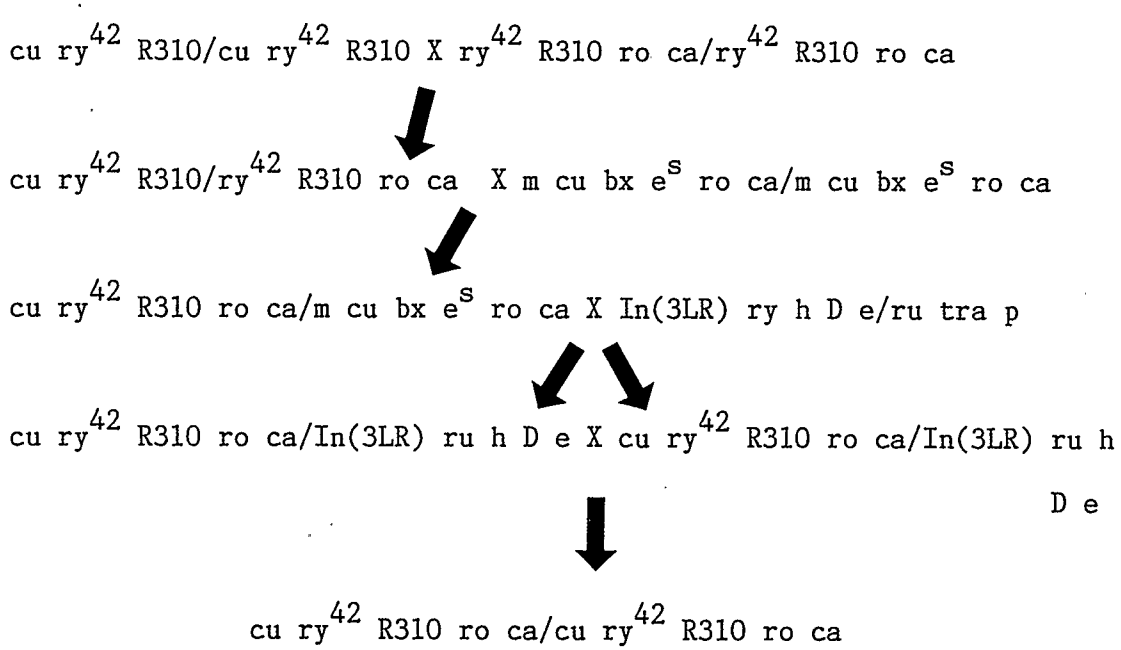


Figure 3. Mating scheme for the insertion of the cu recessive marker to the left side of ry ^{42} and the ro and ca recessive markers to the right side of R310 tandem duplication of ry $^{+}$. Because their flies have one copy of the four tandem duplications of ry $^{+}$ on each of this two third chromosomes, I designated these flies as the E stock (eight ry $^{+}$ copies).

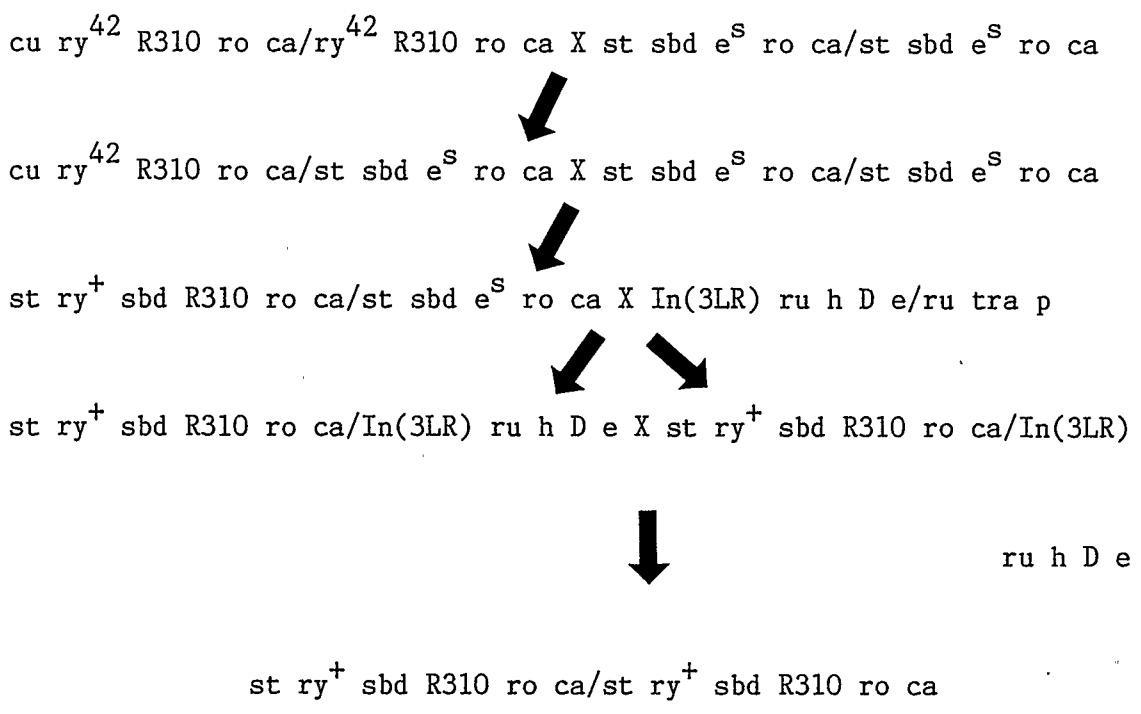


Figure 4. Mating scheme for the insertion of sbd to the left side and ro and ca to the right side of the four tandem ry $^{+}$ copies in R310 and for replacing ry ^{42} with a ry $^{+}$ allele in the normal position, 87D.

These flies have two copies of the R310 four tandem repeats of ry $^{+}$ at the 93AB position plus two copies of ry $^{+}$ at

Table 1. Crosses designed to produce progeny with selected numbers of rosy $^{+}$ gene copies.

	6380 F	OR F	R310 F	E F	T F
6380 M			6380*R310 4 ry C.	6380 * E 4 ry C.	6380 * T 5 ry C.
OR M		OR 2 RY C.	OR * R310 5 ry C.	OR * E 5 ry C.	OR * T 6 ry C.
R310 M	R310*6380 4 ry C.	R310 * OR 5 ry C.	R310 8 ry C.		R310 * T 9 ry C.
E M	E * 6380 4 ry C.	E * OR 5 ry C.		E 8 ry C.	E * T 9 ry C.
T M	T * 6380 5 ry C.	T * OR 6 ry C.	T * R310 9 ry C.	T * E 9 ry C.	T 10 ry C.

'M' and 'F' designates male or female parents, respectively. 'C.' refers to the number of ry $^{+}$ gene copies. All ry in this Table refers to ry $^{-}$. The descriptions for stocks 6380, R310 and OR are in the DROSOPHILA MELANOGASTER STOCKS section of this chapter. The descriptions of the E and T stocks are presented in Figures 3 and 4 and accompanying text.

AO ASSAY:

Single male or female flies were homogenized with a teflon hand homogenizer in a 1.5 ml centrifuge tube with 30 ul of grinding buffer (0.1M Tris-HCl, pH 7.5; 0.001M ethylenediamine tetraacetic acid). Homogenates were then centrifuged at 4°C at 16,900g for ten minutes. Ten ul of supernatant was added to 3 ml of assay buffer (0.02M Potassium Phosphate, pH 7.6, 0.001M ethylenediamine tetraacetic acid, 0.05M Acetaldehyde, 0.02 mg/ml 2,6-Dichloroindophenol, 0.04 mg/ml Phenazine Methosulfate(PMS)). The changes in absorbance at 600 nm and 30°C were measured by the use of a Pye Unicam PU 8800 UV/VIS spectrophotometer (Dickinson, 1970). On the same homogenate, duplicate protein assays were done.

PO ASSAY:

Individual males or females were homogenized using a teflon hand homogenizer in 30ul of 0.1 M Tris borate (pH 8.7), 0.001M EDTA. The supernatant, which came from centrifuging the homogenate at 4°C at 16,900g for 10 min, was used for the activity assay as well as the soluble protein assay. For the activity assay, 10 ul of supernatant were added to 990 ul of assay buffer consisting of 0.1 M Tris-HCL (pH 8.0), 0.15 mM phenazine methosulfate (PMS), 2.42mM 2,4,5-trimethoxy benzaldehyde (TMB), and 0.01 mM 3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyl tetrazolium bromide (MTT). The reaction was monitored at 550nm and 30°C by the same spectrophotometer (Cypher at al., 1982). The same homogenates were used for duplicate soluble protein assays.

SO ASSAY:

The SO activity was measured by the method of Bentley at al. (1989). For a SO assay, a single fly was ground in a 1.5 ml centrifuge tube with 40 ul of grinding buffer (100mM Tris, pH 8.05, 0.12 mg/ml sodium cyanide, 2 mg/ml deoxycholate, 5 mg/ml norite). After a 15 min centrifugation at 16900g at 4°C, 20 ul of fly extract were added to 980 ul of assay solution consisting of 920 ul of 100 mM Tris (pH 8.05), 40 ul of 12 mg/ml cytochrome-C (beef heart) and 20 ul of 100 mM sodium sulfite. The reaction catalyzed by sulfite oxidase transfers two electrons from SO_3^{2-} to SO_4^{2-} and was followed spectrophotometrically at 550 nm and 30°C. The same homogenate was used for duplicate soluble protein assays.

XDH ASSAY:

Fourteen male plus fourteen female flies were homogenized together using a teflon hand homogenizer in 210 ul of grinding buffer, 0.1 M Tris-HCL (pH 8.0), and then centrifuged twice with an intervening 45 mg florisil treatment. 100 ul of the resulting supernatant were added to 1650 ul of 0.1 M Tris-HCl (pH 8.0), 50 ul of nicotinamide adenine dinucleotide (NAD, 20 mg/ml), 200 ul of 2-amino-4-hydroxypterine (AHP, 4.9 mg/100ml). The production of isoxanthopterin (IXP) was monitored by the use of a Turner model 430 recording spectrofluorometer and a Haake D3 water circulator at 30°C. Change of fluorescence was converted to umoles of IXP produced by comparison to a mixed dilution curve of measured amounts of AHP and IXP (Bentley and Williamson,

1979b).

PROTEIN ASSAY:

The soluble protein concentrations of samples were determined by the method of Bradford (1976). The assay solution consists of 0.12 mM Coomassie Brilliant Blue G dissolved in 25 ml 95% ethanol, 50 ml 85% phosphoric acid and 425 ml double distilled water. The absorbance of 5 ul of sample in this solution was determined spectrophotometrically at 595 nm after mixing and a ten to fifteen minute incubation period at room temperature. A standard curve was constructed using selected concentrations of bovine serum albumin and sample results were compared to this standard curve. Duplicate or triplicate determinations were performed on each sample and the mean protein concentration used in calculations.

STATISTICAL ANALYSES:

Nonparametric statistical analyses were performed on XDH, AO, PO, and SO data. For two sample comparisons, the Mann-Whitney U test was used (Mann and Whitney, 1947). For more than two sample comparisons, the Kruskal-Wallis test was used (Kruskal and Wallis, 1952). For all the statistical analyses, the enzymatic activities for XDH, AO, PO, and SO from different stocks and different cross progenies were used to compare to that of OR flies, which had been assayed at the same time. If significant differences were observed between OR flies and some stocks or some cross progenies with respect to one of the four molybdoenzymes, comparisions among these stocks and cross progenies were

performed. In order to make comparisions between the different stocks and different cross progenies, the specific activities of molybdoenzymes assayed during each session were converted to a percentage of average OR values obtained in controls of the same sex and age which were assayed on the same day.

SOUTHERN HYBRIDIZATION ANALYSIS OF GENOMIC DNA:

Genomic DNA was prepared from adult flies essentially as described by Davis et al. (1986). Generally, 100 flies were ground in buffer (100mM NaCl, 10mM EDTA, 20mM Tris-HCl, pH 8.0) and then treated with DNase-free RNase and Proteinase K, followed by phenol and chloroform extraction and then ethanol precipitation. Genomic DNA was subjected to Hind III digestion. After transferring to nitrocellulose paper, ^{32}P oligolabelled rosy probe, protein kinase-C (PKC) probe and Drosophila ribosomal protein 49 (rp49) probe were used for the hybridization. The filter, after washing, was exposed to Kodak XAR 5 Diagnostic film at $-80^{o}C$ for some time and developed.

The plasmid Pryl was used as a source of ry $^{+}$ genomic DNA and carried an 8.1Kb Sal I Drosophila ry $^{+}$ DNA fragment including a 7.2Kb Hind III ry $^{+}$ fragment (Rubin et al., 1982; Rubin and Spradling, 1982). The plasmid Pryl was first used to transform JM109 bacterial cells, and then the plasmids were extracted and subjected to Hind III digestion. After digestion the 7.2 Kb rosy $^{+}$ fragment was purified. This DNA fragment was used for the oligolabelling. Plasmid HR0.6, which carries the ribosomal protein 49 gene of Drosophila

melanogaster (O'Connell and Rosbash, 1984) was linearized by EcoR I digestion and then subjected to oligolabelling. The maps for plasmid Pryl (Rubin and Spradling, 1982), for plasmid HRO.6 (Wong et al., 1981) and for pSBll, which carries a partial PKC cDNA (constructed in our lab.), are presented in Figures 5, 6 and 7, respectively.

PURIFICATION OF DNA FRAGMENTS FROM PLASMIDS:

DNA fragments were purified from plasmids by the method described in the Geneclean Kit protocol (Bio101 Inc.) after restriction endonuclease digestion and electrophoresis. Generally, 20ug of plasmid DNA were digested with Hind III for pryl and then loaded onto a 1% agarose gel for electrophoresis. The DNA band of the correct size was excised from the ethidium bromide stained agarose gel with a razor blade using long wave UV light. The agarose gel fragment was transferred to a 1.5 ml microcentrifuge tube and 2 to 3 volumes of NaI stock solution (Bio101 kit) was added and the tube placed in a 45-55^{o}C waterbath for about 5 minutes with occasional mixing by inverting the microcentrifuge tube. The appropriate amount of Glassmilk suspension (5 ul of Glassmilk suspension for 5 ug or less of DNA and 1 additional ul for each 0.5 ug of DNA) was added to the solution and placed on ice for 5 minutes. After 5 seconds centrifugation, the supernatant was discarded and 200 to 700 ul of NEW (NaCl/Ethanol/Water; Bio101 kit) wash solution was added to the pellet. The pellet was resuspended and the mixture was centrifuged for 5 seconds and the supernatant was discarded. This step was repeated twice. 5 ul or more of TE buffer (10 mM Tris-HCl, pH 7.4; 1 mM EDTA) (Maniatis et al., 1982) was added to the

Figure 5. The map of pryl (Rubin and Spradling, 1982). An 8.1 Kb Sal I DNA fragment containing the $rosy^+$ gene was inserted into the single Xho I site in the plasmid p6.1 (Rubin et al., 1982). The resulting junctions between these two DNA's are not cleaved by either Sal I or Xho I. The *rosy* $^+$ gene was inserted in the middle of a 1.2 Kb P element, which is between two Sal I sites in the small part of the circle not containing the insertion (black and white checkerboard). The pBR322 vector sequences in P6.1 are indicated by the larger open bar. The remainder of DNA sequences in p6.1 are nonrepetitive sequences derived from the white locus of *D. melanogaster* .

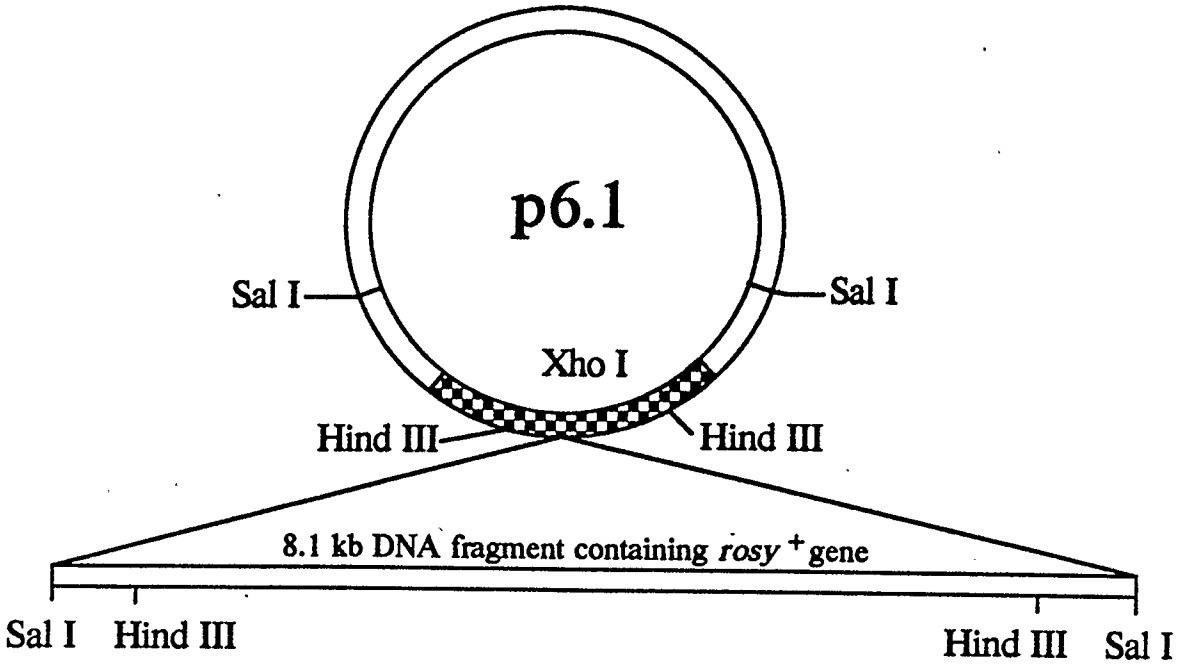

p6.1
Sal I
Sal I
Xho I
Hind III
Hind III
8.1 kb DNA fragment containing rosy + gene
Sal I
Hind III
Hind III
Sal I

Figure 6. The map of plasmid HRO.6 (Wong et al.,1981). The open bar is plasmid pBR322 sequences. The black bar is part of the Drosophila ribosomal protein 49 genomic DNA. The cross area is Drosophila DNA (Vaslet et al., 1980). This plasmid was subcloned from phage 14 (Vaslet et al., 1980).

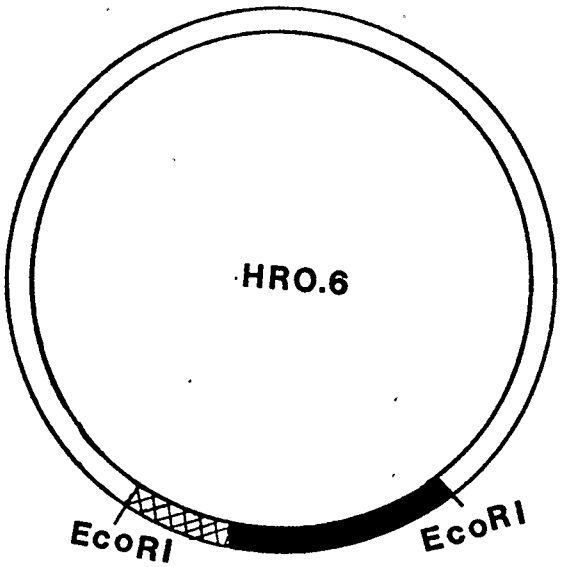
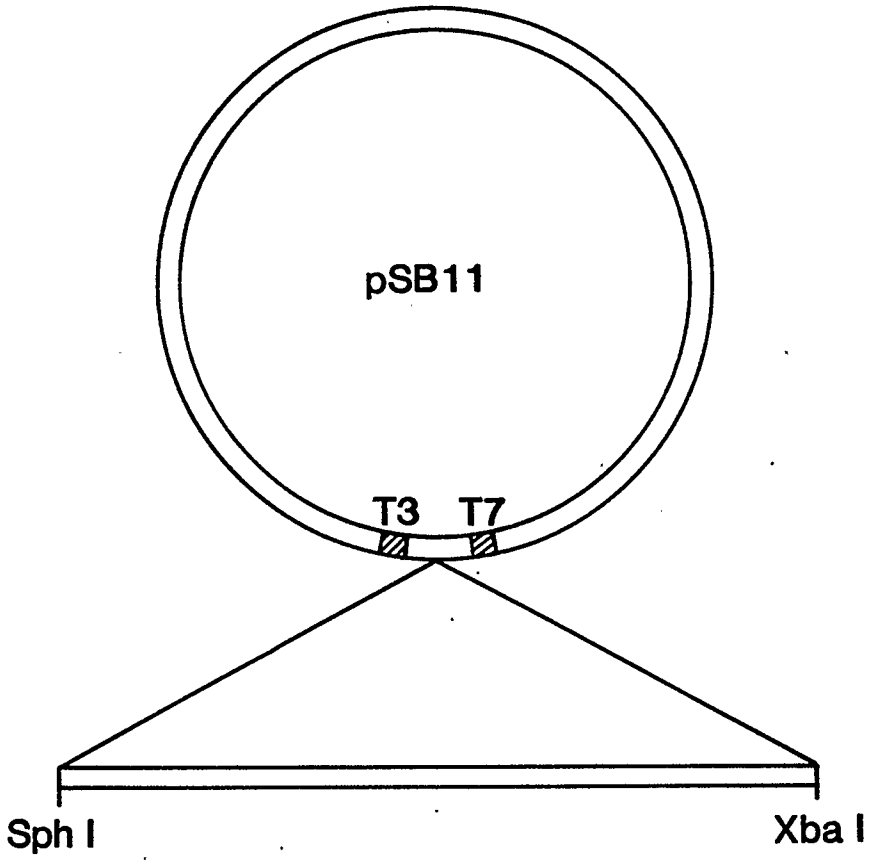

HRO.6
EcoRI
EcoRI

Figure 7. The map of plasmid pSB11 (constructed in our lab.). The 1.8 Kb Sph I-Xba I fragment from the plasmid p101 covering a 1.9 Kb partial cDNA sequence of Drosophila protein kinase-C was inserted into the multiple cloning site of a Bluescript vector (pBM^+) between T3 and T7 promotors.


pSB11
T3
T7
Sph I
Xba I

pellet and then incubated in a 45-55^{o}C waterbath for 3 minutes and centrifuged for 30 seconds and the DNA containing supernatant collected. This step was repeated twice. The purified DNA fragments were stored in TE Buffer at 4^{o}.

OLIGOLABELLING:

The oligolabelling of DNA fragments was performed by the method described by Feinberg and Vogelstein (1984). The DNA was denatured by boiling for 15 minutes and cooled on ice for 5 minutes. At room temperature, 29 ul of water was combined with 2 ul of heat denatured DNA (about 50 ng), 10 ul reagent mixture, 2 ul of BSA (bovine serum albumin), 5 ul of (^{32}P)dCTP (3000 Ci/mmole) or 5 ul of biotinylated dUTP, 2 ul of Klenow. The total volume is 50 ul. The reagent mixture consists of 2 parts solution A (1.25 M Tris-HCl, pH 8.0, 0.125 M $MgCl_2$), 5 parts of solution B (2 M N-2-hydroxy ethyl piperazine-N'-2-ethane sulsonic acid (Hepes) titrated to pH 6.6 with 4 M NaOH), and 3 parts of solution C (90 OD/ml of random hexadeoxyribonucleotides) (Feinberg and Vogelstein, 1984).

TRANSFORMATION OF BACTERIA:

JM109 cells were grown in 40 ml of LB (Lurial-Bertani) medium at 37^{o}C with shaking for several hours until the O.D. of the growing cells was 0.3 to 0.5. The cells were centrifuged for 5 minutes at 1500 g at room temperature. After gently resuspending the cells in 3 ml of 50 mM $CaCl_2$, the volume was brought to 20 ml with 50 mM $CaCl_2$ and then incubated on ice for 30 minutes. Following a 5 min

centrifugation at 1500 g, the cells were resuspended in 4 ml of ice cold 50 mM $CaCl_2$. To the 0.2 ml of this cell solution 0.1 ug of plasmid was added, which contained the DNA fragment of interest. After a 30 min incubation on ice, the cells were heat shocked at 42°C in a water bath for 2 minutes and added to 1 ml of LB medium (1% bacto-tryptone, 0.5% bacto-yeast extract, 1% NaCl) (Davis et al., 1986; Maniatis et al., 1982; Ausubel et al., 1987). The cells were allowed to grow 45 minutes at 37°C. After this treatment, the cells were plated on the surface of LB/agar/ampicillin plates and grown overnight at 37°C. The colonies were picked for plasmid extraction and for storage (Davis et al., 1986).

PLASMID EXTRACTION:

Cells were grown overnight in 30 ml of sterile LB medium. After a 10 minute centrifugation at 3000 rpm, 0.8 ml of lysozyme solution was added (50 mM glucose, 25 mM Tris, pH 8.0, 10 mM EDTA, 2 mg/ml lysozyme (chicken egg white from Sigma) which is freshly prepared; Davis et al., 1986). The pellet was then vortexed to resuspend it in the lysozyme solution and incubated 30 minutes on ice. After incubation, 1.6 ml of 0.2 N NaoH, 0.5% SDS was added to the solution and incubated for 5 minutes on ice. 1 ml of 3 M sodium acetate (pH 4.8) was added and the solution was incubated on ice for 30 minutes. The solution was centrifuged for 20 minutes and the supernatant was transferred to another tube. This step was repeated several times until no precipitate was observed. Then the solution was subjected to phenol/chloroform/IAA (25:24:1) extraction. After this treatment, the plasmid DNA was

precipitated with ice cold 95% ethanol and washed with 70% ethanol. The plasmid DNA was resuspended in distilled water or TE buffer(Davis et al., 1986; Ausubel et al., 1987).

ELECTROPHORESIS:

A one percent agarose gel was prepared. 50 mg of agarose was added to 50 ml of TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA; Ausubel et al., 1987) and then melted in a microwave oven. After cooling to 55^{o}C, ethidium bromide was added to a final concentration of 0.5 ug/ml and then melted agarose was poured into a minigel platform sealed with tape at the open ends and with an appropriate comb (well former) in place. The platform box is 10.5 cm long x 8 cm wide x 2 cm high. Gels were poured to a thickness of between 0.5 and 1 cm. After the gel had hardened, the tape was removed from the open ends and the comb was withdrawn. The gel platform was set in the electrophoresis tank, which was filled with 350 ml of TAE buffer. 20 ul of DNA sample containing about 10 ug of DNA was loaded into each well. The DNA sample was prepared by adding 2 ul of loading buffer (0.25% Bromphenol Blue, 20% Ficoll 400, 1% SDS, 0.1 M Na_2EDTA, pH8.0) to 18 ul of DNA solution. The DNA marker used is Hind III digested lambda genomic DNA. The gel was allowed to run at 90 V for 2 to 3 hours and then the DNA was visualized by placing under a 260 nm UV light source and photographed.

IN SITU HYBRIDIZATION:

The polytene chromosomes of D. melanogaster were prepared essentially as described by Atherton and Gall (1972). Drosophila larvae

were harvested from well-yeasted uncrowded culture bottles. The chromosome squashes were performed on salivary glands from late third instar larvae that had crawled up the side of the bottle and had stopped moving. Larvae were dissected in Drosophila Ringer Solution (7.5g NaCl, 0.35g KCl, and 0.21g $CaCl_2$ per litre of water; Ransom, 1982) and one pair of salivary glands, from which most of the attached fat bodies were teased away, was transferred to one drop of 45% acetic acid placed on a very clean coverslip for a three minute fixation. A clean microscope slide was lowered onto the coverslip and used to pick it up. The slide was turned over and tapped after blotting the excess acetic acid with Kimwipe paper by pressing the edges of the coverslip. The slide was dried at 45°C on a slide warmer for three minutes and then placed in liquid nitrogen for 10 seconds after squashing again. The frozen coverslip was removed immediately by inserting a razor blade under a corner of the coverslip and flipping it off. The slide was then dipped into 95% ethanol for ten minutes. After air drying, the slide was examined under a phase contrast objective (40 times) of a Nikon microscope (104) and were stored for weeks at room temperature or 4°C.

After preparation of polytene chromosomes, the slide was treated in 2 x SSC (3M NaCl, 0.3M sodium citrate) (Maniatis et al., 1982; Davis et al., 1986; Ausubel et al., 1987) at 58 to 65°C for 30 minutes and then washed twice in 2 x SSC at room temperature for 2 minutes each. The polytene chromosomes were acetylated in 250 ml of freshly prepared 0.1 M triethanolamine-HCl with 0.31 ml of acetic anhydride for 10

minutes and then washed twice in 2 x SSC for 5 minutes each and twice in 70% ethanol for 5 minutes each and once in 95% ethanol for 5 minutes at room temperature. After air drying, the chromosome DNA was denatured in freshly prepared 0.07 M NaOH for 3 minutes and subjected twice to 2 x SSC, twice to 70% ethanol, and once to 95% ethanol, each for 5 minutes. The denatured chromosomes were hybridized with biotinylated rosy DNA probe for 12-18 hours. Biotinylation of rosy DNA probe was prepared by oligolabelling with biotinylated dUTP (from BRL). The hybridization buffer is 0.6M NaCl, 50mM Na_2PO_4, 1 x Denhardt's solution (0.1g polyvinylpyrrolidone, 0.1g bovine serum albumin and 0.1g Ficoll 400 in 500 ml water; Denhardt, 1966), 5 mM $MgCl_2$, and 5% dextran sulfate (Kennison and Tamkun, personal communication).

After hybridization, the chromosomes were washed three times in 2 x SSC for 20 minutes each at 53°C and two times in 2 x SSC for 10 minutes each at room temperature and once in buffer 1 (0.1 M Tris, pH 7.5, 0.1 M NaCl, 2 mM $MgCl_2$, 0.05% Triton X-100) at room temperature for 10 minutes and washed once in buffer 2 (buffer 1 plus 2% BSA) at 42°C for 20 minutes. After air drying, the chromosomes were stained with streptavidin (1 mg/ml in 50 mM Tris-HCl, pH 7.5, 0.2 mg/ml sodium azide) first for 10-15 minutes and then washed three times with buffer 1 each for 3 minutes. After the washes, the chromosomes were stained with biotin(AP) (biotinylated calf intestinal alkaline phosphatase, 1 mg/ml in 3 M NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 30 mM triethanolamine, pH 7.6) for 10-15 minutes. After two times of 3 minutes each washing with buffer 1 and two times of 3 minutes each with buffer 3 (0.1 M Tris, pH

9.5, 0.1 M NaCl, 50 mM $MgCl_2$), the chromosomes were stained with NBT (nitro-bluetetrazolium 75 mg/ml, in 70% dimethylformamide) mixed with BCIP (5-bromo-4-chloro-3-indoyl phosphate 50 mg/ml in 70% dimethylformamide) for at least 2 hours. After staining, the slide was immersed in 20 mM Tris pH 7.5, 5 mM EDTA for 5 minute and then stained in Giemsa for 4 to 5 minutes. The Giemsa solution was made by adding 6 ml of Giemsa stock solution (0.5 g Giemsa in 33 ml of glycerol heated to 60°C for two hours with stirring and then 33 ml of absolute Methanol added) in 100 ml of 10 mM $NaPO_4$, pH 7.0 (Pardue, 1986). After washing in distilled water and air drying, the slides were mounted by putting one drop of immersion oil between the coverslip and the slide and then sealing the edges with nail-polish oil. The chromosomes were examined under the microscope (Kennison and Tamkun, personal communication; Pardue, 1986; Pliley et al., 1986; Whiting et al., 1987; the protocol in BRL biotinylation kit).

RESULTS

For the purpose of studying the competitive regulation of XDH, AO, PO, and SO in the molybdoenzyme system of D . melanogaster , eleven Drosophila stocks and cross progeny genotypes were constructed having different numbers of wild-type rosy gene copies. Enzymatic activity assays for XDH, AO, PO, and SO were performed on individuals (AO, PO and SO) or groups of organisms (XDH) of these genotypes from reciprocal crosses. All the data were subjected to nonparametric statistical analyses.

The results of nonparametric statistical analyses, Mann-Whitney U tests and Kruskal-Wallis tests, of the AO enzymatic activities are presented in Tables 2 and 3. The data used for these analyses are presented in Appendix I. These Tables are a statistical comparision of AO specific activities between individuals of different stocks and cross genotypes from reciprocal crosses. The data in Tables 2 and 3 show that, for AO specific activities, there is no significant difference between cross progeny from reciprocal crosses as indicated by all the numbers in Tables 2 and 3 being greater than 5%, the level of significant difference. There are also no significant differences between male and female progeny of a stock or any cross involved in this experiment (the percentage immediately to the right or below the 'S's). Because there are no observed differences between males and females of a

Table 2. Statistical analyses of AO specific activities for reciprocal crosses of 6380 * R310, 6380 * E, OR * E, OR * R310 and 6380 * T.

			6380*R310			6380 * E			OR * E			OR*R310			6380*T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	12	12	24	12	7	19	12	12	24	12	6	18	12	9	21
R310 * 6380	F	12	30		30			7			30			11			8
	M	12		30													
	S	24	30		>30												
E * 6380	F	12				30											
	M	5					30										
	S	17	7					7									
E * OR	F	12							30								
	M	12								30							
	S	24	30								30						
R310 * OR	F	12										30					
	M	10											30				
	S	22	11											7			
T * 6380	F	6													30		
	M	11														30	
	S	17	>30														30

All the data in this Table are presented as a percentage. For all reciprocal crosses, male parents are listed first. n: number of independent AO enzyme assays. M and F refer to males and females respectively. S refers to total. *: indicates a cross between individuals from the two stocks mentioned.

Table 3. Statistical analyses of AO specific activities for reciprocal crosses of OR * T, R310 * T and E * T as well as the stocks R310, E and T.

			OR * T			R310			E			R310*T			E * T			T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	12	12	24	12	12	24	11	7	18	12	12	24	12	9	21	12	11	23
T * OR	F	12	30		30			30			9			20			28			21
	M	12		30																
	S	24	30		6															
R310	F	12					30													
	M	12				30														
	S	24	30					30												
E	F	11								9										
	M	7							9											
	S	18	9								9									
T * R310	F	12										30								
	M	12											29							
	S	24	20											19						
T * E	F	12													27					
	M	12														17				
	S	24	28														28			
T	F	12																	21	
	M	11																21		
	S	23	21																	21

Legend same as Table 2.

given genotype and no observed differences between the progeny of one cross and its reciprocal cross, the data for AO specific activities of males and females with a single genotype were combined. The final combined data of AO enzymatic activities from different stocks and cross progenies were compared to that of OR flies by the use of Mann-Whitney U tests. The results of these analyses are presented in Table 4 and show that, with respect to AO specific activity, there are no significant differences between wild-type OR flies and various Drosophila genotypes that have different numbers of ry$^+$ gene copies.

Tables 5 and 6 are the result of nonparametric statistical analyses of SO specific activities between individuals from different stocks and from different genotypes of reciprocal crosses. The data used for these analyses are presented in Appendix II. There are no significant differences between males and females of a given genotype (as shown immediately below or to the right of the 'S's). Similar to the results presented in Tables 2 and 3 for AO enzymatic activities, there are no significant differences between different reciprocal crosses for SO enzymatic activity. Again, all the data from males and females of a given genotype from reciprocal crosses were combined and compared to the SO specific activity data of OR flies. Table 7 shows that the level of SO specific activity found in OR flies does not differ significantly from that of flies, which have different numbers of ry$^+$ gene copies.

Tables 8, 9 and 10 are similar to Tables 2, 3 and 4, respectively,

Table 4. Statistical analyses of AO specific activity in different genotypes compared to individuals of the OR genotype.

Progeny of crosses or stocks	hypothesized ry^+ copies	N	n	P value from statistical test
R310 * 6380	4	48	24	>30
E * 6380	4	36	12	>30
E * OR	5	48	24	>30
R310 * OR	5	40	18	>30
T * 6380	5	38	18	7
T * OR	6	48	24	9
R310	8	24	12	30
E	8	18	15	>30
T * R310	9	48	18	>30
T * E	9	48	16	13
T	10	23	12	31

OR individuals have 2 ry^+ gene copies and are a standard wild type control strain. 6380 individuals have one selected third chromosome carrying the ry^{42} null mutant allele. E and T individuals were constructed to have 8 and 10 ry^+ gene copies, respectively. N: number of separate AO assays from each genotype. n: number of separate AO assays of OR individuals.

Table 5. Statistical analyses of SO specific activities for reciprocal crosses of 6380 * R310, 6380 * E, OR * E, OR * R310 and 6380 * T.

			6380*R310			6380 * E			OR * E			OR*R310			6380*T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	18	18	36	12	12	24	6	6	12	12	12	24	12	12	24
R310 * 6380	F	18	30		30			30			30			30			30
	M	18		30													
	S	36	30		30												
E * 6380	F	12				30											
	M	12					30										
	S	24	30					>30									
E * OR	F	6							30								
	M	6								11							
	S	12	30								30						
R310 * OR	F	12										30					
	M	12											30				
	S	24	30											30			
T * 6380	F	12													30		
	M	12														30	
	S	24	30														>30

Legend same as Table 2 except that the results of SO assays are compared.

Table 6. Statistical anlyses of SO specific activities for reciprocal crosses of OR * T, R310 * T and E * T as well as the stocks R310, E and T.

			OR * T			R310			E			R310*T			E * T			T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	12	12	24	12	12	24	6	6	12	6	6	12	12	6	18	6	6	12
T * OR	F	12	30		30			30			30			10			8			30
	M	12		30																
	S	24	30		>30															
R310	F	12					30													
	M	12				30														
	S	24	30					30												
E	F	6								30										
	M	6							30											
	S	12	30								30									
T * R310	F	6										30								
	M	6											7							
	S	12	7											11						
T * E	F	12													30					
	M	12														19				
	S	24	27														20			
T	F	6																	30	
	M	6																30		
	S	12	30																	>30

Legend same as Table 2 except that the results of SO assays are compared.

Table 7. Statistical analyses of SO specific activity in different genotypes compared to individuals of the OR genotype.

Progeny of crosses/stocks	ry $^{+}$copies	N	n	P value from statistical test
R310 * 6380	4	72	36	16
E * 6380	4	48	24	>30
E * OR	5	24	12	18
R310 * OR	5	48	24	>30
T * 6380	5	48	24	>30
T * OR	6	48	24	25
R310	8	24	12	>30
E	8	24	12	30
T * R310	9	24	12	8
T * E	9	42	24	22
T	10	12	12	30

Legend the same as Table 4 except that the results of SO assays are compared.

and present nonparametric statistical analyses, Mann-Whitney U tests and Kruskal-Wallis tests, of PO specific activity data. The data used for these analyses are presented in Appendix III. The same methods were used to analyse the data in Tables 8, 9 and 10, respectively, as used to produce Table 2, 3 and 4. For a given genotype, males do not differ significantly from females with respect to PO enzymatic activity as shown by the P value immediately below or to the right of the 'S's. Tables 8 and 9 show that, for each genotype, the reciprocal crosses have the same levels of PO specific activity. Table 10 shows that the levels of PO specific activities of OR wild-type flies and the flies with altered numbers of $\underline{ry}^{+}$ gene copies are all within the 95% confidence level and show no significant differences.

The results of statistical analyses of XDH specific activity data dramatically differ from those found for AO, SO, and PO. Tables 11 and 12 present the results of statistical analyses of XDH enzymatic activity data between individuals from different reciprocal crosses. The data used for these analyses are presented in Appendix IV. There are no significant differeces between males and females for each genotype as shown by the P values immediately below or to the right of 'S's. These Tables show that there are no significant differences between reciprocal crosses, except for the progeny of the crosses of E with 6380 and E with T. The progeny of the crosses of E with 6380 and the crosses of E with T differ significantly from their respective reciprocal crosses with respect to XDH enzymatic activity. Table 13 shows that the levels of XDH enzymatic activity from different D . melanogaster stocks and cross

Table 8. Statistical analyses of PO specific activities for reciprocal crosses of 6380 * R310, 6380 * E, OR * E, OR * R310 and 6380 * T.

			6380*R310			6380 * E			OR * E			OR*R310			6380*T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	6	6	12	12	12	24	6	6	12	12	12	24	10	10	20
R310 * 6380	F	6	30		30			22			30			12			30
	M	6		30													
	S	12	30		30												
E * 6380	F	12				30											
	M	12					25										
	S	24	22					22									
E * OR	F	6							30								
	M	6								30							
	S	12	30								30						
R310 * OR	F	12										30					
	M	12											15				
	S	24	12											13			
T * 6380	F	12													30		
	M	12														30	
	S	24	30														30

Legend same as Table 2 except that the result of PO assays are compared.

Table 9. Statistical analyses of PO specific activities for reciprocal crosses of OR * T, R310 * T and E * T as well as stocks R310, E and T.

			OR * T			R310			E			R310*T			E * T			T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	12	12	24	12	11	23	6	6	12	12	12	24	17	17	34	12	12	24
T * OR	F	12	6		6			10			30			18			30			8
	M	12		6																
	S	24	6		6															
R310	F	12					10													
	M	11				10														
	S	23	10					10												
E	F	6								30										
	M	6							30											
	S	12	30								30									
T * R310	F	12										18								
	M	12											18							
	S	24	18											17						
T * E	F	16													15					
	M	14														30				
	S	30	30														>30			
T	F	12																	8	
	M	12																8		
	S	24	8																	8

Legend same as Table 2 except that the results of PO assays are compared.

Table 10. Statistical analyses of PO specific activity in different genotypes compared to individuals of OR genotype.

Progenies of crosses/stocks	ry $^{+}$copies	N	n	P value from statistical test
R310 * 6380	4	24	12	>30
E * 6380	4	48	24	>30
E * OR	5	24	12	20
R310 * OR	5	48	24	>30
T * 6380	5	44	24	7
T * OR	6	48	24	10
R310	8	23	24	7
E	8	12	12	10
T * R310	9	48	24	20
T * E	9	64	26	25
T	10	24	24	>30

Legend same as Table 4 except that the results of PO assays are compared.

Table 11. Staticsical analyses of XDH specific activities for reciprocal crosses of 6380 * R310, 6380 * E, OR * E, OR * R310 and 6380 * T.

			6380*R310			6380 * E			OR * E			OR*R310			6380*T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	12	11	23	12	12	24	12	12	24	12	12	24	12	12	24
	F	11			30			22			30			31			6
R310																	
*	M	10															
6380																	
	S	21	30		29												
	F	12															
E																	
*	M	12															
6380																	
	S	24	10					1									
	F	12															
E																	
*	M	12															
OR																	
	S	24	17								>30						
	F	12															
R310																	
*	M	12															
OR																	
	S	24	>30											>30			
	F	12													30		
T																	
*	M	12														18	
6380																	
	S	24	30														6

Legend same as Table 2 except that the result of XDH assays are compared.

Table 12. Statistical analyses of XDH specific activities for reciprocal crosses of OR * T, R310 * T and E * T as well as stocks R310, E and T.

			OR * T			R310			E			R310*T			E * T			T		
			F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S
		n	12	12	24	6	6	12	12	12	24	12	12	24	12	12	24	12	12	24
T * OR	F	12	11		6			20			30			36			7			30
	M	12		30																
	S	24	6		13															
R310	F	6					20													
	M	6				20														
	S	12	20					20												
E	F	12								30										
	M	12							30											
	S	24	30								30									
T * R310	F	12										36								
	M	12											36							
	S	24	36											36						
T * E	F	12																		
	M	12																		
	S	24	29													0.4				
T	F	12																	30	
	M	12																30		
	S	24	30																	>30

Legend same as Table 2 except that the results of XDH assays are compared.

Table 13. Statistical analyses of XDH specific activity in different genotypes compared to individuals of the OR genotype.

Progeny of crosses/stocks	*ry* $^{+}$copies	N	n	P value from statistical test
R310 * 6380	4	44	24	< 1
E * 6380	4	24	24	< 1
6380 * E	4	24	24	< 1
E * OR	5	48	24	< 1
R310 * OR	5	48	24	< 1
T * 6380	5	48	24	< 1
T * OR	6	48	24	< 1
R310	8	12	12	< 1
E	8	24	24	< 1
T * R310	9	48	24	< 1
T * E	9	24	24	< 1
E * T	9	24	24	< 1
T	10	24	24	< 1

Legend same as Table 4 except that the results of XDH assays are compared.

progeny, which were constructed to have 4, 5, 6, 8, 9, and 10 wild-type rosy gene copies differ significantly from that of OR wild-type flies, which have 2 copies of $\underline{ry}^{+}$.

Table 14 presents the detailed analyses of XDH specific activity levels found in different stocks and cross progeny genotypes. Columns 4 and 7 are the observed and predicted percentages found for the XDH enzymatic activity levels. The percentage data were obtained by the comparison of XDH activity found in each stock and each cross progeny genotype to the average of that of OR flies. Row 3 is the stock, R310, which has four tandemly repeated $\underline{ry}^{+}$ gene copies inserted at the 93AB position by P element mediated gene transformation (Rubin and Spradling, 1983). The level of XDH enzymatic activity found in R310 flies was 3.3 times (330%) of that found in OR control flies measured by Spradling and Rubin in 1983. In this study, the XDH specific activity level of R310 is 3.02 times (302%) that of OR control flies, roughly the same as the XDH enzymatic activity level of R310 determined by Rubin and Spradling. For calculation of predicted values, I assumed that the R310 stock has two units of R310 XDH activity and that one of those units is genetically one tandem repeat of four $\underline{ry}^{+}$ gene copies. Therefore, one R310 unit should give 151% of the XDH enzymatic activitiy found in OR controls. Because four $\underline{ry}^{+}$ genes in R310 flies give 151% of control XDH activity, one $\underline{ry}^{+}$ in R310 flies will give 38% (151/4) of XDH activity. One copy of $\underline{ry}^{+}$ from OR flies produces 50% of XDH activity. When analyzing the high levels of XDH activity in Table 14, the differences (12%) between one copy of $\underline{ry}^{+}$ from R310 and from OR

Table 14. Statistical analyses of XDH activity in different cross progeny and stocks.

Case	Cross	N	XDH ACT.	Ob. R310 U.	P. R310 U.	P.XDH ACT.
1	R310 * OR	48	200.0	1.3	1.25	188.5
2	R310 * 6380	44	141	0.94	1	151
3	R310	12	302	2	2	302
4	T * R310	48	510	3.4	2.25	339.8
5	T * OR	48	603	4.0	1.5	226.5
6	T * 6380	48	664	4.4	1.25	188.5
7	T	24	1161	7.7	2.5	377.5
8	E * OR	48	708	4.7	1.25	188.5
9	T * E	24	1141	7.4	2.25	339.8
10	E * T	24	995	6.6	2.25	339.8
11	E * 6380	24	961	6.4	1	151
12	6380 * E	24	1130	7.5	1	151
13	E	24	1629	10.9	2	302

Column 3 is the number of individual assays used in analyses. Columns 4 and 5 are observed XDH activities and R310 units, respectively. Columns 6 and 7 are predicted XDH activities and R310 units, respectively.

will not be significant. Therefore, the $\underline{ry}^{+}$ genes from R310 and from OR will not be treated separately. By using 151% to divide all the XDH activity data in column 4, the observed R310 units for different stocks and cross progeny genotypes were obtained and are shown in column 5. Column 6 is the predicted R310 XDH activity units based on how the stocks and cross progeny genotypes were constructed (Figures 3 and 4, Table 1 in Materials and Methods). Column 7 is the expected level of XDH specific activity for the different stocks or cross progeny genotypes obtained by multiplying the predicted R310 units of different stocks or cross progeny genotypes (column 6) with 151 (the level of XDH enzymatic activity in one unit of R310). In rows 1 and 2 of Table 14 for the cross of R310 with OR and R310 with 6380, the data in columns 4 and 5 are quite consistent with the data in columns 6 and 7. For all other crosses and stocks, except the R310 parental stock itself, the data in columns 4 and 5 are all larger than in columns 6 and 7 and all of these crosses and stocks included either E or T stock parents. This comparison indicates that the wild-type rosy gene is either amplified or the predicted eight and ten rosy gene copies in the E and T stocks, respectively, are being overexpressed by some amount. For all of the crosses involving the stock 6380, the third chromosome was selected, which has the $\underline{ry}^{42}$ mutant at position 52.0 (the natural location). In rows 11 and 12, progeny of the cross E with 6380 and its reciprocal cross are presented. The progeny of this cross are predicted to have half of R310 units of the stock E. But their observed levels of XDH specific activity, 961% for the progeny of E * 6380 and 1130% for the progenies of 6380 * E, are much more than half of that, 814.5%

(1629%/2), found in the E stock. This indicates that all $\underline{ry}^{+}$ gene copies in the progeny of the E * 6380 are overexpressed if it is assumed that all the $\underline{ry}^{+}$ gene copies in E flies are normally expressed. If it is assumed that all $\underline{ry}^{+}$ genes in the progeny of E * 6380 are normally expressed, then, the $\underline{ry}^{+}$ genes in E flies are underexpressed. For the cross of E with OR in row 8, the predicted level of XDH activity is 864.5% (1629%/2 for half of the R310 units present in the E stock + 50% for the one copy of $\underline{ry}^{+}$ from OR flies). The observed level of XDH activity, 708%, found in this cross is less than 864.5%. Because the progeny of E * OR have more $\underline{ry}^{+}$ gene copies than the progeny of E * 6380 and its reciprocal cross, the XDH activity levels of progeny from E * OR should be higher than those of the progeny from E * 6380 and its reciprocal cross. This is not the case. By comparison of row 8 for the cross of E * OR with rows 11 and 12 for cross of E with 6380 and its reciprocal cross, it is clear that the R310 units of $\underline{ry}^{+}$ in the progeny of crosses of E with OR are underexpressed. The expected levels of XDH activity for the cross of T with E and its reciprocal cross in rows 9 and 10 are 1395% (1629%/2 + 1161%/2). The observed XDH activity for these flies in Table 14 is about 1100% (1141%/2 + 995%/2). Therefore, the R310 units in these cross progeny are underexpressed. The R310 units of the cross of T with 6380 in row 6 are roughly normal because the observed 664% level of XDH specific activity of these flies is about the same as the predicted 580.5%(1161%/2) level of XDH activity. The predicted levels of XDH specific activity for crosses of T with R310 and T with OR are 731.5% (1161%/2 + 302%/2) and 630.5% (1161%/2 + 50%), respectively. The

observed XDH activities for the progenies of T * R310 and T * OR are 510% and 630%, respectively. Therefore, the R310 units in the progeny of T * R310 are underexpressed while R310 ry $^{+}$ DNA in the progeny of T * OR is normally expressed. The ry $^{+}$ genes in the progeny of T * R310, E * OR, T * E and E * T are underexpressed by these same criteria.

Table 14 shows that either the ry $^{+}$ gene was amplified during construction of the E and T stocks or the ry $^{+}$ gene is overexpressed in these stocks. Figure 8 is the Southern analysis for OR, R310, E and T flies. Total genomic DNA was extracted from adults, digested with Hind III and 18 ug of DNA was loaded into each lane. Figure 9 presents a picture of the ethidium bromide-stained gel. For this gel, ethidium bromide was added to the DNA samples with a little more ethidium bromide in the T sample, therefore, giving the stronger signal in this lane. The gel was run, the DNA transferred to nitrocellulose membrane and the filter was probed with ^{32}P labelled rosy DNA fragment. Densitometer scanning of the autoradiograph in Figure 8 indicates that E and T flies have more ry $^{+}$ DNA than that of R310 or the OR control. The amount of ry $^{+}$ DNA in the E and T stocks is about 5.4 and 5.5 times of that in R310, respectively. The amount of ry $^{+}$ DNA in E and T stocks is 8.0 and 8.2 times that of OR flies. This indicates that the ry $^{+}$ gene in these two stocks was amplified more than would be expected by the method of stock construction. Figure 10 is a picture of the filter in Figure 8 stripped and reprobed with a Protein Kinase-C (PKC) DNA fragment that was oligolabelled with ^{32}P. It is clear that the PKC gene has changed and may also be amplified by comparison to lanes 1 and

Figure 8. Southern analyses of rosy DNA. Drosophila genomic DNA was digested with Hind III and then transferred to nitrocellulose paper and hybridized with radiolabelled rosy DNA. The rosy DNA fragment was cut from plasmid pryl.


OR
R310
E
T
7.2

Figure 9. Drosophila genomic DNA stained with ethidium bromide. Genomic DNA of Drosophila was digested with Hind III and then electrophoresized in a 1% agarose gel. The gel was stained with ethidium bromide.

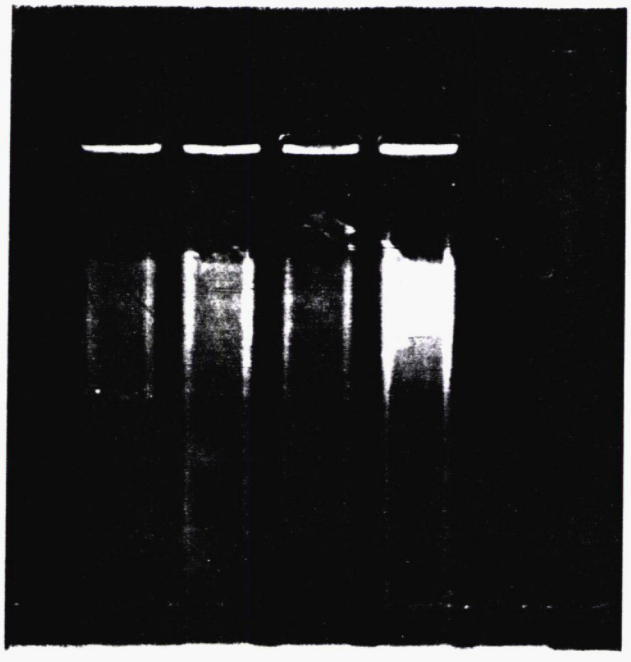


Figure 10. Southern analysis of the Drosophila protein kinase-C (PKC) gene. Drosophila genomic DNA was prepared, digested with Hind III, transferred to nitrocellulose paper and hybridized with ^{32}P radiolabelled PKC DNA fragment.

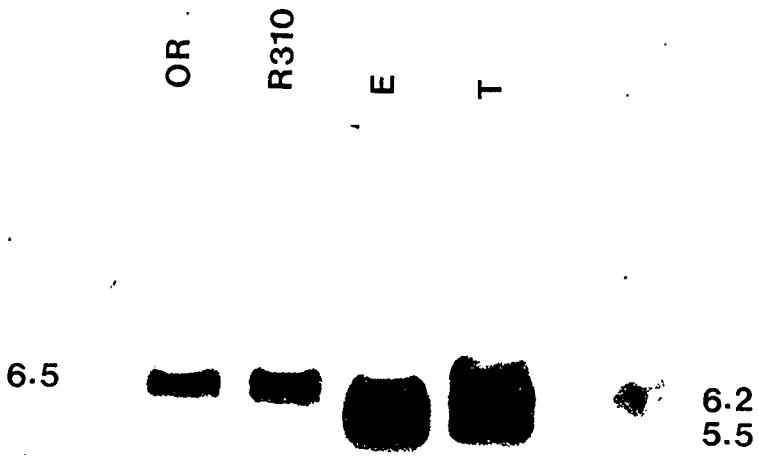

OR
R310
E
T
6.5
6.2
5.5

Figure 11. Southern analysis of the Drosophila ribosomal protein 49. Gene genomic DNA was prepared, digested with Hind III, transferred to nitrocellulose paper and Hybridized with radiolabelled rp49 DNA fragment.


OR
R310
E
T
6.5
4.2

2 for OR and R310. Figure 11 is Southern analysis of Drosophila genomic DNA that was subjected to Hind III digestion and then probed with a P^{32}-labelled ribosomal protein 49 DNA fragment (p^{rp49}). The amount of DNA in each lane is 11 ug for R310, 10 ug for OR and T, 8 ug for E flies. The Drosophila ribosomal protein 49 gene has been sequenced by O'Connell and Rosbash in 1984. This gene is about 0.6 Kb long (O'Connell and Rosbash, 1984). Figure 11 displays that the 6.5 Kb band is of equal or greater intensity compared to the 4.2 Kb band with respect to OR and R310. In the E and T lanes, the 4.2 Kb band is much more intense than the 6.5Kb band, especially in the E lane. By comprison of lanes 2, 3 and 4, there is more 6.5 Kb DNA fragment in lane 4 and less 6.5 Kb DNA fragment in the lane 3. This indicates that the 6.5 Kb DNA is less in E flies, even taking into consideration the reduced DNA loaded in this lane.

Figures 12, 13, 14, 15, 16, 17, 18 and 19 are the in situ hybridizations to salivary gland chromosomes for OR flies, R310 flies, E flies, and T flies which are probed with biotinylated rosy DNA fragment. Twenty two in situ hybridizations were performed on E and T larvae. Figure 12 displays clearly that an OR fly has a rosy gene at the 87D position (arrow). The R310 flies in Figure 13 have rosy gene copies on two different positions (arrows) One location is at 87D of chromosome three. The other location in R310 is at 93AB, which consists of four tandemly repeated wild type rosy gene copies (Spradling and Rubin, 1983). Figures 14, 15 and 16 are pictures of in situ hybridizations to

Figure 14. Figure 15 shows that one band consists of three bands. Another band in Figures 14 and 15 may consist of two bands or a duplication of the insertion by comparison of the intensity of this band to that of the other band, which consists of three bands. This phenotype indicates that the R310 unit or truncated R310 unit has been moved to other places in the E stock during the stock construction. There are partial P elements attached at each end of every ry^+ gene copy of the R310 unit, the four tandemly repeated ry^+ gene copies at the 93AB position (Rubin and Spradling, 1983). Therefore, it is possible that the ry^+ gene copies in the R310 unit moved as a whole or truncated the R310 unit of ry^+ genes. Figure 16 shows that two hybridizations (arrows) may be located on the left arm of the second chromosome. Figure 17, 18 and 19 are the *in situ* hybridizations to salivary gland chromosomes of T larvae probed with biotinylated rosy DNA fragments. Figure 17 shows two rosy bands for T flies (arrows). These two bands consist of two small bands each. Figure 18 is a higher magnification of the lower part in Figure 19. Figure 18 shows clearly two hybridizations on the same arm of one chromosome (arrows). Figure 19 displays that these two hybridizations may be located on the right arm of the second chromosome. Therefore, the R310 unit or truncated R310 rosy DNA unit in the T stock has been moved and has been amplified during the stock construction.

Figure 12. In situ hybridization to OR larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by oligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.


85

Figure 13. In situ hybridization to R310 larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by oligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.


3R

Figure 14. In situ hybridization to E larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by bioligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.

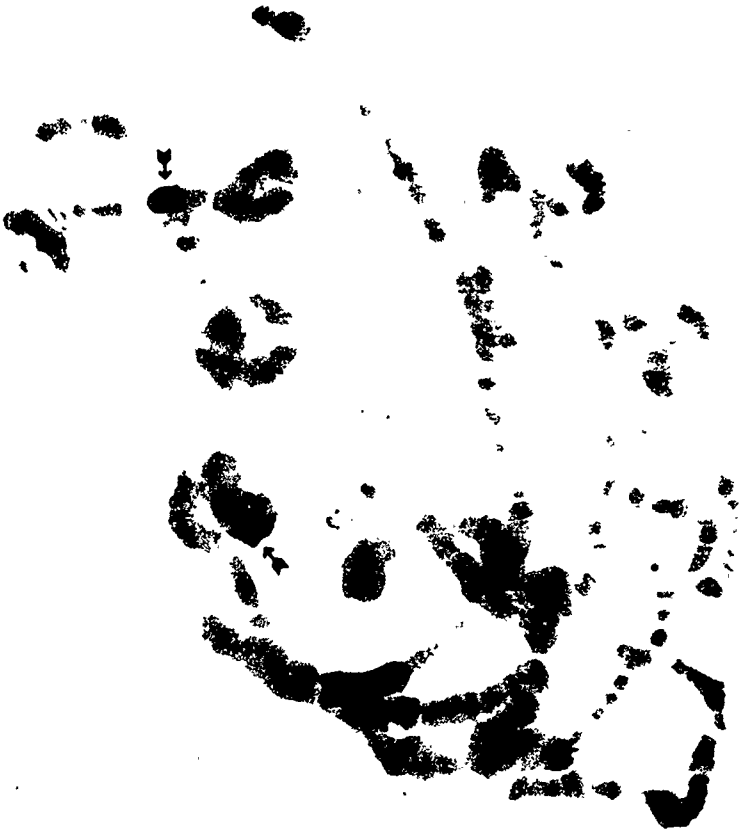


Figure 15. In situ hybridization to E larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by bioligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.

Figure 16. In situ hybridization to E larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by oligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.


X
3R
4
68
89

Figure 17. In situ hybridization to T larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by oligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.

Figure 18. In situ hybridization to T larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by oligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.

Figure 19. In situ hybridization to T larva polytene chromosomes. The chromosomes were prepared from a third instar larva and hybridized with biotinylated Drosophila rosy probe. The probe was made by oligolabelling the rosy fragment with biotinylated dUTP. The rosy DNA template was purified from the plasmid pryl.


3L
68
39

DISCUSSION

It has been hypothesized that different molybdoenzymes have a different affinity for the MoCo. It has been suggested by the study of lxd that the affinities of the different molybdoenzymes for MoCo are XDH > AO > SO > PO (Schott et al., 1986; Bentley et al., 1981; Meidinger and Bentley, 1986). The levels of enzymatic activity for XDH, AO, SO, and PO in homozygous lxd^{1} is 25%, 12%, 2%, and 0% , respectively (Schott et. al., 1986). This has also been demonstrated in studies of molybdoenzyme activity in ma-1 mutants. Homozygous ma-1 mutants are usually associated with the elimination of all activity for XDH, AO, and PO without affecting SO activity. A leaky ma-1 mutant, isolated by Bentley and Williamson (1982b), which expresses measurable levels of enzymatic activity, shows that the specific activity and CRM levels are in the order of XDH > AO > PO. A similar effect is also observed in complementing ma-1 allelic heterozygotes (Bentley and Williamson, 1982b). In complementing cin allelic heterozygotes, the specific activity and CRM levels for different molybdoenzymes follow the same order and suggest a differential affinity of the enzymes for the MoCo (Bentley and Williamson, 1979b; Browder et al., 1982a and b; Warner et al., 1980). This differential affinity of molybdoenzymes for the MoCo is also suggested by the study of the aldox-2 gene (Meidinger and Bentley, 1986; Bentley et al., 1989).

If the hypothesis of different affinities of the molybdoenzymes for the MoCo is true, it might be expected that the PO activity would be decreased first during the serial amplification of the XDH structural gene, rosy $^{+}$. In this experiment, the ry $^{+}$ gene was amplified serially. From Table 14, it is clear that although the number of wild-type rosy gene copies for different stocks was designed to be from 4 to 10, the levels of XDH activity in these stocks are far higher than two (4/2) times or five (10/2) times that of OR flies. In row 13 of Table 14 the E stock is shown to have an XDH activity level about 5.5 (1629/302) times that of R310 flies, which is three times the XDH activity level of OR flies. The XDH activity level of E flies is around 16 times that of OR flies. If the supply of MoCo is the rate limiting factor in the E stock, the PO activity level should be reduced by the competition of four molybdoenzymes for the MoCo. This was not the case. The PO activity level of E flies does not differ significantly from that of OR flies (Table 10). This result is also seen in other stocks in Table 10 for PO activity, for AO activity (Table 4) and for the SO activity (Table 7).

There are several possible explanations for these results. One possible explanation for these results is that, the XDH activity does not reach a high enough level to compete for the MoCo supply of the other three molybdoenzymes, especially for PO. This may indicate that the wild-type rosy gene needs even greater amplification to begin to compete for MoCo with the other three molybdoenzymes. In a study of the

Aldox gene, it was found that heterozygotes containing an Aldox $^{+}$ allele with a deficiency for the Aldox region produce 74.2% of the AO-CRM found in Aldox $^{+}$ homozygotes (Bentley, 1986). Bentley proposed the presence of trans-acting factors, which serve to activate gene expression in a system in which each gene copy can not maximally express (Bentley, 1986). It has been shown that the molybdoenzyme CRM levels and MoCo levels are mutually dependent. Without the molybdoprotein, the MoCo does not apparently survive; without the MoCo, some of the molybdoproteins may be degraded. This may indicate that D. melanogaster has the ability to make an apparently large excess of new MoCo so that the XDH activity of E flies can attain 16 times that of OR wild type flies while other molybdoenzymes still express normally.

Secondly, it may be that there is no differential affinity of XDH, AO, SO, and PO for the MoCo. The evidence that supports this idea is found in Table 14. By comparison of row 13 for E flies with rows 11 and 12 for progeny of cross E with 6380 and cross 6380 with E, it is clear that the flies from E * 6380 or 6380 * E have higher XDH activity levels than half of the XDH activity level of E flies. If the ry $^{+}$ gene can be normally expressed in the cross of E with 6380 and its reciprocal cross, then the ry $^{+}$ gene of E flies is underexpressed, perhaps because the flies cannot make enough MoCo for all the rosy genes present to express normally. This indicates that the MoCo supply may already be the limiting factor in E flies. Alternatively, it may be that the ry $^{+}$ gene in cross E with 6380 and 6380 with E is overexpressed while

the rosy gene of E flies is expressed normally. A Northern analysis and the analysis of XDH-CRM levels for the progeny from E * 6380, 6380 * E, and E stocks may help to distinquish these two possibilities. If the Northern Blot and XDH-CRM analyses indicate that E flies have twice as much RNA as E * 6380 or 6380 * E flies and two times the XDH-CRM levels found in E * 6380 or 6380 * E, then the $\underline{ry}^{+}$ gene of E flies is underexpressed because of insufficient MoCo. If E flies have two times the RNA of the flies from E * 6380 or 6380 * E and less than twice the XDH-CRM of E * 6380 or 6380 * E, there is(are) some factor(s) affecting translation. If the RNA level of E * 6380 and 6380 * E is higher than half of that of E flies, there may be some trans-acting factor regulating the transcription level so that all the $\underline{ry}^{+}$ genes of E flies cannot maximally express.

The differences in tissue distribution of these four molybdoenzymes may also affect these results. It was found that AO and PO were present in many tissues in larvae and adults (Cypher et al., 1982; Dickinson and Gaughan, 1981). But, XDH mainly exists in fat body and Malpighian tubules in larvae and mainly in hemolymph and Malpighian tubules in adults (Spradling and Rubin, 1983). It may be that the Malpighian tubules are not the main tissues for AO and PO. If so, then when the $\underline{ry}^{+}$ gene was amplified to a certain level, either PO or both AO and PO activity was reduced insufficiently to affect the overall level of AO and PO enzymatic activities. This may explain why these experiments did not find decreased PO or AO activity in any stocks or

cross progeny. One way to investigate this is by using Malpighian tubules from the flies which contain different numbers of rosy gene copies and performing enzymatic activity assays for XDH, AO and PO. If differential affinity in malpighian tubules is true, then PO or both AO and PO activities will be decreased in E flies.

Table 14 shows that many genotypes including either E or T have higher levels of XDH activity than predicted from the supposed number of ry $^{+}$ gene copies. This can either mean that there are more rosy gene copies in these flies than the number of rosy gene copies predicted from the method of stock construction or that the rosy gene of these flies can transcribe or translate ry $^{+}$ message more efficiently. A Southern analysis can help to differentiate between these possibilities. Figure 8 is a picture of a Southern Blot for the OR, E, T and R310 flies. Figure 8 reveals that the E and T flies in lanes three and four have more rosy $^{+}$ DNA than equal numbers of R310 flies. This indicates that E flies and T flies or the genotypes including either one of them as parents have more rosy DNA than originally expected. R310 flies were used for the construction of the E and T stocks. Therefore, it is possible that the R310 rosy unit (four tandem repeats of the ry $^{+}$ gene inserted at the 93AB position) has been moved during the stock construction because the R310 stock was made by P element mediated gene transformation. It has been shown that the P element transposase will increase when the P element transformed line has been maintained for an extended period of time (Robertson et al., 1988). Table 14

predicts that the E and T stocks should have ten or eleven and eight R310 units, respectively.

Figures 12, 13, 14, 15, 16, 17, 18 and 19 are the in situ hybridizations for OR, R310, E, and T flies probed with a biotin labeled rosy DNA fragment cut from plasmid Pry1. Figure 12 is the in situ hybridization to OR chromosomes in agreement with previous results (Glassman and Mitchell, 1959a). It displays one hybridized area on 87D of the third chromosome. The in situ hybridization for R310 flies, Figure 13, shows two hybridization bands, also in agreement with previous results (Spradling and Rubin, 1983). Figures 14, 15 and 16 are the in situ hybridizations to the E larvae salivary chromosomes. Figure 14 clearly shows two large bands. Figure 15 shows that one of the hybridization bands consists of three small bands. Figure 16 shows that the two hybridizations may be located on the left arm of chromosome 2. The in situ hybridizations to T larvae salivary gland chromosomes, Figures 17, 18 and 19, show two hybridization bands which consist of two separate bands and may be located on the right arm of chromosome 2. Figure 17 indicates that there are about four R310 units or truncated R310 units of ry^+ DNA in the half genome of the T flies. Figure 14, the in situ hybridization to the E larvae salivary gland chromosomes, shows that by comparing the intensities of the two bands, the weaker band may consist of two small bands. Therefore, I conclude that the E flies have five R310 units of rosy DNA per haploid genome. These results indicate that the R310 unit of rosy DNA has been moved

during the construction of the E and T stocks. Because the R310 unit of rosy DNA consists of four tandemly repeated rosy gene copies, the moved R310 units of *rosy* DNA in the E and T stocks are either whole R310 units or truncated R310 units of *rosy*$^{+}$ DNA. Figures 14, 15, and 17 show that R310 units of *ry*$^{+}$ DNA have been moved several times during construction of the E and T stocks. From these Figures, it appears that the R310 unit (whole or truncated) of *ry*$^{+}$ DNA has a tendency to move near another R310 unit that was already inserted in the genome or that they tend to move to one position and be amplified there. It has been shown that the P element transformed lines of *D. melanogaster* have increased their P elements during the propagation process. Daniels et al. (1987) found that by month 42 of propagation, the number of P elements in P element transformed lines approached that of the Harwich strain (Daniels et al., 1987), a very strong P strain (Kidwell and Novy, 1979). By monitoring the mutability of the hypermutable sn^{w} allele, Robertson et al. (1988) found that a *D. melanogaster* line made by the insertion of a single P element, P(ry^{+2-3}; 99B), increased its transposase activity so that its transposase activity was higher than that of an entire P strain. This transformed *D. melanogaster* line can cause mobilization of *w*$^{+}$ and *ry*$^{+}$ from the X chromosome to autosomal sites at unusually high frequencies (Robertson et al., 1988). The R310 stock was made by P element mediated gene transformation in the early 1980's and has been propagated since that time. Therefore, it is possible that the P element transposase activity has increased since the stock construction.

An interesting finding was that the protein kinase-C (PKC) gene and ribosomal protein 49 (rp49) gene of D. melanogaster have been amplified in the E and T flies (Figure 10 and 11). The DNA was digested with Hind III and the amount of DNA loaded in each well was about equal. One possible explanation for this result is that there are polymorphisms for the PKC gene and for the rp49 gene in the D. melanogaster stocks from which the recessive markers for the E and T stocks came. This possibility can be checked by Southern Analysis of the marker Drosophila stocks. The chance for two gene polymorphisms in the E and T stocks for two independently chosen probes is not high. The PKC gene is on 53E of second chromosome (Rosenthal et al., 1987) and rp49 gene is on the tip of sex chromosome (Ritossa and Spiegelman, 1965; Pardue et al., 1970). It has been suggested that the copia mobile element can mobilize in response to P-M hybrid dysgenesis (Clark et al., 1986b); so it is possible that the alterations seen in the PKC and rp49 genes of D. melanogaster are due to the copia mobile element.

Another possiblility for the amplification of the PKC gene and the rp49 gene in the E and T flies is that the P element causes these two genes to be transposed and be amplified. In theory, only a gene introduced by P element microinjection or a gene enclosed in a P element at both ends will be affected during the construction of E and T stocks. It is known that the number of P elements in a P element transformed D. melanogaster line will increase with time during the propagation

(Daniels et al., 1988). It is possible that the P element in R310 flies has multiplied during the propagation. The P element has inserted at some place around these two genes and during the crosses these two genes have been mobilized and amplified. This idea may be supported by Figure 10, which shows the pattern of PKC bands in E and T flies has changed. There are two PKC bands in the E and T lanes and their molecular weights are lower than those in the OR and R310 lanes. This may indicate that P elements inserted in the PKC gene and changed the PKC gene restriction sites. One way to check whether P elements are attached to the PKC gene or the rp49 gene is by using a P element probe to hybridize multiple digestions of R310, E and T DNA to see if the PKC gene band and rp49 gene band were picked up by this probe. Figures 10 and 11 also indicate that the PKC gene and rp49 gene of D. melanogaster in the OR and R310 flies are stable. A possibility is that the R310 flies have the P cytotype instead of the M cytotype because the P element transposase is suppressed in the P cytotype. Another possible method to test this is to do in situ hybridization using the P element, PKC, and rp49 genes as probes. This will determine whether these genes are amplified at the same place or by moving to some other position and whether the P element was involved in these amplifications.

The rp49 gene has been cloned by Vaslet et al. (1980). The rDNA of Drosophila melanogaster has been mapped to the bb locus (Ritossa and Spiegelman, 1965; Pardue et al., 1970). In genetically normal Drosophila melanogaster males, there are two clusters of tandemly

repeated rRNA genes (rDNA), each containing approximately 250 genes. One of these arrays is located in the proximal heterochromatin of the X chromosome and the other on the short arm of the Y chromosome. It has been shown that the rDNA of D. melanogaster will undergo magnification in the progeny of bb/Ybb- males (Ybb- means the Y chromosome carries a deletion of the bb gene; Ritossa, 1976). This kind of magnification has been found to be controlled by some autosomal modifiers (Marcus et al., 1986). That the rp49 gene was amplified as shown in Figure 11 may be due to some kind of autosomal modifiers present in E and T flies. In situ hybridization using the P element probe and rp49 gene probe would help show whether this is a possible explanation. That the in situ hybridization shows a mobile rp49 gene may not preclude the presence of an autosomal modifier. A definitive method to test this is by using E and T flies for successive crosses for several generations with flies, which do not have the autosomal modifiers and whose chromosomes 2, 3 and 4 can be visually differentiated from the second, third and fourth chromosomes of E and T flies. If it is the autosomal modifiers that cause the rp49 gene to be magnified, the degree of magnification will decrease after a few generations of crosses.

The genomes of E and T flies are constructed to have ten and eight copies of R310 ry^+ DNA, if it is assumed that all the R310 ry^+ DNA in E and T stocks are whole R310 units. Therefore, the levels of XDH activity in E and T flies should be 5 (10/2) and 4 (8/2) times of that of R310 flies. Activity levels are shown in rows 13 and 7 of Table

14. The observed XDH activity levels for E and T flies are 5.3 (1629/302) and 3.8 (1161/302) times that of R310 flies, respectively, in this Table. The number of R310 units of $\underline{ry}^{+}$ DNA in flies from crosses of E with T and its reciprocal cross is 9 (5 + 4). The expected XDH activity level for these flies is around 4.5 (9/2) times that of R310. Rows 9 and 10 in Table 14 indicate that the XDH activity levels for flies from E * T and its reciprocal cross are around 3.6 (1100/302) times that of R310 flies. Therefore, the $\underline{ry}^{+}$ genes in the progeny of T * E are underexpressed. In row 8 of Table 14 for the crosses of E with OR, the $\underline{ry}^{+}$ gene level for these flies is 5 R310 units plus one normal rosy gene copy, which indicates that the expected XDH should be around 3 (5/2 + 0.5) times of that of R310 flies. The two copies of a $\underline{ry}^{+}$ gene in OR flies give 100% of XDH activity. One copy of a $\underline{ry}^{+}$ gene from OR flies should give 50% XDH activity. The $\underline{ry}^{+}$ gene level of progeny from E * 6380 is 5 R310 units. The predicted XDH activity for the progeny from E * 6380 is 2.5 (5/2). The observed XDH activity levels are 2.3 (708/302) times the R310 flies for E * OR and 3.4 (1050/302) times R310 flies for E * 6380 which indicate that the $\underline{ry}^{+}$ gene in E * OR flies is underexpressed and the $\underline{ry}^{+}$ in E * 6380 flies is overexpressed. According to rosy gene levels that are 5 R310 units (4 + 1) for T * R310 , 4 R310 units for T * 6380 and 4 R310 units plus one copy of normal rosy gene for T * OR, the predicted XDH activities for these flies should be 2.5 (5/2) times, 2 (4/2) times and 2.5 (4/2 +0.5) times of R310 flies, respectively. The observed XDH activity levels for these flies are 1.7 (510/302) times, 2.2 (664/302)

times and 2.0 (603/302) times of that of R310 flies, respectively. This indicates that the ry $^{+}$ genes in T * R310 and T * OR are underexpressed while the rosy genes of T * 6380 flies are about normally expressed. In this discussion, the XDH activity levels from different stocks and cross progeny are predicted based on the gene level. The results are that the ry $^{+}$ genes in the progeny from crosses of E * OR, T * E, T * R310, and T * OR are underexpressed while the ry $^{+}$ gene in progeny from E * 6380 are overexpressed.

The results from Table 14 discussed above, may be subject to positional effect. Position effects have been known in Drosophila for a long time. There are two kinds of position effects in D. melanogaster . One is heterochromatic position effect, which is the effect upon the expression of a euchromatic gene placed adjacent to or in heterochromatin. Heterochromatic effect is modified by the Y chromosome (Lewis, 1950; Baker, 1968). Heterochromatic position effect variegation is affected by histone levels (Mottus et al., 1980; Moore et al., 1979; Moore et al., 1983). The rosy locus is subjected to heterochromatic effect when the heterochromatin is placed ajacent to the rosy region of chromosome 3 (Rushlow and Chovnick, 1984). Another position effect recently found is the euchromatic position effect in studies of stable transformants of the rosy locus in D. melanogaster (Clark and Chovnick, 1986; Daniels et al., 1986). The characteristic of the heterochromatic position effect is that the gene is underexpressed and the modifer genes (Y chromosome and histone genes) are nonspecific and

can act in trans. The euchromatic position effect can cause the gene to be either underexpressed or overexpressed and this position effect is site specific. The modifiers for euchromatic position effect should be located immediately adjacent to the gene that is euchromatin-affected. Therefore, the expectation is that the E flies are subjected to some euchromatic position effects so that the wild-type rosy genes in E * 6380 and its reciprocal cross are overexpressed. There may be some trans modifier present in T and OR flies so that the ry^{+} gene of E flies cannot be expressed as highly in the cross E * T and its reciprocal cross and cross E * OR (Table 14). If the E flies are subjected to some euchromatic position effect, the XDH activity level of E flies in row 13 of Table 14 should be higher than observed in that Table. The reason for not getting higher levels of XDH activity for E flies may be due to supply-limited MoCo.

This experiment cannot support the hypothesis of a differential affinity of molybdoenzymes for MoCo. Many previous results support this hypothesis (Schott et al., 1986; Bentley et al., 1981; Bentley and Williamson, 1982b and 1979b; Browder et al., 1982a and b; Warner et al., 1980). The MoCo has been demonstrated to be transferable among the molybdoenzymes (Nason et al., 1974). Further enzyme assays for XDH, AO and PO using Malpighian tubules from third instar larvae, where XDH, AO and PO can each express (Cypher et al., 1982; Dickinson and Gaughan, 1981) and which have different numbers of ry^{+} gene copies, will unambiguously test the hypothesis. Because AO and PO are present in

many identical tissues in larvae and adults (Cypher et al., 1982), amplification of the AO structural gene, Aldox , may decrease PO specific activity if the hypothesis is true. The ry $^{+}$ genes that are bracketed at each end by P element sequences in the R310 unit were moved and amplified in the E and T stocks. These ry $^{+}$ genes may be subject to some autosomal positional effects. The PKC and rp49 DNA sequences exist at higher levels in the E and T stocks than in OR and R310 flies. The most probable explanation is that the change of PKC and rp49 genes in the E and T flies are due to P element effects. Because PKC and rp49 genes are on the second and X chromosomes, respectively, (Rosenthal et al., 1987; Ritossa and Spiegelman, 1965; Pardue et al., 1970) and only a short region of the right arm of the third chromosome is selected during the E and T stock construction, the possibility of keeping a certain unselected chromosome 2 and an X chromosome from marker strains, which were used to construct the E and T stocks, is less than 5% ($1/2^5$). Therefore, the probability of higher levels of PKC and rp49 DNA in the E and T stocks coming from one of the strains from which the recessive markers for these two stocks were obtained is very small.

BIBLIOGRAPHY

Andres, R.Y. (1976). Aldehyde oxidase and xanthine dehydrogenase from wild type Drosophila melanogaster and immunologically cross-reacting material from ma-1 mutants. Eur. J. Biochem. 6, 591-600.

Atherton, D. and J. Gall(1972). Technical notes. DIS. 49, 131-133.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moone, J.A. Smith, J.G. Seidman and K. Struhl (1987). Current Protocols in Molecular Biology . John Wiley and Sons. Toronto.

Baker, B. (1973). The maternal and zygotic control of development by cinnamon a new mutant in Drosophila melanogaster . Dev. Biol. 33, 429-440.

Baker, W.K. (1968). Position-effect variegation. Adv. Genet. 14, 133-169.

Barbara, A. and L. Diana and E. Johnson (1983). Hidden electrophoretic variation at the xanthine dehydrogenase locus in a natural population of Drosophila melanogaster . Genetics 104, 301-305.

Bender, W., P. Spierer and D.S. Hogness (1983). Chromosomal walking and jumping to isolate DNA from the Ace and rosy loci and the bithorax complex in Drosophila melanogaster . J. Mol. Biol. 168, 17-33.

Bentley, M.M. (1986). Analyses of aldox^{n} alleles isolated from natural population of Drosophila melanogaster . Biochem. Genet. 24, 291-308.

Bentley, M.M., R.G. Meidinger and A.C. Braaten (1989). The aldox-2 locus of Drosophila melanogaster affects sulfite oxidase and molybdenum metabolism. Biochem. Genet. in press.

Bentley, M.M. and J.H. Williamson (1979a). A new mutant affecting aldehyde oxidase in Drosophila melanogaster . Z. Naturforsch 34c, 304-305.

Bentley, M.M. and J.H. Williamson (1979b). The control of aldehyde oxidase and xanthine dehydrogenase activities by the cinnamon gene in Drosophila melanogaster . Can. J. Genet. Cytol. 21, 457-471.

Bentley, M.M. and J.H. Williamson (1982a). The developmental analysis of aldehyde oxidase activity in cin allelic heterozygotes of Drosophila melanogaster . Can. J. Genet. Cytol. 24, 1-9.

Bentley, M.M. and J.H. Williamson (1982b). The control of

aldehyde oxidase and xanthine dehydrogenas activities and CRM levels by the ma-1 locus in Drosophila melanogater . Can. J. Genet. Cytol. 24, 11-17.

Bentley, M.M., J.H. Williamson and M.J. Oliver (1981). The effect of molybdate, tungstate and lxd on aldehyde oxidase and xanthine dehydrogenase in Drosophila melanogaster . Can. J. Genet. Cytol. 23, 597-609.

Birchler, J.A. and K.J. Newton (1981). Modullation of protein levels in chromosomal dosage series of maize: The biochemical basis of an anenploid syndromes. Genetics 99, 177-182.

Bingham,P.M., M.G. Kidwell and G.M. Rubin (1982). The molecular basis of P-M hybrid dysgenesis: The role of the P element, a P-strain-specific transposon family. Cell 29, 995-1004.

Bogaart, A.M. and L.F. Bernini (1981). The molybdoenzyme system of Drosophila melanogaster . I. sulfite oxidase: Identification and protein expression of the enzyme in maroon-like (ma-1), low-xanthine dehydrogenase (lxd), and cinnamon (cin) flies. Biochem. Genet. 19, 833-946

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Browder, L.W., L. Tucker and J. Wilkes (1982a). Xanthine dehydrogenase (XDH) cross-reacting material in mutants of Drosophila melanogaster deficient in XDH activity. Biochem. Genet. 20, 125-132.

Browder, L.W., J. Wilkes and L. Tucker (1982b). Aldehyde oxidase cross-reacting material in the Aldox (N), cin , mal and lxd mutants of Drosophila melanogaster . Biochem. Genet. 20, 111-124.

Browder, L.W., and J.H. Williamson (1976). The effects of cinnamon on xanthine dehydrogenase, aldehyde oxidase and pyridoxal oxidase activity during development in Drosophila melanogaster . Dev. Biol. 53, 241-249.

Chovnick, A., W. Gelbart, M. McCarron, B. Osmond, E.P.M. Candido and D.L. Baillie (1976). Organization of the rosy locus in Drosophila melanogaster : Evidence for a control element adjacent to the xanthine dehydrogenase structural element. Genetics 84, 233-255.

Chovnick, A., M. McCarron, A. Hilliker, J. O'Donnell, W. Gelbart, and S. Clark (1978). Gene organization in Drosophila . Cold. Spring Harbor Symp. Quant. Biol. 42, 1011-1021.

Clark, S.H. and A. Chovnick (1986). Studies of normal and position-affected expression of rosy region genes in Drosophila

melanogaster . Genecics 114, 819-840.

Clark, S.H., S. Daniels, L.A. Rushlow, A.J. Hilliker and A. Chovnick (1984). Tissue specific and pretranslational character of the rosy locus control element in Drosophila melanogaster . Genetics 108, 953-968.

Clark, S.H., A,J, Hilliker and A. Chovnick (1986a). Genetic analysis of the right (3') end of the rosy locus in Drosophila melanogaster . Genet. Res. Camb. 47, 109-116.

Clark, S.H., A.J. Hilliker and A. Chovnick (1986b). Recombination can initiate and terminate at a large number of sites within the rosy locus of Drosophila melanogaster . Genetics 118, 261-266.

Clark, S.H., M. McCarron, C. Love and A. Chovnick (1986). On the identification of the rosy locus DNA in Drosophila melanogaster : intragenic recombination mapping of mutations associated with insertion and deletions. Genetics 112, 755-767.

Collins, J.F., E.J. Duke and E. Glassman (1971). Multiple molecular forms of xanthine dehydrogenase and related enzymes. IV. The relations of aldehyde oxidase to xanthine dehydrogenase. Biochem. Genetics 5, 1-13.

Collins, J.E. and E. Glassman. (1969). A third locus (lpo) affecting pyridoxal oxidase in Drosophila melanogaster . Genetics 61, 833-839.

Corces V, R. Holmgren, R. Frevind, R. Morimoto and M. Meselson (1980). Four heat shock proteins of Drosophila melanogaster Coded within a 12kb region in chromosome subdivision 67B. Proc. Watl. Acad. Sci. USA 77: 5390-5393.

Cote, B., W. Bender, D. Curtis and A. Chovnick. (1986). Molecular mapping of the rosy locus in Drosophila melanogaster . Genetics 112, 769-783.

Courtright, J.B. (1967). Polygenic control of aldehyde oxidase in Drosophila . Genetics 57, 25-39.

Covington, M., D. Fleener and R.B. Denlin (1984). Analysis of xanthine dehydrogenase mRNA level in mutants affecting the expression of the rosy locus. Nucleic Acids Research 12, 4559-4573.

Cove, D.J. (1979). Genetic studies of nitrate assimilation in Aspergillus nidulans . Biol. Rev. 54, 291-237.

Cypher, J.J., J.L. Tedesco, J.B. Courtright and A.K. Kumaran. (1982). Tissue-specific and substrate-specific detection of aldehyde and pyridoxal oxidase in larval and imaginal tissues of Drosophila

melanogaster . Biochem. Genet. 20, 315-332.

Daniels, S.B., S.H. Clark, M.G. Kidwell and A. Chovnick (1987). Genetic transformation of Drosophila melanogaster with an autonomous P element: phenotypic and molecular analysis of long-established transformed line. Genetics 115, 711-723.

Daniels, S.B., M. McCarron, C.L. Stephen, H. Clark and A. Chovnick (1986). The underlying bases of gene expression difference in stable transformants of the rosy locus in Drosophila melanogaster . Genetics 113, 265-285.

Davis, L.G., M.D. Dibner and J.F. Battey (1986). Basic Methods in Molecular Biology. Elsevier Science Publishing Co. Inc., New York, N.Y. 10017, U.S.A.

Denhardt, D.T. (1966). A membrane-filter technique for detection of complementary DNA. Biochem. Biophys. Res. Com. 23, 641-646.

Devlin, R.H., D.G. Holm and T.A. Grigliatti (1982). Autosomal dosage compensation in Drosophila melanogaster strains trisomic for the left arm of chromosome 2. Proc. Natl. Acad. Sci. USA 79, 1200-1204.

Devlin, R.H., T.A. Grigliatti and D.G. Holm (1984). Dosage compensation is transcriptionally regulated in autosomal trisomies of Drosophila . Chromosoma 91, 65-73.

Devlin, R.H., T.A. Grigliatti and D.G. Holm (1985). Gene dosge compensation in trisomies of Drosophila melanogaster . Dev. Genet. 6, 39-58.

Dickinson, W.J. (1970). The genetics of aldehyde oxidase in Drosophila melanogaster . Genetics 66, 487-496.

Dickinson, W.J. (1971). Aldehyde oxidase in Drosophila melanogaster : A system for genetic studies on developmental regulation. Dev. Bio. 26, 77-86.

Dickinson, W.J. (1975). A genetic locus affecting the developmental expression of an enzyme in Drosophila melanogaster . Dev. Biol. 42, 131-140.

Dickinson, W.J. (1978). Genetic control of enzyme expression in Drosophila : A locus influencing tissue specificity of aldehyde oxidase. Dev. Biol. 67, 333-342.

Dickinson, W.J. (1980a). Complex cis-acting regulatory genes demonstrated in Drosophila hybrids. Dev. Genet. 1, 229-240.

Dickinson, W.J. (1980b). Tissue specificity of enzyme expression regulated by diffusible factors: Evidence in Drosophila hybrids. Science

207, 995-997.

Dickinson, W.J. and S. Gaughan (1981). Aldehyde oxidase of Drosophila : Contribution of several enzymes to observed activity patterns. Biochem. Genet. 19, 567-583.

Dickinson, W.J. and E. Weisbrod (1976). Gene regulation in Drosophila melanogaster : Independent expression of closely linked related structural loci. Biochem. Genet. 14, 709-721.

Engels, W.R. (1983). The P family of transposable elements in Drosophila Ann. Rev. Genet 17, 315-344.

Feinberg, A.P. and B. Vogelstein (1984). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 133, 266-267.

Finnerty, V. and G. Johnson (1979). Post-translational modification as a potential explanation of high levels of enzyme polymorphism: xanthine dehydrogenase and aldehyde oxidase in Drosophila melanogaster . Genetics 91, 695-722.

Gardner, E.J. and C.M. Woolf (1949). Maternal effect involved in the inheritance of abnormal growths in the head region of Drosophila melanogaster . Genetics 34, 573-585.

Gardner, E.J. and C.M. Woolf (1950). The influnce of high and low temperatures on the expression of tumorous-head in Drosophila melanogaster . Genetics 35, 44-55.

Gausz, J., M.G. Hall, A. Spierer and P. Spierer (1986) Molecular genetics of the rosy-Ace region of Drosophila melanogaster . Genetics 112, 65-78.

Gelbart, W., M, McCarron and A. Chovnick (1976). Expression of the XDH structural element in Drosophila melanogaster . Genetics 84, 211-232.

Glassman, E. (1965). Genetic regulation of xanthine dehydrogenase in Drosophila melanogaster . Fed. Proc. 24, 1243- 1252.

Glassman, E., and H.K. Mitchell (1959a). Mutants of Drosophila melanogaster deficient in xanthine dehydrogenase. Genetics 44, 153-162.

Glassman E. and H.K. Mitchell (1959b). Maternal effect of ma-1 $^{+}$ on xanthine dehydrogenase of Drosophila melanogaster . Genetics 44, 547-554.

Glassman, E., T. Shinoda, E.J. Duke and J.F. Collins (1968). Multiple molecular forms of xanthine dehydrogenase and related enzymes. An New York Acad. Sci. 151, 263-273.

Glassman, E., T. Shinoda, H.M. Moon and J.D. Karam (1966). In vitro complementation between non-allelic Drosophila mutants deficient in xanthine dehydrogenase IV. molecular weights. J. Mol. Biol. 20, 419-422.

Grell, E.H. (1962). The dose effect of ma-l $^{+}$ and ry $^{+}$ on xanthine dehydrogenase activity in Drosophila melanogaster . Z. Vererbungsl. 93, 371-377.

Hadorn, E. (1956). Patterns of biochemical and development pleiotropy. Cold Spring Harbor Symp. Quant. Biol. 21, 363-373.

Hadorn, E. and I. Schwinck (1956). Feblen Von Isoxanthoptein Und Nicht-Autonomie in der Bildung der roten Augenpignoente be: Einer Mutante (rosy^{2}) Von Drosophila melanogaster . Z. Veerbungsi. 87, 528-553.

Hille, R. and V. Massey (1985). Molybdenum containing hydrogenase: xanthine oxidase, aldehyde oxidase and sulfite oxidase. In Spiro, T.G. (ed.) Molybdenum enzymes . John Wiley and Sons, Toronto. PP. 443-518.

Hilliker, A.J. and A. Chovnick (1981). Further observations on intragenic recombination in Drosophila melanogaster Genet. Res. Camb. 38, 282-297.

Johnson, J.L., B.E. Hainline and K.V. Rajagopalan. (1980a). Characterization of the molybdenum cofactor of sulfite oxidase, xanthine oxidase and nitrate reductase. J.Biol. Chem. 255, 1783-1786.

Johnson, J.L. and K.V. Rajagopalan. (1982). Structural and metabolic relationship between the molybdenum cofactor and urothione. Proc. Natl. Acad. Sci. USA 79, 6856-6860.

Johnson, J.L., W.R. Waud, K.V. Rajagopalan, M. Duran, F.A. Beemer and S.K. Wadman (1980b). Inborn errors of molybdenum metabolism: combined deficiencies of sulfite oxidase and xanthine dehydrogenase in a patient lacking the molybdenum cofactor. Proc. Natl. Acad. Sci. USA 77, 3715-3719.

Keith, T.P., A. M. Riley, M. Kreitman, R.G. Lewontin, D. Curtis and G. Chambers (1987). Sequence of the structural gene for xanthine dehydrogenase (rosy locus) in Drosophila melanogaster . Genetics 116, 67-73.

Keller E.C., Jr. and E. Glassman. (1964a). Xanthine dehydrogenase: difference in activity among Drosophila stains. Science 143, 40-41.

Keller, E.C., Jr. And E. Glassmen. (1964b). A third locus (lxd

) affecting xanthine dehydrogenase in Drosophila melanogaster . Genetics 49, 663-668.

Kidwell, M.G. (1986). P-M mutagenesis in Roberts, D.B. (ed.). Drosophila A Practical Approach. pp 59-81. IRL Press Limited. Washington DC.

Kidwell, M.G. and J.F. Kidwell (1977). Hybrid dysgenesis in Drosophila melamegaster : A syndrome of aberrant traits including mutation, sterility and male recombination. Genetics 86, 813-833.

Kidwell, M.G. and J.B. Novy (1979). Hybrid dysgenesis in Drosophila melanogaster : Sterility resulting from gonadal dysgenesis in the P-M system. Genetics 92, 1127-1140.

Klose, J. and B. Putz (1963). Analysis of two-dimensional protein patterns from mouse embryos with different brisonies. Proc. Natl. Acad. Sci. USA 80, 3573-3575.

Kruskal, W.H., and W.A. Wallis (1952). Use of ranks in one-criterion analysis of variance. J. Amer. Statist. Assoc. 47, 583-621.

Kuhn, D.T. and G.N. Cunningham (1976). Aldehyde oxidase activity in the tumorous-head strain of Drosophila melanogaster . Dev. Biol. 52, 43-51.

Kuhn, D.T. and G.N. Cunningham (1977). Aldehyde oxidase compartmentalization in Drosophila melanogaster wing imaginal discs. Science 196, 875-877.

Kuhn, D.T., S.C. Fogerty, A.A.C. Eskens and Th. E. Sprey (1983). Developmental compartments in the Drosophila melanogaster wing disc. Dev. Biol. 95, 399-413.

Kuhn, D.T. and F.C. Walker (1978). Region-specific aldehyde oxidase pattern in imaginal discs of Drosophila melanogaster . Molec. Gen. Genet. 163, 125-129.

Kurnit, D.M. (1979). Down syndrome: Gene dosage at the transcriptional level in skin fibroblasts. Proc. Natl. Aca. Sci. USA 76, 2372-2375.

Langley, C.H., R.A. Voelker, A.J.L. Brown, S. Ohnishi, B. Dickson, and E. Montgomery (1981). Null allele frequencies at allozyme loci in natural populations of Drosophila . Genetics 99, 151-156.

Lee, C.S., C. Daniels, M. McCarron, C. Love, M. Gray, W. Bender and A. Chovnick (1987). Mutations affecting expression of the rosy locus in Drosophila melanogaster . Genetics 116, 55-65.

Lewis, E.B. (1950). The phenomenon of position effect. Adv. Genet. 3, 73-115.

Lewis, E.B. (1960). A new standard for food medium. DIS 34, 117.

Lindsley, D.L. and E.H. Grell (1967). Genetic variations of Drosophila melanogaster . Carnegie Institution of Washington Publication 522, USA.

Lindsley, D.L. and G. Zimm (1985). The genome of Drosophila melanogaster . Part 1: Gene A-K. DIS. 62.

Magenis, R.E., R.D. Koler, E. Lourien, R.H. Bigley, M.C. Duval, and K.M. Overton. Gene dosage: Evidence for assignment of erythrocyte acid phosphatase locus to chromosome 2. Proc. Natl. Acad. Sci. USA. 4526-4530.

Maniatis, T., E.F. Fritsch and J. Sambrook (1982). Molecular cloning . Cold Spring Harbor Laboratory, New York, N.Y. 10017, USA.

Mann, H.B., and D.R. Whitney (1947). On a test of whether one of two random variables is stochastically larger than the other. Ann. Math. Statist. 18, 50-60.

Marcus, C.H., A.E. Zitron, D.A. Wright and R.S. Hawley (1986). Autosomal modifiers of the bobbed phenotype are a major component of the rDNA magnification paradox in Drosophila melanogaster . Genetics 113, 305-319.

Marino, B. and F. Giannelli (1975). Gene dosage effect in human trisomy 16. Nature 256, 204-206.

McCarron, M., J. O'Donnell, A. Chovnick, B.S. Bhullar, J. Hewitt and E.P.M. Candido. (1979). Organization of the rosy locus in Drosophila melanogaster : Further evidence in support of a cis-acting control element adjacent to the xanthine dehydrogenase structural element. Genetics 91, 275-293.

Meidinger, E.M. and J.H. Williamson (1978). Genetic control of aldehyde oxidase activity and cross-reacting material in Drosophila melanogaster . Can. J. Genet. Cytol. 20, 489-497.

Meidinger, R.G. and M.M. Bentley (1986). Genetic and developmental characterization of the aldox-2 locus of Drosophila melanogaster . Biochem. Genet. 24, 683-699.

Mitchell, H.K., E. Glassman and E. Hadorn. (1958). Hypoxanthine in rosy and maroon-like mutants of Drosophila melanogaster . Science 129, 268-269.

Moore, G.D., J.D. Procunier, D.P. Cross and T.A. Grigliatti (1979). Histone gene deficiencies and position effect variegation in Drosophila . Nature 282, 312-314.

Moore, G.D., D.A. Sinclair and T.A. Grigliatti (1983). Histone gene multiplicity and position effect variegation in Drosophila melanogaster . Genetics 105, 327-344.

Mottus, R., R. Reeves and T.A. Grigliatti (1980). Butyrate suppression of position-effect variegation in Drosophila melanogaster . Molec. Gen. Genet. 178, 465-469.

Nason, A., K.Y. Lee, S.S. Pan, and R.H. Erickson (1974). Evidence for a molybdenum cofactor common to all molybdenyum enzymes based on the in vitro assembly of assimilatory NADP-nitrate reductase using the Neurospora mutant nit-1 . Proc. Climax first Int. Conf. on Chemistry and Uses of Molybdenum, Univ. of Reading, England, Sept., 1973 (Mitchell, P.C.H. Ed). Climax Molybdenum Company, Ltd., London, 1974.

Nolte, H. (1955). The eye-pigment system of Drosophila melanogaster . IV. the pigment of the ruby and red groups of mutants. J. Genet. 53, 1-10.

O'Connell, P. and M. Rosbash (1984). Sequence, structure, and codon preference of the Drosophila ribosomal protein 49 gene. Nuc. Ac. Res. 12, 5495-5513.

Osley, M.A. and L.M. Herefort (1981). Yeast histone genes show dosage Compensation. Cell 24, 377-384.

Pardue, M.L. (1986). In situ hybridization to DNA of chromosomes and nuclei. In Roberts, D.B. (ed.). Drosophila , A Practical Approach. pp 118. IRL Press. Washington, D.C.

Pardue, M.L., S.A. Gerbi, R.A. Eckardt and J.G. Gall (1970). Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of Diptera . Chromosoma 29, 268-290.

Pateman, J.A., D.J. Cove, B.M. Rever and D.B. Roberts (1964). A common cofactor for nitrate reductase and xanthine dehydrogenase which also regulates the synthesis of nitrate reductase. Nature 201, 58-60.

Pearson, N.J., H.M. Fried and J.R. Warner (1982). Yeast use translational control to compensate for extra copies of a ribosomal protein Gene. Cell 29, 347-355.

Pliley, M.D., J.L. Farmer and D.E. Jeffery (1987). Technical notes. DIS. 66, 170-171.

Ransom, R. (1982). Techniques. In Robert Ransom (ed.). A handbook of Drosophila development . Elsevier Biomedical Press, New

York. pp. 19.

Reardon, J.T., C.A. Liljestrand-Golden, R.L. Dusenbery and P.D. Smith (1987). Molecular analysis of Diepoxybutane-Induced mutation at the rosy locus of Drosophila melanogaster . Genetics 115, 323-331.

Ritossa, F. (1976). In The Genetics and Biology of Drosophila . Vol. 1b. Ashurner, M. and E. Novitski Ed. Academic Press Inc. Ltd. New York.

Ritossa, F.M. and S. Spiegelman (1965). Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of Drosophila melanogaster . Proc. Natl. Acad. Sci. U.S.A. 53, 737-745.

Robertson, H.M., R.Preston, R.W. Phillips, D.M. Johnson-Schlits, W.K. Benz and W.R. Engles (1988). A stable genomic source of P element transposase in Drosophila melanogaster . Genetics 118, 461-470.

Rosenthal, A., L. Rhee, R. Yadegari, R. Paro, A. Ulrich and D.V. Goeddel (1987). Structure and nucleotide sequence of a Drosophila melanogaster protein kinase C gene. The EMBO Journal 6, 433-441.

Rubin, G.M., M.G. Kidwell and P.M. Bingham (1982). The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations. Cell 29, 987-994.

Rubin, G.M. and A.C. Spradling. (1982). Genetic transformation of Drosophila with transposable element vectors. Science 218, 348-353.

Rubin, G.M. and A.C. Spradling. (1983). Vector for P element-mediated gene transfer in Drosophila . Nucleic Acids Res. 18, 6341-6351.

Rushlow, C.A., W. Bender and A. Chovnick (1984). Studies on the mechanism of heterochromatic position effect at the rosy locus of Drosophila . Genetics 108, 603-615.

Rushlow, C.A., and A. Chovnick. (1984). Heterochromatic position effect at the rosy locus of Drosophila melanogaster : cytological, genetic and biochemical characterization. Genetics 108, 589-602.

Schott, D.R., M.C. Baldwin, and V. Finnerty (1986). Molybdenum hydroxylases in Drosophila . III. further characterization of the low xanthine dehydrogenase gene. Biochem. Genet. 24, 509-527.

Snyder, M., J. Hirsh and N. Davidson (1981). The cuticle genes of Drosophila : a developmentally regulated gene cluster. Cell 25, 165-177.

Snyder, M., M. Hunkapiller, D. Yuen, D. Siwer, J. Fristrom and N. Davidson (1982). Cuticle protein genes of Drosophila : structure,

organization and evolution of four clustered genes. Cell 29, 1027-1040.

Spradling, A.C. and G.M. Rubin (1982). Transposition of cloned P elements into Drosophila germ line chromosomes. Science 218, 341-347.

Spradling, A.C. and G.M. Rubin. (1983). The effect of chromosomal position on the Drosophila xanthine dehydrogenase gene. Cell 34, 47-57.

Sprey, Th. E., D. Segal, H.E. Sprey-Pieters, and D.T. Kuhn (1981). Influence of homoeotic genes on the aldehyde oxidase pattern in imaginal discs of Drosophila melanogaster . Dev. Genet. 2, 75-87.

Stewart, B., and J. Merriam (1977). Dosage compensation. In Ashburner, M. and T.R.F. Wright (ed.). The Genetics and Biology of Drosophila . volume 2d , pp. 107-140.

Ursprung, H. and E. Hadorn (1961). Xanthine dehydrogenase in organen von Drosophila melanogaster . Experimentia 17, 230-232.

Vaslet, C.A., P. O'Connell, M. Izquierdo and M. Rosbash (1980). Isolation and mapping of a cloned ribosomal protein gene of Drosophila melanogaster Nature 285, 674-676.

Voelker, R.A., C.H. Langley, A.J. Langley, A.J. Leigh Brown, S. Ohnishi, B. Dickson, E. Montgomery and S.C. Smith. (1980). Enzyme null alleles in natural populations of Drosophila melanogaster : frequencies in a North Carolina population. Proc. Natl. Acad. Sci. USA 77, 1091-1095.

Wahl, R.C., C.K. Warner, V. Finnerty and K.V. Rajagopalan (1982). Drosophila melanogaster ma-1 mutants are defective in the sulfuration of desulfo Mo hydroxylase. J. Biol. Chem. 257, 3958-3962.

Warner, C.K. and V. Finnerty (1981). Molybdenum hydroxylases in Drosophila . II. Molybdenum cofactor in xanthine dehydrogenase, aldehyde oxidase and pyridoxal oxidase. Mol. Gen. Genet. 184, 92-96.

Warner, C.K., D.T. Watts and V. Finnerty. (1980). Molybdenum hydroxylases in Drosophila . I. Preliminary studies of pyridoxal oxidase. Mol. Gen. Genet. 180, 449-453.

Whiting, J.H. Jr., J.L. Farmer and D.E. Jeffery (1987). Technical notes. DIS. 66, 170-171.

Wong, Y.C., P. O'Connell, M. Rosbash and S.C.R. Elgin (1981). DNase hypersensive sites of the chromatin for Drosophila melanogaster ribosomal protein 49 gene. Nucleic Acids Research 24, 6749-6762.

Yen, T.T. and E. Glassman (1965). Electrophoretic variants of xanthine dehydrogenase in Drosophila melanogaster . Genetics 52, 977-981.

Appendix I. The data for AO specific activity.

Cross progeny	Sex	n	A.S.A.
R310 x 6380	M	12	46.3
	F	12	41.2
Total		24	43.7
6380 x R310	M	12	51.1
	F	12	49.7
Total		24	50.4
E x 6380	M	5	61.5
	F	12	56.4
Total		17	57.9
6380 x E	M	7	59.9
	F	12	54.2
Total		19	56.3

n: number of separate assays

A.S.A.: average specific activity expressed as the change of absorbance A600/min./10 g soluble protein.

Appendix I. (continued).

Cross progeny	Sex	n	A.S.A.
OR x E	M	12	60.2
	F	12	55.6
Total		24	57.9
E x OR	M	12	54.4
	F	12	45.0
Total		24	49.8
R310 x OR	M	10	40.5
	F	12	38.9
Total		22	39.6
OR x R310	M	6	44.4
	F	12	39.3
Total		18	41.0

Appendix I. (continued).

Cross progeny	Sex	n	A.S.A.
T x 6380	M	11	54.4
	F	6	49.3
Total		17	52.6
6380 x T	M	9	56.7
	F	12	49.4
Total		21	52.5
T x OR	M	12	41.1
	F	12	38.1
Total		24	39.6
OR x T	M	12	42.2
	F	12	39.1
Total		24	40.7

Appendix I. (continued).

Cross progeny	Sex	n	A.S.A.
T x E	M	12	31.0
	F	12	31.9
Total		24	31.5
E x T	M	12	24.7
	F	12	30.4
Total		24	27.6
R310 x T	F	12	39.2
	M	12	33.7
Total		24	36.5
T x R310	F	12	38.4
	M	12	35.4
Ttotal		24	36.9

Appedix I. (continued).

Stock	Sex	n	A.S.A.
R310	M	12	57.1
	F	12	56.4
Total		24	56.7
E	M	7	37.5
	F	11	33.4
Total		18	35.0
T	M	11	40.5
	F	12	35.3
Total		13	37.8
OR	M	73	44.7
	F	75	40.8
Total		148	42.7

Appendix II. The data for SO specific activity.

Cross progeny	Sex	n	A.S.A.
R310 x 6380	M	18	34.5
	F	18	36.0
Total		36	35.3
6380 x R310	M	18	35.0
	F	18	35.9
Total		36	35.5
E x 6380	M	12	37.5
	F	12	38.0
Total		24	37.8
6380 x E	M	12	37.3
	F	12	39.5
Total		24	38.4

n: number of separate assays.

A.S.A.: average specific activity expressed as the change of absorbance A550 nm/min./100 mg soluble protein.

Appendix II. (continued).

Cross progeny	Sex	n	A.S.A.
OR x E	M	6	36.1
	F	6	36.1
Total		12	36.1
E x OR	M	6	33.0
	F	6	35.1
Total		12	34.1
R310 x OR	M	12	32.2
	F	12	31.2
Total		24	31.7
OR x R310	M	12	31.7
	F	12	31.2
Total		24	31.5

Appendix II. (continued).

Cross progeny	Sex	n	A.S.A.
T x 6380	M	12	39.8
	F	12	41.3
Total		24	40.6
6380 x T	M	12	40.9
	F	12	42.4
Total		24	41.7
T x OR	M	12	37.9
	F	12	39.1
Total		24	38.5
OR x T	M	12	37.7
	F	12	40.9
Total		24	39.3

Appendix II. (continued).

Cross progeny	Sex	n	A.S.A.
T x E	M	12	41.5
	F	12	42.9
Total		24	42.2
E x T	M	6	38.1
	F	12	42.5
Total		18	40.3
R310 x T	F	6	22.2
	M	6	25.5
Total		12	23.9
T x R310	F	6	25.1
	M	6	28.8
Ttotal		12	27.0

Appedix II. (continued).

Stock	Sex	n	A.S.A.
R310	M	12	44.4
	F	12	44.7
Total		24	44.5
E	M	6	47.1
	F	6	46.6
Total		12	46.9
T	M	6	41.7
	F	6	47.3
Total		12	44.5
OR	M	102	38.5
	F	102	40.3
Total		204	39.4

Appendix III. The data for PO specific activity.

Cross progeny	Sex	n	A.S.A.
R310 x 6380	M	6	26.4
	F	6	24.7
Total		12	25.5
6380 x R310	M	6	21.6
	F	6	23.9
Total		12	22.8
E x 6380	M	12	13.7
	F	12	13.6
Total		24	13.7
6380 x E	M	12	16.5
	F	12	13.2
Total		24	14.9

n: number of separate assays.

A.S.A.: average specific activity expressed as the change Of absorbance A550/min./g soluble protein.

Appendix III. (continued).

Cross progeny	Sex	n	A.S.A.
OR x E	M	6	10.8
	F	6	12.8
Total		12	11.8
E x OR	M	6	10.6
	F	6	12.0
Total		12	11.3
R310 x OR	M	12	24.9
	F	12	18.7
Total		24	21.8
OR x R310	M	12	20.0
	F	12	19.8
Total		24	19.9

Appendix III. (continued).

Cross progeny	Sex	n	A.S.A.
T x 6380	M	12	27.9
	F	12	25.5
Total		24	26.7
6380 x T	M	10	23.9
	F	10	25.7
Total		20	24.8
T x OR	M	12	22.8
	F	12	19.1
Total		24	21.0
OR x T	M	12	16.7
	F	12	16.9
Total		24	16.8

Appendix III. (continued).

Cross progeny	Sex	n	A.S.A.
T x E	M	14	20.3
	F	16	20.0
Total		30	20.2
E x T	M	17	21.3
	F	17	23.2
Total		34	22.3
R310 x T	F	12	25.0
	M	12	21.7
Total		24	23.4
T x R310	F	12	28.5
	M	12	20.7
Ttotal		24	24.6

Appedix III. (continued).

Stock	Sex	n	A.S.A.
R310	M	10	30.0
	F	12	25.0
Total		22	27.3
E	M	6	8.9
	F	6	9.2
Total		12	9.1
T	M	12	31.9
	F	12	22.0
Total		24	27.0
OR	M	108	19.3
	F	107	19.7
Total		215	19.5

Appendix IV. The data for XDH specific activity.

Cross progeny	Sex	n	P.S.A.
R310 x 6380	M	10	158.4
	F	11	138.5
Total		21	148.4
6380 x R310	M	11	141.4
	F	12	129.1
Total		23	135.0
E x 6380	M	12	922.2
	F	12	999.4
Total		24	960.8
6380 x E	M	12	1070.4
	F	12	1190.3
Total		24	1130.4

n: number of separate assays.

P.S.A.: average percent specific activity expressed as a percentage of OR same stage and sex assayed on the same day.

Appendix IV. (continued).

Cross progeny	Sex	n	P.S.A.
OR x E	M	12	732.8
	F	12	683.8
Total		24	708.3
E x OR	M	12	735.6
	F	12	681.0
Total		24	708.0
R310 x OR	M	12	215.3
	F	12	199.2
Total		24	207.3
OR x R310	M	12	207.9
	F	12	177.3
Total		24	192.6

Appendix IV. (continued).

Cross progeny	Sex	n	P.S.A.
T x 6380	M	12	680.3
	F	12	699.9
Total		24	690.1
6380 x T	M	12	617.8
	F	12	671.2
Total		24	644.5
T x OR	M	12	582.7
	F	12	638.4
Total		24	610.5
OR x T	M	12	586.5
	F	12	605.9
Total		24	596.2

Appendix IV. (continued).

Cross progeny	Sex	n	P.S.A.
T x E	M	12	1159.8
	F	12	1123.5
Total		24	1141.7
E x T	M	12	1047.2
	F	12	943.7
Total		24	995.5
R310 x T	F	12	563.9
	M	12	454.4
Total		24	509.2
T x R310	F	12	499.8
	M	12	519.5
Ttotal		24	509.7

Appedix IV. (continued).

Stock	Sex	n	P.S.A.
R310	M	6	324
	F	6	297
Total		12	310.5
E	M	12	1669.7
	F	12	1587.5
Total		24	1628.6
T	M	12	1140.9
	F	12	1181.2
Total		24	1161.1
OR	M	114	20.9
	F	114	22.9
Total		228	(100%)21.9

OR: defined as 100%. Numerical values given are average specific activity expressed as nmol isoxanthpterin produced/min./100 mg soluble protein.