70-14,604

NASH, William Guy, 1941-
EPIGENETIC CLUSTERING OF THE GENES AT THE TIP OF THE X CHROMOSOME IN DROSOPHILA MELANOGASTER.

The University of Michigan, Ph.D., 1969
Zoology

University Microfilms, Inc., Ann Arbor, Michigan

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EPIGENETIC CLUSTERING OF THE GENES AT THE TIP OF THE X CHROMOSOME IN *DROSOPHILA MELANOGASTER*

by

William Guy Nash

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in
The University of Michigan
1969

Doctoral Committee:

Professor Tahir M. Rizki, Chairman
Professor Rowland H. Davis
Professor Morris Foster
Professor Norman E. Kemp

ABSTRACT

EPIGENETIC CLUSTERING OF THE GENES AT THE TIP OF THE X CHROMOSOME IN DROSOPHILA MELANOGASTER

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William Guy Nash

Chairman: Tahir M. Rizki

Experiments were designed to test if a functional-linkage relationship exists between the gene loci represented in the region corresponding to the deficiency Df(1)260-2 (bands 1A1-8) and the closely located deep orange (dor) gene in Drosophila melanogaster. A functional-linkage relationship was initially suggested by the proximity of dor (0.3 on the X chromosome) to Df(1)260-2 (0.0 on the X chromosome) and the discovery that both dor and Df(1)260-2 result in a similar phenotypic expression designated f^e (failure of late embryos to eclode) Nash (1966). Both f^e and Df(1)260-2 zygotes display only weak, ineffective hatching motions at the time of eclosion and their tracheal systems fail to fill with gas. Some of the f^e zygotes artificially removed from the egg cases managed to survive to maturity, but the majority of them were subjected to rapid desiccation.

Specific attached XY chromosomes were constructed to examine the question of a functional-linkage relationship among the gene loci in the vicinity of dor as well as the

effect of extra heterochromatin on the f^e syndrome. Extra heterochromatin showed a generalized detrimental effect on all stages of development in these studies.

The incidence of the f^e phenotype among dor/+ zygotes is conditioned by the maternal genotype. Crosses of a dor/dor female X dor^+ male produced f^e zygotes whereas F_1 zygotes from a dor^+/dor^+ female X dor male were normal.

A duplication y^+ (= band 1A1-8) genetically coextensive with the region represented by Df(1)260-2) was either absent or present on the tip of the Y fragment associated with an attached XY chromosome. Using the appropriate marker genes, dor was incorporated in the attached XY chromosome, and thus dor/dor females were constituted which had either one or two y^+ duplications, or none. The frequency of f^e in the progeny of dor females carrying two y^+ duplications was significantly higher (three to four times) than the frequency of this phenotype among the progeny of females lacking the duplication. The synergistic effect on the f^e phenotype observed when dor and the y^+ duplication are present can be quantitatively modified by altering the allelic state of the gene yellow. Further evidence for a structural relationship was suggested by the finding that the phenotype of the adults affected by the particular allele of yellow (y^N) was qualitatively altered by the presence of the tightly linked mutant dor allele.

Interaction of dor with the mutant rosy (ry) and also with the carnation (car) gene results in lethality of the zygotes. The stage of lethality and morphological syndromes

were quite distinct from the $\underline{fe}$ syndrome. Thus interacting genetic variability remotely located from the dor gene apparently fails to contribute to the common syndrome displayed by the mutants located at the tip of the X chromosome.

This study indicates that the structural and functional integrity of the genes at the tip of the X chromosome is significantly important for maintaining the normal physiologic state of late embryos. Since the morphological structures influenced by these gene loci are differentiated at different stages of development, this thesis proposes that the functional relationship indicates the existence of an epigenetic clustering of genes in multicellular forms.

ACKNOWLEDGEMENTS

I wish to acknowledge the inspiration and encouragement provided throughout the several years of this investigation by my Chairman, Dr. Tahir M. Rizki of the Department of Zoology. Through his own enthusiasum he has infected me with a happy attachment to the sometimes perplexing but rewarding investigation of Drosophila genetics. I also wish to thank Professor Morris Foster and Professor Norman E. Kemp of the Zoology Department for their many beneficial criticisms and suggestions. I wish to thank Professor Rowland Davis of the Department of Botany for his help in correcting this manuscript and also for allowing me the use of his laboratory to participate in the rearing of Neurospora.

Dr. Charles Sing of the Department of Human Genetics has contributed helpful suggestions in the statistical manipulation of the data.

Dr. William K. Baker of the Zoology Department of The University of Chicago provided me with much helpful information concerning my initial experiments as well as several of the stocks used in this investigation.

Throughout the course of this study I have received support as a Public Health Service pre-doctoral trainee in genetics (USPHS 5T1 GM 71-08) administered through the Department of Human Genetics, The University of Michigan.

Finally I wish to thank my wife Carol, without whom this thesis would not have been possible.

CONTENTS

ACKNOWLEDGEMENTS. ii

CONTENTS. iii

TABLES. iv

ILLUSTRATIONS v

INTRODUCTION. 1

METHODS AND MATERIALS 13

EXPERIMENTAL PROCEDURES 23

RESULTS 25

DISCUSSION. 57

SUMMARY 77

BIBLIOGRAPHY. 80

LIST OF TABLES

Table		Page
1.	Complementation properties of several *y*, *ac* and *sc* alleles.....	10
2.	Effect of extra heterochromatin on the embryonic development of male and female zygotes from the cross *dor* x *Ore-R*.....	27
3.	Genotypically different females crossed to *Ore-R* males and their characteristic progenies.....	31
4.	*Dor* females with no or two extra y^+ duplications crossed to *Ore-R* males and their characteristic progenies.....	35
5.	The dosage effect of y^+ duplications in the *dor* maternal genotype on the percentage of f^e zygotes found among her progeny.....	41
6.	*ydor* females crossed to *Ore-R* males and their characteristic progenies.....	44
7.	Genotypically different females crossed to *Ore-R* and their characteristic progenies.....	47
8.	The effect of several eye color mutants in combination with *dor*.....	50
9.	Ovary transplants between y^N, *Ore-R*, ry^2, *car* and y^N*dor* late 3rd instar larvae.....	55

ILLUSTRATIONS

Figure		Page
1.	Distal tip of the X-chromosome (modified from Genetic Variation of *Drosophila melanogaster*).....	11
2.	Synthesis of attached-XY chromosomes with a specific mutant marker.....	16
3.	Attached-XY chromosomes used in the present investigation.....	18
4.	Third instar neuroblast stained with aceto-orcein (X2600).....	20
5.	Photograph of late embryos enclosed within the vitelline membrane of wild type and heterozygous *dor/+* females.....	28
6.	Effect of maternal age on the developmental stage reached by zygotes from *dor* mothers....	38

INTRODUCTION

The clustering of genes that act in the same metabolic pathway was first detected in Salmonella typhimurium by Demerec (1955). By means of the transduction technique he was able to show that the order of four genes on the linkage map (tryA → tryB → tryC → tryD) coincided exactly in sequence in their role in the synthesis leading to tryptophane (precursor → anthranilic acid → compound "B" → indole → tryptophane). Demerec thus demonstrated the colinearity between the genes controlling enzymes in a metabolic pathway and the metabolic pathway itself.

At the time of its discovery, gene clustering of this sort was quite puzzling. With the demonstration of the operon model of regulation in E. coli (Jacob and Monod, 1961) this phenomenon was rationalized. The clustering of genes offered to the organism the ability to control coordinately all of the genes in the cluster by means of a single operator gene.

The question naturally arose as to the general occurrence and nature of gene clustering. In bacteria it was found to be frequent in some genera and non-existent or rare in others and showed no phylogenetic trend. The conclusion was drawn that the clustering of genes represents an individual species adaptation and is not a phenomenon of general importance to the genetic function of all bacteria (Demerec, 1964).

Gene clustering in bacteria can be divided into two types: "metabolic" gene clustering, already discussed, and what one might call "functional" gene clustering as exemplified by the lac operon in E. coli. The structural genes of this operon do not act sequentially to modify the structure of a precursor molecule; yet the functioning of the z gene product (β-galactoside permease) is vital to the functioning of the y gene product (β-galactosidase). That is to say, without β-galactoside permease E. coli cells cannot hydrolyze β-galactosides even though they possess β-galactosidase.

Jacob and Monod's brilliant genetic analysis of the regulatory mechanism of the lac operon led investigators to search for parallel systems in higher organisms. Evidence for a kind of genetic regulation analogous to the bacterial operon is rare in higher organisms. Nonetheless, it may be much more common than present techniques allow us to investigate.

"Controlling elements" (McClintock, 1951) in corn, already well established prior to the detection of the bacterial operon, seemed to have many properties similar to the regulator and operator genes in the lac system. It should be emphasized though, that the regulator- and operator-like elements in corn, in addition, have a number of properties not found in bacterial controlling systems. It would appear that in the course of evolution genetic regulatory systems have changed in nature with the increasing complexity of the organism.

In Drosophila the most compelling evidence to date for

an operon-like cluster is that put together by Lewis (1963) using the bithorax pseudoallelic series. In an attempt to fit the extensive data on the bithorax mutants of Drosophila melanogaster to the operon model he has emphasized those properties which are common to both systems. By hypothesizing three "bithorax substances" he can explain how the gene in the bithorax cluster act in a co-ordinated manner to determine the development of certain body segments. In addition, recombination analysis shows cis-trans effects which suggest that the action of the genes of the bithorax clusters are polarized in a manner similar to the bacterial operon (Lewis, 1963).

Higher multicellular organisms face a whole set of problems not encountered by bacteria. The genetic regulatory systems of bacteria are geared to respond in an essentially on or off fashion to an external and internal environment fluctuating with respect to essential growth metabolites. In a differentiating multicellular organism with many cell types, genetic regulation has to be co-ordinated also with respect to the time and place of its control in development. In higher organisms, therefore, one might expect to find genes which are clustered "epigenetically." In this model, genes that have to function together or sequentially at a specific time or place in development may be clustered to provide a genetic arrangement favorable for co-ordinated physiological control.

In Drosophila two aspects of genetic regulation are of

particular interest. The first concerns the relationship of the structural organization of the chromosome to the mechanism of supragenic regulation. Although a molecular explanation of this relationship remains obscure, several examples are known where genetic regulation is exerted along a rather substantial length of the chromosome. The second aspect of genetic regulation deals with the functional relationship between the genes that make up a co-ordinately controlled linkage unit or cluster. This can be contrasted to the functional interaction of unlinked genes which are not co-ordinately controlled.

Functional-linkage relationships in Drosophila may be detected using the cis-trans test (Lewis, 1955). Originally "cis" and "trans" positions were used operationally to distinguish whether closely linked genes functioned independently or belonged to the same pseudoallelic complex. The importance of position effect pseudoallelism was realized first by Lewis in his study of the tightly linked Star, asteroid genes and later also with the Stubble, stubbloid genes (Lewis, 1945, 1949). Genes in the cis position may be referred to as being in coupling, and in the trans position as being in repulsion. Coupling-repulsion position effects are not restricted to closely linked genes as is the case with Star, asteroid and Stubble, stubbloid position effects. Hannah-Alava (1964) has shown that the expression of the extra sex comb (Scx) and Polycomb (Pc) phenotypes are stronger when Scx and Pc are in coupling with the bithorax mutants than when in repulsion.

Scx and Pc are more than ten map units from the bithorax group.

The Scx, Pc-bithorax case differs from the cis-trans effects in bacteria in that the mutant genes in coupling rather than repulsion cause the greater departure from the normal phenotype. The explanation of intracistronic cis-trans effects in micro-organisms which occur only between very short distances of the chromosome obviously is not adequate to explain coupling-repulsion effects in Drosophila. Variegation position effect (Schultz, 1941; Russell and Bangham, 1959) observed only in organisms with complex chromosome structure supports the theory that an organizational pattern of the chromosome itself is necessary for normal gene function.

There is further suggestive evidence that regulation occurs at the chromosome level of organization rather than at the individual gene level in higher organisms as demonstrated by studies of chromosome rearrangements in Drosophila pseudoobscura and D. persimilis. Dobzhansky and Sturtevant (1938) and Dobzhansky and Epling (1944) have shown that several rearrangements often occur in a species in one geographic region as a balanced polymorphism, and that their relative frequencies change from one region to the next. Laboratory experiments show that the carriers of these different gene arrangements have different adaptive values under different experimental conditions (Dobzhansky, 1947), suggesting that this balanced polymorphism is maintained because certain gene

arrangements are adapted to different geographic environments. It may be concluded that natural selection has stabilized the existence of these gene arrangements in these populations. Although direct experimental evidence is lacking it seems possible that the inverted portion of the chromosome might modify the physiology of the carrier partially by position effect phenomena.

The evidence to date leaves little doubt that the kind of supragenic regulation found in Drosophila is of a very different nature from that found in bacteria where clustering involves only very short lengths of the chromosome. This does not argue against the existence of operon-like units in higher organisms, but it does demonstrate the existence of a higher level of genetic integration.

The second aspect of genetic regulation referred to earlier concerns the nature of clustering in higher organisms. One would like to know what kind of genes make up a cluster. Evidence at hand suggests that some genes cluster according to the "epigenetic" model presented earlier. In looking at the puffing patterns of the dipteran Acricotopus, Mechelke (1961) observed a distal to proximal sequential movement of a large puff along the chromosome over a distance of several bands (Band 78 to 58 in the BR_4 region). Puff formation and movement begins at the end of the last larval instar and ends in the prepupal stage. This pattern of puffing activity is exactly what one would expect if genes are clustered according to their epigenetic function in development, although in no

case has a cluster of genes of known function been localized to specific bands in a puffing pattern. The current theory that puffing indicates increased gene activity suggests that a number of clustered genes of the BR_4 region undergo sequential activation during a specific period of development. This model predicts that closely linked genes, whose common function is known to affect a specific stage of development, will likely be co-ordinately regulated. The present investigation proposes to test this prediction by examination of genes located at the very distal end of the X chromosome in Drosophila melanogaster. A brief history of some of the genetic information known about this region suggests it is appropriate for such an investigation.

A study of the gene homologies of the chromosome elements in the genus Drosophila shows that certain groups of genes have remained in close association for a long period of time in the history of the evolution of these species (Sturtevant and Novitski, 1941). One chromosomal element rarely broken up by inversions is the yellow (y) scute (sc) region; this section exists intact in species of both the subgenera studied by Sturtevant and Novitski (1941). In melanogaster this region also includes achaete (ac) and occupies the distal tip of the X chromosome at map distance 0.0. It has been investigated intensively both cytologically and genetically. Dubinin (1932) in his classic experiments on step-allelomorphisms demonstrated a functional-linkage relationship between ac and sc. He found that trans combinations of several ac

and sc alleles failed to complement one another in the production of a wild phenotype. Green (1961) has shown that y^{c4}/y^{2} is wild type; however, $y^{c4}/y^{2}sc$ is like y^{2}. Further investigation conclusively demonstrated that sc interacted with y^{2} in preventing the complementation observed between y^{c4} and y^{2}. This evidence suggests that y, ac, and sc all belong to a single structural-functional unit. See Table 1 and Figure 1.

A series of deletions of this region including the genes chlorotic, 1(1)J-1, y, ac, and sc have been studied and are found to give phenotypes ranging from those that are essentially normal to those which are embryonic lethal (Demerec and Hoover, 1936). One of these small deletions, deficiency 260-2 (Df(1)260-2) is missing the distal-most eight bands of the X-chromosome including y and ac but not sc (Kaliss, 1939). Male embryos hemizygous for this deficiency fail to hatch, even though they appear to develop normally. Poulson (1940) has shown that the semi-lethal scute-B also produces a class of fully-formed larvae which fail to hatch.

The gene deep orange (dor) is closely linked to the region of the chromosome represented by Df(1)260-2. Counce (1956) reported earlier that dor male zygotes from the cross dor x dor die later than dor female zygotes from this cross. (To see how parent dor flies are obtained see page 23). This investigation was originally designed to repeat these observations and investigate the effect of extra heterochromatin on the development of dor embryos. Preliminary observations

resulted in the discovery of a class of lethals very much resembling those of the Df(1)260-2 syndrome. The proximity of dor (0.3 on the X chromosome) to Df(1)260-2 suggested that a functional-linkage relationship might be the basis for this similarity in phenotypic expression (Nash, 1966).

The present study is designed to test whether there is a functional-linkage relationship between the gene loci represented in the region corresponding to Df(1)260-2 and the closely located deep orange (dor) gene and whether the lethality produced by the interaction of dor with rosy (ry) as described by Lucchesi (1963) is also like the Df(1)260-2 syndrome.

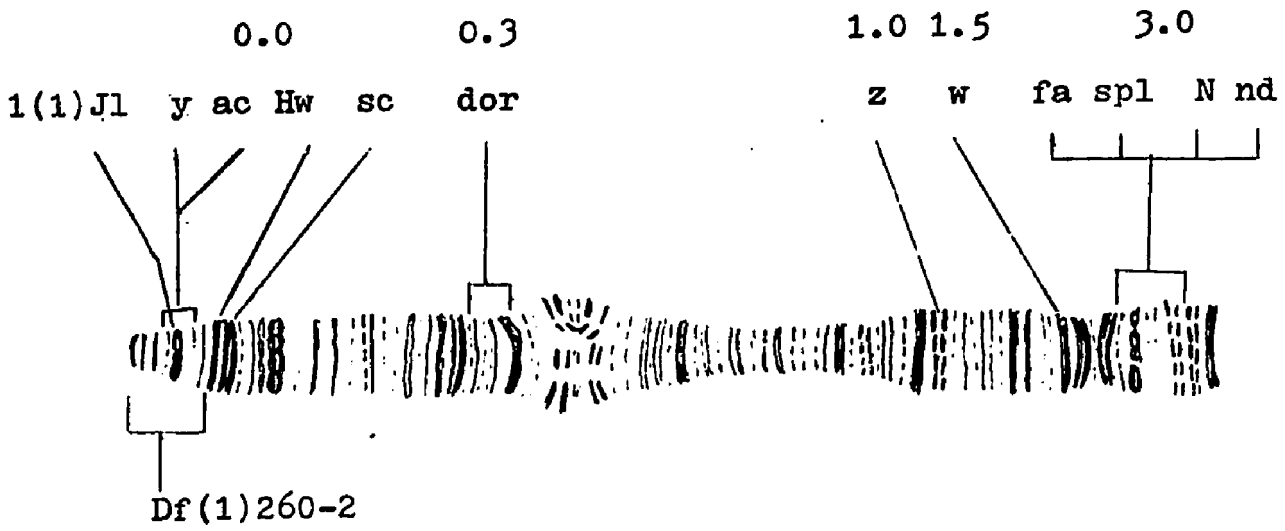
TABLE 1

COMPLEMENTATION PROPERTIES OF SEVERAL *y*, *ac*, and *sc* ALLELES

Alleles	Phenotype	Reference
ac^2/sc^3	mutant	Dubinin (1932)
ac^2/sc^2	wild type	Dubinin (1932)
y^2/y^2	mutant	Green (1961)
y^2/yc^4	wild type	Green (1961)
y^2sc/yc^4	mutant	Green (1961)

The genes *y*, *ac*, and *sc* are all very tightly linked, mapping at 0.0 on the X chromosome. Some alleles of *ac* and *sc* fail to complement in the *trans* configuration indicating that the *ac* and *sc* genes belong to the same complementation group. In the *cis*-configuration *sc* alters the complementation properties of y^2 such that it no longer complements with y^{c4} to produce a wild phenotype.

Figure 1. Distal Tip of the X-Chromosome (Modified from Genetic Variations of Drosophila melanogaster).



Key

Df(1)260-2	missing all of bands 1A
1(1)7e*	specific modifier of dor
1(1)J1	lethal (1) of Jacobs Muller
y	yellow - body color mutant
ac	achaete - bristle mutant
Hw	Hairy wing - wing mutant
sc	scute - bristle mutant
dor	deep orange - eye color mutant, female sterile
z	zeste - eye color mutant
w	white - eye color mutant
fa	facet - eyes rough
spl	split - eyes small, rough
N	Notch - wing mutant
nd	notchoid - wing mutant

* Not cytogenetically located - genetically maps at 0.

METHODS AND MATERIALS

Hatching and Progeny Survival

All stocks were maintained on live yeasted, standard cornmeal molasses food and kept at 25°C. Paper spoons with cream of wheat-molasses food were used in the hatching experiments and again the temperature was kept at 25°C. The procedure used to assay hatching and progeny survival was carefully repeated each time. About 10 virgin females and 10 newly emerged males were placed in a pint bottle with honey yeasted cream of wheat spoons. Honey yeast was used to encourage maximum egg laying. When dor flies were used as parental females three or four replicate bottles were run simultaneously, to insure including a representative sample of the population. Replicate testing suggested that between 20 and 40 dor females were required to obtain statistically reproducible results. The spoons were changed every 24 hours, and upon commencement of egg laying were transferred to bottles containing no flies. From any given bottle of flies, eggs were collected only for five successive days.

It was found that 24 hours after the eggs had been laid, any that had not hatched failed to do so even if watched for an additional 24 hours. Therefore, the eggs laid in a 24-hour period were set aside for 24 more hours before scoring hatching.

All unhatched eggs were picked off the spoons with a dissecting needle and placed in a dilute clorox solution.

This removed the opaque chorion; and made it possible to look through the transparent vitelline membrane to determine the approximate developmental stage of the unhatched embryo. Individuals that hatched were transferred to yeast cornmeal bottles and allowed to continue development to the adult stage. Thus pre- and post-embryonic lethality could be recorded and quantified.

Synthesis of Genetically Marked XY-Chromosomes

Stocks used to synthesize genetically marked attached-XY chromosomes were obtained from The University of Chicago and The University of Michigan. In describing these stocks only the genetic constitution of the attached-XY chromosome will be given since no autosomal markers were present in any of the stocks.

In each case, the desired mutant markers were moved to an appropriate attached-XY chromosome by the same kind of stepwise procedure. Females with the desired marker(s) and others to determine necessary cross-over points were crossed to males carrying the attached-XY chromosome. F_1 females from this cross were heterozygous for the X-chromosome carrying only markers and the attached-XY chromosome, to which the desired markers were to be moved by crossing over. These F_1 females were crossed to Ore-R males, and the F_2 males by virtue of the markers they carried could be classified on genetic grounds as being either X/Y or XY/Y. The presence of the attached-XY chromosome was also confirmed cytologically, by looking at neuroblast preparations stained

with aceto-orcein. Figure 2 and 3 illustrate the procedure used to recover an appropriately marked attached-XY chromosome, and Figure 4 presents a neuroblast cell preparation showing an attached-XY chromosome. Sex linked, female sterility genes like dor are normally maintained with an attached-X or balanced stock. Therefore, all synthesized compound dor-Y chromosomes were maintained both ways. A similar genetic background was provided by using the same attached-X and FM-3 stock to maintain all compound dor-Y chromosomes. Also the dor gene used in these investigations was originally extracted from the same FM-4 stock.

Ovary Transplants

Reciprocal ovary transplants were performed between dor and Ore-R females. Dor ovaries were also transplanted into carnation (car) and rosy (ry) females.

The special apparatus used for Drosophila tissue transplants was developed by T. M. Rizki (unpublished). The Drosophila Ringer's in which larva were dissected was prepared according to the formula originally prepared by Rizki (1965).

Late 3rd instar larvae were used both as donors and recipients in these ovary transplantation studies. The recipient larvae were first washed in Ringer's and then placed in 80% EtOH for a minute or so to reduce the hazard of infection. The larvae were removed from the EtOH and blotted dry on paper toweling. Then they were placed dorsal side up on a microscope slide covered with "double sticky Scotch" tape. Usually, ten larvae at a time were readied in this way

Figure 2. Series of crosses utilized to show how an attached-XY chromosome with a specific mutant marker, in this case deep orange, is synthesized from an attached XY chromosome carrying the markers vermilion (v), forked (f) and Bar (B). X's between chromosomes indicate cross-over points.

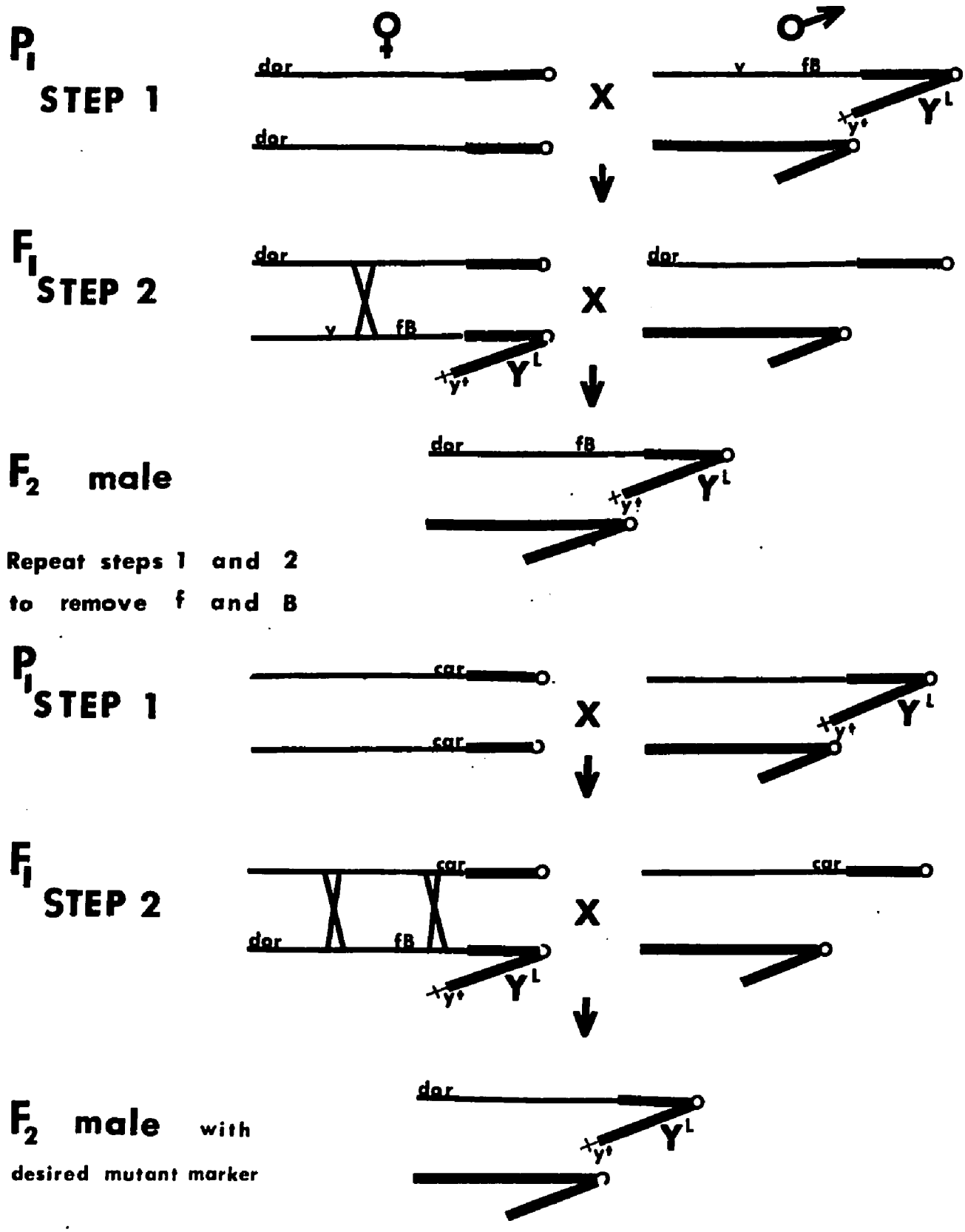

P1
STEP 1
dor
dor
X
y
fB
Y^L
+y+
F1
STEP 2
dor
y
fB
+y+
Y^L
X
dor
F2 male
dor
fB
+y+
Y^L
Repeat steps 1 and 2
to remove f and B
P1
STEP 1
car
car
X
+y+
Y^L
F1
STEP 2
car
dor
fB
+y+
Y^L
X
car
F2 male with
desired mutant marker
dor
+y+
Y^L

Figure 3. The attached-XY chromosomes on the left exist in balanced stocks obtained from the University of Chicago and The University of Michigan. The attached-XY chromosomes on the right were synthesized from these on the left by the method illustrated in Figure 1. The $\underline{y^+}$ symbol at the tip of the $\underline{Y^L}$ and $\underline{Y^S}$ chromosome fragment represents a terminal X duplication shown to include the genes yellow and achaete but not scute (see text).

Attached XY - Chromosomes From Stock Culture

Synthesized Attached XY- Chromosomes

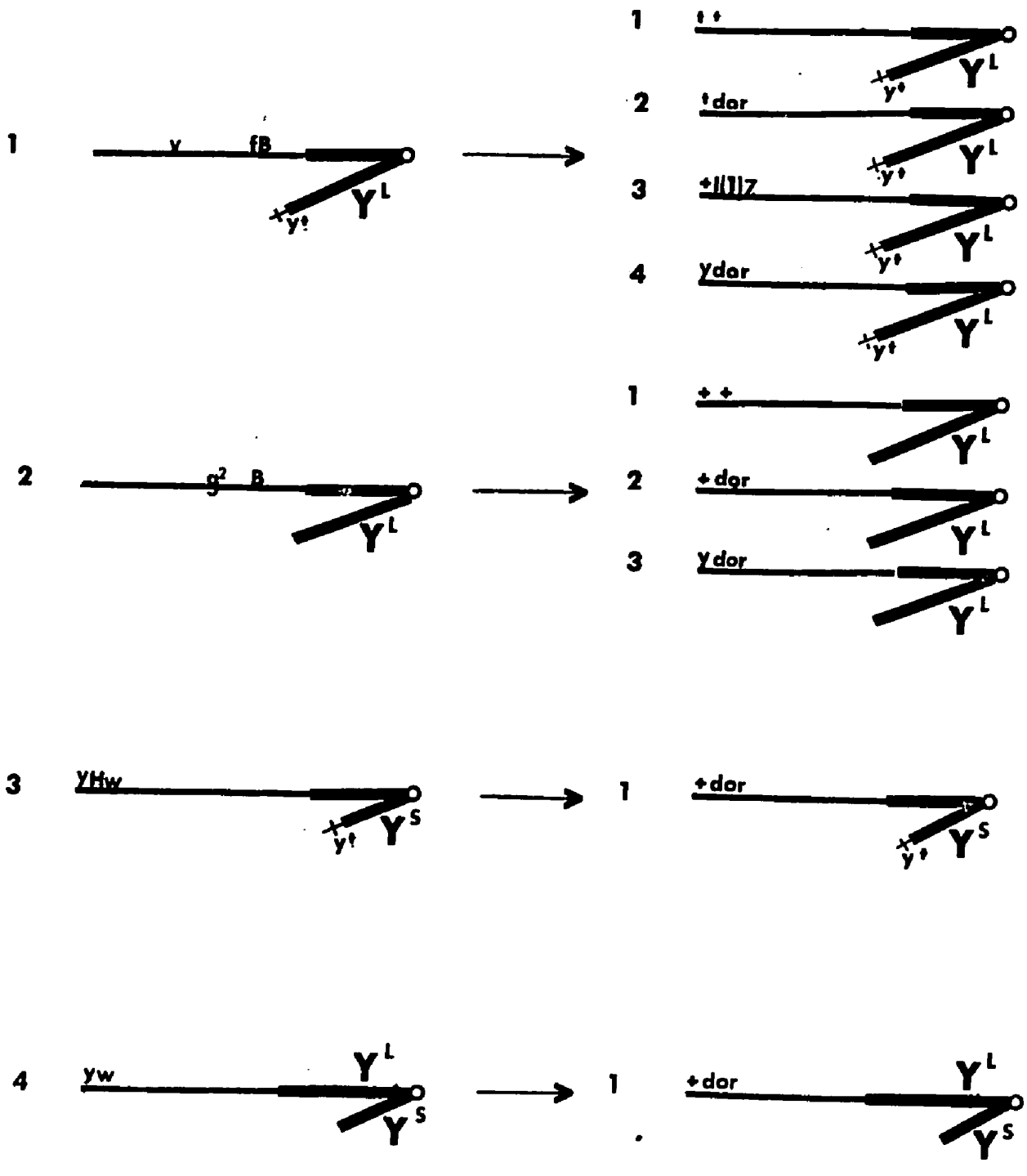
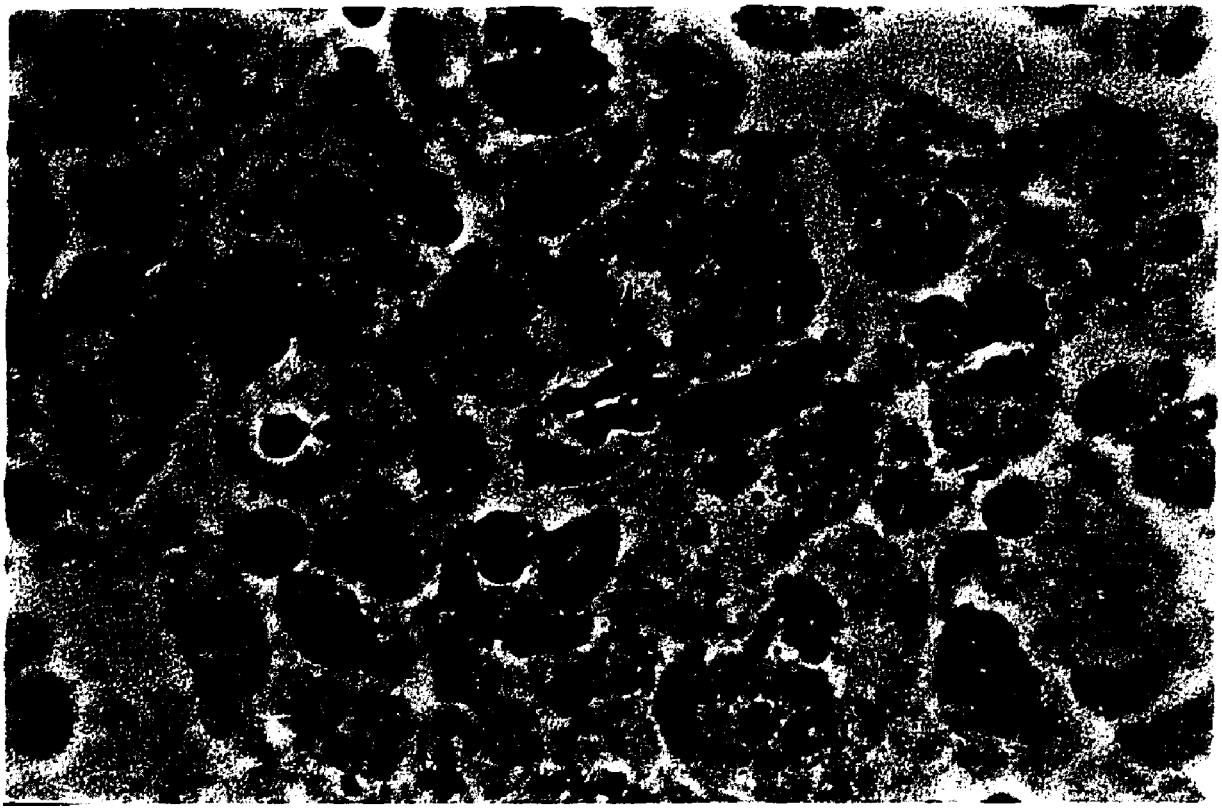


Figure 4. Third instar neuroblast stained with aceto-orcein from the brain of a homozygous dor 3rd instar larva. The arrows point to the centromere regions of the two X-chromosomes. The X-chromosome to the right has the long arm of the Y-chromosome attached at the centromere (X2600).



for transplantation. The donor larvae were operated on one at a time in a drop of Ringer's solution. The two ovaries were each transplanted to female host larvae before a second larva was operated on.

The puncture made by the transplant needle was always just above the left ovary of the host larva. When the needle was removed, after injection of the ovary, hemolymph from the wound usually was adequate to release the larvae from the tape. The larvae were then immediately placed in a non-yeasted bottle with cornmeal molasses food, wet only with distilled water. This method insured about 70% adult survival.

Adult survivors were single pair mated to determine if the transplanted ovary was functional. The presence or absence of a yellow mouth part marker expressed in the hatching progeny, signalled the success or failure of a functional ovary transplant. After five days of egg laying each female was sacrificed to determine the condition of her ovaries.

EXPERIMENTAL PROCEDURES

Deep orange is one of several sex-linked female sterility genes found in Drosophila melanogaster. The types of inheritance shown by these genes, illustrated by a sex-linked sterility gene m, is as follows:

Case I $m/m♀ \times m/Y♂$
$m/m♀$'s die $m/Y♂$'s die (as embryo's)

Case II $m/m♀ \times +/Y♂$
$m/+♀$'s some live all $m/Y♂$'s die

Case III $m/+♀ \times m/Y♂$
$m/m♀$; $m/+♀$, $m/Y♂$; $+/Y♂$, all live

The above mode of inheritance may be explained if we assume that eggs produced by mutant mothers are in some way maternally conditioned to die as developing embryos. To allow for the fact that some female zygotes survive in Case II, we may further postulate that the normal allele of dor upon entering the egg at fertilization reverses in some cases the maternally conditioned lethality. Since the female parent in Case III carries a normal allele of dor, her eggs are all perfectly viable and therefore from this cross all her progeny live.

Counce (1956) reported that male zygotes from the cross dor x dor die later in embryonic development than female zygotes from the same cross. It seemed possible that this

might be another case of a heterochromatic effect, where the greater amount of heterochromatin in the male genome delayed the onset of lethality. To test this hypothesis, attached dor·XY^L chromosomes were synthesized (see Methods and Materials). Females with the genotype dor·XY^Ly^+/ dor·XY^Ly^+ were crossed to Ore-R males (see Case II above). This cross was chosen for the following reasons: (1) the reported developmental stage reached by male embryos in Case I and Case II was the same (Counce, 1956); (2) from the cross a certain percentage of female zygotes survived and therefore one could test the effect of extra heterochromatin on two kinds of mortalities, those which resulted from the conditioned lethality passed on maternally to all male zygotes, and those which resulted from the failure of the paternally derived normal dor allele to reverse the lethality in the female zygotes.

RESULTS

Effect of Extra Heterochromatin on the Development of Male Zygotes from the Cross dor x Ore-R

Preliminary experiments (Table 2) showed that the number of male zygotes that reached an advanced stage of development decreased with increasing amounts of extra heterochromatin. The percentages under the heading "Undeveloped" in Table 1 refer to eggs that have no demonstrable nuclei detectable by aceto-orcein staining. Percentages under the heading "developed" include eggs that reached an advanced stage of embryonic development as well as those continuing beyond hatching, i.e., female zygotes. Some female zygotes are included in the "undeveloped" column, but the majority are male zygotes. This can most easily be seen by comparing row 1 with row 4. In row 4, 33% of the "developed" class are known to be females. Thus even if the remaining 17% female zygotes (assuming a 1:1 sex ratio) are included under the "undeveloped" heading, this leaves 20% of the "undeveloped" zygotes which must be males. If the 10% of "undeveloped" zygotes in row 1 are all males, still row 4 has twice as many undeveloped males.

These results do not support the original hypothesis, but rather are consistent with the finding that extra heterochromatin generally lowered viability in all the stocks where it was present. It is not surprising that extra hetero-

chromatin had this effect even in deep orange flies. After the present study was underway Hildrith and Lucchesi (1966) demonstrated that Counce's (1956) original observation was incorrect. They found that the "early-early" lethals (classified as females) observed by Counce were probably unfertilized eggs and that "early-early" lethals did not exist typically in the dor stocks.

Developmental Arrest Among Female Zygotes from the Cross dor x Ore-R

The mortality in female zygotes with extra-heterochromatin followed the same general trend as that found for males. Fewer female zygotes from the cross dor·$Y^{L}y^{+}$/dor·$Y^{L}y^{+}$ x +/Y reached the adult stage than female zygotes from the cross dor/dor x +/Y. A more detailed inspection of the stages of developmental arrest in these female zygotes revealed that a certain percentage of them characteristically died just prior to hatching. Among female zygotes from dor·$Y^{L}y^{+}$/dor·$Y^{L}y^{+}$ mothers this class predominated over all other stages of lethality. Furthermore, the array of phenotypic anomalies characteristic of this class of embryos parallel to a remarkable degree the syndrome in hemizygous male zygotes carrying the short terminal X deficiency (Df(1)260-2). Both classes of zygotes at the time of normal hatching produce only weak, ineffective hatching movements. They fail to escape from the enclosing extraembryonic membranes. If hatched artificially, they show a marked tendency to dehydrate. The great tracheal trunks fail to fill with

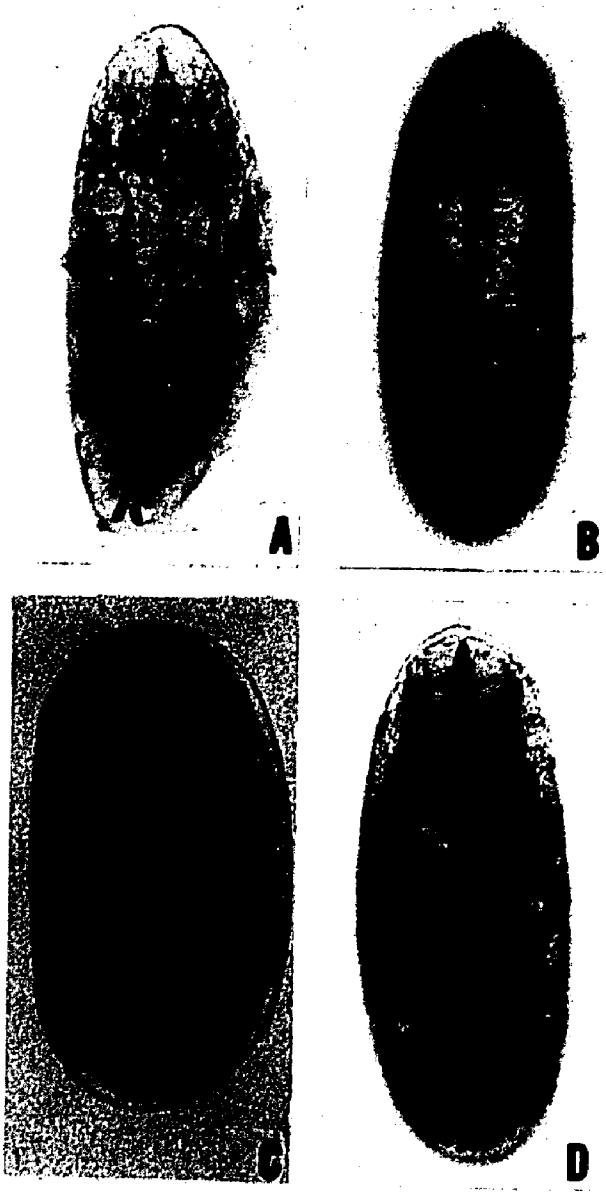
TABLE 2

EFFECT OF EXTRA HETEROCHROMATIN ON THE EMBRYONIC DEVELOPMENT OF MALE AND FEMALE ZYGOTES FROM THE CROSS dor x Ore-R

Maternal Genotype	Eggs	
	% Developed (% known to be females)	% Undeveloped
1. dor / dor	90 (38)	10
2. dor / dor Y^L	72 (41)	28
3. dor Y^L / dor Y^L	78 (34)	22
4. dor / dor S	63 (33)	37
5. dor S / dor S	38 (16)	62

The addition of increased amounts of extra heterochromatin can be seen to increase the number of undeveloped zygotes. Undeveloped eggs are defined as those with no detectable nuclei demonstrable 24 hours after being laid using aceto-orcein staining. The developed class include eggs showing an advanced stage of development determined by the presence of many nuclei and those which continue development to the hatching stage and beyond (these latter zygotes are known to be female zygotes).

Figure 5. Photograph of four late embryos enclosed within the vitelline membrane. (A) An Ore-R late embryo just prior to hatching. Notice the great treacheal trunks are completely filled with gas. (B-C-D) Heterozygous dor/+ female embryos from dor mothers demonstrating the range of abnormal gas filling of the tracheal trunks. These embryos are all 24+ hours old. The unfilled portions of the tracheal trunks appear to be morphologically normal.


A
B
C
D

gas but morphologically they appear quite normal. Artificially hatched male larvae carrying Df(1)260-2 fail to grow and usually die within a short period of time (Kaliss, 1939). A few female zygotes from the dor x Ore-R cross, when artificially harched, grow and become fertile adults. The survivors always result from individuals whose trachael trunks are partly filled with gas. The ability of these larvae to withstand dehydration appears to parallel the extent to which the tracheae are filled with gas, since individuals with no filling dehydrate immediately while those with some filling resist dehydration better. The inability of certain portions of the tracheal trunks to fill with gas is carried over from one larval instar stage to the next.

Because these late embryos normally fail to hatch they are abbreviated f^e (failure to eclode). Figure 5 illustrates the range of the f^e phenotype with respect to filling of the tracheal trunks with gas.

The Effect of the Small y^+ Duplication on the Appearance of f^e Zygotes

The design of the original experiments took into consideration only the effect of extra heterochromatin on zygote mortality. The small y^+ duplication appended to the end of the long arm of the Y chromosome was not included in the analysis. Upon discovering the f^e phenotype, it appeared desirable to re-evaluate the importance of this small duplication since it includes those genes which are deleted with

TABLE 3

GENOTYPICALLY DIFFERENT FEMALES CROSSED TO Ore-R MALES AND THEIR CHARACTERISTIC PROGENIES

Maternal Genotype	Total Number of Eggs	Mortality Expressed as a Fraction of the Total Observed Eggs			Fraction Adults
		Failed to Complete Embryonic Development	Failed to Eclode as Larvae	Post Embryonic Mortality	
1. Ore-R / Ore-R	546	0.05	---	0.06	0.89
2. Ore-R / Y^L / Ore-R / Y^L	872	0.08	---	0.11	0.81
3. Ore-R / Y^L / Ore-R / Y^L	1120	0.10	---	0.10	0.80
4. dor / dor	2618	0.25	0.11	0.21	0.43
5. dor / Y^L / dor / Y^L	1934	0.24	0.08	0.41	0.27
6. dor / Y^L / dor / Y^L	1904	0.32	0.46	0.18	0.04

The percentages shown in rows 4-6 represent only female zygotes.
X^2 values and associated probabilities of comparison between rows.
Row 2-3 df = 3, X^2 = 4.03, $p > 0.05$ not significantly different
Row 1-(2+3) df = 3, X^2 = 37.2, $p < 0.0001$
Row 4-5-6 df = 6, X^2 = 1989, $p < 0.0001$

Df(1)260-2. New experiments were designed to determine the separate effects on mortality of both the extra long arm of the Y chromosome and the y^+ duplication. The results and χ^2 values for different sets of data are presented in Table 3.

A comparison of the progeny from Ore-R and dor mothers with and without the extra long arm of the Y chromosome (rows 1 and 2, and 4 and 5) demonstrates the generalized detrimental effect extra heterochromatin has on all states of development.

A comparison of the progeny from Ore-R and dor mothers with just the extra Y^L and with the Y^L plus the y^+ show the separate effects of the Y^L and y^+ chromosome fragments. In Ore-R zygotes the y^+ duplication has no effect. Neither extra heterochromatin nor the y^+ duplication have the effect of stopping development in Ore-R zygotes at the f^e stage. When the y^+ duplication is present in parent dor females (row 6) the numbers of female f^e zygotes resulting is about four times as large as when it is absent. Extra heterochromatin alone (row 4 and 5) in parent dor females does not affect the frequency of f^e zygotes. Among these zygotes extra heterochromatin preferentially increases post-embryonic mortality. Chi square tests demonstrate that all of the above comparisons are statistically significant. The probability of these inferences being in error is small ($p < 0.01$).

The results in Table 3 do not distinguish whether the y^+ duplication acts only maternally during oogenesis, or

after fertilization in the F_1 zygote. The other possibility is that it acts at both stages of the life cycle. These postulates can be tested by comparing F_1 zygotes which receive the dor·$Y^L y^+$ chromosome maternally to those which receive it paternally. Crosses representing these two cases respectively are: dor·$Y^L y^+$/dor·$Y^L y^+$ x Ore-R and Ore-R x dor·$Y^L y^+$/Y^+. In both cases female F_1 zygotes will carry one normal chromosome and one dor·$Y^L y^+$ chromosome. Forty-six percent of the female zygotes stop at the f^e stage of development when dor·$Y^L y^+$ is received maternally. No F_1 female zygotes stop at the f^e stage when dor·$Y^L y^+$ is received paternally at the time of fertilization. One can conclude that the influence of the y^+ duplication on the production of f^e phenotypes is maternally inherited and not affected by its presence in the F_1 zygote.

Since it is the added y^+ duplication in dor females which specifically increases the number of f^e phenotypes in the F_1 zygotes, it seemed desirable to determine the genetic boundaries of this chromosome fragment. This parameter can be determined by testing whether or not the y^+ duplication masks the phenotypic effects of mutant genes just proximal to the y gene. Achaete (ac) and scute (sc) are two such genes which, along with y, map at 0.0 on the X chromosome. The following compound chromosomes were synthesized: ac·$Y^L y^+$ and sc·$Y^L y^+$. Males hemizygous for these chromosomes should be mutant if the y^+ duplication does not genetically cover ac and sc, and wild type if it does. The combination ac·$Y^L y^+$

is wild type and sc.YLy+ shows the sc phenotype. Therefore the y+ duplication includes y and ac but not sc. This is interesting because Df(1)260-2, which gives rise to an "fe"-like phenotype is missing the distal-most eight bands of the X chromosome and includes y and ac but not sc (Kaliss, 1939). Genetic tests, therefore, indicate that Df(1)260-2 and the y+ duplication are co-extensive.

Effect of Genetic Background on the Interaction Between dor and the y+ Duplication

The data at hand suggest that the y+ duplication acts maternally in dor female parents (it is to be understood that all dor female parents are crossed to Ore-R males) to arrest development of the F_1 female zygotes at the f^e stage. The influence of the residual genetic background has not been considered. Every attempt has been made in the mating scheme to keep the genetic backgrounds as nearly alike as possible, but because a pure homozygous dor stock can not be maintained, it is virtually impossible to get isogenic dor females. One would like to know how much of the effect being measured is a result of a direct interaction between the y+ duplication and dor, and how much is contributed by the residual genetic background. Short of using isogenic stocks one can determine this by purposely varying the genetic background. If the parameter being measured is independent of the genetic background it should remain constant when these manipulations are made.

TABLE 4

dor FEMALES WITH NO OR TWO EXTRA y^+ DUPLICATIONS CROSSED TO Ore-R MALES AND THEIR CHARACTERISTIC PROGENIES

Maternal Genotype	Total Number of Eggs	Mortality Expressed as a Fraction of the Total Observed Eggs: Failed to Complete Embryonic Development	Failed to Eclode as Larvae	Post Embryonic Mortality	Fraction Adults
1	2	3	4	5	6
* 1. dor ___. dor ___.	2618	0.25	0.11	0.21	0.43
FM-4 2. dor ___ $\cdot$ L $\times y^+Y$ dor ___ $\cdot$ L $\times y^+Y$	1904	0.32	0.46	0.18	0.04
3. dor ___. dor ___.	862	0.47	0.18	0.19	0.16
FM-3 4. dor ___ $\cdot$ L $\times y^+Y$ dor ___ $\cdot$ L $\times y^+Y$	1312	0.20	0.43	0.27	0.10
* 5. dor ___. dor ___.	2618	0.25	0.11	0.21	0.43
FM-4 6. dor ___ S $\times y^+Y$ dor ___ S $\times y^+Y$	1987	0.21	0.47	0.22	0.10

* Duplicates

X^2 values and associated probability of comparison between rows of progeny failing to complete embryonic development (Rows 1 & 3) and progeny failing to eclode as larvae (Rows 2 & 4 & 6)

Rows 1 & 3 d.f. = $X^2 = 33.9$ $p < 0.0001$

Rows 2 & 4 & 6, d.f. = $X^2 = 2.8$ $p > 0.05$ not significantly different

Performances of the dor females with diverse genetic backgrounds may be seen in Table 4. Each female genotype, with the exception of duplicates 1 and 5 has several sources of variability. All the flies with attached-XY chromosomes required a number of out-crossings for their synthesis (see Methods and Materials). The dor females come from two different stock cultures which have been maintained separately for many years. The different attached-XY chromosomes used for synthesis come from genetically diverse stocks. Finally, since it is primarily the effect of y^+ duplication that is being tested, the extra pieces of heterochromatin themselves can be considered part of the residual genetic background.

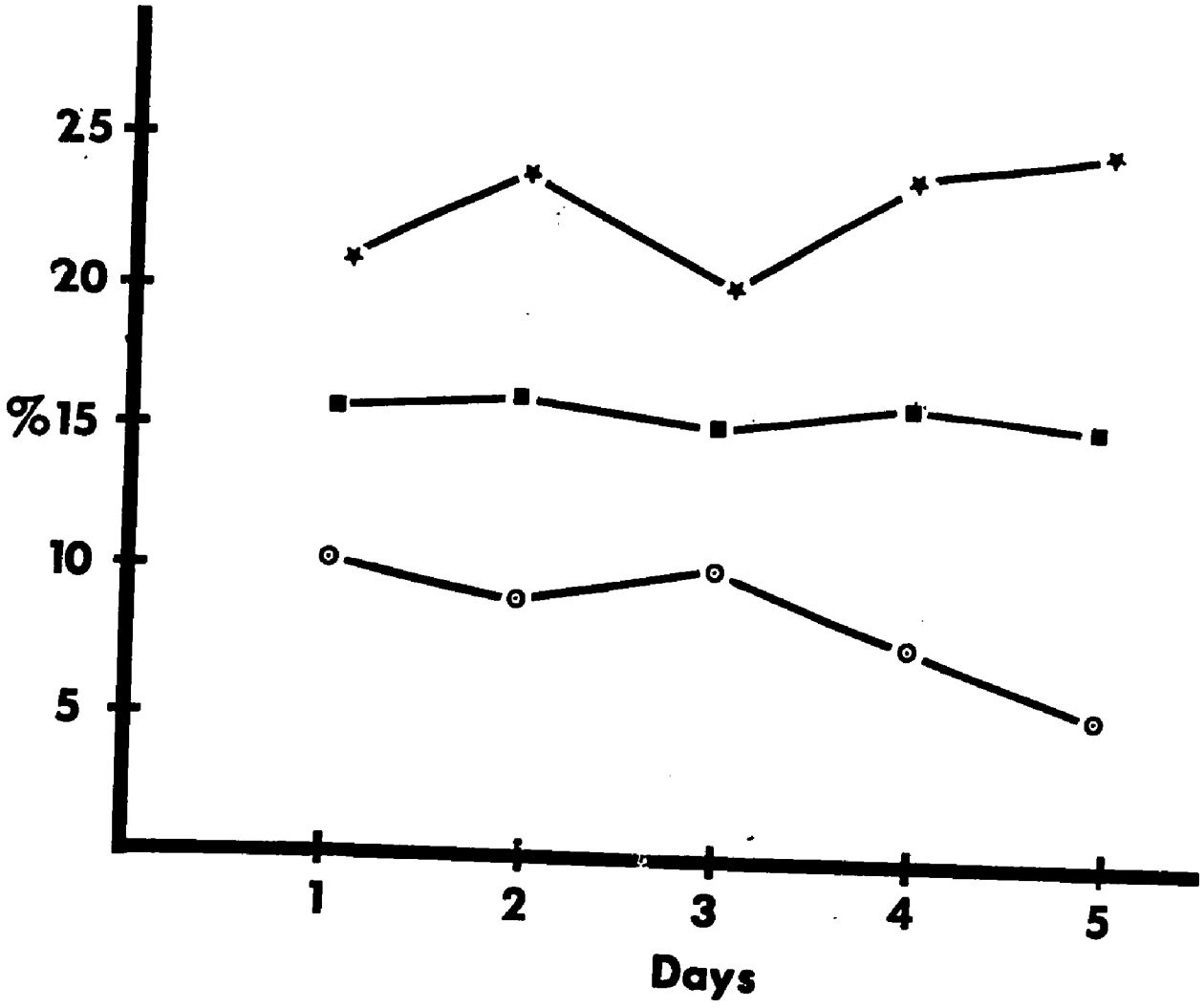
The data in rows 1 and 3, column 3 demonstrate that embryonic mortality in zygotes from dor mothers, varies significantly with the genetic background. The data in rows 2, 4, and 6 of the same column demonstrate that neither extra heterochromatin nor the y^+ duplication affects embryonic mortality in a predictable way. The data in rows 1 and 3 of column 4 demonstrate that the zygotes from dor mothers that stop development at the f^e stage, in the absence of extra heterochromatin and the y^+ duplication varies significantly with different genetic backgrounds, but is low (1/10 to 1/5 of female zygotes). The data in rows 2, 4 and 5 of the same column demonstrate that this same class of zygotes (f^e class) from dor mothers with extra heterochromatin and the y^+ duplication makes up almost half of all zygote mortality. This result is independent of the genetic background. In all cases where two doses of the y^+ duplication are present

in the dor mother the number of f^e individuals comprise almost half of all her F_1 female zygotes. In the only case where extra heterochromatin alone (no y^+ duplication) was present in the dor mother (Table 3) it did not increase the percentage of f^e individuals among the F_1 female zygotes. The conclusion can be drawn that extra heterochromatin is not directly involved in the interaction between dor and the y^+ duplication leading to an increased percentage of f^e zygotes. This conclusion is further supported by the fact that there is no significant difference in the percentage of f^e zygotes observed when either Y^L or Y^S is used to carry the y^+ duplication.

The evidence above suggests that the percentage of female zygotes from dor mothers that reach the f^e stage of development is determined largely by the genetic background and not by the presence of the y^+ duplication or extra heterochromatin. However, in any given maternal background the interaction between dor and the y^+ duplication leads to a constant and predictable number of zygotes that stop at the f^e stage of development. The relationship between the number of zygotes that reach the f^e stage of development and those which continue development beyond this point can be more precisely determined by plotting the daily mortality and hatching data for these zygotes. Figure 6 is a representative example. The center set of points represents one-half of the total percentage of female zygotes from dor mothers that reach the f^e stage of development. Over the 5-day period investigated

Figure 6. The relationship between the number of zygotes from dor mothers that reach the f^e stage of development and those which stop at this stage. Although the absolute percentages vary depending on the dor female genotype being tested, the general relationship below is always observed. The total number of zygotes reaching the f^e stage (middle line below) is essentially constant over the five day period observed.

dor·Y^{L}/dor·Y^{L} X Ore-R



* = % of female zygotes that hatch

⊙ = % of female zygotes with $\underline{f}^{e}$ phenotye

■ = ½% of * + ⊙

the percentage of zygotes reaching this stage remains constant. There is a fluctuation in the daily percentages of zygotes which reach the $\underline{f^e}$ stage and stop, and those which hatch and continue larval development. This fluctuation is not random; on any given day if the percentage of $\underline{f^e}$ phenotypes is low the percentage of hatching is comparatively high and vice-versa. Also it may be seen in Figure 6 that as the parent dor female ages, fewer F_1 female zygotes stop development at the $\underline{f^e}$ stage. This is not a consistent trend observed among the progeny of dor females with varying genetic backgrounds.

Dosage Effect of Extra y^+ Duplication on the Occurrence of f^e Phenotypes Among the Progeny of dor Mothers

Only the progeny of dor mothers with either no or two $\underline{y^+}$ duplications have been compared. In order to determine the effect of different doses of the $\underline{y^+}$ duplication on the percentage of $\underline{f^e}$ phenotypes observed, mothers heterozygous for the $\underline{y^+}$ duplication must be synthesized. The synthesis of these stocks introduces a residual genetic heterogeneity that can not be avoided. It seems reasonable to assume, however, that the residual background diversity is randomly distributed within the different genotypes. Three dor stocks individually maintained for many years are shown in Table 5. Each of these three stocks was used to obtain dor mothers with either 0, 1 or 2 $\underline{y^+}$ duplications, yielding a matrix of nine different genotypes. Only the three dor mother genotypes with no $\underline{y^+}$ duplications present can be legitimately

TABLE 5

THE DOSAGE EFFECT OF y^+ DUPLICATIONS IN THE dor MATERNAL GENOTYPE ON THE PERCENTAGE OF f^e ZYGOTES FOUND AMONG HER PROGENY

The numbers of y^+ duplications is varied from zero to two in three different stock backgrounds. Stock 3 is a combination of stocks Fm-4 and Fm-6.

	0	1	2
Stock 1 FM-4	12	14	46
Stock 2 FM-3	18	33	43
Stock 3 FM-6 FM-4	11	17	45

Number of y^+ duplications present in the dor female genotype.

Analysis of Variance

F value between treatments (i.e., different doses of y^+ duplication with 2/4 d.f. = 22.7 $p < 0.01$.

F value between stocks (genotypes) with 2/4 d.f. = 1.51 $p > 0.05$ not significantly different.

Duncan's Multiple Range Test

Rank	1		2
Treatment mean values	13.7	21.3	44.6

Bar indicates treatment means which are not significantly different at 0.05 level. The interval between two and three treatment means necessary to establish a significant difference at the 0.05 level is 17.05 and 19.92 respectively.

compared with respect to stock differences. The other six genotypes due to the nature of their synthesis are mixtures of the three stock genotypes, an attached-X stock and an Ore-R stock. Therefore, we are not really testing the effect of stock differences on the interaction between the y^+ duplication and dor but rather the dosage response of the y^+ duplication in dor flies with essentially random backgrounds. A two way analysis of variance reveals that at the 1% level, the difference between treatments, i.e., different doses of y^+ duplication, is significant. At the 5% level of significance there is no detectable difference between stocks (genotypes). Applying Duncan's multiple range test to the data allows one to see which differences among the treatments are significant (Duncan, 1955). At the 5% level there is no significant difference between the means of the two treatments represented by zero and one y^+ duplication present in the dor female genotype. There is a significant difference between the means of treatments represented by two y^+ duplications and either one or zero y^+ duplications.

Evidence for a Structural Relationship Between the Df(1)260-2 Region and dor

The first gene known to be common to both Df(1)260-2 and the y^+ duplication was the y gene itself. Therefore, the y gene was selected for manipulation in dor mothers to test for a structural relationship between a gene (y) in the

Df(1)260-2 region and dor. At its normal locus on the chromosome the y gene can occur in either the wild or mutant allelic state. Independent of its allelic state at the normal locus its wild type allele can be either absent or present as part of a duplication appended to the long arm of the Y chromosome. Thus one can independently substitute the allelic state and position of the y gene in dor mothers.

The presence of the y mutant allele in dor mothers has a pronounced deleterious effect on zygote viability among her progeny as may be seen in Table 6, row 1. More than three-fourths of all female zygotes die prior to the completion of embryonic development. Only one percent of her progeny survives to the adult stage. Note that adult survival is consistently low among the progeny of all y dor mothers, even those which are phenotypically normal with respect to body color due to the presence of the wild type y allele in the y^+ duplication. Since the residual genetic background is diverse in the different y dor mothers shown in Table 6, it seems likely that the low adult survival is a property of the y dor mutant combination itself. A marked improvement in embryonic development occurs among the progeny from y dor mothers with one or two doses of extra heterochromatin. This will be discussed later.

Rows 2 and 3 of Table 6 may be compared with rows 4 and 5 to determine if the interaction between dor and the y^+ duplication is altered by the presence of y in the dor mother's genotype. The y dor mothers in rows 2 and 3 differ

TABLE 6

ydor FEMALES CROSSED TO *Ore-R* MALES AND THEIR CHARACTERISTIC PROGENIES

	Maternal Genotype	Total Number of Eggs	Mortality Expressed as a Fraction of the Total Observed Eggs			Fraction Adults
			Failed to Complete Embryonic Development	Failed to Eclode as Larvae	Post Embryonic Mortality	
1.	$\frac{ydor}{ydor}$	1016	0.78	0.14	0.07	0.01
2.	$\frac{ydor}{ydor}$ $\times y^+Y^L$	3770	0.25	0.34	0.27	0.13
3.	$\frac{ydor}{ydor}$ $\times y^+Y^L$ $\times y^+Y^L$	1560	0.32	0.40	0.23	0.05
4.	$\frac{ydor}{ydor}$ $/Y^L$	2212	0.31	0.25	0.34	0.10
5.	$\frac{ydor}{ydor}$ $/Y^L$ $/Y^L$	2440	0.31	0.19	0.42	0.08

X^2 and associated probability of combined progeny in rows 2 and 3 as compared with combined progeny in rows 4 and 5, which failed to eclode as larvae.

$$d.f. = 1,\ X^2 = 139.4 \qquad p < 0.0001$$

from those in rows 4 and 5 only by the presence of the y^+ duplication. The combined percentages of f^e zygotes among the progeny of y dor mothers with and without the y^+ duplications are respectively 74 and 44 percent. One may conclude that the y mutant gene in dor mothers does not qualitatively alter the interaction between dor and the y^+ duplication.

Position Effect of the Yellow Alleles on the dor-y^+ Duplication Interaction

Although the percentage of the f^e phenotype is relatively high among the progeny of y dor mothers with extra heterochromatin (rows 4 and 5), no cause and effect relationship is indicated since a higher percentage of f^e phenotypes occurs when one rather than two doses of extra heterochromatin is present in the mothers.

The single most distinguishing characteristic of the progeny of y dor mothers is their low adult survival. This feature indicates a specific interaction between y and dor, but not necessarily at the structural level of organization of the chromosome. The hypothesis of a structural relationship between y and dor may be tested in the following way. Two special attached dor·Y^L chromosomes are synthesized in such a way that one has the wild type allele of y in its normal position and the other has this allele in the y^+ duplication. These chromosome types may be seen in rows 1 and 2 of Table 7. One can now ask whether these two chromosomal types are functionally equivalent in homozygous dor females.

If not, what is the effect of having the wild type allele of *y* in the y^+ duplication rather than at its normal position next to *dor*? It may be seen in rows 1 and 2 of Table 7 that having the wild type allele of *y* in the y^+ duplication in *dor* mothers, has two effects on the zygotes among her progeny. First the fraction of adult survivors is lowered, and second, the percentage of f^e phenotypes is doubled. Note that it is not the absence of the y^+ duplication itself in the *dor* mothers in row 2, Table 7, which increases adult survival among her progeny, since including the y^+ duplication in her genotype (row 3, Table 7) does not change the result. This evidence supports the model that *y* and *dor* are not only functionally related but that their normal functioning depends in part on their structural proximity.

Added Note of Supporting Evidence

A fine structure analysis of the *y* gene shows that most *y* mutants map in two regions called y^1 and y^2 (Green, 1961). Mutant y^1 is to the left of y^2 and mutants in this region usually result in flies with a yellow body color as well as yellow hairs, bristles and wings. Mutants of the y^2 region are usually characterized by a yellow body color with various degrees of darkening of the bristles and wings toward the wild type condition.

The *y* allele used in these investigations was a y^1 mutant. In one of the *y dor* stocks a male was found with black bristles and dark grey wings, almost as dark as in

TABLE 7

GENOTYPICALLY DIFFERENT FEMALES CROSSED TO Ore-R MALES AND THEIR CHARACTERISTIC PROGENIES

Maternal Genotype	Total Number of Eggs	Mortality Expressed as a Fraction of the Total Observed Eggs			Fraction Adults
		Failed to Complete Embryonic Development	Failed to Eclode as Larvae	Post Embryonic Mortality	
1. ydor / ydor $\times y^{+}Y^{L}$	3770	0.25	0.34	0.27	0.13
2. ydor / ydor $\times Y^{L}$	1811	0.24	0.17	0.29	0.30
3. ydor / ydor $\times y^{+}Y^{L}$	3859	0.21	0.18	0.34	0.27

X^2 and associated probability between rows 2 and 3

d.f. = 3, $X^2 = 5.20$ $p > 0.05$ not significantly different.

wild type flies. This male was first crossed to an attached-X female to multiply the new male's genotype. Further matings revealed that this new yellow phenotype was the result of a second spontaneous mutation in the y^2 region which could be separated from the y^1 mutation by crossing over. This new yellow mutant was designated y^N

It was discovered that $y^N dor/Y$ males homozygous for ry^2, reverted back toward the y^1 phenotype. The bristles of the $y^N dor/Y$; ry^2/ry^2 males are completely yellow and the wings are light yellow grey. By contrast y^N/Y; ry^2/ry^2 males with respect to the yellow phenotype are y^N, i.e., have black bristles and wings almost as dark as wild type flies. A direct interaction between dor and ry^2 in the production of the yellow bristle phenotype can be ruled out in that dor/Y; ry^2/ry^2 males have normal bristles. In the ry^2 background it is possible that the dor locus is interacting with the y locus directly along the chromosome to alter the y^N phenotype.

The Interaction of a Number of Eye Color Mutants With dor

The mutant car was used as a marker to determine cross over points on the X-chromosomes (see Fig. 2 Methods and Materials). An expected class of dor car males failed to appear among the adult progeny of these crosses. Further testing revealed that the dor car double mutant was a lethal combination.

Lucchesi (1963, 1968) in an attempt to ameliorate the effect of dor, combined this mutant, which accumulated

isoxanthopterin, (Counce 1957) with several eye color mutants known to be low or lacking in isoxanthopterin. He found that dor is lethal in combination with ry and semi-lethal with cinnabar brown (cn bw). In addition he found dor males to be sterile in combination with bw, sepia (se), and claret (ca). Lucchesi's results suggest that the amount of isoxanthopterin is not the critical factor in determining whether or not a mutant is lethal in combination with dor since ry and ma-1 both lack isoxanthopterine but only ry and car are similar in their lethal interaction with dor. Other phenotypically related eye color genes, i.e., ma-1, ca, pr, and garnet (g) were not lethal with dor. The eye color of a mutant was not a reliable indicator of its viability in combination with dor. Therefore, a wide spectrum of eye color mutants (shown at the bottom of Table 8) were combined with dor to determine their viabilities.

The combination of v bw is viable and male fertile with dor, whereas Lucchesi (1954, 1968) reports cn bw to be semi-lethal and bw males viable but sterile in combination with dor. Since cn bw and v bw flies both have white eyes yet behave differently, w and several pseudoalleles of w were combined with dor. All combinations were viable and male fertile.

The double mutant dor pr was unusual in several ways. The eye color of pr dor flies at 25°C was variable and ranged from ivory to yellow-orange. At 18°C the eye color ranged from ivory to deep orange-red. In this interaction the pr gene behaved as it it were a dominant since all eye colors

TABLE 8

THE EFFECT OF SEVERAL EYE COLOR MUTANTS IN COMBINATION WITH dor

Mutant	Symbol	Viability	Phenotype
*ry^1 $rosy^1$	ry^1	1	--
*ry^2 $rosy^2$	ry^2	1	--
*cinnabar	cn	v	--
*cinnabar, brown	cnbw	s.l.	--
*brown	bw	v.s	--
*sepia	se	v.s	--
*claret	ca	v.s	--
*maroon-like	ma-l	v	--
carnation	car	1	--
vermilion	v	v	light yellow
vermilion-brown	vbw	v	white
purple	pr	v	ivory--deep orange
raspberry	ras	v	deep orange
red Malpighian tubules	red	v+	light orange
garnet	g	v+	light orange
chocolate	cho	v+	light orange
lightoid	ltd	v+	light yellow
white	w	v	white
white honey	wh	v	white
white apricot	w^a	v	ivory
white eosine	w^e	v	light yellow
white coral	w^{co}	v	dilute red
white satsuma	w^{sat}	v	dilute red

* alleles tested by Lucchesi

+ male fertility not tested

KEY l = lethal
s.l. = semi lethal
v.s. = viable but sterile
v = viable and fertile

from ivory to yellow-orange and ivory to deep orange-red were observed in dor flies heterozygous for purple at 25° and 18°C respectively. Single pair matings of the general type yf:=;pr/pr x dor/Y;pr/pr were performed to see if the patroclinous male progeny of dor pr males would have the same eye color phenotypes as their father. Both dark and light eyed pr dor males gave rise to light, intermediate and dark eyed patroclinous males. This result suggests that dor is not the factor responsible for the variable eye color observed, since in passing patroclinously all the sex linked male genes from a given cross including dor should be identical.

Non-Allelic Double Mutant Interaction Between ry^2, car and dor

The phenotypically related eye color mutants ry^2 and car are characterized by dull red rather than bright red eyes. Car is located at 62.5 on the X-chromosome and ry^2 is located at 52.4 on the 3rd chromosome. The two lethal interactions between dor; ry and dor, car serve as a contrast to the interaction between dor and the y^+ duplication. The comparison is not direct in the sense that the y^+ duplication acts maternally in dor mothers to condition lethality among her heterozygous dor/+ female progeny. Yet one may consider the possibility that the lethal interaction in homozygous dor; ry^2 or dor car zygotes occurs in the embryo such that development stops at the f^e stage. A preliminary investigation of the hatchability of eggs from the following two crosses (dor/+;

$ry^2/+$♀ x +/Y; ry^2/ry^2♂ and dor/car♀ x car/Y♂) shows that hatching is normal and that no individuals stop at the f^e stage of development. One-fourth of the male zygotes from either cross should be lethal combinations and therefore since hatching is normal we may conclude that these lethal combinations die post-embryonically. These preliminary results indicate that the functional interaction between dor and the y^+ duplication is different from that between dor with either ry^2 or car.

A Study of the Specific Time and Syndrome of Developmental Arrest in dor; ry^2 Zygotes

In order to recognize dor; ry^2 zygotes as larvae some reliable phenotypic marker must be present. The mutant y is an autonomous gene whose phenotypic effect can be observed from the time of hatching through adult development. Since crossing-over between y and dor occurs with a frequency of only 0.003, the y marker can be used as a reliable indicator of the presences of the dor gene in the larval genotype. From the cross y^Ndor/+; ry^2/ry^2♀ x +/Y; ry^2/ry^2♂ one can be virtually certain that all hatching males among the progeny with yellow mouth parts are carrying dor and therefore have the following genotype (y^Ndor/Y; ry^2/ry^2). These males can be separated from the rest of the progeny and their post-embryonic dvelopment followed.

At 25°C all the larval molts of these zygotes are on time. Pupation and metamorphosis appear to proceed normally

up to the time of eye pigment deposition. From this point on metamorphosis appears to be abnormal. At 25°C no yNdor/Y; ry2/ry2 male zygotes have ever emerged as adults. (At 18°C some do emerge as adults (Clancy, unpublished).) When these zygotes are artificially removed from the puparium their external morphology appears quite normal with the exception of yellow bristles, which as previously mentioned are not characteristic of the y^N phenotype. Internally several abdominal tissues are observed to be abnormal. An excessive accumulation of fat body cells is present (a characteristic dor phene). The Malphigian tubes are chalky white with grey granular accumulations. They are also morphologically abnormal. The testes are morphologically ovoid, reminiscent of the 3rd instar larval condition.

Ovary Transplants Between 3rd Instar Ore-R, ry^2, car and y^N dor Larvae

Ovary tissue was chosen as transplant material for two reasons: (1) during metamorphosis the ovaries have to attach to the adult oviducts and therefore, the presence of more than two ovaries creates competition for attachment; (2) using hosts and donors of different genotypes allows one to test for gene specific tissue interactions measured by the differential ability of transplanted ovaries to compete successfully for oviduct attachment in reciprocal transplants. For this purpose ovaries from y^Ndor late 3rd instar larvae were transplanted into Ore-R, ry^2 and car host larvae of

approximately the same age. Potentially, one might expect either a ry^2 or car host larva to be a lethal environment for dor tissue since the double mutant zygotes are lethals. Experiments were designed to test whether dor ovaries would survive in ry^2 and car hosts and metamorphose normally. If they metamorphosed normally they should in some cases compete successfully for the host oviduct and become functional ovaries. To facilitate the testing of this hypothesis the autonomous y^N marker was included in the donor dor genotype. If a y^Ndor ovary becomes functional in the adult host the X-bearing egg fertilized by a y^N male will produce larvae with yellow mouth parts at the time of hatching. The test for the presence of a functional transplanted y^Ndor ovary in Ore-R, ry^2 or car host simply involves looking for larvae with yellow mouth parts among the hatching host progeny. In all cases a y^N male is mated to the female host. The percentage of donor ovaries that became functionally attached to host oviducts may be seen in Table 9. The computed percentages are based only on the total number of hosts shown to have three ovaries by adult dissection after the completion of progeny testing. Sometimes progeny testing established the presence of a functional transplanted ovary when adult dissection revealed the presence of only two ovaries. These individuals were also included in computing the percentages in Table 9.

Ovaries from y^N were transplanted to Ore-R hosts to test the independent effect of this mutant allele on the ability of the ovaries to compete for the host oviducts.

TABLE 9

OVARY TRANSPLANTS BETWEEN y^N, Ore-R, ry^2, car and $y^N dor$ LATE 3rd INSTAR LARVAE

Host Genotype	Donor Genotype	Number of Transplants	Ratio of Functional Transplanted Ovaries: Non-Functional Transplanted Ovaries	Percent of Transplanted Ovaries that Become Functional
1. Ore-R	y^N	58	36 : 22	62
2. $y^N dor$	Ore-R	50	34 : 16	68
3. Ore-R	$y^N dor$	45	18 : 27	40
4. car	$y^N dor$	36	12 : 24	33
5. ry^2	$y^N dor$	52	16 : 36	30

X^2 values and associated probabilities of comparisons between rows. The number of functional transplanted ovaries to non-functional transplanted ovaries are compared for different genotypes.

Rows 1 and 2, d.f. = 1, $X^2 = 0.71$ $p > 0.05$ not significantly different

Rows 2 and 3, d.f. = 1, $X^2 = 8.72$ $p < 0.05$

Rows 4 and 5, d.f. = 1, $X^2 = .0004$ $p > 0.05$ not significantly different

Rows 3 and(4&5), d.f.=1, $X^2 = 1.28$ $p > 0.05$ not significantly different

Sixty-two percent of the $\underline{y^N}$ donor ovaries became functionally attached to the adult oviduct at metamorphosis. By contrast only forty percent of the $\underline{y^N dor}$ donor ovaries were successful in Ore-R hosts. In the reciprocal transplant experiment sixty-eight percent of the donor Ore-R ovaries competed successfully for attachment to $\underline{y^N dor}$ host oviducts. In this case success of attachment was measured by the presence of larvae with wild type mouth parts among the hatching progeny. Donor ovaries from $\underline{y^N dor}$ attached successfully only thirty and thirty-three percent of the time in $\underline{ry^2}$ and car host respectively. A chi-square test at the 0.05 level of significance shows that there is no difference in the performance of $\underline{y^N dor}$ ovaries in Ore-R, $\underline{ry^2}$ and car hosts. There is a significantly greater number of Ore-R ovaries that became functional in $\underline{y^N dor}$ hosts than vice-versa.

DISCUSSION

The interpretation of the mechanism(s) of genetic regulation in higher organisms is still largely an open question. In the few cases where genetic regulation has definitely been demonstrated in higher organisms, the biochemical events occurring between the genotype and the phenotype are not very well understood. This has complicated any attempt to devise models explaining events at the molecular level based on observations made at the phenotypic level. With the elucidation of the operon model of regulation in E. coli (Jacob and Monod, 1961) it became increasingly popular for workers to try to fit the data on genetic regulation from higher organisms to the molecular model demonstrated in bacterial systems (Lewis, 1963, Baker, 1963, McClintock, 1964 and Pelling, 1966). This had had the effect of directing thinking around a model not adequate to explain the many examples of genetic regulation whose mechanism seems to reside in the complex structural organization of the chromosome itself. Therefore, in elucidating the mechanism of genetic regulation in higher organisms, it will be necessary to consider the manner in which genes are structurally and functionally organized into larger complex units on the chromosome. Grant, (1956) citing examples from a variety of organisms, and Lindsley (1964) considering examples largely from Drosophila, have emphasized the importance of chromosome structure for gene function.

Genetic regulation as understood in micro-organisms probably does not require gross chromosomal conformational changes. This does not seem to be the case in chromosomes of higher organisms, where the chromomere, thought to be the site of genetic transcription, is either tightly coiled (transcription inactive) or in the form of an unwound loop (puffed or transcription active) (Beerman, 1952; Eddstrom and Beerman, 1962; Keyl and Pelling, 1963; and Pelling, 1966). Thus in higher organisms, possibly in place of, but probably in addition to the "operon like" genetic unit, there seems to be a qualitatively different mechanism of genetic regulation.

Based on Lewis' cis-trans comparison, Benzer has developed the concept of the cistron in microorganisms (Lewis, 1951 and Benzer, 1957). It is not yet certain how applicable this test is to an organism like Drosophila where the existence of position effects implies a functional, structural patterning more extensive than that covering a single gene. Green (1961) finds that while y and sc alleles always complement each other, y^2 no longer complements with y^{c4} to give a wild phenotype when sc is in the cis position with y^2. The demonstration of the existence of this kind of supragenic organizational unit suggested by evidence requires new genetic techniques.

The present investigation has not been concerned so much with the mechanism of genetic regulation but rather with ways of genetically testing for structural and functional

gene relationships. Hopefully this can lead to the formulation of testable models of how genes in an organizational pattern are physiologically related to each other.

Evidence on the Time of Action of the dor Gene and the Df(1)260-2

Although puffing patterns provide indirect evidence that gene activation does occur at specific times in development, it is very difficult to establish for any phenotypically known gene the precise point at which it becomes active in transcription. This difficulty results from the fact that the biochemical events occurring between transcription and the resulting phenotype in higher organisms are not known. In most cases, one can only say that a given gene becomes active sometime between fertilization and the first appearance of its phenotypic effect. Determining the point at which a gene becomes active in this manner, gains some degree of precision only when the gene is known to function fairly soon after fertilization. Dor is such a gene. For details of its mode of inheritance, refer to page 23. Hildreth and Lucchesi (1966) have established that both male and female dor zygotes from a mutant-mutant cross stop embryonic development just prior to gastrulation. Some heterozygous dor female zygotes (dor/+) among the progeny from dor mothers survive. The most likely explanation is that the normal allele of dor, upon entering the egg at the time of fertilization, becomes active and reverses the pattern of predetermined lethality which would normally be expressed just

prior to gastrulation.

A small but significant percentage of the dor/+ female zygotes complete embryonic development but fail to hatch. A preliminary histological examination of these f^e zygotes revealed no readily observable morphological defects, only a failure of the tracheal trunks to fill completely with gas. The fact that some artificially hatched f^e zygotes reached the adult stage supports the observation that these zygotes are morphologically normal. Apparently the action of the dor gene is necessary for a zygote to complete gastrulation normally and also to maintain normal physiology just prior to hatching.

The loss of the distal-most eight bands in the Df(1)260-2 hemizygous male zygotes is not expressed phenotypically until the end of embryonic development, when, like the f^e zygotes, their tracheal trunks fail to fill with gas. It is impossible to pick out the individual gene(s) whose loss is responsible for the breakdown of the physiological functioning of these late embryos. Probably y and ac alone are not responsible since the two deficiency (y ac) is viable (Muller, 1935). However, they may be involved in the total effect of the Df(1)260-2), since the way genes act together physiologically may be quite different from the sum of the actions of the individual genes. In the intact X-chromosome a mutant gene product from the region, represented by Df(1)260-2, could conceivably lead to an earlier embryonic lethal effect, than a deficiency of this whole region. The

gene 1(1)X-10, thought to be included in the Df(1)260-2 region, according to the "Genetic Variations of Drosophila melanogaster" (Lindsley and Grell, 1967), results in three "types" of lethality, one of which acts very early in embryonic stage of development. Since the mouth parts of y embryos are mutant, y is assumed to act embryonically. 1(1)7e seems to be a specific modifier of dor in that $1(1)7^{e}$ dor^{1} zygotes die earlier than dor^{1} zygotes (Oftedal, 1953). It is not known how many reside in the Df(1)260-2. All one can say is that the loss of all the genes in concert in the Df(1)260-2 results in a genetic lesion expressed phenotypically as a late physiological malfunction similar to that found in f^{e} late embryos (Kaliss, 1937).

Test for the Functional Relationship of dor and the Df(1)260-2 Region

The Df(1)260-2 was not saved after being studied by Kaliss and therefore was not available to be used in experiments designed to test the functional relationship between dor and the Df(1)260-2 region. A duplication of this region exists attached either to the short arm or the long arm of the Y-chromosome (YSy^{+} or YLy^{+}). Most of the attached XY dor stocks originally synthesized to test the effect of extra heterochromatin on embryonic development in dor zygotes utilized the $XY^{L}y^{+}$ chromosome. Because these stocks were already available, most of the subsequent work also made use of the y^{+} duplication appended to the Y^{L} arm. The f^{e} phenotype was first observed among the progeny of dor mothers with the

three following genotypes: dor/dor, dor $Y^L y^+$/dor, and dor $Y^L y^+$/dor $Y^L y^+$. The preponderance of f^e phenotypes among the progeny of dor mothers carrying two y^+ duplications, coupled with the information that this duplication was genetically coextensive with Df(1)260-2 known to produce an "f^e like" phenotype, raised the possibility that a specific interaction was occurring between dor and the y^+ duplication.

Dosage Effect

Further investigation revealed that in every dor stock tested, the presence of two y^+ duplications in the genotype of dor mothers caused a large and very consistent increase in the percentage of f^e zygotes among their female progeny (about 50%). In every case tested, a single y^+ duplication in the maternal dor genotype caused a rise in the percentage of f^e zygotes observed. The increase over that observed with no y^+ duplication present was small, except in the FM-3 stock shown in Table 5 from this we may conclude that the residual background of the FM-3 stock has a low genetic buffering capacity with respect to suppressing the deleterious effect of the single y^+ duplication on embryonic development.

Synergistic Maternal Effect

The y^+ duplication results in the appearance of f^e zygotes only maternally from dor females. If genotypically identical zygotes receive the y^+duplication from a dor male, these zygotes develop normally. Three lines of evidence suggest that it is this specific maternal interaction between

the dor gene and the y^+ duplication which increases the percentage of f^e zygotes among the progeny of dor mothers. (1) The f^e phenotype is specific to the female progeny of dor mothers and male zygotes hemizygous for Df(1)260-2. No "f^e-like" zygotes were observed among the unhatched eggs of many recessive mutants tested. Genes y, sc and su^2-s are some of the mutants in or near the Df(1)260-2 region tested. (2) The y^+ duplication present either in Ore-R stocks or in the original stocks from which the attached-XY^Ly^+ chromosomes were extracted never resulted in any "f^e-like zygotes. (3) Agents other than the y^+ duplication known to affect the viability of the female progeny of dor mothers did not act specifically to increase the percentage of f^e zygotes. Neither the added Y^L nor the $Y^{L.S}$ arm of the Y chromosome in the dor mother's genotype increased the percentage of f^e zygotes among her progeny. The combination of various mutant alleles in the maternal dor genotype such as y, v, f and Bar also had no specific effect on the percentage of f^e zygotes observed. It seems safe to conclude that the interaction between dor and the y^+ duplication is quite specific and favors the interpretation that the genes of the y^+ duplication (= the genes in the Df(1)260-2) and the dor gene are functionally related in a synergistic fashion.

Position Effect as a Test for Structural Relationships Between Genes

Both the synergistic nature of the interaction between *dor* and the y^+ duplication and the tight linkage between *dor* and the *Df(1)260-2* argue against the fortuitous association of the genes in this region of the chromosome. To test for an obligate structural ordering of genes in the *Df(1)260-2* - *dor* region, the wild type allele of *yellow* was located at its normal position on the chromosome or in a distantly linked duplication. The results of the position effect studies are incomplete for reasons which will require a short digression to explain.

During the course of the position effect work, all the *dor* stocks one by one, began to show morphological abnormalities of the legs, wings and abdominal segments. Breeding experiments of the affected flies indicated that this abnormality resulted in almost complete sterility among the *dor* females in these stocks. Although the morphological syndrome was expressed in both males and females, male sterility was not observed. The heterozygous *dor* female flies and male flies carrying any balancer chromosome were normal morphologically and in the breeding test.

In an attempt to discover the cause for these abnormalities, several different food recipes were tried with no success. New *dor* stocks were obtained from Pasadena, Bowling Green, Yale and from Dr. S. Counce personally. At The University of Michigan in Ann Arbor, despite careful precautions

to avoid any possibility of contamination, all these stocks produced flies showing the syndrome just described. Several other investigators have had similar kinds of problems maintaining healthy dor stocks (Counce, 1968, personal communication). To date, no method short of raising flies at 18°C has proved successful in the rearing of morphologically normal dor flies. Fortunately most of the data-collecting involving a position change of the y gene was completed prior to the appearance of "sick" flies. Nonetheless, the inability to rear healthy dor flies has prevented more extensive investigations involving the systematic manipulation of several other genes in the Df(1)260-2 region.

The design of the position effect experiment involves genetically synthesizing two kinds of dor females which are phenotypically and genotypically identical, except that in one case the y^+ gene is at its normal position and in the other the y^+ gene is located in the y^+ duplication. The genotypes of the two flies are $ydor \cdot Y^L y^+/ydor$ and $y^+ dor Y^L/ydor$. Phenotypically, both kinds of flies have mutant dor eyes and wild type progeny. Flies of the former genotype behave essentially like a homozygous ydor female and the latter like a homozygous dor female. The progeny of ydor females consistently show low adult survivorship, independently of the residual genetic background, i.e., stock differences, the presence of extra heterochromatin and y^+ duplications. Curiously, the addition of extra heterochromatin to the ydor parent female genotype greatly increased the percent of zygotes among her progeny which completed embryonic development.

All four homozygous ydor genotypes with at least one extra Y^L in Table 6 show approximately a fifty percent rise in the number of zygotes completing embryonic development as compared to the homozygous ydor genotype without a Y^L. In these four genotypes the residual background is randomly heterogeneous, and the y^+ duplication is absent in as many cases as it is present. Presumably, therefore, it is the extra heterochromatin which is "switching" the ydor female genotype to an essentially y^+dor genotype with respect to the embryonic development of her progeny.

The percentage of f^e zygotes among the progeny of ydor mothers with different residual genetic backgrounds was quite variable, but large duplication runs of progeny tests using the genotype of primary interest, i.e., ydorY^Ly^+/ydor, gave results which were statistically the same. The percentage of f^e zygotes among the progeny of ydor mothers with the y^+ gene in the small translocated duplication was twice the percentage observed when the y^+ gene was in its normal position. It seems reasonable to conclude that in the genotypes just described, having the y^+ gene in a small translocated duplication as compared to its normal position leads to an increase in f^e zygotes among the progeny and a reciprocal decrease in adult survivorship.

Cis-Position Effect as a Test for a Functional-Linkage Relationship Between y and dor

The cis-position effect mentioned earlier between Pc, Scx, and bithorax involves mutants with dominant effects,

allowing one to observe the greater mutant phenotype of the cis heterozygote. In this case one can readily distinguish between a chromosomal (structural) as opposed to an extra-chromosomal (biochemical) interaction as an explanation of this phenomenon. In the following possible cis-position effect, involving only recessive mutants, it is not possible to distinguish clearly between a chromosomal and an extra-chromosomal interaction. Green (1961) has shown that y^2, which normally complements with y^{c4} to give a wild phenotype, in the cis-configuration with sc fails to complement y^{c4} and gives a y^2 mutant phenotype. Since y^{c4} is present is present only in an inverted chromosome, a $y^{c4}sc/y^{2+}$ combination has not been tested. This combination would distinguish whether sc is acting biochemically in a single dose or acting along the chromosome to change the complementation properties of y^2.

The interaction between y^N and dor in $y^N dor/Y$; ry^2/ry^2 males, resulting in yellow rather than black y^N bristles, resembles Green's y^2-sc example above. Again, a clear distinction cannot be made between a chromosomal and extra-chromosomal interaction with the data available. This is because to date, the interaction has been tested only in males. The crucial test would be to compare a $y^N dor/y^{1+}$; ry^2/ry^2 and a $y^{N+}/y^1 dor$; ry^2/ry^2 female. If the former has yellow bristles and the latter has black bristles, then this would conclusively demonstrate a cis-chromosomal interaction. If dor is required to be homozygous in order for the y^N-dor

interaction to be expressed then both genotypes would yield flies with y^N bristles. In this event it would not be possible to distinguish between a chromosomal and an extrachromosomal interaction. If the interaction between y^N and dor is chromosomal, this would be very interesting in that a third recessive mutant is required for the phenotypic expression of this interaction.

Possible Structural Similarities Between Zeste-White and the Df(1)260-2 - Deep Orange Region

A consideration of the white(w)-zeste(z) interaction is instructive to the discussion of closely linked functionally related genes. The genes z and w are located at 1.0 and 1.5 respectively on the X-chromosome. Several lines of evidence support the idea that there is an homology between these two genes. Reciprocal duplication and deficiencies for the region between z and w are regularly recovered among the progeny of w^{a2}/w^{a} females. This presumably results from pairing of the bands 3A3(z) and 3C1(w) accompanied by unequal crossing over (Green, 1965). In 1953 Gans demonstrated that the expression of the z phenotype is dependent on the dose of the white locus, two doses of white being necessary for the expression of zeste. More recently, McCarron and Fuscaldo (1968) have isolated a protein (w-1) associated with the interacting zeste-white region. They conclude that w-1 is "(a) a product of both the zeste and the white region or (b) the primary product of the zeste region modified by interacting with the white region."

The w gene is composed of at least the two chromosomal bands 3C1 and 3C2 and perhaps also 3C3. All the alleles of w show a close physiological interdependence, yet with respect to such properties as dosage compensation, homogeneity of eye coloration, and the ability to suppress the mutant z, each of the three sites in the white locus is functionally discrete. Genes y, ac and sc which all map at 0.0 on the X-chromosome and overlap into the Df(1)260-2 region may be compared to the three sites in the white pseudoallelic complex. These three genes plus Hairy Wing (Hw) are all thought to be contained in a single band (Muller and Prokofyeva, 1935). Although these three genes do not appear to be physiologically interdependent to the same degree as are the sites within the w locus, they are closely related, both structurally and functionally. A similar structural homology as that suggested to exist between z and w (0.5 map units apart) could explain the functional relationship shown to exist between the Df(1)260-2 region and dor (0.3 map units apart).

Judd (1961) has suggested that the white region may have evolved as a series of tandem duplications resulting from unequal cross overs. The idea of unequal crossing over as a possible mechanism for the evolution of new genetic material in Drosophila is not new (Bridges, 1935; Muller, 1935; Lewis, 1945, 1951). The homology between zeste and white suggests a common origin for these two genes with a subsequent physical and functional separation. Extending the analogy, one can postulate that, like zeste-white, the Df(1)260-2 - deep orange region represents, at least in part, tandem

duplications, one of which (the dor gene) has been physically separated from the genes of the Df(1)260-2 region. The z-w region may serve as a useful model for the evolution of functionally complex related gene loci, however, the genes in the Notch locus more closely resemble the developmental relationship existing between the genes in the Df(1)260-2 - dor region. In contrast to z-w the obligate structural continuity which seems to be necessary for the normal functioning of the distal tip of the X-chromosome including dor suggests this clustering is an adaptive property of these genes.

Notch (N) as a Model of Epigenetic Clustering

Welshons (1958) and Welshons and Von Halle (1962) have shown that the recessive mutants facet, split and notchoid all belong to the Notch pseudoallelic system. Phenotypically these mutants are rather different and would not immediately suggest a pseudoallelic relationship. Even the complementation properties of these recessive mutants are deceiving. The trans heterozygote of fa and spl, for example, complements to give a completely wild type fly. Yet dominant N mutants occur along the entire length of the Notch locus (0.11 map units). Furthermore all the recessive mutants of this complex locus shows pseudodominance in heterozygous combination with any N allele, even those alleles which appear to be point mutation.

The diverse phenotypic effects shown by the mutants of this pseudoallelic series suggest a separation of function

in both time and space. The fa mutants have rough eyes, spl mutants have small eyes and many bristles doubled and some missing; nd mutants have cut wings and thick veins. At the same time in interspersed N mutants show that the entire locus has a common influence on development.

The tip of the X-chromosome including dor (a map distance of 0.30 units) seems to be functionally organized in much the same manner as the Notch region. Historically forty separate mutable loci have been found to occur between the distal tip of the X an dor. Twenty-one of these mutants have been lost. Sixteen of the forty are lethals and three suppressor genes are interspersed along this region of the chromosome. Of the remaining loci having known mutants existing at the present, six are bristle mutants, two are body color mutants, and two are female steriles. Like Notch it is suggestive that the genes at tip of the X are separated functionally in both time and space. Yet a common phenotype, the f^e phenotype, is interspersed along this region, reminiscent of the dominant Notch pseudoalleles.

It is suggested that the genes clustered at the tip of the X-chromosome, including dor, have a common influence on the developmental physiology of late embryos and may represent a case of structural and functional regionalization of genetic material on the chromosome. The further study of similar functional-linkage units should serve to emphasize that the chromosome of higher organisms is a highly differentiated organelle allowing for the controlled release of genetic information during development.

The Functional Interaction of ry^2 and car with dor

Three lines of evidence presented earlier suggested that the maternal action of the y^+ duplication in dor females is quite specific. To test this proposition further, the ry^2 and car genes were separately introduced into the dor genotype. Genes ry^2 and car interact directly in the dor genotype and result in lethality of the double mutant zygotes. It is important to know whether or not lethal f^e phenotypes would be observed among the dor;ry^2 and dor, car double mutant zygotes. In both zygotes lethality occurred postembryonically, supporting the conclusion that the interaction of the y^+ duplication in the dor genotype is functionally different from the interaction between either ry^2 or car and dor.

Glassman et al. (1966) have shown that the ry mutant can be distinguished biochemically from maroon-like (ma-l) and low xanthine dehydrogenase (lxd) by the presence of normal amounts of pyridoxal oxidase activity, which is deficient in both ma-l and lxd. All three mutants either lack (ry, ma-l) or are low (lxd) in xanthine dehydrogenase (XDH) activity. Genetically these mutants can be distinguished in that ry is lethal in combination with dor, while ma-l and lxd are not (Lucchesi, 1968). The apparent specificity of the biochemical interaction between ry and dor makes it potentially very interesting. The fact that the combination of two eye colors can result in lethality demonstrates again that the intermediates in pigment metabolism are far more important than incidental steps leading to the decoration of the adult

eye with pigment. It is known that XDH is active on two classes of substrates; one is a pterine and the other a purine (Glassman, 1965). The involvement of XDH in both pterine and purine metabolism points to the interrelatedness of the pigment and purine pathways.

Interestingly, dor is the only mutant known to increase the level of isoxanthopterin above the wild type level (Counce, 1957) while ry flies are completely deficient in isoxanthopterin (Glassman, 1952). Because dor; ry^2 zygotes reach an advanced stage of development, dying only after the beginning of eye pigment deposition, a biochemical analysis of this system in combination with feeding experiments could be very helpful in elucidating the intermediary metabolism in the pigment pathway leading to this genetic lesion. The stage of development reached by lethal dor, car zygotes had yet to be tested.

Ovary Transplants Between Ore-R, ry^2, car and dor

Ovary transplants were performed to answer two questions. The first involved the nature of the genetic lesion resulting in lethality of the dor; ry^2 and dor, car double mutants. The second question concerned whether or not ovaries of different genotypes have differential competitive abilities for the attachment to host oviducts.

The lethal interaction between either ry^2 or car and dor could be the results of the interaction of diffusible metabolic products or could result from the absence of two metabolic products whose combination is vital for survival.

In the former case, transplanted dor ovaries would not be expected to develop normally in ry^2 or car hosts. In light of the abnormal development of gonadal tissue in dor;ry^2 flies, this was a likely possibility. In the latter case both the host and transplanted tissue would lack only one metabolic product and therefore both would develop according to their own genotype. The results indicate that the second alternative is more likely since dor ovaries competed with equal success for the attachment of host oviducts in ry^2, car and Ore-R flies.

With a selective technique at hand for recovering y^Ndor/Y; ry^2/ry^2 male larvae, it is now possible to perform the crucial transplant experiments which can more precisely establish the nature of dor;ry^2 lethal interaction. For example, transplanting dor; ry^2 3rd instar testes into wild type larvae of a comparable age should establish whether or not the dor; ry^2 interaction is cell lethal. Also, various wild type tissues can be implanted in dor; ry^2 3rd instar larvae in an attempt to restore normal pupal development.

With one important exception, the design and results of the ovary competition experiments were quite similar to experiments performed by Clancy and Beadle (1937). In both experiments, female sterile mutant ovaries were transplanted into wild type host and vice-versa to test host and transplant responses. Clancy and Beadle (1937) found the ovaries of three female sterile mutants singed (sn), fused (fu) and female-sterile (fes) all showed autonomous development in

wild type host and vice-versa.

The dor ovaries also show autonomous development but this was to be expected since transplanted dor eye disks are known to develop autonomously (Hadorn and Counce, unpublished). Clancy (1964) also found that transplanted dor^1 ovaries, although functional, produced no offspring.

Using sn and two wild type stocks, Clancy and Beadle found that the percentage of transplanted ovaries which became functional varied with the genic constitution of the stock (75.0 - 41.7 percent). However, for any constitution, reciprocal transplants gave approximately equal frequencies. In the present study the transplanted dor ovaries became functional in wild type hosts significantly less frequently than did the reciprocal transplants. Evidence in the literature suggests a possible explanation for the behavior of transplanted dor ovaries. Pantelouris (1954) has found that if one ovary is removed in the 3rd instar larvae, the oviduct on that side usually fails to develop. This suggests that the oviduct develops in response to something produced by the ovary. Doane (1161) using the adipose (adp) mutant has shown that the hypertrophied corpus allatum in adp flies is an allophenic response to the mutant ovaries. She presents further evidence as well as a model for hormonal feedback control between the ovary and corpus allatum. It is tempting to speculate that female sterility expressed by the dor mutant is a result of a hormonal deficiency occurring in the corpus allatum, or, more probably, in the ovary itself. If

the oviducts are responding to an ovarian hormone, this would explain the decreased ability of dor ovaries to compete successfully with wild type ovaries for host oviducts.

SUMMARY

Genetic investigation of the gene loci at the tip of the X chromosome, with particular emphasis on the gene *deep orange* (*dor*) resulted in the following conclusions.

1) The hypothesis that the greater amount of heterochromatin in the *dor* male genome might explain the later developmental stages reached by male versus female *dor* embryos, reported by Counce (1956), was rejected. This is consistent with the finding of Hildreth and Lucchesi (1966) that the "early-early" female lethals reported by Counce were in fact unfertilized eggs.

2) Among the female progeny of *dor* mothers crossed to *Ore-R* males there is a class of zygotes which develop normally but fail to eclode (f^e). This class of zygotes very closely resembles late embryos carrying the deficiency *Df(1)260-2* missing the band 1A1-8. Both f^e and *Df(1)260-2* zygotes produce only weak, ineffective hatching motions at the time of hatching. Their tracheal branches fail to fill with gas and upon being artificially hatched these embryos show a marked tendency to dehydrate.

3) The percentage of f^e zygotes among the progeny of *dor* mothers with two duplications of the chromosome region 1A1-8 increases three to five times that occurring when this duplicated region is absent. Agents other than the y^+ duplication (= bands 1A1-8) known to affect the viability of the progeny of *dor* mothers did not act specifically to

increase the percentage of f^e zygotes. Furthermore, a study of the specific time and syndrome of developmental arrest produced by the interaction of dor with rosy (ry) and with carnation (car) demonstrated these lethal interactions to be distinct from the lethal interaction between dor and the y duplication.

4) Transplanted ovaries of late 3rd instar donor dor larvae successfully attached to the host oviducts of ry^2 and car host. One may conclude that while ry^2 and car are lethal in combination with dor neither ry^2 or car provides a lethal host environment for dor tissue.

5) Reciprocal transplants between Ore-R and dor of ovaries of late 3rd instar show that donor ovaries compete less successfully in Ore-R hosts than vice-versa.

6) The y^+ duplication was shown to be genetically coextensive with the gene loci represented in the region of Df(1)260-2.

7) The gene loci in the region 1A1-8 and dor are responsible for maintaining the normal physiological state of late embryos. When the region 1A1-8 is present as a duplication (i.e., the y^+ duplication) in the maternal dor gene type it acts in a synergistic fashion with dor during oogenesis to increase the percentage of progeny expressing the f^e phenotype.

8) The synergistic interaction between dor and the y^+ duplication was quantitatively modified by altering the allelic state of the gene yellow located in the region 1A1-8.

9) The phenotype of a particular allele of yellow (y^N) was qualitatively altered by the presence of the tightly linked mutant dor allele.

10) Statements 2, 3, 6 and 7 suggest a close functional relationship between the gene loci in the region 1A1-8 and dor. Statements 8 and 9 further suggest that a structural relationship exists between the gene loci at the tip of the X chromosome including dor.

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