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AND IN OXYGEN OF THE SUPPRESSOR-ERUPT SYSTEMS
IN SEVERAL STRAINS OF DROSOPHILA MELANOGASTER.

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A COMPARISON BY MEANS OF X-IRRADIATION IN AIR AND IN OXYGEN OF THE SUPPRESSOR-ERUPT SYSTEMS IN SEVERAL STRAINS OF DROSOPHILA MELANOGASTER

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Audrey Marie Aubele, B. A.

* * * * * * *

The Ohio State University
1966

Approved by

Henry L. Blair
Adviser
Department of Zoology and Entomology
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June 19, 1930  Born — Pittsburgh, Pennsylvania

1960  B. A., College of Saint Mary of the Springs, Columbus, Ohio

1960 - 1962  Secondary School Teacher, Newark Catholic High School, Newark, Ohio


1962  Instructor, College of Saint Mary of the Springs, Columbus, Ohio

1963  National Science Foundation Summer Fellowship for Secondary School Teacher, The Ohio State University, Columbus, Ohio

1964 - 1965  Graduate Assistantship, Department of Zoology and Entomology, The Ohio State University, Columbus, Ohio

1965 - 1966  American Association of University Women Grace Ellis Ford Fellowship for American Women, The Ohio State University, Columbus, Ohio

1965 - 1966  Graduate Assistantship, Department of Zoology and Entomology, The Ohio State University, Columbus, Ohio

FIELD OF STUDY

Major Field: Genetics
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INTRODUCTION

The suppressor-erupt system (symbolized Su-er; er) of *Drosophila melanogaster* has been the subject of a large number of investigations. An introduction to the literature can be found in Glass (1957) and Plaine (1955a). The erupt alleles and their specific suppressor alleles are widespread in both wild and laboratory strains of *Drosophila melanogaster* (Glass, 1949, 1957; Plaine, 1955b).

Analyses of strains having the suppressor-erupt system (Glass, 1944) indicate that these strains carry the mutant gene erupt (er) on the third chromosome and the suppressor of erupt (Su-er) on the second chromosome. The only difference in these strains is the strength of the alleles at these two loci. In each naturally occurring strain, the suppressor allele is always effective against the erupt allele with which it is in combination. However, when the suppressor and erupt loci of different strains are recombined, the degree of effectiveness of a suppressor allele varies, depending upon its strength and the strength of the erupt allele with which it is in new combination. In addition to the suppressor-erupt system, Glass (1954) reported the reciprocal location of the suppressor-melanotic tumor system (symbolized tu; su-tu) in a known suppressor-erupt strain of *D. melanogaster* which had originated as the brown-scarlet (bw; st) mutant strain of an original Amherst wild-type stock.
The genetic composition of strains having both suppressor systems is represented as

\[
\begin{array}{ccc}
\text{Su-er} & \text{tu} & \text{su-tu} & \text{er} \\
\_1 & \_2 & \_3 & \\
\end{array}
\]

where \( l \) symbolizes the position of the centromere.

The presence of effective mutant alleles may be revealed despite the presence of their specific suppressor alleles by treatments with X-rays (Glass, 1944), oxygen and hydrogen peroxide (Glass and Plaine, 1952; Plaine, 1955a), and certain compounds related to tryptophan (Glass and Plaine, 1955; Plaine, 1955c; Plaine and Glass, 1955). These same agents affect the melanotic tumor system of \textit{D. melanogaster} in a parallel manner (Chelélovitch, 1961; Plaine and Glass, 1952, 1955; Plaine, 1955a). The widespread distribution of the suppressor-erupt and suppressor-tumor systems together with their sensitivity to a wide range of treatments, including X-irradiation in air and in an atmosphere of pure oxygen, make them highly suitable for the study of gene action in general and of suppressor action in particular.

Suppressor genes comprise one of the major categories of modifying genes, and their presence has been studied in several organisms, most notable of which are \textit{Drosophila melanogaster} and \textit{Neurospora crassa} (Strauss and Pierog, 1954; Suskind and Kurek, 1959; Suskind, Ligon, and Carsiotis, 1962; Yorno and Suskind, 1964). A substantial number of suppressors have been carefully studied in microbial systems including yeast, bacteria, and phage (Brody and Yanofsky, 1964; Capecchi and Gussin, 1965; Fincham, 1958; Gartner and Orias, 1965; Orias and
Gartner, 1964). The suppressor-erupt and suppressor-tumor systems, as well as the suppressor of vermilion studied by Marzluf (1965), differ from many suppressors that occur in microbial systems because of their dominant-recessive relationships and because they concern non-lethal characters.

According to Marzluf (1965), a suppressor gene, in the strict sense, is one which brings about the complete or partial restoration of the wild-type phenotype in the presence of another nonallelic mutant gene that alone causes a mutant phenotype. The term "second site reversion" has been introduced by Helinski and Yanofsky (1963) to distinguish intragenic "suppressors" from the more common nonallelic suppressors. Suppression is a phenomenon observed and defined at the phenotypic level and it arises from many diverse mechanisms. Several mechanisms of action for suppressors which have been demonstrated include (1) the removal of a metabolic inhibitor accumulated because of the mutant block (Strauss and Pierog, 1954); (2) the removal of an inhibitor normally present in all strains which inhibits a mutant enzyme but not the wild-type enzyme (Suskind and Kurek, 1959; Suskind, Ligon, and Carsiotis, 1962); (3) the alteration or elimination of a repressor substance so that a mutant operator no longer irreversibly binds the repressor and can therefore function (Mukai and Margolin, 1963); (4) the alteration in the primary structure of a mutant enzyme (Brody and Yanofsky, 1963, 1964); and (5) the synthesis of a small amount of normal enzyme (Marzluf, 1965).

Abnormal growths are associated with the mutant phenotypes of both the suppressor-erupt and suppressor-tumor systems in D. melanogaster.
The abnormal growth characteristic of erupt affects the structure of the eye. A series of drawings depicting the phenotypes characteristic of erupt is given in Figure 1. Since erupt is a homoeotic mutant, many of which are known in D. melanogaster (Villee, 1942) and in D. virilis (Fujii et al., 1964), the expressivity of any erupt allele is variable; that is, the range of phenotypes for genetically identical individuals may be divided into normal or wild-type (A), weak erupt characterized by a disarrangement of the facets of the eye (B), and extreme erupt (C and D). Extreme erupt may be characterized by breaks or holes of varying sizes in the surface of the eye (C) or by a palp-like structure protruding through the break (D). In rare cases weaker manifestations of erupt may have only an extra bristle at the anterior margin of the eye.

In addition to variable expressivity, homoeotic mutants are characterized by variable penetrance; that is, there are great variations in the percentage of individuals that show the character at all. Although all individuals in a given strain are genetically homozygous for the mutant factor, many will appear completely normal. However, the penetrance, or percentage of individuals having the mutant erupt phenotype, varies from strain to strain; but for any given strain the penetrance is fairly constant and characteristic for the suppressor and erupt alleles present in that strain. As in most homoeotic mutants, penetrance rarely reaches 100%.

Although a number of workers (Berseth and Gardner, 1961; Gardner, 1948; Ghébélovitch, 1961; Goldschmidt and Lederman-Kein, 1958; Hansen
Figure 1. Phenotypes associated with strains having the suppressor-erupt system. A - normal or wild-type eye; B - weak erupt characterized by disarrangement of facets; C - extreme erupt characterized by a break or hole in the eye surface; D - extreme erupt characterized by a palp-like protrusion from surface of eye.
and Gardner, 1962; Hartung, 1950; Hillman, 1961; Marzluf, 1960; Russell, 1942; Turner and Gardner, 1960; Woolf et al., 1964) have found and studied genetic modifiers of abnormal growths in D. melanogaster, perhaps more is known about the distribution, nature, and time of action of the suppressor-erupt gene than about any other specific gene in this species (Glass, 1949, 1957; Glass and Plaine, 1950; Hildreth, 1965). Since genetic modifiers have an important role in the expression of many mutants, any contribution to a further knowledge of the nature of one of these closely integrated genetic systems, such as the suppressor-erupt system in D. melanogaster, is fundamental to a growth in our knowledge of the nature of gene action. On account of the sensitivity of the suppressor-erupt system and the amount of information already gained concerning it, this system is particularly suitable for further studying the nature of suppressor action and the relationship between components of a coadapted genetic system.

Recent studies by Burnet and Sang (1964a,b) involving the suppressor-tumor system in a tumor strain which possesses the mutant tumor gene (tu) but which presumably lacks the specific suppressor of tumor (i.e., theoretically possesses the normal or wild-type allele symbolized by $su-tu^+$) led the authors to conclude that X-irradiation directly enhances the effect of the mutant allele rather than inhibiting the action of the suppressor gene as earlier studies had indicated, since a higher frequency of tumor incidence followed X-irradiation in the assumed absence of the specific suppressor of tumor ($su-tu$).
Since the suppressor-tumor and suppressor-erupt systems have such a striking reciprocal relationship and parallel responses to X-irradiation and other agents known to affect these systems (Plaine and Glass, 1955), this study was undertaken to investigate the response of the suppressor-erupt system in this tumor strain and in the two parent strains from which it was originally derived. Theoretically, at least, this strain does not possess the erupt mutant allele (i.e., it possesses the normal or wild-type allele symbolized by er\textsuperscript{+}) although it does have the specific suppressor of erupt (Su-er). Since the mutant erupt allele is theoretically lacking in this strain, it should not, therefore, express the erupt phenotype. Analysis of the erupt response induced in these three strains by X-irradiation in air and in an atmosphere or pure oxygen was undertaken.

In order to test for variations in response to X-ray treatment which might be found between different genotypes, certain strains derived by chromosome substitution from the three parent strains were also utilized in this study. The strains having chromosome substitutions were chosen on the basis of genetic differences at the two major loci comprising the suppressor-erupt system.
INDUCTION OF Erupt EYES IN THREE STRAINS OF DROSOPHILA MELANOCASTER
BY X-IRRADIATION IN AIR AND IN AN ATMOSPHERE OF PURE OXYGEN

Materials and Methods

The flies used in these experiments were from cultures maintained in the Genetics Laboratory at The Ohio State University. These parent strains, from which additional strains used in this study were derived by chromosome substitutions, are closely inbred descendents of the original strains used in the work of Glass and Plaine.

All irradiations were carried out with a Norelco MG 150/10 industrial X-ray unit, manufactured by the Philips Electronic Instrument division of Philips Electronic and Pharmaceutical Industries, Incorporated, Mount Vernon, New York. This unit has a 215 mm beryllium window and a maximum output of 150 kv. For the purpose of this investigation, this instrument was operated at 70 kv and 5 ma with no additional filtration. The TSD (target-shin-distance) was 48 cm and the HVL of the incident beam was estimated to be 0.1 mm Al. The delivered dose in air was 1000 roentgens (±5%) at 200 r/min as measured with a Victoreen thimble chamber dosimeter. These settings, allowing for differences in individual X-ray machines, are the same as those used in studies of the suppressor-tumor and suppressor-erupt systems by Glass and Plaine (1952).

X-irradiation of each strain was carried out both in air and in an atmosphere of pure oxygen. Since the presence of oxygen is known
to enhance the effect of X-irradiation in biological systems in general (Bychkovskaya and Ochinskaya, 1964; Gerschman et al., 1954; Oster, 1961; Slater et al., 1964), and the erupt response of the suppressor-erupt system in particular (Glass and Plaine, 1952), an atmosphere of pure oxygen was used during X-irradiation in order to assure the maximum erupt response from each strain.

Adult flies for the control counts were taken from each of the four subculture bottles maintained for each strain used in the study. These flies represent the phenotypic frequency found under standard culture conditions in any given strain. Eggs to be X-rayed were collected over twenty-four hour periods on the surface of dishes of standard cornmeal-dextrose-agar media. At the time of treatment embryos were estimated to be 12±12 hr of embryonic development. Since studies by Glass and Plaine (1950) indicate the inactivation of the suppressor-erupt effect is maximum from the time of fertilization up to 24 hours of larval development, the mean embryonic age of 12 hours was chosen as the most suitable for purposes of this study.

All irradiations both in air and in 100% oxygen were carried out in a plastic chamber. The chamber allowed for simultaneous irradiation of four disks containing embryos from each of the four subculture bottles of any given strain. This chamber was equipped with openings to allow for oxygen flow during those treatments requiring an atmosphere of pure oxygen. Oxygen flow was started prior to X-irradiation in order to flush the chamber of any air present, and was then continuous for the duration of the X-ray treatment. X-irradiations in air were
also carried out in this same chamber, but without the flow of oxygen. After treatment, all disks containing irradiated embryos were placed on the surface of standard media in culture bottles and incubated at 25±1°C. Control bottles were kept at room temperature. Variations in temperature do not affect the response of the suppressor-erupt system.

All adults which developed in the irradiated cultures were examined upon emergence, or were extracted from the pupal cases; and the phenotype of the eyes of each fly was scored as normal, weak erupt, or extreme erupt. Since erupt is a typical homoeotic mutant, flies manifest bilateral asymmetry of the erupt phenotype. This being the case, each fly was classified according to the strongest expression of erupt on either the right or left side, and was classified only once.

The proposed genotypes, with respect to the suppressor-erupt and suppressor-tumor systems, of the three parent strains used in this study are given in Table 1. By convention, homologous pairs of chromosomes are represented as single lines. Genotypic constitution is represented above each line. The number below each line designates the genetic source by laboratory strain number of each homologous pair of chromosomes. Each strain used is homozygous for the major loci involved in this study.
TABLE 1

PROPOSED GENOTYPES WITH RESPECT TO SUPPRESSOR-ERUPT AND SUPPRESSOR-TUMOR SYSTEMS IN THREE STRAINS OF *DROSOPHILA MELANOGASTER*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressor-erupt</td>
<td>31</td>
<td>( X_{31} Su-er tu bw_{31} st er su-tu_{31} IV )</td>
</tr>
<tr>
<td>Su-er; ( er^+ )</td>
<td>36</td>
<td>( X_{36} Su-er tu bw_{36} er^+ su-tu^+_{36} IV )</td>
</tr>
<tr>
<td>al b c sp^2</td>
<td>13</td>
<td>( X_{13} al b Su-er^+ c tu^+ sp^2_{13} er^+ su-tu^+_{13} IV )</td>
</tr>
</tbody>
</table>

The loci of the suppressor-tumor and suppressor-erupt systems are on the second and third chromosomes of *D. melanogaster*, and have a reciprocal relationship to each other. The suppressor-erupt strain, strain 31, is descended from the original brown-scarlet (bw;st) mutant strain of the Amherst wild-type stock described by Glass (1954). This strain which carries the recessive genes for eye color, brown (bw) on the second chromosome and scarlet (st) on the third chromosome, gave frequencies of erupt eyes approaching 90% after treatment with 1000r of X-rays in an atmosphere of air (Glass, 1944). Further tests involving outcrossing of the second or third chromosomes of the brown-scarlet strain into other strains also indicated the presence in the
bw;st strain of strong suppressor alleles and strong mutant alleles for
both the erupt and tumor systems (Glass, 1954). The bw;st strain which
was originally designated as the suppressor-erupt strain, was later des-
ignated as a double suppressor strain. For purposes of this study, this
strain will be designated by its original description as the suppressor-
erupt strain. This suppressor-erupt strain will hereafter be referred
to in this study by its laboratory stock number, strain 31. Strain 31
is represented as having the suppressor of erupt (Su-er) and the tumor
gene (tu) together with brown (bw) on the second chromosome, and the
suppressor of tumor (su-tu) and the erupt gene (er) together with
scarlet (st) on the third chromosome.

Another laboratory strain, strain 13, carrying the genes arista-
less (al), black (b), curved (c), and speck (sp$^2$) on the second chromo-
some, gave no indication of the erupt phenotype after X-irradiation in
air; and Glass (1944) designated the allele at the erupt locus as being
a wild-type, non-effective one (er$^+$). Since an incidence of erupt
approaching 80% was obtained after substitution of the second chromo-
some from the al b c sp$^2$ strain into the suppressor-erupt strain
(strain 31), the al b c sp$^2$ strain was thought to lack an effective
suppressor of erupt. Thus, the al b c sp$^2$ strain has been assumed to
be a non-suppressor, non-erupt strain and has been designated as pos-
sessing the wild-type, non-effective alleles for both erupt and the
suppressor of erupt (Su-er$^+$; er$^+$). The al b c sp$^2$ (Su-er$^+$; er$^+$)
strain will hereafter be referred to as strain 13.
A third laboratory strain, strain 36, was derived from strain 31 (suppressor-erupt, Su-er;er) and strain 13 (al b c sp² or Su-er⁺; er⁺). Strain 36 has the second chromosome of strain 31 and the third chromosome of strain 13. Therefore, strain 36 possesses a strong allele of the suppressor of erupt (Su-er) on the second chromosome, but is thought to have a wild-type, non-effective allele of erupt (er⁺) on the third chromosome. Reciprocally, then, this strain would possess a mutant allele for melanotic tumors (tu) on the second chromosome, but have the non-suppressor allele (su-tu⁺) on the third chromosome. This Su-er;er⁺ strain will hereafter be referred to as strain 36.

Working with this strain, relative to tumor incidence after X-irradiation in air, Burnet and Sang (1964a,b) concluded that the increased tumor incidence resulted from the direct enhancement of the effect of the mutant tumor locus rather than from an indirect inhibition of the action of the suppressor locus.

Each of these three parent strains was maintained in four subculture bottles during the course of this investigation. Each treatment (i.e., 1000r of X-irradiation in air, and 1000r of X-irradiation in 100% oxygen) was carried out on eggs and embryos collected from each of the four subcultures simultaneously and irradiated at the same time. All four subcultures for each strain were represented in at least three separately replicated experiments.

Except for the sex difference in the expression of erupt noted by Plaine (1955b) in the Swedish-b wild-type strain, no difference in the level of incidence for the erupt phenotype between sexes has been
observed either in previous studies or in the present investigation. The results for both sexes, while counted separately, are here combined in the data presented. No significant difference between subculture bottles or replicated series within a given strain was observed in this investigation. Results from the four subcultures and replicated series are, therefore, pooled to obtain mean response for each strain.

The data obtained from the subcultures and replicated series within a strain, as well as the results obtained with all strains and treatments, were analyzed by means of two-way analyses of variance and Duncan's multiple range test (Freund, Livermore, and Miller, 1960; Steel and Torrie, 1960). The analyses of variance were used to indicate the existence of a difference between subcultures, series, and sexes within strains, and among the various strains and treatments. Duncan's multiple range test (DMR, hereafter) at the 1% level of significance was used to distinguish the difference between strains and specific treatments.

Computations were accomplished by an IBM 7094 high speed digital computer. Free time on the computer was furnished by the Numerical Computation Laboratory of The Ohio State University.
ANALYSES OF RESULTS

The results obtained with strain 36 (Su-er;er\(^+\)) at all treatment levels are summarized in Table 2. No case of extreme erupt was found out of the total 1511 flies examined from the stock subcultures in the absence of X-irradiation. A very low number of these flies, amounting to 0.3% of the total examined, were observed to have facet disarrangement.

The lack of extreme erupt in flies under standard culture conditions is in accord with earlier findings for the suppressor-erupt system (Glass, 1949, 1957). As described in the introduction, the suppressor-erupt system is a genetically coadapted one whose presence is revealed only when chromosome substitution or outcrossing engenders a genetic imbalance between the mutant erupt allele and its specific suppressor allele, or when specific treatments, such as X-irradiation, someway result in a manifestation of the mutant erupt phenotype.

Facet disarrangements, particularly those involving only two or three facets, may be due to other factors, but it seems safe to regard the majority of them as slight expressions of erupt. Most of the facet disarrangements that occur are usually in the center of the eye where the extrusion takes place in extreme erupt individuals; moreover, the weak erupt category which is characterized by these slight facet disarrangements is observed to vary in the same manner.
as extreme erupt. However, evidence for the presence of erupt in any strain is not considered conclusive unless the phenotype characteristic of extreme erupt as described in the introduction (Figure 1) is observed to occur in that strain.

**TABLE 2**

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 36 (Su-er; er+) OF DROSOPHILA MELANOGASTER

<table>
<thead>
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<th>GENOTYPE:</th>
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<td></td>
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</tr>
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<td>1129</td>
<td>42.0</td>
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<td>1027</td>
<td>74.6</td>
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<td>2</td>
<td>594</td>
<td>74.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1087</td>
<td>75.8</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>2708</td>
<td>75.0c</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1511</td>
<td>99.7</td>
</tr>
</tbody>
</table>

a All treatments were applied to 12±12 hr embryos.

b The ± values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942).
c Frequency of phenotypes based on total number of flies for three experiments.

** Significant from control at 1% level of significance.

Strain 36 has been thought to lack an effective erupt allele;
However, a definite incidence of extreme erupt (5.4%) was observed in the first 3 series of 1027 flies of this strain which were tested with 1000r of X-irradiation in air (Table 2). In order to determine whether this incidence of extreme erupt was accidental or whether it was really a consistent and repeatable phenomenon associated with strain 36, two additional experiments each based on the four subcultures and composed of separately replicated series were conducted with this strain. The results of all three experiments with X-irradiation in air, as well as the total based on pooled data from all three experiments, are given in Table 2. A two-way analysis of variance indicates no significant difference at the 1% level for extreme or total erupt among the three experiments. The occurrence of the erupt response in strain 36 (Su-er; er$t$) is, thus, a consistent and repeatable phenomenon.

All series irradiated in air and in an atmosphere of pure oxygen differ significantly for both extreme and total erupt at the 1% level from the non-irradiated controls. In addition, a highly significant increase is observed in both the extreme and total erupt categories between X-irradiation in air and in 100% oxygen.

Experiments with the role of oxygen concentration in determining the effectiveness of X-rays on the response of the suppressor-erupt system (Glass and Plaine, 1952) indicated a statistically significant increase in the penetrance of erupt in the interval between 20 per cent $O_2$ (air) and 0 per cent $O_2$ (100% $N_2$). The effect of X-rays in pure oxygen, however, was not much greater than that in air although there was some increase.
Not only is there a striking increase in the penetrance of extreme erupt between treatment with X-rays in air (5.6%) and in pure oxygen (28.7%), but the expressivity of erupt is affected. After X-irradiation in air, extreme erupt accounts for approximately one-fifth (5.6/25.0) of the total erupt class, whereas, after X-irradiation in 100% oxygen, extreme erupt accounts for approximately one-half (28.7/58.0) of all flies having the erupt phenotype (Table 2). It is evident that the erupt response can be induced in strain 36 (Su-er; er^+) by X-irradiation despite the theoretical lack of the mutant erupt allele.

The remainder of this study is an attempt to analyze the nature of this erupt response in strain 36 and in the strains from which it was derived. Since the third chromosome of strain 36, having been derived from strain 13 (al b c sp or Su-er^+; er^+), possesses the wild-type or non-effective allele of erupt (er^+), strain 13 was likewise subjected to treatments with X-irradiation in air and in an atmosphere of pure oxygen. The results obtained in irradiated and non-irradiated series are summarized in Table 3.

A low frequency of flies with the characteristic breaks and palp-like protrusions of extreme erupt were found among flies of strain 13 after treatment with X-rays in air. The percentage of extreme erupt (1.8%) after X-irradiation in air differed significantly from the control series at the 5% level of significance, but not at the 1% level. A slight but not significant increase in extreme erupt is observed between X-irradiation in air and in 100% oxygen. The percentage of
extreme erupt (5.3%) after X-irradiation in pure oxygen does, however, differ significantly from the control at the 1% level of significance.

**TABLE 3**

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 13 (al b c sp2) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Normal</th>
<th>Extreme Erupt</th>
<th>Total Erupt</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray; 100% O₂ 1000r</td>
<td>3</td>
<td>1509</td>
<td>67.8</td>
<td>5.3 (3.81)</td>
<td>32.2±1.20**</td>
</tr>
<tr>
<td>X-ray; Air 1000r</td>
<td>3</td>
<td>1268</td>
<td>81.2</td>
<td>1.8 (0.85)</td>
<td>18.8±1.10**</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1250</td>
<td>99.4</td>
<td>0.0 (0.00)</td>
<td>0.6 (0.16)</td>
</tr>
</tbody>
</table>

a All treatments were applied to 12±12 hr embryos.

b The * values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942)

** Significant from control at 1% level of significance.

* Significant from control at 5% level of significance, but not at 1% level.

The total erupt category does not differ significantly between air and 100% oxygen, although some increase is observed. However, the percentage of flies with any expression of erupt both after X-irradiation in air and in an atmosphere of pure oxygen differs significantly from
the control at the 1% level. While the increase in extreme erupt in strain 13 is not so striking as that in strain 36, the shift in expressivity toward the extreme erupt category after X-irradiation in 100% oxygen is still observed. Whereas approximately one-tenth (1.8/18.8) of all flies having the erupt phenotype after X-irradiation in air belong to the extreme erupt class, one-sixth (5.3/32.2) of the total erupt category are extreme erupt after X-irradiation in pure oxygen (Table 3).

The occurrence of extreme erupt in strain 13 (Su-er+; er+) is significantly lower than that observed in strain 36 (Su-er; er+) after the respective treatments; however, there is no doubt that the erupt response can be induced in strain 13 as well as in strain 36 after X-irradiation. The maximum penetrance of extreme erupt in strain 13 after X-irradiation in pure oxygen is at the same level as the occurrence of extreme erupt in strain 36 after X-irradiation in air. The striking increase in the penetrance of extreme erupt in strain 36 after X-irradiation in pure oxygen has no counter-part in the response of strain 13.

While the third chromosome of strain 36 was originally derived from strain 13, the second chromosome of strain 36 was derived from strain 31 (Su-er; er). Since this second chromosome is known to carry a strong suppressor of erupt (Glass, 1944; Glass and Plaine, 1952), strain 31 was subjected to treatments with X-irradiation in air and in an atmosphere of pure oxygen in order to compare better and analyze
its response with that reported by Glass and Plaine and with those obtained for strains 36 and 13.

The results obtained with strain 31 after X-irradiation in air and in an atmosphere of 100% oxygen are summarized in Table 4.

### Table 4

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 31 (Su-er; er) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Phenotype of Eyes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray; 100% O₂ 3 1000r</td>
<td>3</td>
<td>139</td>
<td>Normal: 1.4, Extreme Erupt: 71.2 ± 3.84b**, Total Erupt: 98.6 ± 0.99**</td>
</tr>
<tr>
<td>X-ray; Air 3 1000r</td>
<td>3</td>
<td>729</td>
<td>Normal: 7.7, Extreme Erupt: 65.0 ± 1.77**, Total Erupt: 92.3 ± 0.99**</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1227</td>
<td>Normal: 94.1, Extreme Erupt: 0.1 (0.00), Total Erupt: 5.9 (4.27)</td>
</tr>
</tbody>
</table>

a All treatments were applied to 12±12 hr embryos.
b The values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942)
** Significant from control at 1% level.

A high frequency of extreme erupt which differs significantly from the control (0.1%) at the 1% level is observed both after X-irradiation in air (65.0%) and in an atmosphere of 100% oxygen (71.2%). The increases observed in extreme and total erupt between X-irradiation in air and in an atmosphere of pure oxygen are not significant (Table 4).
For total erupt, the control series for strain 31 (5.9%) does not
differ significantly from that of strain 13 (0.6%), and differs signif­
icantly from strain 36 (0.3%) only at the 5% level. Strain 31 is the
only one of these strains tested in which a low percentage of extreme
erupt individuals was found in the control count; however, there is no
significant difference among the controls of the three strains tested
with respect to extreme erupt.

The shift in expressivity of erupt toward the extreme erupt cate­
gory after X-irradiation in 100% oxygen is not observed in strain 31
as it was in strains 36 and 13. Approximately five-sevenths of all
flies having the erupt phenotype belong to the extreme erupt class
after X-irradiation either in air (65.0/92.3) or in 100% oxygen (71.2/
98.6). The highest levels of both penetrance and expressivity for the
erupt phenotype are observed in strain 31 (Su-er; er).

Considering the short generation time of D. melanogaster and the
number of years intervening between the early work with the suppressor­
erupt strain (strain 31) and the present investigation, one may assume
that some change has taken place which would alter the response of this
strain with respect to the suppressor-erupt system. The results of
the early studies of Glass (1944) and of Glass and Plaine (1952) with
strain 31 are compared with those obtained with this same strain in
the present investigation (Table 5). The response of strain 31 with
respect to total erupt, after either X-irradiation in air or in an
atmosphere of 100% oxygen, does not differ significantly among the
several investigations.
TABLE 5

COMPARISON OF RESULTS FROM PREVIOUS STUDIES WITH THE PRESENT RESULTS OBTAINED AFTER X-IRRADIATION OF STRAIN 31 (Su-er;er)

<table>
<thead>
<tr>
<th>Author</th>
<th>Total Counted</th>
<th>Total Erupt</th>
<th>Extreme Erupt</th>
<th>Total Counted</th>
<th>Total Erupt</th>
<th>Extreme Erupt</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass, 1944</td>
<td>89</td>
<td>93.3</td>
<td>83.2**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glass and Plaine, 1952</td>
<td>41</td>
<td>92.7</td>
<td>75.6</td>
<td>255</td>
<td>98.0</td>
<td>85.9**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>435</td>
<td>91.5</td>
<td>83.7**</td>
<td></td>
<td></td>
<td></td>
<td>Glass and Plaine, 1952</td>
</tr>
<tr>
<td>Aubele, present study</td>
<td>729</td>
<td>92.3</td>
<td>65.0</td>
<td>139</td>
<td>98.6</td>
<td>71.2</td>
<td></td>
</tr>
</tbody>
</table>

** Significant: from present study at 1% level.

The investigation of Glass (1944) was conducted only with X-irradiation in air. The frequency of extreme erupt obtained in his study, as well as the frequency of extreme erupt for one of the series irradiated in air reported by Glass and Plaine (1952), is significantly higher at the 1% level than the frequency of extreme erupt obtained after X-irradiation in air in the present study. However, the other series of the 1952 study does not differ significantly for extreme erupt from the present study although a slight decrease in the extreme erupt category is observed (Table 5).

The significant decrease in the extreme erupt class in the present study from that obtained in the earlier studies is also observed after X-irradiation in an atmosphere of pure oxygen. The percentage of total erupt, however, still remains the same, and the high levels
of penetrance and expressivity of the erupt phenotype in strain 31 are still observed. Thus, the response of the suppressor-erupt system in strain 31 has not undergone any drastic change during the interim between the successive investigations.

Figure 2 is a graphic comparison of the results obtained in this study with strain 31 (Su-er;er), strain 36 (Su-er;er^t), and strain 13 (Su-er^t;er^t). Data obtained in the study by Glass and Plaine (1952) with the wild-type Oregon-R strain are included as an additional basis for comparison. Each strain has a characteristic response to X-ray treatment with respect to the level of incidence of erupt individuals observed. This level of penetrance of the mutant erupt phenotype for any given combination of second chromosome (suppressor-erupt locus) and third chromosome (erupt locus) after treatment, with 1000r of X-irradiation either in air or in 100% oxygen, is observed to be consistent and repeatable in each of the strains studied.

Analyses of data indicate no significant difference in the incidences of extreme erupt after X-irradiation in air among strains 36, (5.6%), 13 (1.8%), and the Oregon-R (3.5%). The percentage of extreme erupt individuals in strain 31 (65.0%) after X-irradiation in air differs significantly at the 1% level from the other three strains tested. Likewise, the incidence of total erupt after X-irradiation in air does not differ significantly in strains 36 (25.0%), 13 (18.8%), and the Oregon-R (29.7%). Strain 31 (92.3%) still differs significantly from the other three strains reported.
Figure 2. Comparison of the erupt response in four strains of *Drosophila melanogaster* after X-irradiation in air and in 100% oxygen. Column on left side represents X-ray in air. Column on right represents X-ray in 100% oxygen. The lower portion of each column represents extreme erupt. The upper portion of each column represents weak erupt. An entire column represents total erupt for the specified treatment.
While no significant difference is observed in the frequency of extreme erupt after X-irradiation in 100% oxygen between strain 13 (5.3%) and the Oregon-R strain (8.1%), there is a highly significant difference between the response of these two strains and that of strain 36 (28.7%). Strain 36 also differs significantly from strain 31 (71.2%) in the level of incidence of extreme erupt after X-irradiation in 100% oxygen. In spite of the possession of the same third chromosome (er\textsuperscript{+}), strain 13 (Su-er\textsuperscript{+};er\textsuperscript{+}) and 36 (Su-er;er\textsuperscript{+}) differ significantly from each other and from strain 31 (Su-er;er\textsuperscript{+}) which has the same second chromosome (Su-er) as strain 36.

On the basis of total erupt no difference is observed between strain 13 (32.3%) and the Oregon-R (34.1%) after irradiation in pure oxygen. However, strains 31 (98.6%) and 36 (58.0%) differ significantly from strain 13 and from each other. There is, therefore, a differential enhancement of the erupt response among these strains after X-irradiation in 100% oxygen which is not observed after X-irradiation in air. While the enhancement of the erupt response is differential among the strains, the only strain tested in which the level of erupt obtained in oxygen is significantly different from the level of erupt obtained in air is strain 36 (Su-er;er\textsuperscript{+}).

The response for both extreme and total erupt of strain 36 after X-irradiation in 100% oxygen lies approximately midway between the responses of the two strains from which it was derived, while after X-irradiation in air, the response of strain 36 is not distinguishable from that of strain 13 (Figure 2).
Strain 13 (Su-er⁺;er⁺) is observed to have the same range of low level incidence of the erupt phenotype as is observed in several wild-type strains of which the Oregon-R strain is representative (Glass, 1949, 1957). The same shift towards increased expressivity of extreme erupt after X-irradiation in 100% oxygen that is observed in the other low penetrance strains (strain 13 and strain 36) is also observed in the Oregon-R strain. After X-irradiation in air, extreme erupt in Oregon-R accounts for approximately one-eighth (3.5/29.7) of the total erupt class, whereas after X-irradiation in 100% oxygen, approximately one-fourth (8.1/34.1) of all individuals having the erupt phenotype are extreme erupt (Glass and Plaine, 1952).

The difference between the frequencies of total erupt after X-ray in air and X-ray in oxygen is greater in strain 13 (18.6%, 32.2%, resp.) than it is in Oregon-R (29.7%, 34.1%, resp.) (Figure 2). This same difference is found in strain 36 (Su-er⁺;er⁺) to an even greater degree (25.0% in air, 58.0% in O₂). No marked difference in total erupt is observed between X-irradiation in air and X-irradiation in 100% oxygen for either strain 31 (92.3%, 98.6%, resp.) or Oregon-R.

Figure 3 is a graphic representation of the enhancement of the expression of erupt after X-irradiation in an atmosphere of 100% oxygen. The frequency of each class of erupt (extreme and total) obtained in 100% oxygen was divided by the frequency of the same class obtained after X-irradiation in air to calculate and oxygen/air index for each class. An index of 1 on the scale indicates an equivalent response in air and in pure oxygen.
The fivefold increase in the frequency of extreme erupt in strain 36 (Su-er;er\(^+\)) is significantly higher than in any of the other three strains (Figure 3). The major effect of the increased oxygen concentration seems to be on the expressivity rather than on the penetrance of the erupt response. In each of the low penetrance strains (Oregon-R, strain 13 and strain 36), the frequency of extreme erupt in relation to total erupt (expressivity) is observed to increase much more than the overall incidence of total erupt (penetrance). Since both penetrance and expressivity in strain 31 (Su-er;er) are near the maximum after X-irradiation in air, it is not possible to detect an appreciable enhancement of either after X-irradiation in oxygen.
Figure 3. $O_2$/air indices for effectiveness of X-rays in revealing erupt in several strains of Drosophila melanogaster. An $O_2$/air index was calculated for each category of erupt by dividing the frequency of each class of erupt after X-irradiation in 100% $O_2$ by the frequency of the same class after X-irradiation in air.
INDUCTION OF ERUPT EYES BY X-IRRADIATION IN AIR AND IN AN ATMOSPHERE OF PURE OXYGEN IN SEVEN STRAINS OF DROSOPHILA MELANOGASTER DERIVED BY CHROMOSOME SUBSTITUTION

Materials and Methods

The experiments with strains 36, 13, and 31 indicated a consistent, low incidence of erupt in strains 36 (Su-er;er⁺) and 13 (Su-er⁺; er⁺), and the same high penetrance and expressivity in strain 31 (Su-er;er) that had characterized the response of the suppressor-erupt system in this strain from the time of its earliest analysis by Glass (1944). Certain strains, chosen on the basis of genetic differences at the two major loci comprising the suppressor-erupt system, were derived by chromosome substitution from the three parent strains (i.e., strains 36, 13 and 31). These derived strains with chromosome substitutions were subjected to treatments with X-irradiation in air and in an atmosphere of pure oxygen in the same manner as were the three parent strains in order to (1) confirm the presence of weak, though effective, mutant alleles of erupt in strains 36 and 13; (2) analyze the relationship between the two major loci of this coadapted genetic system (i.e., the suppressor of erupt and erupt); and (3) test for variations in response to X-ray treatment which might be found between different genotypes with respect to the suppressor-erupt system.

Control counts and all irradiations in air and in 100% oxygen were carried out in the same manner as described for the three parent
strains. Disks containing irradiated embryos (12±12 hr of embryonic development) were incubated at 25±1°C, and all adults were examined and classified with respect to the erupt phenotype as described for the three parent strains.

Upon completion of the chromosome substitutions, four single pair matings from each synthesized strain were set up. The descendants of each single pair mating were used as the basis for one of the four subcultures of each substitution strain. Experimental studies with the strains having chromosome substitutions were begun any time after the third generation following the establishment of the four subcultures of any given strain.

The results obtained from the two sexes, four subcultures, and replicated series for any given strain were grouped in the same manner as those obtained with the three parent strains, since analyses of variance indicated no significant difference between the sexes, subcultures, and replications. Duncan's multiple range test (MR) at the 1% level of significance was used to measure the existence of differences and to distinguish the differences between strains and specific treatments.

Each synthesized strain derived by chromosome substitution resulted from two major steps: (1) preparation of the parent strain for substitution of chromosomes II and III; and (2) substitution of chromosomes II and III from any specified strain into the parent.
strain so prepared (i.e., replacing own chromosome of parent strain with one from another strain). Figure 4 is a generalized mating scheme for the preparation of the respective parent strains for substitution of chromosomes II and III.

The mating scheme employed makes use of a marked inversion strain to aid in selection of the desired chromosomal combinations and to prevent recovery of cross-over products. This marked inversion strain (H-41, see Dros. Inf. Serv. 31:28, 1954) was procured from Purdue University and involves a system of inversions and balanced dominant lethals in each of the three major chromosome pairs (X, II, and III). The small fourth pair of chromosomes was not controlled directly although it does carry the recessive gene poliert (pol). Only non-poliert strains were selected for use in the final steps of the mating schemes. Although the fourth pair of chromosomes so far is not known to have any effect on the erupt response, selection was continued against poliert for at least three generations. None of the substitution strains used in this investigation produced any poliert individuals even after the tenth generation. The substitution strains used in this study are, therefore, assumed to be homozygous for the fourth chromosome of the original parent strain.

The source and genetic composition of the X chromosome in each strain was controlled, and is that of the original parent strain. The X chromosome of the H-41 strain carries
Figure 4. Generalized mating scheme for preparing parent strains for substitution of chromosomes II and III. Chromosome IV is not controlled in this mating scheme, ps indicates chromosome from parent strain.
an inversion and is marked by the recessive gene, white-apricot \( (w^a) \),
and the dominant gene, Bar eye \( (B) \), which differs in phenotype between
the homozygous and heterozygous state. The second chromosome pair
carries an inversion and the dominant lethal Curly \( (Cy) \) in one member
and the dominant lethal Plum \( (Pm) \) in its homologue. Stubble \( (Sb) \) to­
gether with an inversion, and Ultrabithorax \( (Ubx) \) form the balanced
lethal combination in the third chromosome pair.

Each of the three parent strains (viz., strains 36, 13 and 31)
was prepared according to the scheme outlined in Figure 4. The stock
for chromosome II substitution which results from this mating scheme
(left side, Figure 4) has a balanced lethal combination \( (Cy/Pm) \) in
place of its own homologous second pair of chromosomes. The resulting
stock for chromosome III substitution (right side, Figure 4) also has
a balanced lethal combination \( (Sb/Ubx) \) in place of its own homologous
third pair of chromosomes.

The mating scheme is so designed that the two stocks from any
parent strain may be prepared simultaneously (Figure 4). Since the
new stocks resulting from the mating scheme are balanced genetically,
they may be maintained indefinitely through inbreeding and be used
at any time for the second step of chromosome replacement.

The next major step in preparing the strains having chromosome
substitutions was to replace the balanced lethal combinations in the
prepared stocks of the parent strains with the second or third chromo­
some, respectively, from the desired genetic source. Figure 5 is an
outline of the mating scheme used to replace the balanced lethal combinations in prepared stocks from parent strain 36 with the second and third chromosomes, respectively, of strain 13. This same general procedure was used to derive all the other substitution strains used in this study.

Completing the mating scheme on the left side of Figure 5 results in a strain having the X and third chromosome of strain 36, but with the second chromosome of strain 13. Such a substitution strain is designated as 36 II13. Since the third chromosome of strain 36 (Su-er; er+) was originally derived from strain 13 (Su-er+; er+), this substitution should give a strain with the same genotype as strain 13 (Su-er+; er+) with respect to the suppressor-erupt system, and theoretically with the same response as strain 13 to X-irradiation. The mating scheme on the right side of Figure 5 results in a strain with the X and second chromosome of strain 36, but with the third chromosome of strain 13. This substitution strain would be designated as strain 36 III13. Theoretically, this strain should give the same response as strain 36 (Su-er; er+) to X-irradiation, since it has the same type of third chromosome (er+) as that in strain 36 in combination with the second chromosome and X chromosome of strain 36.

Table 6 lists the three parent strains and the strains derived from them by chromosome substitution which were used in this investigation. The genotypic constitution of each strain with respect to the suppressor-erupt (and suppressor-tumor) system is given. Each
Figure 5. Mating scheme for substitution of chromosomes II and III of strain 13 (Su-er⁺;er⁺) into Strain 36 (Su-er;er⁺). Chromosome IV is not controlled in this mating scheme.
A homologous pair of chromosomes is represented as a single line. Symbols above each line represent genotypic constitution. The number below each line designates the genetic source by laboratory strain number of each homologous pair of chromosomes. Each strain is homozygous for the major loci (suppressor of erupt and erupt) involved in this study.
TABLE 6

GENOTYPIC CONSTITUTION WITH RESPECT TO SUPPRESSOR-ERUPT AND SUPPRESSOR-TUMOR SYSTEMS OF THREE PARENT STRAINS AND SEVEN STRAINS DERIVED FROM THEM BY CHROMOSOME SUBSTITUTION

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parent Strains:</strong></td>
<td></td>
</tr>
<tr>
<td>36 (Su-er;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>36 Su-er tu bw er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>13 (Su-er⁺;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>13 al b Su-er⁺ c tu⁺ sp² er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>31 (Su-er;er)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>31 Su-er tu bw st er su-tu IV</td>
</tr>
<tr>
<td><strong>Substitution Strains:</strong></td>
<td></td>
</tr>
<tr>
<td>36 III₁₃ (Su-er;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>36 Su-er tu bw er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>36 II₃₁ (Su-er;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>36 Su-er tu bw er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>31 III₃₆ (Su-er;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>31 Su-er tu bw er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>13 II₃₆ (Su-er;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>13 Su-er tu bw er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>13 II₃₁ (Su-er;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>13 Su-er tu bw er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>36 II₁₃ (Su-er⁺;er⁺)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>36 al b Su-er⁺ c tu⁺ sp² er⁺ su-tu⁺ IV</td>
</tr>
<tr>
<td>36 III₃₁ (Su-er;er)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>36 Su-er tu bw st er su-tu IV</td>
</tr>
</tbody>
</table>
ANALYSES OF RESULTS

The results obtained with the strains which were derived by chromosome substitution from the three parent strains are presented according to the parent strain genotype which they most closely resemble (Table 6). The suppressor-erupt;erupt genotype of strain 36 (Su-er; er\(^+\)) is represented by five of the seven substitution strains listed in Table 6 (i.e., strain 36 III\(_{13}\), 36 II\(_{31}\), 31 III\(_{36}\), 13 II\(_{36}\), and 13 II\(_{31}\)). The difference in genetic source of the second and third chromosomes, as well as the difference in residual background (X and fourth chromosomes), forms a basis for comparison among the various substitution strains and between these strains and strain 36.

Table 7 is a summary of the erupt response induced in strain 36 III\(_{13}\). No extreme erupt was found in the control series, and only a very low percentage (0.6%) of all flies examined had facet disarrangement. Extreme erupt flies were found both after X-irradiation in air (3.6%) and in 100% oxygen (3.3%). These frequencies of the extreme erupt class differ significantly from the control at the 1% level of significance. The percentages of total erupt after X-irradiation in air (22.9%) and in 100% oxygen (37.0%) also differ significantly from the control at the 1% level.

Neither the decrease in the incidence of extreme erupt between X-irradiation in air (3.6%) and in 100% oxygen (3.3%), nor the increase
TABLE 7

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 36 III_{13} (Su-er;er^{+}) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>GENOTYPE: X</th>
<th>Su-er tu bw</th>
<th>er^{+} su-tu^{+}</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>36</td>
<td>13</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Phenotype of Eyes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray; 100% O_{2} 1000r</td>
<td>3</td>
<td>92</td>
<td>Normal: 63.0, Extreme Erupt: 3.3 (0.37), Total Erupt: 37.0±5.03**</td>
</tr>
<tr>
<td>X-ray; Air 1000r</td>
<td>2</td>
<td>83</td>
<td>Normal: 77.1, Extreme Erupt: 3.6 (0.41), Total Erupt: 22.9±4.61**</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1250</td>
<td>Normal: 99.4, Extreme Erupt: 0.0 (0.00), Total Erupt: 0.6 0.19</td>
</tr>
</tbody>
</table>

\[ a \text{All treatments were applied to 12±12 hr embryos.} \]
\[ b \text{The ± values are standard errors; bracketed figures are confidence limits at the 1\% level of significance (Stevens, 1942).} \]
\[ ** \text{Significant from control at 1\% level of significance.} \]

In total erupt from 22.9\% after X-irradiation in air to 37.0\% after X-irradiation in 100\% oxygen, is significant. The enhancement of the manifestation of extreme erupt after X-irradiation in 100\% oxygen is not apparent in strain 36 III_{13}.

Another strain which duplicates the genotype of strain 36 (Su-er; er^{+}) with respect to the suppressor-erupt system is strain 36 II_{31}. Strain 36 II_{31} has the X and third chromosomes of strain 36 in new combination with the second chromosome of strain 31 (Table 6). This second chromosome of strain 31 is theoretically the same as that in...
parent strain 36 since it was originally derived from strain 31. Table 8 summarizes the results obtained with strain 36 II 31.

**TABLE 8**

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 36 II 31 (Su-er;er+) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>GENOTYPE:</th>
<th>X 36</th>
<th>Su-er tu bw 31</th>
<th>er+ su-tu+ 36</th>
<th>IV 36</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Phenotype of Eyes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>X-ray; 100% O2 1000r</td>
<td>3</td>
<td>121</td>
<td>67.8</td>
</tr>
<tr>
<td>X-ray; Air 1000r</td>
<td>3</td>
<td>165</td>
<td>43.5</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1374</td>
<td>99.4</td>
</tr>
</tbody>
</table>

*aAll treatments were applied to 12±12 hr embryos.

bThe * values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942).

** Significant from control at 1% level of significance.

No extreme erupt flies were found in the control counts, and only 0.6% of the flies examined had disarranged facets. The frequency of extreme erupt after X-irradiation in 100% oxygen (2.5%), however, does not differ significantly from the control, and is significantly lower than the frequency of extreme erupt after X-irradiation in air at the
5% level of significance. The frequencies of total erupt after X-irradiation in air (57.5%) and after X-irradiation in pure oxygen (32.2%) differ significantly from the control. A definite decrease in the incidence of total erupt, as well as of extreme erupt, after treatment with X-rays in pure oxygen is observed in strain 36 II. This decrease is significant at the 1% level with respect to the total erupt category, and at the 5% level for extreme erupt.

Strain 31 III resynthesizes the Su-er;er+ genotype of strain 36 by replacing the third chromosome of strain 31 with that of strain 36 (Table 6). Strain 31 III (Su-er;er+) also has the same combination of second and third chromosomes as strain 36 II. Only the X and fourth chromosomes differ between the two strains (Table 6). Results obtained with the control series and after X-irradiation of strain 31 III are given in Table 9.

The incidences of extreme erupt after X-irradiation in air (4.6%) and after X-irradiation in 100% oxygen (5.5%) differ significantly from the non-irradiated control (Table 9). In addition, the frequencies of total erupt after treatment with X-rays in air (46.1%) and in pure oxygen (59.6%) differs significantly from the control. The increase in extreme erupt from 4.6% after X-irradiation in air to 5.5% after X-irradiation in pure oxygen, as also the increase in total erupt from 46.1% with X-rays in air to 59.6% with X-rays in 100% oxygen, is not significant.

The proportion of extreme erupt individuals out of all erupt flies
classified (4.6/46.1) after X-irradiation in air is approximately one-tenth. About this same proportion of extreme erupt individuals out of the total erupt class (5.5/59.6) is found after X-irradiation in 100% oxygen.

<table>
<thead>
<tr>
<th>TABLE 9</th>
</tr>
</thead>
</table>

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 31 III (Su-er;er⁺) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>GENOTYPE:</th>
<th>X</th>
<th>Su-er tu bw</th>
<th>er⁺ su-tu⁺</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>31</td>
<td>36</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Phenotype of Eyes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray; 100% O₂ 1000r</td>
<td>3</td>
<td>146</td>
<td>Normal 40.4</td>
</tr>
<tr>
<td>X-ray; Air 1000r</td>
<td>3</td>
<td>364</td>
<td>Normal 53.9</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>2161</td>
<td>Normal 99.3</td>
</tr>
</tbody>
</table>

a All treatments were applied to 12±12 hr embryos.
bThe * values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942).
** Significant from control at 1% level of significance.

The genotype of strain 36 (Su-er;er⁺) is also represented by two strains having chromosome substitutions in the genetic background of strain 13. The results obtained with strain 13 II₃₆ (Su-er;er⁺) after treatment with X-rays in air and in an atmosphere of 100% oxygen are given in Table 10.
TABLE 10

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 13 II_{36} (Su-er;er^{+}) OF DROSOPHILA MELANOGASTER

GENOTYPE: \( \frac{X}{13} \quad Su-er \quad tu \quad bw \quad \frac{er^{+} \quad su-tu^{+}}{13} \quad IV \quad \frac{13}{13} \)

<table>
<thead>
<tr>
<th>Treatment(^{a})</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Normal</th>
<th>Extreme Erupt</th>
<th>Total Erupt</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray; 100% O(_{2})</td>
<td>3</td>
<td>178</td>
<td>39.9</td>
<td>5.1 (1.76)</td>
<td>60.2 ± 3.67(^{b})**</td>
</tr>
<tr>
<td>X-ray; Air</td>
<td>3</td>
<td>205</td>
<td>41.5</td>
<td>3.4 (0.99)</td>
<td>58.5 ± 3.26**</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1332</td>
<td>97.7</td>
<td>0.0 (0.00)</td>
<td>2.3 (1.39)</td>
</tr>
</tbody>
</table>

\(^{a}\)All treatments were applied to 12±12 hr embryos.

\(^{b}\)The ± values are standard errors; bracketed figures are confidence limits at the 1\% level of significance (Stevens, 1942).

** Significant from control at 1\% level of significance.

The incidences of extreme (3.4\%) and total erupt (58.5\%) in strain 13 II_{36} after X-irradiation in air differ significantly from the control at the 1\% level. A significant difference also exists between the control series and the frequency of extreme (5.1\%) and total erupt (60.2\%) after X-irradiation in 100\% oxygen. However, the increase in the incidence of extreme and total erupt after X-irradiation in 100\% oxygen over that in air is not significant (Table 10).
A shift in expressivity of erupt is again observed in strain 13 II\textsubscript{36}. After treatment with X-rays in air, extreme erupt accounts for one-seventeenth (3.4/58.5) of the total erupt class, whereas after treatment with X-rays in 100% oxygen, approximately one-twelfth (5.1/60.2) of all erupt flies are of the extreme erupt phenotype. (Table 10).

The only difference between strains 13 II\textsubscript{36} (Su-er;er\textsuperscript{+}) and strain 13 II\textsubscript{31} (Su-er;er\textsuperscript{+}) is in the source of the second chromosome. The results obtained with strain 13 II\textsubscript{31} after treatments with X-rays in air and in 100% oxygen are summarized in Table 11.
TABLE 11

THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 13 II 31 (Su-er;er+) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>GENOTYPE: X Su-er tu bw er+ su-tu+ IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 31 13 13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Phenotype of Eyes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>X-ray; 100% O2 1000r</td>
<td>3</td>
<td>113</td>
<td>55.8</td>
</tr>
<tr>
<td>X-ray; Air 1000r</td>
<td>3</td>
<td>123</td>
<td>58.5</td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1214</td>
<td>98.1</td>
</tr>
</tbody>
</table>

aAll treatments were applied to 12-12 hr embryos.
bThe ± values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942).

** Significant from control at 1% level of significance.

Here, as in all the strains having the Su-er;er+ genotype, no case of extreme erupt was found in the control series. The frequency of extreme (4.1%) and total erupt (41.5%) after X-irradiation in air differ significantly from the control at the 1% level. There is also a significant difference between the control and the frequencies of extreme (3.5%) and total erupt (44.2%) after X-irradiation in pure oxygen. Neither the increase in total erupt between treatment in air (41.5%) and in oxygen (44.2%), nor the decrease in extreme erupt (4.1% in air, 3.5% in oxygen) are significant.
Whereas, the parent and other substitution strains tested tended to shift toward a higher expressivity of erupt after X-irradiation in oxygen (i.e., Oregon-R, strains 13, 36, 13 II 36), or to remain at approximately the same level of expressivity of erupt after X-irradiation in oxygen (i.e., strains 31 and 31 III 36), three strains were observed to shift toward the lower level of expressivity of extreme erupt after X-irradiation in oxygen. These three strains are strain 36 III 13 (viz., 3.6/22.9 in air; 3.3/37.0 in oxygen), strain 13 II 31 (viz., 4.1/41.5 in air; 3.5/44.2 in oxygen), and strain 36 II 31 (viz., 6.7/57.5 in air; 2.5/32.1 in oxygen).

One strain having the genotype of strain 13 (Su-er 13 ;er 13 ) was derived by substitution of the second chromosome from strain 13 into the genetic background of strain 36. The results obtained with strain 36 II 13 after X-irradiation in air and in 100% oxygen are given in Table 12.

The incidence of extreme erupt (5.2%) after X-irradiation in pure oxygen differs significantly from the control series, but the frequency of extreme erupt (1.7%) after X-irradiation in air does not. The frequencies of total erupt after X-irradiation in air (24.3%) and in 100% oxygen (42.3%) both differ significantly from the control at the 1% level of significance. There is, however, no significant difference in the frequency of extreme erupt between X-irradiation in air and in oxygen, but the frequency of total erupt after X-irradiation in oxygen is significantly higher than the frequency obtained after X-irradiation in air.
TABLE 12
THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 36 II13 (Su-er\textsuperscript{+}; er\textsuperscript{+}) OF DROSOPHILA MELANOGASTER

<table>
<thead>
<tr>
<th>GENOTYPE:</th>
<th>X</th>
<th>al b</th>
<th>Su-er\textsuperscript{+}</th>
<th>tu\textsuperscript{+}</th>
<th>sp\textsuperscript{2}</th>
<th>er\textsuperscript{+}</th>
<th>su-tu\textsuperscript{+}</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatmenta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray; 100% O\textsubscript{2} 1000r</td>
<td>3</td>
<td>116</td>
<td>57.7</td>
<td>5.2</td>
<td>(13.44\textsuperscript{**})</td>
<td>1.33</td>
<td>42.3\textsuperscript{+4.59}\textsuperscript{b\textsuperscript{**}}</td>
<td></td>
</tr>
<tr>
<td>X-ray; Air 1000r</td>
<td>3</td>
<td>701</td>
<td>75.7</td>
<td>1.7</td>
<td>(2.06)</td>
<td>0.71</td>
<td>24.3\textsuperscript{+1.62}\textsuperscript{**}</td>
<td></td>
</tr>
<tr>
<td>Non-irradiated Control</td>
<td>1</td>
<td>1155</td>
<td>99.2</td>
<td>0.0</td>
<td>(0.46)</td>
<td>0.00</td>
<td>0.8 (0.27)</td>
<td></td>
</tr>
</tbody>
</table>

\*All treatments were applied to 12\textsuperscript{+12} hr embryos.
\textsuperscript{b}The \( \pm \) values are standard errors; bracketed figures are confidence limits at the 1\% level of significance (Stevens, 1942).
\textsuperscript{**} Significant from control at 1\% level of significance.

A shift toward increased expressivity of extreme erupt is observed in strain 36 II\textsubscript{13}. After treatment with X-rays in air, extreme erupt accounts for approximately one-fourteenth (1.7/24.3) of the total erupt class, whereas, after treatment with X-rays in 100% O\textsubscript{2}, approximately one-eighth (5.2/42.3) of all erupt flies are extreme erupt. This shift in expressivity in strain 36 II\textsubscript{13} is to the same extent and in the same direction as that in the parent strain with the same genotype, strain 13, (Su-er\textsuperscript{+}; er\textsuperscript{+}).

A strain (36 III\textsubscript{31}) with the genotype of strain 31 (Su-er; er) in the genetic background of strain 36, was derived by substitution of
the third chromosome of strain 31 into strain 36. Table 13 summarizes the results obtained with strain 36 III^31.

**Table 13**

**THE ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF STRAIN 36 III^31 (Su-er;er) OF DROSOPHILA MELANOGASTER**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Series</th>
<th>Total Counted</th>
<th>Phenotype of Eyes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>X-ray; 100% O_2</td>
<td>3</td>
<td>134</td>
<td>4.5</td>
</tr>
<tr>
<td>X-ray; Air</td>
<td>3</td>
<td>133</td>
<td>5.3</td>
</tr>
<tr>
<td>Non-irradiated</td>
<td>1</td>
<td>1660</td>
<td>96.4</td>
</tr>
</tbody>
</table>

^aAll treatments were applied to 12±12 hr embryos.

^bThe ± values are standard errors; bracketed figures are confidence limits at the 1% level of significance (Stevens, 1942)

**Significant from control at 1% level of significance.**

Strain 31 (Su-er;er) was the only parent strain in which extreme erupt flies were found among the control series (0.1%). So too, strain 36 III^31 (also Su-er;er) is the only substitution strain in which extreme erupt individuals were found among the controls (0.2%). There is, however, no significant difference among the control series of all strains examined for extreme and total erupt.

In strain 36 III^31, the frequencies of extreme (72.9%) and total erupt (94.7%) after X-irradiation in air, as well as the frequencies
of extreme (73.9%) and total erupt (95.5%) after X-irradiation in 100% oxygen, differ significantly from the control at the 1% level. There is no significant difference between the incidence of extreme erupt after X-irradiation in air (72.9%) and after X-irradiation in 100% oxygen (73.9%), or between the incidence of total erupt after X-irradiation in air (94.7%) and after X-irradiation in 100% oxygen (95.5%) (Table 13).

As with strain 31, no shift in the level of expressivity of extreme erupt is observed between X-irradiation in air and in oxygen with strain 36 III_31. Extreme erupt accounts for approximately five-sixths of the total erupt class both after X-irradiation in air (72.9/94.7) and in oxygen (73.9/95.5). Strain 36 III_31 has the highest level of penetrance and expressivity for the erupt response of all the substitution strains tested. Only strain 36 III_31 has the genotype of the suppressor-erupt strain (strain 31) and its response is in all ways the same as that in strain 31 (Table 14).

A graphic comparison of the responses of the seven substitution strains with respect to the suppressor-erupt system after treatment with X-rays in air and in 100% oxygen is given in Figure 6.

All five strains with the Su-er; er+ genotype (i.e., strains 36 III_31, 36 II_31, 31 III_36, and 13 II_31) have a low incidence of extreme erupt after X-irradiation in air and in 100% oxygen. There is no significant difference among these five strains with respect to the incidence of extreme erupt either after X-irradiation in air or in 100% oxygen. A low incidence of extreme erupt after X-irradiation in air and in 100% oxygen is also observed in strain 36 II_13.
TABLE 14
SUMMARY OF ERUPT EYE RESPONSE IN X-IRRADIATED AND NON-IRRADIATED SERIES OF THREE PARENT STRAINS AND SEVEN STRAINS DERIVED FROM THEM BY CHROMOSOME SUBSTITUTION

<table>
<thead>
<tr>
<th>Strain Genotype</th>
<th>Non-irradiated Control</th>
<th>X-ray; Air 1000r</th>
<th>X-ray; 100% O₂ 1000r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Count</td>
<td>Extreme Erupt %</td>
<td>Total Erupt %</td>
</tr>
<tr>
<td>Su-er; er⁺</td>
<td>1511</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>13 Su-er⁺; er⁺</td>
<td>1250</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>31 Su-er; er</td>
<td>1227</td>
<td>0.1</td>
<td>5.9</td>
</tr>
<tr>
<td>36 III₁₃ Su-er; er⁺</td>
<td>1250</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>36 II₃₁ Su-er⁺</td>
<td>1374</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>31 III₃₆ Su-er⁺</td>
<td>2161</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>13 II₃₆ Su-er⁺</td>
<td>1332</td>
<td>0.0</td>
<td>2.3</td>
</tr>
<tr>
<td>13 II₃₁ Su-er⁺</td>
<td>1214</td>
<td>0.0</td>
<td>1.9</td>
</tr>
<tr>
<td>36 II₁₃ Su-er⁺</td>
<td>1155</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>36 III₃₁ Su-er⁺</td>
<td>1660</td>
<td>0.2</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Figure 6. Comparison of the erupt response in seven strains derived by chromosome substitution after X-irradiation in air and in 100% oxygen. Column on left side represents X-ray in air. Column on right represents X-ray in 100% oxygen. The lower portion of each column represents extreme erupt. The upper portion of each column represents weak erupt. An entire column represents total erupt for the specified treatment.
(Su-er\(^+\);er\(^+\)) which has the same genotype as parent strain 13. The highest levels of extreme and total erupt after X-irradiation in air and in 100% oxygen are found in strain 36 III\(_{31}\) (Su-er;er) which has the same genotype as the original suppressor-erupt strain (strain 31). LMR tests at the 1% level of significance based on the percentages of extreme erupt in the seven substitution strains after X-irradiation in air and in 100% oxygen rank these strains as follows:

**Extreme Erupt - X-rays; Air**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>36II(<em>{13}) 13II(</em>{36}) 36III(<em>{13}) 13II(</em>{31}) 31III(<em>{36}) 36II(</em>{31}) 36III(_{31})</td>
<td>1.7% 3.4% 3.6% 4.1% 4.6% 6.7% 72.9%</td>
</tr>
</tbody>
</table>

**Extreme Erupt - X-rays; 100% O\(_2\)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>36II(<em>{31}) 36III(</em>{13}) 13II(<em>{31}) 13II(</em>{36}) 36II(<em>{13}) 31III(</em>{36}) 36III(_{31})</td>
<td>2.5% 3.3% 3.5% 5.1% 5.2% 5.5% 73.9%</td>
</tr>
</tbody>
</table>

There is no significant difference at the 1% level in the incidence of extreme erupt after X-irradiation in air or in oxygen between these five strains with the Su-er;er\(^+\) genotype and strain 36 II\(_{13}\) (Su-er\(^+\); er\(^+\)). Only strain 36 II\(_{31}\) differs significantly at the 5% level from strain 36 II\(_{13}\) for extreme erupt after X-irradiation in air. Strain 36 III\(_{31}\) (Su-er;er) differs significantly at the 1% level from the other six strains for extreme erupt after X-irradiation in air and in 100% oxygen.

The incidence of total erupt after X-irradiation in air is higher in strains 36 II\(_{31}\), 31 III\(_{36}\), 13 II\(_{36}\), and 13 II\(_{31}\) (all with the Su-er; er\(^+\) genotype but with third chromosomes from either strain 36 [er\(^+\)]
or strain 13 [er+]），than the incidence of total erupt after the same
treatment in strain 36 II13 (Su-er+;er+) and strain 36 III13 (Su-er;er+
but with third chromosome [er+] from parent strain 13) (Figure 6). A
DNR test based on the percentages of total erupt after X-irradiation in
air ranks the seven substitution strains as follows,

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incidence of Total Erupt</th>
</tr>
</thead>
<tbody>
<tr>
<td>36III13</td>
<td>22.9%</td>
</tr>
<tr>
<td>36II13</td>
<td>24.3%</td>
</tr>
<tr>
<td>31III36</td>
<td>46.1%</td>
</tr>
<tr>
<td>13II31</td>
<td>41.5%</td>
</tr>
<tr>
<td>36II31</td>
<td>57.5%</td>
</tr>
<tr>
<td>13II36</td>
<td>58.5%</td>
</tr>
<tr>
<td>36III31</td>
<td>94.7%</td>
</tr>
</tbody>
</table>

Strains 36 III13 and 36 II13, which do not differ from each other,
differ significantly at the 1% level from the four strains 31III36,
13 II31, 36 II31, and 13 II36, which, likewise, do not differ from one
another. Strain 36 III31 differs significantly from these four strains
and from strains 36 III13 and 36 II13.

Due to the differential effect of the presence of oxygen on the
response of the suppressor-erupt system in the various substitution
strains, a comparison of the incidences of total erupt after X-irradi-
ation in 100% oxygen presents a somewhat different picture from that
observed after X-irradiation in air. Enhancement of the manifesta-
tion of extreme erupt and enhancement of the incidence of total erupt
in strain 36 II13, after X-irradiation in oxygen, raises the incidence
of total erupt in this strain to the same level as the incidence of
total erupt in strain 13 II31 after the same treatment (Figure 6).
The striking decrease in the incidence of both extreme and total
erupt in strain 36 II31 after X-irradiation in 100% oxygen, relative
to the results obtained in air, lowers the incidence of total erupt
for this strain to the same level as that found in strain 36 III\textsubscript{13} (Figure 6). The incidence of both extreme and total erupt in strain 36 III\textsubscript{31} are significantly higher than those in any of the other six substitution strains (Figure 6). Based upon the frequencies of total erupt following X-ray in oxygen, a DMR test at the 1\% level of significance ranks the strains as follows:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>36II\textsubscript{31}</td>
<td>36III\textsubscript{13}</td>
</tr>
<tr>
<td>32.2%</td>
<td>37.0%</td>
</tr>
</tbody>
</table>

Strains 36 II\textsubscript{31} and 36 III\textsubscript{13} (both Su-er;er\textsuperscript{+}) differ significantly from strains 31 III\textsubscript{36} and 13 II\textsubscript{36} (also Su-er;er\textsuperscript{+}). Strain 36 II\textsubscript{13} (Su-er\textsuperscript{+};er\textsuperscript{+}) and strain 13 II\textsubscript{31} (Su-er;er\textsuperscript{+}) do not differ significantly at the 1\% level from strains 36 II\textsubscript{31} and 36 III\textsubscript{13}, or from strains 31 III\textsubscript{36} and 13 II\textsubscript{36}. At the 5\% level, strains 36 II\textsubscript{13} and 13 II\textsubscript{31} do differ from strain 36 II\textsubscript{31} and strain 13 II\textsubscript{36}.

This differential response between X-irradiation in air and in 100\% oxygen with respect to the extreme and total erupt categories among the various substitution strains was also observed among the parent strains (Figure 3). Since the major difference among these strains is in the combination of second and third chromosomes (Suppressor of erupt and erupt loci), it seems that different combinations of suppressor of erupt and erupt alleles at these loci vary in their response to X-irradiation in 100\% oxygen. In order to present more clearly the effect of X-irradiation in an atmosphere of 100\% oxygen...
on the expression of erupt, $O_2/air$ indices were calculated for extreme and total erupt for each of the seven substitution strains (Figure 7). An $O_2/air$ index was calculated for each strain by dividing the frequency of extreme and total erupt after X-irradiation in 100% oxygen by the frequency of the comparable phenotype after X-irradiation in air. An index of 1 indicates an equivalent response in air and in pure oxygen.

No enhancement of either the incidence of extreme or total erupt after X-irradiation in oxygen over that obtained in air is observed in strains 36 $III_{31}$ (Su-er;er) and 31 $III_{36}$ (Su-er;er$^+$) (Figure 7). Enhancement of both the incidence of extreme erupt (expressivity) and to a lesser degree the incidence of total erupt (penetrance) is observed in strain 36 $II_{13}$ (Su-er$^+$;er$^+$). The inverse of the response of strain 36 $II_{13}$ after X-irradiation in 100% oxygen is observed in strain 36 $II_{31}$. In other words, in strain 36 $II_{31}$ (Su-er; er$^+$) the incidence of extreme erupt (expressivity) and total erupt (penetrance) are lower than those obtained after X-irradiation in air (Figure 7).

In strain 13 $II_{31}$ (Su-er;er$^+$) which has the same second chromosome as strain 36 $II_{31}$ (Su-er;er$^+$), but with different X and third chromosomes (Table 6), there is also a decrease in incidence of extreme erupt (expressivity) after X-irradiation in oxygen. The penetrance in strain 13 $II_{31}$ after X-irradiation in oxygen is, however, the same as that obtained after X-irradiation in air (Figure 7).
Figure 7. $O_2$/Air Indices for effectiveness of X-rays in revealing erupt in seven strains derived by chromosome substitution. An $O_2$/air index was calculated for each category of erupt by dividing the frequency of each class of erupt after X-irradiation in 100% $O_2$ by the frequency of the same class after X-irradiation in air.
A reverse response is observed between strains 13 II₃₆ (Su-er;er⁺) and 36 III₁₃ (Su-er;er⁺) after X-irradiation in 100% oxygen (Figure 7). In strain 13 II₃₆ there is an enhancement of the incidence of extreme erupt (expressivity) without a change in incidence of total erupt (penetrance), while in strain 36 III₁₃ there is an enhancement of the incidence of total erupt (penetrance) without an appreciable change in the incidence of extreme erupt (expressivity). Strains 36 III₁₃ and 13 II₃₆ have the same second and third chromosomes and differ only in their X and fourth chromosomes (Table 6).

Table 14 is a summary of the results obtained for the three parent strains and all seven substitution strains used in this investigation. A frequency profile of the erupt response for all parent and substitution strains used in this investigation is given in Figure 8. This profile is based on the incidences in percentage for extreme and total erupt after treatment with X-rays in air and in oxygen, respectively.

With respect to the suppressor-erupt system, the only difference between the genotypes of strain 36 and strain 36 III₁₃ is in the source of the third chromosome (Table 6). Strain 36 III₁₃ possesses the third chromosome of strain 13, from which the third chromosome in strain 36 was originally derived about 1954. The incidence of extreme erupt after X-irradiation in air (3.6%) for strain 36 III₁₃ is lower than the frequency of extreme erupt after comparable treatment (5.6%) in strain 36 (Table 14). There is, however, no significant
Figure 8. Frequency profiles for extreme and total erupt after X-irradiation in air and in 100% oxygen for the three parent strains and the seven substitution strains. Breaks in the ordinate separate groups between which significant differences exist and within which differences are not significant, according to Duncan's multiple range test at the 1% level of significance.
difference in the incidence of extreme erupt after X-irradiation in air between strain 36 and strain $36 \text{III}_{13}$ (Figure 8). Likewise, the incidence of total erupt after X-irradiation in air in strain $36 \text{III}_{13}$ (22.9%) is equivalent to the incidence of total erupt after the same treatment in strain 36 (25.0%) (Table 14 and Figure 8).

Only with respect to the incidence of both extreme and total erupt after X-irradiation in 100% oxygen is the response in strain $36 \text{III}_{13}$ significantly different from that of strain 36 (Figure 8). Strain 36 has a striking increase in the enhancement of the extreme erupt class after X-irradiation in 100% oxygen over that obtained after irradiation in air (from 5.6% to 28.7%), while strain $36 \text{III}_{13}$ has a non-significant decrease in extreme erupt after X-irradiation in 100% oxygen (from 3.6% to 3.3%) (Table 14). The incidence of total erupt after X-irradiation in oxygen in strain $36 \text{III}_{13}$ (37.0%) is significantly lower at the 1% level than that in strain 36 (58.0%) for the same treatment (Table 14 and Figure 8).

Strain $36 \text{II}_{31}$ has the X and third chromosome of strain 36 in new combination with the second chromosome of strain 31 (Table 6). This second chromosome of strain 31 is theoretically the same as that in parent strain 36 since it was originally derived from strain 31 about 1954. There is no significant difference between the occurrence of extreme erupt after X-irradiation in air (6.7%) in strain $36 \text{II}_{31}$ and that in strain 36 (5.6%) (Table 14). However, a significant difference in the response of the suppressor-erupt system
between these two strains becomes evident after X-irradiation in oxygen (Figure 8). In strain 36, the incidence of extreme erupt increases significantly (5.6% to 28.7%), while in strain 36 II_{31} the incidence of extreme erupt decreases significantly (6.7% to 2.5%) (Table 14). This same type of response is observed with respect to total erupt after treatment with X-rays in 100% oxygen; in strain 36 total erupt increases from 25.0% in air to 58.0% in O_2 as opposed to a decrease from 57.5% in air to 32.2% in O_2 in strain 36 II_{31} (Table 14). Both the increase in total erupt in strain 36 after X-irradiation in oxygen over that obtained in air, and the decrease in total erupt after irradiation in oxygen in strain 36 II_{31} are significant at the 1% level (Figure 8). However, on the basis of the frequency of total erupt after X-irradiation in air, strain 36 II_{31} has a significantly higher incidence (57.5%) than strain 36 (25.0%) (Table 14 and Figure 8).

In strain 31 III_{36}, the third chromosome of the original parent strain 31 has been replaced by the third chromosome from strain 36. This substitution gives the same genotype, Su-er;er^+, as that of strain 36, but with the second and X chromosomes of strain 31 (Table 6).

Although strains 31 III_{36} (4.6%) and 36 (5.6%) have approximately the same incidence of extreme erupt after X-irradiation in air, the incidence of total erupt after the same treatment differs between these two strains (Table 14). The incidence of total erupt after X-irradiation in air in strain 31 III_{36} (46.1%) is significantly
higher at the 1% level than the incidence of total erupt after the same treatment in strain 36 (25.0%) (Figure 8). Thus, the suppressor-erupt system in strain 31 III \textsubscript{36} is characterized by a higher penetrance (i.e., higher incidence of total erupt) and a lower expressivity (i.e., smaller proportion of extreme erupt out of total erupt observed) than the suppressor-erupt system in parent strain 36.

After treatment with X-rays in 100% oxygen, the manifestation of extreme erupt (28.7%) in strain 36 is enhanced significantly over that obtained after irradiation in air (5.6%), while there is no significant difference between the manifestation of extreme erupt (5.6%) after irradiation in oxygen and that obtained in air (4.6%) for strain 31 III \textsubscript{36} (Table 14 and Figure 8). Therefore, while the incidence of total erupt after X-irradiation in oxygen (58.0%) in strain 36 does not differ significantly from that in strain 31 III \textsubscript{36} (59.6%), the manner of response of the suppressor-erupt systems leading to this result is not the same in these two strains. In the case of strain 36, the incidence of total erupt after X-ray in oxygen involves a significant increase in both penetrance and expressivity of the suppressor-erupt system; whereas, in the case of strain 31 III \textsubscript{36} the incidence of total erupt after X-ray in oxygen involves no significant change in either penetrance or expressivity of the suppressor-erupt system (Figure 8).

The same genotype as that of strain 36 (Su-er;er\textsuperscript{+}) was also derived by replacing the second chromosome (Su-er\textsuperscript{+}) of parent strain 13 with the second chromosomes (Su-er) from strains 36 and 31 giving
strains 13 II\textsubscript{36} and 13 II\textsubscript{31}, respectively (Table 6). The incidences of extreme erupt after X-irradiation in air in strain 13 II\textsubscript{36} (3.4%) and in strain 13 II\textsubscript{31} (4.1%) do not differ significantly from the frequency of extreme erupt in strain 36 (5.6%) after the same treatment (Table 14 and Figure 8). However, the incidences of total erupt after X-irradiation in air for strain 13 II\textsubscript{36} (58.5%) and strain 13 II\textsubscript{31} (41.5%) are significantly higher than the incidence of total erupt after the same treatment in strain 36 (25.0%) (Table 14 and Figure 8).

In strain 13 II\textsubscript{36}, an increase in the incidence of extreme erupt is observed after X-irradiation in oxygen (3.4% to 5.1%), while in strain 13 II\textsubscript{31} a decrease in the incidence of extreme erupt (4.1% to 3.5%) is observed after the same treatment (Table 14). Neither the increase in strain 13 II\textsubscript{36}, nor the decrease in strain 13 II\textsubscript{31} are statistically significant (Figure 8). However, this enhancement of extreme erupt in strain 13 II\textsubscript{36} after irradiation in oxygen (Figure 7) parallels at a lower level the enhancement of extreme erupt observed after X-irradiation in oxygen in strain 36 (Figure 3). The response for extreme erupt after irradiation in oxygen in strain 13 II\textsubscript{31} parallels to a less degree the response of strain 36 II\textsubscript{31} after X-irradiation in oxygen (Figure 7).

On the basis of total erupt after X-irradiation in 100% oxygen, there is no significant difference between strain 36 (58.0%) and strain 13 II\textsubscript{36} (60.2%) (Table 14 and Figure 8). Total erupt in
13 II₃₁ (44.2%) differs significantly at the 5% level from both strain 36 and strain 13 II₃₆ for the same treatment.

Although there is no significant difference in the incidence of total erupt after X-irradiation in oxygen between strains 36 and 13 II₃₆, the manner of response of these two strains is different. In strain 36, the higher level of total erupt after X-irradiation in oxygen is due to a significant enhancement of the incidence of both extreme and total erupt after irradiation in oxygen over that obtained in air, while in strain 13 II₃₆, the level of total erupt after X-irradiation in oxygen is not significantly higher than the existing high level of total erupt obtained after X-irradiation in air.

Analyses of relationships among the remaining parent strains (strain 13 and strain 31) and the seven substitution strains by means of a DMR test of significance indicate no significant difference at the 1% level among any of the strains with the Su-er;er⁺ or Su-er⁺;er⁺ genotypes for extreme erupt after X-irradiation in air (Figure 8). Strain 36 III₃₁ (Su-er;er) and strain 31 (Su-er;er) differ consistently from the other two parent and six substitution strains but not from each other (Figure 8). The strains, excluding strains 31 and 36 III₃₁, are grouped as follows:

<table>
<thead>
<tr>
<th>Strain</th>
<th>%</th>
<th>Strain</th>
<th>%</th>
<th>Strain</th>
<th>%</th>
<th>Strain</th>
<th>%</th>
<th>Strain</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>36II₁₃</td>
<td>1.7%</td>
<td>13</td>
<td>1.8%</td>
<td>13II₃₆</td>
<td>3.4%</td>
<td>36III₁₃</td>
<td>3.6%</td>
<td>13II₃₁</td>
<td>4.1%</td>
</tr>
<tr>
<td>31III₃₆</td>
<td>4.6%</td>
<td>36</td>
<td>5.6%</td>
<td>36II₃₁</td>
<td>6.7%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only strains 36 and 36 II₃₁ differ at the 5% level from strains 36 II₁₃ and 13.
After X-irradiation in oxygen a DMR test, based on the percentage of extreme erupt, groups the strains, excluding strain 31 and strain 36 III31, as follows:

<table>
<thead>
<tr>
<th>36II31</th>
<th>36III13</th>
<th>13II31</th>
<th>13II36</th>
<th>36II13</th>
<th>13</th>
<th>31III36</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5%</td>
<td>3.3%</td>
<td>3.5%</td>
<td>5.1%</td>
<td>5.2%</td>
<td>5.3%</td>
<td>5.5%</td>
<td>28.7%</td>
</tr>
</tbody>
</table>

Five substitution strains have the same Su-er;er+ genotype as strain 36, but none of these substitution strains have the same combination of second and third chromosomes as that found in strain 36. After X-ray in oxygen, strain 36 differs significantly from all the other strains tested. The response for extreme erupt after irradiation in oxygen found in strain 36, is not observed in any other strain tested.

Analyses on the basis of total erupt after X-irradiation in air by means of a DMR test group the strains, excluding strains 31 and 36 III31, as follows:

<table>
<thead>
<tr>
<th>13</th>
<th>36III13</th>
<th>36</th>
<th>36II13</th>
<th>13II31</th>
<th>31III36</th>
<th>36II31</th>
<th>13II36</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.8%</td>
<td>22.9%</td>
<td>25.0%</td>
<td>24.3%</td>
<td>41.5%</td>
<td>46.1%</td>
<td>57.5%</td>
<td>58.5%</td>
</tr>
</tbody>
</table>

while on the basis of total erupt after X-irradiation in oxygen the grouping of the strains, excluding strains 31 and 36 III31 as previously, is

<table>
<thead>
<tr>
<th>36II31</th>
<th>13</th>
<th>36III13</th>
<th>36II13</th>
<th>13II31</th>
<th>36</th>
<th>31III36</th>
<th>13II36</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.1%</td>
<td>32.2%</td>
<td>37.0%</td>
<td>42.3%</td>
<td>44.2%</td>
<td>58.1%</td>
<td>59.6%</td>
<td>60.2%</td>
</tr>
</tbody>
</table>
For each strain an overall mean response was calculated, based on the summation of the mean incidences of extreme and total erupt for the control, the series irradiated in air, and the series irradiated in oxygen. Since this analysis of overall response includes a measure of penetrance as well as a measure of expressivity, it is a useful measure of relationship among the various strains tested with respect to the suppressor-erupt system. The DMR test at the 1% level of significance, based on the overall mean response for each strain indicates the following relationships:

\[
\begin{align*}
13 & 36III_{13} 36II_{13} 36II_{31} 13II_{31} 31III_{36} 36 13II_{36} 36III_{31} 31 \\
9.9% & 11.1% 12.7% 16.5% 16.9% 19.8% 20.0% 21.7% 52.7% 55.4%
\end{align*}
\]

The overall mean response obtained by replacing the third chromosome (er*) of strain 36 with the third chromosome of strain 31 and its erupt allele (er) is significantly different from the response in strain 36. The response of this substitution strain (36 III_{31}; i.e., Su-er;er) in no way differs from that of the original suppressor-erupt strain (31; i.e., Su-er;er).

The DMR test on the basis of overall mean response confirms the similar patterns of response between strain 13 and strain 36 II_{13}.

The second chromosome of strain 13, when in new combination with the third chromosome of strain 36, is an effective suppressor of the erupt response. The overall percentage of response for strain 36 III_{13} (12.7%) differs significantly from that for strain 36 (20.0%).
The third chromosome (er$^+$) of strain 13, when substituted against the suppressor of erupt present in strain 36, still produces erupt at a level significantly lower (11.1%) than that in strain 36 (20.0%), but not significantly different from that in strain 13 (9.9%). The responses of strains 13, 36 III$_{13}$, and 36 II$_{13}$ differ significantly from those of strains 31 III$_{36}$, 36, and 13 II$_{36}$.

Strains 36 II$_{31}$ and 13 II$_{31}$ differ significantly at the 5% level from strains 31 III$_{36}$, 36, and 13 II$_{36}$.
DISCUSSION

Throughout this investigation, a consistent low incidence of the phenotype characteristic of extreme erupt was observed among flies of strains 36 (Su-er;er\(^{+}\)) and 13 (Su-er\(^{+}\);er\(^{+}\)) which had been exposed to 1000r of X-rays in air or in 100% oxygen. This occurrence of the extreme erupt phenotype after X-irradiation was also observed in each of the various substitution strains which had either the third chromosome of strain 36 or that of strain 13, regardless of the type of second chromosome present (Su-er or Su-er\(^{+}\)). The occurrence of any erupt response indicates some genetic basis for the mutant phenotype in the strain. By convention, the designation of an allele as wild-type (symbolized by \(+\)) means that the allele is normally non-effective with respect to production of the mutant phenotype. The alleles of erupt present in the third chromosomes of strains 36 and 13 are not "non-effective" with respect to production of the mutant erupt phenotype and should not, therefore, be designated as er\(^{+}\) or wild-type alleles.

Historically, the designation of the erupt allele in the al b c sp\(^{2}\) strain (strain 13) as a wild-type, non-effective allele of erupt dates back to the work Glass (1944) carried out with the suppressor-erupt strain, strain 31 (Su-er;er), and the al b c sp\(^{2}\) strain, strain 13, in 1941.

The response of the suppressor-erupt system in strain 31 at the time of Glass' study was just as striking as it is today. The high
levels of penetrance and expressivity which are characteristic of the suppressor-erupt -- erupt combination in strain 31 still stand in sharp contrast to the low penetrance and expressivity of the al b c sp² strain. Strain 36 had not yet been derived at the time of these studies and was not, therefore, involved in the work reported in 1944. Furthermore, since the role of the presence of oxygen as an enhancer of the effect of X-irradiation in general was not well known at this time, the treatments of strains 31 (suppressor-erupt) and 13 (al b c sp²) with X-rays were carried out only in air. The first work indicating the role of oxygen in the response of the suppressor-erupt system to X-irradiation was conducted much later (Glass and Plaine, 1952; Plaine, 1955a).

The actual number of flies involved in the exposure of the al b c sp² strain to X-irradiation in air was not given (Glass, 1944). However, judging from the total numbers of flies of the suppressor-erupt strain which were used in 1941 (221 flies) and in 1942 (89 flies), one may assume that the total number was not greater than 200 to 300 flies. This number may have been much smaller since viability in the al b c sp² strain is low under standard culture conditions and is even lower after X-irradiation. While in this present investigation extreme erupt flies did occur in the al b c sp² strain after X-irradiation in air, the incidence of extreme erupt was quite low (1.8% out of 1268 flies) and was not statistically significant from the control (0.0% out of 1250 flies). On sheer basis of numbers, then, it would have been highly probable to miss extreme erupt in the
al b c sp\(^2\) strain after X-irradiation in air. In fact, with a frequency of extreme erupt ranging between 1 to 2\%, a total count of between 1183 and 2053 flies are required to come within an 80 to 90\% confidence level of obtaining any extreme erupt flies at all. With a total count of less than 345 flies the probability of finding extreme erupt is lower than 50\%.

Moreover, the erupt phenotype in the suppressor-erupt strain after X-irradiation in air is characterized by a high expressivity of the erupt response (i.e., the majority of individuals have extreme manifestations of erupt characterized by palp-like protrusions and extra bristles). In the al b c sp\(^2\) strain, on the other hand, palp-like protrusions and extra bristles are rare. Extreme erupt in this strain is usually characterized by a break or hole in the surface of the eye without extrusion of facial material through the break.

That Glass did not regard facet disarrangement as a weak manifestation of erupt at the time of the 1941 studies of the suppressor-erupt and al b c sp\(^2\) strains is evident from his 1944 paper. The suppressor-erupt strain, after treatment with X-rays in air, was classified only according to extreme erupt in the 1941 series. Glass also states, with respect to outcrossing experiments conducted after the original irradiation experiments in 1941, "In examining flies of this generation, it was noticed for the first time that many of them had slight disarrangements of the ommatidia, usually in the center of the eye where the extrusion takes place in erupt individuals" (Glass, 1944; italics mine). The first classification by Glass of
flies as weak erupt after X-irradiation is found with respect to the suppressor-erupt strain during the experiments in 1942. No further experiments were conducted, however, with the al b c sp\(^2\) strain in 1942.

Since no further experiments using X-irradiation in air, or in 100% oxygen, were reported for the al b c sp\(^2\) strain until the present study, the original designation of the erect gene as er\(^+\) in this strain continued to be accepted. Strain 36 (Su-er;er\(^+\)), its third chromosome originally derived from the al b c sp\(^2\) strain (Su-er\(^+\); er\(^+\)), inherited the er\(^+\) designation at its erupt locus. The first report of X-irradiation of strain 36 is that of Burnet and Sang (1964b), and they considered only the melanotic tumor locus and its specific suppressor locus. Burnet and Sang did not examine the flies of this strain for erupt after X-irradiation.

Since outcrossing of the second chromosome of the al b c sp\(^2\) strain against the third chromosome of the suppressor-erupt strain gave an incidence of 88% extreme erupt, Glass (1944) concluded that the al b c sp\(^2\) strain carried in its second chromosome a gene (or genes) permitting strong manifestation of erupt. Exactly why this gene (or genes) in the second chromosome came to be designated as a wild-type, non-effective allele (Su-er\(^+\)) (Glass, 1944) of the suppressor of erupt is not entirely clear; but the designation has remained until the present time, and has been used as a basis for analysis of the response of the suppressor-erupt system (Glass, 1944, 1949, 1957; Glass and Plaine, 1950), and also of the melanotic tumor system (Burnet and Sang, 1964a,b).
The designation of the wild-type alleles for the suppressor of tumor and the tumor loci was transferred to the reciprocal melanotic tumor system in strain 13 (Su-er\textsuperscript{+} tu\textsuperscript{+}; er\textsuperscript{+} su-tu\textsuperscript{+}), and later to strain 36 (Su-er tu bw; er\textsuperscript{+} su-tu\textsuperscript{+}) due to its chromosome derivation, although Glass (1944) states, "The emerging flies (al b c sp\textsuperscript{2}) were feable, frequently crippled, and tumorous, as in the case of the bw;st (suppressor-erupt; suppressor-tumor) treated flies, but in not a single individual was the erupt characteristic to be observed" (parentheses and italics added).

In the present investigation, the second chromosome of the al b c sp\textsuperscript{2} strain, supposedly carrying a wild-type, non-effective suppressor of erupt (Su-er\textsuperscript{+}), was substituted into strain 36 (Su-er;er\textsuperscript{+}). The resulting substitution strain, 36 II\textsubscript{13}, has the second chromosome (theoretically Su-er\textsuperscript{+}) of strain 13 in new combination with the third chromosome (theoretically er\textsuperscript{+}) and the X-chromosome of strain 36, reconstituting strain 13 with respect to the suppressor-erupt duplex. If the substituted second chromosome actually carries a non-effective allele of the suppressor of erupt, one would expect either no change at all in the response of erupt or an increase in the erupt response after irradiation, were the action of the erupt gene directly enhanced, since a non-effective suppressor would not be effective against the erupt allele. The overall mean response of erupt in strain 36 II\textsubscript{13} was, however, observed to be significantly lower than that of strain 36, and was the same as that of strain 13. It would seem, therefore, that the second chromosome of strain 13 carries a gene (or genes) that does exert an effect on the erupt
response, when substituted into strain 36, which is different from
the effect exerted on the erupt response in strain 36 by its own
second chromosome (Su-er).

It is possible that the response of strain 13 with respect to
the suppressor of erupt and erupt loci has changed in the interim
between 1941 and the present, so that alleles which were non-effec-
tive in 1941 are now effective. Considering the comparable results
obtained in the present investigation with the suppressor-erupt
strain as compared with those obtained for the same strain by Glass
(1944), and Glass and Plaine (1952), one would not expect any more
of a change in strain 13 with respect to the suppressor-erupt system
than would be expected in strain 31. These comparisons indicate no
highly significant change in the suppressor-erupt system in strain
31, and the slight difference observed was toward a decrease, not an
increase, in the frequency of extreme erupt while total erupt re-
mained at the same level. While some change during the interim can-
not be ruled out completely with respect to the erupt response in
strain 13, it seems more likely that the low number of flies examined,
combined with lowered viability after X-irradiation, and a low pene-
trance of extreme erupt, accounted for Glass' failure to observe
extreme erupt in the original experiments with strain 13 (al b c sp^2).
Failure to recognize disarranged facets as an expression of weak erupt
in these first irradiation experiments would account for the lack of
this category and that of total erupt in the original experiments.
Because results obtained in the present investigation indicate the presence of effective alleles of the suppressor-erupt -- erupt system in strain 13 and strain 36, the relationship of the two major loci with respect to the production of the mutant phenotype after X-irradiation was analyzed. Since, however, whole chromosomes were substituted, the effect of the specific loci cannot be distinguished from the presence of additional modifying loci which may also be present on the same chromosomes as the suppressor-erupt (second chromosome) and erupt (third chromosome) loci.

It is clear from the present investigation that X-irradiation in air alone is not a sufficient basis for comparison of the erupt response among the various suppressor-erupt -- erupt combinations. X-irradiation in air reveals the initial levels of expressivity and penetrance for any given strain with respect to the suppressor-erupt system, but these levels of penetrance overlap to a great extent. It is only after X-irradiation in pure oxygen that finer differences in the response of the suppressor-erupt systems in various strains for specific combinations of second, third, and X chromosomes become apparent.

Previously, it was thought that the already high level of penetrance and expressivity of the suppressor-erupt -- erupt combination in strain 31 (Su-er;er) after X-ray in air made it difficult to distinguish any further enhancement of the penetrance or expressivity in this strain after X-irradiation in oxygen. Substitution of the second chromosome and its suppressor (Su-er) from strain 31, in
combination with the low penetrant erupt alleles (er+), in strains 36 and 13, revealed a general lack of enhancement of penetrance and expressivity after X-irradiation in oxygen (refer to strain 31 III 36, strain 13 II 31, and strain 36 II 31, Figure 7). In strain 36 II 31, where the second chromosome of strain 31 (Su-er) is present with the third and the X chromosomes of strain 36, there is an actual decrease in the levels of expressivity and penetrance of the erupt response between X-irradiation in air and in oxygen (refer to 31 III 36 and 13 II 31 vs. 36 II 31, Figure 7). It also seems that the second chromosome of strain 31 (Su-er) is more effective in preventing the expression of extreme and total erupt than is the second chromosome of strain 36 (also Su-er) (refer to 36 vs. 36 II 31, Figure 8). These two second chromosomes, with respect to their suppressor of erupt, differ in their ability to suppress the manifestation of erupt after X-irradiation in oxygen. These two chromosomes are theoretically the same since that in strain 36 was originally derived from strain 39 about 1954. Genetically though, they differ in their effect against the same erupt allele (er+) in the same genetic background (i.e., that of strain 36) (refer to 36 vs. 36 II 31, Figure 8).

Enhancement of the effect of the suppressor of erupt from strain 31 in combination with the X chromosome of strain 36, as well as differences in the effect of the suppressor of erupt of strain 36 when in combination with its own X chromosome and when in combination with the X chromosome of strain 13 (refer to 36 III 13 vs. 13 II 36, Figure 7), suggests that the X chromosome of strain 36 may possess
modifiers which parallel the action of the second chromosome suppressor of erupt. Using backcross tests to investigate the constitution of 15 wild-type strains with respect to the suppressor-erupt system, Glass (1957) found no evidence for any suppressors of erupt in the X chromosome. However, Plaine (1957), using chromosome substitution tests, found evidence that the X chromosome in the Swedish-b strain carries a modifier of erupt but it does not act differently in the two sexes.

There does not seem to be any appreciable change between the effect of the third chromosome (erupt locus) of strain 36 and that of strain 13 after the interim of about 12 years (refer to 13 vs. 36 13 and 36 13, Figure 8). This comparison may be deceptive since the presence of the X chromosome of strain 36 may enhance the effect of the suppressor of erupt upon the response of the erupt allele present with it. That this might be the case is suggested by the higher penetrance of erupt after X-irradiation in air and in oxygen associated with strains 13 36 and 13 36, which have the same erupt allele as strains 13 and 36 13, but which do not have the X chromosome of strain 36 and strain 36 13.

Burnet and Sang (1964a,b), working with the melanotic tumor system, observed an increased incidence of tumors after X-irradiation in air in both the suppressor-erupt strain (Su-er tu bw; st er su-tu) and in strain 36 (Su-er tu bw; er su-tu). Based on the reports of Glass (1944, 1957), Glass and Plaine (1952), Plaine and Glass (1952), and Plaine (1955a), the mode of action of X-irradiation in revealing
erupt and melanotic tumors in *D. melanogaster* was through an indirect effect due to interference with the action of the specific suppressor. This being the case, no enhancement of the penetrance of tumors or of erupt would be expected unless an effective allele of the suppressor were present together with an effective allele of the specific mutant gene. Since, at the time of the work of Burnet and Sang (1964a,b), it was assumed that strain 36 (Su-er tu bw; er⁺ su-tu⁺) did not have an effective suppressor of tumor, this increase in tumor penetrance in strains homozygous for the tu second chromosome (irrespective of the allelic combination present at the suppressor locus) was considered to be due to a direct effect of X-rays on the penetrance of the tumor gene rather than to an indirect effect on the action of the suppressor. Studies with the al b c sp² strain (Su-er⁺ tu⁺; er⁺ su-tu⁺) and with strain 36 by Gretchen Brooks at The Ohio State University (personal communication), with respect to the tumor system in these strains, seem to confirm my findings concerning the effectiveness of the suppressor of erupt and erupt alleles of the suppressor-erupt system in these same strains. Thus, the suppressor of tumor (formerly designated su-tu⁺) is an effective suppressor of the tumor allele which occurs in the al b c sp² strain just as the suppressor of erupt (formerly designated Su-er⁺) in this same strain is an effective suppressor of the erupt allele which occurs with it in this strain. While these suppressors are effective against the alleles which normally occur with them in the al b c sp² strain, they may not be completely effective against the tumor allele from any other strain.
Results obtained in this investigation with the suppressor-erupt system indicate that the greater part of the effectiveness of X-rays, either in air or in oxygen, is directed more against the action of the suppressor of erupt than against the action of the mutant alleles. Six of the strains used in the present study may be divided into two groups (strains 36, 36 II₁₃, and 36 II₃₁) and (strains 13, 13 II₁₃, and 13 II₃₁). The strains within each group have the same allele of erupt (i.e., the same third chromosome) and the same X chromosome, but have a different suppressor of erupt (i.e., different second chromosome) in each strain. Results obtained, particularly with respect to total erupt after X-irradiation in oxygen, indicate that even with the same allele of erupt and the same X chromosome there are differences in response of the suppressor-erupt system among the strains in each group which can be accounted for only by the difference in suppressor alleles present (Figure 8).

Demonstration that the reputed wild-type, non-effective Su-er⁺ and er⁺ alleles of the suppressor-erupt system, particularly in the al b Su-er⁺ c tu⁺ sp²; er⁺ su-tu⁺ strain and in strains derived from it, are in reality low penetrant though effective alleles offer additional opportunities for further and more detailed analyses not only of the suppressor-erupt system but of the suppressor-tumor system as well. Interpretations and conclusions arrived at from previous studies of both the suppressor-erupt and the suppressor-tumor systems, which were based on the fallacious assumption that the alleles
at the suppressor loci and the mutant loci, which are symbolically designated as "+" (i.e., Su-er<sup>+</sup>;er<sup>+</sup> and tu<sup>+</sup>;su-tu<sup>+</sup>), were in fact normal, wild-type, non-effective alleles, need be subject to re-examination.

Moreover, assuming that the genes, either at the suppressor loci or the mutant loci, exist in only two alternative allelic states (i.e., Su-er vs. Su-er<sup>+</sup>; er vs. er<sup>+</sup>; tu vs. tu<sup>+</sup>; and su-tu vs. su-tu<sup>+</sup>) has led to erroneous interpretations. As Glass (1957) has already concluded, there exist numerous wild-type isoalleles of erupt of differing potency and at least the same number of significantly different suppressor of erupt alleles. So too, the mutant form of the erupt gene must exist in several allelic states having different potencies, some of which undoubtedly approach the threshold of response of very weak "normal" alleles of erupt which produce the erupt phenotype under certain conditions. That this same kind of situation probably exists for the suppressor-tumor system has been found by Gretchen Brooks (personal communication).

The existence of alleles and isoalleles which vary in dominance as well as in potency is a very common genetic phenomenon and Glass (1957) further concluded that suppressor-mutant systems composed of various combinations of alleles at each locus which preserve the wild-type phenotype are more common that the polygenic type of modifier system. Nevertheless, while the major response of the suppressor-erupt system may be attributable primarily to the various allelic combinations present at the suppressor and the mutant loci, the
response and the analysis of the response must be considered relative to the complete genetic background under any given set of environmental conditions.

Among drosophila workers, an expedient though not particularly accurate method for analyzing the relative roles of specific genes or of whole chromosomes depends upon the segregation of backcross progeny. According to this method, a given (unknown) strain is crossed to a tester strain which carries recessive genetic markers on whatever chromosomes one wishes to test in the former strain. The F₁ males are then backcrossed to the tester strain. The resulting backcross progeny segregate into different types. The only chromosomes engendered homozygous among the progeny are those of the tester strain; therefore, unless the genes and chromosomes of the unknown strain being tested are effective in the heterozygous state, no response may be detected. Moreover, if the initial cross is between unknown males and tester females, the X chromosome of the former is not present in the F₁ males and its backcross progeny. If the reciprocal cross is made, the unknown X chromosome is heterozygous in backcross females but absent in backcross males. Consequently, no evidence relative to an effect of the sex chromosome, or a lack thereof, can be inferred. Lastly, because classification of the backcross progeny into their respective types must await the emergence of adults, it is not possible selectively to irradiate or otherwise treat embryos of a given genotype to the complete exclusion of other genotypes among the backcross progeny.
Certainly, then, more efficacious is the method of actual chromosome substitution or replacement, whereby a given chromosome from one strain replaces its homologue in another strain and all chromosomes are rendered homozygous in the process. Comparisons may then be made between chromosomes within strains as well as between strains, under given environmental conditions. It is also possible to test more accurately whether or not additional modifiers exist on the X or fourth chromosomes. In light of the results obtained by Plaine (1957) and of those reported in my study, the X chromosome deserves more consideration and requires more study than has heretofore been afforded it. Known parental as well as substituted and reconstituted genotypes may be selectively irradiated and compared with other irradiated or non-irradiated genotypes.

Relative to penetrance and expressivity, the erupt response falls into four major categories - the enhancement of neither, the enhancement of both, or the enhancement of one but not the other. Which of these responses is achieved undoubtedly reflects the genic interaction of the alleles at the two major loci against the residual genetic background. Irradiation in oxygen provides more insight into these finer differences of penetrance and expressivity which are associated with the various combinations at the suppressor of erupt and erupt loci than does irradiation in air alone.
A specific suppressor system, the suppressor-erupt — erupt, was analyzed in several strains of *Drosophila melanogaster*. The mutant phenotype associated with the erupt gene is characterized by breaks or holes in the surface of the eye, frequently with an abnormal growth or protrusion. Weaker manifestations of erupt may be characterized only by disarrangement of the facets of the eye.

Embryos of three parent strains (the Su-er tu bw; er\(^+\) su-tu\(^+\) strain, the al b c sp\(^2\) or Su-er\(^+\);er\(^+\) strain, and the Su-er bw; ster or suppressor-erupt strain) and seven strains derived from them by chromosome substitution were exposed to X-irradiation in air and in 100% oxygen at 12±12 hr of embryonic development.

Flies with the phenotype characteristic of extreme erupt were found consistently in irradiated series of each strain tested. The er\(^+\) alleles in the third chromosomes of the al b c sp\(^2\) and Su-er tu bw;er\(^+\) su-tu\(^+\) strains are equally effective in producing extreme erupt after X-irradiation in air, even when in combination with the effective Su-er allele in the second chromosome from the suppressor-erupt strain. The supposed wild-type, non-effective er\(^+\) alleles in third chromosomes of the Su-er tu bw; er\(^+\) su-tu\(^+\) strain and the al b c sp\(^2\) strain are found to be effective erupt alleles, although of lower penetrance and expressivity than the mutant er allele present in the suppressor-erupt strain.
There is no significant difference between the frequencies of total erupt produced after X-irradiation in the suppressor-erupt strain used in this investigation and those found originally in earlier studies on this strain. A decrease in the expressivity of the suppressor-erupt system in the suppressor-erupt strain may be indicated by the lower frequency of extreme erupt out of the total erupt class which was obtained in the present study of this strain.

Differences in penetrance and expressivity, particularly after X-irradiation in 100% oxygen, obtained in the Su-er tu bw; er+ su-tu+ and al b c sp2 strains after replacement of their respective second chromosomes by the second chromosome of the suppressor-erupt strain indicate some change between the original suppressor-erupt second chromosome and that now present in the Su-er tu bw; er+ su-tu+ strain.

Additional modifiers on the X-chromosome of the Su-er tu bw; er+ su-tu+ strain which act to enhance suppression of the erupt response are also suggested by results obtained with several substitution strains.

The Su-er+ allele in the second chromosome of the al b c sp2 strain is an effective suppressor of the third chromosome er+ allele present with it in this strain and is equally effective against the er+ allele present in the third chromosome of the Su-er tu bw; er+ su-tu+ strain.

The penetrance and expressivity of the er+ allele after X-irradiation differed among substitution strains having the same er+
allele together with different suppressor of erupt alleles in the second chromosome. Action of X-rays by direct enhancement of the effect of the erupt alleles is not supported from the results obtained in this investigation. The major action of X-rays in producing the erupt phenotype appears to be an interference with the action of the suppressor of erupt associated with any given er or er\textsuperscript{+} allele.

The presence of oxygen during irradiation exerts a differentiating effect on the expression of erupt in the various strains tested. Combinations of second and third chromosomes with the same levels of extreme and total erupt after X-irradiation in air were found to differ from each other after X-irradiation in 100% oxygen.
LITERATURE CITED


